CRANFIELD UNIVERSITY

PAUL BABAJIDE OLADIMEJI

DISC1 AND NEUROGENESIS IN SCHIZOPHRENIA AND OTHER MAJOR PSYCHIATRIC DISORDERS: A POST-MORTEM STUDY OF THE HUMAN HIPPOCAMPUS

CRANFIELD HEALTH
Translational Medicine

PhD
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ABSTRACT

Psychiatric illnesses are disorders that affect millions worldwide. Evidence from quantitative and molecular genetics analysis suggests a strong genetic component to these disorders. There is also evidence that embryonic neurodevelopment is a key period in the progression schizophrenia. The aim of the present study was to use post-mortem human hippocampus from subjects of a variety of psychiatric phenotypes to investigate neurodevelopmentally-relevant gene expression in this region of the adult human brain. Particular interest is paid to schizophrenia risk gene DISC1; it has been shown to exhibit linkage and association to schizophrenia and is highly involved in embryonic and post natal neurodevelopmental processes. The results reported in this study indicate that DISC1 binding partners, and genes used to mark neurogenesis, can be found aberrantly expressed in schizophrenia and bipolar disorder, relative to controls. The results also suggest that *DISC1* genotype may predict expression patterns of DISC1 binding partners and neurogenesis markers, irrespective of diagnosis. This may provide clues to the timing and nature of abnormal brain development in this illness and aid in development of treatment strategies.

Keywords:

Psychiatry, Gene Expression, Neurodevelopment, Dentate Gyrus

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I would like to dedicate this thesis to a dear friend, Zoe Anderson, who passed whilst I was studying at Cranfield. Here's to you!

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1 INTRODUCTION

1.1 Schizophrenia and Other Major Psychiatric Disorders

The term ‘schizophrenia’ was introduced into the psychiatric lexicon by Paul Eugen Bleuler to describe what he saw as a psychiatric condition characterised by a splitting of psychic functions[1]. Originally conceived as dementia praecox, a mental illness of deterioration and abnormal behaviour characterised by an early onset, Bleuler developed on the earlier ideas proposed by Benedict A. Morel and Emil Kraeplin by envisaging a group of syndromes of mental dysfunction distinct from dementias[2]. In doing so, Bleuler used the term schizophrenia to progress from the observational to a more explanatory terminology. When translated from the ancient Greek, schizophrenia alludes to a splitting of the mind. It is believed that this ‘splitting’ is a reference to a loss of coherence and co-ordination between aspects of mental function, i.e. the cognitive and the affective[2].

Schizophrenia has since become the term used to describe a disease that is estimated to affect millions of people worldwide with a point prevalence of 0.46%[3,4]. Schizophrenia has a complex symptomatology. Symptoms can range from delusions to depression, apathy to anhedonia and lethargy to loss of insight, all encompassed within a spectrum of positive and negative symptoms. The positive symptoms are those that indicate a supra-normal behavioural state i.e. beyond the range of what is deemed normal (delusions, hallucinations, thought disorders etc.). The negative symptoms are those characterised by a behavioural deficit (anhedonia, alogia, aboulia etc.).

Diagnosis of schizophrenia requires symptom manifestation over a minimum period of 6 months. This must be to such an extent that it impairs one’s ability to lead a productive lifestyle. At least one of the core positive symptoms, i.e. hallucinations, delusions or disorganised speech, must be present. These core symptoms can present at varying degree of severity, but at least one other symptom must also be present over the 6 month period (Diagnostic and Statistical Manual of Mental Disorders-V, DSM-V).

The most recent DSM eliminated schizophrenia subtypes and the special exceptions for ‘bizarre delusions’ and ‘schneiderian first-rank auditory hallucinations’, which were previously accepted alone as sufficient for diagnosis.

DSM-V also made further considerations in the delineation between the major psychiatric disorders, bipolar disorder (BPD) and major depression disorder (MDD, also known as unipolar depressive disorder). Major depressive disorder (10-15% lifetime prevalence[5]) is a debilitating disorder of recurring major depressive episodes with a high mortality and morbidity[6]. Depression is required to sustain for 2 weeks, impinging significantly on the patient’s life, for the clinical classification as a major depressive episode (DSM-V).

Bipolar disorder is a recurring pathological mood disorder, of manic and hypomania. Mood and activity, over the period of a week, are accepted specifiers for the manic and hypomanic episodes, however cognitive disturbances and psychosis also feature. Bipolar subtypes include depressive symptoms with either; mania (Subtype I) or hypomania (Subtype II), bipolar type schizoaffective disorder is now only diagnosed if either mania or hypomania are present for the majority of the schizophrenia illness. The recent addition of ‘other specified bipolar and related disorder’ accounts for cases where the bipolar symptoms are not sufficient for a bipolar disorder diagnosis (DSM-V).

1.2 Neurobiological Findings

In an attempt to extend our knowledge of schizophrenia, researchers have sought to identify its neuroanatomical, neurochemical and cytoarchitectural correlates. Though there has been no distinct diagnostic pathology found to date, there are some neurobiological findings that have proven quite robust when studying the brains of patients with schizophrenia.

A general reduction in brain volume and an increase in ventricle size were reported in the initial attempts to find an anatomical basis for schizophrenia through pneumoencephalography[7]. With the development of imaging techniques such as magnetic resonance imaging (MRI) a greater

degree of detail has now become available. While providing more evidence for the earlier volumetric findings, imaging studies have also enabled researchers to particularise brain volume reductions to structures such as the prefrontal and superior temporal gyri[5], the hippocampus[8] and the thalamus[9]. The amygdala has also been shown to exhibit significant reductions in patients[10] while the third and lateral ventricles have been shown to be significantly increased[11]. Cerebral asymmetry has also been reported[12].

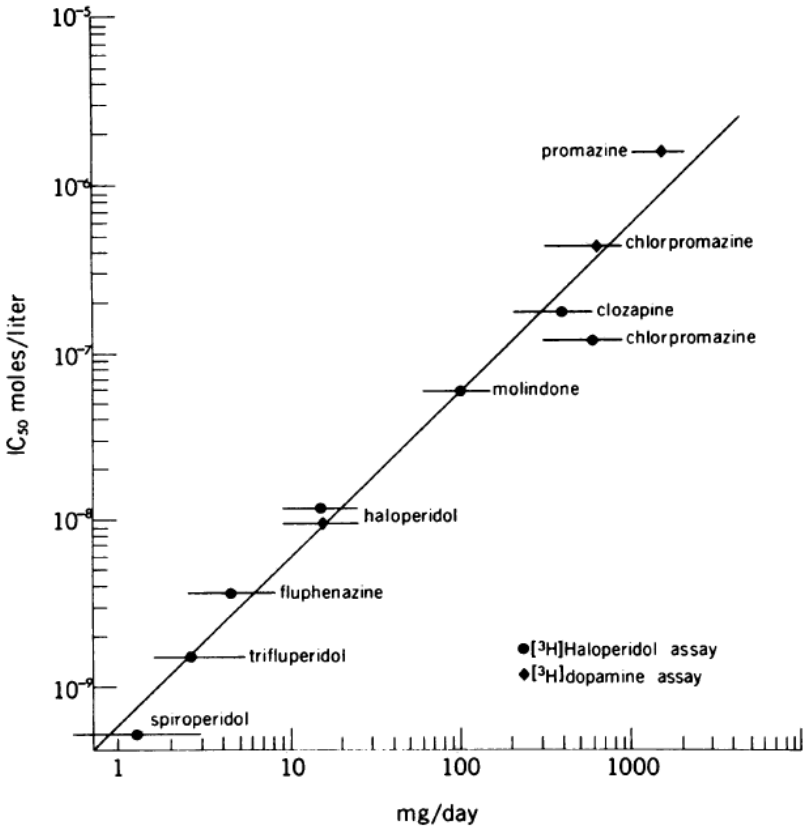
Some more recent studies have attempted to correlate these neuroanatomical alterations to the specific neurocognitive deficits sometimes seen in schizophrenia[13]. Nevertheless, it is important to remember that many of the neuroanatomical alterations reported in schizophrenia are extremely subtle, showing considerable overlap with non-psychiatric controls. For example, Steen and colleagues[14] reported the average reduction in the brain volume of schizophrenia patients to be only 3%; this was only just above the resolution limits of detection for the MRI methods of the time.

Neuroanatomical abnormalities have also proven an important correlate of major depressive disorder. The dorsolateral prefrontal cortex (DLPFC), the anterior cingulated cortex (ACC), the lateral orbital prefrontal cortex, the ventral striatum and the nucleus accumbens are all brain regions where neuroanatomical abnormalities have been shown to persist in brains of major depressive disorder patients[15; 16]. A full review of the early neuroanatomical reports concerning major depressive and bipolar disorders was compiled by P. Harrison[17].

An increase in dopaminergic signalling has been proposed as a neurochemical feature of schizophrenia on the basis of pharmacological findings (e.g. Carlsson, 1978[18]). Seeman and colleagues[19] found that the first generation antipsychotics treated schizophrenia with a clinical potency that directly correlated to dopamine receptor D2 blockade (see Figure 1-1). An important role for dopamine in schizophrenia is supported by Laruelle and colleagues[20] who used Single Photon Emission Tomography (SPET) to show that patients with schizophrenia exhibited a significantly greater level of

dopamine release in response to an amphetamine challenge. These findings helped to provide the rationale for the dopamine hypothesis of schizophrenia[21].

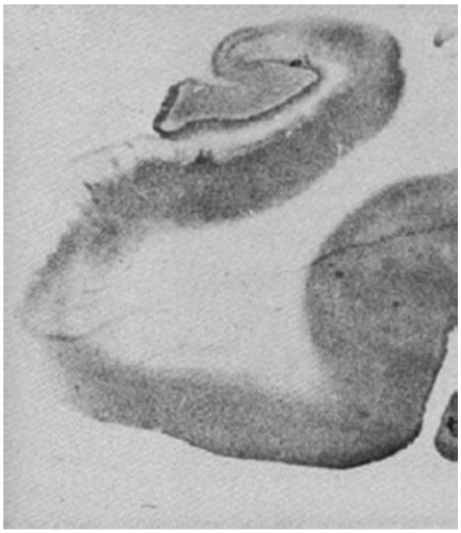
Contrary to first generation antipsychotics, second generation antipsychotics do not have such high affinity for D2 receptors but have comparable clinical potencies. Ergo, other neurotransmitter systems have been implicated in schizophrenia pathophysiology[22]. The evidence for a role of glutamatergic neurotransmission includes Krystal and colleagues[23] who reported that the symptoms seen in subjects treated with a non-competitive N-methyl-D-aspartate (NMDA) antagonist resembled much of schizophrenia's positive and negative symptomatology. There have also been reports of decreased levels of glutamate in the cerebrospinal fluid of patients with schizophrenia[24].



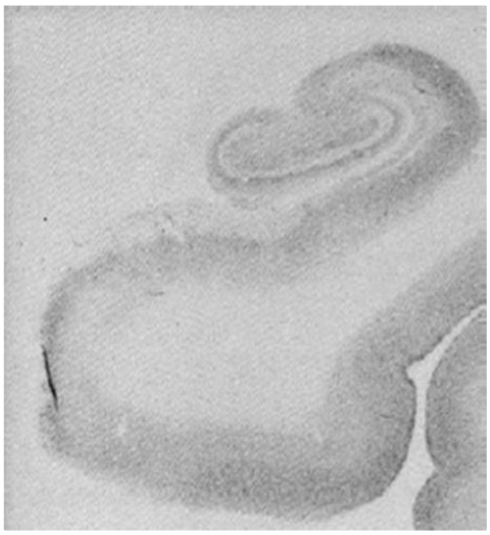
1-1 From Seeman et al.[19] A graph demonstrating the correlation between D2 receptor affinity and clinical potency of first-generation antipsychotic medication. The x-axis shows the recommended clinical dosage; the y-axis describes the half maximal inhibitory concentration.

Numerous neurotransmitters abnormalities have been reported in the brains of bipolar disorder patients. Elevated levels of noradrenaline turnover have been particularised to the frontal and occipital cortices as well the thalamus[25]. Contrarily, the researchers also detected reductions in metabolites associated with serotonin neurotransmission[25]. For major depressive disorder, prominent amongst the neurochemical findings has been data implicating the brain-derived neurotrophic factor (*BDNF*). Animal studies simulating stress have reported reductions in BDNF mRNA expression[26], sera levels of BDNF have been demonstrated to undergo reductions in untreated patients[27] and post-mortem analyses have reported reduced BDNF and neurotrophin 3 (NT-3) expression levels in the brains of subjects who commit suicide in comparison to non-suicide counterparts[26].

The cytoarchitectural changes that have been observed in schizophrenic patients include aberrant clustering of neurons in neocortical white matter[28] and in the entorhinal cortex (especially in lamina II)[29; 30]. Neuronal size reductions in the neocortex[31] and the hippocampus[32] have also been reported, along with a reduction in the number of dendritic spines[33] and oligodendrocytes present[34]. Additionally, reductions in the abundance of synaptic markers such as synaptophysin[35], synaptosomal-associated protein 25 (SNAP-25)[35] and complexin II[36] have been shown to be present in the brains of patients suffering from schizophrenia (see Figure 1-2). This has proven consistent with The Feinberg hypothesis which states that schizophrenia is induced by aberrant synaptic pruning. First episodes of schizophrenia usually present in the late puberty / early adulthood[37,38], this coincides with the synaptic maturational process of cortical pruning.



Control Subject



Schizophrenia patient

1-2 From Eastwood and Harrrrison[39] Immunoautradiograph of the synaptic marker protein, synaptophysin in the hippocampus. In this study, the control subjects had on average more of this protein than did the patients with schizophrenia reflected in the paler second image.

Both bipolar disorder and major depressive disorder have been demonstrated to associate with reductions in glial density[40]. However, cortical neuron numbers, which have been demonstrated to be relatively stable in bipolar disorder, have been shown to be reduced in Brodmann areas (BA) 6 and 47 in major depressive disorder[40]. Regarding bipolar disorder, recent reports have intimated that reduced white matter integrity may serve as an endophenotype in this illness[41].

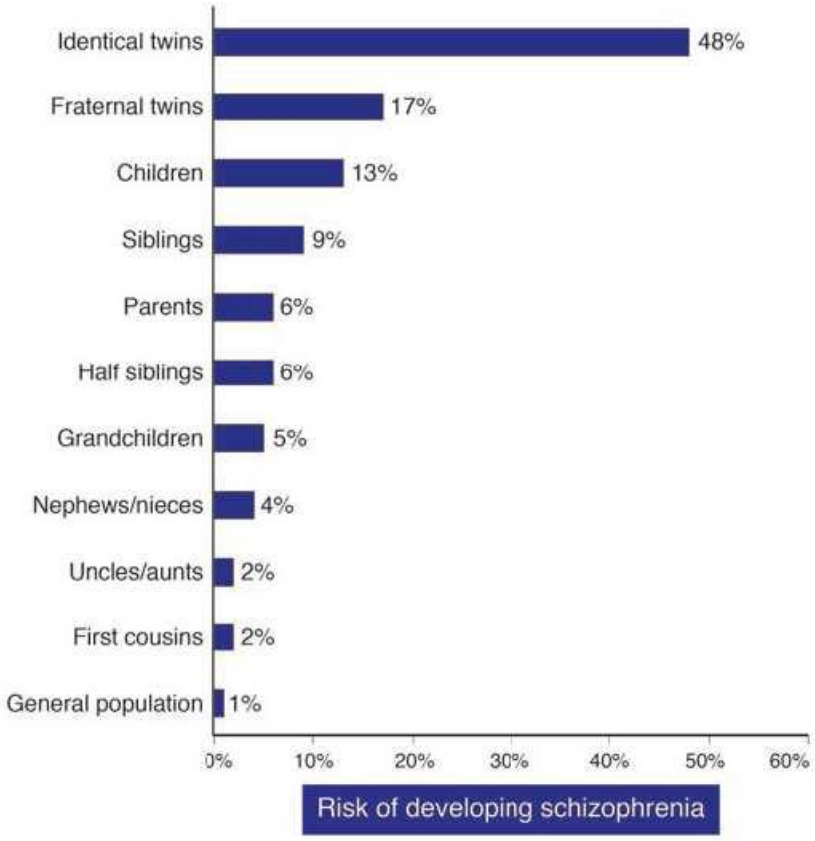
It is important to always consider that neurobiological findings of this nature may represent adaptive changes caused by the disorder and not be causative of the disorder. Many of these measures may also be liable to confounds such as pharmacological intervention. Consequently, the study of genes at the molecular level is likely to provide a unique insight into the aetiology of schizophrenia. *The genetic code is conserved throughout the course of an individual's life, irrespective of disease progression. Consequently, variations in the genome that can be statistically associated to schizophrenia can be directly attributed causative, though not necessarily deterministic, status*.

1.3 Genetic Literature

1.3.1 Quantitative Genetics

Genetic epidemiological studies suggest that in comparison with the general population, there is a 10-fold increase in the risk of developing schizophrenia for the children and dizygotic siblings of schizophrenia patients (Fig. 1-3). Furthermore, a review of the epidemiological data performed by Gottesman[42] estimated the average monozygotic concordance rate at 48% as opposed to the dizygotic concordance rate of 17%. Adoption studies have shown this risk to be significantly maintained even when the twins undergo separate upbringings[43].

Current estimates now put the heritability at around 80%[44]. Among the non-Mendelian genetic disorders it is thought that schizophrenia has one of the highest heritabilities[45]. As well as supporting the idea of schizophrenia's strong genetic component, the epidemiological data also suggests that there are other influences impacting on schizophrenia's manifestation. The environmental contribution is made implicit by the aforementioned monozygotic twin concordance being estimated at considerably less than 100%.



1-3 From Kirov et al.[45] Risk of developing schizophrenia in relatives of probands. The data are based on the review of studies compiled by Gottesman[42]**.**

For major depressive disorder, heritability has been estimated at 37%, with a 2-4-fold increase in the risk of developing the disorder for first degree relatives of probands[46]. There is also the evidence of a gender-difference in the prevalence of the disorder, with females twice as more likely to present with symptoms than males[47; 48]. The epidemiology also strongly suggests a genetic component to bipolar disorder. Patients commonly belong to families with a history of psychiatric illness, and the family history has proven an important clinical predictor of disease progression[49]. Twin and adoption studies evidence a bipolar disorder monozygotic concordance of 40-70%[49].

The manifestation of complex disorders such as schizophrenia and bipolar disorder is now understood to be the result of a complex interplay between environmental influences and numerous genes of small contributory effect[50; 51]. These genes are thought to have a combined effect lowering the threshold with which environmental influences are able elicit disease onset; an

increased susceptibility theory. The total numbers of genes that play a role are as of yet unknown.

Unlike Huntington's and the familial form of Alzheimer's, the hereditary pattern of major psychiatric disorders do not conform to Mendelian patterns of inheritance; the molecular genetic studies have failed to produce a single genetic marker for disease. Risch[52] concluded that, for schizophrenia, the probability of a genetic locus conferring a sibling relative risk (λ) of more the 3 was unlikely on the basis of the epidemiological findings.

1.3.2 Molecular Genetics

The proposition of a gene as a candidate for genetic association with psychiatric disorders is generally made on the following bases; functional candidate genes are proposed due to the nature of the protein product, cytogenetic candidate genes are proposed through their connection to a chromosomal abnormality that has been linked to disease, while positional candidate genes are proposed on the basis of linkage studies[45].

For a disorder such as schizophrenia, where limited aetiological information is available, linkage studies have proven informative in determining which chromosomal loci contain susceptibility genes. This is because they require no extra knowledge of the disorder other than its potential genetic basis. Furthermore, a particular type of linkage study, the nonparametric linkage analysis, makes no assumptions about the nature of the genetic inheritance.

Model-free linkage studies use multigenerational genetic data from families with numerous affected individuals to investigate the coinheritance between cases. These analyses hypothesise that genomic loci containing one or more susceptibility genes will be consistently inherited by affected relatives. These loci are demarcated by polymorphic markers, usually microsatellites, spaced a minimum of 10cM apart[53].

In keeping with the polygenic theory proposed by Gottesman and Shields[54], initial linkage analysis failed to produce any results of statistical significance. This failure is likely to be due to small sample sizes and

inadequate genetic marker density, all conferring insufficient statistical power on the studies. However, in the early 2000s two meta-analyses were published with greatly increased sample bases allowing for more rigorous statistical analysis[55,56]. Unfortunately there was minimal convergence in the genomic areas that these two papers proposed as susceptibility loci. Two loci that were both put forward by both papers were 8p and 22q.

Association studies offer a means detecting genes of smaller effect sizes than is possible with linkage analysis. They ascribe disease association to individual polymorphisms or haplotypes, comparing the alleles of affected and unaffected individuals in either population samples (case-control studies) or families (transmission disequilibrium tests, TDT). A significant difference in the 2 groups regarding the frequency of any particular allele or haplotype is deemed suggestive of an association between the allele and the disease. This means that the marker allele, or a marker in close linkage disequilibrium, confers a risk of disease upon carriers.

Of the positional candidates, Dysbindin-1 (*DTNBP1*) was found to contain several single nucleotide polymorphisms (SNPs) associated to schizophrenia after association mapping of the putative linkage region 6p22.3[57]. Associated SNPs were also found in D-amino acid oxidase activator (*DAOA*) after mapping in the 13q22-34 linkage region[57]. The region of linkage on chromosome 8p was found to contain the gene Neuregulin-1 (*NRG1*), a gene which has also exhibited associations to schizophrenia[58].

Some of the functional candidate genes include the neurotransmitter receptor genes dopamine receptor D3 (*DRD3*), dopamine receptor D2 (*DRD2*) and 5-hydroxytryptamine receptor 2A (*HTR2A*). The meta-analyses conducted by Glatt[59], Jonsson[60], Abdolmaleky[61], and their respective colleagues suggest that these genes are implicated in schizophrenia susceptibility. Though the odds ratios are small (< 1.2), the data suggest that variants within these genetic loci contribute to schizophrenia susceptibility. A recently implicated functional candidate gene is glutamate receptor, metabotropic 3 (*GRM3*). This gene codes for a G protein-linked glutamatergic receptor. Egan and colleagues[62] reported

over-transmission of a *GRM3* haplotype to schizophrenia cases versus controls. They also detected a weak association between a SNP marker and schizophrenia. Though these findings were unsubstantiated in a repeat sample; the authors reported that this added to the evidence for altered glutamatergic neurotransmission in schizophrenia.

Genome wide association studies (GWAS) extend the principle of association mapping. They treat the whole genome as a single chromosomal locus, investigating multiple markers simultaneously across the genome; as such they are hypothesis-free[63]. A sex-specific association of reelin (*RELN*) to schizophrenia has been suggested by a genome wide scan[64]. Recent genome wide association studies have implicated zinc finger protein 804A (*ZNF804A*) as a schizophrenia susceptibility gene[65], along with neurogranin (*NRGN*), Transcription Factor 4 (*TCF4*) and loci in the major histocompatibility complex (MHC) region on chromosome 6[66]. Further GWAS studies with increased statistical power are serving to increase our knowledge of the disorder, including quantifying the extent to which common polygenic variation contributes to schizophrenia and bipolar disorder risk[67].

Whole genome scans have also allowed researchers to identify uncommon (<5%) mutations existing below the resolution of light microscopy in disease populations. These mutations, generally copy number variants, have been found in greater numbers in schizophrenia[68] and autism[69], with effect sizes that are highly variable[70], The investigation of these uncommon, and sometimes rare (<1%) variants, has added much to our understanding of the genetic architecture of psychiatric disorders. Foremost has been the reconciliation of hypotheses focussing singularly on either common or rare variants[71]. CNV studies have also highlighted several chromosomal loci as important in psychiatric disorders, including 3q29[72], 17q12[73], 16p13.1[74], 16p11.2[75] 15q11-q13[76], as well as the vasoactive intestinal receptor 2 (*VIPR2*)[77] and neurexin (*NRXN1*)[78] genes.

Mutations above the resolution of light microscopy (cytogenetic abnormalities) have also implicated genes in schizophrenia. These include

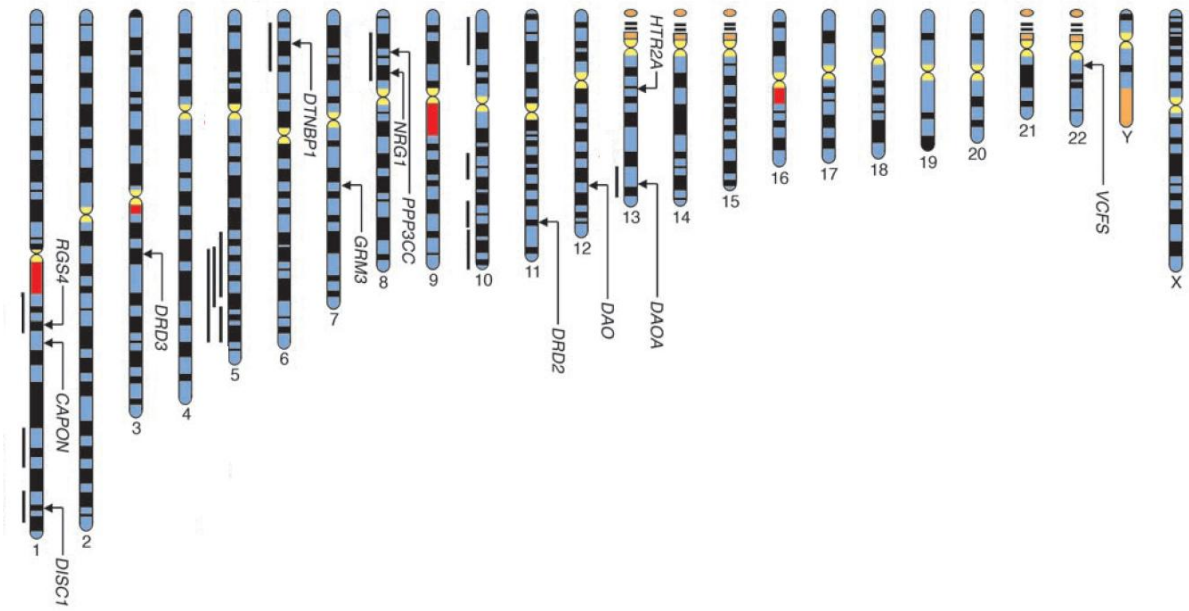
catechol-o-methytransferase (*COMT*), proline dehydrogenase (*PRODH*) and disrupted-in-schizophrenia-1 (*DISC1*) and disrupted-in-schizophrenia-2 (*DISC2*). The 22q11 chromosomal locus is deleted in patients with Velo-Cardio-Facial Syndrome. Patients with deletions in this locus have a higher risk of developing schizophrenia than the general population[79]. *COMT*, *PRODH* and zinc finger, DHHC-Type Containing 8 (*ZDHHC8*) are genes which map to this region. Association studies have also suggested that *COMT* may contribute to schizophrenia risk[80]. The existence of a *PRODH* schizophrenia susceptibility variant has been proposed due to animal data suggesting that it regulates reductions in prepulse inhibition similar to those seen in human patients with schizophrenia[81]. There have also been positive schizophrenia association findings from studies on both *PRODH*[82] and *ZDNNC8*[83].

A t(1:11) translocation within an extended Scottish pedigree was reported to co-segregate with schizophrenia, bipolar disorder or depression[84]. It was found that this translocation disrupted 2 genes; *DISC1* and *DISC2*. There is now also evidence for association between *DISC1* and psychiatric disorders in multiple population sets[85].

For major depressive disorder, linkage studies have largely failed produce consistent reports; although the 15q locus produced a linkage signal of relative note[86]. The investigations of candidate genes, such as brain-derived neurotrophic factor (*BDNF*); Solute Carrier Family 6 (Neurotransmitter Transporter), Member 4 (*SLC6A4*); 5-hydroxytryptamine receptor (*HTR2A*) and Tryptophan hydroxylase 2 (*TPH2*); produced similarly mixed results[87]. Initial GWAS studies also failed to identify loci of genome wide significance, though piccolo pre-synaptic cytomatrix protein (*PCLO*)[88] and cyclin D2 (*CCND2*)[89] were highlighted as genes worthy of further consideration. More interesting have been the reports of a joint increased risk for both major depressive and bipolar disorder linked to the chromosomal loci of 12q23[90] and 18q[91]. Further to this, a study jointly investigating major depressive and bipolar disorder identified 15 SNPs in a 248kb high linkage disequilibrium block on 3p21.1 as exhibiting linkage[92].

For bipolar disorder, linkage studies and candidate gene studies again failed to produce clear and consistent results. Some have argued that this may be due to poor SNP / gene choices and low sample sizes[93]. The GWAS have implicated numerous genomic regions; however the largest GWAS with an initial sample size of 16,731 and a replication sample of 46,912 subjects, detected a genome wide significance of one previously indicated gene (CACNA1C) and implicated another for the first time (ODZ4), a member of the teneurins family, thought to be involved in cell surface signalling and neuronal pathfinding[94]. Using this cohort, researchers have used an aggregated effect of risk variants to estimate the common polygenic variation of bipolar disorder to be 20-30%[67; 95]. Furthermore, pathway analysis of this cohort implicated the gene ontology category of 0015270, which relates to voltage gated calcium channel activity[96].

Recent data indicates an overlap in the genetic susceptibility for bipolar disorder and schizophrenia[67; 97]. In an interesting example of this overlap, family studies have reported an increased risk of bipolar spectrum disorders with the 22q11 deletion linked to schizophrenia[98]. Other notable evidences of a role for CNVs in the bipolar disorder include the reported risk conferred by the 3q29 locus, and the 4.8-fold increase in de novo CNVs in bipolar subjects[99]. Though SNPs and rare CNVs appear to be main contributory factors in bipolar disorder risk, dynamic mutations, mitochondrial DNA (mtDNA) and epigenetic variation have all also been implicated[100].



1-4 Adapted from Kirov et al.[45] A diagram depicting the human karyotype; rectangular bars and black arrows highlight a selection of chromosomal loci with reported linkage to schizophrenia.

1.3.3 Genetic Contribution To Disease

"Simplistically, genetic variation affects disease susceptibility in one of two ways. Either it changes the structure of the encoded protein (e.g. by an amino-acid substitution of frame-shift mutation) or it alters the expression of the gene (e.g. by altering some parameter of transcription or translation) and thereby the amount or distribution of the protein"[101]

Point mutations, such as SNPs, that have no effect on the amino acid sequence of the resultant protein are known as silent mutations. Many silent mutations occur within introns. When present within exons they exist on degenerate sites of triplet codons; this means that though the mature messenger RNA (mRNA) transcript will harbour the point mutation, it will have no effect on the amino acid sequence of the protein produced. These silent mutations are known as synonymous silent mutations. Conversely, non-synonymous (also known as missense) mutations can have a marked effect on protein structure as they alter the amino acid sequence of the protein. When non-synonymous mutations are associated to a disease, they are thought to confer disease susceptibility in large part due to an effect on the protein

structure and consequent function. When silent mutations are associated to disease they are thought to act wholly through an effect on gene expression. The majority of genetic associations to schizophrenia are intronic.

1.4 Evidence of a Neurodevelopmental Component

1.4.1 Cytoarchitectural, Neuroanatomical and Structural Findings

Important in the perceived neurodevelopmental component to psychiatric illness has been the lack of any consistent findings of neurodegenerative markers reported in the cytoarchitectural literature. Prominent amongst the subtle abnormalities found in the neuroanatomy of schizophrenia patients are the general and regionalised volumetric reductions (1.2 Neurobiological Findings). Without neurodegeneration to explain these abnormalities, researchers have proposed that they are the result of disturbances in the neurodevelopmental process[102].

There is now also growing evidence that points to a disproportionate number of minor physical abnormalities in schizophrenic patients[103]. These structural abnormalities, of which the minor craniofacial malformations figure prominently, develop during the gestational process. These premorbid findings predict that indicators of illness presage schizophrenia onset, suggesting a neurodevelopmental aspect to schizophrenia aetiology. The following will focus on a full account of some of the factors which suggest a schizophrenia neurodevelopmental model.

The neurodevelopmental component of bipolar and major depressive disorder is less clear, some of the neurodevelopmentally-relevant environmental risk factors for schizophrenia, do not appear to operate on bipolar risk. Additionally, children who later go on to develop bipolar perform similar to the general population in neuromotor and cognitive tests[104]. This has led researchers to broach the possibility that whereas schizophrenia may associate with neurodevelopmental abnormalities, bipolar disorder may not[104]. For major depressive disorder, researchers continue to argue that a better understanding

of disease neurobiology can be reached with a full appreciation of the proposed neurodevelopmental models of the disorder[105].

1.4.2 Environmental Factors

Obstetric complications (OCs) have been found to produce a small but significant increase in schizophrenia risk. Meta-analysis of the available data has concluded that the obstetric complications with the highest schizophrenia risk ratios were diabetes in pregnancy, low (<2000g) birth weight and emergency caesarean section ($p<0.03$)[106]. More recent retrospective studies have identified significant increases in the number of obstetric complications in the histories of high-risk individuals who later go on to develop schizophrenia[107].

Nevertheless, as with neurobiological findings, obstetric complications represent multi-factorial events that may not be directly contributory to schizophrenia but serve only to mark an underlying causative process. Thusly, researchers have also sought to identify the molecular mechanism behind this putative risk. It has been proposed that certain obstetric complications can promote foetal hypoxia and/or elevate maternal cytokine levels, attenuating the neurodevelopmental process and imbuing schizophrenia risk[108; 109].

1.4.3 Premorbid Findings

Retrospective studies have suggested that there are significant behavioural and cognitive disturbances in the childhoods of patients who later go on to develop schizophrenia. Premorbid deficits in cognitive performance, reductions in academic performance, lower IQ scores and a higher prevalence of attentional deficit disorders have all been reported[110;111]. The premorbid social skills of schizophrenia patients have also been shown to suffer significant deficits; anti-social behaviour and social withdrawal are significantly more prevalent in the childhoods of schizophrenia cases when compared to non schizophrenia controls[110].

1.4.4 Neurogenesis and Schizophrenia

In concordance with the theory of schizophrenia as a developmental disorder, connections between neurogenesis, and the genes involved in its regulation, have been reported with schizophrenia. For example, lithium, a mood stabilising drug used in psychiatric therapy, has been shown to stimulate neurogenesis[112].

1.4.4.1 Wingless-Type MMTV Integration Site Family (WNT)/β-catenin

The WNT family of secreted glycoproteins are involved in a variety of developmental processes in both vertebrates and invertebrates. There is growing evidence as to their specific importance to neurogenesis. Hall and colleagues[113] showed WNT7A to be involved in numerous processes including axonal modelling and synaptic differentiation. β-catenin, a downstream effector of WNT in the canonical WNT signalling pathway, has been shown to promote undifferentiated precursor cell proliferation and the development of dendrites[114]. It is also thought to recruit scaffolding molecules to developing synapses in order to aid in synaptic development[115].

Components of WNT signalling pathways have been reported as exhibiting distorted expression levels in schizophrenia. Reductions in glycogen synthase kinase-3β (GSK-3β), a downstream effector of WNT in the canonical signalling pathway, have been reported in the cerebrospinal fluid (CSF)[116], hippocampus[117] and also the prefrontal cortex[118] of schizophrenia patients when compared to controls.

1.4.4.2 Reelin

Reelin is an extracellular matrix glycoprotein expressed during embryonic corticogenesis. It is synthesised by Cajal-Retzius cells in the cortical marginal zone of the telencephalon and external granule cells[119]. During development, reelin has been shown to be involved in neural migration[120], laminar positioning[121] and dendritic development[122]. In adulthood, reelin is synthesised and secreted by almost all cortical and hippocampal γ-aminobutyric acid

(GABA) interneurons. It is postulated to modulate neurotransmission, memory formation and synaptic plasticity[122].

In schizophrenic patients, post-mortem reelin mRNA and protein levels have been found reduced by 50% in the hippocampus, temporal lobe and cortex[123,124]. DNA analyses of the *RLN* promoter have found an increased occurrence of methylation at positions 134 and 139 in schizophrenics as opposed to controls[125]. This has been corroborated by findings of increased expression of the methylating enzyme DNA methyltransferase 1 (DNMT1) in schizophrenic patients[126]. A direct genetic association of *RLN* with schizophrenia has also been suggested on the basis of case-control and TDT association studies[127; 128].

1.4.4.3 Retinoids

Since before the 1950s, vitamin A has been implicated in neurodevelopment[129]. In the early 1980s vitamin A's acid derivative, retinoic acid, was discovered to be an inducing signal in the chick embryo[130]. The retinoids have now been accepted as key regulators of the neurodevelopment process, acting on members of the steroid / thyroid hormone receptor superfamily. These activated receptors act as transcription factors to regulate the expression of other neurodevelopmental genes, including sonic hedgehog (*SHH)* and homeobox (*HOX)*, playing a central role in aspects of neurodevelopment ranging from neurogenesis to axon extension[131].

There is evidence of abnormal retinoid receptor levels in the dentate gyrus (see 1.6.1). Furthermore, the clinical and pathological anomalies observed in patients suffering retinoid dysfunction are similar to those seen in schizophrenia[132].

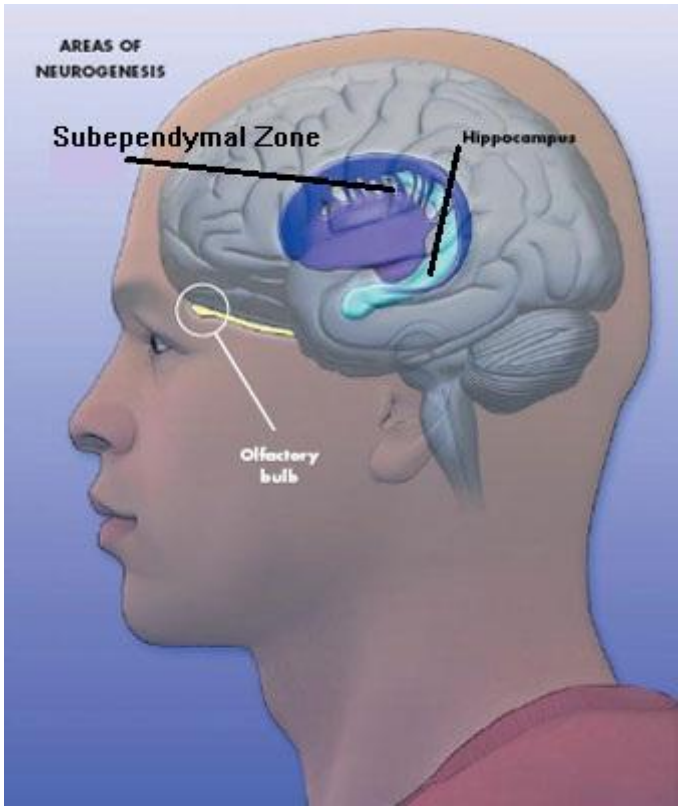
1.5 Adult Neurogenesis

By the time a psychiatric diagnosis is made, much of neurogenesis has expired. This is a considerable hindrance to attempts to analyse the role of neurogenesis in schizophrenia. Originally described by Altman and colleagues[133], adult neurogenesis refers to the process by which multipotent

neural precursor pools produce fully mature neurons in the adult brain. These precursor pools are preserved in two neurogenic ‘niches’ of the post-natal mammalian brain; the subependymal zone (SEZ) and the subgranular zone (SGZ). The developing neurons display differing migration dynamics contingent upon, though not necessarily determined by, the niche from which they derive. These neurons integrate into the neural circuitry, contributing to the brain’s neural architecture over an individual’s life and offering a means of potentially investigating neurogenesis in schizophrenic patients post-diagnosis.

1.5.1 Adult Olfactory Neurogenesis

The subependymal zone lines the anterior portion of the lateral ventricles; it is a region immediately apical to the ependymal cell bodies lining the ventricular space. Neuroblasts deriving from the SEZ migrate along channels collectively known as the rostral migratory stream (RMS). This stream traverses the SEZ to the olfactory bulb and is defined by a subgroup of specialized glial cells, the elongated walls of which form the channels developing neuroblasts migrate along. Upon reaching the olfactory bulb the developing neurons differentiate into interneurons, integrating into the local circuitry[134].



1-5 Adapted from the Society for Neuroscience website (www.sfn.org) Diagram showing critical regions in the brain for adult neurogenesis.

1.5.2 Adult Hippocampal Neurogenesis

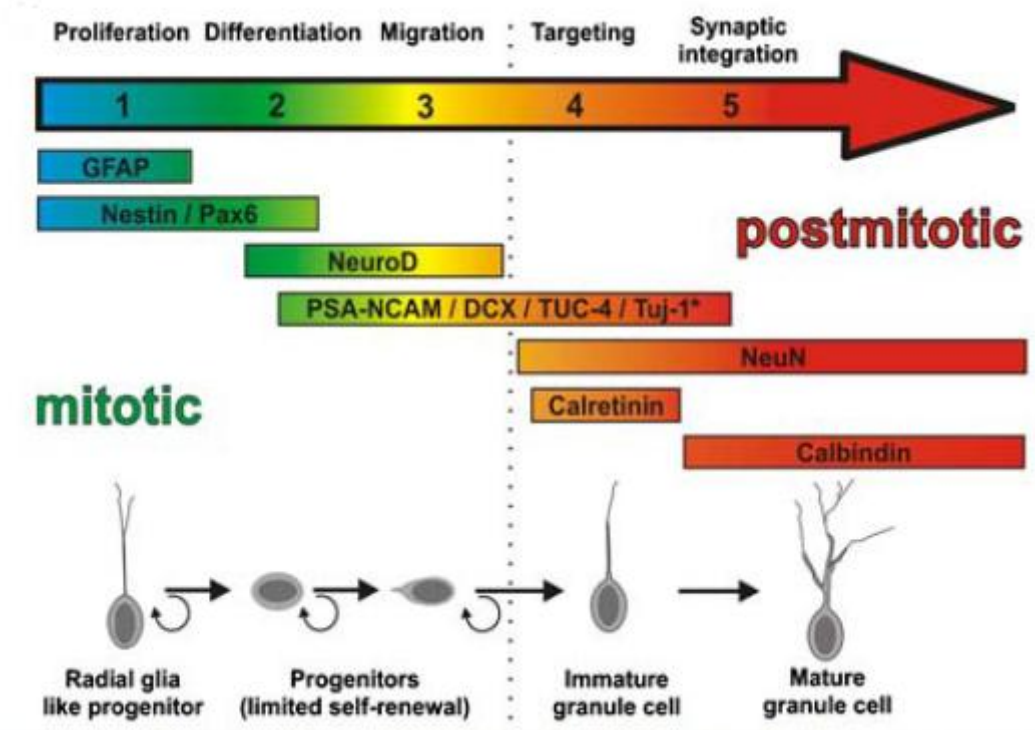
Adult hippocampal neurogenesis occurs with the dentate gyrus which is a subfield of the hippocampal formation. The subgranular zone (SGZ) is a thin layer of cells approximately 3 nuclei in thickness between the granule layer and the hilus of the dentate gyrus[135]. These cells give rise to dentate granule cells which, having migrated tangentially as neuroblasts, elaborate processes, including axons extending to the cornu ammonis 3 (CA3) layer of the hippocampus.

1.5.3 Immunohistological Markers

With the development of thymidine analog incorporation techniques [136], researchers were able to label cells undergoing mitosis, indicating erstwhile instances of neurogenesis in animal tissue. However, this technique is prohibited from use in schizophrenia patients due to the inherent mutagenic

properties of the utilised analogues. They also indiscriminately mark neurons and glia, further limiting their use experimentally.

When using tissue from post-mortem samples, immunohistological markers are used to indicate neurogenesis (Appendix A.3). These markers are preferably proteins that are expressed transiently during adult neurogenesis only; and not in either mature neurons or neurons regenerating after injury. Though numerous proteins have been used as neurogenesis markers, researchers caution against the use of any single marker as a sole determinant of neurogenesis [137].



1-6 From Von Bohlen and Halbach[138] A diagram showing the generation and development of new neurons within the granular layer of the DG divided into five stages. At each stage, different specific molecules are expressed in the developing neuron, some of these markers have the potential to be used to quantify and qualify adult neurogenesis[NeuN = neuron-specific nuclear protein; GFAP = glial fibrillary acidic protein; PSA-NCAM = polysialylated neural cell adhesion molecule; TUC-4 = CRMP4 (collapsin response-mediated protein4); Tuj-1 = class III β-tubulin].

1.5.3.1 Doublecortin (*DCX*)

Doublecortin is the founding member of a superfamily of genes which includes doublecortin-like kinase (*DCLK*) genes *DCLK1*, *DCLK2* and *DCLK3*, retinitis pigmentosa-1 (*RP1*) and doublecortin-domain containing 2 (*DCDC2*). The common feature between all *DCX* superfamily members are microtubule-binding properties of the gene products conferred by their *DCX* domain. Though the primary function of this domain is microtubule association[139], the superfamily gene products are involved in a variety of cellular processes; DCLKs are proteins with kinase activity, RP1 is a photoreceptor linked to the retinitis pigmentosa disease and DCDC2 is linked to dyslexia.

The founding *DCX* gene is located on the X chromosome, it encodes a product 360 amino acids (aa) in length and contains two *DCX* domains and a serine, threonine and proline-rich (SP-rich) domain. As determined by its molecular domain, the DCX gene product is a microtubule-associated protein (MAP). Classic MAPs bind using a random coil structure, whereas DCX-domain containing proteins utilise ubiquitin-like folding patterns. The DCX domains are 80 amino acids in length and at least two are required for sufficient microtubule binding function[140]. Upon binding, DCX has been demonstrated to stabilise microtubules, counteracting their dynamic depolymerisation.

DCX is thought to function transiently in the extremities of migrating neurons. Its cellular expression has been localised to migrating neurons of the RMS and tangentially migrating neurons of the developing embryonic cortex[141]. It is transiently expressed in immature post-mitotic migrating neurons. Expression begins 1 day after the process of mitosis ceases and lasts for a period of approximately 2-3 weeks, at this point the abundance of the protein in the cell begins to subside[142]. It is on this basis that DCX has been proposed as an endogenous marker of neurogenesis.

1.5.3.2 Neurogenic Differentiation Factor 1 (*NeuroD1*)

The *NEUROD1* gene codes for a member the basic-helix-loop-helix (bHLH) family of transcription factors, characterised by the structural motif which shares its name. *NEUROD1* has been demonstrated to be proneural,

transiently-expressed, and critical to normal neurodevelopment, especially in hippocampal and cerebellar granule cell development[143; 144]. In adult hippocampal neurogenesis, *NEUROD1* expression is enough to promote neuronal differentiation and to some degree maintain newborn neuron numbers[145].

The *NEUROD1* gene contains transcription factor / lymphoid enhancer-binding factor (TCF/LEF) regulatory elements, downstream effectors of the WNT pathway. WNTs are also a regulators of neurogenesis[146]. Nevertheless, until recently an interaction between WNT and NEUROD1 had yet to be characterised. *NEUROD1* activation is now understood to reflect the WNT regulation of intracellular β-catenin, accumulation of which leads directly to the transcription of *NEUROD1* via its TCF/LEF elements[147].

Furthermore, Gao and colleagues[145] presented evidence for the direct interaction of β-catenin with *NEUROD1* regulatory elements. β-catenin and/or *NEUROD1* silencing also produced similar effects on the generation and survival of newborn neurons. Upon NEUROD1 silencing, an absence of WNT-mediated neurogenesis was also observed.

1.6 Dentate Gyrus Gene Expression

Adult hippocampal neurogenesis is of particular relevance psychiatric research. The hippocampus harbours some of the particularised volumetric reductions associated with schizophrenia. It also plays a role in memory and behaviour, neuropsychological indices shown to exhibit marked deficits in affected individuals[148]. The hippocampal size reductions have been observed in both prodromal and first episode cases; somewhat suggestive of a relevance to the primary aetiology involved. A full review into the role of the hippocampus in psychiatric illness is provided by Harrison[149].

Though much of this focus on the hippocampus in schizophrenia research is borne out of a perceived active contribution to disease, some researchers have sought to identify more subtle mechanisms by which this region may contribute to the disease state. Using a novel approach to analyse

the role of the hippocampus in schizophrenia, Altar and colleagues[150] utilised laser-capture micro-dissection to isolate populations of dentate granule neurons. The authors demonstrated reductions in the expression of genes associated with “protein turnover and mitochondrial energy metabolism” in the brains of patients with schizophrenia.

Interestingly, they were also able to report reductions in genes involved in “neurite extension, and synaptic plasticity”. These results suggest that, at least to some degree, schizophrenia patients are subject to significant disturbances in postnatal neurodevelopmental processes. It is an intriguing possibility that this also applies to the embryonic neurodevelopment process (see 9Appendix A).

1.6.1 Neurodevelopmental Genes

POU domain, class 3, transcription factor 1 (*POU3F1*, also known as Oct-6), is a developmentally active POU-III domain transcription factor. Best known for its role in Schwann cell myelination, Oct-6 is expressed in the central nervous system during neuronal identity specification. Ilia and colleagues[151] reported a drastic increase in the Oct-6 immunoreactivity of neurons in the granule cell layer of the dentate gyrus when comparing schizophrenics to normal controls, which displayed “little or no staining”. This expression disparity also extended to the CA3 region, where the pyramidal neurons of the schizophrenic cohort exhibited clear immunoreactivity, in stark contrast to their control counterparts. Western blots from the temporal cortex extracts of an independent sample further corroborated this expression disparity.

As aforementioned, the retinoids are a group of molecules that are crucial to the neurodevelopmental process. Rioux and Arnold[131] demonstrated a twofold increase in the proportion of granule cells expressing retinoic acid receptor subtype alpha (RARα) protein in schizophrenia. Further to this, Miyaoka and colleagues[152] present data suggestive of a significant 2.5 times increase in the WNT1 immunoreactive cell density levels in CA3 region of patients with schizophrenia. A member of the aforementioned WNT family of secreted glycoproteins, WNT1 specifically, has been implicated in cell adhesion

and synaptic plasticity. Miyoaka and colleagues[152] also found WNT1 cell density levels in the neighbouring non-neurogenic CA4 region to also be significantly increased.

1.6.2 Hormone Receptors

The expression of genes involved in endocrine signalling has also been found to be dysregulated in this neurogenic region of the hippocampus in schizophrenia. Oestrogen signalling is thought to influence the growth, maturation and differentiation of a variety of tissues including the central nervous system[153]. In situ hybridization (ISH) analysis has revealed significant reductions in the dentate gyrus expression of oestrogen receptor-α (ERα) mRNA in slides from schizophrenia patients are compared with controls[154]. This would appear to cohere with oestrogen's status as an environmental risk-factor for schizophrenia *in utero*[155]; especially as higher-than-normal levels of oestrogen during development are thought to predict aberrant ERα expression[156].

In another ISH study, Webster and colleagues[157] also found the mRNA expression of the nuclear receptor subfamily 3 member, glucocorticoid receptor (GR), to be reduced in dentate gyrus, CA3, frontal cortex and inferior temporal cortex of schizophrenia patients. This is intriguing as glucocorticoids, such as cortisol, are molecules primarily elicited as part of the physiological stress response which has been tentatively linked to schizophrenia as both a developmental and non-developmental risk factor. This correlates with the findings of reduced *GR* mRNA expression in both neurogenic and non-neurogenic regions.

1.6.3 Regulators of Neurotransmission

Pro-psychotic and neuroleptic drug activity has provided much of the rationale for the neurotransmitter theories of schizophrenia. Both glutamate NMDA receptor antagonists and dopamine agonists have been shown to produce effects similar to those seen in schizophrenia patients. Gene expression findings in the dentate gyrus implicate neurotransmission in the

post-natal neurodevelopmental context. For example, an ISH study found dopamine type 1 receptor (*DRD1*) mRNA to be decreased by 21% in the CA3 brain region of subjects with schizophrenia[158].

A variety of glutamate receptor subtypes and subunits have also been shown to exhibit aberrant expression levels in the postnatal neurogenic brain regions of schizophrenia sufferers. Kerwin and colleagues[159] used quantitative auto-radiographic analysis of slides from schizophrenia patients and controls to identify differences in expression of glutamate receptor binding sites. From this study they were able to report bilateral reductions in kainate receptor levels (via kainic acid binding) in the dentate gyrus and CA3 of schizophrenia patients. These reports of reduced kainite receptor-binding levels were supported by findings at the mRNA level. Porter and colleagues[160] demonstrate that mRNAs corresponding to glutamate receptor, ionotropic kainate 5 (*KA2*) and glutamate receptor, ionotropic kainate 2 (*GluR6*) both exhibit significant reductions in the dentate gyrus and CA3 of schizophrenia patients.

AMPA glutamate receptor subunits (GluR1-2) have also been to shown exhibit schizophrenia-related expression disturbances in the neurogenic context. Both *GluR1* and *GluR2* subunit mRNAs have been shown to undergo reductions of approximately 50% or more in the dentate gyrus and CA3[161]. For *GluR1*, these reductions were also found to be significant as a measure of abundance per neuron. The same applies for the analysis of NMDA receptor subunit 1 mRNA (*NMDAR1*) which Law and colleagues[162] have found to exhibit lateralised reductions in left side dentate gyrus and CA3 in schizophrenia.

Other participants in glutamate neurotransmission include vesicular glutamate transporters (VGLUTs); responsible for maintaining glutamate pools in the vesicles of pre-synaptic terminals. Type 1 VGLUT has been shown, by use of regionalized immunohistochemical analysis, to exhibit an 84% increase in the post-mortem dentate gyrus of schizophrenic patients[163].

Reductions in the expression of other glutamate processing molecules have also been observed. Glutamic acid decarboxylases (GADs) are the signature molecules of GABAergic interneurons in the dentate gyrus;

responsible for the conversion of glutamate into GABA in inhibitory pre-synaptic terminals. The expression of the GAD_{67} isoform, encoded by the *GAD1* gene, was shown to be reduced by 22% in the dentate gyrus of schizophrenia sufferers when compared to controls[164].

Another study into the cellular expression of both GAD mRNA isoforms (GAD_{65} and GAD_{67}) failed to detect any differences between schizophrenia sufferers and controls in the dentate gyrus; it is important to note that this study did find significant decreases in the density of GAD65 mRNA stained neurons in the dentate and a combination of the CA2/3 regions when comparing controls with bipolar disorder sufferers[165].

Other receptors shown to exhibit abnormal expression levels in the dentate gyrus of patients with schizophrenia include acetylcholinergic receptors. Crook and colleagues[166] used radio-labelled binding assays to measure the abundance of cholinergic receptor, muscarinic 1 (M1) and cholinergic receptor, muscarinic 4 (M4) receptor binding sites and thus receptor levels. They reported a decrease in muscarinic receptor expression levels in the dentate gyrus and CA3.

These results were substantiated in 2007 by Scarr and colleagues[167], who also found expression reductions in this neurogenic region of the postnatal brain. They were able to show, using quantitative auto-radiographic analysis and in situ hybridization of post-mortem slides, concurrent reductions in *M4* mRNA across whole sections and M1/4 receptor binding sites in CA3 and dentate gyrus (molecular and polymorphic layers, delineated by high resolution specific binding readings).

Other cholinergic receptors that have been analysed in the dentate include low-affinity nicotinic acetylcholinergic receptors that bind α-bungarotoxin (i.e. Homomeric CNS-type (α7)5 nicotinic receptors). As a proxy measure of the expression of these receptors, radio-labelled α-bungarotoxin binding was found to be decreased in the CA3 region of patients with schizophrenia[168].

Dean and colleagues[169] investigated signalling molecules operating downstream from G-protein coupled receptors. After analysing the radio-ligand binding capacity of sections taken from individuals with schizophrenia, they reported a trend towards a decrease in protein kinase C expression and a significant decrease in the expression of adenylate cyclase in the dentate gyrus; no significant differences were observed in CA3.

Growth-associated protein 43 (GAP43), a substrate of protein kinase C, has been shown to exhibit reduced expression in the dentate gyrus. Two studies analysing the expression of this protein[170,171], using slides from controls and patients with schizophrenia, compared antibody labelling intensities. Tian and colleagues[170] revealed a trend towards a correlation between a schizophrenia diagnosis and reduced GAP43 levels in the dentate gyrus. Chambers and colleagues[171] found that optical density readings for GAP43 antibody were reduced when the inner molecular layer of the dentate gyrus was analysed separately.

1.6.4 Synaptic Markers

Theories connecting schizophrenia and the synapse have endured, at least in part, on the basis of neurobiological findings, such as those highlighted by Harrison and Eastwood[172]. A possible role for synaptic abnormalities in a neurodevelopmental phase of schizophrenia aetiology has also been proposed[173]. Synaptophysin, a putative synaptic marker, has been shown to exhibit lateralized decreases in the dentate gyrus of schizophrenia patients[39].

When Young and colleagues[35] looked at synaptophysin and another synaptic protein, 2SNAP-25, they also reported expression disturbances. However, they reported an increase in the dentate gyrus granule cell layer expression of synaptophysin in schizophrenia; contrary to the findings of Eastwood and colleagues[39], and a specific reduction of SNAP-25. The findings of reduced SNAP25 expression were specific in that they remained significant even when the synaptophysin expression levels were used as a covariate.

Nevertheless, the findings of Eastwood and colleagues[39] have since been substantiated by Chambers and colleagues[171] . In their study they demonstrate synaptophysin protein levels to be decreased in both the inner and outer layers of the dentate gyrus. This is concordant with the additional reports of reduced synaptic protein levels in patients with schizophrenia. For example, Nowakowski and colleagues[174] report the expression of synapse I, a protein associated with synaptic vesicles, to be reduced in the inner and outer layers of the dentate gyrus in schizophrenia.

Weickert and colleagues[175] demonstrated that the expression of Dysbindin-1; protein is decreased in the dentate of schizophrenics. Dysbindin is a constituent of the dystrophin-associated protein complex concentrated at the postsynaptic density. Weickert and colleagues[175] present data demonstrating that, in the brain of subjects with schizophrenia, the mRNA expression of Dysbindin-1 is decreased by 25% in the dentate gyrus, and 39% in CA3, when compared with healthy controls.

At the protein level, a similar decrease in expression was reported. Talbot and colleagues[163] found that Dysbindin-1 protein neuropil immunoreactivity was reduced by 42% in the inner molecular layer of the dentate gyrus. This decrease was 14% in the parent cells of neurons responsible for the intrinsic glutamatergic input in the inner molecular layer. Decreases in Dysbindin-1 expression were also reported when CA2 and CA3 regions were pooled; a 30% decrease in the stratum oriens and 37% in the stratum radiatum.

1.7 The Duality of Adult and Embryonic Neurogenesis

A role for adult neurogenesis in the recovery from illness has been previously proposed as it is increased in response to brain injury[176] and antidepressant stimulation[177]. In schizophrenia research, adult neurogenesis may also prove to be informative as an indicator of abnormalities with bearings upon neurodevelopmental processes of the ante- and peri-natal state. This requires the assumption that any analogy between adult and embryonic neurogenesis is valid. Gene expression disturbances can be highly dependent

on context, whether regional, temporal or otherwise. Disturbances observed in postnatal neurogenesis may, at best, only hint at clues to the nature of the embryonic neurodevelopmental abnormalities. Nevertheless, the mechanisms underpinning both adult and embryonic neurogenesis have been shown to exhibit intriguing similarities.

1.7.1 Molecular Overlap

The expression of genes, that are integral to the development and characterisation of neurons, can help elucidate the link between adult and embryonic neurogenesis. Researchers have found many of the transcription factors and guidance molecules that are active in embryonic neurogenesis to be expressed post-natally in developing neural precursors. In part, this can be interpreted as evidence of a conserved developmental program.

1.7.1.1 Transcription Factors

1.7.1.1.1 *Empty Spiracles Homeobox1 and 2 (Emx1* and *Emx2)*

There is evidence to suggest that these transcription factors, postulated to play a role in forebrain regionalization, are also involved in adult neurogenesis. Adult *Emx1* mutants have been shown to exhibit reduced levels of adult hippocampal neurogenesis; they exhibit smaller dentate gyri with fewer proliferating cells[178]. These observations may be a reflection the developmental impact of *Emx1* expression depletion. Kohwi and colleagues[179] provide more evidence of a role for *Emx1* in postnatal neurogenesis; in their studies they found that Emx1 lineage-derived embryonic progenitors were able to produce GABAergic olfactory bulb interneurons when transplanted into adult mouse brain SEZ.

Emx2 is also thought to play a role in forebrain regionalization; however it has also been suggested to be involved in area patterning and the maintenance of progenitor pools in the embryonic brain. There is also evidence to suggest that *Emx2* is involved in adult neurogenesis. *Emx2* was found to be up-regulated in the SEZ of adult monkeys, concomitant with increased levels of neurogenesis[180]. The neurogenesis was stimulated by transient global cerebral

ischemia and confirmed by bromodeoxyuridine (BrdU) staining. An up-regulation of numerous other developmental transcription factors, including *Ngn1*, was observed.

1.7.1.1.2 *Neurogenin 1 and 2 (Ngn1* and *Ngn2)*

The neurogenin genes are thought to influence neural differentiation, marking the dorsal telencephalon during embryonic development. Numbers of Ngn1-expressing cells were found to be increased in adult monkeys in response to transient global cerebral ischemia by Tonchev and colleagues[180]. However, Vergano-Vera and colleagues[181] found that embryonic adult olfactory bulb precursor cells expressed *Ngn1* and *Ngn2* while their adult counterparts expressed very little. This is suggestive of a neurogenic role that is biased towards embryonic neurogenesis.

1.7.1.1.3 *Hairy and Enhancer of Split (Hes)* Genes

This family of genes are activated by Notch signalling and have been postulated to play a role in the proliferation and maintenance of progenitor pools in embryonic neurodevelopment. *Hes1* and *Hes5* expression has been found to be increased in the adult rat/mouse hippocampus in response to increased hippocampal neurogenesis[182]. In this instance, neurogenesis was stimulated by chronic fluoxetine administration and validated by immunohistochemistry. However, *Hes* gene up-regulation has also been detected upon an increase in neurogenesis stimulated by mild stress[183] and simulated stroke[184].

1.7.1.1.4 Inhibitor Of DNA Binding 1, Dominant Negative Helix-Loop-Helix (Id) Genes and Paired Box 6 (*Pax6)*

Similar to the Hes genes, the Id family are thought to be involved in the proliferation of progenitor pools and differentiation suppression. There is however evidence to suggest that members the Id gene family play varying roles in neurogenesis dependent on the type of cell in which they are expressed[185].

Pax6 is a multi-functional regulator of neurodevelopment found to be active in embryonic and post-natal neurogenesis. It is quite conceivable that its

pre-natal function is maintained in the neurogenic regions of the adult brain; Osumi and colleagues[186] present a review that provides a convincing rationale for this hypothesis.

1.7.1.1.5 *NeuroD1*; T-box, Brain, 1 and 2 (*Tbr1* and *Tbr2)*

These transcription factors have also been shown to be involved in the neurogenesis observed post-natally and in the developing mammalian neocortex[187,188]. The aforementioned *NeuroD1* (see 1.5.3.2) is a bHLH transcription factor thought to play a critical role in neural differentiation. *Tbr1* is a T-box transcription factor involved in layer-related differentiation.

Also known as *EOMES*, *Tbr2* is also expressed in the developing embryonic cortex. There has been evidence to suggest that it is involved in radial glial diifferention[189]. Intriguingly, mutated *Tbr2* has been shown to evoke an abrogation of adult neurogenesis in the adult dentate gyrus[190]. Thusly, not only is Tbr2 expressed post-natally[191] but the evidence suggests that it is critical to the process in the adult dentate.

1.7.1.2 Guidance Molecules

1.7.1.2.1 Polysialylated Neural Cell Adhesion Molecule (*PSA-NCAM*)

PSA-NCAM is a developmental cell surface marker, key to neural adhesion and involved in events such as neurite outgrowth[192]. Seki and colleagues[193] investigated its expression in young and adult mice injected with BrdU. They found co-expression of both PSA-NCAM and BrdU in the hippocampus of rats aged 2-3 months and 15 months, with no qualitative difference in the morphology of the presumptive developing neurons. This is suggestive of a similar role for PSA-NCAM in adult and embryonic neurogenesis.

1.7.2 Functional and Morphological Overlap

The similarities in genes expressed during adult and embryonic neurogenesis are indicative of a well-conserved process. These similarities notwithstanding, there is still scope for these expression profiles to represent divergent processes. Transcription factor expression is postulated to be context-

dependent[194]. This suggests that the observance of a gene being expressed in both instances of neurogenesis is not enough to confirm a genuine homogeneity of process.

The temporal profile of gene expression in adult and embryonic neurogenesis has also been shown to exhibit intriguing similarities. Immuno-labelling of the inside-out laminal progression of the mouse neocortex has allowed researchers to characterize the sequence of transcription factor expression at the embryonic cortical plate.

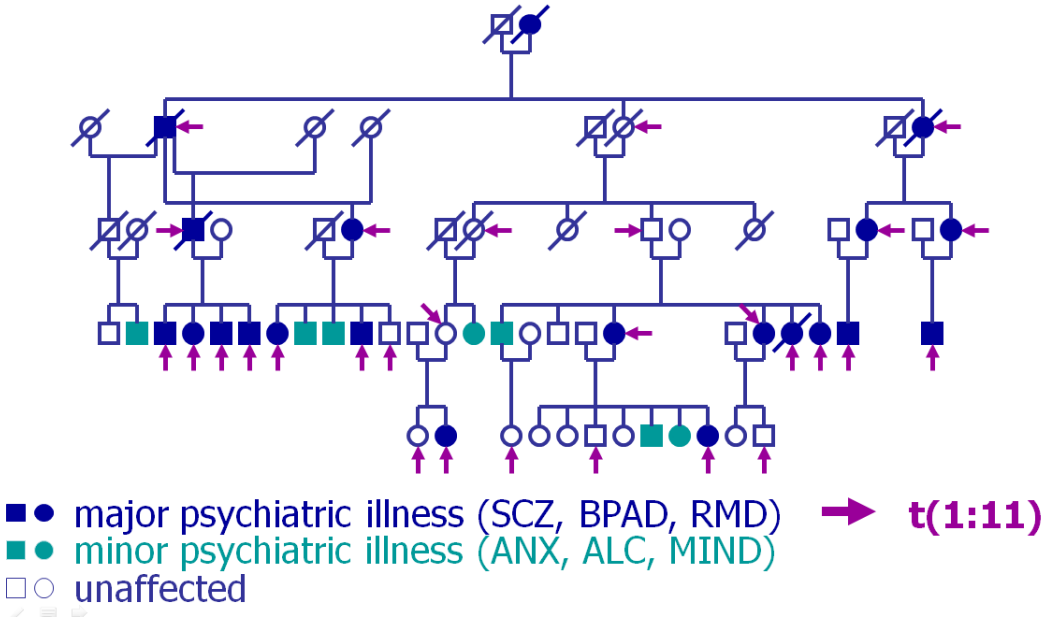
This has proven to be directly parallel to the transcription factor expression sequence observed during the hippocampal neurogenesis of adult mice. Transcription factor expression during mouse embryonic neurogenesis has been shown to proceed from Pax6 to Tbr2, then NeuroD and finally Tbr1[195]. The same sequence been observed during adult neurogenesis studied in the dentate[196]. In total, the evidence is highly suggestive of adult neurogenesis recapitulating much of what is observed during embryonic neurodevelopment.

1.8 Disrupted-in-schizophrenia- (*DISC1*)

DISC1 became a gene of particular interest in schizophrenia research when its truncation was observed in a balanced translocation of loci 1q42 and 11q14.3 which co-segregated with major psychiatric disorders in an extended Scottish family[84; 197]. Genetic association of *DISC1* to schizophrenia has since been demonstrated in Finnish[198], North American[199], Chinese[200] and Japanese[201] population samples. Nevertheless, a recent meta-analysis has indicated no overall association of common polymorphisms with schizophrenia[202]. Chubb and colleagues[85] present a full review into the role of the DISC1 locus in psychiatric disorders.

DISC1 expression has been shown to be significantly reduced in lymphoblastoid cell lines from members of the original *DISC1* translocation family [203]. However these results have not been supported by studies analysing expression in brain. Lipska and colleagues[204] presented data suggesting a trend

towards reduced DISC1 protein expression in the brains of schizophrenic individuals. However, these results did not reach a statistical significance.



1-7 Taken from St. Clair et al.[84] and Blackwood et al.[205] A pedigree showing the co-segregation of a balanced t(1:11) translocation with psychiatric illness

The *DISC1* gene produces an immature mRNA transcript of 414457 nucleotides; the translation of which produces a protein that is 854aa in length. This corresponds to the full-length L isoform DISC1 protein. There are 4 isoforms consistently reported, though recent data has suggested the existence of further alternative splice variants[206].

The full-length DISC1 protein contains a number of domains that have been characterised. Leliveld and colleagues[207] show that its N-terminus region, up to approximately amino acid 320, is mostly non-structured. From amino acid 320 to the C-terminal region, the DISC1 protein contains numerous binding domains that have been described in the literature [85]. A self-association domain has been described and four predicted coiled-coil domains (CCDs) are reported as existing between residues 400-830[207].

1.8.1 *DISC1* Expression

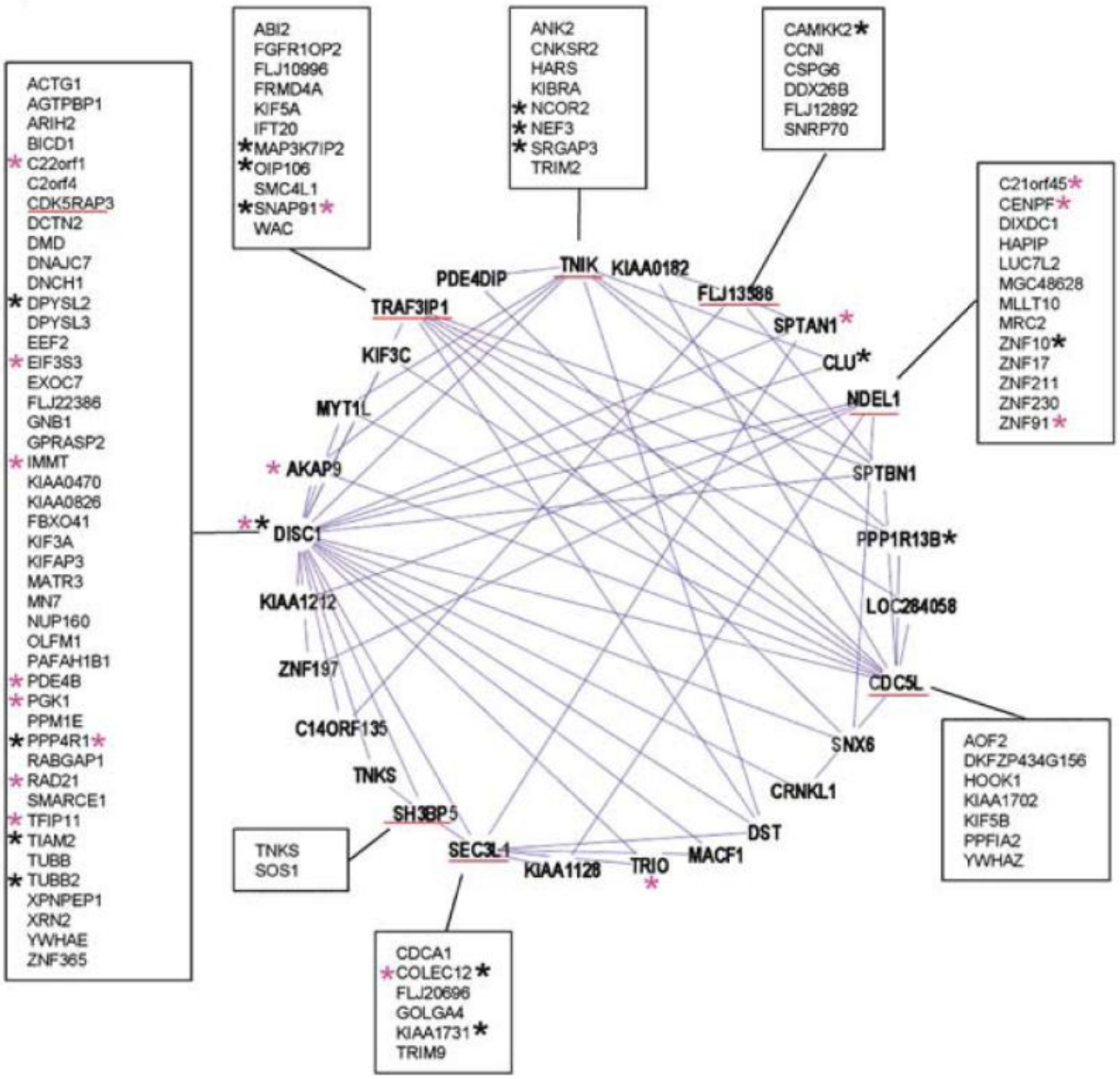
DISC1 is generally expressed at higher levels during embryonic and perinatal periods than in adulthood[204]. As a neurodevelopmentally active

schizophrenia risk gene, *DISC1* has been investigated for roles in neurogenesis. One of the sub-cellular locations where DISC1 expression is pronounced is the growth cone of developing neurites [208]. It is also expressed at the centrosome[209] and is thought to be critical to neurite outgrowth and neuronal migration[210]. The cortical neurons of mice designed to express a truncated DISC1 protein were shown to exhibit significantly less well developed dendrites[211]. These findings have led researchers to propose that *DISC1* is a positive regulator of the neurogenic process.

Intriguingly, when *DISC1* has been analysed for roles in adult neurogenesis, results divergent from those seen during embryonic neurogenesis have been observed. *DISC1* knockdown in the adult mammalian hippocampus has been shown to increase neuronal migration and arborisation[212]. Morphological and physiological signs of precocious synaptic maturation were also observed. This is strongly suggestive of a negative regulatory role for *DISC1* in adult neurogenesis, as opposed to its positive embryonic regulatory role. The data appears to present evidence of a context-dependent role for *DISC1* in neurogenesis.

1.8.2 *DISC1* Function

DISC1 requires particular attention, not only because of its aforementioned neurodevelopmental role, but it has also been described as a protein that acts as a 'molecular hub' for many other neurodevelopmental proteins[213]. Camargo and colleagues[213] identified numerous DISC1-interacting proteins using iterative yeast-two hybrid (Y2H) screens. They also conducted pathway and functional analyses of the observed 'DISC1 interactors'; upon which the biological processes over-represented included regulation/organisation of actin and microtubule cytoskeleton (neurite morphology), cell cycle, cell division (neurogenesis) and intracellular transport (communication). These are highly significant processes in neurodevelopment, and supplement the previously described evidence of DISC1's role in the developing nervous system.

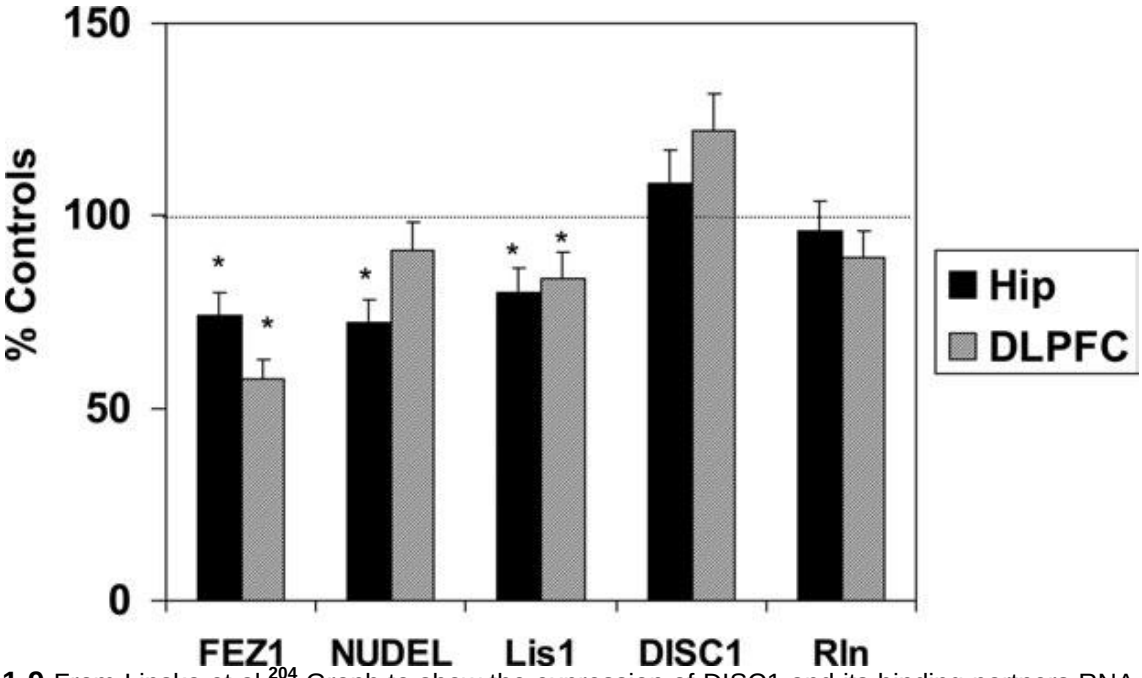


1-8 From Camargo et al.[213] DISC1 network of protein-protein interactions. Underlined in red are proteins that interact with DISC1 and were used in further yeast-two hybrid (Y2H) screens to derive the protein interaction network. Connecting lines indicate an interaction between molecules. In boxes are proteins that interact with the protein screened in further Y2H screens. A black asterisk indicates a protein that is located in a schizophrenia risk locus. A purple asterisk indicates a protein that is found in regions where chromosomal abnormalities have been linked to schizophrenia

Accumulating evidence suggests that DISC1 acts in a facilitatory capacity. For example, it regulates neuronal migration through its interaction with Lissencephaly-1 (LIS1, also known as Platelet-Activating Factor Acetylhydrolase 1b, Regulatory Subunit 1, 45kDa; PAFAH1B1) and NudE

Neurodevelopment Protein 1-Like 1 (NDEL1) proteins. DISC1 co-localises with NDEL1 and LIS1 at the centrosome and mutations in LIS1 and NudE Neurodevelopment Protein 1 (NDE1) can precipitate defects in neurogenesis and neuronal migration[214; 215]. DISC1 is also thought to facilitate growth cone extension. It co-localizes with LIS1, NDEL1, kinesin-1 and 14-3-3ε at the developing growth cone in a functional complex that is critical to the successful development of axons.

An association between *DISC1* genotype and the expression of DISC1 binding partners was recently demonstrated in a study by Lipska and colleagues [204]. Post-mortem tissue from the hippocampus and dorsolateral prefrontal cortex (DLPFC) of 43 schizophrenia patients and 79 controls was dissected. Gene expression was analysed using real-time polymerase chain reaction (PCR). The authors found the expression of the DISC1 binding partners *NDEL1*, fasciculation and elongation protein Zeta-1 (*FEZ1*)and *LIS1* to be significantly reduced in the schizophrenia patients in comparison to the controls. This reduced expression was found in both brain regions; except in *NUDEL*'s case where the reduction in the DLPFC mRNA was not found to be significant.



1-9 From Lipska et al.[204] Graph to show the expression of DISC1 and its binding partners RNA

in the DLPFC and the hippocampus in brains of schizophrenic patients as percentages of the average expression of normal controls. NUDEL = NDEL1, asterisks denote significance, $P<0.05$

Intriguingly, they also found an effect of *DISC1* genotype on the expression of *NUDEL*, *FEZ1* and *LIS1*. Reductions in the hippocampal expression of the DISC1 binding partners were predicted by homozygosity for the *DISC1* risk SNPs hCV219779, rs821597 and rs821616. Even more intriguingly, this effect was not found to be as robust in the DLPFC.

Somewhat surprisingly, two of these risk variants (hCV219779 and rs821597) are produced by silent mutations. This suggests that, in the case of hCV219779 and rs821597, the associations with reduced expression are likely the result of regulatory variants and not the result of a change in protein structure. Further interrogations of the molecular processes with which *DISC1* is involved are only likely to contribute to our understanding of schizophrenia aetiology. This will potentially aid in the development of therapies and diagnostic capabilities.

1.8.3 *DISC1* Binding Partners

The following chapters offer a partial focus on schizophrenia. For a full review into *DISC1* binding partners in psychiatric disorders readers are directed to the recent paper by Bradshaw and Porteous[216].

1.8.3.1 Fasciculation and elongation protein Zeta-1 (*FEZ1*)

Fez1 is the mammalian orthologue of the *Unc76* of the nematode worm *Caenorhabditis elegans*. *Unc76* has been shown to be involved in the development and elongation of worm axons[217]. It appears that this role is conserved in *Fez1*; rat phaeochromocytoma PC12 cells subjected to *Fez1* RNA interference exhibit reduced neurite outgrowth[218]. FEZ1 protein has also been shown to be involved in microtubule transport, complexing with the microtubule motor protein kinesin[218]. It is thought that this function is critical to FEZ1's role in neurite development[219].

FEZ1 is a protein of 392 amino acids in length. Small angle X-ray analysis of FEZ1 suggests that it is mainly an unfolded protein and exists as a

stable dimer[220]. Lanza and colleagues[220] confirms FEZ1's C-terminal coiled-coil domain as critical for protein-protein interactions. This concords with the findings that a large number of the proteins postulated as FEZ1 binding partners have been found to associate with this C-terminal coiled-coil region[221]. The N-terminal region appears to be important for the protein dimerisation and this had led to speculation that FEZ1 acts as a bivalent transport adaptor protein[220].

FEZ1 has been show to associate with schizophrenia independently of DISC1[222]. However further association analyses have failed to corroborate these findings[223; 224]. FEZ1 is thought to bind to DISC1 close to the DISC translocation point; this suggests the possibility that the interruption of the FEZ1-DISC1 interaction plays a role in the molecular mechanism of Schizophrenia[225]. The postulated binding region of FEZ1 on the DISC1 protein is interrupted by the non-synonymous SNP rs6675281.

1-10 Annotated DISC1 protein. Grey area denotes reported FEZ1 binding region, red arrows show relative position of non-synonymous polymorphisms.

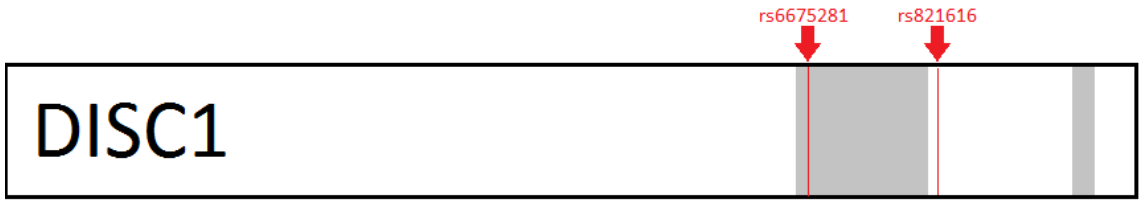
1.8.3.2 NudE Neurodevelopment Protein 1-Like 1 (*NDEL1*)

Two mammalian orthologs of the nuclear distribution pathway first identified in *Aspergillus nidulans* are *Nde1* and *Ndel1*. *NDEL1* codes for the Nuclear Distribution Factor E Homolog 1 which localises at the centrosome[226]. It is thought to play a role in the positioning of the nucleus and has been shown to be critical for neuronal migration and neurite outgrowth[227]. Located within chromosome locus of 16p13.11, the gene codes for a protein of 345aa. Splice variants have been recently described[228].

There is evidence indicating that *NDEL1* impacts on schizophrenia risk through its interaction with DISC1[229]. A reduction in *NDEL1* expression in the brains of schizophrenics has also been suggested[204]. Furthermore, the

interaction between NDEL1 and DISC1 would appear to be critical to neuronal integration in the adult hippocampus[212].

NDEL1 has been postulated to bind to amino acids 598-696 of the DISC1 protein[230]. This region is interrupted by the non-synonymous *DISC1* SNP rs6675281. However, the binding domain more consistently reported spans the amino acids 802-835 of DISC1 protein [227]. It is possible that both binding domains are valid.

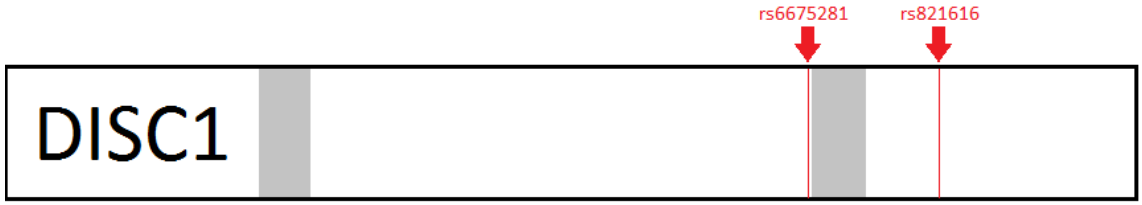


1-11 Annotated DISC1 protein. Grey area denotes reported NDEL1 binding region, red arrows show relative positions of non-synonymous polymorphisms.

1.8.3.3 Phosphodiesterase-4B (*PDE4B*)

The genes that code for the phosphodiesterase 4 family of genes are mammalian orthologs of the *Drosophila melanogaster* Dunce gene. The phosphodiesterase 4 family consists of 4 members; A-D. They all have intrinsic cAMP hydrolytic activity. Dunce is thought to play important roles in both learning and memory in *D.melanogaster*. Inhibition of PDE4s has been demonstrated to enhance cognition[231]. PDE4s have also been shown to partake in axon regeneration after injury[232].

The 4 family members are responsible for over 16 different isoforms; all containing 1 or both of 2 different domains, upstream conserved regions (UCR1 and UCR2). UCRs perform key regulatory functions on the catalytic unit of the PDE4 enzyme. UCR2 is also thought to harbour a DISC1 binding region[203]. A sex-specific effect of 4 *PDE4B* tagSNPs in female schizophrenia patients has been reported[233]. Direct binding of PDE4B/D to DISC1 has been described; the regions of DISC1 thought to be critical to this binding include amino acids 191-230 and 611-650[234]. This postulated binding region of the PDE4s lies in close proximity to the non-synonymous SNP rs6675281.



1-12 Annotated DISC1 protein. Grey area denotes reported PDE4B/D binding region, red arrows show relative positions of non-synonymous polymorphisms.

1.8.3.4 Dihydropyrimidinase-like 2 (*DPYSL2*)

DPYSL2 codes for a dihydropyrimidinase related protein. This is a member of the DPYSL family of proteins. Though the binding of DPYSL2 with DISC1 protein has been reported[213], the potential binding region is yet to be described, The yeast-two hybrid (Y2H) screen in which it was indicated elucidated a detailed protein-protein interaction network of proteins binding both with DISC1, and each other. The DPYSL2 protein was identified as binding only to the DISC1 bait protein. DYPSL2 has been shown to have >95% homology with chick CRMP2 (collapsing response mediator protein), mouse Ulip-2 (Unc-33 like phosphoprotein 2) and rat TOAD-64[235]. These orthologs have all been reported as being mediators of axonal outgrowth[236,237].

DPYSL2 is a gene of 14 exons and approximately 100kbp encoding a protein of 572 amino acids. Though the chicken ortholog (CRMP62) is neuron specific, DPYSL2 is thought to be expressed in a wide variety of tissues including brain[235]. The chromosomal loci of 8p21, in which *DPYSL2* resides, is a putative schizophrenia risk loci[238,239]. Furthermore, numerous studies have identified significant SNP associations with schizophrenia in Japanese[240] and Caucasian[241] populations.

2 RATIONALE AND AIMS

2.1 Rationale

In the context of the postnatal neurogenic niche, post-mortem analysis of gene expression has the potential to provide novel insights. The data presented by Lipska and colleagues[204] shows that the expression of DISC1's binding partners can be affected by mutations in the *DISC1* gene. They also present evidence to suggest that this effect varies in the context of brain region. In the Lipska study, the effect of *DISC1* genotype was manifest in the hippocampus. This brain region contains the subgranular neurogenic niche. It is intriguing to speculate as to whether this effect will endure when the neurogenic niche is specifically targeted.

The Lipska study analysed a set of 12 single nucleotide polymorphisms. Two of these polymorphisms were non-synonymous in nature. Of these two non-synonymous SNPs, one was found to affect DISC1 binding partner expression. Non-synonymous SNPs have a particular relevance to indirect effects on gene expression; that is to say effects not on the expression of the genomic locus, but on the binding partners of gene products. However, it is also intriguing to speculate that any previously undetected effect of DISC1 SNPs (non-synonymous or otherwise) may be revealed by the specific targeting of neurogenic regions of the brain.

2.2 Aims

The potential for post-mortem studies to investigate neurogenic gene expression requires either; the accurate targeting of tissue, and/or measurements of neurogenesis marker expression. This project therefore aims to measure the effect of diagnosis and *DISC1* genotype on the expression of DISC1 binding partners and neurogenesis genes in hippocampal subfield dissections. The genes chosen for this study are:

Gene	Rationale
DCX	• A MAP which has proven useful in marking neurogenesis[142].

DPYSL2
- A putative DISC1 binding partner identified by a Y2H screen[213].
- Independent evidence of genetic association to schizophrenia[240].

FEZ1
- A DISC1 protein binding partner strongly indicated by Y2H[208].
- Independent evidence of genetic association to schizophrenia[222].

NDEL1
- A DISC1 protein binding partner indicated by both Y2H[213] and co-immunoprecipitation (Co-IP)[230].
- Evidence of an epistatic interaction with *DISC1* in schizophrenia risk[229].

NEUROD1
- A putative immunohistological marker of adult neurogenesis[138].

PDE4B
- A DISC1 protein binding partner indicated by both Y2H[213] and Co-IP[234].
- A pharmacological target of rolipram, which has been demonstrated to enhance long term potentiation[231].

2.3 Hypotheses

i. The expression of DISC1 binding partners and genes used to mark neurogenesis will be aberrant relative to controls in the brains of schizophrenics.

ii. DISC1 genotype will affect the expression of DISC1 binding partners and the neurogenesis markers, irrespective of diagnosis.

2.4 Objectives

The key steps in this process can be summarised as follows:

i. Extract DNA and perform allelic discrimination analyses on DISC1 SNPs associated to schizophrenia and/or residing on reported binding regions for DISC1 binding partners.
ii. Dissect dentate region from hippocampal sections and extract RNA
iii. Measure gene expression in schizophrenia for DISC1 binding partners.
iv. Measure expression of postulated markers of neurogenesis.

v. Assess the cellular expression of the utilised neurogenesis markers through immunohistochemistry.

3 MATERIALS AND METHODS

3.1 Subjects

Post-mortem brain samples from the Stanley Consortium were used for the present study. This sample consists of 60 subjects with mixed diagnostic statuses (based on DSM-IV criteria) at time of death; 15 suffering from major depressive disorder (MDD), 15 suffering from bipolar disorder (BPD), 15 suffering from schizophrenia (SCZ) and 15 controls with no history of mental illness (CON). Frozen sections (10um) of anterior hippocampus were used for gene expression studies and immunohistochemistry, paraffin-embedded fixed sections (14um) of frontal cortex were used for immunohistochemistry pilot studies. Frozen blocks of cerebellum were used for DNA extraction and subsequent genotype analysis. All experiments were performed blind to diagnosis. The availability of all tissue for the proposed study was approved by The Stanley Medical Research Institute.

3.2 Dentate Dissections

Dentate dissection protocol consisted of air drying the slides, immersing the tissue in RNA*later* stabilisation reagent (AM7024M, Ambion, www.ambion.com) to increase pliancy, then scraping desired region into an aliquot of RNA later using a sterile scalpel. Using enlarged images of Nissl stained sections for guidance, frozen sections of anterior hippocampus were manually dissected into three regions. The hippocampal formation (HF, also described as the pes hippocampi) was identified and dissected in two portions; a neurogenic portion (the dentate gyrus and CA3) and a non-neurogenic portion (CA1, CA2, and the subiculum). The remainder of the slides, largely encompassing the anterior part of the parahippocampal gyrus, was also dissected separately.

Frozen slides of anterior hippocampus were not available for 9 of the 60 samples requested for analysis. Of the 51 samples dissected, variations in size and position on the antero-posterior axis necessitated the graphical recording of each dissection. Scans of haematoxylin and eosin stained slides, comprised of

each Stanley Consortium subject, were annotated for this purpose (Fig 3-1). These coronal sections were contiguous with the frozen sections of anterior hippocampus used in the current study.

3-1 Picture of anterior hippocampus coronal section with dissected regions annotated (DG = dentate gyrus molecular layer, CA1 = cornu ammonis 1, CA2 = cornu ammonis 2, CA3 = cornu ammonis 3

3-2 Picture of anterior hippocampus coronal section without annotations

3.3 Extraction Procedures

DNA was extracted from the frozen blocks of cerebellum using a variant of standard phenol-chloroform extraction procedures outlined in Freeman and colleagues [242]. Yield and quality, indicated by the absorbance ratio (1.7 ≤ A260/A280 ≤ 1.9), was assessed via a Picodrop® spectrophotometer (Picodrop Ltd., Cambridge Bioscience, Cambridge, UK).

The RNA was extracted from the dissected tissues with TRIzol reagent (#VX15596-026, Life Technologies Inc., Grand Island, NY, USA) and Qiagen RNeasy mini kits (#74106, QIAgen, UK). Tissues were pelleted prior to pulverization in TRIzol reagent and the chloroform phase separation step was conducted. The resulting aqueous phase was then mixed with an equal volume of 70% and purified using Qiagen RNeasy mini kits (Qiagen Ltd., Manchester, UK). Extractions provided an average yield per mg staring tissue of 0.20µg ± 0.07; the maximum expected yield per mg starting tissue is 1.5µg. During the dentate dissection procedure, tissues subjected to RNA*later* treatment produced an average yield of 0.14µg ± 0.04 per mg starting tissue. This translated to an average yield of 1.76µg ± 0.43 per whole slide.

Quality control (QC) assessment of the micro-dissected hippocampal RNA was performed using the Picodrop® spectrophotometer (RNA: 1.9 ≤ A260/A280 ≤ 2.1) and a micro-scale electrophoresis system, the Bio-Rad Experion® (Bio-Rad, Hercules, CA, USA). Electropherograms of each RNA extract enabled the assessment of RNA quality using the proprietary RNA Quality Indicator (RQI) analysis metric. Extracts of RQI ≥ 3.8 were required for downstream applications[204]. Of the 51 samples dissected, 27 satisfied the RNA quality threshold. However, insufficient RNA yield prevented the accurate determination of RQI in the neurogenic HF extractions; precluding further analysis in this region.

RNA extracts from each region were diluted to standard concentrations and treated with TURBO DNA-free kit (Applied Biosystems, Cheshire, UK) in order to remove trace amounts of DNA. Reverse transcription experiments were conducted, 22.5 - 180ng RNA in 20µl reactions, using a mixture of oligo dT and

random primers in the nanoScript reverse transcriptase system (PrimerDesign, Southampton, UK). DNA extractions were performed on the frozen blocks of cerebellum using a phenol-chloroform extraction procedure outlined by Freeman and colleagues[242]. Nucleic acid yield and purity was assessed using the Picodrop spectrophotometer (DNA: 1.7 ≤ A260/A280 ≤ 1.9).

3.4 Quantitative Polymerase Chain Reaction (qPCR)

The expression of target genes of interest (GOIs) were assessed via pre-designed TaqMan assays (Applied Biosystems). Assays for Reference (REF) genes; hydroxymethylbilane synthase (*HMBS*) and succinate dehydrogenase complex, subunit A (*SDHA*); were custom-designed via the Roche ProbeLibrary® (available at www.universalprobelibrary.com). REF genes were pre-validated across diagnostic categories from a panel of 8 candidate REF genes using the Genorm software as described by Vandesompele and colleagues[243]. The software highlighted that these two reference genes were necessary and sufficient for normalisation of target gene data. Probes for the custom-designed assays were purchased from Roche (Hertfordshire, UK); primers were purchased separately (Invitrogen, Life Technologies, Paisley, UK). The qPCR plate design (see Table 3-B) followed the gene maximisation method[243] and incorporated 3 technical replicates from which mean Cq values for each case were derived (*SD* < 0.2). Experiments were performed on the BIO-RAD CFX96 Real Time System.

3-A Table showing qPCR assay information

Gene	Assay Info. (ABI Assay ID / Primer-probe details)	Isoform coverage	RefSeq Ids
DPYSL2	Hs00265851_m1	3/3	NM_001197293.2, NM_001244604.1, NM_001386.5
DCX	Hs00167057_m1	5/5	NM_000555.3, NM_001195553.1, NM_178151.2, NM_178152.2,

			NM_178153.3
NEUROD1	Hs00159598_m1	1/1	NM_002500.4
FEZ1	Hs00192714_m1	1/1	NM_005103.4
NDEL1	Hs01092620_m1	2/2	NM_001025579, NM_030808.4
PDE4B	Hs00277080_m1	4/4	NM_0010324116.1, NM_001037340.1, NM_001037341.1, NM_002600.3
SDHA	Primer(L): aagtccctccaattaaaccaaac Primer(R): gcagcatgattttgcattga Roche Probe 60 Cat No.: 04688589001	1/1	NM_004168.2
HMBS	Primer(L): caccacaggggacaagattc Primer(R): gtgaacaaccaggtccacttc Roche Probe 37 Cat No.: 04687957001	3/3	NM_001258209.1, NM_000190.3, NM_001258208.1

Based on Applied Biosystems' assay re-evaluations in August 2010, a synonymous PDE4B SNP (rs34492439) was found to be located under the PDE4B assay probe/primer sequence. The NCBI website (dbSNP) reports the heterozygosity of this SNP to be 0.027, with the minor allele frequency undetermined. Furthermore, the latest genome build (Genome Build GRCh37.1), determined that the NDEL1 assay may detect NDEL1 genomic DNA. Due to experimental constraints, the possible impact of these findings on the data was not evaluated,

Normalised relative quantity (NRQ) values were obtained by calculating the ratio of the GOI copy number to the geometric mean of REF gene copy numbers[243]. Copy numbers were extrapolated from the assay reaction efficiency and the mean Cq value of samples, relative to a calibrator oligonucleotide. Amplification efficiencies were verified by plotting standard curves for each primer pair using serial dilutions of the calibrator oligonucleotides (see 3.4.2.2). Calibrator oligonucleotides were synthetic template oligonucleotides, of known concentration, used to protect against the inter-run variability inherent across qPCR plates. Specific calibrator oligonucleotides required for each assay, and run across all qPCR plates (see 3.4.2.1).

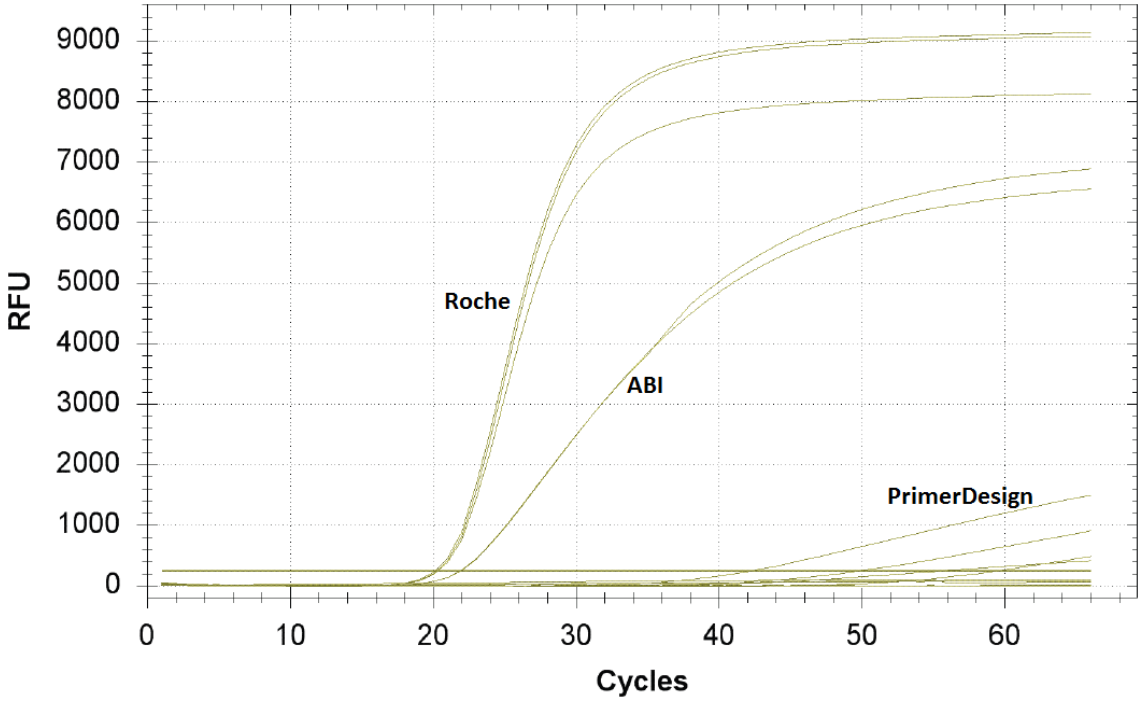
3-B Schematic representation of the 96-well gene-maximised qPCR plate design (CO = calibrator oligonucleotide, NTC = non template control)

		1st SUBJECT			2nd SUBJECT			3rd SUBJECT			CO		NTC
		1	2	3	4	5	6	7	8	9	10	11	12
DCX	A												
DPYSL2	B												
FEZ1	C												
PDE4B	D												
NDEL1	E												
NEUROD1	F												
HMBS	G												
SDHA	H												

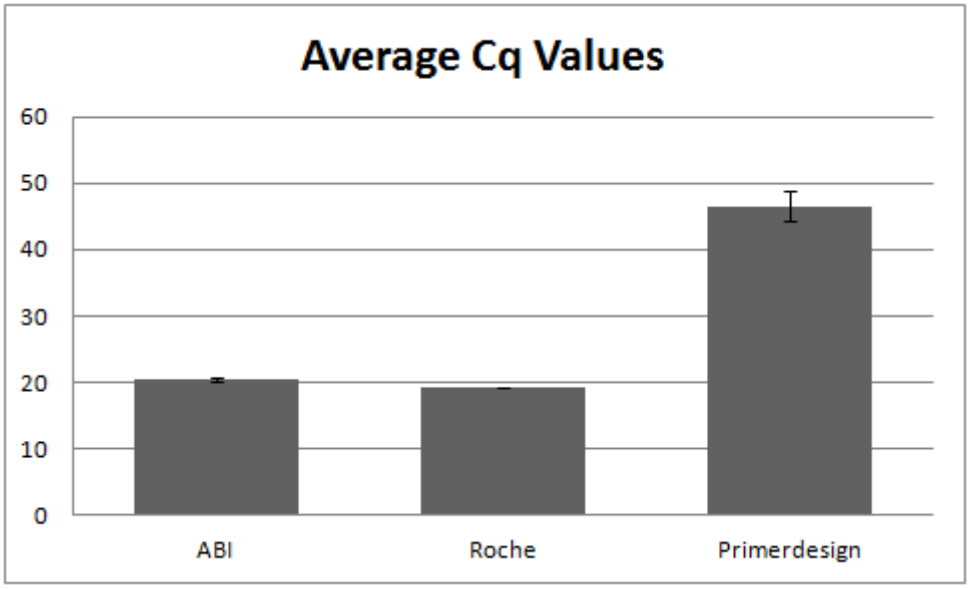
3.4.1 Enzyme Selection

During qPCR allelic discrimination experiments, sub-optimal efficiencies were observed. These did not affect the qualitative genotype assays, however they were prohibitive to qPCR. Therefore, comparisons of the commercially

available enzymes were performed. Across all the assays analysed, the Taq Polymerase supplied by Roche produced the most effective amplification. This is evidenced by an increased inclination of the sigmoid curves (Fig 3-3), increased maximum fluorescence, and slightly lower Cq value (Fig 3-4), all indicating increased reaction efficacy.



3-3 Graph showing real-time amplification of a DISC1 locus using a TaqMan assay with VIC probe. The 3 sets of curves represent the 3 Taq polymerase enzymes tested; Roche = Roche Universal Probe Master (#04913957001), ABI = TaqMan Universal Mastermix (#4364341), PrimerDesign = PrimerDesign Precision Mastermix (#Precision)



3-4 Graph showing the average threshold cycle (c_q) number from 20ng DNA, obtained using DNA polymerase from a variety of suppliers. Roche = Roche Universal Probe Master (#04913957001), ABI = TaqMan Universal Mastermix (#4364341), PrimerDesign = PrimerDesign Precision Mastermix (#Precision)

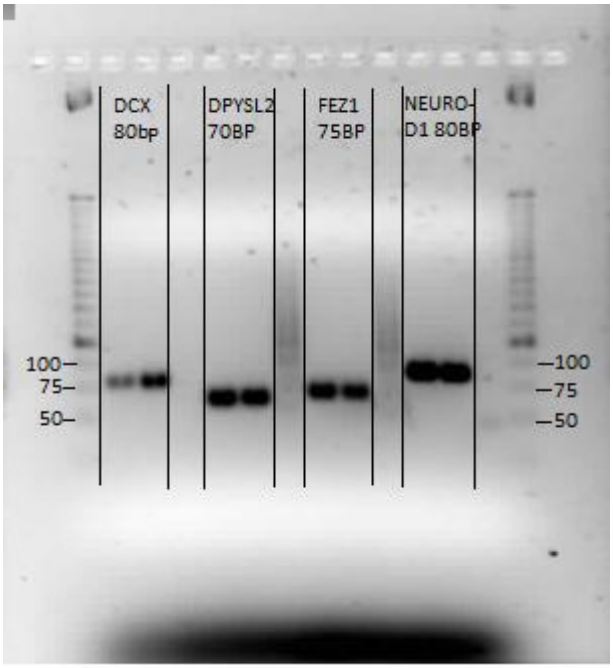
3.4.2 Standard Curves

Quantification of genes of interest (GOIs) with qPCR requires the simultaneous measurement of either; a stably expressed reference gene, or a control sample with a known number of copies of the gene (or gene locus) in question. In absolute quantification, standard curves, serial dilutions of known quantities, are used as a comparative tool to estimate quantity. In relative quantification studies, they are used to calculate the efficiency of a reaction and determine whether the assay is suitable for further analysis. This study utilized a combination of both absolute and relative quantification strategies. Standard curves were used to validate calibrator oligonucleotides. This allowed expression levels to be quantified relative to a known sample (a form of absolute quantification). Standard curves were also used to determine the amplification efficiencies for each assay, therefore allowing expression levels to be further normalised by REF genes (relative quantification).

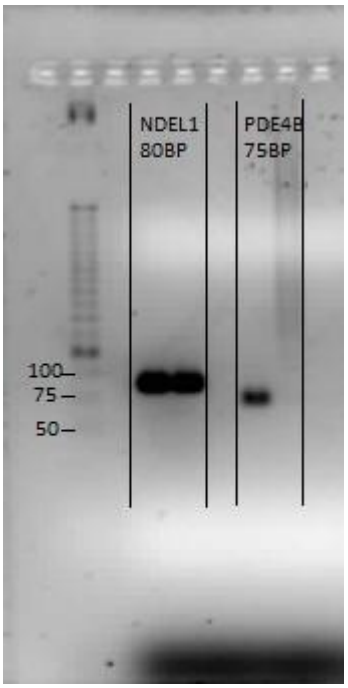
3.4.2.1 Calibrator Oligonucleotide Design

Calibrator oligonucleotides were designed to replicate qPCR assay amplicon sequences. For the REF gene assays, amplicon sequences were provided by the Roche ProbeLibrary® software. For the predesigned assays, very limited sequence information was available except for a 'context sequence' (a selected 25bp sequence from the predicted amplicon). Agarose gels were therefore utilised to reveal the actual size of GOI amplicons (Figs. 3-5, 3-6). This allowed the lengths of the designed GOI calibrators to reflect the possibility that the context sequence maybe positioned at either the 5' or 3' end of the amplicon (3-C Table).

Consequently, the sequence of the GOI calibrators represent; the context sequence, plus the necessary flanking sequence information from the gene (available from www.ncbi.nlm.nih.gov/genbank). Due to their size, GOI calibrator oligonucleotides were purchased from Integrated DNA Technologies (Ultramer DNA, IDT, Coralville, IA). REF gene calibrator oligonucleotides (<100bp) were procured from Invitrogen®.



3-5 3% agarose gel with 25bp ladder comparing size of PCR products obtained from the gene expression assay for DCX, DPYSL2, and FEZ1, NEUROD1. Image is annotated with estimated amplicon sizes



3-6 3% agarose gel, with 25bp ladder, comparing size of PCR products obtained from the gene expression assays for NDEL1 and PDE4B. Image is annotated with estimated amplicon sizes

3-C Table showing the results of the calibrator oligonucleotide design. Estimated amplicon sizes were derived from gel electrophoresis experiments, calibrator oligonucleotide sequences equal the 25bp context sequence (highlighted in red) extended at both the 5' and 3' ends in accordance with the estimated amplicon size estimations. Extensions of the calibrator oligonucleotides were rounded up to the nearest 25bp to provide required oligonucleotide size values, sequence information can be found at www.ncbi.nlm.nih.gov/genbank.

Gene	Estimated Amplicon Size (bp)	Required Oligonucleotide Size (bp)	Calibrator Oligonucleotide Sequence (25bp context sequence highlighted in red)
DPYSL2	70	125	GGACATCAGTGAGTGGCATAAGGGCATCCAG GAGGAGATGGAAGCGCTTG**TGAAGGATCACG GGGTAAATTCCTT**CCTCGTGTACATGGCTTTC AAAGATCGCTTCCAGCTAACGGATTGCCAGA
DCX	80	175	GATTTCTTTGGTGATGATGATGTGTTTATTGCC TGTGGTCCTGAAAAATTTCGCTATGCTCAGGA TGATTTTTCT**CTGGATGAAAATGAATGCCGAG TCA**TGAAGGGAAACCCATCAGCCACAGCTGG CCCAAAGGCATCCCCAACACCTCAGAAGACTT CAGCCAAGAGCCCTG
NEUROD1	80	175	GAGGAATTCGCCCACGCAGGAGGCGCGGCG TCCGGAGGCCCCAGGGTTATGAGACTATCAC TGCTCAGGACCTAC**TAACAACAAAGGAAATC GAAACATG**ACCAAATCGTACAGCGAGAGTGG GCTGATGGGCGAGCCTCAGCCCCAAGGTCCT CCAAGCTGGACAGACGAGTGT
FEZ1	75	125	ACCTAGCTCCCGTGAAGAACCAGTTACAGATC CAAGAGGAGGAGGAGACC**CTTCAGGACGAG GAGGTTTGGGATG**CTCTGACAGACAATTACAT CCCTTCACTCTCAGAAGACTGGAGGGATCCA
NDEL1	80	175	AGTTGGAGGCACAATTAGTACAGGCTGAACAA AGAAATAGAGACTTGCAGGCTGATAACCAAAG ACTGAAATATG**AAGTGGAGGCATTAAAGGAG AAGCT**AGAGCATCAATATGCACAGAGCTATAA GCAGGTCTCAGTGTTAGAAGATGATTTAAGTC AGACTCGGGCCATTAA
PDE4B	75	175	TGGCAGACCTGAAGACAATGGTAGAAACGAA GAAAGTTACAAGTTCAGGCGTTCTTCTCCTAG ACAACTATACCG**ATCGCATTCAGGTCCTTCGC AACAT**GGTACACTGTGCAGACCTGAGCAACC CCACCAAGTCCTTGGAATTGTATCGGCAATGG ACAGACCGCATCATGGA

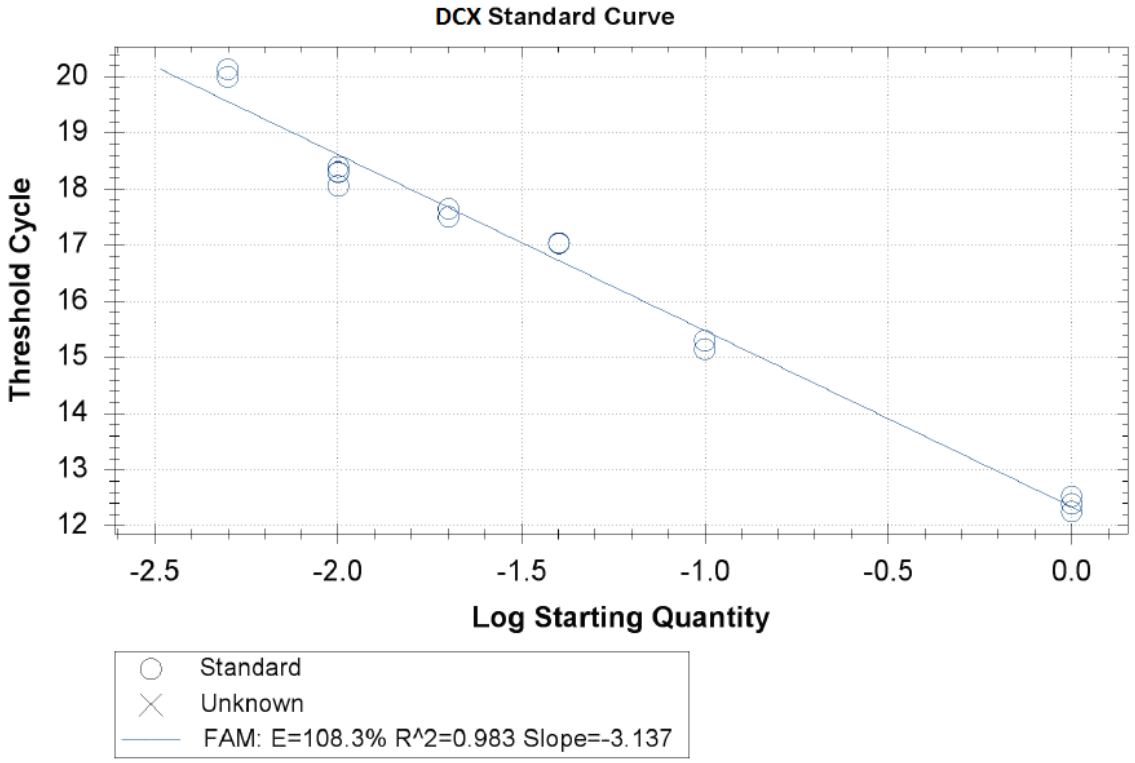
3.4.2.2 Reaction Efficiencies

The exponential nature of a PCR reaction predicts that the quantity of the target locus present in the reaction will be increased by a factor of 2 with every cycle. Simply put, the reaction efficiency (E) is a percentage calibration of the amplification success rate; 100% indicating successful target duplication each

cycle. In order to accurately quantify expression of genes across all samples, a PCR must demonstrate an optimal efficiency (90%≤E≤110%, R^2≥0.980).

3.4.2.2.1 DCX

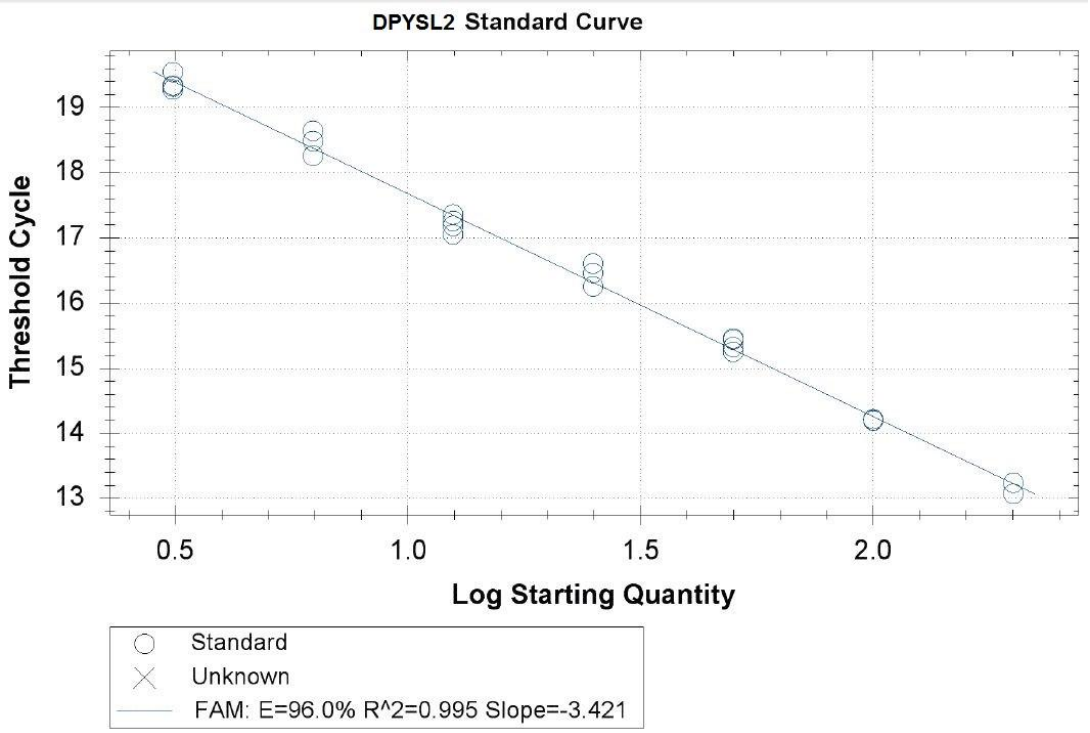
A sufficient reaction efficiency was observed for the assay designed to measure the expression of the *DCX* gene (E = 108.3%, R^2 = .983).



3-7 Standard curve of *DCX* assay representing the threshold cycles of standard dilutions prepared from a synthetic oligonucleotide designed from the *DCX* gene

3.4.2.2.2 DPYSL2

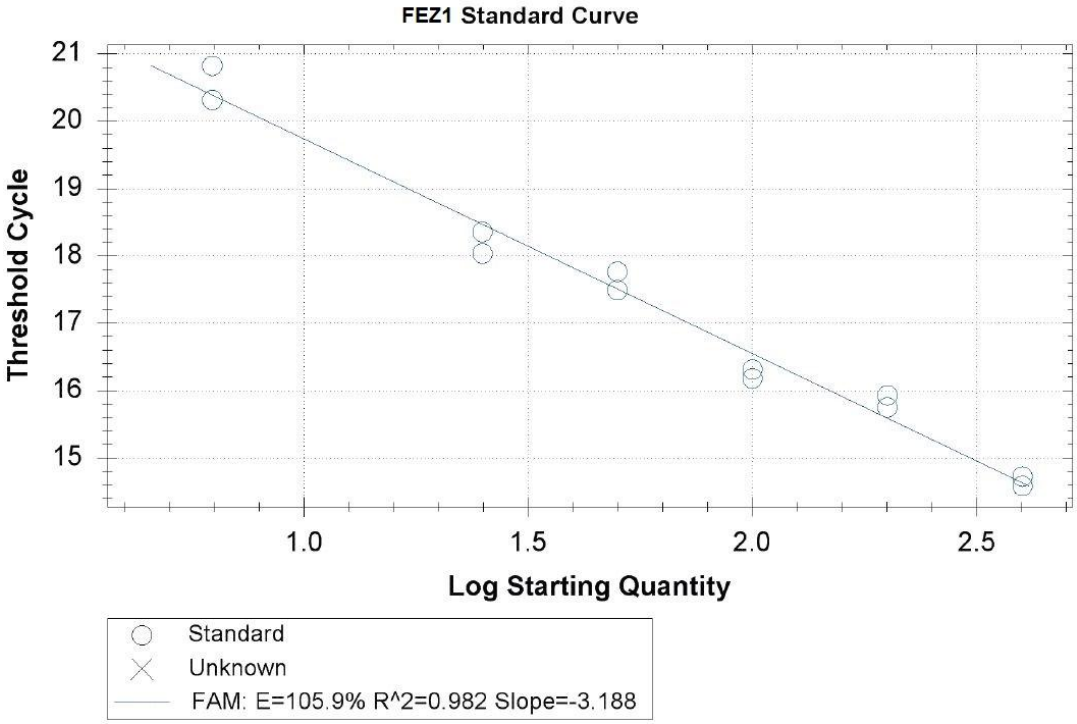
A sufficient reaction efficiency was observed for the assay designed to measure the expression of the *DPYSL2* gene (E = 96.0%, R^2= .995).



3-8 Standard curve of *DPYSL2* assay representing the threshold cycles of standard dilutions prepared from a synthetic oligonucleotide designed from the *DPYSL2* gene

3.4.2.2.3 FEZ1

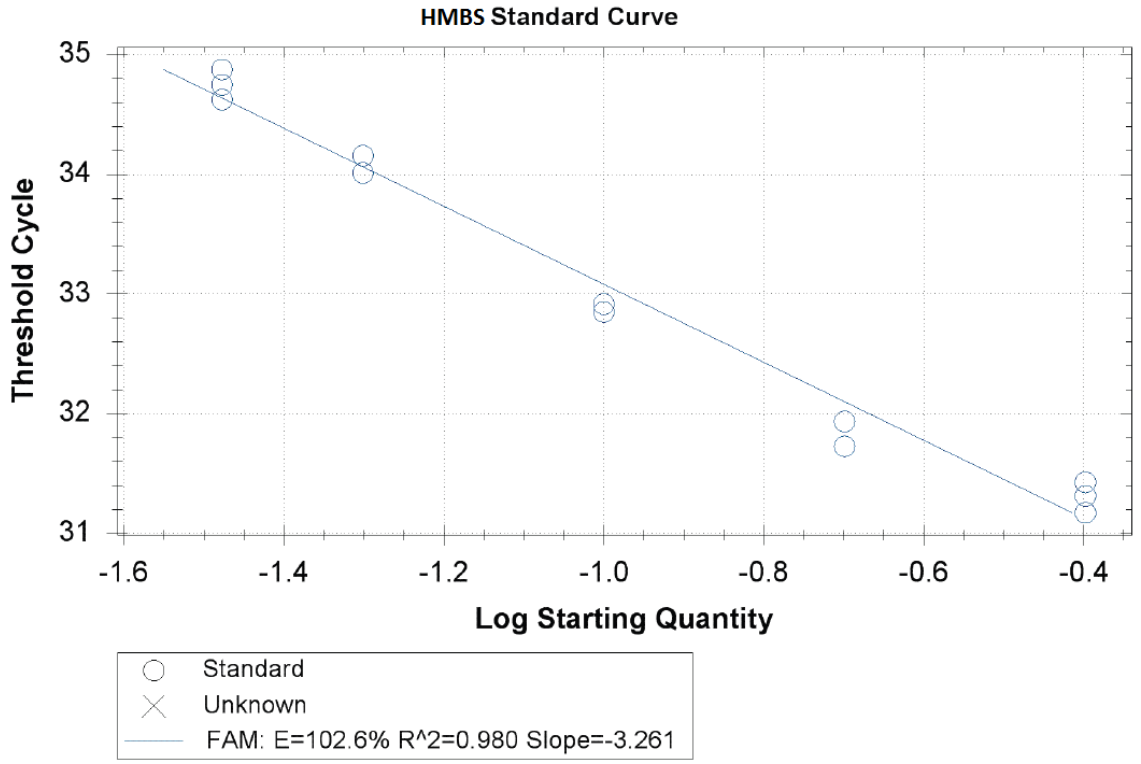
A sufficient reaction efficiency was observed for the assay designed to measure the expression of the *FEZ1* gene (E = 105.9%, R^2 = .982).



3-9 Standard curve of *FEZ1* assay representing the threshold cycles of standard dilutions prepared from a synthetic oligonucleotide designed from the *FEZ1* gene

3.4.2.2.4 HMBS

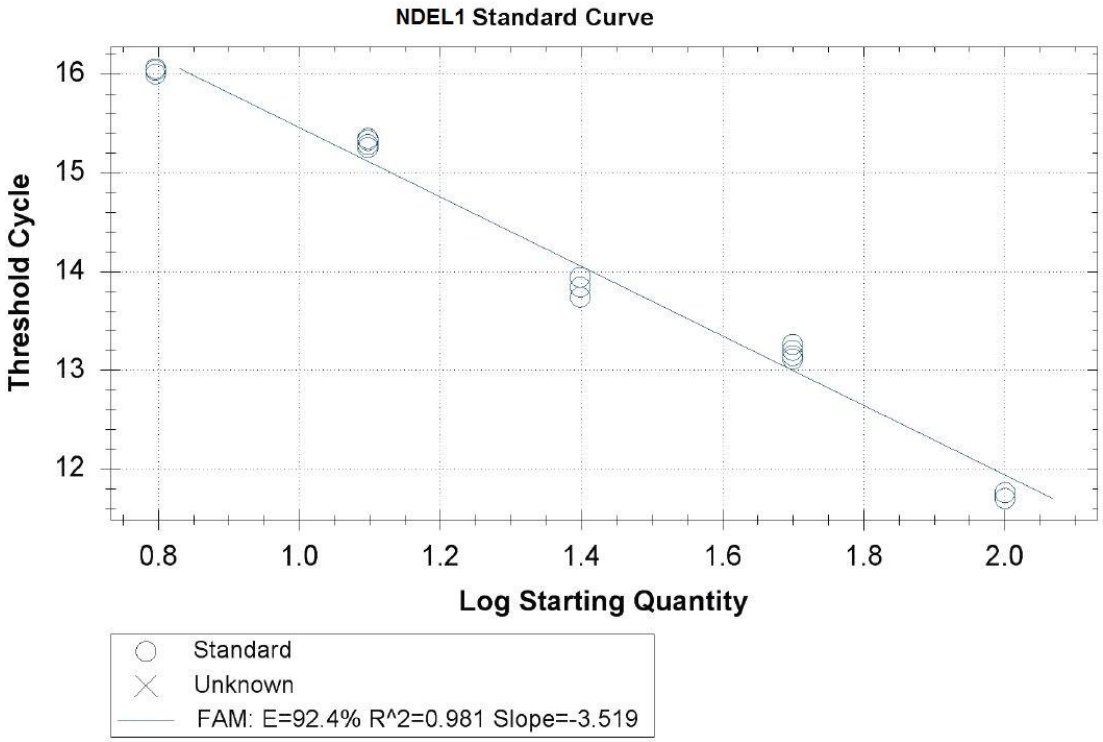
A sufficient reaction efficiency was observed for the assay designed to measure the expression of the reference gene, *HMBS* (E = 102.6%, R^2 = .980).



3-10 Standard curve of *HMBS* assay representing the threshold cycles of standard dilutions prepared from pooled anterior hippocampus cDNA.

3.4.2.2.5 NDEL1

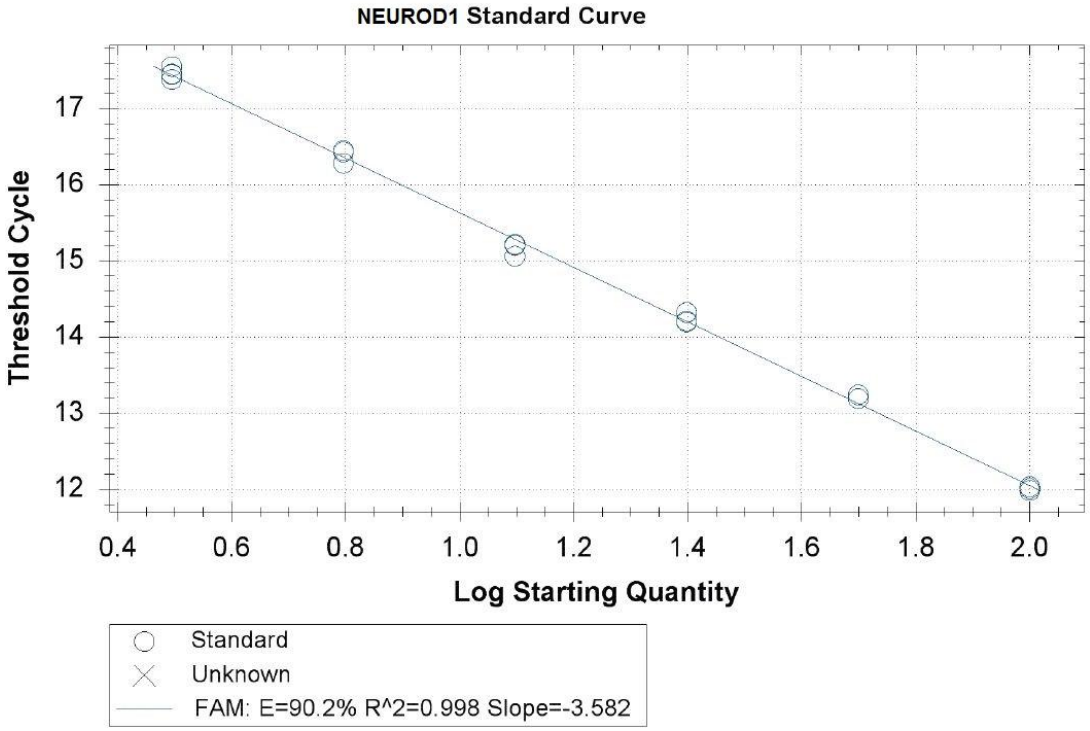
A sufficient reaction efficiency was observed for the assay designed to measure the expression of the *NDEL1* gene (E = 92.4%, R^2 = .981).



3-11 Standard curve of *NDEL1* assay representing the threshold cycles of standard dilutions prepared from a synthetic oligonucleotide designed from the *NDEL1* gene

3.4.2.2.6 NEUROD1

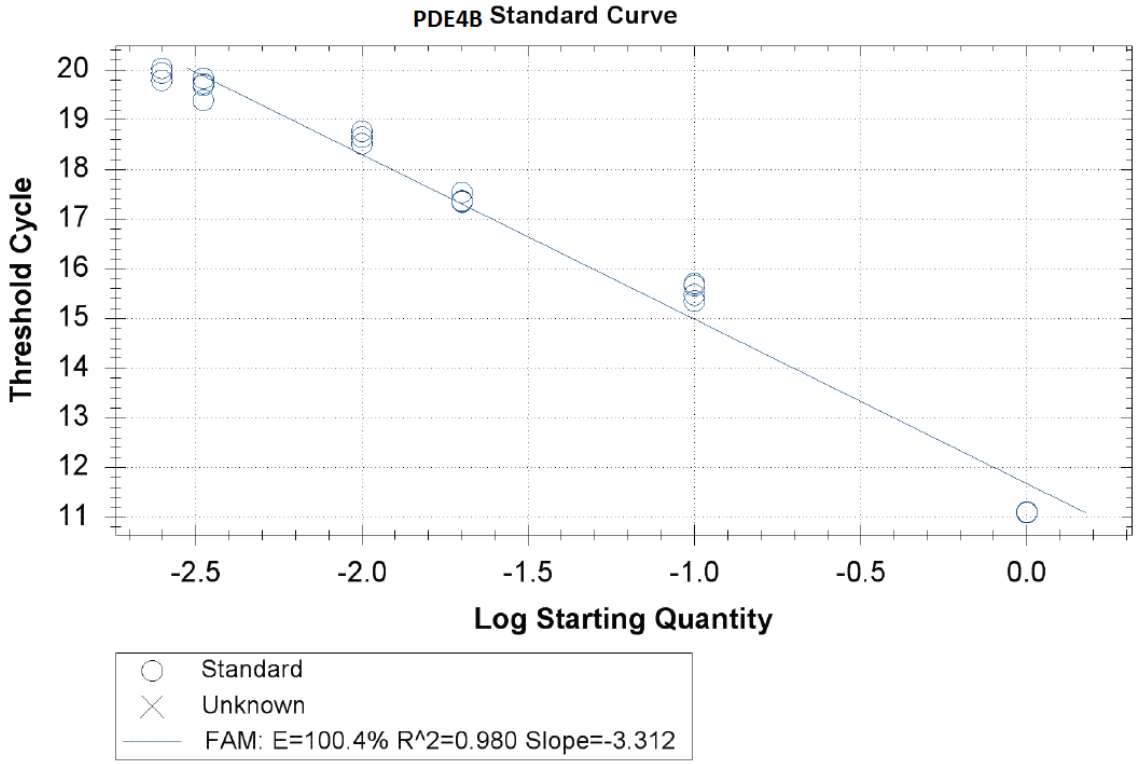
A sufficient reaction efficiency was observed for the assay designed to measure the expression of the *NEUROD1* gene (E = 90.2%, R^2 = .998).



3-12 Standard curve of *NEUROD1* assay representing the threshold cycles of standard dilutions prepared from a synthetic oligonucleotide designed from the *NEUROD1* gene

3.4.2.2.7 PDE4B

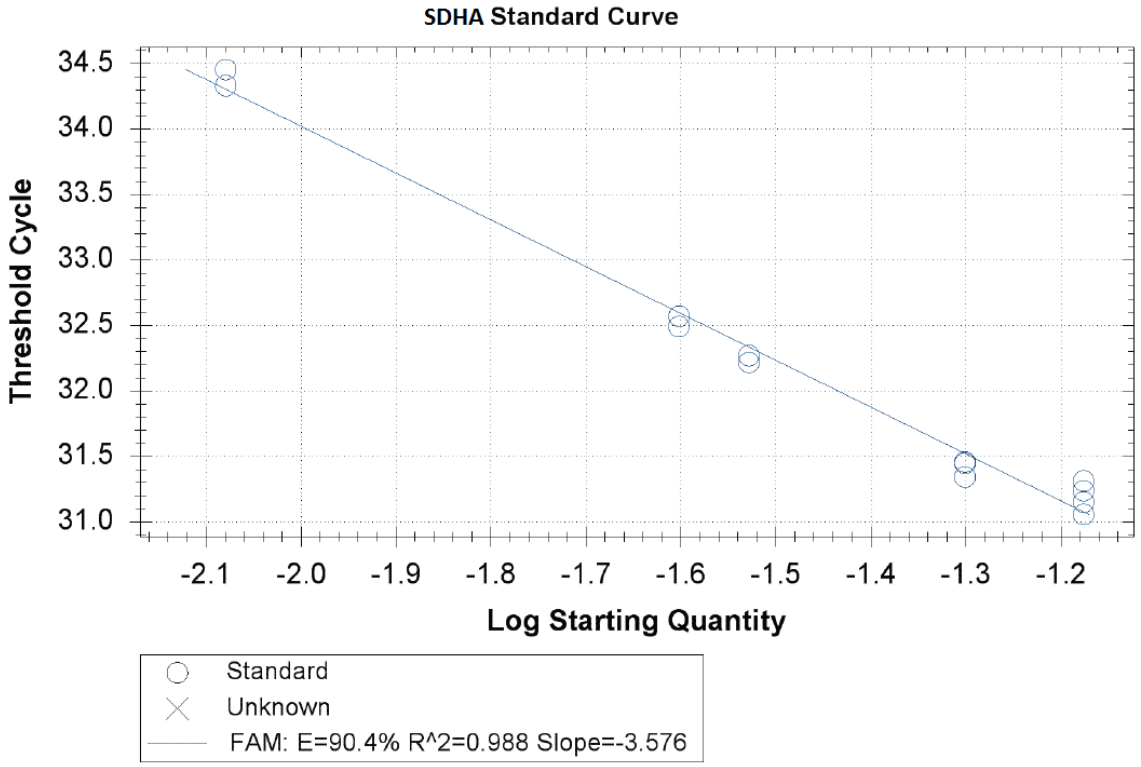
A sufficient reaction efficiency was observed for the assay designed to measure the expression of the *PDE4B* gene (E = 100.4%, R^2 = .980).



3-13 Standard curve of *PDE4B* assay representing the threshold cycles of standard dilutions prepared from a synthetic oligonucleotide designed from the *PDE4B* gene

3.4.2.2.8 SDHA

A sufficient reaction efficiency was observed for the assay designed to measure the expression of the *SDHA* gene (E = 90.4%, R^2 = .988).

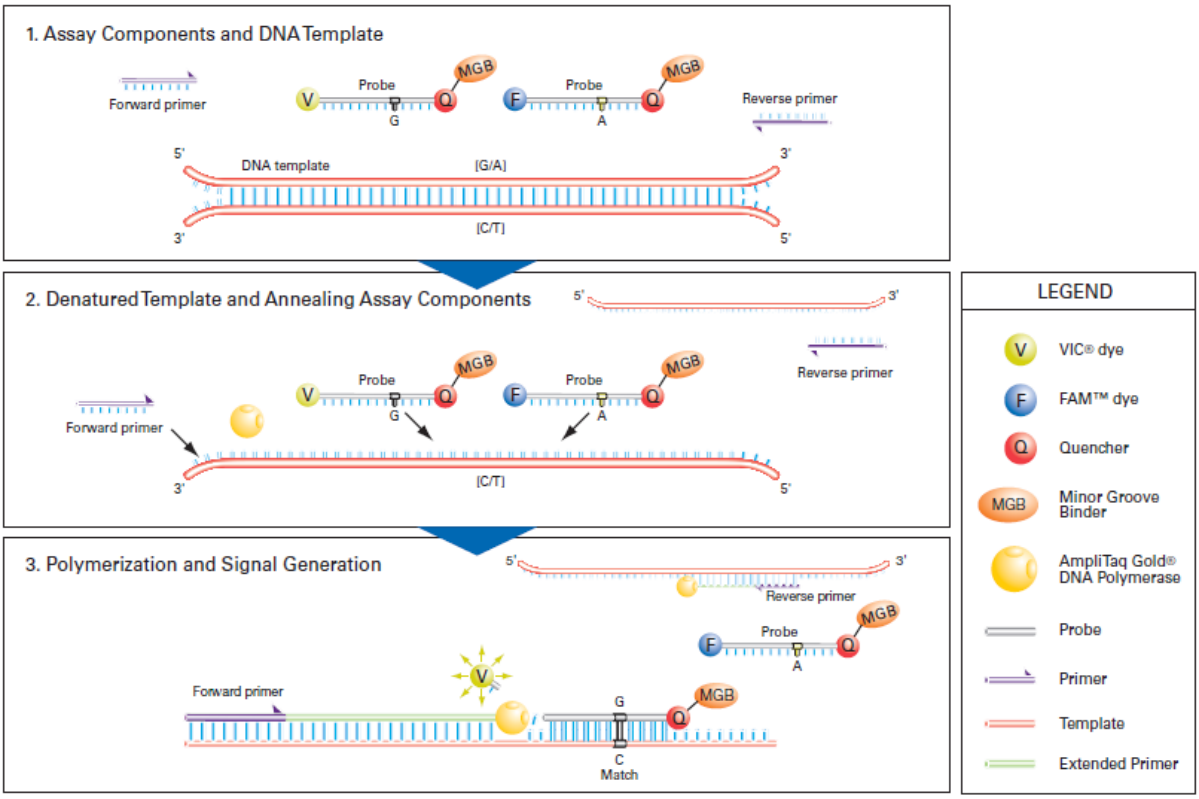


3-14 Standard curve of *SDHA* assay representing the threshold cycles of standard dilutions prepared from pooled anterior hippocampus cDNA

3.5 Allelic Discrimination

Allelic discrimination was carried out (as previously described –Dunham and colleagues[244]) using genomic DNA extracted from Stanley consortium samples and selected TaqMan SNP 5' nuclease assays (4351379, Applied Biosystems, Life Technologies, UK); targeting *DISC1* SNPs rs3738401 (C__25803261_10), rs821616 (C___1433135_20), rs6675281 (C___1650667_10), rs3737597 (C___2747617_30), also SCZ susceptibility gene SNPs BDNF rs6265 (C__11592758_10) and RAPTOR rs2289759 (C__15882533_10). Experiments were performed on the BIO-RAD CFX96 Real-Time System. A control SNP, previously analysed in the sample by a separate laboratory (data not shown), was utilised to fully establish confidence in the genotype determination. The results obtained for this BDNF SNP rs6265 were in 100% concordance with the previously produced results.

The 5’ nuclease assay utilises PCR technology to amplify loci harbouring target SNPs. Quenched reporter probes complimentary to each allelic variant are present with fluorophores attached in an allele-specific manner. The probe fluorophores are cleaved from the quencher during the PCR amplification, increasing fluorescence in an allele-specific manner.



3-15 The 5’ Nuclease process from the ABI TaqMan SNP Genotyping Assays Protocol (4332856C)

In this regard, genotype calls were made on the basis of a minimum of 3 experiments. Post-optimisation, the protocol used for completing the genotype data collection involved dispensing 1µl of wet gDNA, diluted to approximately 40ng/µl, into a 14µl PCR Cocktail for thermo-cycling. The constituent cocktail reagents and thermo-cycling parameters were as follows:

<u>Genotype Thermocycle</u>

1. Closed lid: Lid temperature of 105°C
2. Temperature step: 95°C for 10 min
3. 65 repetitions of 3 steps: a) 92°C for 15 sec
   b) 60.4 for 50 sec

c) Plate read

PCR Cocktail Reagent	Concentration	Volume (µl)
FastStart Universal Probe Master (04913957001, Roche, Hertfordshire, UK)	X2	6.25
SNP Assay	X20	0.625
Water	-	7.125
Total		14

Sample arrangement on each analysis plate is displayed in Fig 3-D, numbers in boxes refer to Stanley Consortium 'AL' identification numbers:

3-D Schematic representation of the 96-well plate setup for allelic discrimination analysis of each SNP. Samples are numbered 1-60 based on an AL identification number (NTC = Non-template control)

	01	**02**	**03**	**04**	**05**	**06**	**07**	**08**	**09**	**10**	**11**	**12**
A	1	2	3	4	5	6	7	8				
B	9	10	11	12	13	14	15	16				
C	17	18	19	20	21	22	23	24				
D	25	26	27	28	29	30	31	32				
E	33	34	35	36	37	38	39	40				
F	41	42	43	44	45	46	47	48				
G	49	50	51	52	53	54	55	56				
H	57	58	59	60	NTC	NTC	NTC	NTC				

3.6 Immunohistochemistry (IHC)

A selection of commercial antibodies for DISC1 binding partners (FEZ1, PDE4B and DPYSL2) and a marker of new-born neurons (NEUROD1) were

tested in tissue sections of hippocampus from Stanley Consortium brains to determine the cellular expression. Due to experimental constraints, only these antibodies were tested. Qualitative assessment was carried out via diaminobenzidine (DAB) immunohistochemistry (VECTASTAIN® ABC kit; VECTOR Laboratories) to determine the types of cells in the dentate hippocampus region expressing these markers. Quantitative assessment of successfully-trialled antibodies were achieved via near-infrared (IR) imaging of slides (LI-COR Odyssey).

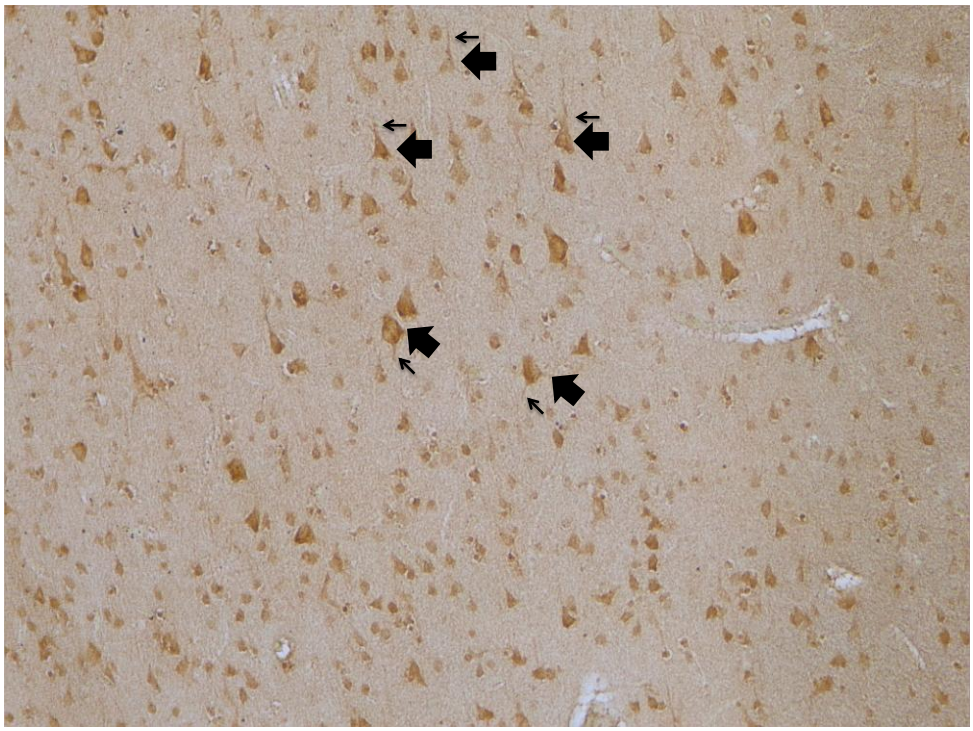
3.6.1 DAB Antibody Trials

FFPE tissue sections from the Stanley Consortium were utilised to demonstrate the binding specificity of the selected primary antibodies. Sections were deparaffinised in xylene (twice for 15 min) and rehydrated in an ethanol series (100% ethanol twice for 3 min, 95% ethanol twice for 3 min, 80% for 3 min, 70% for 3 min, then ddH_20 twice for 3 min). For antigen retrieval, sections were microwaved in citrate buffer (pH 6.0) for 20 min, left to cool until the buffer reached 60°C), then microwaved for a further 5 min. Sections were then incubated in 3% hydrogen peroxide in 0.1 M phosphate-buffered saline (PBS) for 30 min to quench endogenous peroxidise activity. Non-specific binding was blocked using a 10% normal serum in 0.1M PBS for 1.5 h. This was followed by application of the primary antibody diluted in 0.1 M PBS with 1% serum and 0.3% Triton X-100 at 4°C for 20 h.

Sections were then washed in 0.1 M PBS (3 × 10 min) before and after being incubated at room temperature with a biotinylated secondary antibody for 1.5 h. The VECTASTAIN Elite ABC kit (PK-6200, VECTOR Laboratories) was used according to the supplier's instructions to visualise antibody binding. Sections were then dehydrated with ethanol dilutions (ddH_20 for 1 min, 70% for 1 min, 80% for 1 min, 95% ethanol for 1 min and 100% ethanol for 1 min) before being mounted on coverslips. Negative control sections were obtained by omitting the primary antibody.

3.6.1.1 FEZ1 Antibody

FEZ1 immunoreactivity was detected in sections of dorsolateral prefrontal cortex using 1/500 Goat anti-Human FEZ1 antibody (NB100-53816, Novus Biologicus) as a primary antibody and 1/200 biotinylated Rabbit anti-Goat IgG (BA-5000, VECTOR Laboratories) for the secondary antibody. Non-specific binding of the FEZ1 antibody was blocked using 10% normal Rabbit serum (NRS). Discrete cellular FEZ1 staining was observed in both grey and white matter; the labelling of axons and pyramidal-shaped somata (Fig 3-16). corresponded well with previously reported cytosolic expression patterns[219].

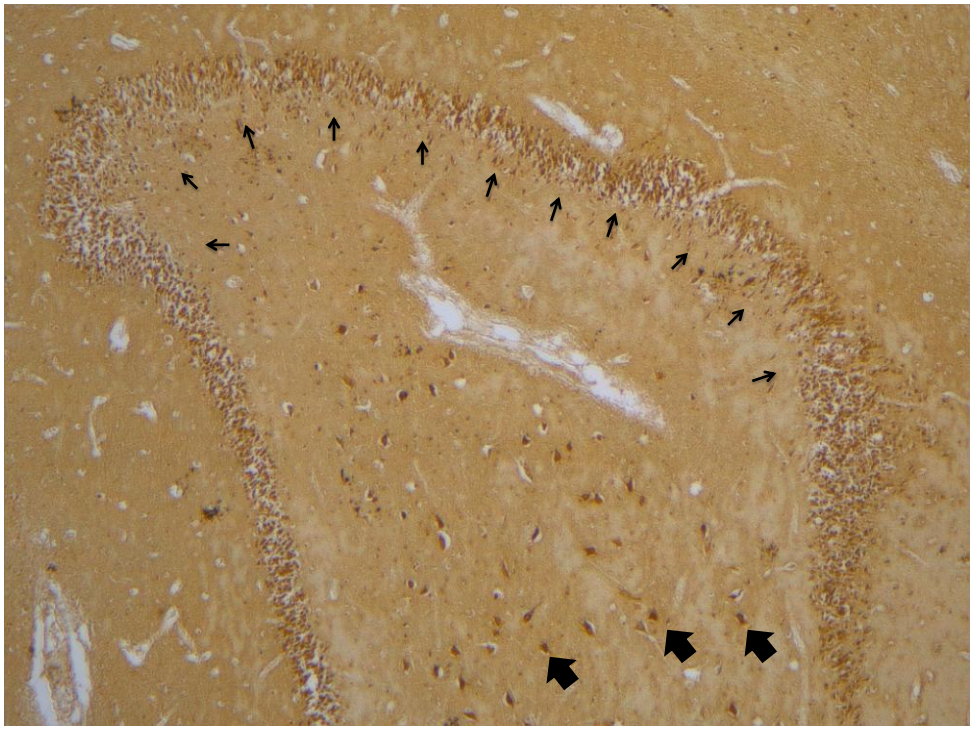


3-16 DAB Immunoreactivity of FEZ1 antibody conjugate in coronal sections of dorsolateral prefrontal cortex grey matter. Staining highlights neurite extensions (small black arrows) and mainly pyramidal-shaped neuronal somata (large black arrows).

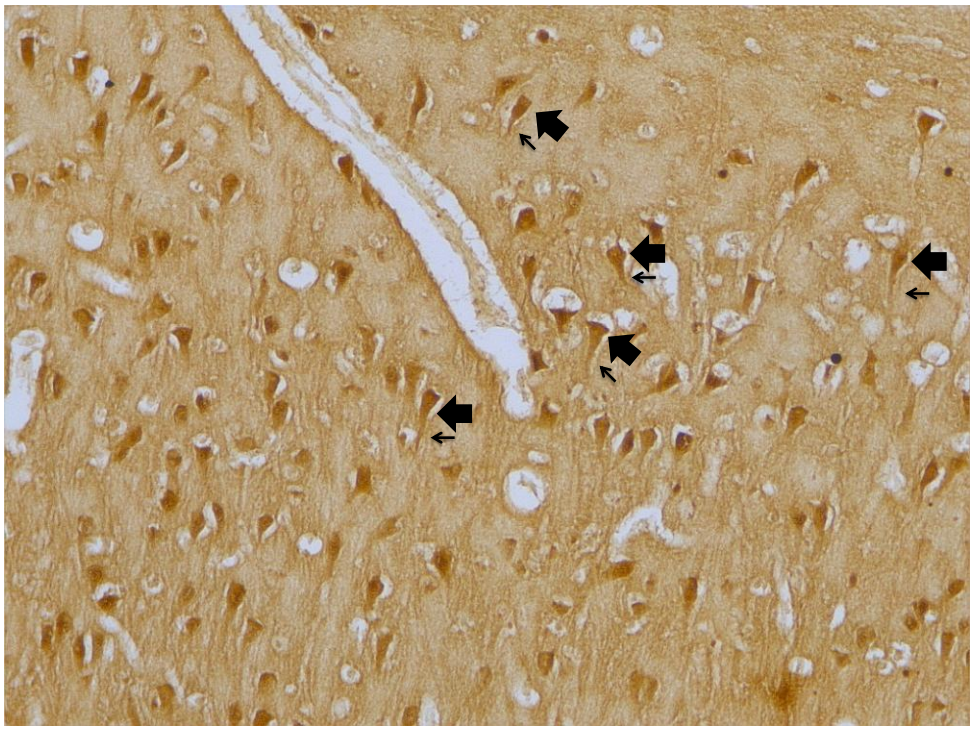
3.6.1.2 NEUROD1 Antibody

NEUROD1 immunoreactivity was detected in sections of anterior hippocampus using 1/200 Mouse anti-Human NEUROD1 antibody (ab60704, abcam, Cambridge, UK) as a primary antibody and a 1/100 dilution of a proprietary biotinylated secondary antibody mix of Horse anti-Mouse and Horse anti-Rabbit IgG supplied as part of the VECTASTAIN Elite ABC kit. Non-specific

binding of the NEUROD1 antibody was blocked using 10% normal Horse serum (NHS). Distinct NEUROD1 immunoreactivity was detected in the dentate gyrus granular layer, with sporadic staining in the hilus (CA4) (Fig 3-17). The morphology of labelled cells was in the granular layer was primarily pyramidal (Fig 3-18). This was consistent with the previous reports of NEUROD1 expression in the dentate gyrus[245]; as well as the expected expression pattern of a putative marker of neurogenesis.



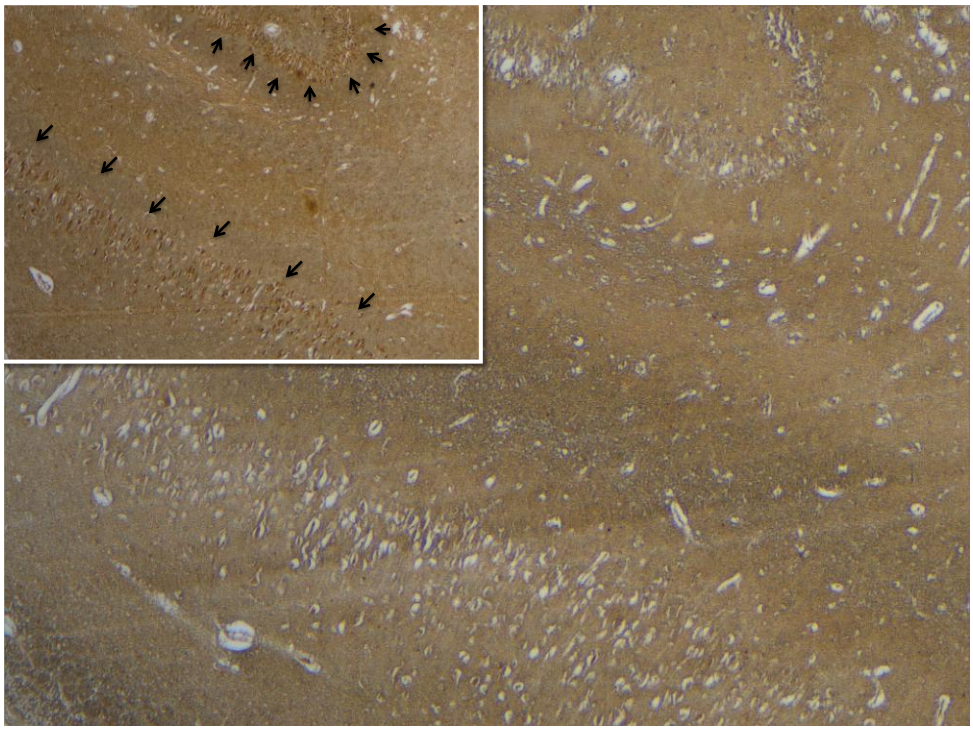
3-17 DAB Immunoreactivity of NEUROD1 antibody conjugate in coronal sections of anterior hippocampus. Staining highlights the dentate gyrus (small black arrows) and the appearance of sporadic staining of spheroid staining in the hilus (CA4) large black arrows).



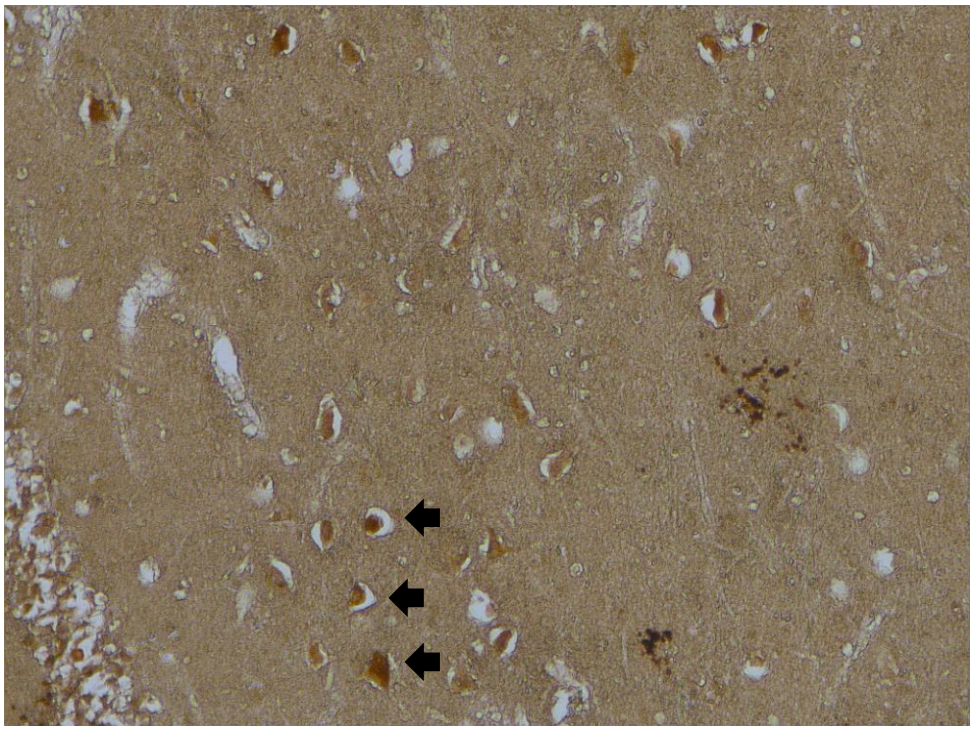
3-18 DAB Immunoreactivity of NEUROD1 antibody conjugate in the dentate gyrus granule cell layer. Staining highlights the mainly pyramidal-shaped somata stained (large black arrows) and their process (small black arrows)

3.6.1.3 PDE4B Antibody

PDE4B immunoreactivity was detected in sections of anterior hippocampus using a 1/100 dilution of Rabbit anti-Human PDE4B antibody (Ab14611, Abcam)1 and a 1/100 dilution of the proprietary mix of biotinylated secondary antibodies (Horse anti-Mouse and Horse anti-Rabbit IgG) supplied as part of the VECTASTAIN Elite ABC kit. This antibody was not selected for quantitative analysis. This was because no discrete staining was observed to would allow for accurate quantification (Fig 3-19, 3-20). Furthermore, the lack of obvious staining in the anterior hippocampal sections ran in direct contradiction to the previous reports of widespread distribution in the hippocampus[246]. The limited quantitative IHC resources were therefore redirected to the analysis of more effective primary antibodies.



3-19 DAB Immunoreactivity of PDE4B antibody conjugate in sections of anterior hippocampus. The clear lack of discrete cellular staining is highlighted by comparisons with NEUROD1 staining trials in the same region of the hippocampus where staining of the dentate gyrus can be observed (inset, small black arrows)

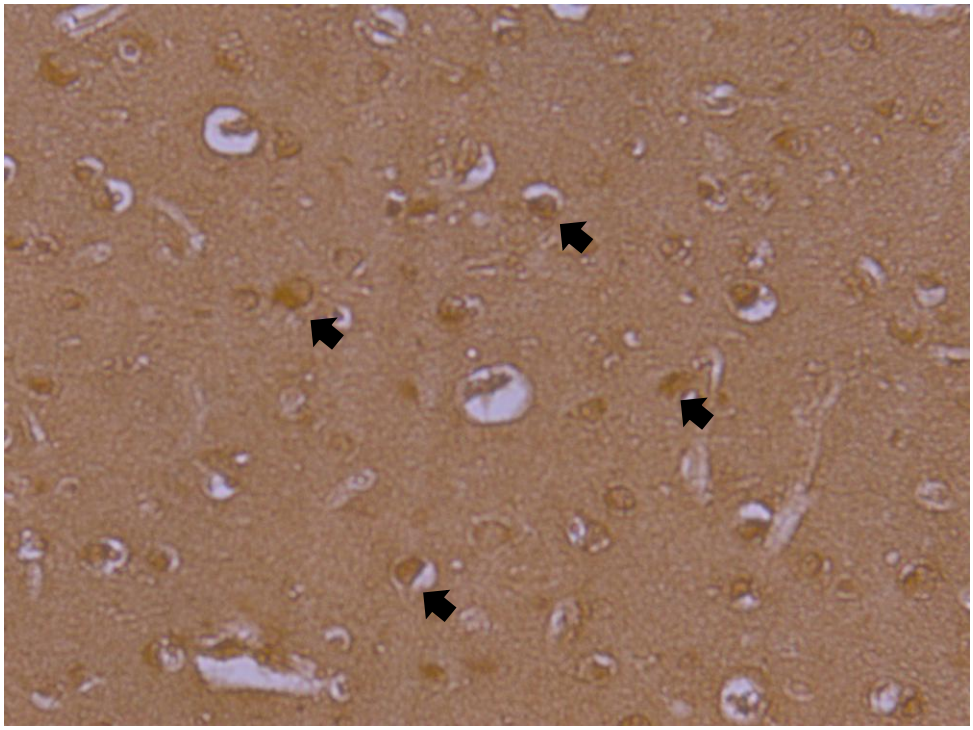


3-20 DAB Immunoreactivity of PDE4B antibody conjugate in the hilus (CA4). The appearance of sparse staining of spheroid cell somata can be observed (large black arrows)

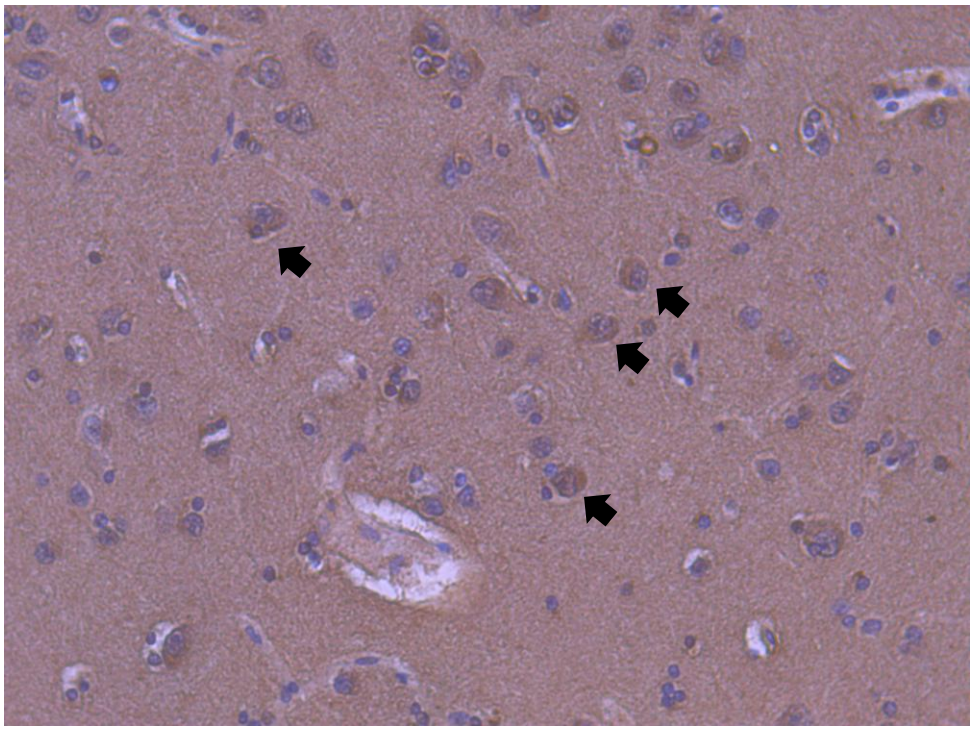
3.6.1.4 DPYSL2 Antibody

Detection of the DPYSL2 immunoreactivity was conducted in sections of dorsolateral prefrontal cortex using a 1/200 dilution of Goat anti-Human CRMP2 (AB9892, Millipore UK, Watford, UK) as a primary antibody; and a 1/200 of Rabbit anti-Goat Biotinylated IgG for the secondary. Expression was expected to be specific to neuronal populations with the capacity for differentiation and neuroplasticity modifications[247]. During the optimisation of the IHC assay, it was determined that the staining produced by the antibody was mainly non-specific especially in the white matter (Fig. 3-21). This was investigated further by counter-staining with a Harris modified haematoxylin solution (HHS32, Sigma-Aldrich) for 7 min to stain nuclei, the underside of sections were then run under cold tap water for 1 min) before mounting. Counter-staining was performed after secondary antibody wash steps This demonstrated that stained neurons were sparsely populated in the white matter, and the staining intensity was effectively

masked by the non-specific staining in the neuropil (Fig. 3-22). Quantitative IHC resources were therefore redirected accordingly.



3-21 DAB Immunoreactivity of DPYSL2 antibody conjugate in coronal sections of dorsolateral prefrontal cortex (white matter). The apparent staining of spheroid cell somata (large black arrows) is largely obscured by high non-specific staining in the neuropil.



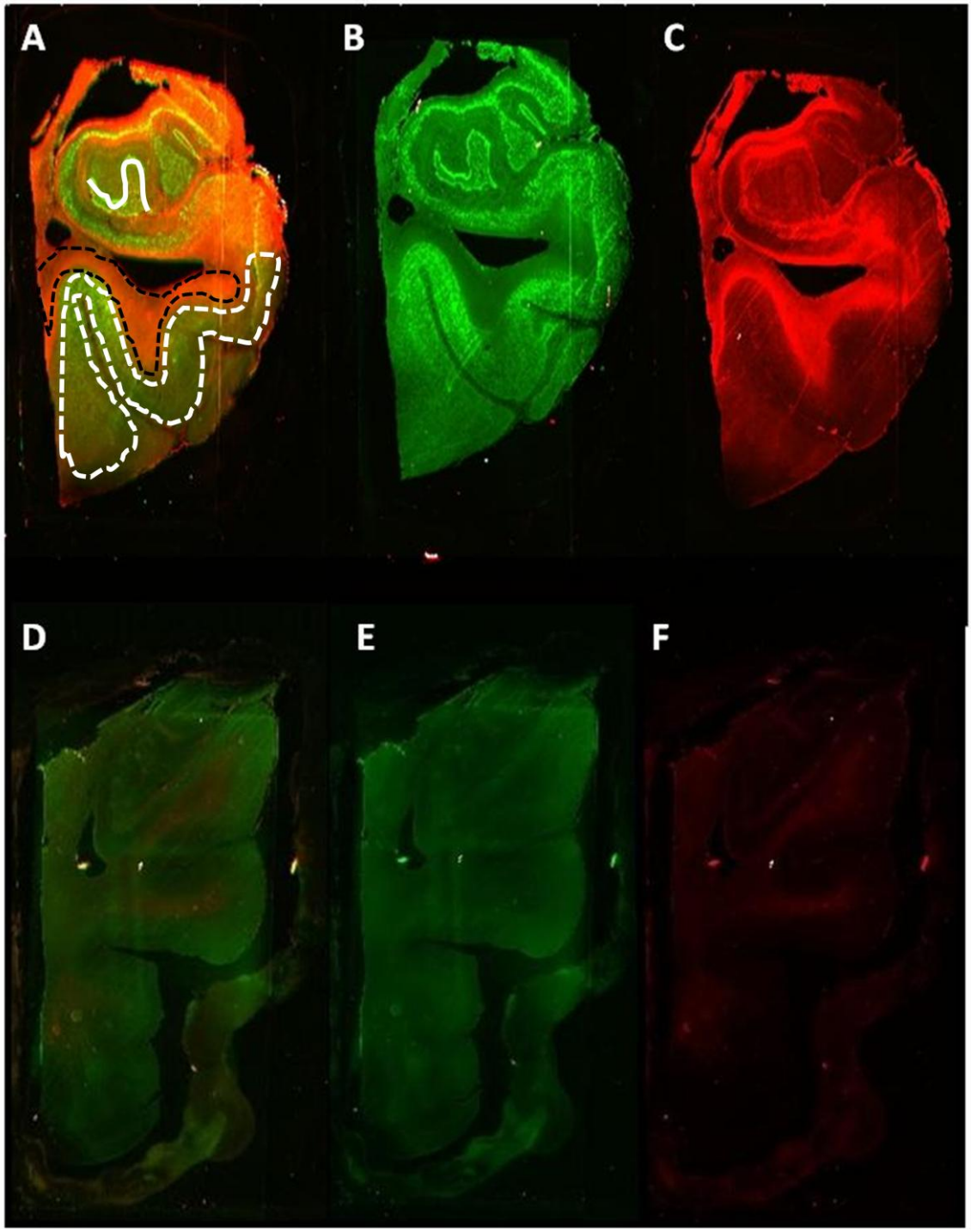
3-22 DAB Immunoreactivity of DPYSL2 antibody conjugate in coronal sections of dorsolateral prefrontal cortex (white matter) counter-stained with haematoxylin. The sparse spheroid staining can be clearly attributed to somata counter-stained with haematoxylin, though this is still obscured by non-specific staining.

3.6.2 Near-IR IHC

As FEZ1 and NEUROD1 antibodies were successfully trialled, quantitative assessments of antibody binding in frozen slides of anterior hippocampus were conducted. Quantitative assessment of FEZ1 protein density was conducted twice (an initial and a repeat experiment). Due to experimental constraints NEUROD1 assessments were carried out once only. In both cases, sections were fixed in 4% paraformaldeyhde (9.5 min), washed in 0.1M PBS (2 × 5 min), and non-specific binding blocked with 10% normal Donkey serum (NDS) in 0.1M PBS for 1.5 h. Primary antibody incubations were performed in 0.1 M PBS and 0.3% Triton X-100, but with 1% NDS at 4°C for 20 h. Sections were then washed in 0.1 M PBS (3 × 10 min) before and after being incubated with a near infrared dye-conjugated secondary antibodies for 1.5 h. Sections were then dehydrated with ethanol dilutions.

FEZ1 expression was estimated using a 1/200 primary antibody dilution and signal intensities emitted from 1:200 dilution of secondary antibody (Donkey anti-Goat IgG, Alexa Fluor® 647 Conjugate, AP180SA6, Millipore, UK). The FEZ1 signal intensity was strongest in the white matter. NEUROD1 expression was estimated using a 1/210 primary antibody dilution and a 1:200 dilution of secondary antibody (IRDye® 800CW Donkey Anti-Mouse IgG (H+L), Highly Cross Adsorbed (926-32212, LI-COR Biosciences). NEUROD1 labelling was primarily located in the dentate gyrus and surrounding grey matter tissue (Fig 3-23).

The fluorescent immuno-complexes were detected with the LI-COR Odyssey (Fig. 2B; 169 µm resolution, 0.9mm offset with high quality). The 700nm channel sensitivity was optimised for each set of stained sections, with 5 being found to be most optimal. Using the associated Odyssey software, regions of interest (ROIs) were defined (Fig 3-23) and independent protein densities (or mean specific intensities, I_{si}), were calculated for the anterior parahippocampal grey matter, white matter and dentate gyrus separately. $I_{si} = I_i - b_i$, where b_i = non-specific and background intensity and I_i = overall mean intensity; b_i was derived from negative primary antibody controls. These calculations were performed according to the Odyssey operating instructions. In short, the protein density reflects the average specific signal intensity of the secondary antibody within the defined ROI.



3-23 Images of FEZ1 and NEUROD1 double immuno-labelling (A, Grey matter ROI = Dashed white line, White matter ROI = dashed black line, Dentate gyrus ROI = solid white line); NEUROD1 immuno-labelling (B); FEZ1 immuno-labelling. No primary antibody negative controls for NEUROD1 and FEZ double immuno-labelling (D), NEUROD1 (E), and FEZ1 (F)

3.7 Statistical Analysis

All statistical analyses were performed using the IBM SPSS statistics package. NRQs and l_{si} values were grouped on the basis of diagnosis for outlier removal prior to statistical testing. Univariate ANOVAs were performed to compare expression levels across diagnostic categories[248]. When Levene's test for Homogeneity of Variance was violated ($p < .05$), in some cases the F value was predicted to undergo a reduction due to sample size, thus making the ANOVA test more conservative. In these cases parametric testing was concluded. Otherwise, when the Levene's Test was violated Kruskal-Wallis testing was utilised; and the non-parametric equivalent of the Levene's test applied.

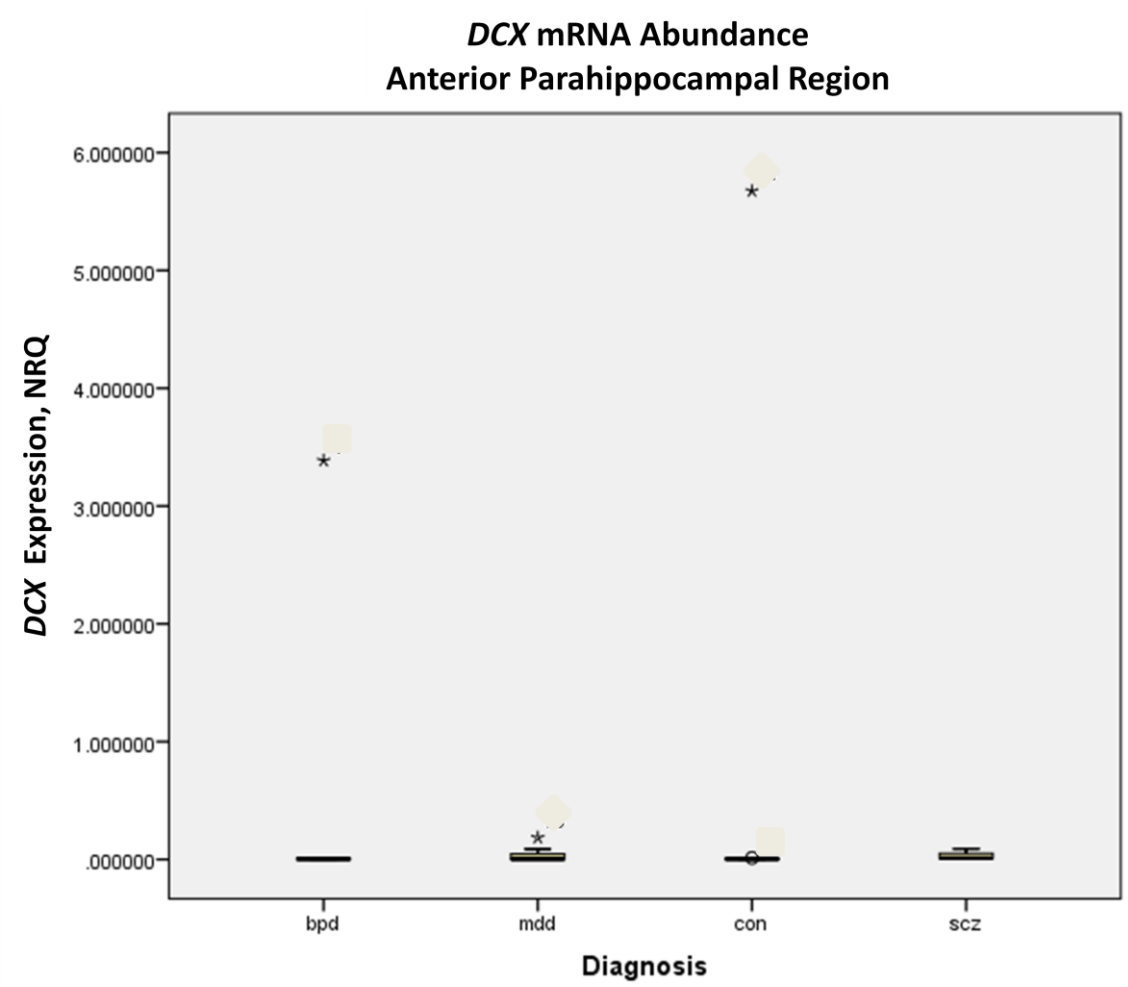
For analysis of genotyping data, the dominant effect model was used by grouping homozygous and heterozygous for the minor allele and comparing with subjects homozygous for the major allele via Mann-Whitney *U* testing. Gene expression data associated with genotype was then further assessed, where possible, using repeated-measures ANOVA (between-subjects factor being genotype) to avoid artefacts of multiple comparisons.

Assessments of demographic and clinical variables were carried out using Mann Whitney *U* tests (dichotomous variables) and Spearman's rank-order correlations (continuous variables). To identify potential dichotomous coviariates; IHC and mRNA data variable were compared between groups of gender, psychosis, and brain hemisphere using Mann Whitney *U* tests. To identify potential continuous covariates, age, pH, PMI, drugs and RQI were correlated with mRNA and IHC variables using Spearman's rank-order correlations (see 3.7.2). Those showing significant correlations were retained as covariates in comparisons between schizophrenia, bipolar disorder, major depressive disorder and controls using Univariate ANCOVAs. Unless stated otherwise, all ANCOVA tests performed conformed to the homogeneity of regression model.

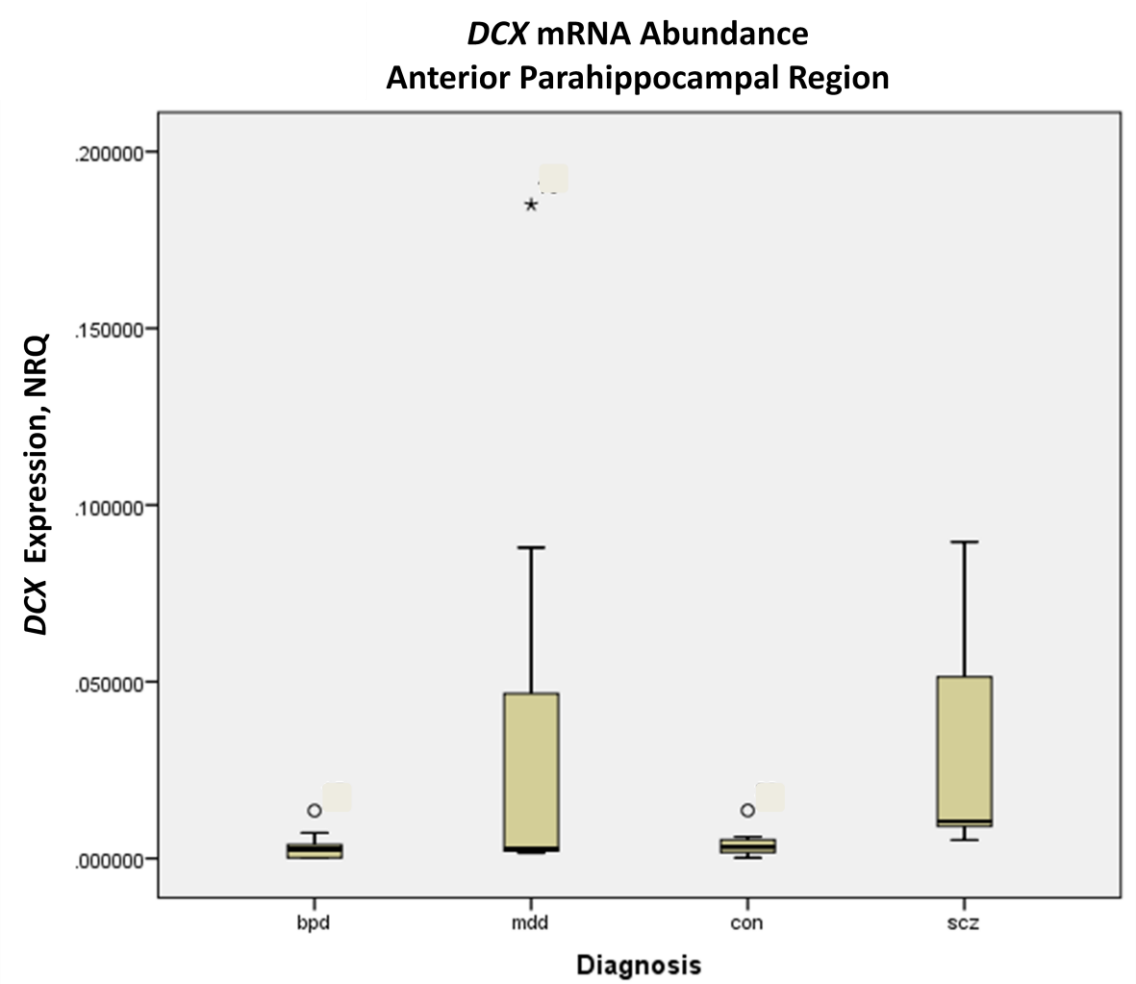
3.7.1 Outlier Removal

Outliers were removed using the Tukey outlier labelling rule[249], (g = 1.5). Sample size precluded the use of Tukey outlier labelling for non-neurogenic HF qPCR, so in this region the limit of 2.5 standard deviations from the mean was utilised instead. In order to maintain outlier removal consistency, a subsequent re-test of all outliers was performed using this 'standard deviation from the mean' rule. These results were in concordance with Tukey outlier labelling. Due to ongoing concerns regarding sample size and statistical power, outlier removal was recorded and detailed below (Figs.3-24 to 3-57) and parallel tests, with outliers maintained, were performed (see 5-A Table, 6-A Table, and 9Appendix C).

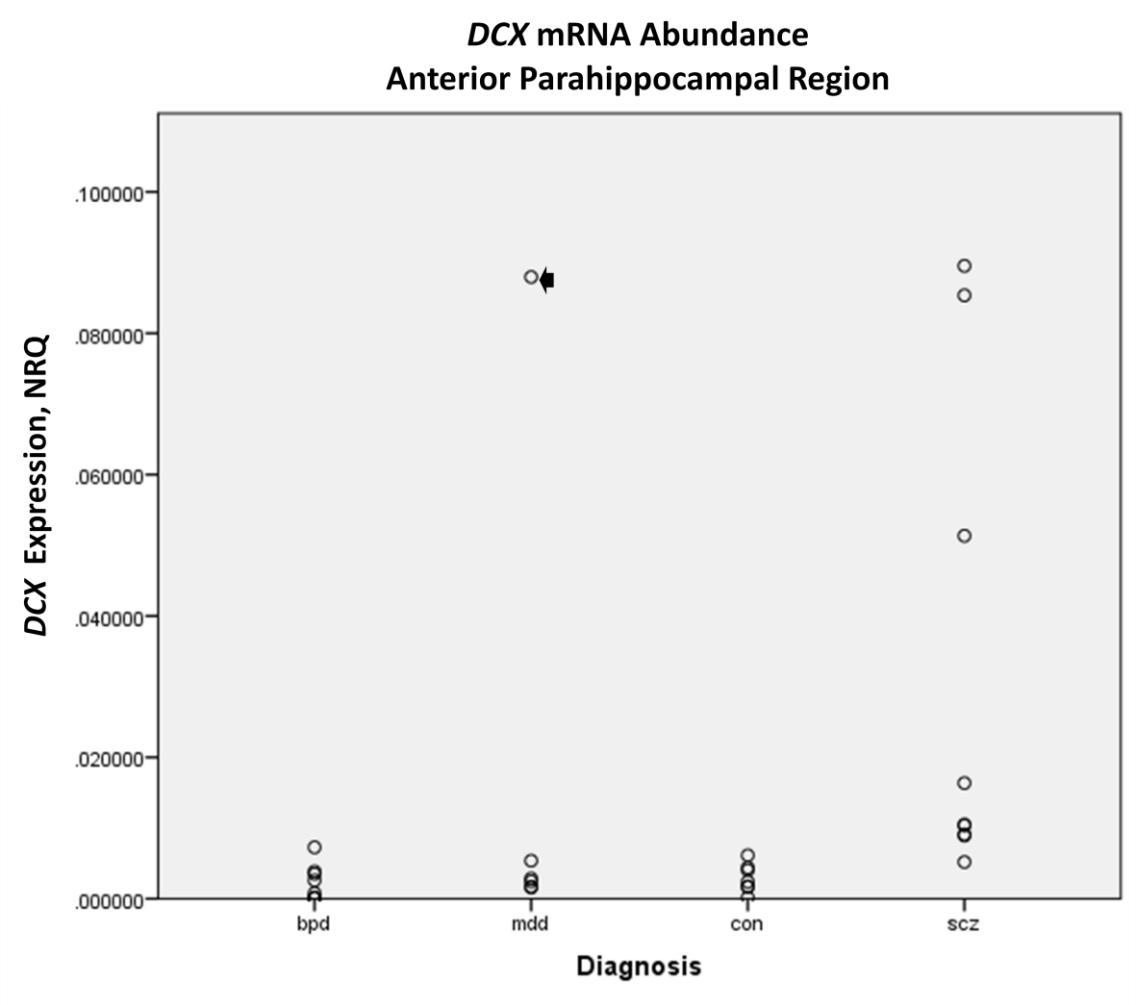
3.7.1.1 *DCX* qPCR Plots



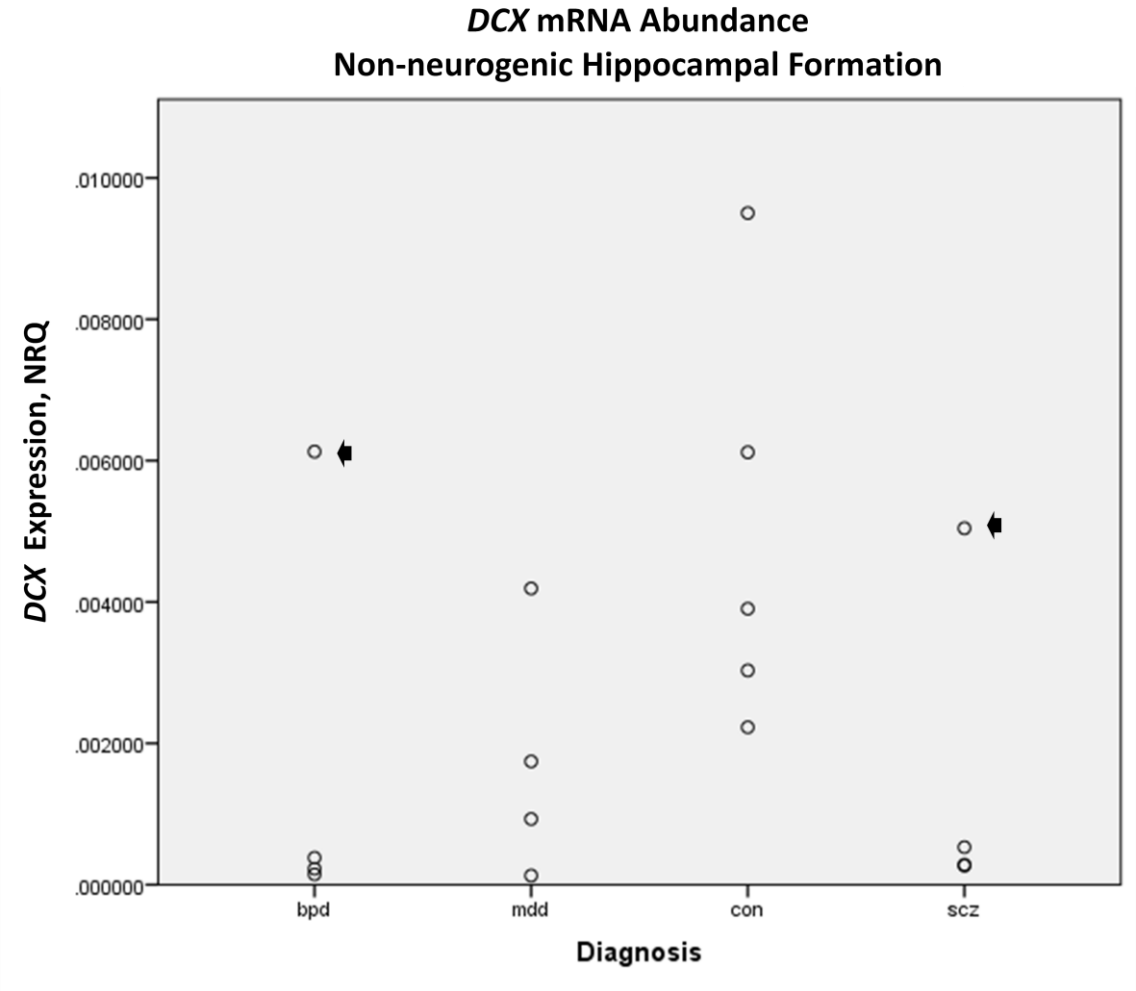
3-24 Box-plots showing distributions of *DCX* mRNA transcript levels in the anterior parahippocampal region and the identified outliers. Asterisk denotes outliers identified using Tukey Outlier Labelling Rule (*g* = 3); circle indicates (*g* = 1.5). All of the indicated Tukey outliers were also highlighted by the '2.5 standard deviations from the mean' test. NRQ = Normalised Relative Quantity, bpd = Bipolar Disorder, mdd = Major Depressive Disorder, scz = Schizophrenia, con = controls,



3-25 Box-plots showing distributions of *DCX* mRNA transcript levels in the anterior parahippocampal region after the initial removal of labelled outliers. Asterisk denotes outliers identified using Tukey Outlier Labelling Rule (*g* = 3); circle indicates (*g* = 1.5). All of the indicated Tukey outliers were also highlighted by the '2.5 standard deviations from the mean' test. NRQ = Normalised Relative Quantity, bpd = Bipolar Disorder, mdd = Major Depressive Disorder, scz = Schizophrenia, con = controls,

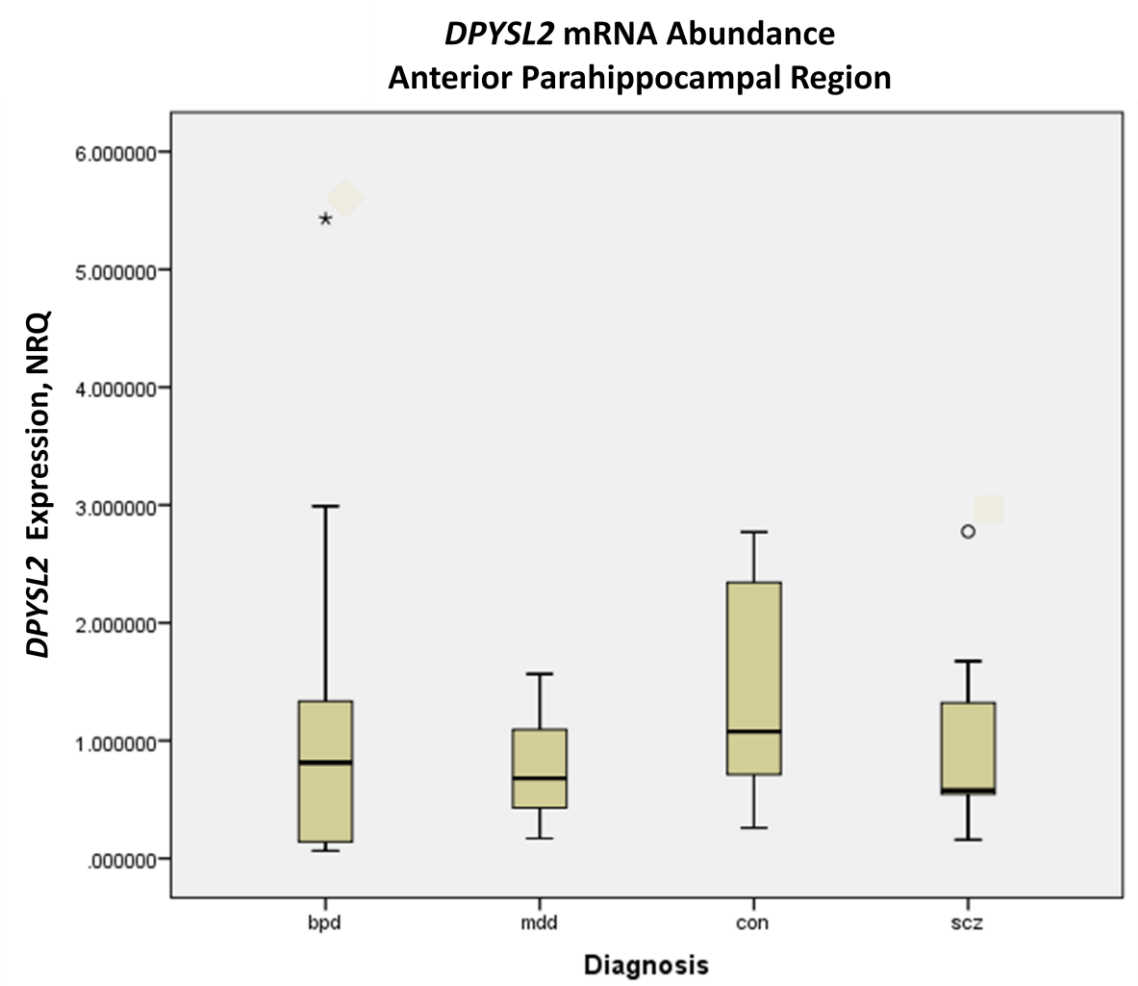


3-26 Scatter plot showing *DCX* mRNA transcript levels in the anterior parahippocampal region after Tukey outlier removal. Black arrows indicate removed outliers that were still highlighted by the '2.5 standard deviations from the mean' test. NRQ = Normalised Relative Quantity, bpd = Bipolar Disorder, mdd = Major Depressive Disorder, scz = Schizophrenia, con = controls.

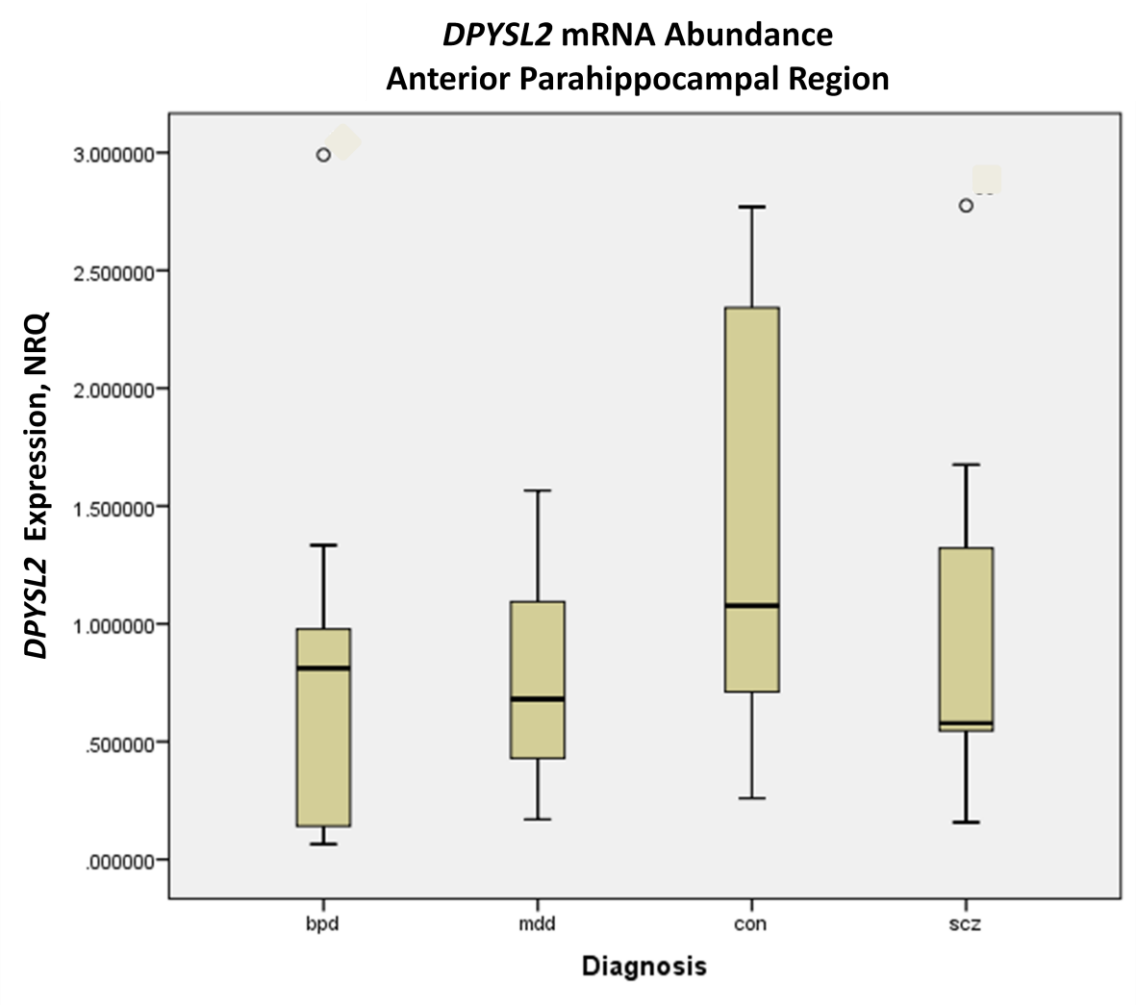


3-27 Scatter plot showing *DCX* mRNA transcript levels in the non-neurogenic hippocampal formation. Black arrows indicate removed outliers highlighted by the '2.5 standard deviations from the mean' test. NRQ = Normalised Relative Quantity, bpd = Bipolar Disorder, mdd = Major Depressive Disorder, scz = Schizophrenia.

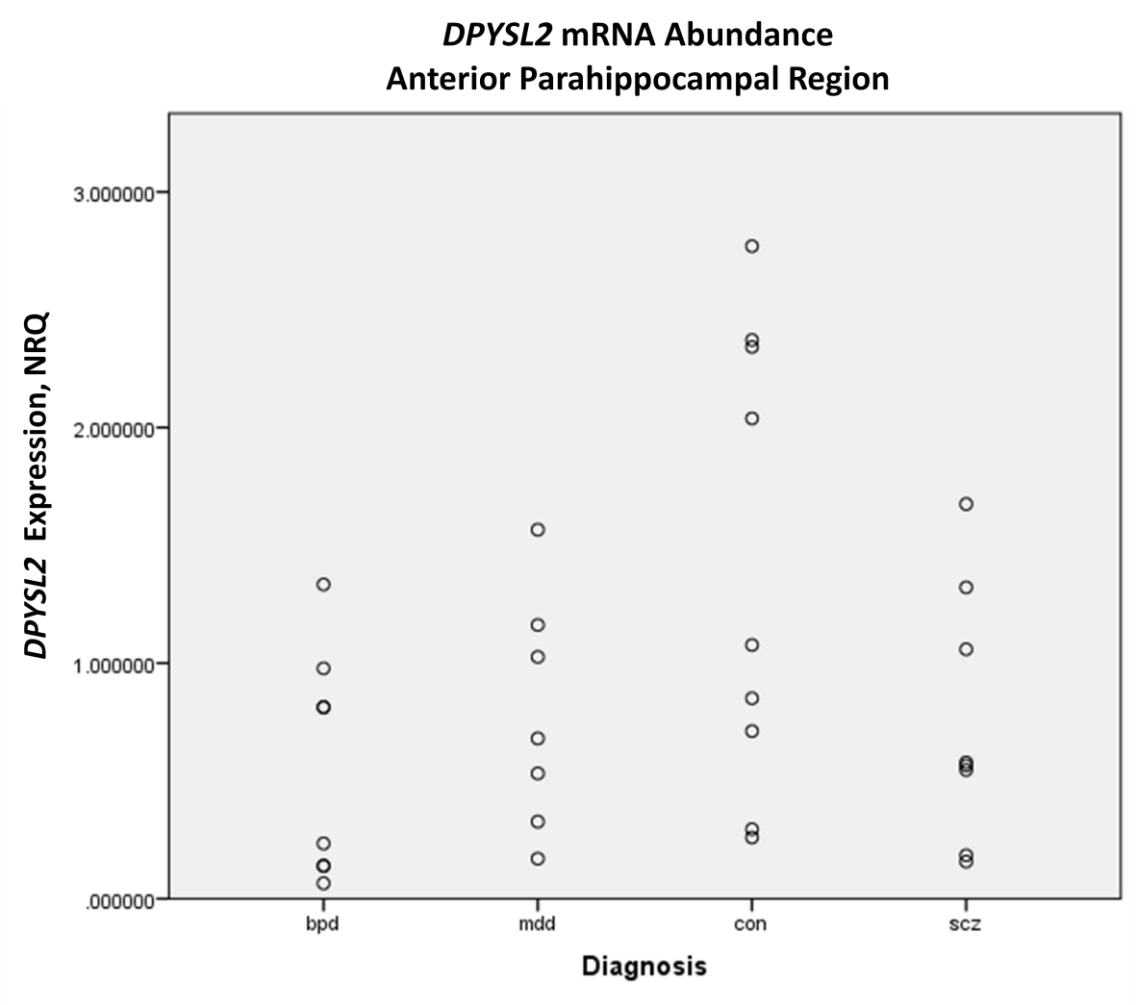
3.7.1.2 *DPYSL2* qPCR Plots



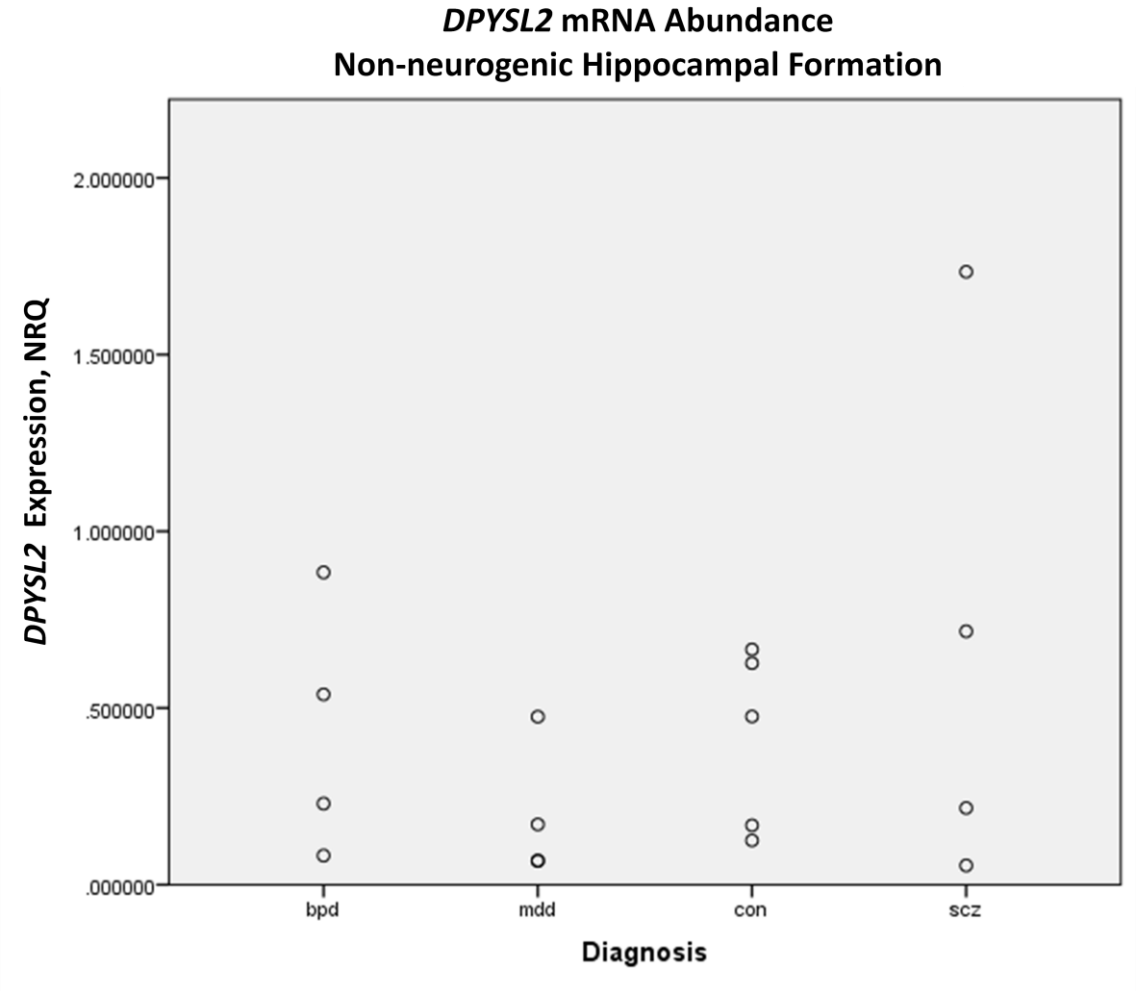
3-28 Box-plots showing distributions of *DPYSL2* mRNA transcript levels in the anterior parahippocampal region and the identified outliers. Asterisk denotes outliers identified using Tukey Outlier Labelling Rule (*g* = 3); circle indicates (*g* = 1.5). All of the indicated Tukey outliers were also highlighted by the '2.5 standard deviations from the mean' test. NRQ = Normalised Relative Quantity, bpd = Bipolar Disorder, mdd = Major Depressive Disorder, scz = Schizophrenia, con = controls,



3-29 Box-plots showing distributions of *DPYSL2* mRNA transcript levels in the anterior parahippocampal region after the initial removal of labelled outliers. Circles denote outliers identified using Tukey Outlier Labelling Rule (*g* = 1.5). All of the indicated Tukey outliers were also highlighted by the '2.5 standard deviations from the mean' test. NRQ = Normalised Relative Quantity, bpd = Bipolar Disorder, mdd = Major Depressive Disorder, scz = Schizophrenia, con = controls,

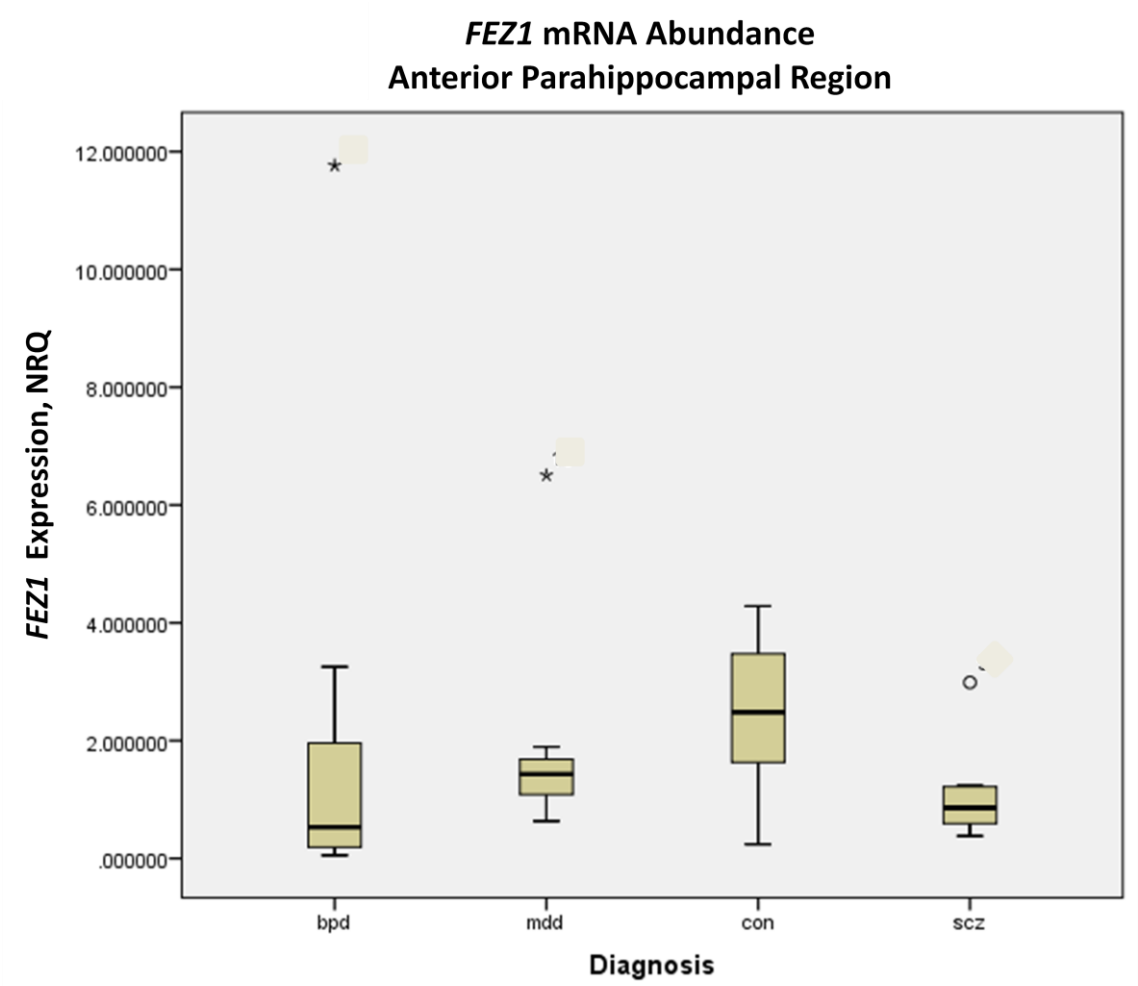


3-30 Scatter plot showing *DPYSL2* mRNA transcript levels in the anterior parahippocampal region after Tukey outlier removal. NRQ = Normalised Relative Quantity, bpd = Bipolar Disorder, mdd = Major Depressive Disorder, scz = Schizophrenia, con = controls.

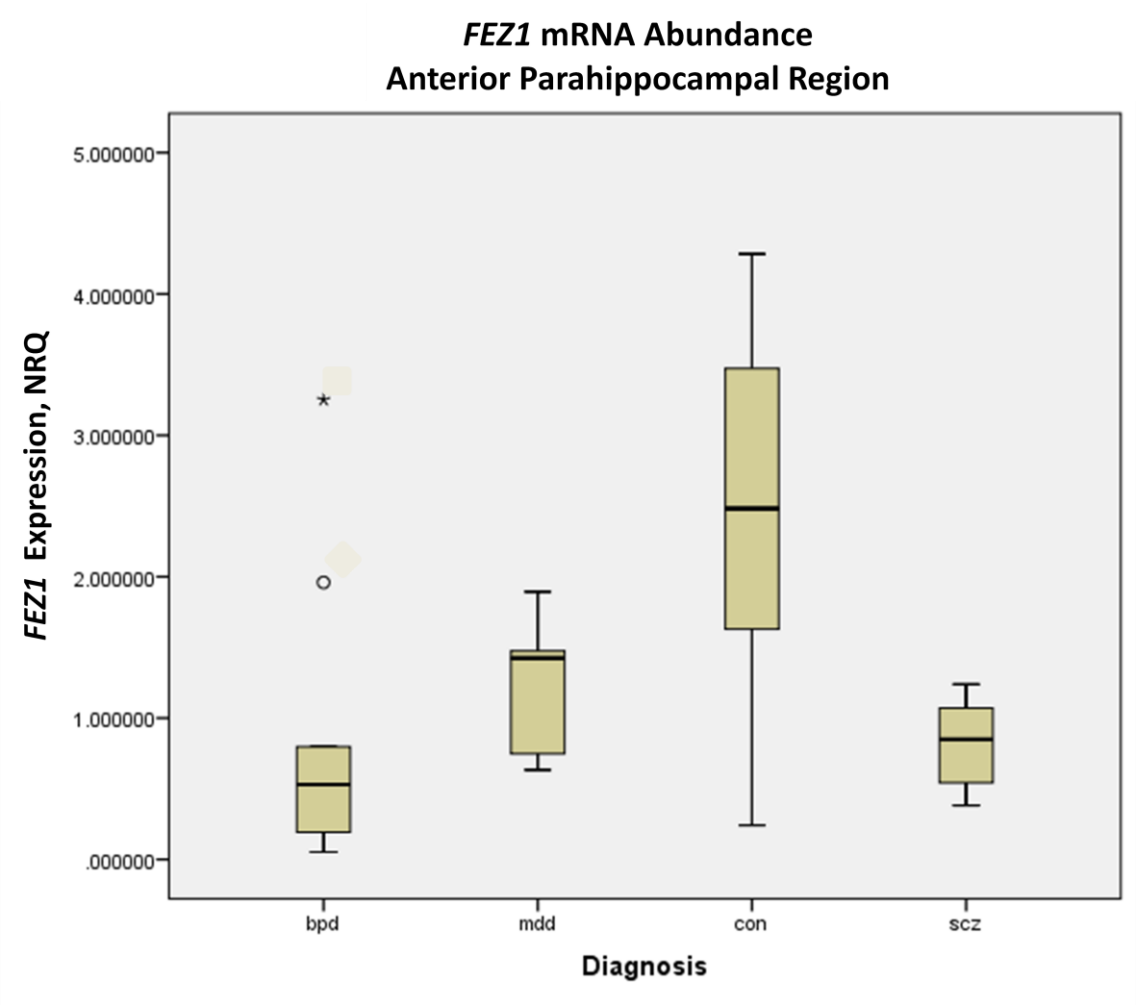


3-31 Scatter plot showing *DPYSL2* mRNA transcript levels in the non-neurogenic hippocampal formation. No outliers were identified. NRQ = Normalised Relative Quantity, bpd = Bipolar Disorder, mdd = Major Depressive Disorder, scz = Schizophrenia.

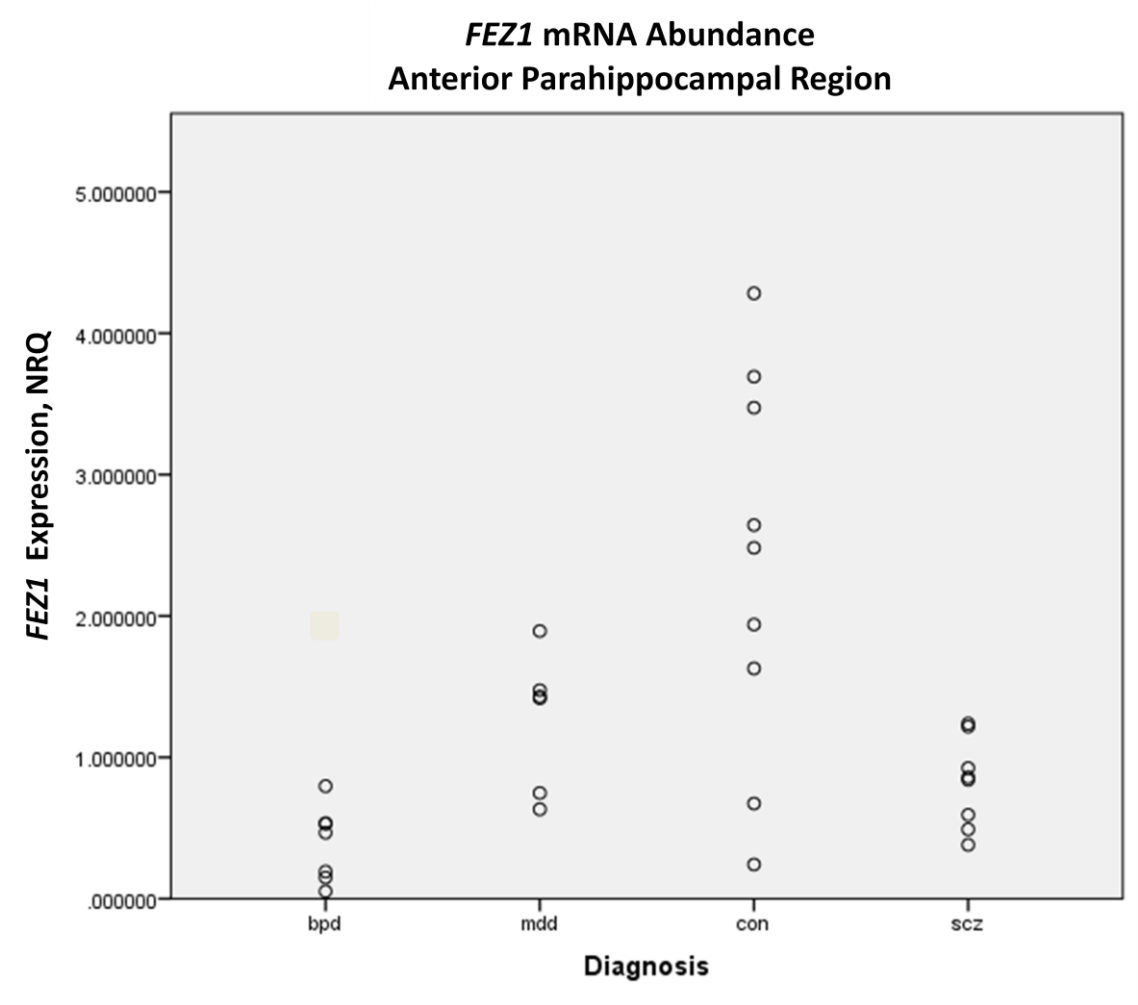
3.7.1.3 *FEZ1* qPCR Plots



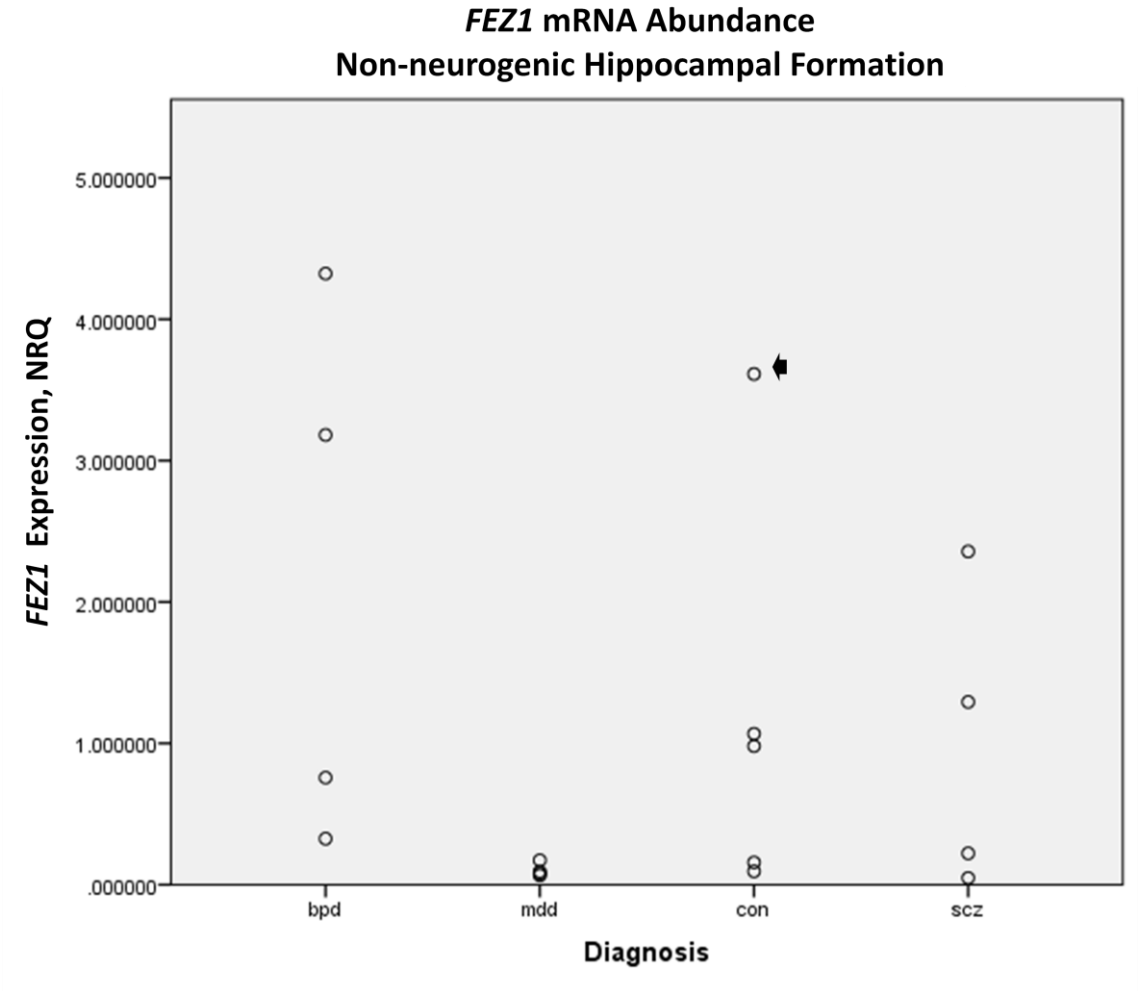
3-32 Box-plots showing distributions of *FEZ1* mRNA transcript levels in the anterior parahippocampal region and the identified outliers. Asterisk denotes outliers identified using Tukey Outlier Labelling Rule (*g* = 3); circle indicates (*g* = 1.5). All of the indicated Tukey outliers were also highlighted by the '2.5 standard deviations from the mean' test. NRQ = Normalised Relative Quantity, bpd = Bipolar Disorder, mdd = Major Depressive Disorder, scz = Schizophrenia, con = controls,



3-33 Box-plots showing distributions of *FEZ1* mRNA transcript levels in the anterior parahippocampal region after the initial removal of labelled outliers. Asterisk denotes outliers identified using Tukey Outlier Labelling Rule (*g* = 3); circle indicates (*g* = 1.5). All of the indicated Tukey outliers were also highlighted by the '2.5 standard deviations from the mean' test. NRQ = Normalised Relative Quantity, bpd = Bipolar Disorder, mdd = Major Depressive Disorder, scz = Schizophrenia, con = controls,

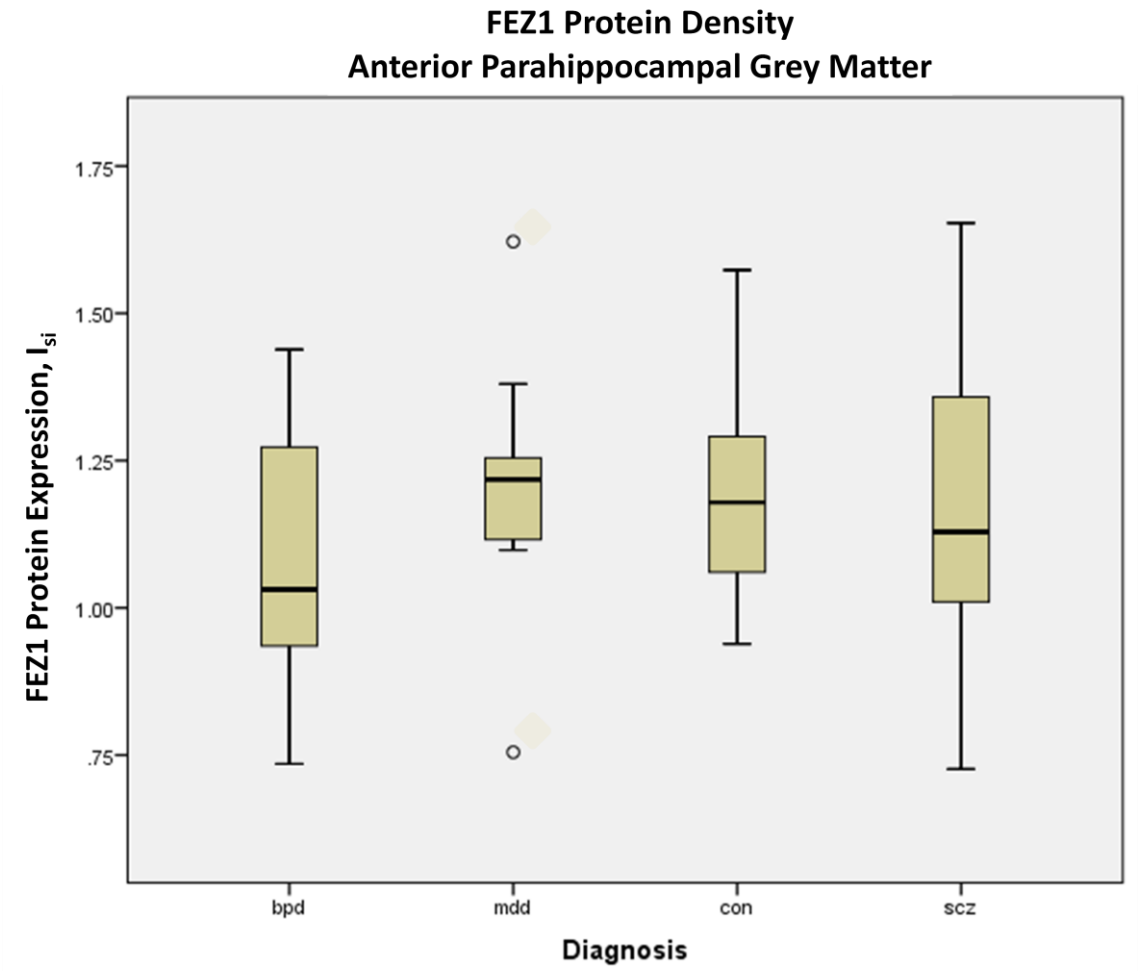


3-34 Scatter plot showing *FEZ1* mRNA transcript levels in the anterior parahippocampal region after Tukey outlier removal. NRQ = Normalised Relative Quantity, bpd = Bipolar Disorder, mdd = Major Depressive Disorder, scz = Schizophrenia, con = controls.

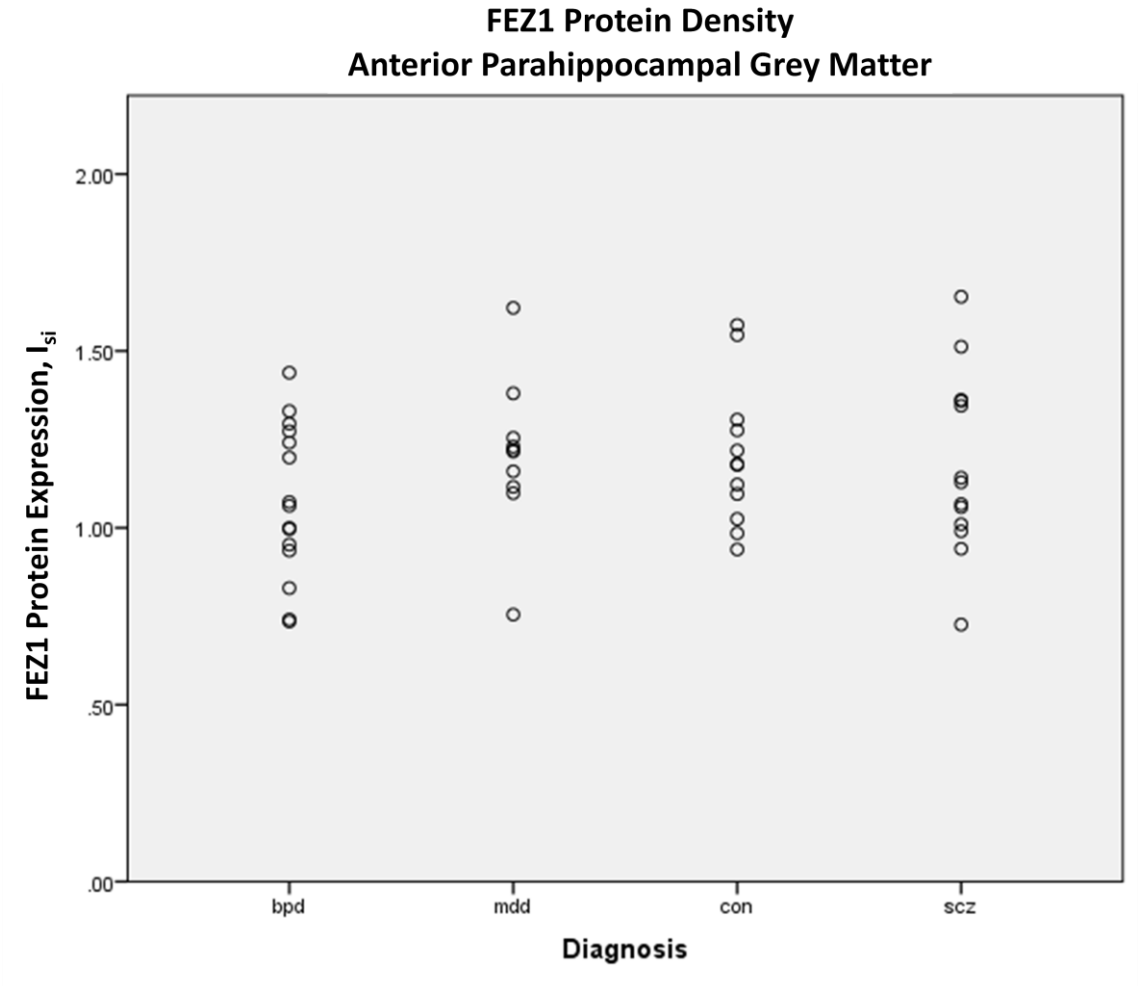


3-35 Scatter plot showing *FEZ1* mRNA transcript levels in the non-neurogenic hippocampal formation. Black arrows indicate removed outliers highlighted by the '2.5 standard deviations from the mean' test. NRQ = Normalised Relative Quantity, bpd = Bipolar Disorder, mdd = Major Depressive Disorder, scz = Schizophrenia.

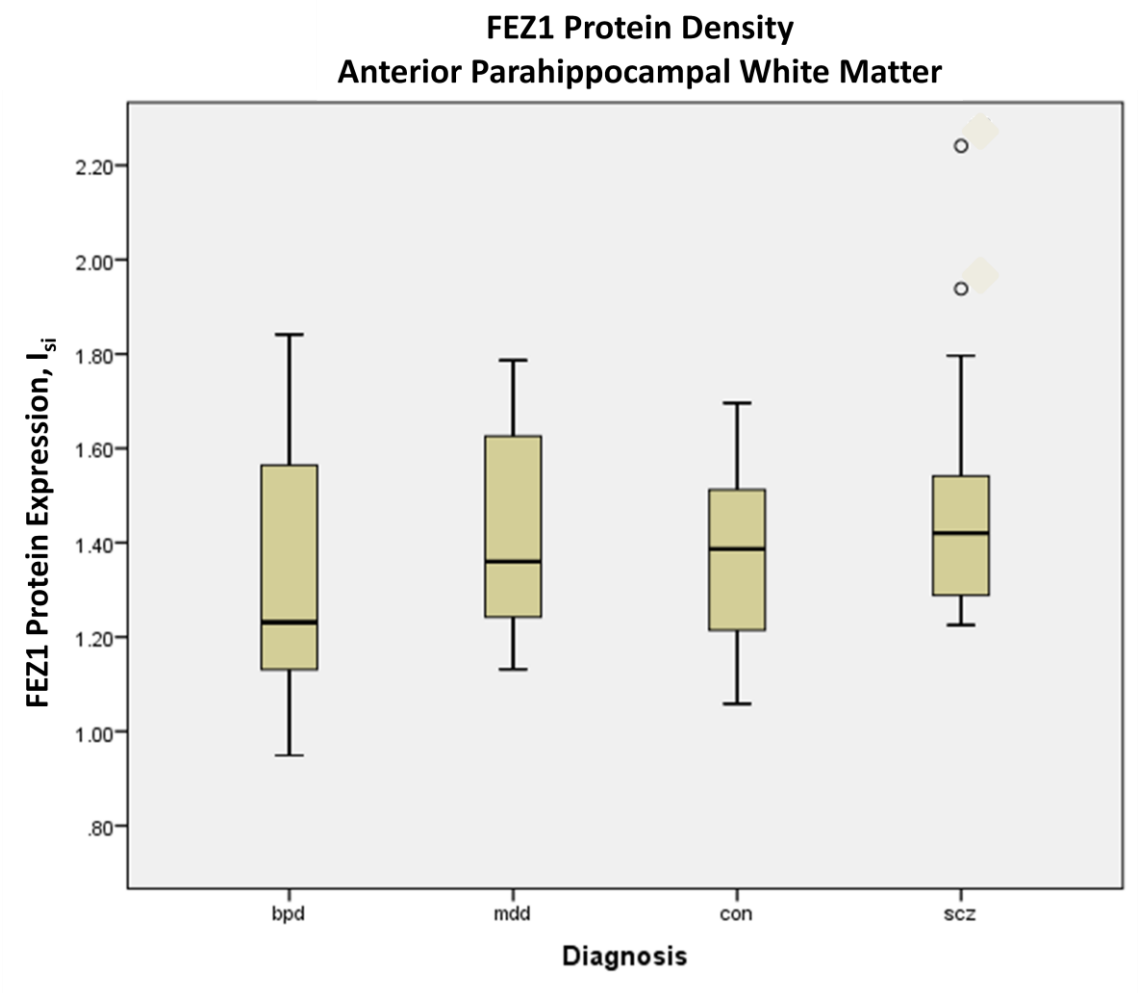
3.7.1.4 FEZ1 IHC Plots



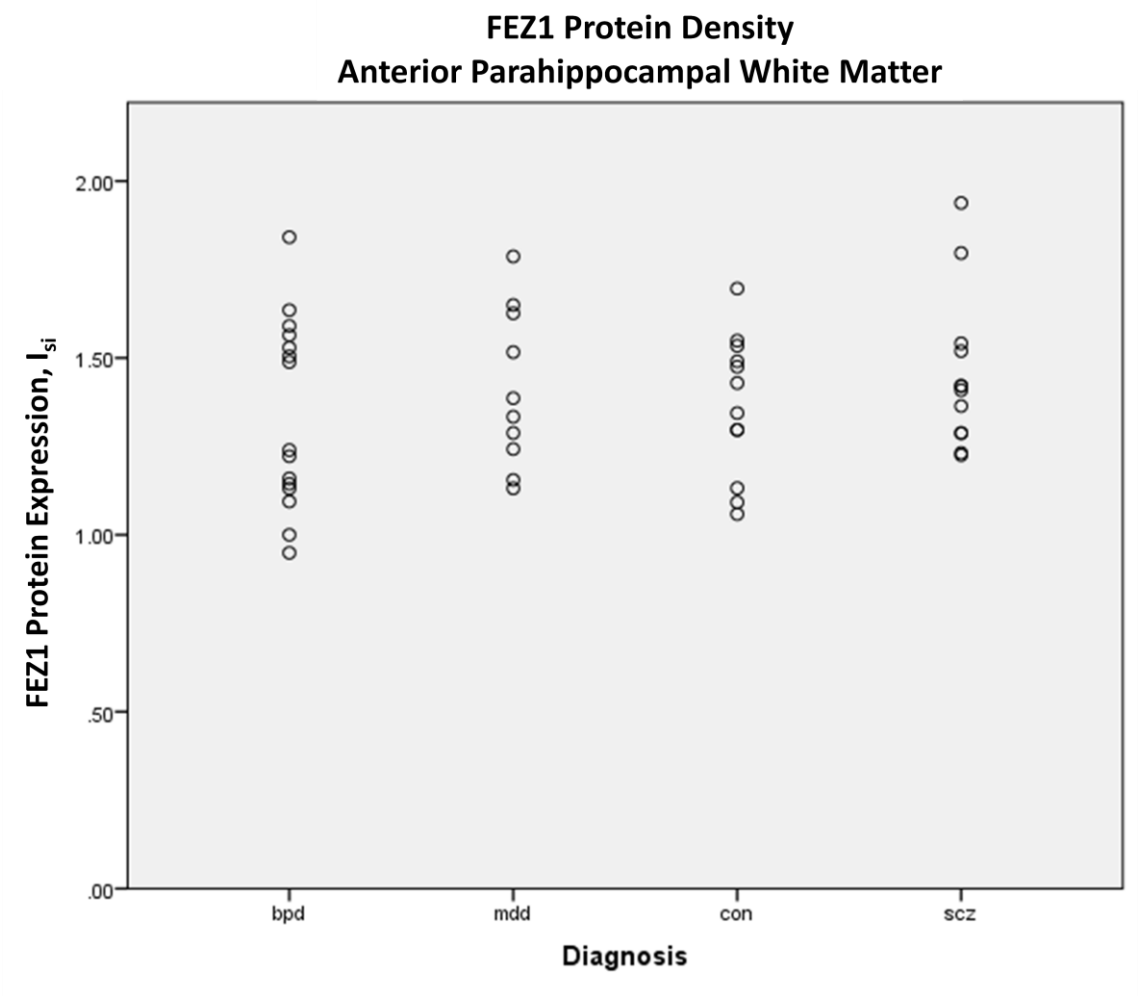
3-36 Box-plots showing distributions of FEZ1 protein density levels across the anterior parahippocampal grey matter and the identified outliers. Circles denote outliers identified using Tukey Outlier Labelling Rule (*g* = 1.5). In this case, the indicated Tukey outliers were not also highlighted by the '2.5 standard deviations from the mean' test, they were therefore not removed. I_{si} = Mean Specific Intensity, bpd = Bipolar Disorder, mdd = Major Depressive Disorder, scz = Schizophrenia, con = controls,



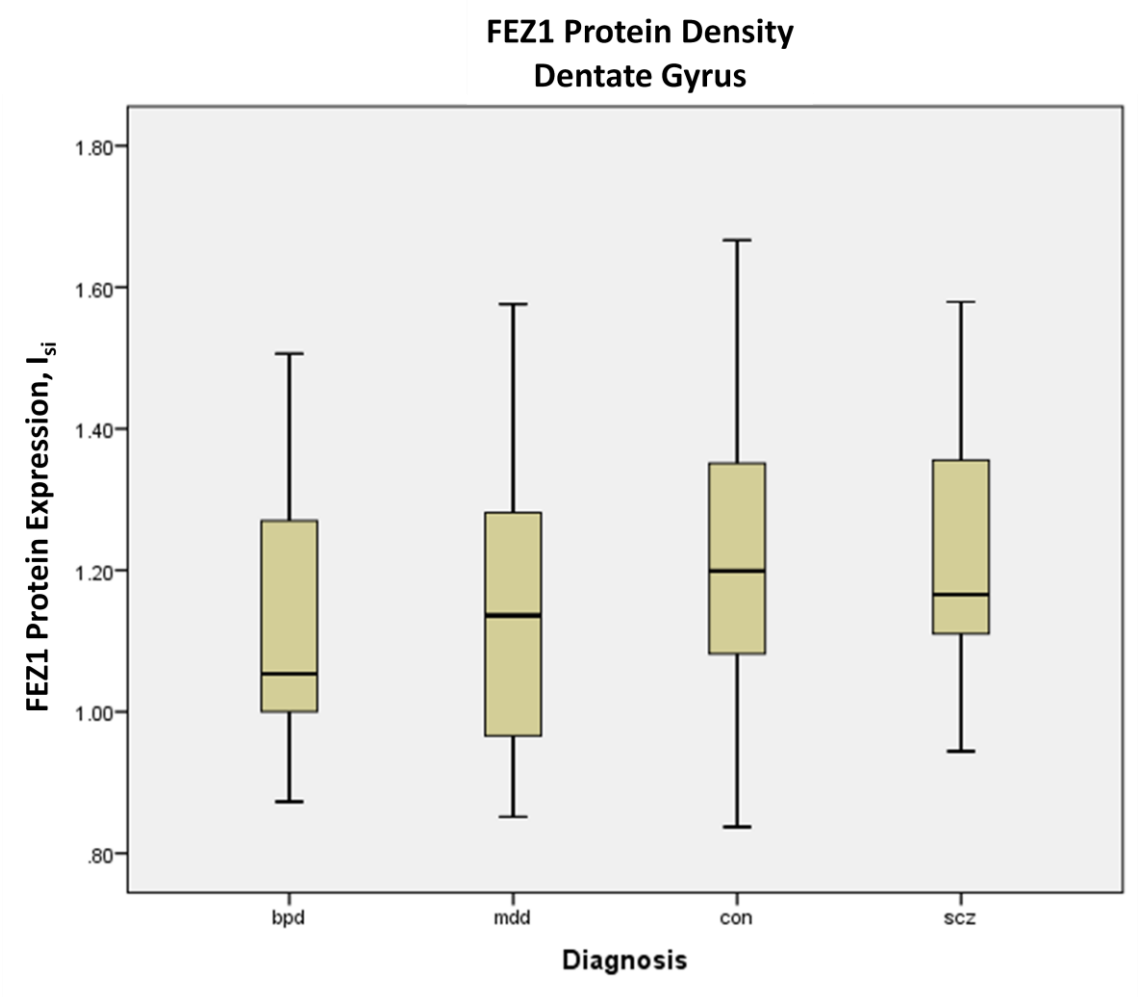
3-37 Scatter plot showing FEZ1 protein density levels across the anterior parahippocampal grey matter after Tukey outlier removal. I_{si} = Mean Specific Intensity, bpd = Bipolar Disorder, mdd = Major Depressive Disorder, scz = Schizophrenia, con = controls.



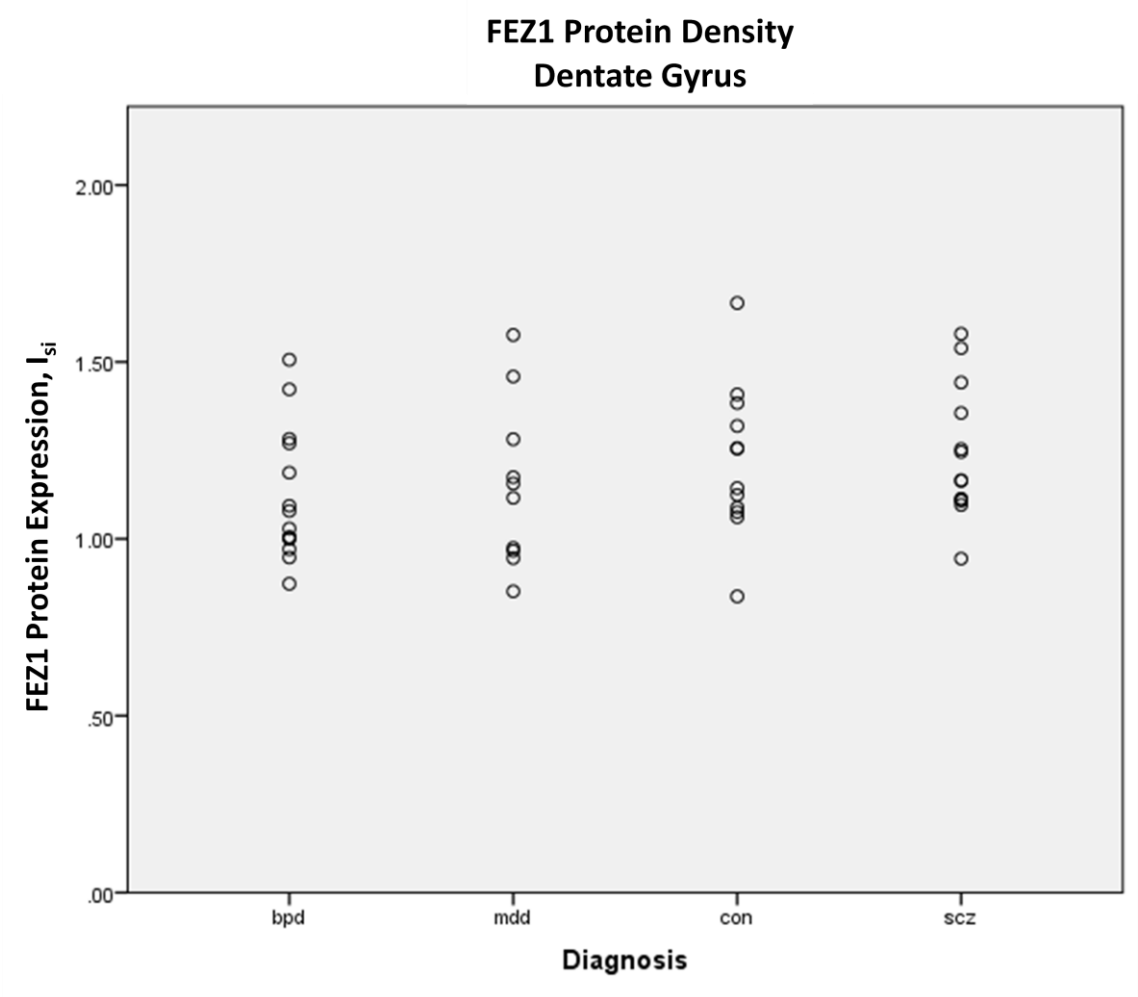
3-38 Box-plots showing distributions of FEZ1 protein density levels across the anterior parahippocampal white matter and the identified outliers. Circles denote outliers identified using Tukey Outlier Labelling Rule (*g* = 1.5). All of the indicated Tukey outliers were also highlighted by the '2.5 standard deviations from the mean' test. I_{si} = Mean Specific Intensity, bpd = Bipolar Disorder, mdd = Major Depressive Disorder, scz = Schizophrenia, con = controls,



3-39 Scatter plot showing FEZ1 protein density levels across the anterior parahippocampal white matter after Tukey outlier removal. I_{si} = Mean Specific Intensity, bpd = Bipolar Disorder, mdd = Major Depressive Disorder, scz = Schizophrenia, con = controls.

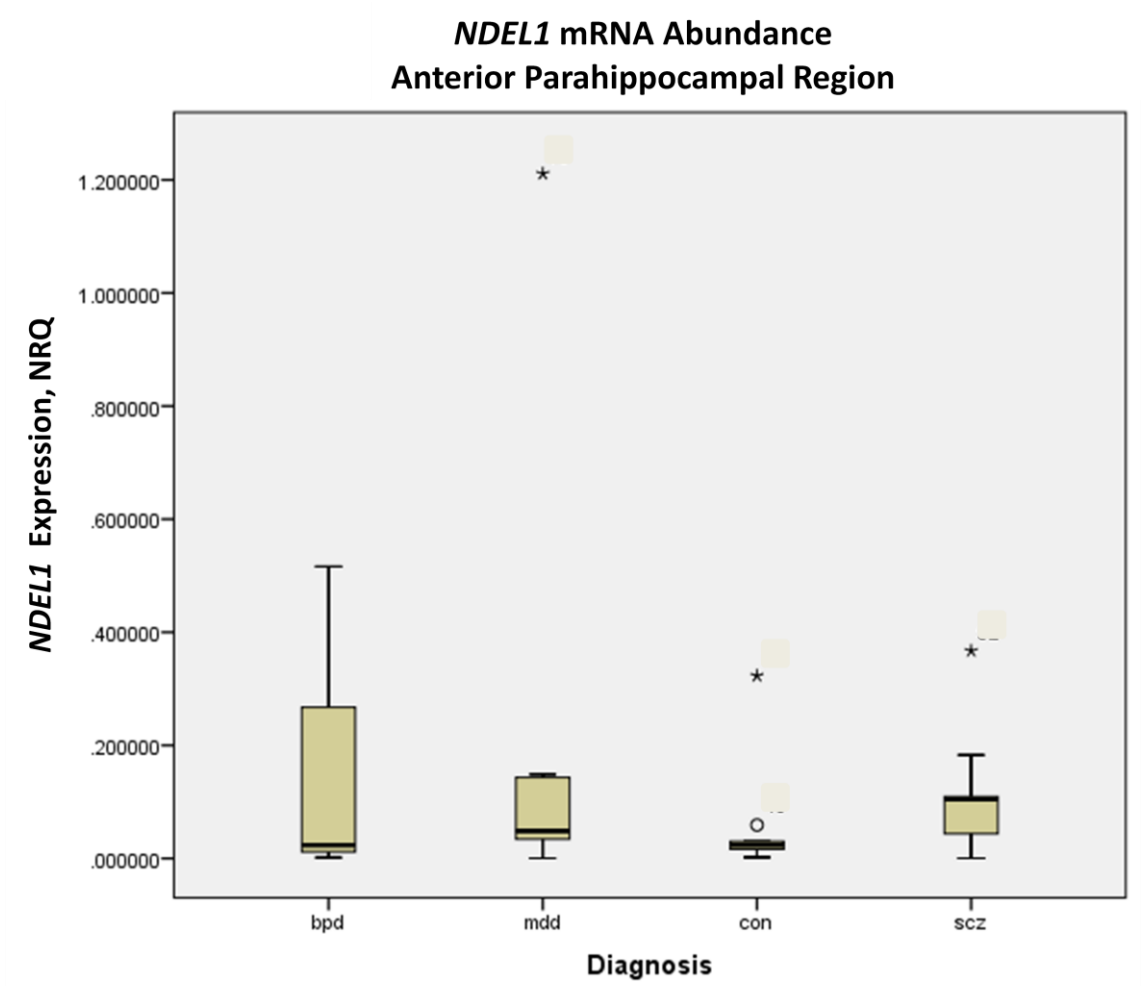


3-40 Box-plots showing distributions of FEZ1 protein density levels in the dentate gyrus. No outliers were identified. I_{si} = Mean Specific Intensity, bpd = Bipolar Disorder, mdd = Major Depressive Disorder, scz = Schizophrenia, con = controls,

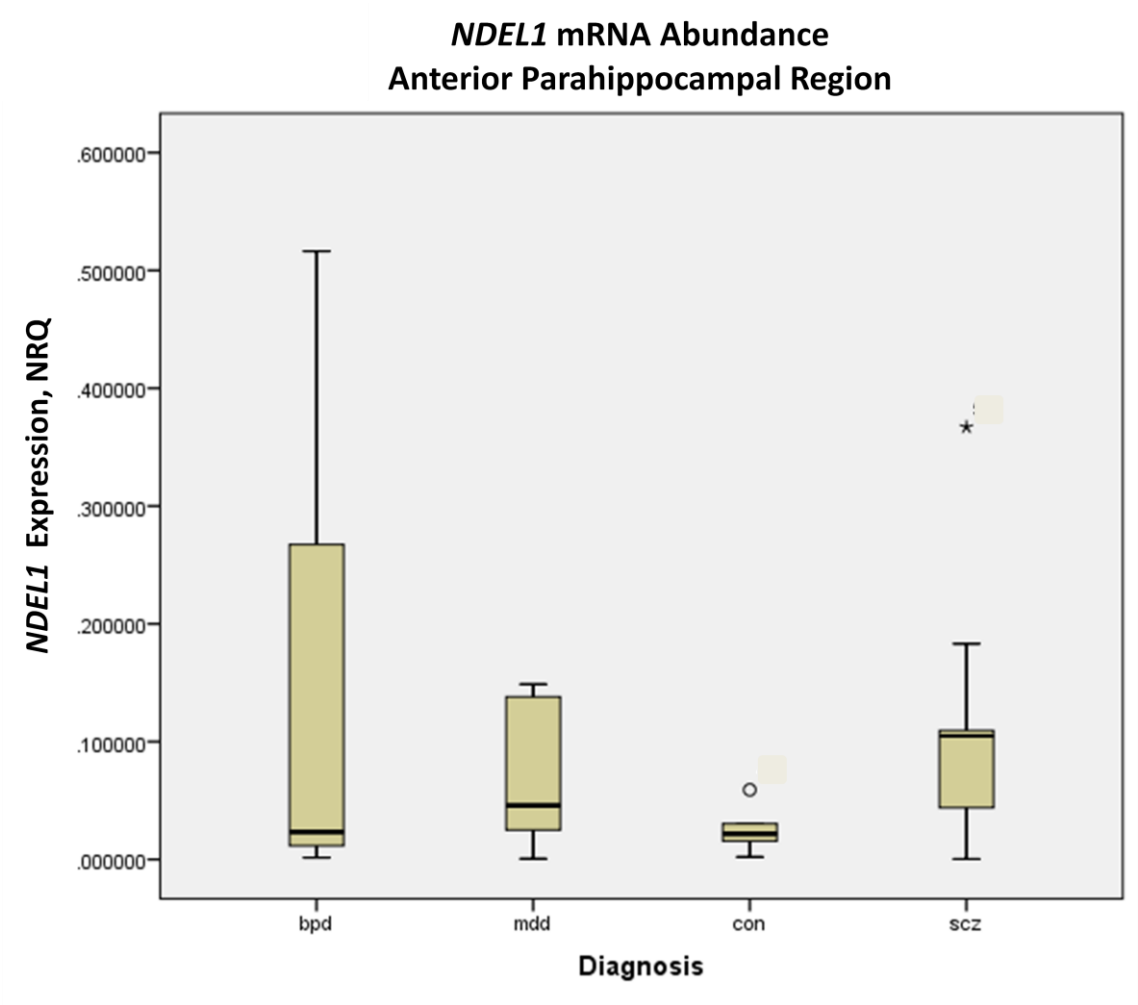


3-41 Scatter plot showing FEZ1 protein density levels in the dentate gyrus. No identifiers were identified in this region. I_{si} = Mean Specific Intensity, bpd = Bipolar Disorder, mdd = Major Depressive Disorder, scz = Schizophrenia, con = controls.

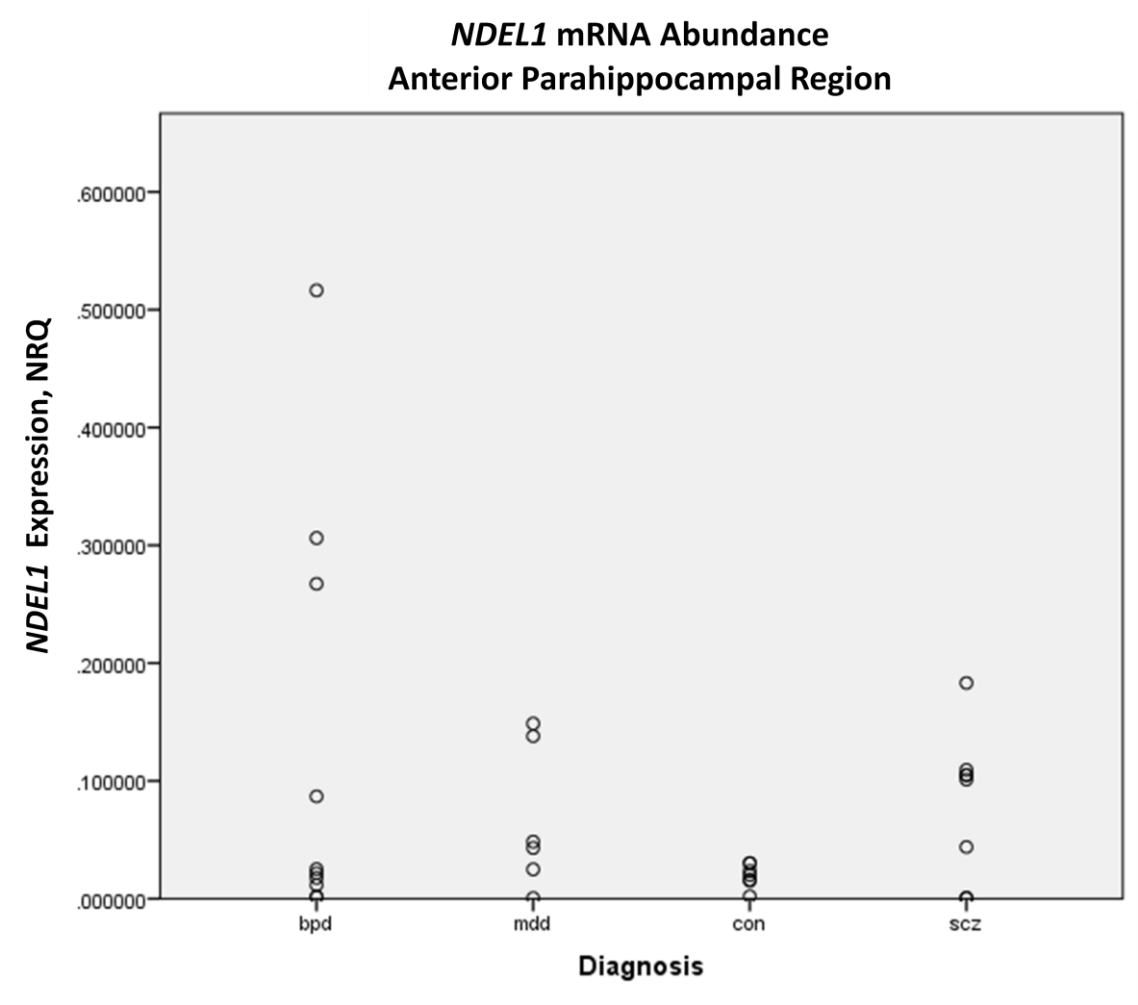
3.7.1.5 *NDEL1* qPCR Plots



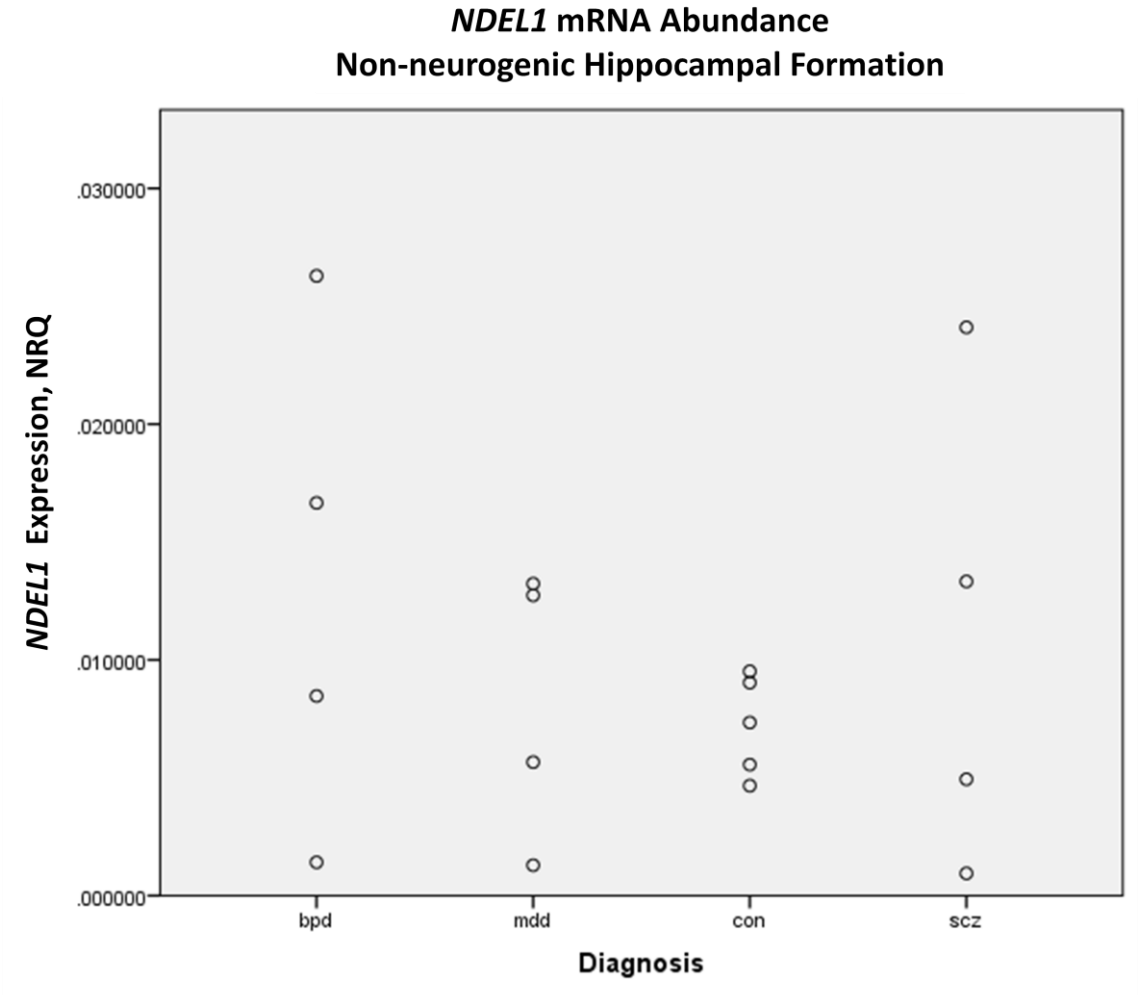
3-42 Box-plots showing distributions of *NDEL1* mRNA transcript levels in the anterior parahippocampal region and the identified outliers. Asterisk denotes outliers identified using Tukey Outlier Labelling Rule (*g* = 3); circle indicates (*g* = 1.5). All of the indicated Tukey outliers were also highlighted by the '2.5 standard deviations from the mean' test. NRQ = Normalised Relative Quantity, bpd = Bipolar Disorder, mdd = Major Depressive Disorder, scz = Schizophrenia, con = controls,



3-43 Box-plots showing distributions of *NDEL1* mRNA transcript levels in the anterior parahippocampal region after the initial removal of labelled outliers. Circles denote outliers identified using Tukey Outlier Labelling Rule (*g* = 1.5). All of the indicated Tukey outliers were also highlighted by the '2.5 standard deviations from the mean' test. NRQ = Normalised Relative Quantity, bpd = Bipolar Disorder, mdd = Major Depressive Disorder, scz = Schizophrenia, con = controls,

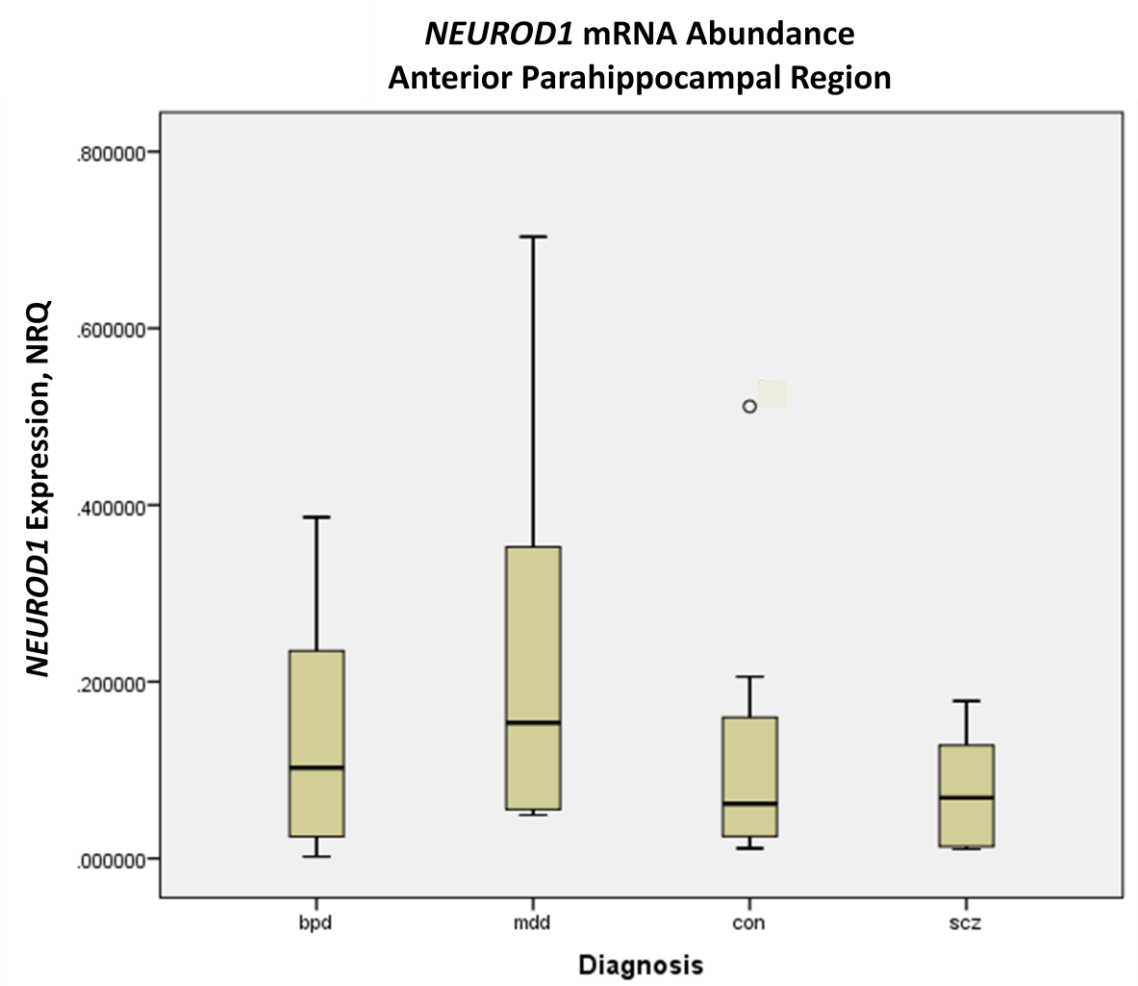


3-44 Scatter plot showing *NDEL1* mRNA transcript levels in the anterior parahippocampal region after Tukey outlier removal. NRQ = Normalised Relative Quantity, bpd = Bipolar Disorder, mdd = Major Depressive Disorder, scz = Schizophrenia, con = controls.

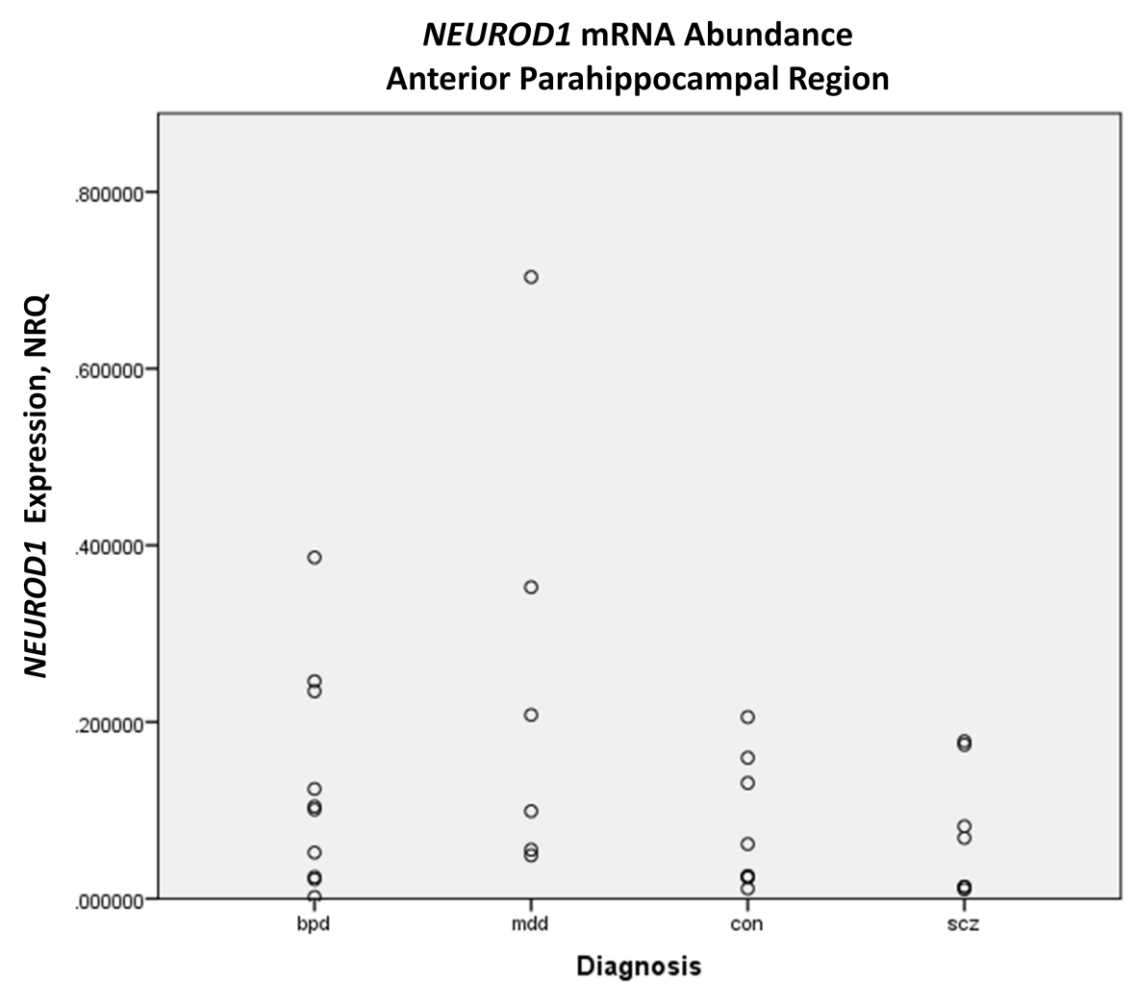


3-45 Scatter plot showing *NDEL1* mRNA transcript levels in the non-neurogenic hippocampal formation. No outliers were identified. NRQ = Normalised Relative Quantity, bpd = Bipolar Disorder, mdd = Major Depressive Disorder, scz = Schizophrenia.

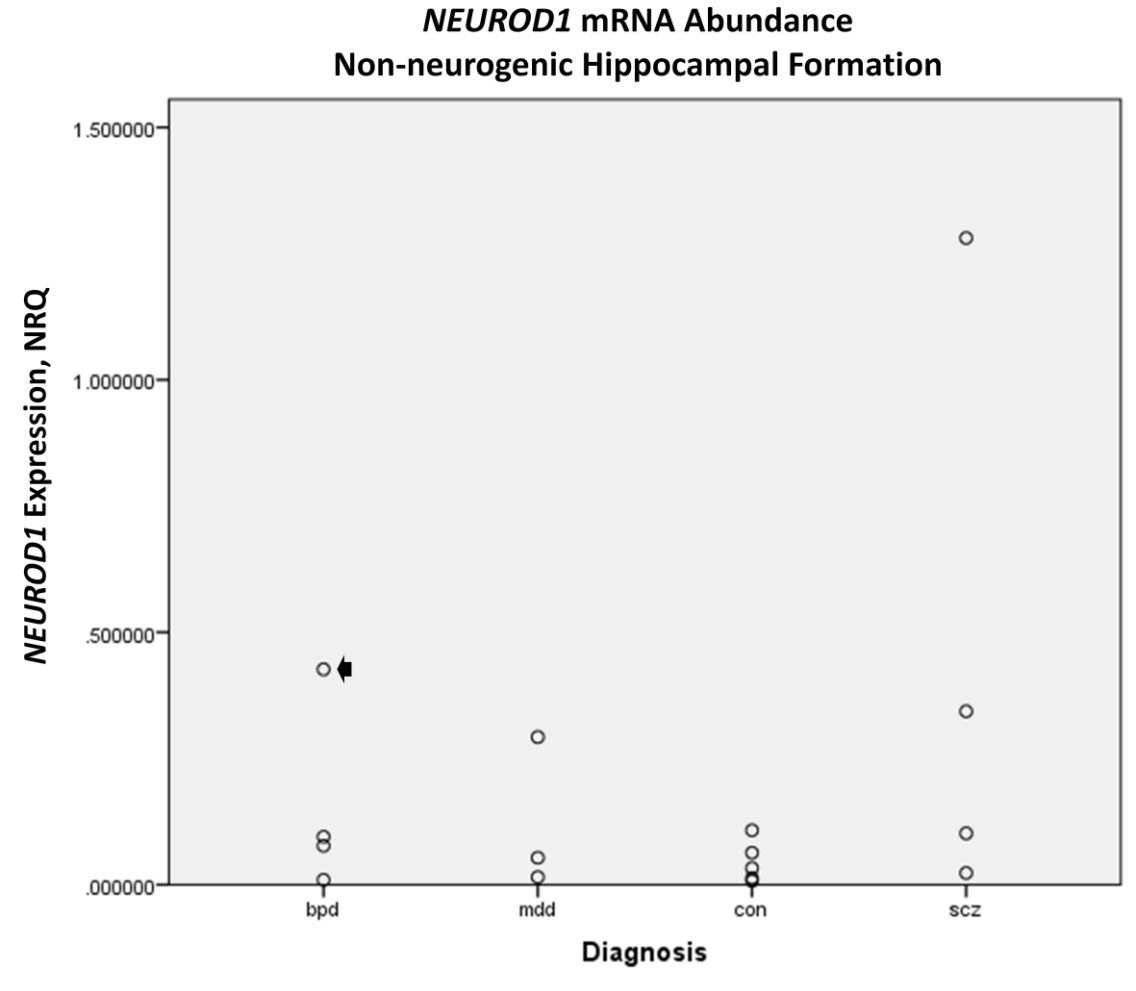
3.7.1.6 *NEUROD1* qPCR Plots



3-46 Box-plots showing distributions of *NEUROD1* mRNA transcript levels in the anterior parahippocampal region and the identified outliers. Circle denotes outliers identified using Tukey Outlier Labelling Rule (*g* = 1.5). All of the indicated Tukey outliers were also highlighted by the '2.5 standard deviations from the mean' test. NRQ = Normalised Relative Quantity, bpd = Bipolar Disorder, mdd = Major Depressive Disorder, scz = Schizophrenia, con = controls,

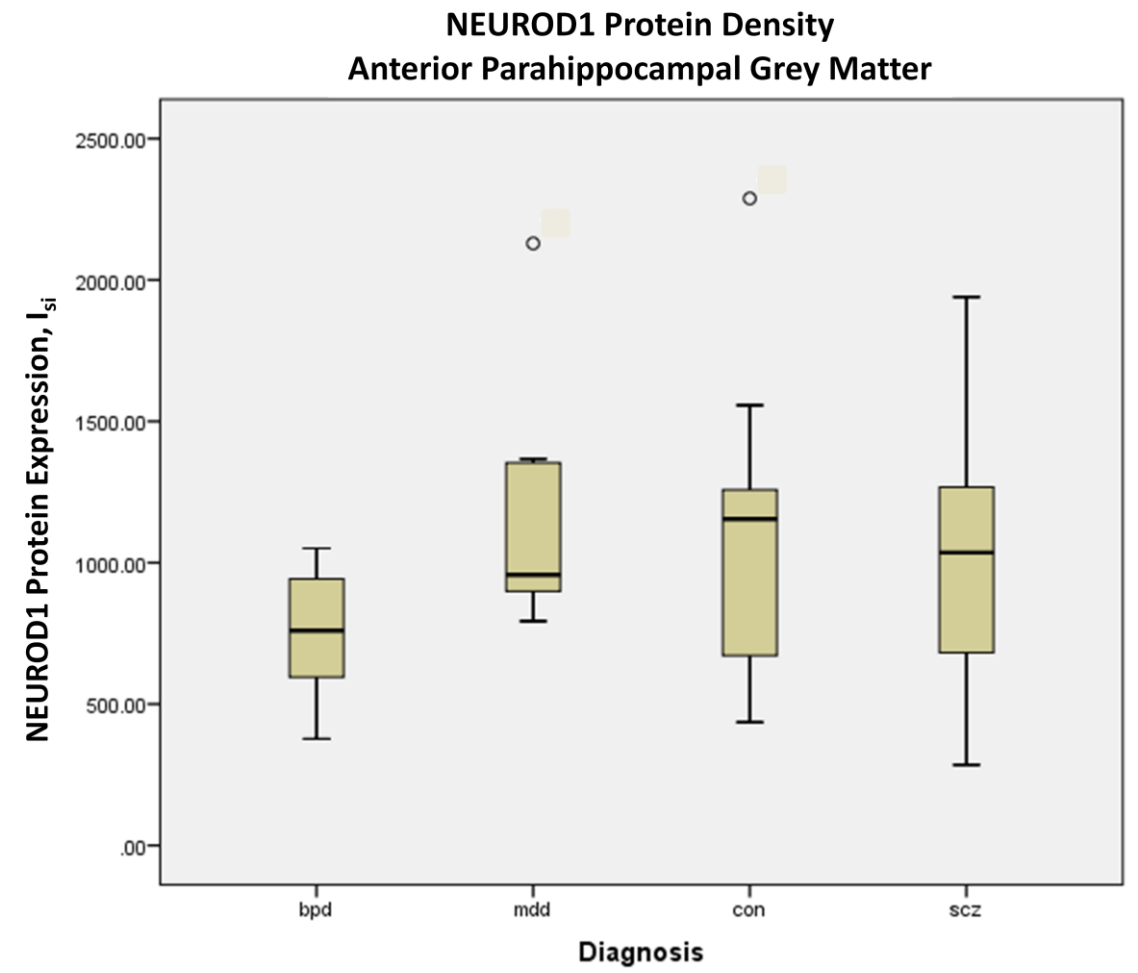


3-47 Scatter plot showing *NEUROD1* mRNA transcript levels in the anterior parahippocampal region after Tukey outlier removal. NRQ = Normalised Relative Quantity, bpd = Bipolar Disorder, mdd = Major Depressive Disorder, scz = Schizophrenia, con = controls.

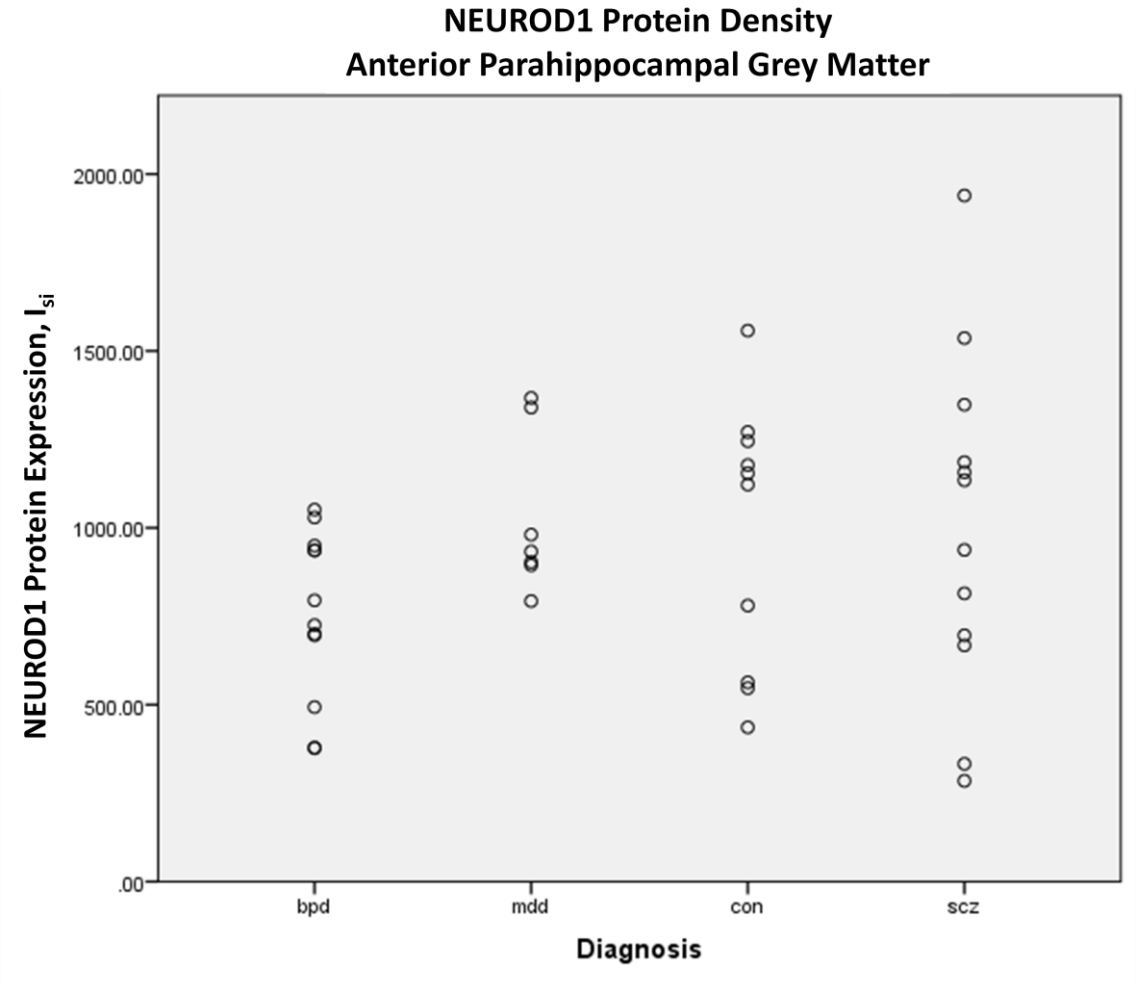


3-48 Scatter plot showing *NEUROD1* mRNA transcript levels in the non-neurogenic hippocampal formation. Black arrow indicates removed outliers highlighted by the '2.5 standard deviations from the mean' test. NRQ = Normalised Relative Quantity, bpd = Bipolar Disorder, mdd = Major Depressive Disorder, scz = Schizophrenia.

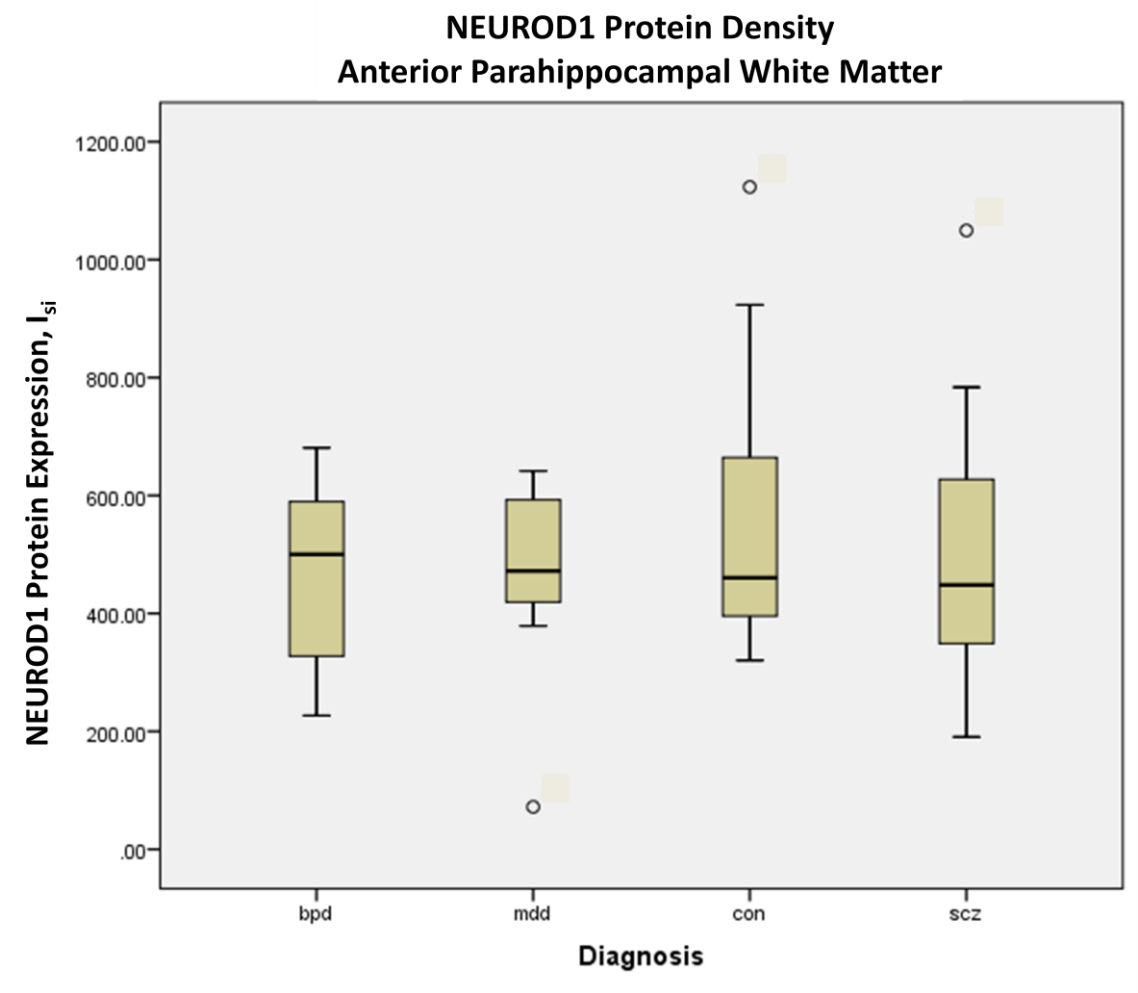
3.7.1.7 NEUROD1 IHC Plots



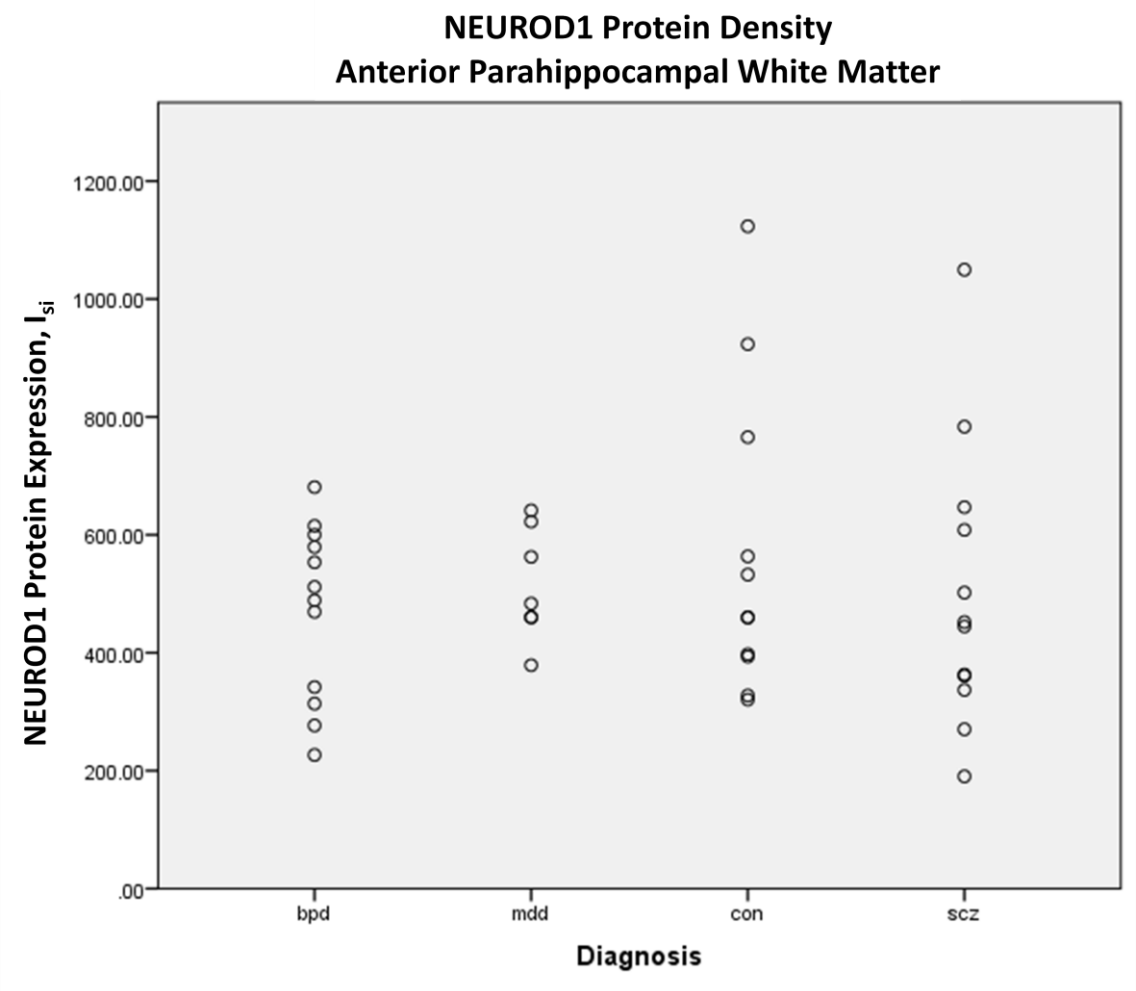
3-49 Box-plots showing distributions of NEUROD1 protein density levels across the anterior parahippocampal grey matter and the identified outliers. Circles denote outliers identified using Tukey Outlier Labelling Rule (*g* = 1.5). All of the indicated Tukey outliers were also highlighted by the '2.5 standard deviations from the mean' test. I_{si} = Mean Specific Intensity, bpd = Bipolar Disorder, mdd = Major Depressive Disorder, scz = Schizophrenia, con = controls,



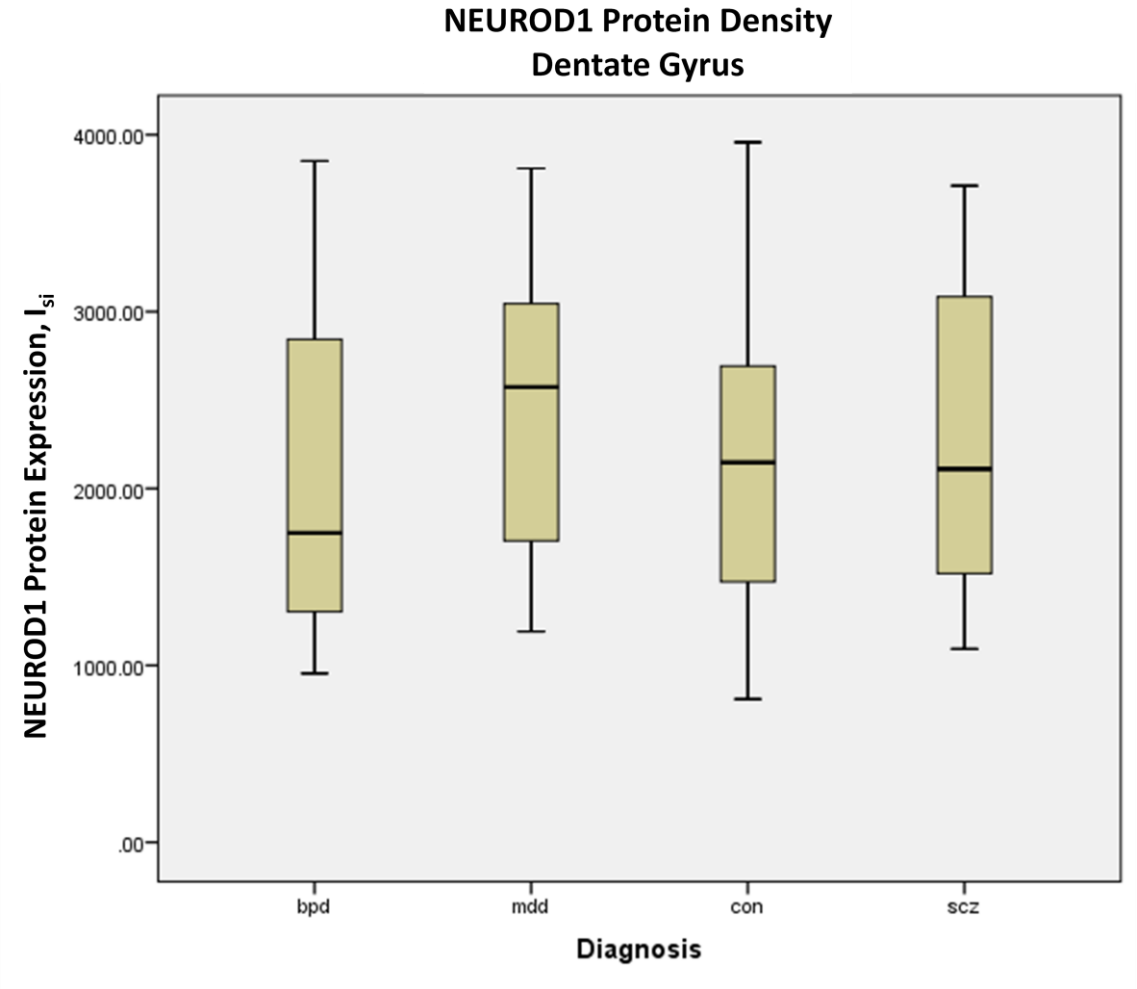
3-50 Scatter plot showing NEUROD1 protein density levels across the anterior parahippocampal grey matter after Tukey outlier removal. I_{si} = Mean Specific Intensity, bpd = Bipolar Disorder, mdd = Major Depressive Disorder, scz = Schizophrenia, con = controls.



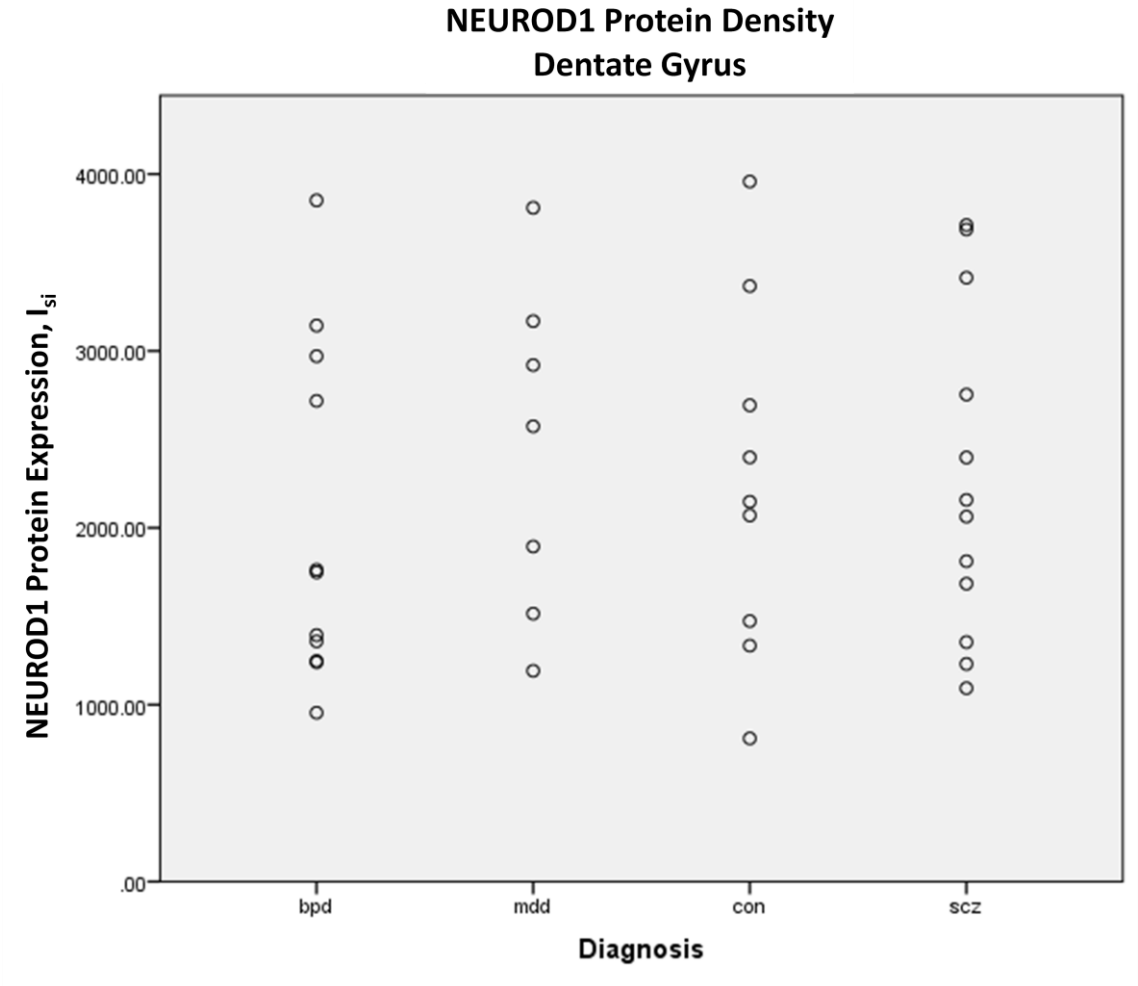
3-51 Box-plots showing distributions of NEUROD1 protein density levels across the anterior parahippocampal white matter and the identified outliers. Circles denote outliers identified using Tukey Outlier Labelling Rule (*g* = 1.5). All of the indicated Tukey outliers were also highlighted by the '2.5 standard deviations from the mean' test. I_{si} = Mean Specific Intensity, bpd = Bipolar Disorder, mdd = Major Depressive Disorder, scz = Schizophrenia, con = controls,



3-52 Scatter plot showing NEUROD1 protein density levels across the anterior parahippocampal white matter after Tukey outlier removal. I_{si} = Mean Specific Intensity, bpd = Bipolar Disorder, mdd = Major Depressive Disorder, scz = Schizophrenia, con = controls.

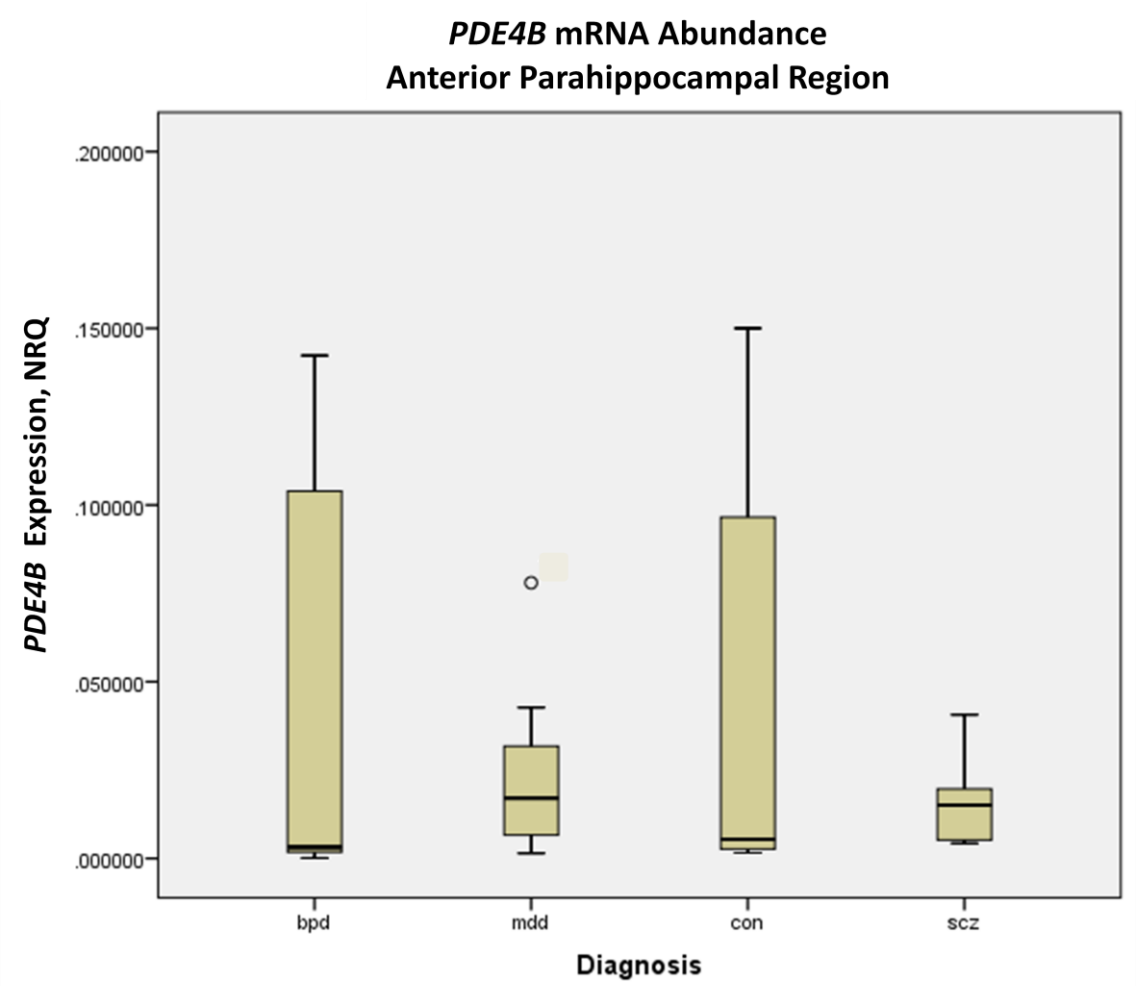


3-53 Box-plots showing distributions of FEZ1 protein density levels in the dentate gyrus. No outliers were identified. I_{si} = Mean Specific Intensity, bpd = Bipolar Disorder, mdd = Major Depressive Disorder, scz = Schizophrenia, con = controls,

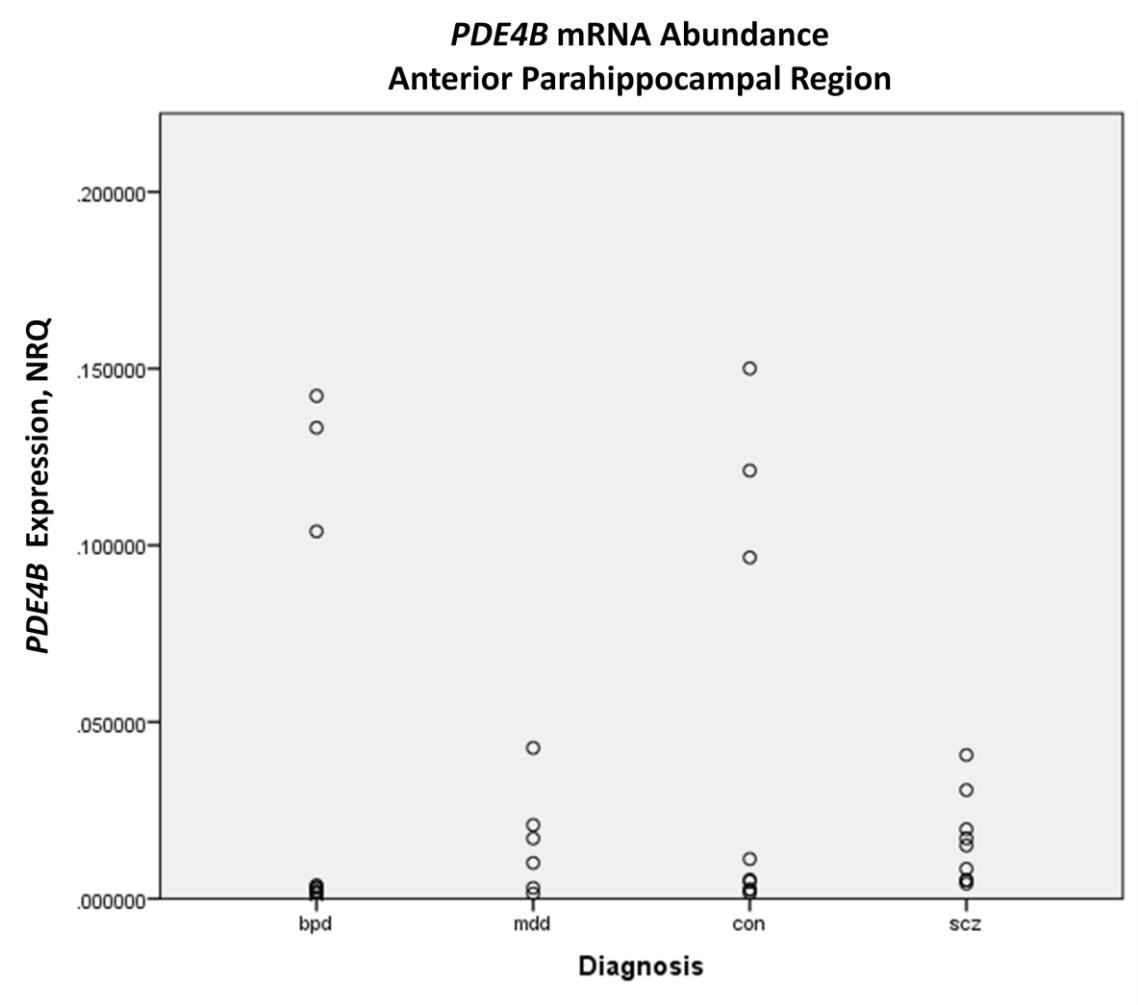


3-54 Scatter plot showing FEZ1 protein density levels in the dentate gyrus. No identified in this region. I_{si} = Mean Specific Intensity, bpd = Bipolar Disorder, mdd = Major Depressive Disorder, scz = Schizophrenia, con = controls.

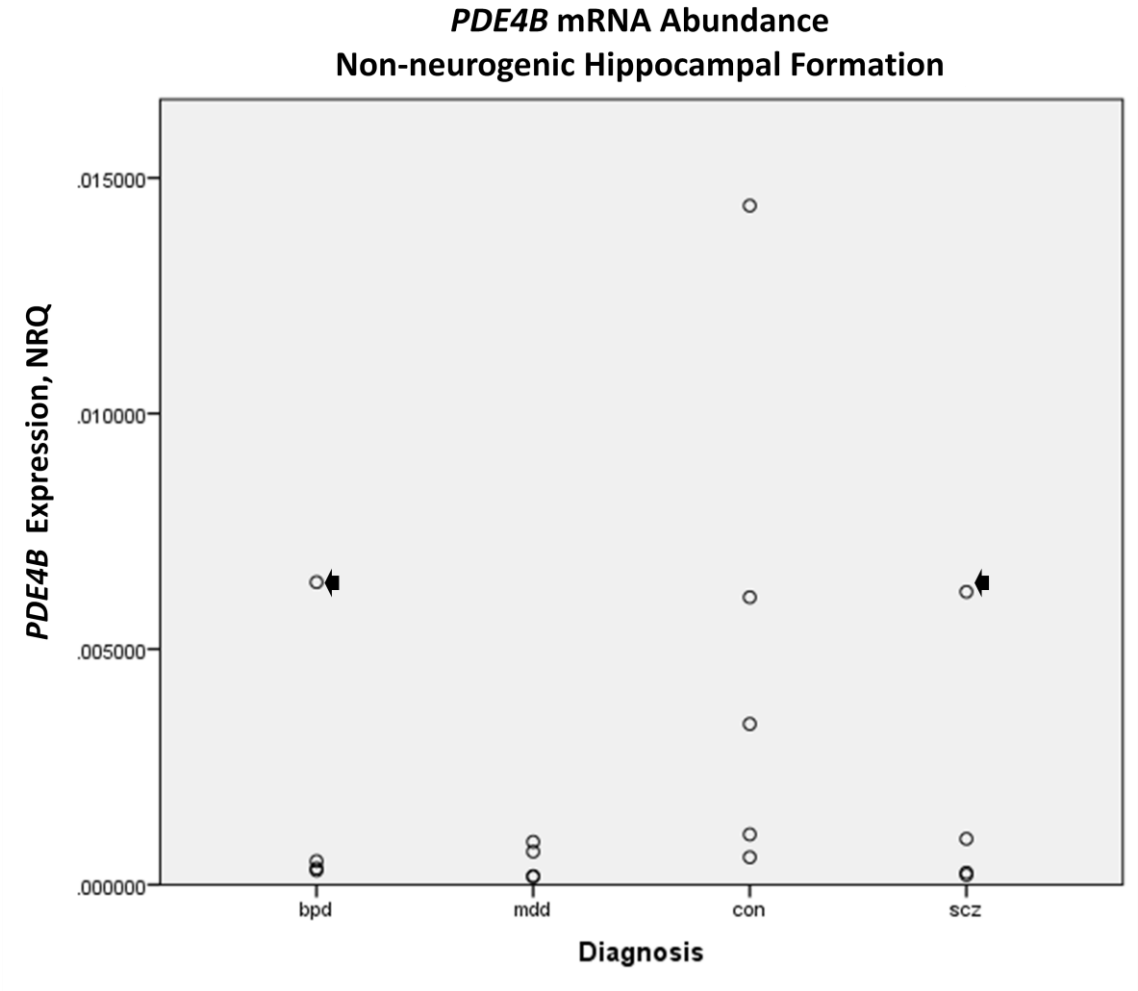
3.7.1.8 *PDE4B* qPCR Plots



3-55 Box-plots showing distributions of *PDE4B* mRNA transcript levels in the anterior parahippocampal region and the identified outliers. Circle denotes outliers identified using Tukey Outlier Labelling Rule (*g* = 1.5). All of the indicated Tukey outliers were also highlighted by the '2.5 standard deviations from the mean' test. NRQ = Normalised Relative Quantity, bpd = Bipolar Disorder, mdd = Major Depressive Disorder, scz = Schizophrenia, con = controls,



3-56 Scatter plot showing *PDE4B* mRNA transcript levels in the anterior parahippocampal region after Tukey outlier removal. NRQ = Normalised Relative Quantity, bpd = Bipolar Disorder, mdd = Major Depressive Disorder, scz = Schizophrenia, con = controls.



3-57 Scatter plot showing *PDE4B* mRNA transcript levels in the non-neurogenic hippocampal formation. Black arrows indicate removed outliers highlighted by the '2.5 standard deviations from the mean' test. NRQ = Normalised Relative Quantity, bpd = Bipolar Disorder, mdd = Major Depressive Disorder, scz = Schizophrenia.

3.7.2 Confounding Factor Detection

In order to control for the effect of potential confounding factors, assessments of demographic and clinical variables were carried out using Mann Whitney *U* tests (dichotomous variables) and Spearman's rank-order correlations (continuous variables). Those demonstrating a significant effect were retained as covariates for the comparisons between diagnostic groups. The full results of the tests are recorded here.

3.7.2.1 Anterior Parahippocampal Data

3-E Table of the Spearman's rank correlations of demographic and clinical variables with the gene expression normalised relative quantities in the anterior parahippocampal region. Asterisk indicates p < 01, double asterisk indicates, p < 05

Continuous Variables: qPCR Non-Parametric Correlations (anterior parahippocampal region)

Spearman's rho		DCX	DPYSL2	FEZ1	NDEL1	NEUROD1	PDE4B
qPCR Plate	Correlation Coefficient	-.175	-.329	-.092	.140	-.001	-.378*
	P (2-tailed)	.364	.066	.628	.460	.996	.027
	N	29	32	30	30	31	34
RQI	Correlation Coefficient	-.313	-.048	-.182	-.506**	-.123	-.276
	P (2-tailed)	.105	.797	.345	.004	.519	.120
	N	28	31	29	30	30	33
age at death (years)	Correlation Coefficient	-.067	.098	.053	.104	-.161	.129
	P (2-tailed)	.729	.592	.782	.583	.387	.467
	N	29	32	30	30	31	34
age of onset of disease (years)	Correlation Coefficient	.130	-.198	-.409*	.272	.220	.031
	P (2-tailed)	.502	.278	.025	.146	.234	.862
	N	29	32	30	30	31	34
duration of disease (years)	Correlation Coefficient	.133	-.346	-.562**	.264	-.008	-.041
	P (2-tailed)	.491	.053	.001	.159	.967	.816
	N	29	32	30	30	31	34
lifetime quantity of fluphenazine or	Correlation Coefficient	.359	-.307	-.600**	.135	.062	-.128

Continuous Variables: qPCR Non-Parametric Correlations (anterior parahippocampal region)

Spearman's rho		DCX	DPYSL2	FEZ1	NDEL1	NEUROD1	PDE4B
equivalent (mg)	*P (2-tailed)*	.056	.087	.000	.478	.739	.472
	N	29	32	30	30	31	34
severity of substance abuse	Correlation Coefficient	-.073	-.153	-.238	-.201	.159	-.173
	P (2-tailed)	.708	.403	.206	.287	.394	.327
	N	29	32	30	30	31	34
severity of alcohol abuse	Correlation Coefficient	-.069	.109	-.155	-.166	.375*	-.170
	P (2-tailed)	.721	.551	.414	.381	.038	.336
	N	29	32	30	30	31	34
PH	Correlation Coefficient	-.186	.186	.001	-.368*	-.369*	-.127
	P (2-tailed)	.334	.308	.994	.045	.041	.474
	N	29	32	30	30	31	34
mass of brain (g)	Correlation Coefficient	.067	.054	.219	-.043	.288	.048
	P (2-tailed)	.732	.769	.245	.823	.117	.789
	N	29	32	30	30	31	34
post-mortem interval (hours)	Correlation Coefficient	.278	-.097	.118	.345	-.044	.198
	P (2-tailed)	.145	.598	.536	.062	.814	.262
	N	29	32	30	30	31	34
STORAGE (DAYS)	Correlation Coefficient	.359	-.233	-.442*	.501**	-.190	-.037
	P (2-tailed)	.056	.199	.014	.005	.307	.835
	N	29	32	30	30	31	34

3-F Table showing the results of Mann-Whitney *U* testing of dichotomous demographic and clinical variables for effects on the gene expression normalised relative quantities in the anterior parahippocampal region

Dichotomous Variables: qPCR Non-Parametric Testing (anterior parahippocampal region)

		DCX	DPYSL2	FEZ1	NDEL1	NEUROD1	PDE4B
female	*N*	11	15	12	13	14	14
	Mean Rank	14.18	14.33	15.17	14.46	14.64	19.29
	Sum of Ranks	156.00	215.00	182.00	188.00	205.00	270.00
male	*N*	18	17	18	17	17	20
	Mean Rank	15.50	18.41	15.72	16.29	17.12	16.25
	Sum of Ranks	279.00	313.00	283.00	277.00	291.00	325.00
Total	*N*	29	32	30	30	31	34
	Mann-Whitney U	90.000	95.000	104.000	97.000	100.000	115.000
	Wilcoxon W	156.000	215.000	182.000	188.000	205.000	325.000
	Z	-.405	-1.227	-.169	-.565	-.754	-.875
	p (2-tailed)	.686	.220	.866	.572	.451	.382
Death by suicide: yes	*N*	7	9	8	8	8	8
	Mean Rank	12.43	12.78	13.00	14.63	14.25	14.63
	Sum of Ranks	87.00	115.00	104.00	117.00	114.00	117.00
no	*N*	22	23	22	22	23	26
	Mean Rank	15.82	17.96	16.41	15.82	16.61	18.38
	Sum of Ranks	348.00	413.00	361.00	348.00	382.00	478.00
Total	*N*	29	32	30	30	31	34
	Mann-Whitney U	59.000	70.000	68.000	81.000	78.000	81.000
	Wilcoxon W	87.000	115.000	104.000	117.000	114.000	117.000
	Z	-.917	-1.404	-.938	-.328	-.632	-.934
	p (2-tailed)	.359	.160	.348	.743	.527	.350
With Psychosis	*N*	17	15	15	15	15	17
	Mean Rank	17.35	13.20	10.00	15.73	15.47	15.35
	Sum of Ranks	295.00	198.00	150.00	236.00	232.00	261.00

Dichotomous Variables: qPCR Non-Parametric Testing (anterior parahippocampal region)

		DCX	DPYSL2	FEZ1	NDEL1	NEUROD1	PDE4B
Without Psychosis	*N*	12	17	15	15	16	17
	Mean Rank	11.67	19.41	21.00	15.27	16.50	19.65
	Sum of Ranks	140.00	330.00	315.00	229.00	264.00	334.00
Total	*N*	29	32	30	30	31	34
	Mann-Whitney U	62.000	78.000	30.000	109.000	112.000	108.000
	Wilcoxon W	140.000	198.000	150.000	229.000	232.000	261.000
	Z	-1.771	-1.869	-3.422	-.145	-.316	-1.257
	p (2-tailed)	.077	.062	.001	.885	.752	.209
Hemisphere: right	*N*	11	11	11	13	12	13
	Mean Rank	13.64	14.82	10.18	17.62	12.00	13.62
	Sum of Ranks	150.00	163.00	112.00	229.00	144.00	177.00
left	*N*	18	21	19	17	19	21
	Mean Rank	15.83	17.38	18.58	13.88	18.53	19.90
	Sum of Ranks	285.00	365.00	353.00	236.00	352.00	418.00
Total	*N*	29	32	30	30	31	34
	Mann-Whitney U	84.000	97.000	46.000	83.000	66.000	86.000
	Wilcoxon W	150.000	163.000	112.000	236.000	144.000	177.000
	Z	-.674	-.734	-2.518	-1.151	-1.947	-1.790
	p (2-tailed)	.500	.463	.012	.250	.052	.074

3.7.2.2 Non-neurogenic Hippocampal Formation Data

3-G Table of the Spearman's rank correlations of demographic and clinical variables with the gene expression normalised relative quantities in the non-neurogenic hippocampal formation. Asterisk indicates p < 01, double asterisk indicates, p < 05

Continuous Variables: qPCR Non-Parametric Correlations (non-neurogenic hippocampal formation)

	DCX	DPYSL2	FEZ1	NDEL1	NEUROD1	PDE4B

Continuous Variables: qPCR Non-Parametric Correlations (non-neurogenic hippocampal formation)

		DCX	DPYSL2	FEZ1	NDEL1	NEUROD1	PDE4B
qPCR Plate	Correlation Coefficient	.928**	-.119	-.391	-.089	-.532*	.397
	P (2-tailed)	.000	.649	.134	.733	.041	.143
	N	15	17	16	17	15	15
RQI	Correlation Coefficient	-.226	-.303	-.404	.004	-.142	-.442
	P (2-tailed)	.417	.237	.121	.989	.614	.099
	N	15	17	16	17	15	15
age at death (years)	Correlation Coefficient	-.025	-.075	-.312	-.428	.175	.136
	P (2-tailed)	.930	.775	.239	.087	.532	.630
	N	15	17	16	17	15	15
age of onset of disease (years)	Correlation Coefficient	-.686**	-.401	-.279	-.070	.238	-.646**
	P (2-tailed)	.005	.111	.295	.791	.392	.009
	N	15	17	16	17	15	15
duration of disease (years)	Correlation Coefficient	-.636*	-.129	-.006	.012	.309	-.582*
	P (2-tailed)	.011	.622	.983	.962	.262	.023
	N	15	17	16	17	15	15
lifetime quantity of fluphenazine or equivalent (mg)	Correlation Coefficient	-.604*	.261	.295	.130	.446	-.395
	P (2-tailed)	.017	.312	.267	.618	.096	.145
	N	15	17	16	17	15	15
severity of substance abuse	Correlation Coefficient	-.510	.110	.298	.162	.020	-.337

Continuous Variables: qPCR Non-Parametric Correlations (non-neurogenic hippocampal formation)

		DCX	DPYSL2	FEZ1	NDEL1	NEUROD1	PDE4B
	P (2-tailed)	.052	.673	.262	.536	.942	.219
	N	15	17	16	17	15	15
severity of alcohol abuse	Correlation Coefficient	-.412	.272	.322	.066	.206	-.085
	P (2-tailed)	.127	.291	.224	.800	.461	.764
	N	15	17	16	17	15	15
PH	Correlation Coefficient	-.369	-.350	-.023	-.031	-.436	-.304
	P (2-tailed)	.176	.169	.934	.905	.105	.271
	N	15	17	16	17	15	15
mass of brain (g)	Correlation Coefficient	.131	.158	.141	.247	-.061	-.061
	P (2-tailed)	.643	.544	.601	.340	.830	.830
	N	15	17	16	17	15	15
post-mortem interval (hours)	Correlation Coefficient	.100	.201	.234	.214	.014	-.011
	P (2-tailed)	.722	.438	.383	.410	.960	.970
	N	15	17	16	17	15	15
STORAGE (DAYS)	Correlation Coefficient	-.900**	.140	.400	.110	.529*	-.411
	P (2-tailed)	.000	.593	.125	.673	.043	.128
	N	15	17	16	17	15	15

3-H Table showing the results of Mann-Whitney *U* testing of dichotomous demographic and clinical variables for effects on the gene expression normalised relative quantities in the anterior parahippocampal region

Dichotomous Variables: qPCR Non-Parametric Testing (non-neurogenic hippocampal formation)

		DCX	DPYSL2	FEZ1	NDEL1	NEUROD1	PDE4B
female	*N*	8	9	8	9	8	8
	Mean Rank	9.00	7.44	6.38	6.89	6.88	9.25
	Sum of Ranks	72.00	67.00	51.00	62.00	55.00	74.00
male	*N*	7	8	8	8	7	7
	Mean Rank	6.86	10.75	10.63	11.38	9.29	6.57
	Sum of Ranks	48.00	86.00	85.00	91.00	65.00	46.00
Total	*N*	15	17	16	17	15	15
	Mann-Whitney U	20.000	22.000	15.000	17.000	19.000	18.000
	Wilcoxon W	48.000	67.000	51.000	62.000	55.000	46.000
	Z	-.926	-1.347	-1.785	-1.828	-1.042	-1.157
	p (2-tailed)	.355	.178	.074	.068	.298	.247
Death by suicide: yes	*N*	6	6	6	6	4	6
	Mean Rank	6.67	8.50	8.00	9.83	7.75	5.50
	Sum of Ranks	40.00	51.00	48.00	59.00	31.00	33.00
no	*N*	9	11	10	11	11	9
	Mean Rank	8.89	9.27	8.80	8.55	8.09	9.67
	Sum of Ranks	80.00	102.00	88.00	94.00	89.00	87.00
Total	*N*	15	17	16	17	15	15
	Mann-Whitney U	19.000	30.000	27.000	28.000	21.000	12.000
	Wilcoxon W	40.000	51.000	48.000	94.000	31.000	33.000
	Z	-.943	-.302	-.325	-.503	-.131	-1.768
	p (2-tailed)	.346	.763	.745	.615	.896	.077
With Psychosis	*N*	6	7	7	7	6	6
	Mean Rank	4.50	11.14	10.86	10.14	10.67	6.00

Dichotomous Variables: qPCR Non-Parametric Testing (non-neurogenic hippocampal formation)

		DCX	DPYSL2	FEZ1	NDEL1	NEUROD1	PDE4B
	Sum of Ranks	27.00	78.00	76.00	71.00	64.00	36.00
Without Psychosis	*N*	9	10	9	10	9	9
	Mean Rank	10.33	7.50	6.67	8.20	6.22	9.33
	Sum of Ranks	93.00	75.00	60.00	82.00	56.00	84.00
Total	*N*	15	17	16	17	15	15
	Mann-Whitney U	6.000	20.000	15.000	27.000	11.000	15.000
	Wilcoxon W	27.000	75.000	60.000	82.000	56.000	36.000
	Z	-2.475	-1.464	-1.747	-.781	-1.886	-1.414
	p (2-tailed)	.013	.143	.081	.435	.059	.157
Hemisphere: right	*N*	9	9	8	9	7	9
	Mean Rank	6.22	8.78	8.25	10.11	9.14	5.56
	Sum of Ranks	56.00	79.00	66.00	91.00	64.00	50.00
left	*N*	6	8	8	8	8	6
	Mean Rank	10.67	9.25	8.75	7.75	7.00	11.67
	Sum of Ranks	64.00	74.00	70.00	62.00	56.00	70.00
Total	*N*	15	17	16	17	15	15
	Mann-Whitney U	11.000	34.000	30.000	26.000	20.000	5.000
	Wilcoxon W	56.000	79.000	66.000	62.000	56.000	50.000
	Z	-1.886	-.192	-.210	-.962	-.926	-2.593
	p (2-tailed)	.059	.847	.834	.336	.355	.010

3.7.2.3 Quantitative IHC Data

3-I Table of the Spearman's rank correlations of demographic and clinical variables with the protein density values in sections of anterior hippocampus. Asterisk indicates p < 01, double asterisk indicates, p < 05

Continuous Variables: IHC Non-Parametric Correlations

Spearman's Rho		Relative FEZ1 (APh Grey Matter)	Relative FEZ1 (APh White Matter)	Relative FEZ1 (Dentate Gyrus)	Absolute NEUROD1 (APh Grey Matter)	Absolute NEUROD1 (APh White Matter)	Absolute NEUROD1 (Dentate Gyrus)
age at death (years)	Correlation Coefficient	-.015	-.326*	-.066	.105	.144	-.098
	P (2-tailed)	.920	.022	.654	.512	.361	.551
	N	50	49	49	41	42	39
age of onset of disease (years)	Correlation Coefficient	-.035	.123	-.198	.027	.054	.111
	P (2-tailed)	.810	.402	.173	.869	.735	.501
	N	50	49	49	41	42	39
duration of disease (years)	Correlation Coefficient	-.150	-.270	-.105	-.056	-.009	-.192
	P (2-tailed)	.298	.061	.471	.728	.956	.242
	N	50	49	49	41	42	39
lifetime quantity of fluphenazine or equivalent (mg)	Correlation Coefficient	-.060	-.098	.108	-.100	-.185	-.132
	P (2-tailed)	.677	.505	.459	.534	.240	.424
	N	50	49	49	41	42	39
severity of substance abuse	Correlation Coefficient	-.157	.025	-.162	-.214	-.053	-.130
	P (2-tailed)	.276	.864	.267	.179	.737	.431
	N	50	49	49	41	42	39
severity of alcohol abuse	Correlation Coefficient	.020	.015	-.068	-.210	-.088	-.030
	P (2-tailed)	.892	.918	.641	.187	.581	.856
	N	50	49	49	41	42	39
PH	Correlation Coefficient	-.040	-.232	.022	-.324*	-.179	.002

Continuous Variables: IHC Non-Parametric Correlations

Spearman's Rho		Relative FEZ1 (APh Grey Matter)	Relative FEZ1 (APh White Matter)	Relative FEZ1 (Dentate Gyrus)	Absolute NEUROD1 (APh Grey Matter)	Absolute NEUROD1 (APh White Matter)	Absolute NEUROD1 (Dentate Gyrus)
	P (2-tailed)	.782	.108	.880	.039	.256	.991
	N	50	49	49	41	42	39
mass of brain (g)	Correlation Coefficient	.002	.124	.047	.038	-.237	.103
	P (2-tailed)	.988	.395	.751	.816	.131	.531
	N	50	49	49	41	42	39
post-mortem interval (hours)	Correlation Coefficient	-.152	.243	.056	.071	-.099	.005
	P (2-tailed)	.292	.092	.704	.658	.532	.976
	N	50	49	49	41	42	39
STORAGE (DAYS)	Correlation Coefficient	.156	-.147	-.111	-.085	.072	-.013
	P (2-tailed)	.278	.315	.449	.596	.653	.936
	N	50	49	49	41	42	39

3-J Table showing the results of Mann-Whitney *U* testing of dichotomous demographic and clinical variables for effects on protein density values in section of anterior hippocampus.

Dichotomous Variables: IHC Non-Parametric Testing

		Relative FEZ1 (APh Grey Matter)	Relative FEZ1 (APh White Matter)	Relative FEZ1 (Dentate Gyrus)	Absolute NEUROD1 (APh Grey Matter)	Absolute NEUROD1 (APh White Matter)	Absolute NEUROD1 (Dentate Gyrus)
female	*N*	22	22	21	17	17	15
	Mean Rank	23.73	25.23	24.52	22.53	24.41	19.33
	Sum of Ranks	522.00	555.00	515.00	383.00	415.00	290.00
male	*N*	28	27	28	24	25	24

Dichotomous Variables: IHC Non-Parametric Testing

		Relative FEZ1 (APh Grey Matter)	Relative FEZ1 (APh White Matter)	Relative FEZ1 (Dentate Gyrus)	Absolute NEUROD1 (APh Grey Matter)	Absolute NEUROD1 (APh White Matter)	Absolute NEUROD1 (Dentate Gyrus)
	Mean Rank	26.89	24.81	25.36	19.92	19.52	20.42
	Sum of Ranks	753.00	670.00	710.00	478.00	488.00	490.00
Total	*N*	50	49	49	41	42	39
	Mann-Whitney U	269.000	292.000	284.000	178.000	163.000	170.000
	Wilcoxon W	522.000	670.000	515.000	478.000	488.000	290.000
	Z	-.762	-.101	-.202	-.688	-1.268	-.289
	p (2-tailed)	.446	.920	.840	.491	.205	.773
Death by suicide: yes	*N*	18	18	17	13	12	11
	Mean Rank	23.56	25.67	24.53	14.46	19.17	18.64
	Sum of Ranks	424.00	462.00	417.00	188.00	230.00	205.00
no	*N*	32	31	32	28	30	28
	Mean Rank	26.59	24.61	25.25	24.04	22.43	20.54
	Sum of Ranks	851.00	763.00	808.00	673.00	673.00	575.00
Total	*N*	50	49	49	41	42	39
	Mann-Whitney U	253.000	267.000	264.000	97.000	152.000	139.000
	Wilcoxon W	424.000	763.000	417.000	188.000	230.000	205.000
	Z	-.707	-.249	-.168	-2.381	-.780	-.468
	p (2-tailed)	.479	.803	.867	.017	.436	.640
With Psychosis	*N*	24	23	23	21	21	20
	Mean Rank	24.79	24.30	27.52	18.95	20.14	18.15
	Sum of Ranks	595.00	559.00	633.00	398.00	423.00	363.00
Without Psychosis	*N*	26	26	26	20	21	19
	Mean Rank	26.15	25.62	22.77	23.15	22.86	21.95
	Sum of Ranks	680.00	666.00	592.00	463.00	480.00	417.00
Total	*N*	50	49	49	41	42	39

Dichotomous Variables: IHC Non-Parametric Testing

		Relative FEZ1 (APh Grey Matter)	Relative FEZ1 (APh White Matter)	Relative FEZ1 (Dentate Gyrus)	Absolute NEUROD1 (APh Grey Matter)	Absolute NEUROD1 (APh White Matter)	Absolute NEUROD1 (Dentate Gyrus)
	Mann-Whitney U	295.000	283.000	241.000	167.000	192.000	153.000
	Wilcoxon W	595.000	559.000	592.000	398.000	423.000	363.000
	Z	-.330	-.321	-1.162	-1.122	-.717	-1.040
	p (2-tailed)	.741	.749	.245	.262	.473	.299
Hemisphere: right	*N*	23	22	22	18	19	18
	Mean Rank	25.48	24.14	21.82	18.94	21.16	21.39
	Sum of Ranks	586.00	531.00	480.00	341.00	402.00	385.00
left	*N*	27	27	27	23	23	21
	Mean Rank	25.52	25.70	27.59	22.61	21.78	18.81
	Sum of Ranks	689.00	694.00	745.00	520.00	501.00	395.00
Total	*N*	50	49	49	41	42	39
	Mann-Whitney U	310.000	278.000	227.000	170.000	212.000	164.000
	Wilcoxon W	586.000	531.000	480.000	341.000	402.000	395.000
	Z	-.010	-.382	-1.407	-.972	-.164	-.704
	p (2-tailed)	.992	.703	.159	.331	.870	.481

4 GENOTYPE ANALYSIS

Once the full complement of cerebellar DNA samples were extracted and verified for purity and yield, discrimination assays were performed (Figs. 4-2 to 4-22). Genotype experiments were repeated twice, except for in the case of the rare variant *DISC1* rs3737597 where only two experiments were performed. For the rest, sample genotype was determined on the basis of at least three technical replicates of normalised relative fluorescence units (RFUs). There were no discrepancies between repeat experiments. In the first round of experiments, some samples did not produce did not produce amplification curves, these samples were re-analysed in a 'missing samples' analysis performed in duplicate (Figs).

Marginal levels of non-specific fluorescence, produced by two of the 'Genotyping Assays', proved to be obstructive towards the sole usage of either threshold cycle number or absolute RFU to determine genotype. An RFU normalization strategy, forwarded by Livak and colleagues[250](Fig 4-1), was therefore utilised. This controlled for the marginal nonspecific fluorescence, and minor variations in starting sample quantity (data not shown). Normalised RFU clusters were then sequestered on an axis plot (membership of the intermediate cluster denoting heterozygosity) in order to make allele calls. In order to check the results, genotype was also determined using threshold cycle during the 2nd experiment for *DISC1* rs6675281 (Fig 4-7).

$$\text{Normalized } A_1 = \frac{A_1}{A_1 + A_2 + \bar{x}(NTC_{A1+A2})}$$

Where:¶

- A_1 represents RFU for Allele 1
- A_2 represents RFU for Allele 2
- $\bar{x}$ represents the mean RFU
- NTC_{A1+A2} represents the sum of RFUs for the NTC sample of Allele 1 and Allele 2

4-1 Adapted from Livak et al.[250] Formula for normalizing RFUs for the purposes of allelic discrimination using the 5' nuclease assay.

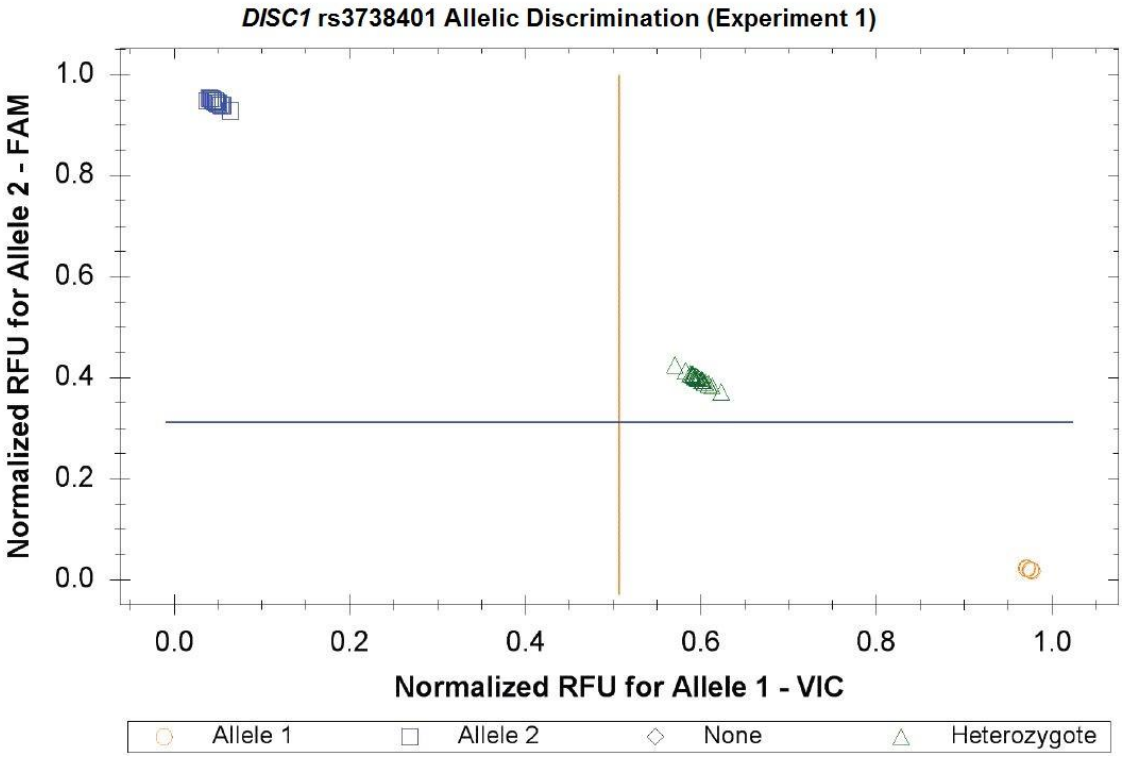
When only 2 clusters were present, 4 non-template control wells were sequentially re-analysed as samples to indicate an expected normalized fluorescence value for probes complimentary to non-present alleles, allowing for a genotype determination between the two groups. The cycle which provided the most distinct clustering across all assays was found to be cycle number 45 (5 extra cycles then recommended by the reagent suppliers).

All SNPs analysed in this study were found to be in Hardy-Weinberg Equilibrium (HWE; χ^2, $p > .05$) with the Stanley Consortium allele frequencies. HWE was therefore also tested using HapMap Central European (CEU) allele frequencies (4-A Table to 4-R Table). This method exploits HWE to 'screen' for distorted SNPs in population samples[251].

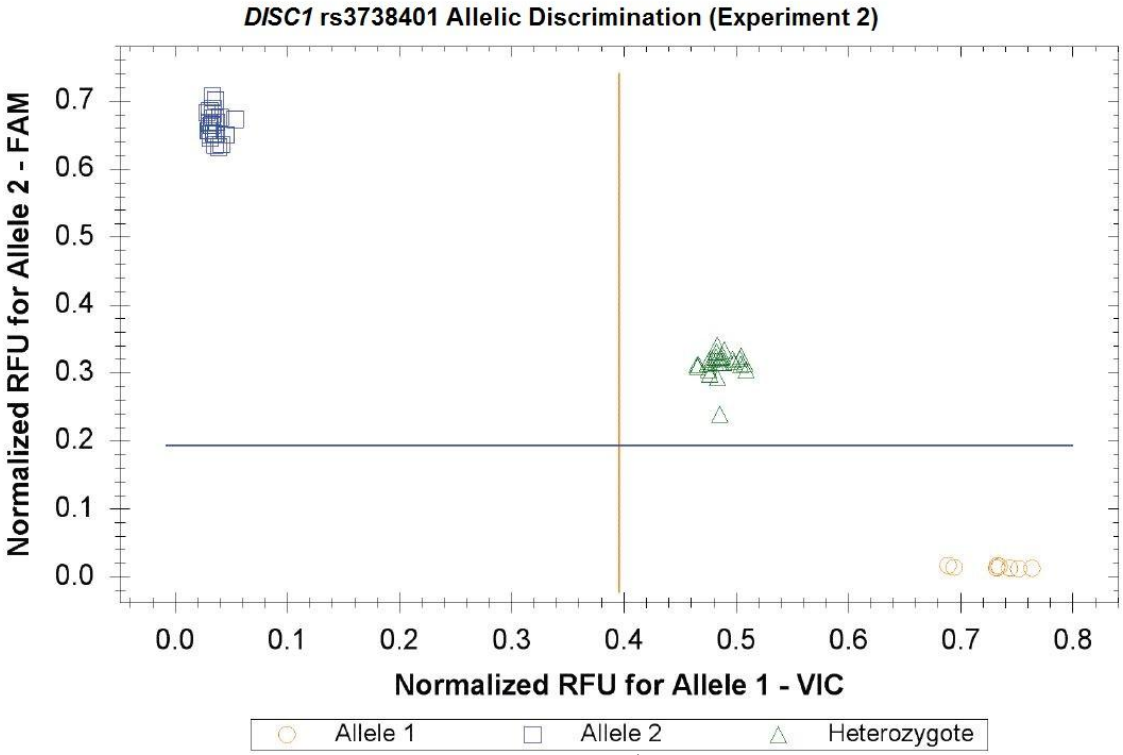
4.1 *DISC1* Locus

4.1.1 rs3738401

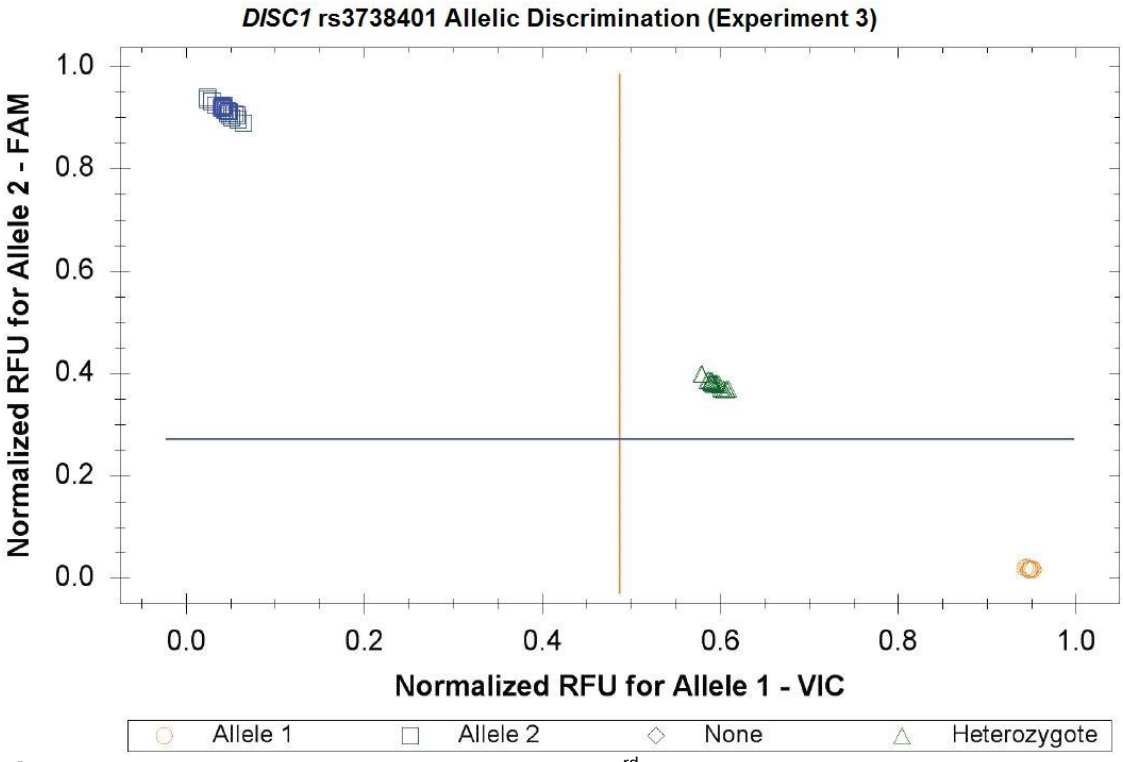
For the rs3738401 assay, the Allele 1 VIC fluorophore was attached to the probe complimentary to the minor A allele; the Allele 2 FAM fluorophore was attached to the major G allele (Fig 4-2 to 4-5). The Stanley Consortium frequencies of the *DISC1* rs3738401 alleles were A = .36, G = .64. The HapMap frequencies of the *DISC1* rs3738401 alleles are A = .36, G = .64. The allelic discrimination analysis results were found to be in HWE with the expected Stanley Consortium allele frequencies for this sample (χ^2, $p = .98$) (4-B Table) and the HapMap allele frequencies (χ^2, $p = 0.96$) (4-C Table). The allelic discrimination repeat experiments for this SNP were in concordance.



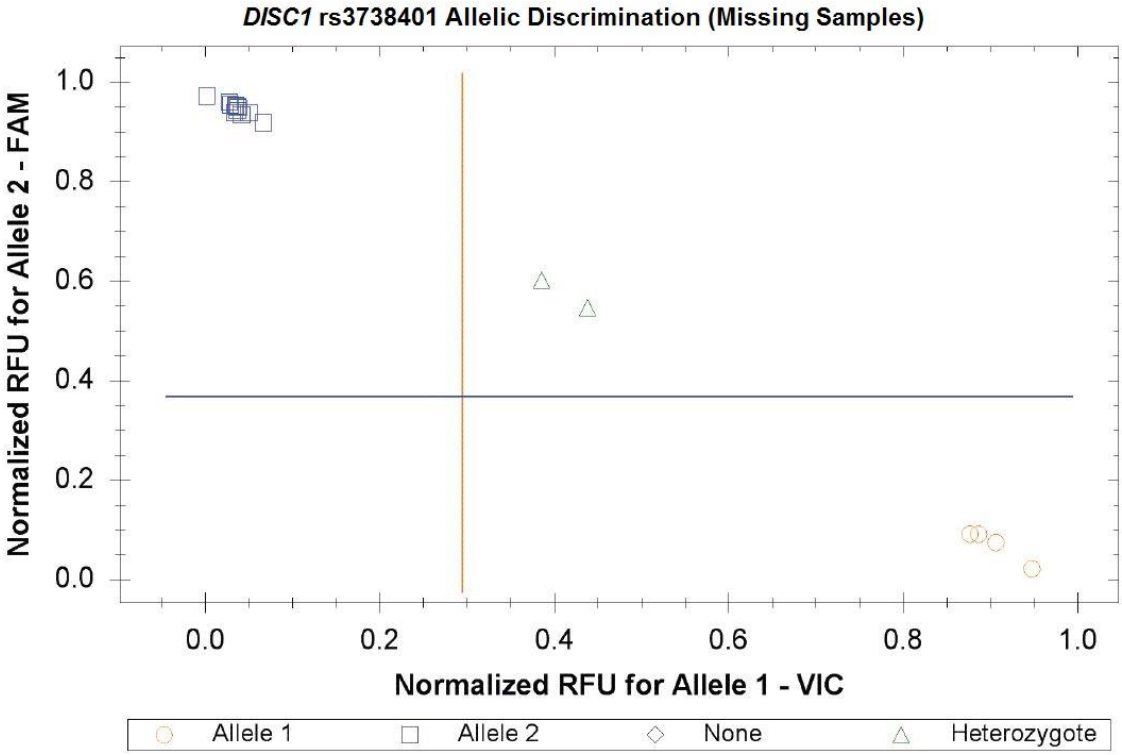
4-2 Scatter plot of the raw data obtained from the 1st analysis of the *DISC1* rs3738401 SNP. The normalised relative fluorescence unit (NRFU) at cycle 45 is demonstrated for each fluorophore.



4-3 Scatter plot of the raw data obtained from the 2nd analysis of the *DISC1* rs3738401. The normalised relative fluorescence unit (NRFU) at cycle 45 is demonstrated for each fluorophore.



4-4 Scatter plot of the raw data obtained from the 3rd analysis of the *DISC1* rs3738401. The normalised relative fluorescence unit (NRFU) at cycle 45 is demonstrated for each fluorophore.



4-5 Scatter plot of the raw data obtained from the analysis of *DISC1* rs3738401 in samples missing from the 1st experiment. Each previously missing sample was run in duplicate, the normalised relative fluorescence unit (NRFU) at cycle 45 is demonstrated for each fluorophore.

4-A Table demonstrating the genotype calls from each experiment for *DISC1* rs3738401, samples that were missing from the 1st experiment are highlighted in grey

AL ID No.	1st	2nd	3rd	Missing Samples (in duplicate)
1	GA	GA	GA	
2	GA	GA	GA	
3		AA	AA	AA
4	GG	GG	GG	
5	GA	GA	GA	
6	GA	GA	GA	
7	GA	GA	GA	
8	GG	GG	GG	
9	GA	GA	GA	
10	GG	GG	GG	
11	GG	GG	GG	
12	GG	GG	GG	
13	AA	AA	AA	

AL ID No.	1st	2nd	3rd	Missing Samples (in duplicate)
14		GG	GG	GG
15	GA	GA	GA	
16	GA	GA	GA	
17	GA	GA	GA	
18	GG	GG	GG	
19		GG	GG	GG
20	AA	AA	AA	
21	GA	GA	GA	
22	GA	GA	GA	
23	GA	GA	GA	
24	GG	GG	GG	
25	GG	GG	GG	
26	GG	GG	GG	
27	GG	GG	GG	
28	GA	GA	GA	
29	GG	GG	GG	
30	GA	GA	GA	
31	AA	AA	AA	
32		AA	AA	AA
33	GA	GA	GA	
34	GA	GA	GA	
35	GA	GA	GA	
36	GG	GG	GG	
37	AA	AA	AA	
38	GA	GA	GA	
39	GA	GA	GA	
40		GG	GG	GG
41	GG	GG	GG	
42	GA	GA	GA	
43	GA	GA	GA	
44	GG	GG	GG	
45	GG	GG	GG	
46		GG	GG	GG
47		GG	GG	GG
48	GG	GG	GG	
49	GG	GG	GG	
50	GA	GA	GA	

AL ID No.	1st	2nd	3rd	Missing Samples (in duplicate)
51	AA	AA	AA	
52		GG	GG	GG
53	GA	GA	GA	
54	GA	GA	GA	
55	AA	AA	AA	
56	GA	GA	GA	
57	GA	GA	GA	
58	GG	GG	GG	
59	GA	GA	GA	
60	GG	GG	GG	

4-B Table showing the Stanley Consortium allele frequency Hardy-Weinberg equilibrium data for *DISC1* rs3738401

***DISC1* rs3738401; *N* = 60; Stanley MAF = .36**

	AA	GA	GG
Observed	8	27	25
Expected	8	28	25

χ^2 test p = .98

4-C Table showing the HapMap allele frequency Hardy-Weinberg equilibrium data for *DISC1* rs3738401

***DISC1* rs3738401; *N* = 60; HapMap MAF = .36**

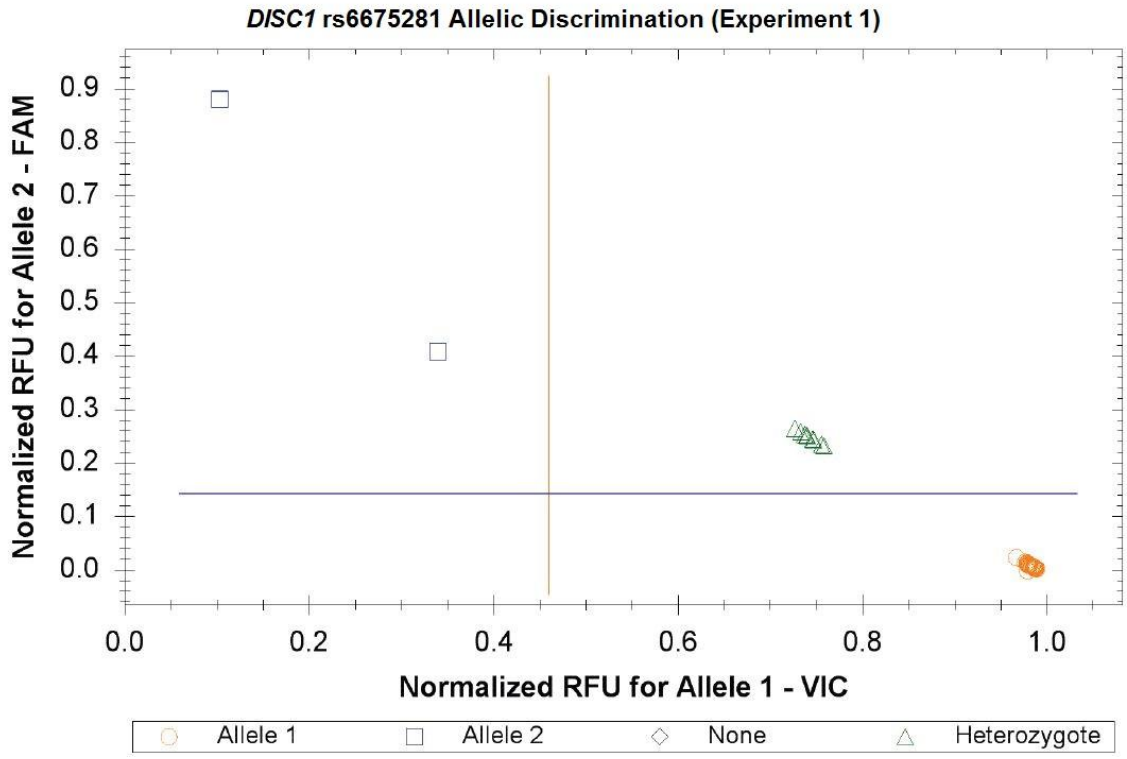
	AA	GA	GG
Observed	8	27	25
Expected	8	28	24

χ^2 test p = .96

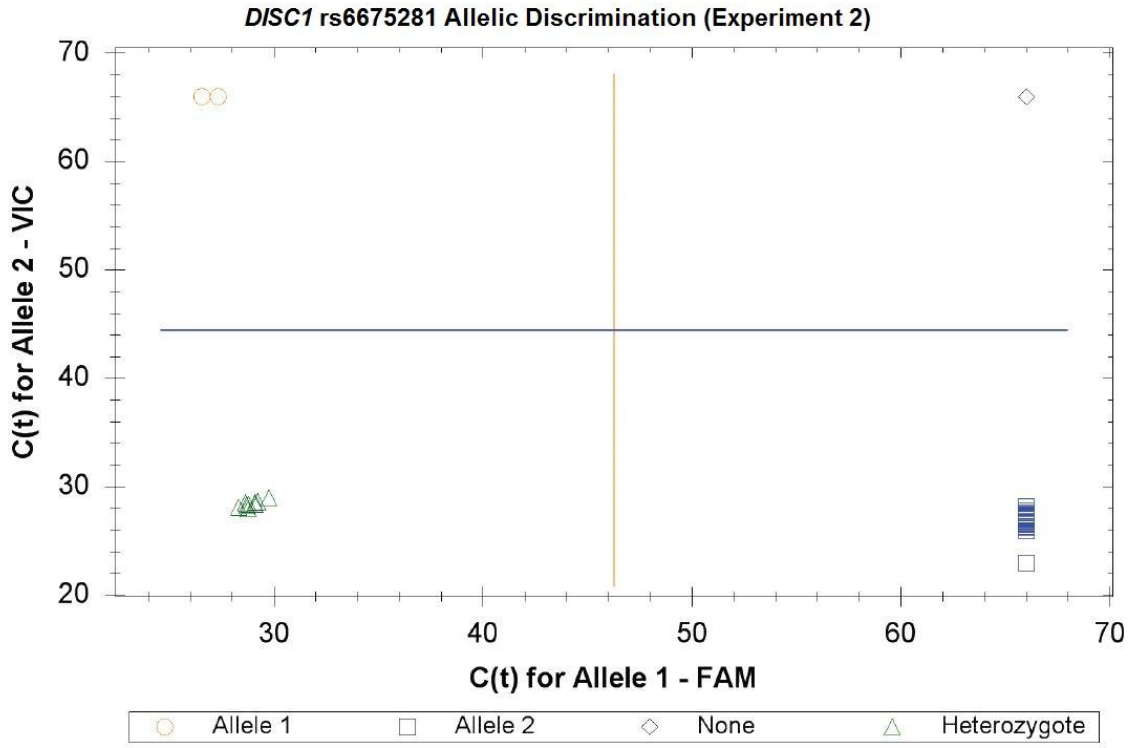
4.1.2 rs6675281

For the rs6675281 assay, the Allele 1 VIC fluorophore was attached to the probe complimentary to the major C allele; the Allele 2 FAM fluorophore

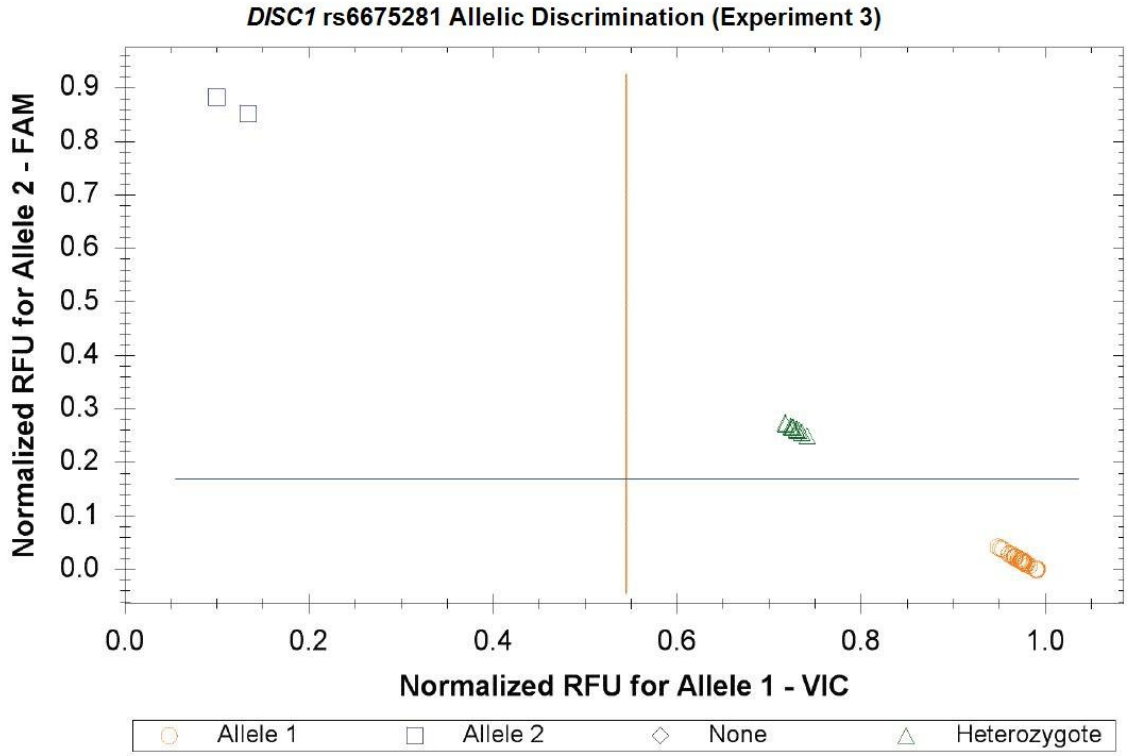
was attached to the minor T allele (Figs. 4-6 to 4-9). The Stanley Consortium frequencies of the *DISC1* rs6675281 alleles were T = .13, C = .87. The HapMap frequencies of the *DISC1* 6675281 alleles are T = .16, C = .84. The allelic discrimination analysis results were found to be in Hardy-Weinberg Equilibrium (HWE) with the expected Stanley Consortium allele frequencies for this sample (χ^2, p = .53) (4-E Table) and the HapMap allele frequencies (χ^2, p = 0.35) (4-F Table). The allelic discrimination repeat experiments for this SNP were in concordance..



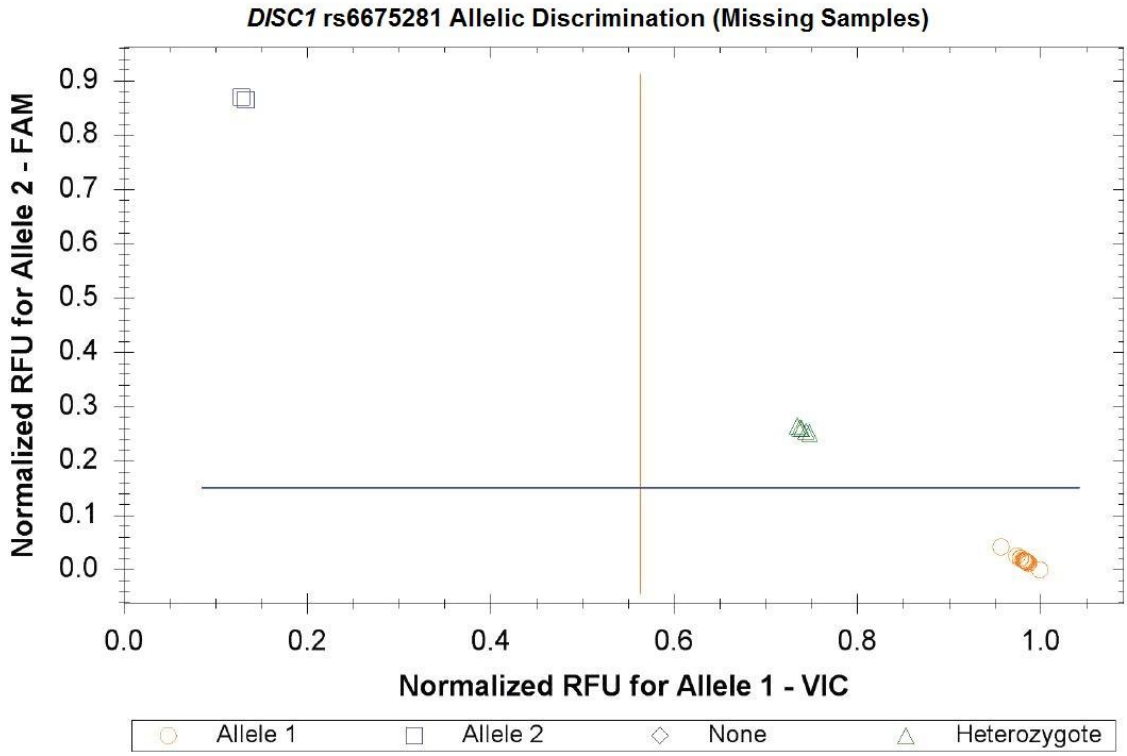
4-6 Scatter plot of the raw data obtained from the 1st analysis of *DISC1* rs6675281. The normalised relative fluorescence unit (NRFU) at cycle 45 is demonstrated for each fluorophore.



4-7 Scatter plot of the raw data obtained from the 2nd analysis of *DISC1* rs6675281. Threshold Cycles (C_q) are demonstrated for each fluorophore.



4-8 Scatter plot of the raw data obtained from the 3rd analysis of *DISC1* rs6675281. The normalised relative fluorescence unit (NRFU) at cycle 45 is demonstrated for each fluorophore.



4-9 Scatter plot of the raw data obtained from the analysis of *DISC1* rs6675281 in samples missing from the 1st experiment. Each previously missing sample was run in duplicate, the normalised relative fluorescence unit (NRFU) at cycle 45 is demonstrated for each fluorophore..

4-D Table demonstrating the genotype calls from each experiment for *DISC1* rs6675281, samples that were missing from the 1st experiment are highlighted in grey

AL ID No.	1st Experiment	2nd	3rd	Missing Samples (in duplicate)
1	CC	CC	CC	
2	CC	CC	CC	
3			CC	CC
4	CC	CC	CC	
5	CT	CT	CT	
6	CC	CC	CC	
7	CC	CC	CC	
8	CC	CC	CC	
9	CC	CC	CC	
10	TT	TT	TT	
11	CC	CC	CC	
12	CC	CC	CC	
13	CC	CC	CC	

AL ID No.	1st Experiment	2nd	3rd	Missing Samples (in duplicate)
14			CT	CT
15	CC	CC	CC	
16	CC	CC	CC	
17	CC	CC	CC	
18	CC	CC	CC	
19			CC	CC
20	CC	CC	CC	
21	CC	CC	CC	
22	CC	CC	CC	
23	CC	CC	CC	
24			CT	CT
25	CC	CC	CC	
26	CT	CT	CT	
27	CT	CT	CT	
28	CC	CC	CC	
29	CC	CC	CC	
30	CC	CC	CC	
31	CC	CC	CC	
32			CC	CC
33	CC	CC	CC	
34	CC	CC	CC	
35	CT	CT	CT	
36	CC	CC	CC	
37	CT	CT	CT	
38	CC	CC	CC	
39	CC	CC	CC	
40	CT	CT	CT	
41	CC	CC	CC	
42	CC	CC	CC	
43	TT	TT	TT	
44	CC	CC	CC	
45	CC	CC	CC	
46			CC	CC
47			CC	CC
48	CC	CC	CC	
49	CT	CT	CT	
50	CT	CT	CT	

AL ID No.	1st Experiment	2nd	3rd	Missing Samples (in duplicate)
51	CC	CC	CC	
52			CC	CC
53	CT	CT	CT	
54	CC	CC	CC	
55	CC	CC	CC	
56	CC	CC	CC	
57			CC	CC
58			CC	CC
59			CT	CT
60			CC	CC

4-E Table showing the Stanley Consortium allele frequency Hardy-Weinberg equilibrium data for *DISC1* rs6675281

***DISC1* rs6675281; *N* = 60; Stanley MAF = .13**

	TT	CT	CC
Observed	2	12	45
Expected	1	14	45
		χ^2 test p =	.53

4-F Table the HapMap allele frequency Hardy-Weinberg equilibrium data for *DISC1* rs6675281

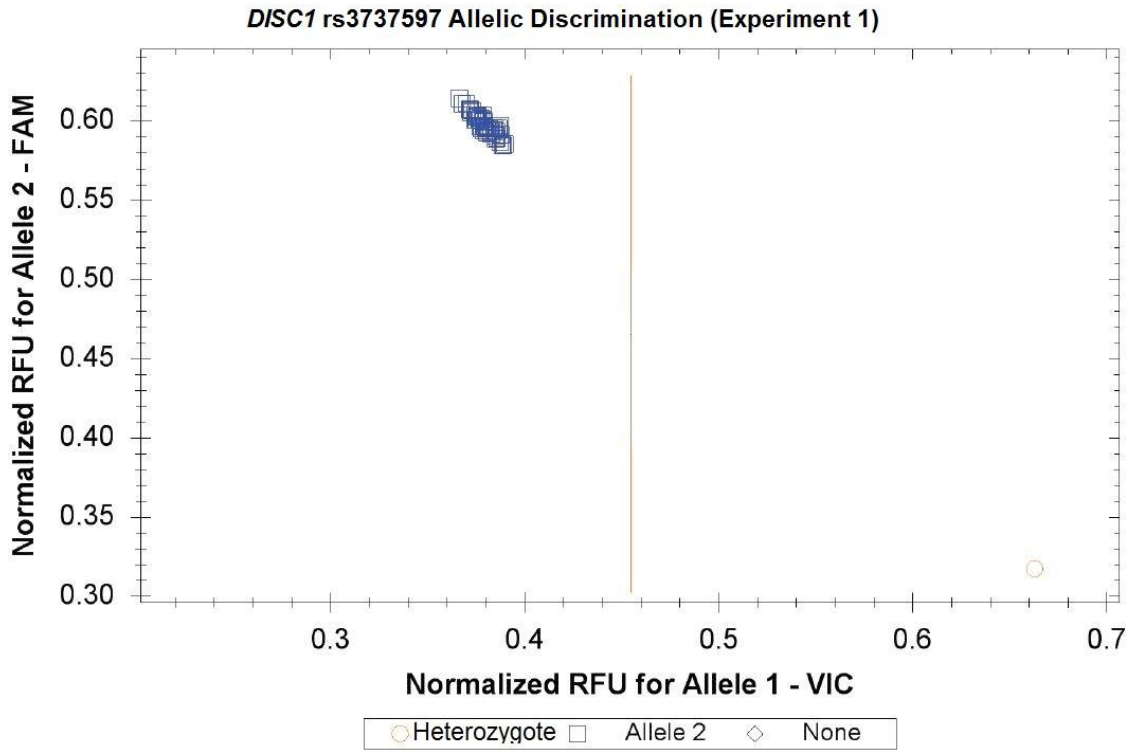
***DISC1* rs6675281; *N* = 60; HapMap MAF = .16**

	TT	CT	CC
Observed	2	12	45
Expected	1	16	43
		χ^2 test p =	.35

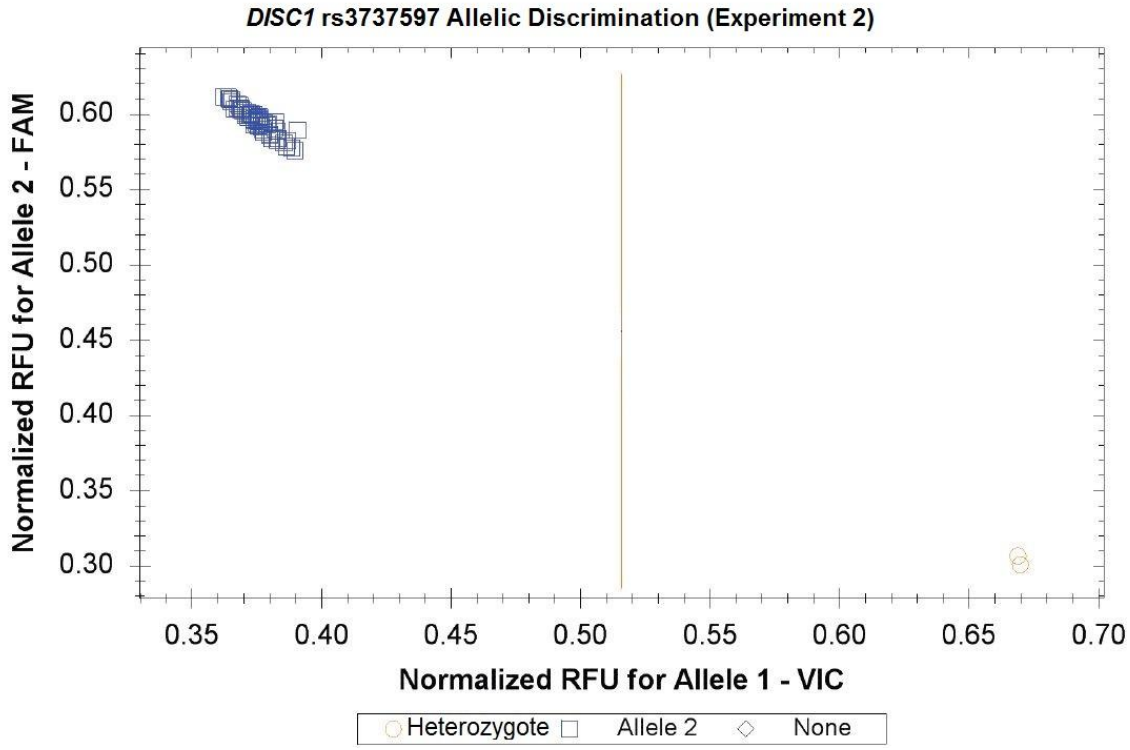
4.1.3 rs3737597

For the rs3737597 assay, the Allele 1 VIC fluorophore was attached to the probe complimentary to the minor A allele; the Allele 2 FAM fluorophore was attached to the major G allele (Fig. 4-10 to 4-11). The Stanley Consortium frequencies of the *DISC1* rs3737597 alleles were A = .02, G = .98. The HapMap frequencies of the *DISC1* rs3737597 alleles are A = .04, G = 0.95. All samples

except two were determined to be homozygous for the major allele(4-G Table). The allelic discrimination analysis results were found to be in Hardy-Weinberg Equilibrium (HWE) with the expected Stanley Consortium allele frequencies for this sample (χ^2, p = .85) (4-H Table) and the HapMap allele frequencies (χ^2, p = .35) (4-I Table). The allelic discrimination repeat experiments for this SNP were in concordance.



4-10 Scatter plot of the raw data obtained from the 1st analysis of *DISC1* rs3737597. The normalised relative fluorescence unit (NRFU) at cycle 45 is demonstrated for each fluorophore.



4-11 Scatter plot of the raw data obtained from the 2nd analysis of *DISC1* rs3737597. The normalised relative fluorescence unit (NRFU) at cycle 45 is demonstrated for each fluorophore.

4-G Table demonstrating the genotype calls from each experiment for *DISC1* rs3737597, samples that were missing from the 1st experiment are highlighted in grey

AL ID No.	1st Experiment	2nd
1	GG	GG
2	GG	GG
3		GG
4	GG	GG
5	GG	GG
6	GG	GG
7	GG	GG
8	GG	GG
9	GG	GG
10	GG	GG
11	GG	GG
12	GG	GG
13	GG	GG
14		GG
15	GG	GG

AL ID No.	1st Experiment	2nd
16	GG	GG
17	GG	GG
18	GG	GG
19		GA
20	GG	GG
21	GG	GG
22	GG	GG
23	GG	GG
24		GG
25	GG	GG
26	GG	GG
27	GG	GG
28	GG	GG
29	GG	GG
30	GG	GG
31	GA	GA
32		GG
33	GG	GG
34	GG	GG
35	GG	GG
36	GG	GG
37	GG	GG
38	GG	GG
39	GG	GG
40	GG	GG
41	GG	GG
42	GG	GG
43	GG	GG
44	GG	GG
45	GG	GG
46		GG
47		GG
48	GG	GG
49	GG	GG
50	GG	GG
51	GG	GG
52		GG

AL ID No.	1st Experiment	2nd
53	GG	GG
54	GG	GG
55	GG	GG
56	GG	GG
57	GG	GG
58	GG	GG
59	GG	GG
60	GG	GG

4-H Table showing the Stanley Consortium allele frequency Hardy-Weinberg equilibrium data for *DISC1* rs3737597

***DISC1* rs3737597; *N* = 60; Stanley MAF = .02**

	AA	GA	GG
Observed	0	2	58
Expected	0.02	2.97	58

χ^2 test p = .85

4-I Table showing the HapMap allele frequency Hardy-Weinberg equilibrium data for *DISC1* rs3737597

***DISC1* rs3737597; *N* = 60; HapMap MAF = .04**

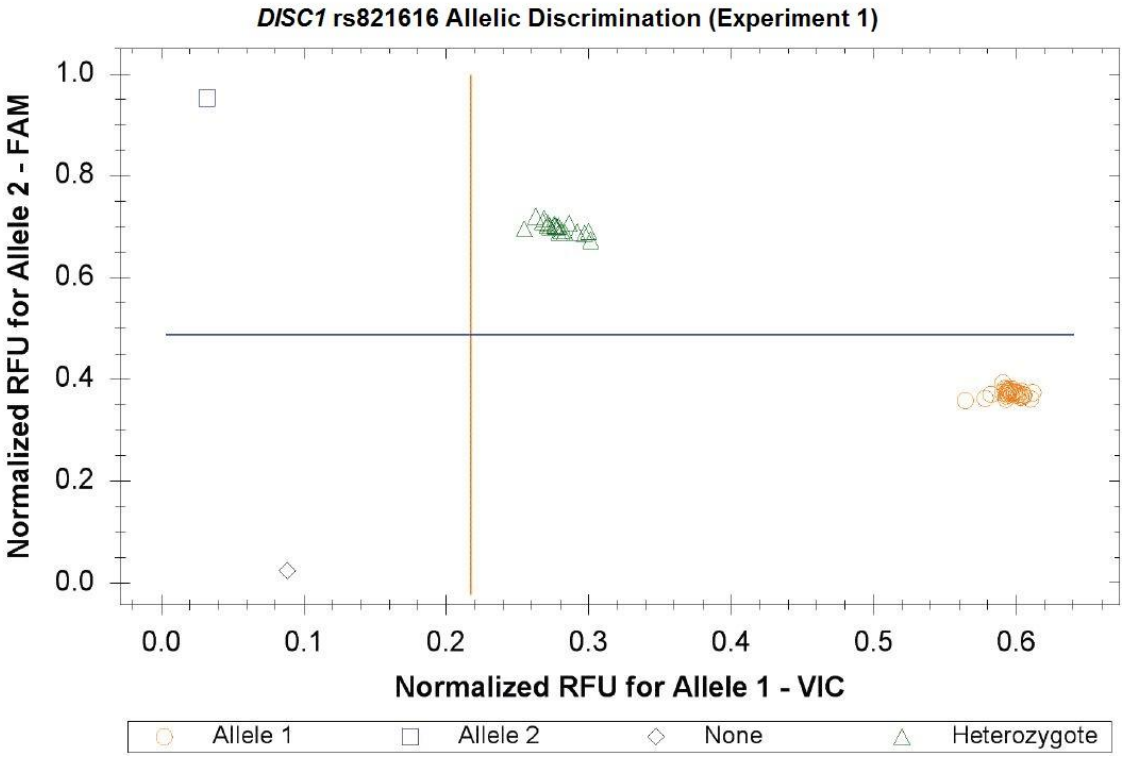
	AA	GA	GG
Observed	0	2	58
Expected	0.1	5	54.8

χ^2 test p = .35

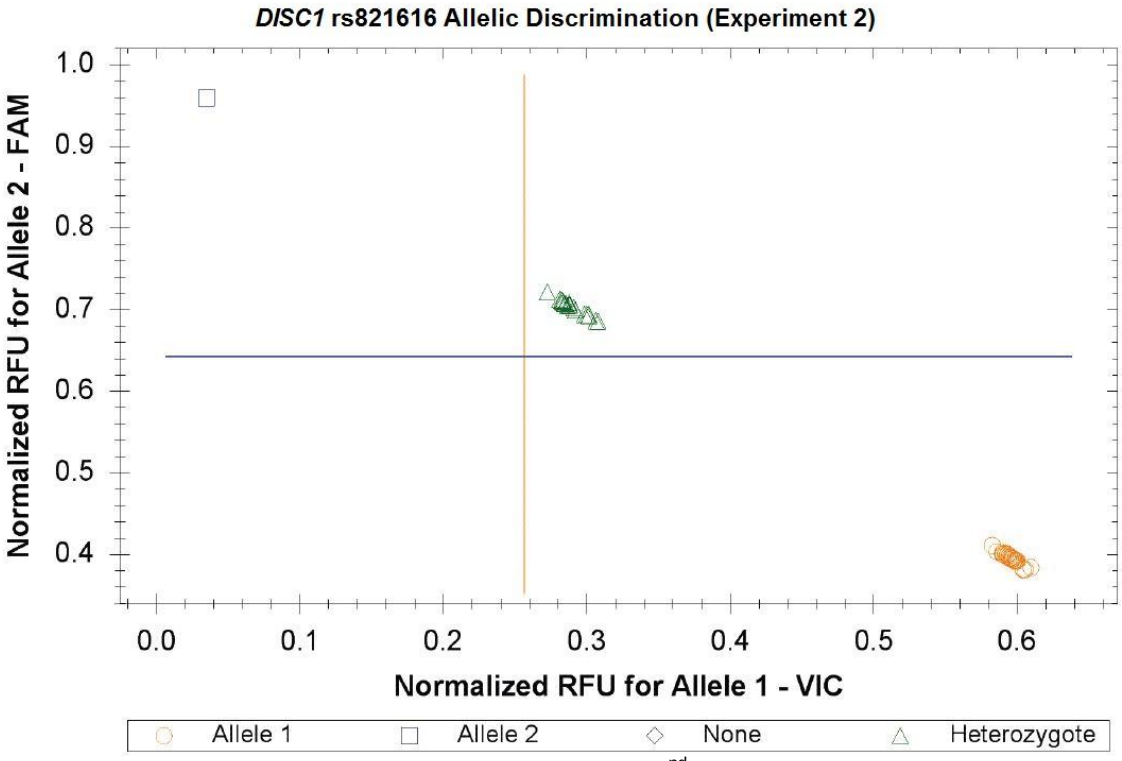
4.1.4 rs821616

For the rs821616 assay, the Allele 1 VIC fluorophore was attached to the probe complimentary to the major A allele; the Allele 2 FAM fluorophore was attached to the minor T allele (Fig. 4-12 to 4-15). The Stanley Consortium

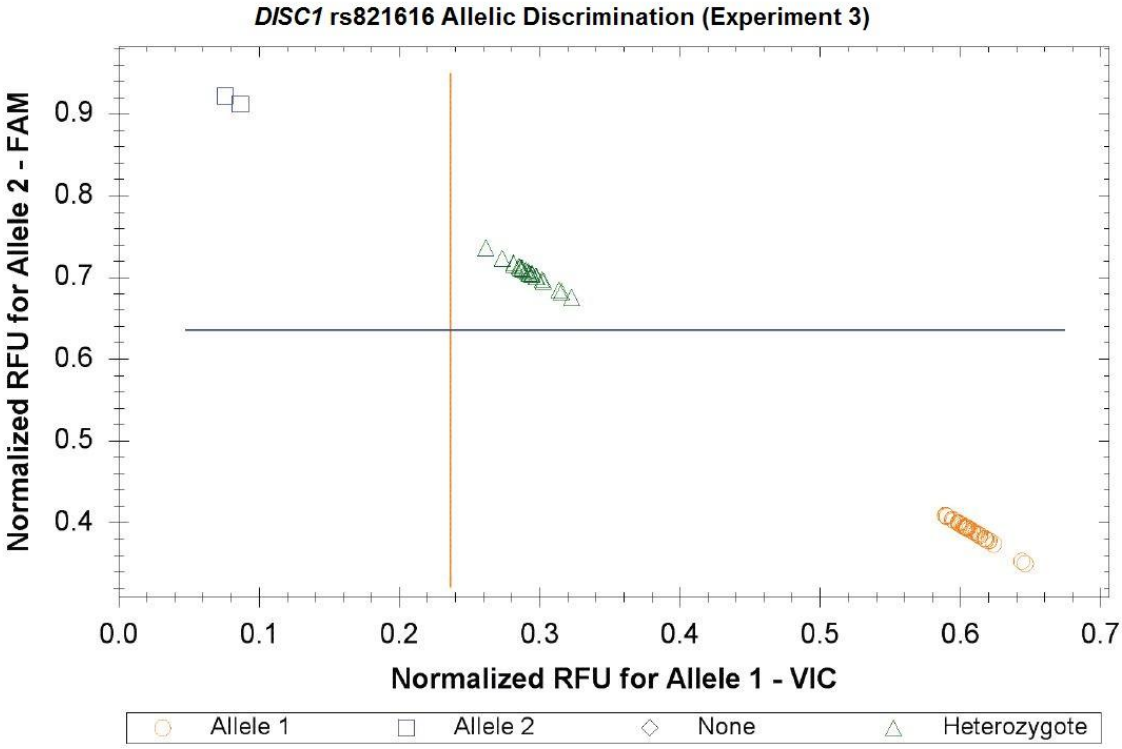
frequencies of the *DISC1* rs821616 alleles were T = .78, A = .23. The HapMap frequencies of the *DISC1* rs821616 alleles are T = 0.31, A = 0.69. The complete allelic discrimination analysis results were found to be in Hardy-Weinberg Equilibrium (HWE) with the expected Stanley Consortium allele frequencies for this sample (χ^2, p = .76) (4-K Table) and the HapMap allele frequencies (χ^2, p = 0.11) (4-L Table). The allelic discrimination repeat experiments for this SNP were in concordance.



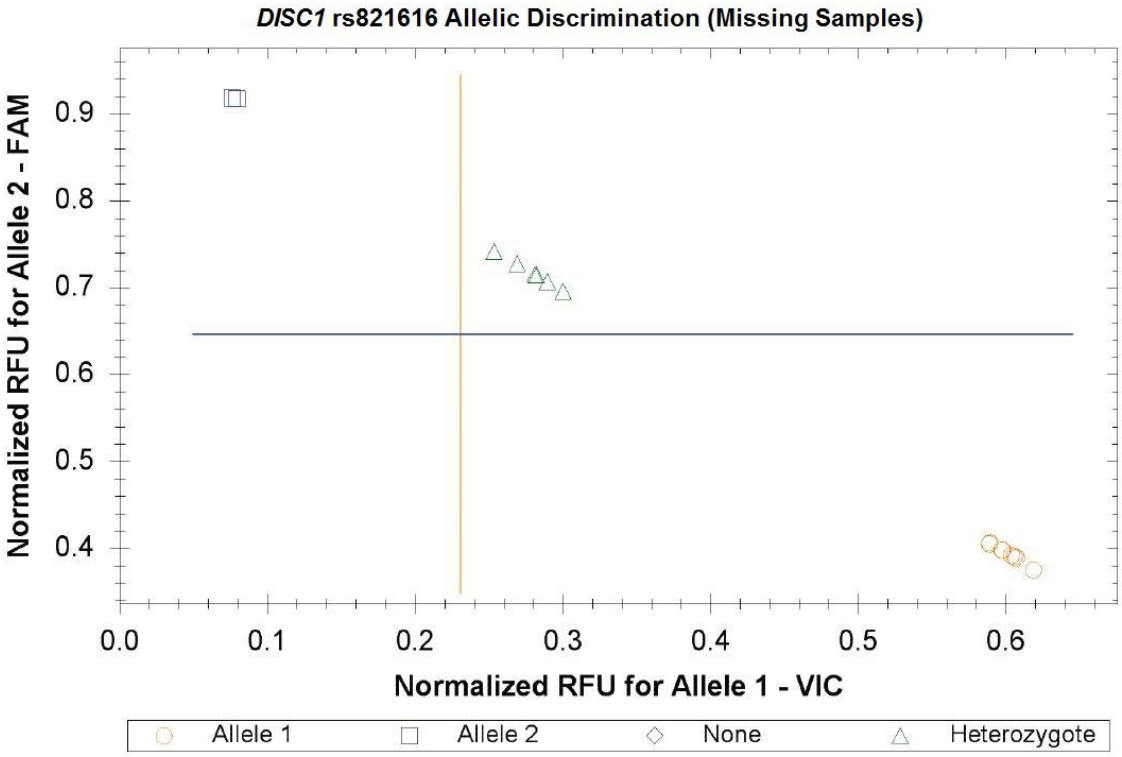
4-12 Scatter plot of the raw data obtained from the 1st analysis of *DISC1* rs821616. The normalised relative fluorescence unit (NRFU) at cycle 45 is demonstrated for each fluorophore.



4-13 Scatter plot of the raw data obtained from the 2nd analysis of *DISC1* rs821616. The normalised relative fluorescence unit (NRFU) at cycle 45 is demonstrated for each fluorophore.



4-14 Scatter plot of the raw data obtained from the 3rd analysis of *DISC1* rs821616. The normalised relative fluorescence unit (NRFU) at cycle 45 is demonstrated for each fluorophore.



4-15 Scatter plot of the raw data obtained from the analysis of *DISC1* rs821616 in samples missing from the 1st experiment. Each previously missing sample was run in duplicate, the normalised relative fluorescence unit (NRFU) at cycle 45 is demonstrated for each fluorophore.

4-J Table demonstrating the genotype calls from each experiment for *DISC1* rs821616, samples that were missing from the 1st experiment are highlighted in grey

AL ID No.	1st Experiment	2nd	3rd	Missing Samples (in duplicate)
1	AT	AT	AT	
2	AA	AA	AA	
3			AT	AT
4	AA	AA	AA	
5	AA	AA	AA	
6	AA	AA	AA	
7	AA	AA	AA	
8	AA	AA	AA	
9	AT	AT	AT	
10	AA	AA	AA	
11	AA	AA	AA	
12	AA	AA	AA	
13	AA	AA	AA	

AL ID No.	1st Experiment	2nd	3rd	Missing Samples (in duplicate)
14			AT	AT
15	AA	AA	AA	
16	AT	AT	AT	
17	AT	AT	AT	
18	AA	AA	AA	
19			TT	TT
20	AA	AA	AA	
21	AT	AT	AT	
22	AT	AT	AT	
23	AT	AT	AT	
24			AA	AA
25	AA	AA	AA	
26	AA	AA	AA	
27	AA	AA	AA	
28	AA	AA	AA	
29	AT	AT	AT	
30	AT	AT	AT	
31	AT	AT	AT	
32			AA	AA
33	AT	AT	AT	
34	AT	AT	AT	
35	AA	AA	AA	
36	AA	AA	AA	
37	AA	AA	AA	
38	AA	AA	AA	
39	AA	AA	AA	
40	AA	AA	AA	
41	AT	AT	AT	
42	AA	AA	AA	
43	AT	AT	AT	
44	AT	AT	AT	
45	AA	AA	AA	
46			AT	AT
47			AA	AA
48	AT	AT	AT	
49	AT	AT	AT	
50	AT	AT	AT	

AL ID No.	1st Experiment	2nd	3rd	Missing Samples (in duplicate)
51	AA	AA	AA	
52			AA	AA
53	AA	AA	AA	
54	AT	AT	AT	
55	AA	AA	AA	
56	AA	AA	AA	
57	AA	AA	AA	
58	AT	AT	AT	
59	TT	TT	TT	
60	AA	AA	AA	

4-K Table showing the Stanley Consortium allele frequency Hardy-Weinberg equilibrium data for *DISC1* rs821616

***DISC1* rs821616; *N* = 60; Stanley MAF = .23**

	TT	AT	AA
Observed	2	23	35
Expected	3	21	36

χ^2 test p = .76

4-L Table showing the HapMap allele frequency Hardy-Weinberg equilibrium data for *DISC1* rs821616

***DISC1* rs821616; *N* = 60; HapMap MAF = .31**

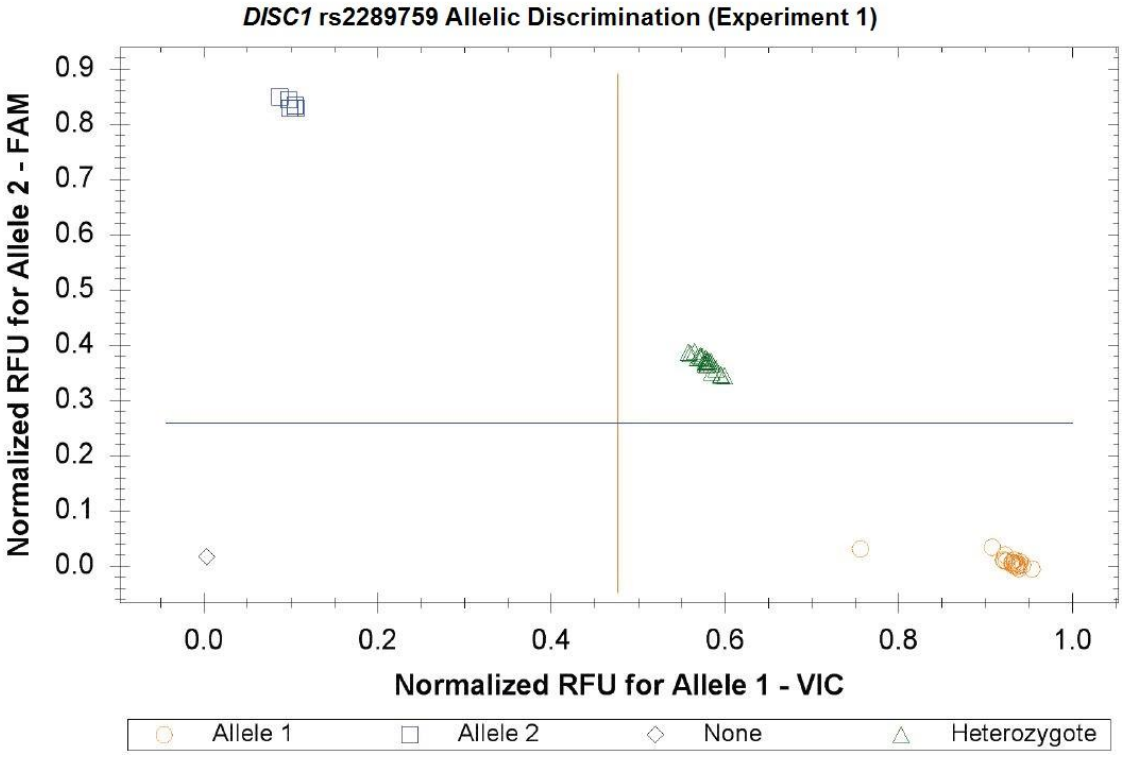
	TT	AT	AA
Observed	2	23	35
Expected	6	26	29

χ^2 test p = .12

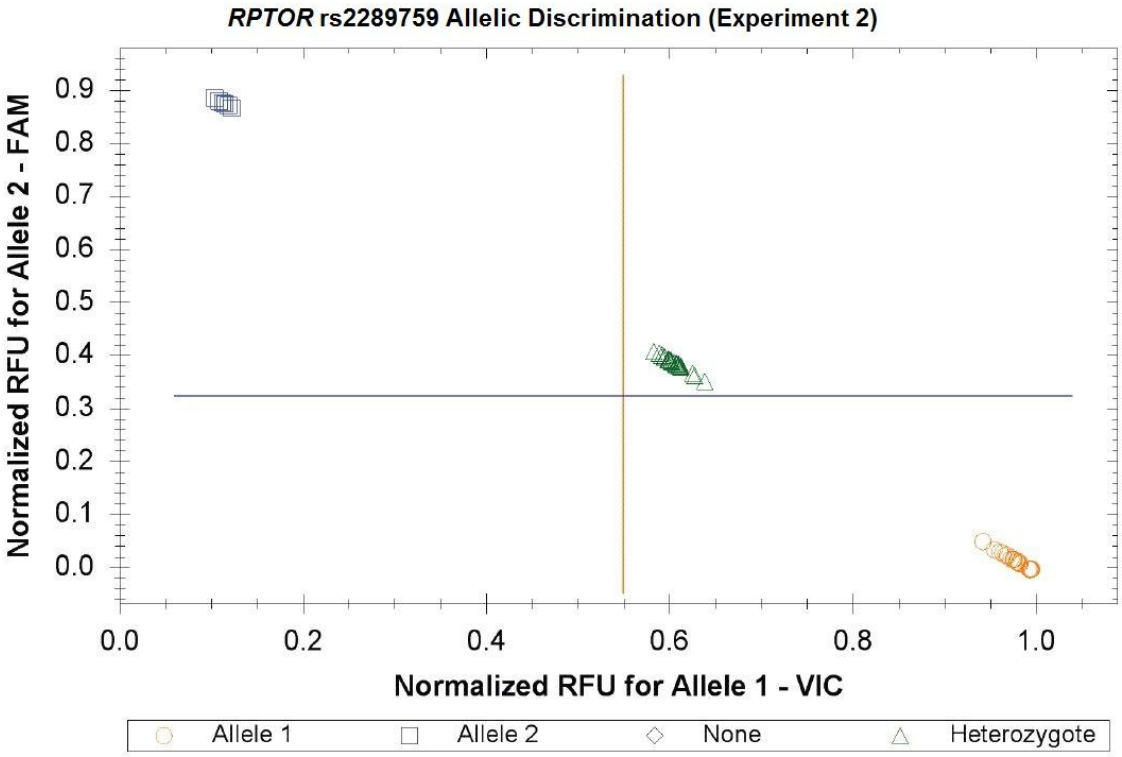
4.2 Schizophrenia-susceptibility Genes

4.2.1 Regulatory-associated Protein of MTOR (*RPTOR*) SNP rs2289759

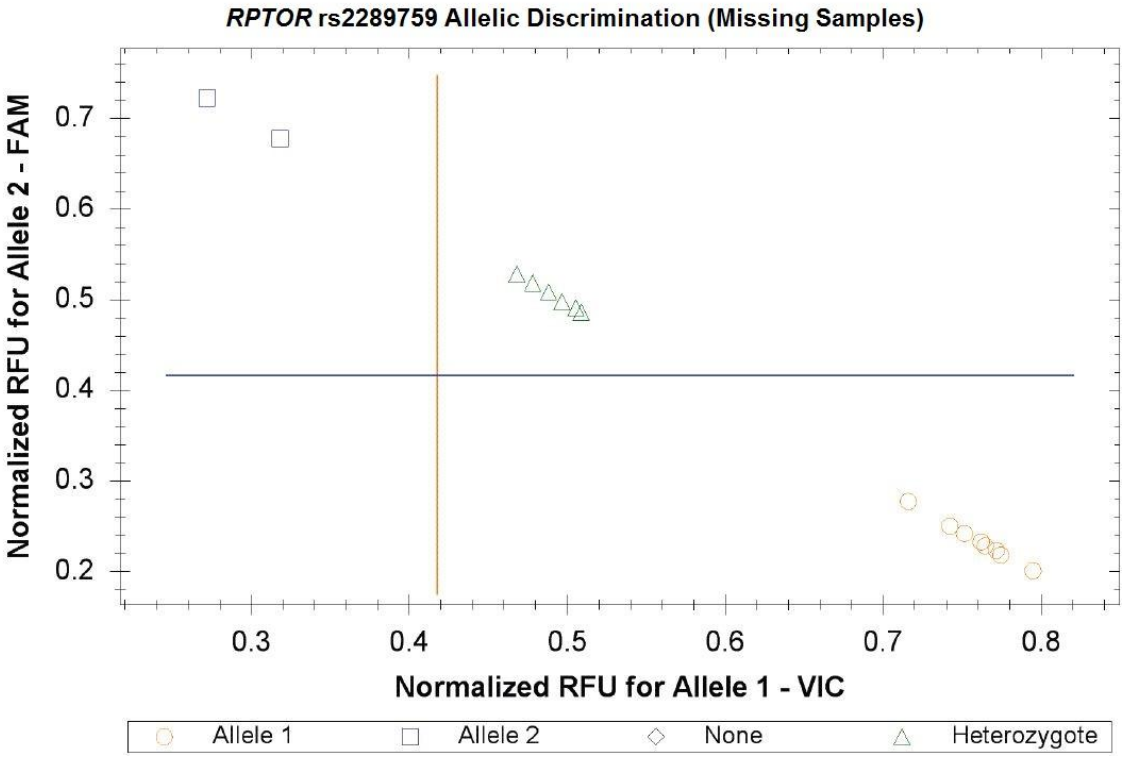
For the *RPTOR* rs2289759 assay, the Allele 1 VIC fluorophore was attached to the probe complimentary to the major A allele; the Allele 2 FAM fluorophore was attached to the minor G allele (Fig. 4-16 to 4-18). The Stanley Consortium frequencies of the *RAPTOR* rs2289759 alleles were G = .33, A = .67. The HapMap frequencies of the rs2289759 alleles are G = 0.22, A = 0.78. The complete allelic discrimination analysis results were found to be in Hardy-Weinberg Equilibrium (HWE) with the expected Stanley Consortium allele frequencies for this sample (χ^2, p = .90) (4-N Table). However the results were not in HWE with the HapMap allele frequencies (χ^2, p = 0.02) (4-O Table). The allelic discrimination repeat experiments for this SNP were in concordance..



4-16 Scatter plot of the raw data obtained from the 1st analysis of *RPTOR* rs2289759. The normalised relative fluorescence unit (NRFU) at cycle 45 is demonstrated for each fluorophore.



4-17 Scatter plot of the raw data obtained from the 2nd analysis of *RPTOR* rs2289759. The normalised relative fluorescence unit (NRFU) at cycle 45 is demonstrated for each fluorophore.



4-18 Scatter plot of the raw data obtained from the analysis of *RPTOR* rs2289759 in samples missing from the 1st experiment. Each previously missing sample was run in duplicate, the normalised relative fluorescence unit (NRFU) at cycle 45 is demonstrated for each fluorophore.

4-M Table demonstrating the genotype calls from each experiment for *RPTOR* rs2289759, samples that were missing from the 1st experiment are highlighted in grey

AL ID No.	1st Experiment	2nd	Missing samples (in duplicate)
1	AG	AG	
2	AG	AG	
3		AA	AA
4	AG	AG	
5	AA	AA	
6	GG	GG	
7	AA	AA	
8	AG	AG	
9	AA	AA	
10	AA	AA	
11	AG	AG	
12	AA	AA	
13	AG	AG	
14		AA	AA
15	AG	AG	
16	GG	GG	
17	AG	AG	
18	AA	AA	
19		AG	AG
20	AA	AA	
21	AA	AA	
22	AA	AA	
23	AG	AG	
24		AG	AG
25	AA	AA	
26	AG	AG	
27	AA	AA	
28	AA	AA	
29	AG	AG	
30	AG	AG	

AL ID No.	1st Experiment	2nd	Missing samples (in duplicate)
31	GG	GG	
32		AG	AG
33	AA	AA	
34	AG	AG	
35	AA	AA	
36	AG	AG	
37	AA	AA	
38	AA	AA	
39	AG	AG	
40	AG	AG	
41	AG	AG	
42	GG	GG	
43	AA	AA	
44	AG	AG	
45	AG	AG	
46		AA	AA
47		GG	GG
48	AA	AA	
49	AA	AA	
50	AG	AG	
51	AG	AG	
52		AA	AA
53	AG	AG	
54	AA	AA	
55	AG	AG	
56	AG	AG	
57	AA	AA	
58	AA	AA	
59	AG	AG	
60	GG	GG	

4-N Table showing the Stanley Consortium allele frequency Hardy-Weinberg equilibrium data for *RPTOR* rs2289759

***RPTOR* rs2289759; *N* = 60; Stanley MAF = .33**

	GG	GA	AA
Observed	6	28	26
Expected	27	27	7

χ^2 test p = .90

4-O Table showing the HapMap allele frequency Hardy-Weinberg equilibrium data for *RPTOR* rs2289759.

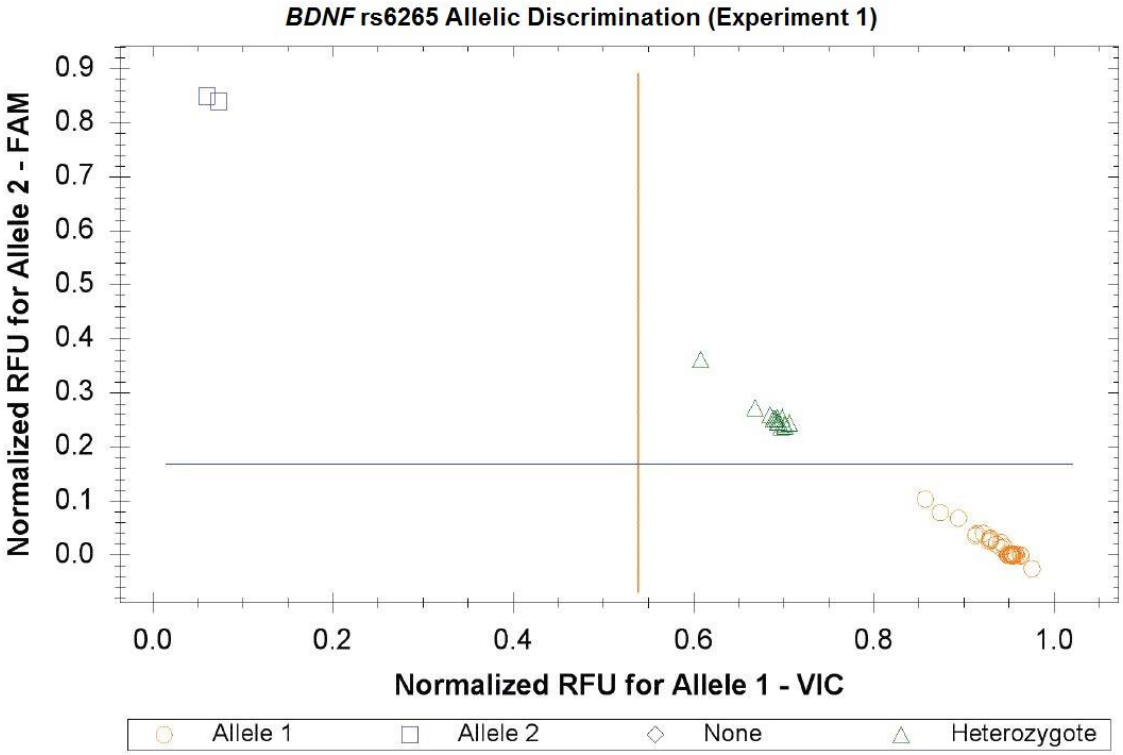
***RPTOR* rs2289759; *N* = 60; HapMap MAF = .22**

	GG	GA	AA
Observed	6	28	26
Expected	3	21	36

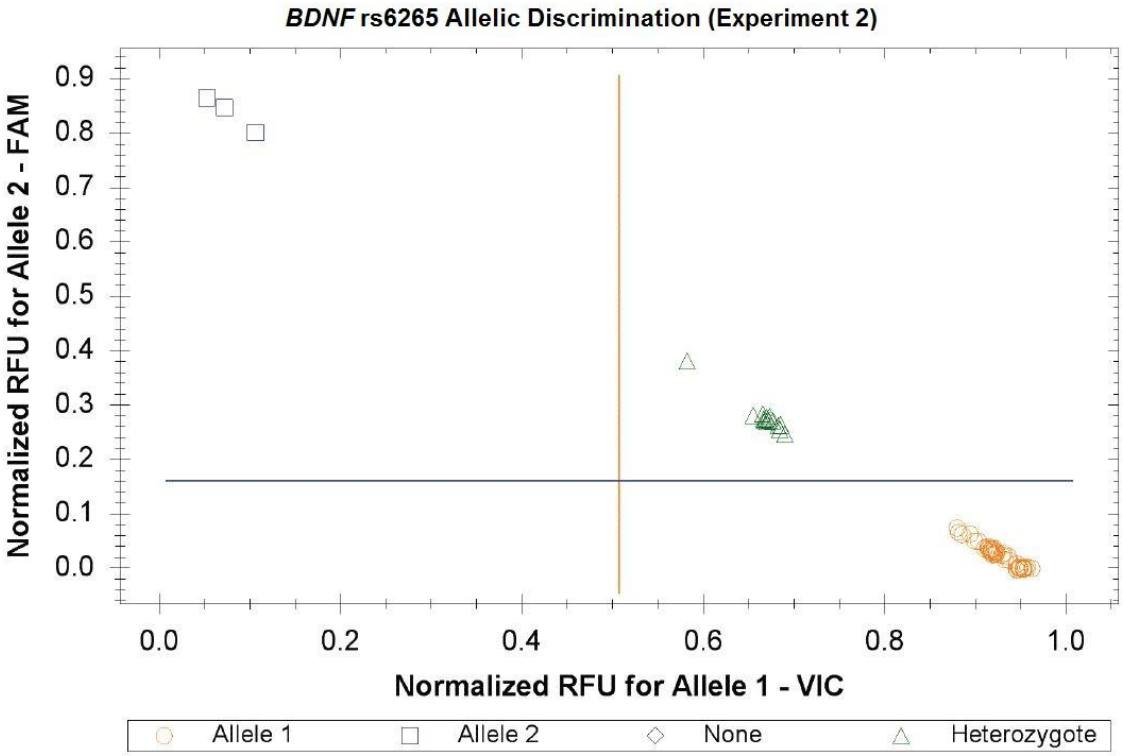
χ^2 test p = .02

4.2.2 Brain-derived Neurotrophic Factor (*BDNF*) SNP rs6265

For the *BDNF* rs6265 assay, the Allele 1 VIC fluorophore was attached to the probe complimentary to the major G allele; the Allele 2 FAM fluorophore was attached to the minor A allele (Fig. 4-19 to 4-22). The Stanley Consortium frequencies of the *BDNF* rs6265 alleles were G = .83, A = .17. The HapMap frequencies of the rs6265 alleles are G = 0.81, A = 0.20. The complete allelic discrimination analysis results were found to be in Hardy-Weinberg Equilibrium (HWE) with the expected Stanley Consortium allele frequencies for this sample (χ^2, p = .59) (4-Q Table) and the HapMap allele frequencies (χ^2, p = 0.33) (4-R Table). The allelic discrimination repeat experiments for this SNP were in concordance.

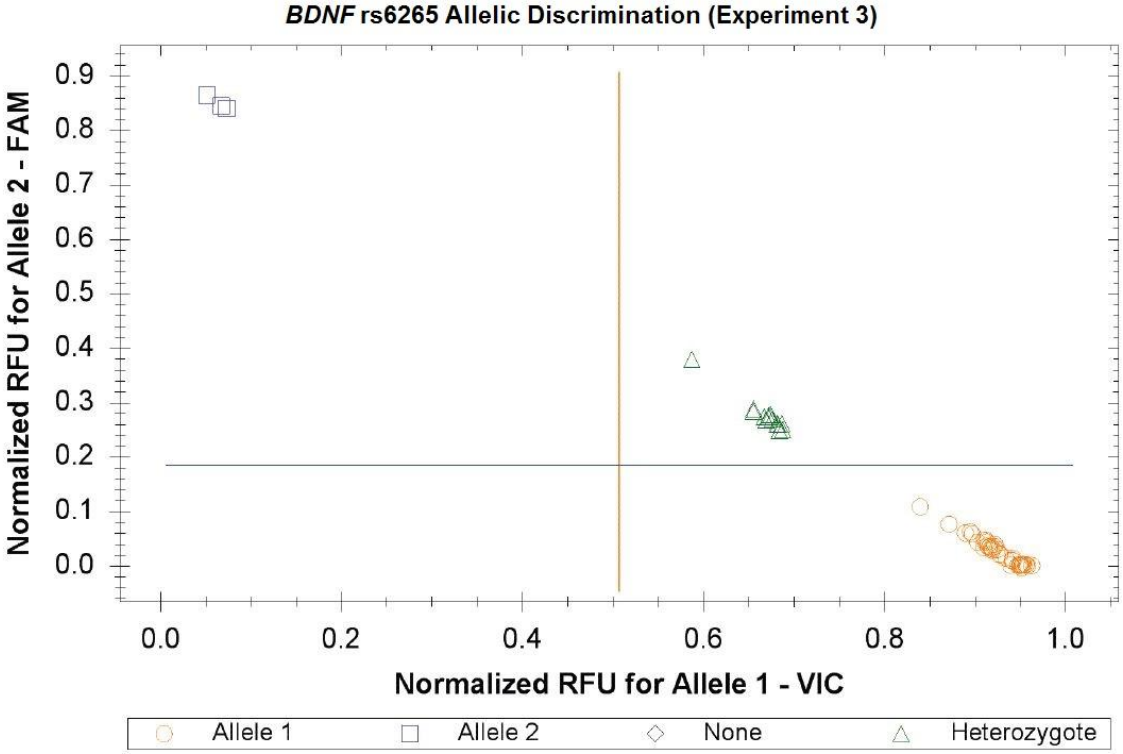


4-19 Scatter plot of the raw data obtained from the 1st analysis of *BDNF* rs6265. The normalised relative fluorescence unit (NRFU) at cycle 45 is demonstrated for each fluorophore. Large annotated bold shapes surround data clusters used to make allele calls.

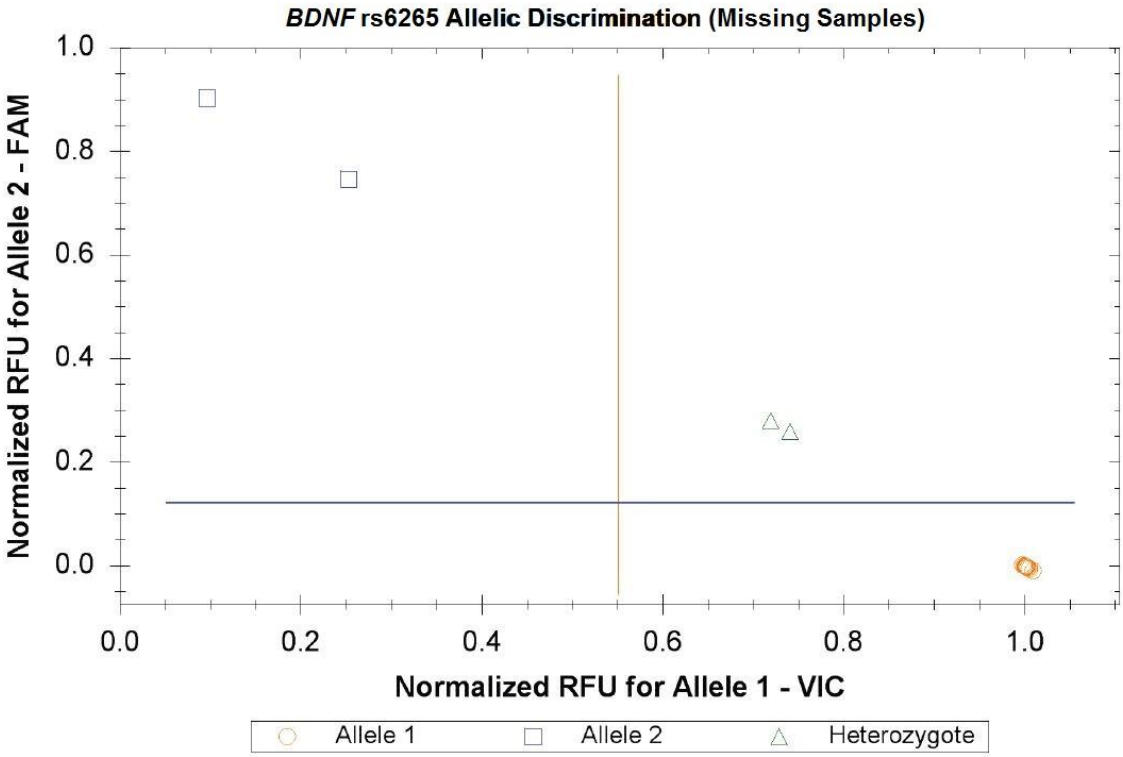


4-20 Scatter plot of the raw data obtained from the 2nd analysis of *BDNF* rs6265. The

normalised relative fluorescence unit (NRFU) at cycle 45 is demonstrated for each fluorophore. Large annotated bold shapes surround data clusters used to make allele calls.



4-21 Scatter plot of the raw data obtained from the 3rd analysis of *BDNF* rs6265. The normalised relative fluorescence unit (NRFU) at cycle 45 is demonstrated for each fluorophore. Large annotated bold shapes surround data clusters used to make allele calls.



4-22 Scatter plot of the raw data obtained from the analysis of *BDNF* rs6265 in samples missing from the 1st experiment. Each previously missing sample was run in duplicate, the normalised relative fluorescence unit (NRFU) at cycle 45 is demonstrated for each fluorophore. Large annotated bold shapes surround data clusters used to make allele calls.

4-P Table demonstrating the genotype calls from each experiment for *BDNF* rs6265, samples that were missing from the 1st experiment are highlighted in grey

AL ID No.	1st Experiment	2nd)	3rd	Missing samples (in duplicate)
1	GG	GG	GG	
2	GG	GG	GG	
3		GG	GG	GG
4	GG	GG	GG	
5	GA	GA	GA	
6	GG	GG	GG	
7	GG	GG	GG	
8	GG	GG	GG	
9	GA	GA	GA	
10	GG	GG	GG	
11	GG	GG	GG	
12	GA	GA	GA	

AL ID No.	1st Experiment	2nd)	3rd	Missing samples (in duplicate)
13	GG	GG	GG	
14		GG	GG	GG
15	GG	GG	GG	
16	GG	GG	GG	
17	GA	GA	GA	
18	GG	GG	GG	
19		GG	GG	GG
20	GG	GG	GG	
21	GG	GG	GG	
22	GG	GG	GG	
23	GG	GG	GG	
24		GG	GG	GG
25	GA	GA	GA	
26	GG	GG	GG	
27	GA	GA	GA	
28	GG	GG	GG	
29	AA	AA	AA	
30	GG	GG	GG	
31	GG	GG	GG	
32		GG	GG	GG
33	GG	GG	GG	
34	AA	AA	AA	
35	GA	GA	GA	
36	GG	GG	GG	
37	GA	GA	GA	
38	GG	GG	GG	
39	GA	GA	GA	
40	GG	GG	GG	
41	GA	GA	GA	
42	GG	GG	GG	
43	GG	GG	GG	
44	GA	GA	GA	
45	GG	GG	GG	
46		GG	GG	GG
47		AA	AA	AA
48	GA	GA	GA	
49	GG	GG	GG	

AL ID No.	1st Experiment	2nd)	3rd	Missing samples (in duplicate)
50	GG	GG	GG	
51	GG	GG	GG	
52		GA	GA	GA
53	GG	GG	GG	
54	GG	GG	GG	
55	GG	GG	GG	
56	GG	GG	GG	
57	GG	GG	GG	
58	GA	GA	GA	
59	GG	GG	GG	
60	GG	GG	GG	

4-Q Table showing the Stanley Consortium allele frequency Hardy-Weinberg equilibrium data for *BDNF* rs6265

***BDNF* rs6265; *N* = 60; Stanley MAF = .17**

	TT	AT	AA
Observed	3	14	43
Expected	2	17	42

χ^2 test p = .60

4-R Table showing the HapMap allele frequency Hardy-Weinberg equilibrium data for *BDNF* SNP rs6265

***BDNF* rs6265; *N* = 60; HapMap MAF = .20**

	TT	AT	AA
Observed	3	14	43
Expected	2	19	39

χ^2 test p = .33

5 QUANTITATIVE PCR (qPCR)

Bar graphs are used to illustrate differences between diagnostic groups as these were assumed to be different based on the sample collection strategy (Stanley Consortium samples are matched across diagnosis). This is also consistent with *a priori* hypotheses of the current study. Scatter plots are used to illustrate data of mixed diagnostic composition i.e. genotype data.

5-A Table of the anterior parahippocampal normalised relative quantity data from each diagnostic category. Asterisk indicates where the Levene's Test of Equality of Error Variances was violated (p < 05), double asterisk indicates, p < 05 when controlling for demographic and clinical variables.; bpd = bipolar disorder, mdd = major depressive disorder, con = controls, scz = schizophrenia

qPCR mRNA Transcript Data (anterior parahippocampal region)

Diagnosis			DCX	DPYSL2	FEZ1	NDEL1	NEUROD1	PDE4B
bpd	*N*	Original	15	15	15	15	15	15
		Received	15	15	15	15	15	15
		Pre-outlier	10	10	10	10	10	10
		No. of Outliers	2	2	3	1	0	0
	N	Valid	8	8	7	9	10	10
	Mean		0.002302	0.564427	0.388825	0.08214	0.129759	0.039532
	Median		0.00169	0.522815	0.467074	0.021545	0.102625	0.003148
	Std. Deviation		0.002537	0.478916	0.265368	0.119132	0.12337	0.060764
post-hoc to controls (Bonferroni) p			1	0.089	< .001	0.756	1	1
mdd	*N*	Original	15	15	15	15	15	15
		Received	13	13	13	13	13	13
		Pre-outlier	7	7	7	7	6	7
		No. of Outliers	2	0	1	1	0	1
	N	Valid	5	7	6	6	6	6
	Mean		0.002794	0.780422	1.266084	0.067273	0.244612	0.015869
	Median		0.00246	0.680171	1.423946	0.045725	0.153497	0.013599
	Std. Deviation		0.001537	0.495491	0.481025	0.061356	0.252577	0.015162
post-hoc to controls (Bonferroni) p			1	0.434	0.112	1	0.231	1
con	*N*	Original	15	15	15	15	15	15
		Received	13	13	13	13	13	13

qPCR mRNA Transcript Data (anterior parahippocampal region)

Diagnosis			DCX	DPYSL2	FEZ1	NDEL1	NEUROD1	PDE4B
		Pre-outlier	9	9	9	9	9	9
		No. of Outliers	2	0	0	2	1	0
	N	Valid	7	9	9	7	8	9
	Mean		0.00294	1.412732	2.33967	0.019646	0.080498	0.043987
	Median		0.002408	1.076593	2.482221	0.020171	0.043837	0.005378
	Std. Deviation		0.002021	0.969235	1.362233	0.009832	0.074491	0.060516
post-hoc to controls (Bonferroni) p			-	-	-	-	-	-
scz	*N*	Original	15	15	15	15	15	15
		Received	2	2	2	2	2	2
		Pre-outlier	9	9	9	9	9	9
		No. of Outliers	0	1	1	1	2	0
	N	Valid	9	8	8	8	7	9
	Mean		0.031855	0.760888	0.818269	0.081087	0.077237	0.016265
	Median		0.010494	0.571892	0.849782	0.10291	0.068748	0.015058
	Std. Deviation		0.034427	0.541494	0.315812	0.062216	0.073327	0.012586
post-hoc to controls (Bonferroni) p			0.042	0.334	0.004	0.854	1	1
Kruskal-Wallis	Pre-outlier	*H*	7.482	1.828	6.194	1.893	2.804	1.695
		p	0.058	0.609	0.103	0.595	0.423	0.638
	Valid	*H*	17.256	5.122	15.036	2.691	3.387	1.797
		p	0.001	0.163	0.002	0.442	0.336	0.616
ANOVA	Valid	*F*	4.616*	2.559*	8.789*	1.036*	2.01*	.856*
		p	0.011**	0.072	<.001**	0.393	0.136	0.474

5-B Table of the anterior parahippocampal normalised relative quantity data from each diagnostic category. Asterisk indicates where the Levene's Test of Equality of Error Variances was violated (p < 05), double asterisk indicates, p < 05 when controlling for demographic and clinical variables.; bpd = bipolar disorder, mdd = major depressive disorder, con = controls, scz = schizophrenia

qPCR mRNA Transcript Data (non-neurogenic hippocampal formation)

Diagnosis			DCX	DPYSL2	FEZ1	NDEL1	NEUROD1	PDE4B
bpd	*N*	Original	15	15	15	15	15	15

qPCR mRNA Transcript Data (non-neurogenic hippocampal formation)

Diagnosis			DCX	DPYSL2	FEZ1	NDEL1	NEUROD1	PDE4B
		Received	15	15	15	15	15	15
		Pre-outlier	4	4	4	4	4	4
		No. of Outliers	1	0	0	0	1	1
	N	Valid	3	4	4	4	3	3
	Mean		0.000252	0.433572	2.146806	0.013207	0.060532	0.000383
	Median		0.000226	0.384002	1.969353	0.012563	0.076985	0.000348
	Std. Deviation		0.00012	0.355288	1.919553	0.010717	0.045039	0.000103
post-hoc to controls (Bonferroni) p			0.048	1	0.436	1	1	0.504
mdd	*N*	Original	15	15	15	15	15	15
		Received	10	10	10	10	10	10
		Pre-outlier	4	4	4	4	3	4
		No. of Outliers	0	0	0	0	0	0
	N	Valid	4	4	4	4	3	4
	Mean		0.001749	0.195381	0.102841	0.008231	0.120305	0.000491
	Median		0.001337	0.119725	0.085903	0.009201	0.053324	0.000444
	Std. Deviation		0.001757	0.192848	0.048234	0.005777	0.150405	0.000373
post-hoc to controls (Bonferroni) p			0.211	1	1	1	1	0.41
con	*N*	Original	15	15	15	15	15	15
		Received	13	13	13	13	13	13
		Pre-outlier	5	5	5	5	5	5
		No. of Outliers	0	0	1	0	0	0
	N	Valid	5	5	4	5	5	5
	Mean		0.004957	0.412305	0.575156	0.007222	0.044911	0.005115
	Median		0.003904	0.475955	0.569149	0.007343	0.032727	0.003411
	Std. Deviation		0.002927	0.253147	0.520381	0.002113	0.041384	0.00564
post-hoc to controls (Bonferroni) p			-	-	-	-	-	-
scz	*N*	Original	15	15	15	15	15	15
		Received	13	13	13	13	13	13
		Pre-outlier	4	4	4	4	4	4
		No. of Outliers	1	0	0	0	0	1
	N	Valid	3	4	4	4	4	3
	Mean		0.000362	0.68069	0.979931	0.010828	0.437323	0.00048
	Median		0.000284	0.467003	0.757242	0.009133	0.222644	0.000257

qPCR mRNA Transcript Data (non-neurogenic hippocampal formation)

Diagnosis			DCX	DPYSL2	FEZ1	NDEL1	NEUROD1	PDE4B
	Std. Deviation		0.002927	0.253147	0.520381	0.002113	0.041384	0.00564
post-hoc to controls (Bonferroni) p			0.054	1	1	1	0.518	0.539
Kruskal-Wallis	Pre-outlier	*H*	4.529	2.537	6.324	0.949	3.044	4.88
		p	0.21	0.469	0.097	0.814	0.385	0.181
	Valid	*H*	8.736	2.537	6.441	0.949	3.263	7.29*
		p	0.033	0.469	0.092	0.814	0.353	0.063
ANOVA	Valid	*F*	5.071*	0.832	2.395*	.522*	1.408*	2.073*
		p	0.019	0.5	0.119	0.675	0.292	0.162

5-C Table showing the results of the non-parametric testing of minor carrier allele status effects on mRNA transcript levels in the anterior parahippocampal region

qPCR Minor Allele Carrier Status Data (anterior parahippocampal region)

		DCX	DPYSL2	FEZ1	NDEL1	NEUROD1	PDE4B
DISC1 rs3738401 minor allele carriers	*N*	20	18	18	20	18	21
	Mean Rank	15.85	14.28	13.00	15.40	14.67	15.71
	Sum of Ranks	317.00	257.00	234.00	308.00	264.00	330.00
Non-carriers	*N*	9	14	12	10	13	13
	Mean Rank	13.11	19.36	19.25	15.70	17.85	20.38
	Sum of Ranks	118.00	271.00	231.00	157.00	232.00	265.00
Total	*N*	29	32	30	30	31	34
	Mann-Whitney U	73.000	86.000	63.000	98.000	93.000	99.000
	Wilcoxon W	118.000	257.000	234.000	308.000	264.000	330.000
	Z	-.801	-1.519	-1.905	-.088	-.961	-1.329
	p (2-tailed)	.423	.129	.057	.930	.337	.184
DISC1 rs6675281 minor allele carriers	*N*	7	7	7	8	8	9
	Mean Rank	16.29	16.86	17.57	17.25	14.25	18.33
	Sum of Ranks	114.00	118.00	123.00	138.00	114.00	165.00
Non-	*N*	22	25	23	22	23	25

qPCR Minor Allele Carrier Status Data (anterior parahippocampal region)

		DCX	DPYSL2	FEZ1	NDEL1	NEUROD1	PDE4B
carriers	Mean Rank	14.59	16.40	14.87	14.86	16.61	17.20
	Sum of Ranks	321.00	410.00	342.00	327.00	382.00	430.00
Total	*N*	29	32	30	30	31	34
	Mann-Whitney U	68.000	85.000	66.000	74.000	78.000	105.000
	Wilcoxon W	321.000	410.000	342.000	327.000	114.000	430.000
	Z	-.459	-.114	-.711	-.657	-.632	-.293
	p (2-tailed)	.646	.909	.477	.511	.527	.770
DISC1 rs821616 minor allele carriers	*N*	11	13	11	12	11	14
	Mean Rank	17.55	20.00	17.55	13.17	13.82	21.07
	Sum of Ranks	193.00	260.00	193.00	158.00	152.00	295.00
Non-carriers	*N*	18	19	19	18	20	20
	Mean Rank	13.44	14.11	14.32	17.06	17.20	15.00
	Sum of Ranks	242.00	268.00	272.00	307.00	344.00	300.00
Total	*N*	29	32	30	30	31	34
	Mann-Whitney U	71.000	78.000	82.000	80.000	86.000	90.000
	Wilcoxon W	242.000	268.000	272.000	158.000	152.000	300.000
	Z	-1.259	-1.746	-.968	-1.185	-.991	-1.750
	p (2-tailed)	.208	.081	.333	.236	.322	.080
DISC1 rs3737597 minor allele carriers	*N*	1	1	1	1	0[a]	1
	Mean Rank	22.00	28.00	19.00	3.00	0.00	26.00
	Sum of Ranks	22.00	28.00	19.00	3.00	0.00	26.00
Non-carriers	*N*	28	31	29	29	31	33
	Mean Rank	14.75	16.13	15.38	15.93	16.00	17.24
	Sum of Ranks	413.00	500.00	446.00	462.00	496.00	569.00
Total	*N*	29	32	30	30	31	34
	Mann-Whitney U	7.000	4.000	11.000	2.000		8.000
	Wilcoxon W	413.000	500.000	446.000	3.000		569.000

qPCR Minor Allele Carrier Status Data (anterior parahippocampal region)

		DCX	DPYSL2	FEZ1	NDEL1	NEUROD1	PDE4B
	Z	-.837	-1.246	-.404	-1.444		-.866
	p (2-tailed)	.403	.213	.686	.149		.386
BDNF rs6265 minor allele carriers	*N*	8	9	8	9	9	10
	Mean Rank	21.25	17.44	15.88	18.67	12.11	21.60
	Sum of Ranks	170.00	157.00	127.00	168.00	109.00	216.00
Non-carriers	*N*	21	23	22	21	22	24
	Mean Rank	12.62	16.13	15.36	14.14	17.59	15.79
	Sum of Ranks	265.00	371.00	338.00	297.00	387.00	379.00
Total	*N*	29	32	30	30	31	34
	Mann-Whitney U	34.000	95.000	85.000	66.000	64.000	79.000
	Wilcoxon W	265.000	371.000	338.000	297.000	109.000	379.000
	Z	-2.440	-.356	-.141	-1.290	-1.523	-1.550
	p (2-tailed)	.015	.722	.888	.197	.128	.121
RAPTOR rs2289759 minor allele carriers	*N*	16	16	16	16	15	17
	Mean Rank	14.88	17.69	18.00	13.63	17.73	16.94
	Sum of Ranks	238.00	283.00	288.00	218.00	266.00	288.00
Non-carriers	*N*	13	16	14	14	16	17
	Mean Rank	15.15	15.31	12.64	17.64	14.38	18.06
	Sum of Ranks	197.00	245.00	177.00	247.00	230.00	307.00
Total	*N*	29	32	30	30	31	34
	Mann-Whitney U	102.000	109.000	72.000	82.000	94.000	135.000
	Wilcoxon W	238.000	245.000	177.000	218.000	230.000	288.000
	Z	-.088	-.716	-1.663	-1.247	-1.028	-.327
	p (2-tailed)	.930	.474	.096	.212	.304	.744

5-D Table showing the results of the non-parametric testing of minor carrier allele status effects on mRNA transcript levels in the non-neurogenic hippocampal formation

qPCR Minor Allele Carrier Status Data (non-neurogenic hippocampal formation)

		DCX	DPYSL2	FEZ1	NDEL1	NEUROD1	PDE4B
DISC1 rs3738401 minor allele carriers	*N*	9	10	9	10	9	9
	Mean Rank	7.00	10.80	9.00	9.00	10.22	8.33
	Sum of Ranks	63.00	108.00	81.00	90.00	92.00	75.00
Non-carriers	*N*	6	7	7	7	6	6
	Mean Rank	9.50	6.43	7.86	9.00	4.67	7.50
	Sum of Ranks	57.00	45.00	55.00	63.00	28.00	45.00
Total	*N*	15	17	16	17	15	15
	Mann-Whitney U	18.000	17.000	27.000	35.000	7.000	24.000
	Wilcoxon W	63.000	45.000	55.000	63.000	28.000	45.000
	Z	-1.061	-1.757	-.476	0.000	-2.357	-.354
	p (2-tailed)	.289	.079	.634	1.000	.018	.724
DISC1 rs6675281 minor allele carriers	*N*	3	3	3	3	2	3
	Mean Rank	10.33	6.67	6.33	8.00	4.50	8.00
	Sum of Ranks	31.00	20.00	19.00	24.00	9.00	24.00
Non-carriers	*N*	12	14	13	14	13	12
	Mean Rank	7.42	9.50	9.00	9.21	8.54	8.00
	Sum of Ranks	89.00	133.00	117.00	129.00	111.00	96.00
Total	*N*	15	17	16	17	15	15
	Mann-Whitney U	11.000	14.000	13.000	18.000	6.000	18.000
	Wilcoxon W	89.000	20.000	19.000	24.000	9.000	96.000
	Z	-1.010	-.882	-.874	-.378	-1.189	0.000
	p (2-tailed)	.312	.378	.382	.705	.234	1.000
DISC1 rs821616 minor allele carriers	*N*	4	5	5	5	5	4
	Mean Rank	9.25	6.40	7.60	8.00	5.80	9.75
	Sum of Ranks	37.00	32.00	38.00	40.00	29.00	39.00

qPCR Minor Allele Carrier Status Data (non-neurogenic hippocampal formation)

		DCX	DPYSL2	FEZ1	NDEL1	NEUROD1	PDE4B
Non-carriers	*N*	11	12	11	12	10	11
	Mean Rank	7.55	10.08	8.91	9.42	9.10	7.36
	Sum of Ranks	83.00	121.00	98.00	113.00	91.00	81.00
Total	*N*	15	17	16	17	15	15
	Mann-Whitney U	17.000	17.000	23.000	25.000	14.000	15.000
	Wilcoxon W	83.000	32.000	38.000	40.000	29.000	81.000
	Z	-.653	-1.370	-.510	-.527	-1.347	-.914
	p (2-tailed)	.514	.171	.610	.598	.178	.361
DISC1 rs3737597 minor allele carriers	*N*	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]
	Mean Rank	0.00	0.00	0.00	0.00	0.00	0.00
	Sum of Ranks	0.00	0.00	0.00	0.00	0.00	0.00
Non-carriers	*N*	15	17	16	17	15	15
	Mean Rank	8.00	9.00	8.50	9.00	8.00	8.00
	Sum of Ranks	120.00	153.00	136.00	153.00	120.00	120.00
Total	*N*	15	17	16	17	15	15
	Mann-Whitney U						
	Wilcoxon W						
	Z						
	p (2-tailed)						
BDNF rs6265 minor allele carriers	*N*	5	6	6	6	6	5
	Mean Rank	7.60	5.83	7.67	8.83	6.17	6.80
	Sum of Ranks	38.00	35.00	46.00	53.00	37.00	34.00
Non-carriers	*N*	10	11	10	11	9	10
	Mean Rank	8.20	10.73	9.00	9.09	9.22	8.60
	Sum of Ranks	82.00	118.00	90.00	100.00	83.00	86.00
Total	*N*	15	17	16	17	15	15
	Mann-Whitney U	23.000	14.000	25.000	32.000	16.000	19.000

qPCR Minor Allele Carrier Status Data (non-neurogenic hippocampal formation)

		DCX	DPYSL2	FEZ1	NDEL1	NEUROD1	PDE4B
	Wilcoxon W	38.000	35.000	46.000	53.000	37.000	34.000
	Z	-.245	-1.910	-.542	-.101	-1.296	-.735
	p (2-tailed)	.806	.056	.588	.920	.195	.462
RAPTOR rs2289759 minor allele carriers	*N*	11	11	10	11	10	11
	Mean Rank	9.64	7.73	6.70	8.36	6.90	8.64
	Sum of Ranks	106.00	85.00	67.00	92.00	69.00	95.00
Non-carriers	*N*	4	6	6	6	5	4
	Mean Rank	3.50	11.33	11.50	10.17	10.20	6.25
	Sum of Ranks	14.00	68.00	69.00	61.00	51.00	25.00
Total	*N*	15	17	16	17	15	15
	Mann-Whitney U	4.000	19.000	12.000	26.000	14.000	15.000
	Wilcoxon W	14.000	85.000	67.000	92.000	69.000	25.000
	Z	-2.350	-1.407	-1.952	-.704	-1.347	-.914
	p (2-tailed)	.019	.159	.051	.482	.178	.361

5.1 DISC1 Binding Partners

5.1.1 *DPYSL2*

5.1.1.1 Confounding Factors

Demographic and clinical variables found to exhibit correlations with *DPYSL2* mRNA transcript levels in the anterior parahippocampal (APh) region, significant or otherwise, included duration of disease (r_s = -.35, p = .053), lifetime fluphenazine dosage (r_s = -.31, p = .087) and qPCR analysis plate (r_s = -.33, p = .066). The negative correlation of *DPYSL2* mRNA transcript levels with disease duration, fluphenazine dosage and qPCR analysis plate were all experimental concerns and were therefore adopted as significant confounding factors for DCX mRNA transcript levels in this region.

When the dichotomous clinical and demographic variables were tested for an effect on *DPYSL2* mRNA transcript levels, subjects with an episode of psychosis in their medical history exhibited a trend towards lower APh mRNA abundance (*Mdn* = .57), when compared to subjects without an episode (*Mdn* = .98), *U* = 78.0, *p* = .062. Psychosis was therefore also adopted as a significant confounding factor for APh *DPYSL2* mRNA transcript levels. No correlations or other significant effects of demographic and clinical variables were detected in the non-neurogenic HF.

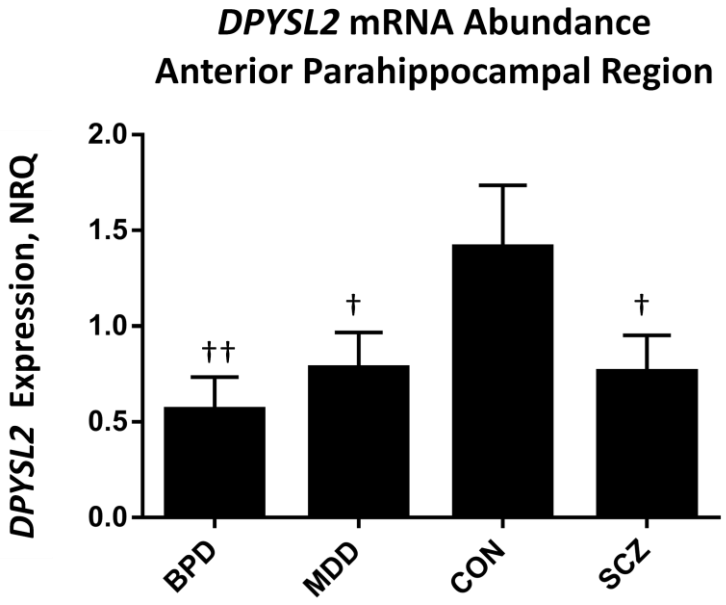
5.1.1.2 Effect of Diagnosis

Upon analysis of variance (ANOVA) testing between diagnostic groups, Levene's Test of Equality of Error Variances was found to be violated in the APh region, $F(3,28) = 6.21$, $p = .002$. Nevertheless, the results of the ANOVA were accepted with confidence. This was because the group exhibiting the largest standard deviation (*SD*) consisted of the largest sample (CON, *N* = 8, *SD* = .97); and smallest sample group exhibited one of the lowest *SD* values (MDD, *N* = 6, *SD* = .50). This pattern of divergent variances has the tendency of reducing the *F* value and thus making the test more conservative.

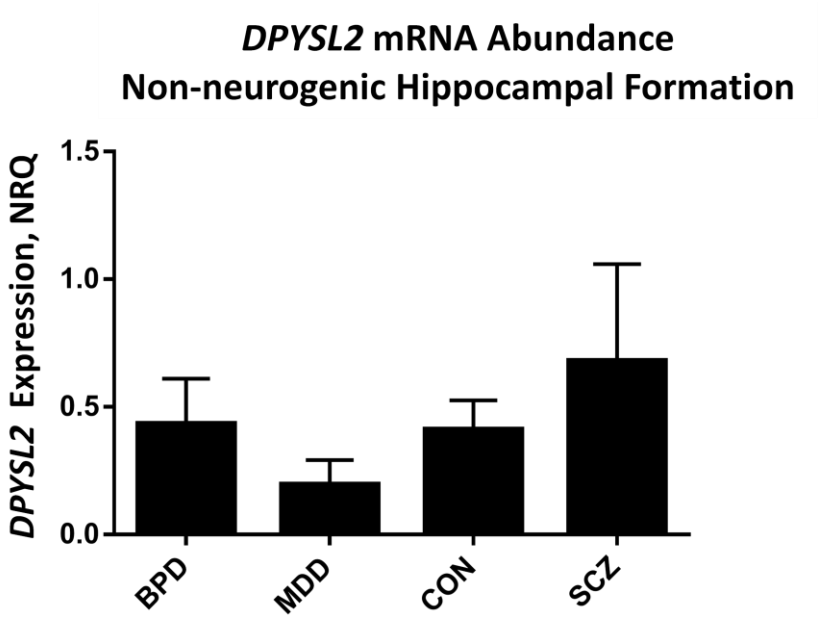
The ANOVA results suggested a trend towards a main effect of diagnosis, $F(3,28) = 2.60$, $p = .072$. Post-hoc analysis using Fisher's Least Significant Difference (*LSD*) revealed this to be a function of lower mean mRNA transcript levels in BPD (*M* = .56, *SD* = .48) when compared to CON (*M* = 1.41, *SD* = .97). However, these results did not survive Bonferroni correction. Trends towards a reduction in both MDD (*M* = .78, *SD* = .50) and SCZ (*M* = .76, *SD* = .54) were also detected when each were respectively compared with CON. However, these also did not survive Bonferroni correction (Fig 5-1). Kruskal-Wallis testing failed to detect a main effect of diagnosis.

Each of the adopted significant confounding factors were included as covariates in an ANCOVA analysis between the diagnostic groups. In all cases, except for qPCR analysis plate, when the identified confounding factor was controlled for, the trend towards a main effect of diagnosis was not evident. When controlling for the effect of qPCR analysis plate, the trend towards a main

effect was detected but slightly adjusted, $F(3,27) = 2.33$, $p = .097$. No main effect was diagnosis was observed when mRNA transcript levels in the non-neurogenic HF were analysed under either ANOVA or Kruskal-Wallis testing conditions (Fig. 5-2).



5-1 Bar graph showing *DPYSL2* mRNA transcript levels across diagnosis in the anterior parahippocampal region. † denotes LSD $p < .1$; †† denotes LSD $p < .05$. BPD = Bipolar Disorder ($N = 8$), MDD = Major Depressive Disorder ($N = 7$), SCZ = Schizophrenia ($N = 8$), CON = controls ($N = 9$).



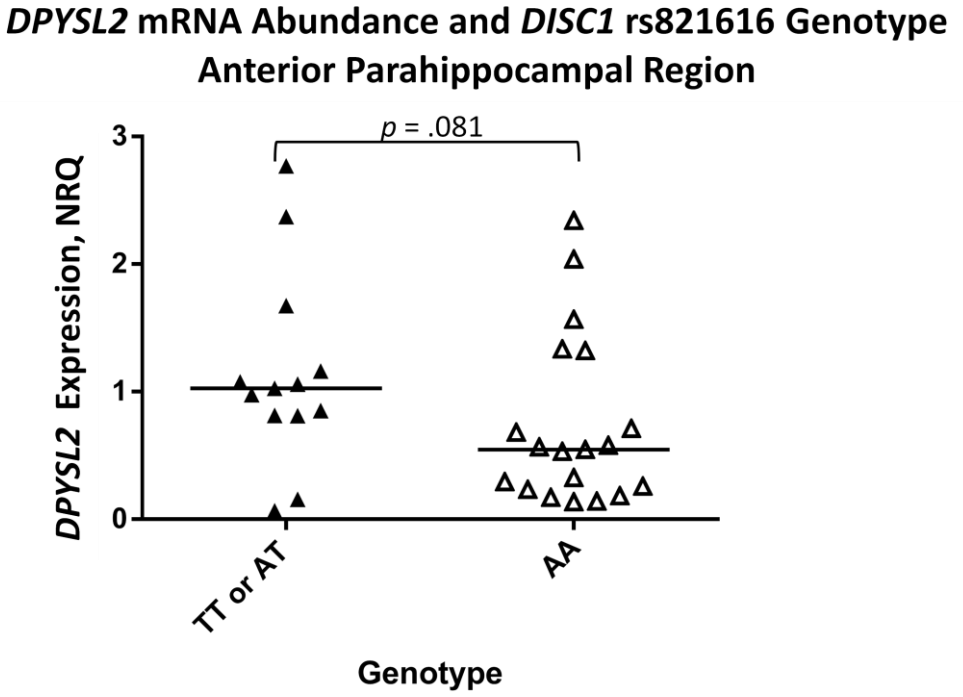
5-2 Bar graph showing *DPYSL2* mRNA transcript levels across diagnosis in the non-neurogenic hippocampal formation. BPD = Bipolar Disorder (*N* = 4), MDD = Major Depressive Disorder (*N* = 4), SCZ = Schizophrenia (*N* = 4), CON = controls (*N* = 5).

5.1.1.3 Genotype Effects

Mann-Whitney *U* tests of each minor allele, using the dominant effect model, suggested marginal effects of *DISC1* rs821616 in the APh region. Minor allele carriers were found to exhibit a trend towards higher mRNA transcript levels in the APh region (*Mdn* = 1.03) when compared to non-carriers (*Mdn* = .55), *U* = 78.0, *p* = .081 (Fig 5-3). As no effect of diagnosis was detected in this region, the rs821616 minor allele was re-analysed under parametric conditions. Upon *t*-testing, no significant difference in *DPYSL2* mRNA transcript levels was detected between *DISC1* rs821616 minor allele carriers (*M* = 1.14, *SD* = .76, *N* = 13) and non-carriers (*M* = .73, *SD* = .67, *N* = 29), *t*(30) = 1.595, *p* = .121.

The results of a 4×2 ANOVA with diagnosis and *DISC1* rs821616 minor allele carrier status as between-subjects factors could not be confidently reported as the Levene's Test for Equality of Error Variances was violated, *F*(7,24) = 3.30, *p* = .013. Notwithstanding this, no significant interaction between the 2 between-subjects factors was detected. Therefore, the results of *t* testing

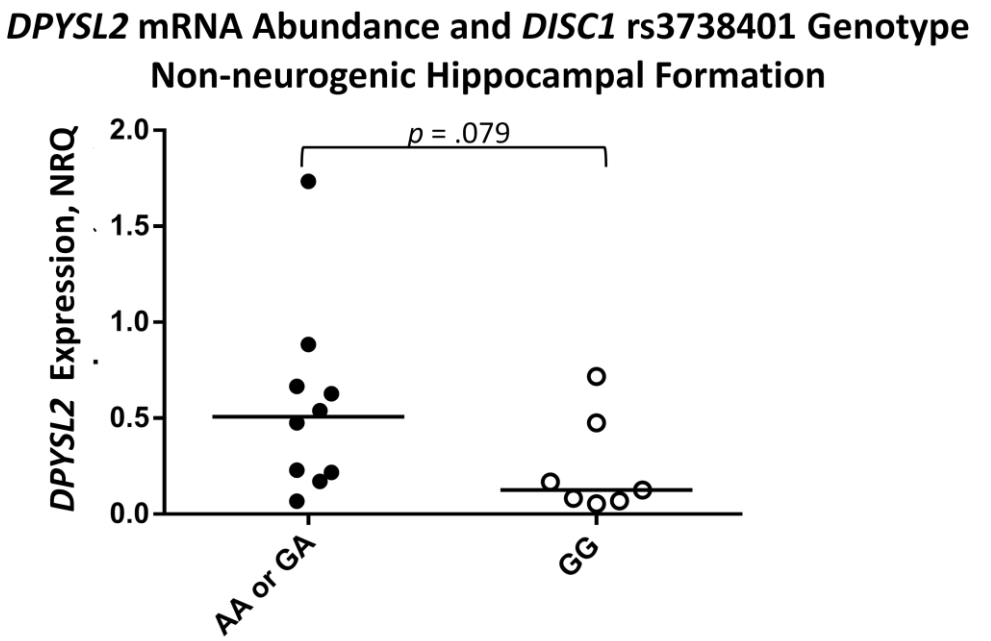
was adopted as representing the effect of the *DISC1* rs821616 minor allele in the APh region. No other significant effects of minor alleles were detected in this region.



5-3 Scatter plot showing the *DPYSL2* mRNA transcript levels of rs821616 minor allele carriers in the anterior parahippocampal region

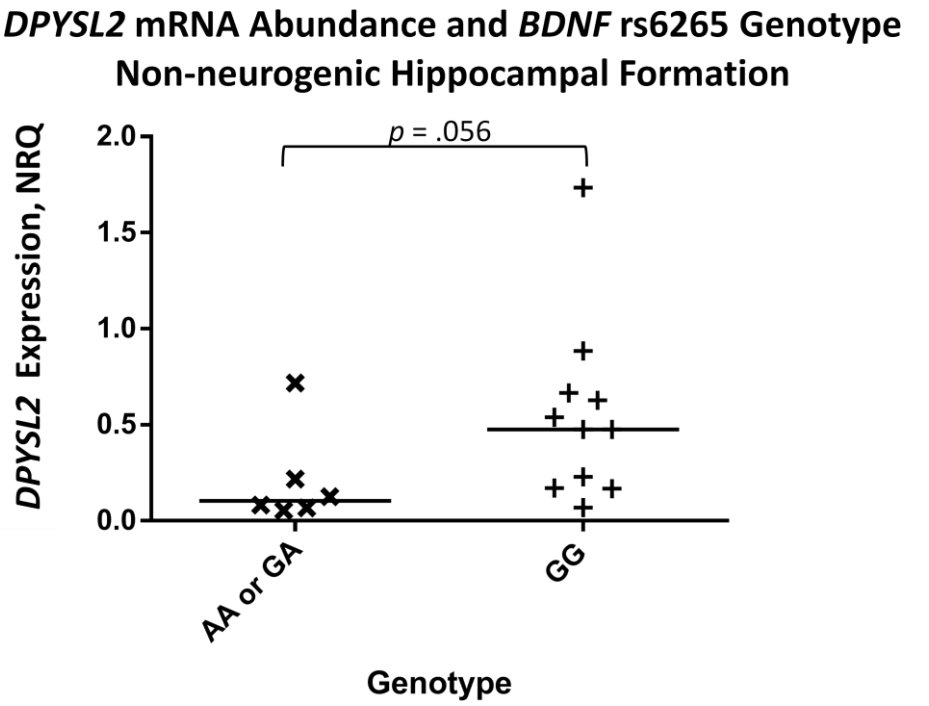
In the non-neurogenic HF, a trend towards an effect of *DISC1* SNP rs3738401 and *BDNF* SNP rs6265 minor allele carrier status was detected. For rs3738401, minor allele carriers were found to exhibit marginally higher mRNA transcript levels (*Mdn* = .51) when compared to non-carriers (*Mdn* = .13), *U* = 17.0, *p* = .079 (Fig 5-4). As no effect of diagnosis was detected in this region, the rs3738401 minor allele was re-analysed under parametric conditions. Upon *t*-testing, no significant difference in *DPYSL2* mRNA transcript levels was detected between *DISC1* rs3738401 minor allele carriers (*M* = .56, *SD* = .49, *N* = 10) and non-carriers (*M* = .24, *SD* = .25, *N* = 7), *t*(15) = 1.58, *p* = .135. In order to protect against any artefacts of multiple comparisons, a 4×2 ANOVA analysis was attempted, However due to inadequate sample size in the non-neurogenic HF, the results of the 4×2 ANOVA, with diagnosis and *DISC1*

rs3738401 as between-subjects factors could not be reported; the principal results of the nonparametric tests were therefore adopted.



5-4 Scatter plot showing the *DPYSL2* mRNA transcript levels of rs3738401 minor allele carriers in the non-neurogenic hippocampal formation

For rs6265, minor allele carriers were found to exhibit marginally lower mRNA transcript levels in the non-neurogenic HF (*Mdn* = .10) when compare to non-carriers (*Mdn* = .48), *U* = 14.0, *p* = .056 (Fig **5-5**). Upon *t*-testing, no significant difference in *DPYSL2* mRNA transcript levels was detected between *BDNF* rs6265 minor allele carriers (*M* = .21, *SD* = .26, *N* = 6) and non-carriers (*M* = .55, *SD* = .47, *N* = 11), *t*(15) = -1.64, *p* = .123. In order to protect against any artefacts of multiple comparisons, a 4×2 ANOVA analysis was attempted, However due to inadequate sample size in the non-neurogenic HF, the results of the 4×2 ANOVA, with diagnosis and *BDNF* rs6265 as between-subjects factors could not be reported; the principal results of the nonparametric tests were therefore adopted. Mann-Whitney *U* testing detected no other significant effects of minor alleles in this region



5-5 Scatter plot showing the *DPYSL2* mRNA transcript levels of BDNF rs6265 minor allele carriers in the non-neurogenic hippocampal formation

5.1.2 *FEZ1*

5.1.2.1 Confounding Factors

Demographic and clinical variables found to exhibit correlations with *FEZ1* mRNA transcript levels in the APh region included duration of disease (r_s = -.56, p = .001), days of storage (r_s = -.44, p = .014), lifetime fluphenazine dosage (r_s = -.60, p < .001) and age of disease onset (r_s = -.41, p = .025). The negative correlations of *FEZ1* mRNA transcript levels with disease duration, storage days, fluphenazine dosage and disease onset were all significant experimental concerns. Therefore, these were all adopted as significant confounding factors for *FEZ1* mRNA transcript levels in this region.

When dichotomous variables were tested for an effect on *FEZ1* mRNA transcript levels in the APh region, psychosis and brain hemisphere were found to exert a significant effect. Subjects with an episode of psychosis in their medical history exhibited significantly lower APh mRNA abundance (*Mdn* = .54), when compared to subjects without an episode (*Mdn* = 1.63), U = 30.0, p =

.001. Additionally, left hemisphere *FEZ1* mRNA abundance (*Mdn* = 1.42) was found to be significantly lower than right hemisphere *FEZ1* mRNA abundance (*Mdn* = .54), *U* = 46.0, *p* = .012. Each were therefore adopted as significant confounding factors for *FEZ1* APh mRNA transcript levels.

In the non-neurogenic HF, no demographic or clinical variables were found to exhibit correlations with *FEZ1* mRNA transcript levels. When the dichotomous clinical and demographic variables were tested for an effect on *FEZ1* mRNA transcript levels in this region, gender and psychosis were found to exert modest effects. Males were found to exhibit a trend towards higher *FEZ1* mRNA transcript levels (*Mdn* = 1.18) than females (*Mdn* = .19), *U* = 15.0, *p* = .074. For psychosis in the non-neurogenic HF, subjects with an episode of psychosis in their medical history exhibited a trend towards higher mRNA transcript levels (*Mdn* = 1.29), when compared to subjects without an episode (*Mdn* = .16), *U* = 15, *p* = .081.

5.1.2.2 Effect of Diagnosis

When homogeneity of variance was tested across diagnostic groups for *FEZ1* mRNA transcript levels in the APh region, The Levene's Test of Equality of Error Variances result was found to be significant, *F*(3,26) = 7.50, *p* = .001. Nevertheless, the analysis of *FEZ1* mRNA abundance was conducted under parametric ANOVA conditions and accepted. This was due to the variance heterogeneity being ascribed to the largest group (CON, *N* = 9, *SD* = 1.362) having a larger standard deviation when compared to the remaining diagnostic groups (BPD, *N* = 7, *SD* = .265; MDD, *N* = 6, *SD* = .481; SCZ, *N* = 8, *SD* = .316). This tends to make the resultant *F* value smaller, and thus the ANOVA test more conservative.

Upon ANOVA testing between diagnostic groups, a main effect of diagnosis on *FEZ1* mRNA transcript levels in the APh region was detected, *F*(3,26) = 8.79, *p* < .001 (Fig 5-6). Post hoc analyses using Bonferroni criterion for significance indicated that average *FEZ1* mRNA abundance was significantly lower in BPD (*M* = .39, *SD* = .27. *p* = .004) and SCZ (*M* = .82, *SD* = .32, *p* < .001) when compared to CON (*M* = 2.34, *SD* = 1.36). This represented

an 83% reduction in BPD *FEZ1* mRNA transcript levels and a 65% reduction in SCZ *FEZ1* mRNA transcript levels when each was compared to CON subjects in the APh region. Kruskal-Wallis testing also detected a main effect of diagnosis, $H(3) = 15.036$, $p = .002$.

Each of the continuous variables adopted as significant confounding factors were included as covariates in an ANCOVA retesting between diagnostic groups. When controlling for the effect of fluphenazine dosage, a significant main effect of diagnosis was still detected, $F(3,25) = 6.50$, $p = .002$. Pair-wise comparisons, using Bonferroni-adjusted alpha levels, indicated *FEZ1* mRNA transcript levels to significantly reduced in BPD ($p = .001$) and SCZ ($p = .036$) when compared to CON subjects. When the effects of storage days were controlled for, a main effect of diagnosis on the *FEZ1* mRNA abundance was also still observed, $F(3,25) = 5.89$, $p = .003$. Furthermore, the significant reduction in both BPD and SCZ, when compared to CON, sustained ($p = .003$ and $p = .030$ respectively).

After controlling for the effects of disease duration, though the significant main effect was detected, $F(3,25) = 3.94$, $p = .020$, not all of the pair wise comparisons sustained upon Bonferroni correction. A significant reduction in BPD mRNA transcript levels when compared to CON was still evident ($p = .013$); however the reduction in SCZ mRNA transcript levels ceased to be significant. Also, when the effects of disease onset were controlled for, though the main effect of diagnosis was still evident, $F(3,25) = 3.50$, $p = .030$, the pair wise comparisons did not survive Bonferroni correction.

This was interrogated further by grouping SCZ and BPD diagnosis categories together by their clinical history of psychosis. The effect of psychosis was then analysed with disease onset as a covariate. Upon ANCOVA analysis, marginal variance heterogeneity was detected by Levene's Test, $F(1,28) = 3.98$, $p = .056$. However, a main effect of psychosis was observed, $F(1,27) = 9.44$, $p = .005$. Subjects with a history of psychosis ($M = .62$, $SD = .36$) exhibited significantly lower *FEZ1* mRNA transcript levels values when compared to subjects without ($M = 1.91$, $SD = 1.20$, $p < .001$) (Fig 5-8).

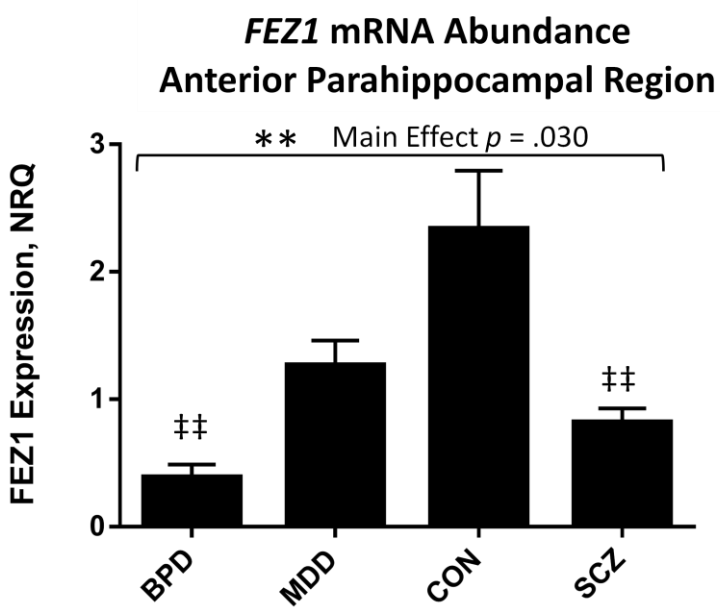
The dichotomous variables identified as significant confounding factors were included as covariates in ANCOVA models to retest *FEZ1* mRNA transcript levels in the APh region. When the effect of brain hemisphere was controlled for, main effect of diagnosis was still evident, $F(3,25) = 7.24$, $p = .001$. When compared to the APh region *FEZ1* mRNA transcript levels in CON, using Bonferroni correction for significance, the reduced mRNA transcript levels in SCZ sustained ($p = .004$), as did the reduced mRNA abundance in BPD ($p = .003$).

The effect of psychosis as a covariate in an ANCOVA test of diagnosis could not be fully analysed. This was due to psychosis being a discriminating factor between BPD and SCZ versus MDD and CON groups. It was established however, that the main effect of diagnosis sustained, $F(2,26) = 3.673$, $p = .039$. Pair-wise comparisons, using Bonferroni-adjusted alpha levels, did indicate lower *FEZ1* mRNA transcript levels in MDD when compared to CON ($p = .037$).

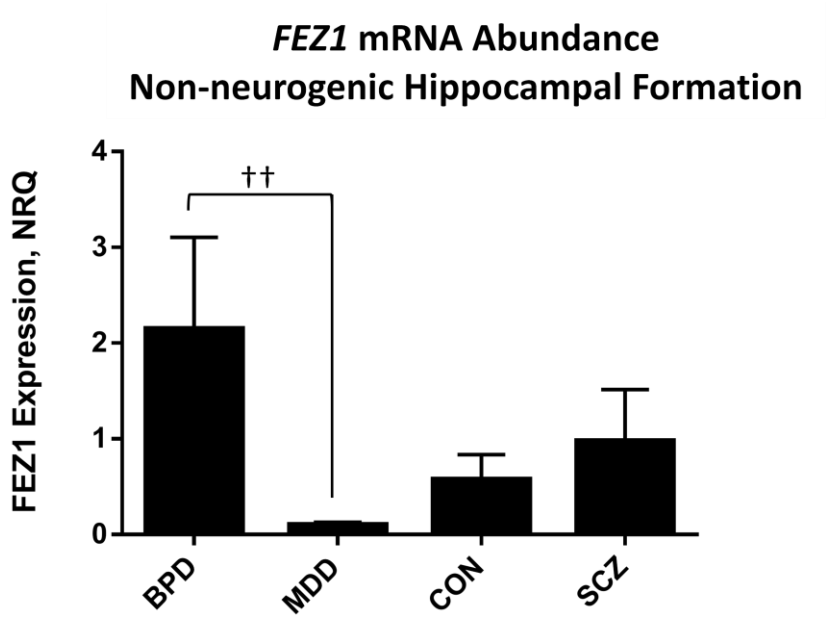
When *FEZ1* mRNA abundance was examined in the non-neurogenic HF, Levene's Homogeneity of Variance Test was not satisfied $F(3,12) = 16.086$, $p < .001$. Under nonparametric conditions, Levene's Test was satisfied and a trend towards a significant difference in *FEZ1* mRNA transcript levels between the diagnostic groups was detected, $H(3) = 6.44$, $p = .092$. When interrogated for pair wise differences using Mann-Whitney testing, this difference was ascribed to *FEZ1* mRNA transcript levels in MDD ($Mdn = .086$), exhibiting significantly lower values when compared to BPD ($Mdn = 1.97$, $U = 0.00$, $p = .021$) (Fig 5-7) and a trend towards lower values when compared to CON ($Mdn = .57$, $U = 2.00$, $p = .083$).

In an attempt to control for the effects of confounding factors on *FEZ1* mRNA transcript levels in the non-neurogenic HF, each of the adopted significant confounding factors in this region were included as covariates in ANCOVA retesting between the diagnostic groups. When controlling for the effects of psychosis, Levene's Testing of Equality of Error Variance detected the variance heterogeneity between diagnostic groups to be a trend, [$F(3,12) = 2.89$, $p = .080$)]. Notwithstanding this, no main effect of diagnosis was detected.

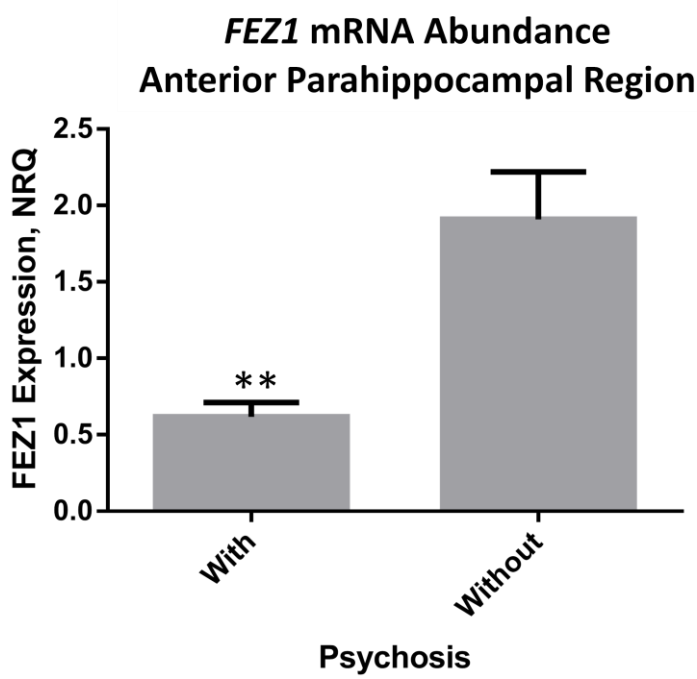
When the effects of gender were controlled for, The Levene's Test of Equality of Error Variances was satisfied and a main effect of diagnosis was detected, $F(3,8) = 11.404$, $p = .003$. *FEZ1* mRNA transcript levels were found to be marginally higher in BPD ($M = 2.14$, $SD = 1.91$) when compared to MDD ($M = .10$, $SD = .05$, $p = .054$). Furthermore, males were found to exhibit a significantly higher level of *FEZ1* mRNA abundance than females, $F(1,8) = 22.953$, $p = .001$., This was qualified by an interaction between gender and diagnosis, $F(3,8) = 8.73$, $p = .007$. Due to inadequate sample size the results of a 4×2 ANOVA with diagnosis and gender as between-subjects factors could not be reported.



5-6 Bar graph showing *FEZ1* mRNA transcript levels across diagnosis in the anterior parahippocampal region. ** denotes $p < .05$ (Bonferroni-adjusted) when controlling for the effects of confounding variables; ‡‡ denotes $p < .05$ (Bonferroni-adjusted). BPD = Bipolar Disorder ($N = 7$), MDD = Major Depressive Disorder ($N = 6$), SCZ = Schizophrenia ($N = 8$), CON = controls ($N = 9$).



5-7 Bar graph showing *FEZ1* mRNA transcript levels across diagnosis in the non-neurogenic hippocampal formation. †† denotes Mann-Whitney *U* and post hoc ANOVA *LSD* $p < .05$. BPD = Bipolar Disorder (*N* = 4), MDD = Major Depressive Disorder (*N* = 4), SCZ = Schizophrenia (*N* = 4), CON = controls (*N* = 4).

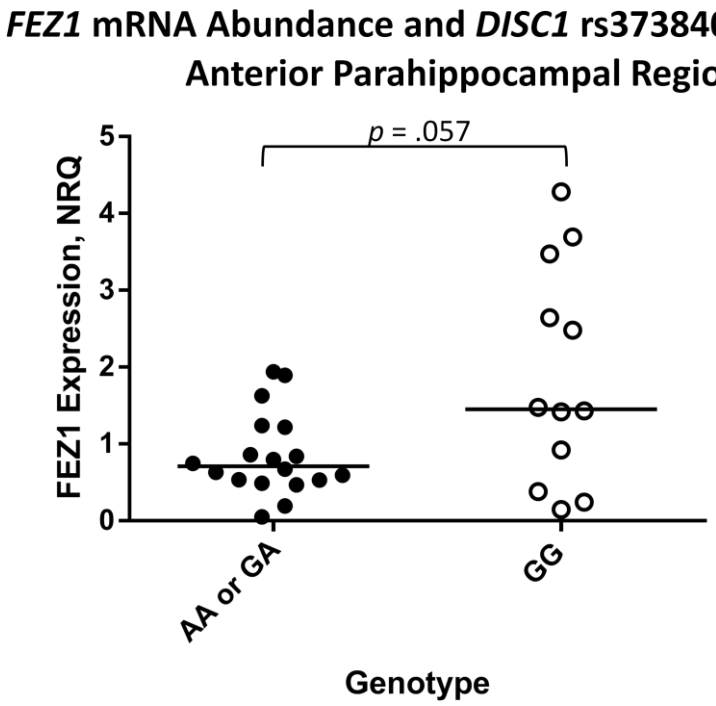


5-8 Bar graph showing *FEZ1* mRNA transcript levels in the anterior parahippocampal region and history of psychosis. ** denotes $p < .05$ (Bonferroni-adjusted) when controlling for the effects of disease onset. With psychosis ($N = 15$), without psychosis ($N = 15$).

5.1.2.3 Genotype Effects

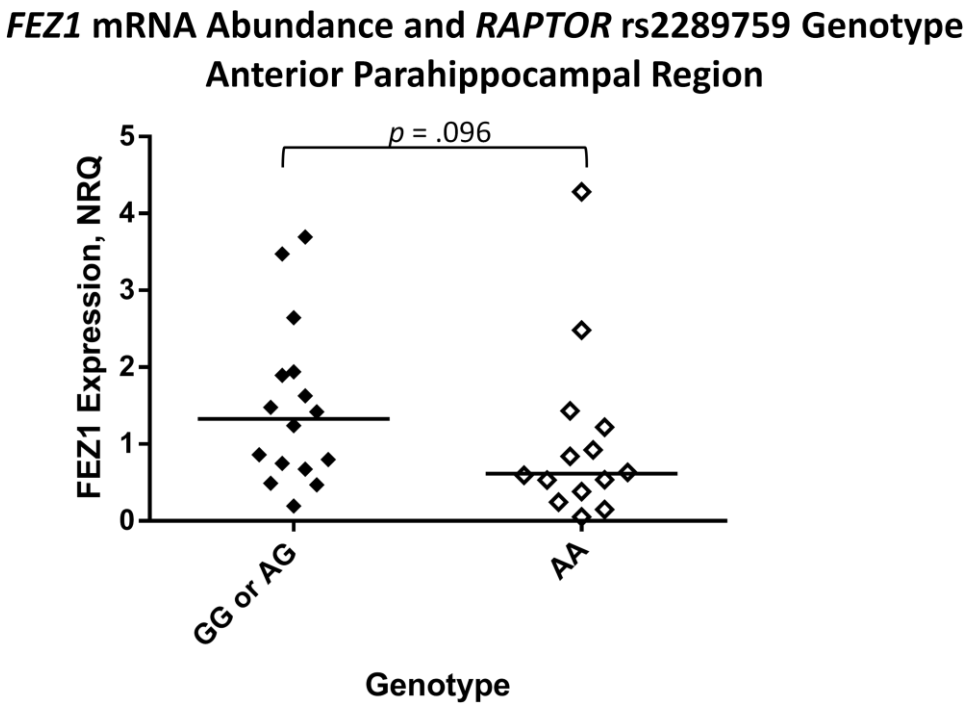
Mann-Whitney *U* testing of minor allele carrier status suggested marginal effects of *DISC1* SNP rs3738401 and *RAPTOR* SNP rs2289759 in the APh region. For rs3738401, minor allele carriers were found to exhibit a trend towards lower mRNA transcript levels (*Mdn* = .71) when compared to non-carriers (*Mdn* = 1.45), $U = 63.0$, $p = .057$ (Fig 5-9). For *RAPTOR* SNP rs2289759, *FEZ1* mRNA abundance was found to be marginally increased in minor allele carriers (*Mdn* = 1.33) when compared to non-carriers (*Mdn* = .61), $U = 72.0$, $p = .096$ (Fig **5**-**10**). Mann-Whitney *U* tests detected no other significant effects of minor alleles in this region.

As a significant effect of diagnosis was previously detected under ANOVA testing in this region, 4×2 ANOVA analyses were performed in order to protect against artefacts of multiple comparisons. In the APh region, the 4×2 ANOVA with diagnosis and *DISC1* rs3738401 minor allele carrier status as between-subjects factors detected the main effect of diagnosis, $F(3,22) = 5.93$, $p = .004$, but not minor allele carrier status, $F(1,22) = .87$, $p = .361$. This was not qualified by an interaction between diagnosis and rs3738401 minor allele carrier status, $F(3,22) = 1.65$, $p = .207$. Although Levene's Test for Equality of Error Variances detected a trend towards heterogeneity of variances between groups, $F(7,22) = 2.36$, $p = .059$, this analysis was adopted as representing the effect of the *DISC1* rs3738401 minor allele in the APh region.



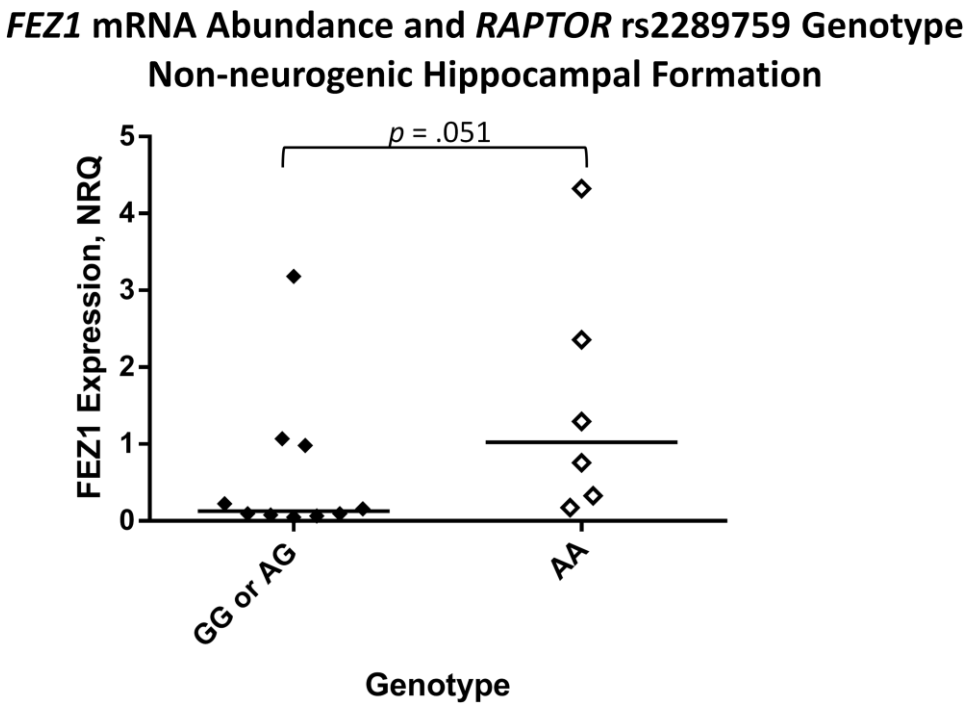
5-9 Scatter plot of *FEZ1* mRNA transcript levels in rs3738401 minor allele carriers in the anterior parahippocampal region

The results of the 4×2 ANOVA with diagnosis and *RAPTOR* rs2289759 minor allele carrier status as between-subjects factors could not be confidently reported as the Levene's test for homogeneity of variances was violated between groups., $F(7,24) = 3.30$, $p = .013$; the principal results of nonparametric testing were therefore adopted. Notwithstanding this, no significant interaction between the 2 between-subjects factors was detected in the APh region.



5-10 Scatter plot of *FEZ1* mRNA transcript levels in RAPTOR rs32289759 minor allele carriers in the anterior parahippocampal region

In the non-neurogenic HF, a trend towards an effect of *RAPTOR* SNP rs2289759 minor allele carrier status was detected. Minor allele carriers (*Mdn* = .13) were found to exhibit a trend towards lower mRNA transcript levels when compared to non-carriers (*Mdn* = 1.02), *U* = 12.0, *p* = .051 (Fig **5**-**11**). As no significant main effect of diagnosis was perceived in this region, the rs2289759 minor allele was re-analysed under parametric conditions. Upon *t*-testing, no significant difference in *FEZ1* mRNA transcript levels was detected between *RAPTOR* rs2289759 minor allele carriers (*M* = .60, *SD* = .99, *N* = 10) and non-carriers (*M* = .1.54, *SD* = .1.58, *N* = 6), *t*(14) = -1.479, *p* = 1.61. Mann-Whitney *U* tests detected no other significant effects of minor alleles in this region.



5-11 Scatter plot of *FEZ1* mRNA transcript levels in RAPTOR rs2289759 minor allele carriers in the non-neurogenic hippocampal formation

5.1.3 *NDEL1*

5.1.3.1 Confounding Factors

Demographic and clinical variables found to exhibit correlations with *NDEL1* mRNA transcript levels in the APh region, significant or otherwise, included RQI (r_s = -.51, p = .004), brain pH (r_s = -.37, p = .045), no. of storage days (r_s = .50, p = .005) and PMI (r_s = .345, p = .062). The negative correlation of *NDEL1* mRNA transcript levels with pH; and the positive correlation with PMI, were both experimental concerns and were thusly adopted as significant confounding factors for *NDEL1* mRNA transcript levels in this region. The positive correlation of *NDEL1* mRNA transcript levels with storage days; and the negative correlation with RQI, were both dismissed on the basis of theoretical invalidity. No effects of dichotomous variables on *NDEL1* mRNA transcript levels were detected in the APh region.

In the non-neurogenic HF, only age at death was found to exhibit a correlation with *NDEL1* mRNA transcript levels (r_s = -.43, p = .087). The

negative correlation of *NDEL1* mRNA abundance with age at death was an experimental concern, and thus adopted as a significant confounding factor for *NDEL1* mRNA transcript levels in the non-neurogenic HF. Of the dichotomous variables, only gender was found to exert a modest effect on *NDEL1* mRNA transcript levels. Males were found to exhibit marginally higher mRNA transcript levels (*Mdn* = .013) than their female counterparts (*Mdn* = .006), *U* =17.0, *p* = .068. The effect of gender was therefore also adopted as a significant confounding factor for *NDEL1* mRNA transcript levels in this region.

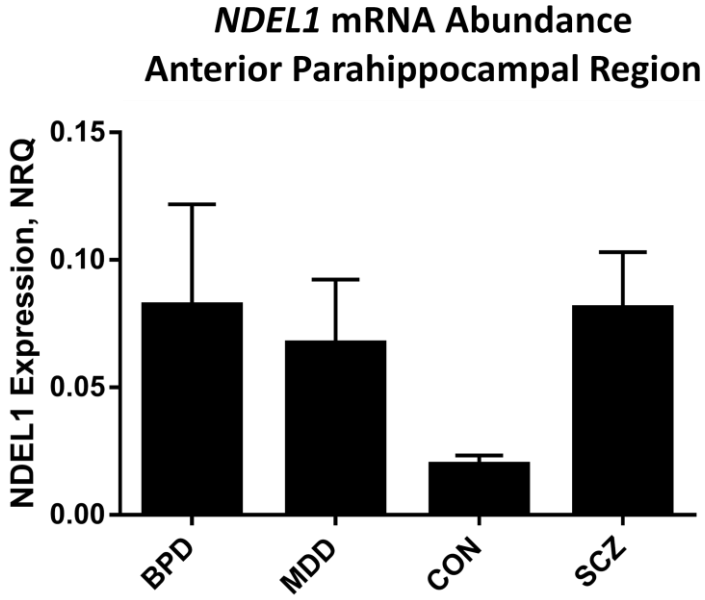
5.1.3.2 Effect of Diagnosis

When *NDEL1* mRNA abundance was examined in the APh region, Levene's Homogeneity of Variance Test was violated, $F(3,26) = 5.08$, $p = .007$. Under nonparametric conditions, The Levene's Test was satisfied. Kruskal-Wallis testing however, revealed no main effect of diagnosis. When attempting to control for the effects of each significant confounding factor on *NDEL1* mRNA transcript levels, the variance heterogeneity, observed in the parametric a priori hypotheses tests, sustained. This precluded confident reporting of ANCOVA tests in this region. Notwithstanding this, when controlling for the effects of the adopted significant confounding factors, no main effect of diagnosis was observed (Fig 5-12).

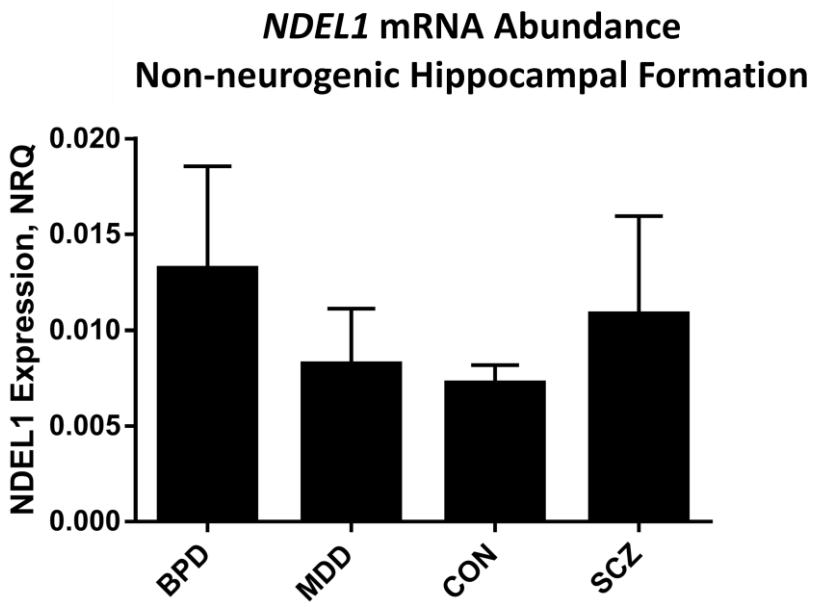
Upon ANOVA testing between diagnostic groups in the non-neurogenic HF, Levene's Test of Equality of Error Variances was found to be violated, $F(3,13) = 3.69$, $p = .040$. Under nonparametric conditions, The Levene's Test detected a trend towards variance heterogeneity, $F(3,13) = 2.98$, $p = .070$. Nevertheless, Kruskal-Wallis testing failed to detect a significant main effect of diagnosis.

In an attempt to control for the effects confounding factors on *NDEL1* mRNA transcript levels in the non-neurogenic HF, each of the adopted significant confounding factors in this region were included as covariates in separate ANCOVA retests of a main effect of diagnosis. When controlling for the effects of either age at death or gender, Levene's Testing of Equality of

Error Variance was satisfied but no main effect of diagnosis was detected (Fig 5-13).



5-12 Bar graph showing *NDEL11* mRNA transcript levels across diagnosis in the anterior parahippocampal region. BPD = Bipolar Disorder (*N* = 9), MDD = Major Depressive Disorder (*N* = 6), SCZ = Schizophrenia (*N* = 8), CON = controls (*N* = 7).



5-13 Bar graph showing N*DEL1* mRNA transcript levels across diagnosis in the non-neurogenic hippocampal formation. BPD = Bipolar Disorder (*N* = 4), MDD = Major Depressive Disorder (*N* = 4), SCZ = Schizophrenia (*N* = 4), CON = controls (*N* = 5).

5.1.3.3 Genotype Effects

Mann-Whitney *U* tests failed to detect significant effects of any minor alleles in either the non-neurogenic HF or APh region.

5.1.4 *PDE4B*

5.1.4.1 Confounding Factors

The only demographic or clinical variable found to exhibit a correlation, significant or otherwise, with APh *PDE4B* mRNA abundance was qPCR analysis plate (r_s = -.38, p = .027). The negative correlation of *PDE4B* mRNA transcript levels with qPCR plate no. was an experimental concern, and thus adopted as a significant confounding factor for *PDE4B* mRNA transcript levels in the APh region. Of the dichotomous variables, only brain hemisphere was found to exert an effect on *PDE4B* mRNA transcript levels in the APh region. Left hemisphere *PDE4B* mRNA abundance (*Mdn* = .011) was found to be significantly higher than right hemisphere *PDE4B* mRNA abundance (*Mdn* = .004), U = 86.0, p = .074. The effect of brain hemisphere was therefore also adopted as a significant confounding factor for *PDE4B* mRNA transcript levels in this region.

In the non-neurogenic HF, significant correlations were detected when *PDE4B* mRNA abundance was compared with age at onset of disease (r_s = -.65, p = .009), duration of disease (r_s = -58, p = .023); a trend was detected with and RQI (r_s = -.44, p = .099). The negative correlation of *PDE4B* mRNA transcript levels with RQI was dismissed on the basis of theoretical invalidity. The negative correlations with disease onset and disease duration on the other hand, were experimental concerns and thus adopted as experimental confounds.

When dichotomous variables were tested for an effect on *PDE4B* mRNA transcript levels in the non-neurogenic HF, subjects who died by suicide exhibited a trend towards lower mRNA transcript levels (*Mdn* = .0003), when compared to subjects who did not (*Mdn* = .0009), $U = 12.0$, $p = .077$. There was also an effect detected when brain hemisphere was tested, left hemisphere *PDE4B* mRNA transcript levels (*Mdn* = .001) was found to be significantly higher than right hemisphere *PDE4B* mRNA transcript levels (*Mdn* = .0003), $U = 5.00$, $p = .010$. The effects of brain hemisphere and death by suicide were therefore adopted as significant confounding factors for *PDE4B* mRNA transcript levels in this region.

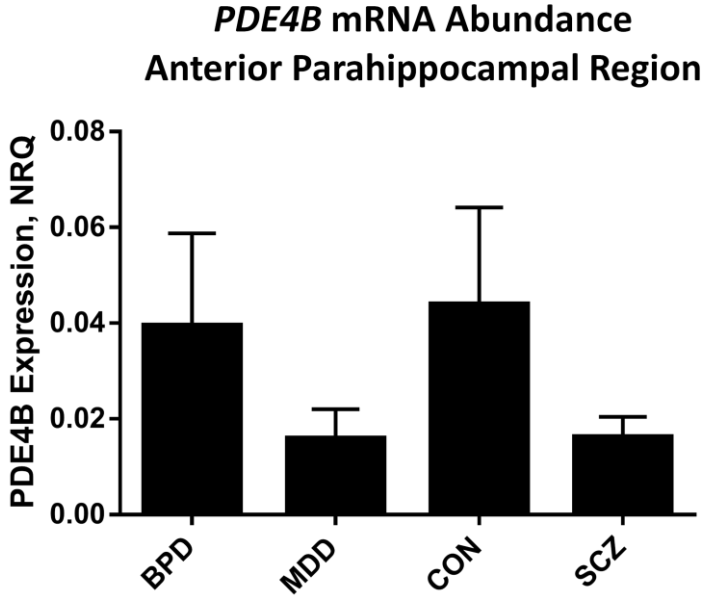
5.1.4.2 Effect of Diagnosis

When homogeneity of variance was tested across diagnostic groups for *PDE4B* mRNA transcript levels in the APh region, The Levene's Test of Equality of Error Variances result was found to be significant, $F(3,30) = 13.0$, $p < .001$. Under nonparametric conditions, the variance heterogeneity was detected as a trend, $F(3,30) = 2.75$, $p = .060$, but no significant effect of diagnosis upon Kruskal-Wallis testing was detected (Fig 5-14).

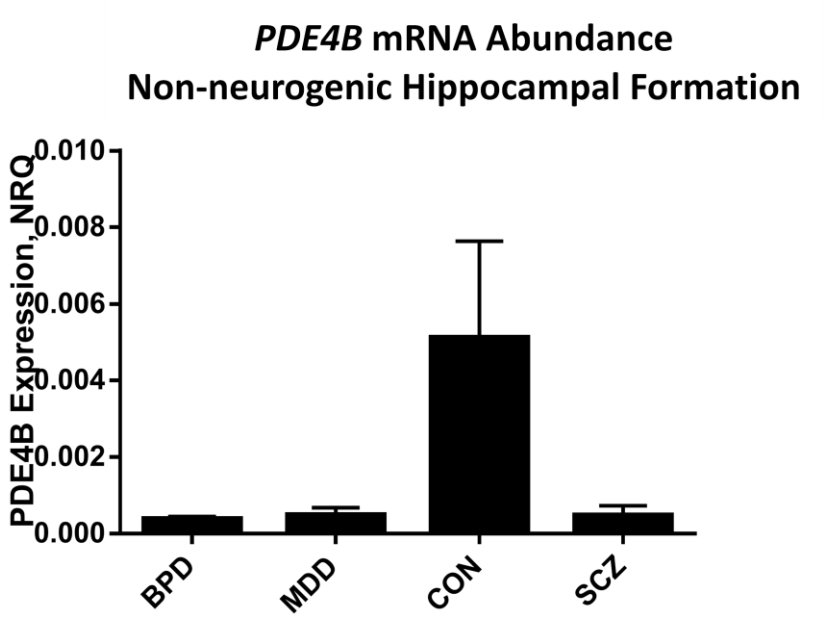
In the non-neurogenic HF, when *PDE4B* mRNA abundance was investigated, The Levene's Homogeneity of Variance Test was not satisfied $F(3,11) = 4.29$, $p = .031$. Kruskal-Wallis testing detected a trend towards an effect of diagnosis, $H(3) = 7.29$, $p = .063$, however the non-parametric equivalent of the Levene's Test was also violated, $F(3,11) = .4.90$, $p = .021$. Therefore, The Brown-Forsythe Test was used to test the effect of diagnosis on *PDE4B* mRNA transcript levels in the non-neurogenic HF. Under these conditions, no significant effect of diagnosis was observed (Fig 5-15).

In an attempt to control for the effects confounding factors on *PDE4B* mRNA transcript levels in the APh region, each of the adopted significant confounding factors in this region were included as covariates in separate ANCOVA retests of a main effect of diagnosis. When controlling for the effects of the adopted covariates, the significant variance heterogeneity observed in the parametric a priori hypotheses tests sustained. This same was observed when

attempting to control for the effects of identified confounding factors for *PDE4B* mRNA transcript levels in the non-neurogenic HF. This precluded confident reporting of ANCOVA test results.



5-14 Bar graph showing *PDE4B* mRNA transcript levels across diagnosis in the anterior parahippocampal region. BPD = Bipolar Disorder (*N* = 10), MDD = Major Depressive Disorder (*N* = 6), SCZ = Schizophrenia (*N* = 7), CON = controls (*N* = 9).



5-15 Bar graph showing *PDE4B* mRNA transcript levels across diagnosis in the non-neurogenic hippocampal formation. BPD = Bipolar Disorder (*N* = 3), MDD = Major Depressive Disorder (*N* = 4), SCZ = Schizophrenia (*N* = 3), CON = controls (*N* = 5).

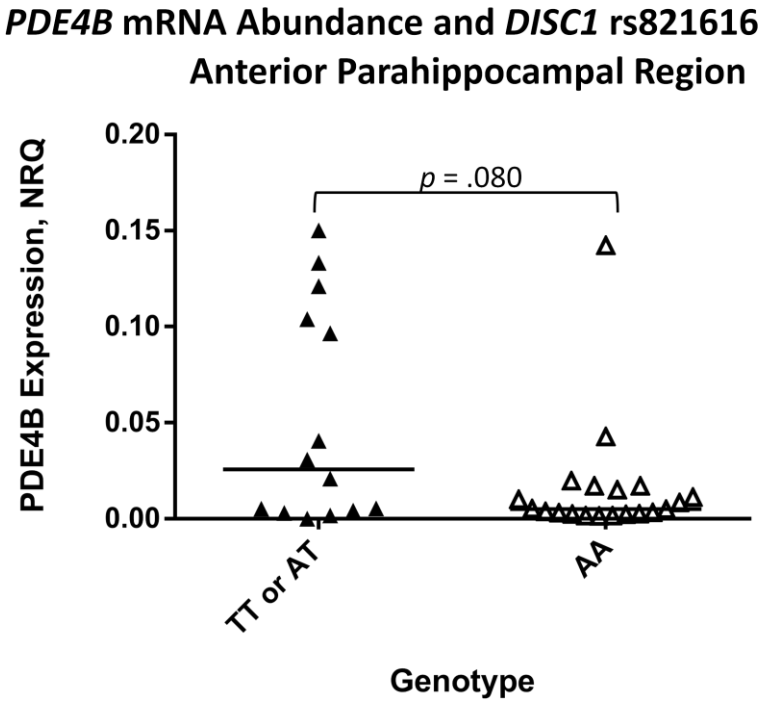
5.1.4.3 Genotype Effects

Mann-Whitney *U* tests of each minor allele, using the dominant effect model, suggested marginal effects of *DISC1* rs821616 in the APh region. Minor allele carriers were found to exhibit a trend towards higher mRNA transcript levels in the APh region (*Mdn* = .026) when compared to non-carriers (*Mdn* = .005), *U* = 90.0, *p* = .080 (Fig 5-16). As no effect of diagnosis was detected in this region, the rs821616 minor allele was re-analysed under parametric conditions. Upon *t*-testing, *PDE4B* mRNA transcript levels of *DISC1* rs821616 minor allele carriers (*M* = .051, *SD* = .056, *N* = 14) were found to be significantly higher than non-carriers (*M* = .016, *SD* = .031, *N* = 20), *t*(19) = 2.4128, *p* = .047 (Fig 5-16); Levene's test indicated unequal variances (*F* = 14.902, *p* = .001), so degrees of freedom were adjusted from 32 to 19.

In order to protect against artefacts of multiple comparisons, a 4×2 ANOVA analysis was performed. However, results of the 4×2 ANOVA, with diagnosis and *DISC1* rs821616 minor allele carrier status as between-subjects

factors, could not be confidently reported as the Levene's Test for Equality of Error Variances was violated, $F(7,26) = 3.30$, $p = .004$; the principal results of nonparametric testing were therefore adopted. Notwithstanding this, no significant interaction between the 2 between-subjects factors was detected. Mann-Whitney *U* testing detected no other significant effects of minor alleles in this region.

In the non-neurogenic HF, no significant effects of the minor alleles were detected upon Mann-Whitney *U* testing.



5-16 Scatter plot showing the *PDE4B* mRNA transcript levels of rs821616 minor allele carriers in the anterior parahippocampal region

5.2 Neurogenesis-related Genes

5.2.1 *DCX*

5.2.1.1 Confounding Factors

The demographic and clinical variables found to exhibit significant correlations with *DCX* mRNA transcript levels in the APh region included PMI ($r_s = .47$, $p = .007$) and no. of storage days ($r_s = .36$, $p = .044$). A correlation trend

was detected between *DCX* mRNA transcript levels and lifetime fluphenazine dosage (r_s = -.36, p = .056). The positive correlations of *DCX* mRNA transcript levels with storage days and PMI were both dismissed on the basis of theoretical invalidity. The negative correlation of *DCX* mRNA transcript levels with fluphenazine dosage was however an experimental concern. Fluphenazine dosage was thusly adopted as a significant confounding factor for *DCX* mRNA transcript levels in the APh region.

Of the dichotomous variables, only psychosis was found to exert a modest effect on *DCX* mRNA transcript levels in the APh region. Subjects with an episode of psychosis in their medical history exhibited a trend towards lower APh mRNA transcript levels (*Mdn* = .007), when compared to subjects without an episode (*Mdn* = .002), U = 62.0, p = .077. Psychosis was therefore also adopted as a significant confounding factor in the analysis of APh *DCX* mRNA transcript levels.

In the non-neurogenic HF, the variables found to exhibit significant correlations with *DCX* mRNA transcript levels included qPCR analysis plate no. (r_s = .93, p < .001), age of onset (r_s = -.69, p = .005), duration of disease (r_s = .64, p = .011), lifetime fluphenazine dosage (r_s = -.60, p = .017) and no. of storage days (r_s = -.90, p < .001); a correlation trend with substance abuse severity (r_s = -.51, p = .052) was also detected. Each of these correlations were deemed to be experimental concerns. They were each therefore adopted as significant confounding factors for *DCX* mRNA transcript levels in the non-neurogenic HF.

When the dichotomous variables were tested for an effect on *DCX* mRNA transcript levels in this region, significant or otherwise, brain hemisphere and psychosis were found to exert effects. Left hemisphere *DCX* mRNA transcript levels (*Mdn* = .003) was found to be marginally higher than right hemisphere *DCX* mRNA transcript levels (*Mdn* = .0004), U = 11.0, p = .059. For psychosis, subjects with an episode of psychosis in their medical history exhibited significantly lower mRNA transcript levels in the non-neurogenic HF (*Mdn* = .0003), when compared to subjects without an episode (*Mdn* = .003), *U*

= 6.00, p = .013. Each of these variables were therefore adopted as significant confounding factors for *DCX* mRNA transcript levels in the non-neurogenic HF.

5.2.1.2 Effect of Diagnosis

When mRNA transcript levels were compared across diagnostic categories, a main effect of diagnosis on APh *DCX* mRNA abundance was detected, $F(3,25) = 4.616$, p = .011. Upon pair wise comparison, this was found to reflect higher *DCX* mean mRNA transcript levels in SCZ (M = .031, SD = .034) in comparison to other diagnostic categories. Mean mRNA transcript levels in was significantly higher in SCZ when compared to both CON (M = .003, SD = .002, p = .042) and BPD (M = .002, SD = .003, p = .028). When compared to MDD (M = .003, SD = .002), the reduced *DCX* mRNA transcript levels in comparison to SCZ was detected as a trend, p = .080. Kruskal-Wallis testing also detected a significant main effect of diagnosis, $H(3) = 17.256$, p = .001.

Before conducting ANOVA tests for *DCX* mRNA transcript levels in the APh region, Levene's Test of Equality of Error Variances was found to be violated, $F(3,25) = .20.30$, $p < .001$. However, this was attributed to the largest group (SCZ, N = 9) having a much larger standard deviation than BPD (N = 8) and SCZ (N = 7); with the smallest group, MDD (N = 5), having the smallest standard deviation. This has the effect of making the reported F value smaller, and thus the ANOVA test more conservative. Therefore, the results of the ANOVA tests on APh region *DCX* mRNA transcript levels were accepted.

Each of the demographic or clinical variables adopted as significant confounding factors were included as covariates in an ANCOVA retesting between diagnostic groups. When fluphenazine dosage was added as a covariate in an ANCOVA test, a trend towards an interaction between hemisphere and diagnosis was detected $F(1,23) = 2.98$, p = .098. Notwithstanding this, the main effect of diagnosis was significant, $F(3,23) = 10.25.$, $p < .001$. Pair-wise comparisons, using Bonferroni-adjusted alpha levels, indicated *DCX* mRNA transcript levels to be significantly reduced in CON (p =

.001), BPD (p = .002) and MDD (p = .002) when compared to SCZ subjects (Fig 5-17).

As psychosis was a discriminating factor between BPD and SCZ versus MDD and CON groups, the effect of psychosis as a covariate in an ANCOVA test of diagnosis could not be fully analysed. It was established however, that the main effect of diagnosis sustained, $F(2,25) = 4.84$, $p = .017$; pair wise comparisons, using Bonferroni-adjusted alpha levels, indicated lower *DCX* mRNA transcript levels in BPD when compared to SCZ (p = .037).

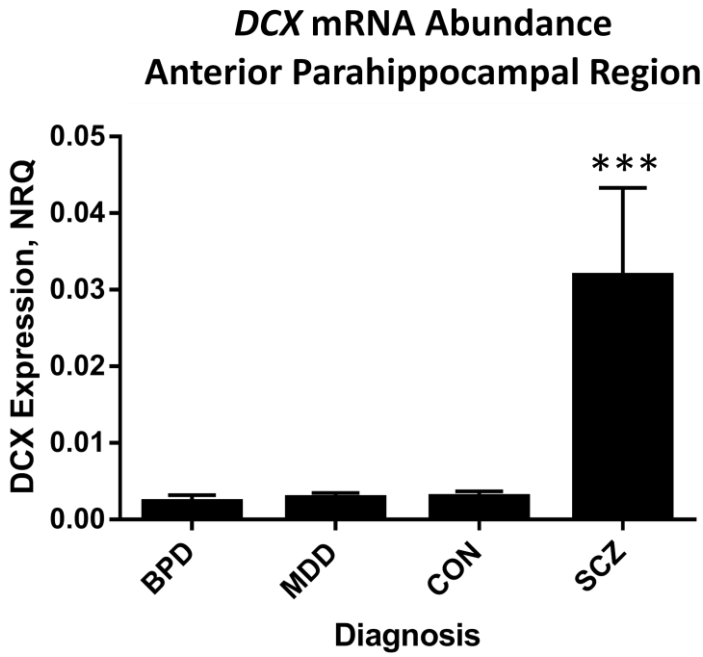
When *DCX* mRNA transcript levels in the non-neurogenic HF were measured, Levene's Test for Homogeneity of Variances was violated, $F(3,11) = 4.15$, $p = .034$. This variance heterogeneity was determined to be caused by the largest group (CON, $N = 5$, $M = .005$, $SD = .003$) having the largest SD and the smallest groups having the smallest SD (BPD, $N = 3$, $M = .0002$, $SD = .0001$; SCZ, $N = 3$, $M = .0003$, $SD = .0001$). Thusly, the main effect of diagnosis, detected upon ANOVA testing, was accepted, $F(3,11) = 5.07$, $p = .019$. Post-hoc analysis, using Bonferroni-adjusted alpha levels, revealed significantly lower *DCX* mRNA transcript levels in BPD (p = .048) and a trend towards lower mRNA transcript levels in SCZ (p = .054) when each were compared to CON (Fig 5-18). Kruskal-Wallis testing also detected a significant main effect of diagnosis, $H(3) = 8.736$, $p = .033$.

Each of the continuous variables adopted as significant confounding factors were included as covariates in an ANCOVA retesting between diagnostic groups. When controlling for the effect of disease onset age, qPCR plate analysis no., no. of storage days and duration of disease, the significant main effect of diagnosis was no longer significant. When the effects of alcohol abuse were controlled for, the main effect of diagnosis on the *DCX* mRNA abundance was also detected as a trend, $F(3,10) = 3.237$, $p = .069$, no significant pair-wise differences upon Bonferroni correction were detected.

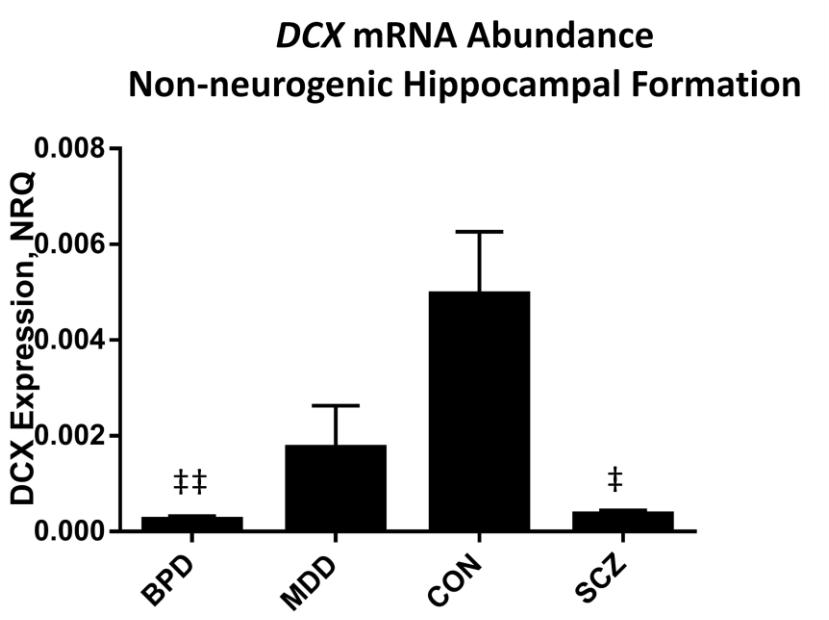
When the effects of fluphenazine dosage were controlled for, the main effect of diagnosis on the *DCX* mRNA abundance was detected as a trend, $F(3,10) = 3.529$, $p = .056$. Pair-wise comparisons, using Bonferroni adjusted

alpha levels, indicated marginally lower non-neurogenic HF *DCX* mRNA transcript levels in BPD when compared to CON (p = .080). The diagnosis-discriminating nature of psychosis notwithstanding, when controlling for its effect on *DCX* mRNA transcript levels, the main effect of diagnosis sustained, $F(2,11) = 2.89$, p = .098. The pair wise comparisons, using Bonferroni-adjusted alpha levels, were able to detect lower *DCX* mRNA transcript levels in MDD when compared to CON (p = .070).

When brain hemisphere was added as a covariate in an ANCOVA test, the interaction between hemisphere and diagnosis was found to be significant $F(2,8) = 12.89$, p = .003; as was the main effect of diagnosis, $F(2,8) = 19.99$, p = .001. Despite the detected interaction, the low sample size in the non-neurogenic HF precluded a 4×2 ANOVA with diagnosis and brain hemisphere.



5-17 Bar graph showing *DCX* mRNA transcript levels across diagnosis in the anterior parahippocampal region. *** denotes $p < .05$ (Bonferroni-adjusted) against all groups when controlling for the effects of confounding variables. BPD = Bipolar Disorder (N = 8), MDD = Major Depressive Disorder (N = 5), SCZ = Schizophrenia (N = 9), CON = controls (N = 7).

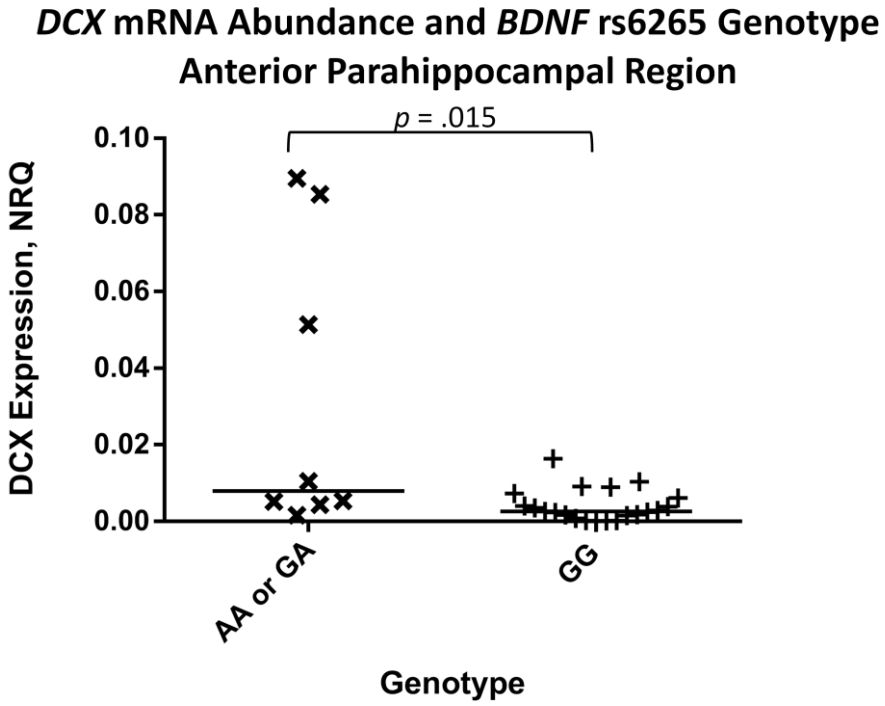


5-18 Bar graph showing *DCX* mRNA transcript levels across diagnosis in the non-neurogenic hippocampal formation. ‡ denotes $p < .1$ (Bonferroni-adjusted), ‡‡ denotes $p < .05$ (Bonferroni-adjusted). BPD = Bipolar Disorder ($N = 3$), MDD = Major Depressive Disorder ($N = 4$), SCZ = Schizophrenia ($N = 3$), CON = controls ($N = 5$).

5.2.1.3 Genotype Effects

In the APh region, Mann-Whitney *U* tests of minor allele carrier status in suggested a significant effect of *BDNF* SNP rs6265. Minor allele carriers were found to exhibit significantly higher mRNA transcript levels in the APh region (*Mdn* = .008) when compared to non-carriers (*Mdn* = .003), $U = 34.0$, $p = .015$ (Fig **5**-**19**). As a significant main effect of diagnosis was detected in this region, the rs6265 minor allele was re-analysed under parametric conditions. Upon *t*-testing, *DCX* mRNA transcript levels of *BDNF* rs6265 minor allele carriers ($M = .032$, $SD = .038$, $N = 8$) were found to be marginally higher than non-carriers ($M = .004$, $SD = .004$, $N = 21$), $t(7) = 2.05$, $p = .079$ (Fig **5**-**19**); Levene's test indicated unequal variances ($F = 84.1$, $p < .001$), so degrees of freedom were adjusted from 27 to 7.

In order to protect against artefacts of multiple comparisons, a 4×2 ANOVA analysis was performed. However, results of the 4×2 ANOVA, with diagnosis and *BDNF* rs6265 minor allele carrier status as between-subjects factors, could not be confidently reported as the Levene's Test for Equality of Error Variances was violated, $F(6,22) = 12.652$, $p < .001$; the principal results of nonparametric testing were therefore adopted. Notwithstanding this, no significant interaction between the 2 between-subjects factors was detected. Mann-Whitney *U* testing detected no other significant effects of minor alleles in this region.

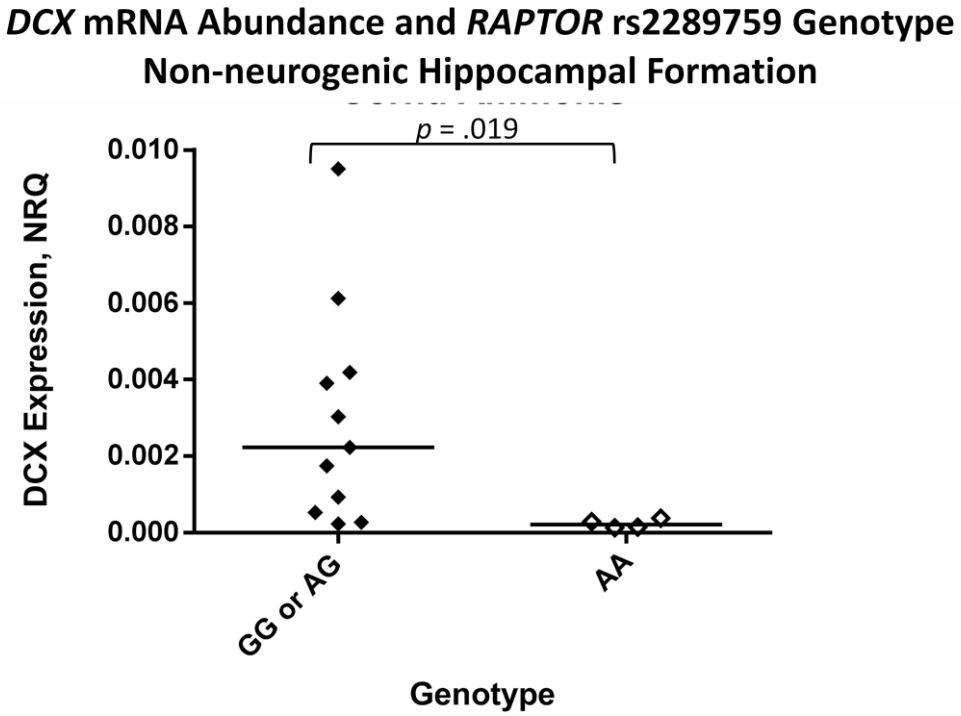


5-19 Scatter plot of *DCX* mRNA transcript levels in BDNF rs6265 minor allele carriers in the non-neurogenic hippocampal formation

In the non-neurogenic HF, Mann-Whitney *U* tests of minor allele carrier status suggested a significant effect of *RAPTOR* SNP rs2289759. The *DCX* mRNA transcript levels of minor allele carriers were found to be significantly increased in minor allele carriers (*Mdn* = .002) when compared to non-carriers (*Mdn* = .0002), $U = 4.00$, $p = .019$ (Fig **5**-**20**). As no significant main effect of diagnosis was perceived in this region, the rs2289759 minor allele was re-

analysed under parametric conditions. Upon *t*-testing, *DCX* mRNA transcript levels of *RAPTOR* rs2289759 minor allele carriers (*M* = .003, *SD* = .003, *N* = 11) were found to be significantly higher than non-carriers (*M* = .0002, *SD* = .0001, *N* = 4), *t*(10) = 3.162, *p* = 010. (Fig **5-20**); Levene's test indicated unequal variances (*F* = 5.332, *p* = .038), so degrees of freedom were adjusted from 13 to 10.

In order to protect against artefacts of multiple comparisons in the non-neurogenic HF, a 4×2 ANOVA analysis was attempted, However, due to inadequate sample size the results of the 4×2 ANOVA with diagnosis and *RAPTOR* SNP rs2289759 as between-subjects factors could not be reported; the principal results of the nonparametric tests were therefore adopted. Mann-Whitney *U* testing detected no other significant effects of minor alleles in this region.



5-20 Scatter plot of *DCX* mRNA transcript levels in RAPTOR rs2289759 minor allele carriers in the non-neurogenic hippocampal formation

5.2.2 *NEUROD1*

5.2.2.1 Confounding Factors

The demographic and clinical variables found to exhibit significant correlations with *NEUROD1* mRNA transcript levels in the APh region included brain pH (r_s = -.37, p = .041) and alcohol abuse severity (r_s = .38, p = .038). The negative correlation of *NEUROD1* mRNA transcript levels with pH and the positive correlation with alcohol abuse were both experimental concerns. They were each therefore adopted as significant confounding factors for *NEUROD1* mRNA transcript levels in the APh region.

When dichotomous variables were tested for an effect on *NEUROD1* mRNA transcript levels, left hemisphere exhibited a trend towards lower APh mRNA transcript levels (*Mdn* = .13), when compared to right hemisphere mRNA transcript levels (*Mdn* = .050), U = 66.0, p = .052. Brain hemisphere was therefore also adopted as a significant confounding factor in the analysis of APh *NEUROD1* mRNA transcript levels.

In the non-neurogenic HF, the continuous variables found to exhibit significant correlations with *NEUROD1* mRNA transcript levels included qPCR analysis plate no. (r_s = -.53, p = .041) and no. of storage days (r_s = .53, p = .043); a correlation trend was detected with lifetime fluphenazine dosage (r_s = -.44, p = .096). The negative correlations of *NEUROD1* mRNA transcript levels with qPCR plate no. and fluphenazine dosage were both experimental concerns. Each was therefore adopted significant confounding factors for *NEUROD1* mRNA transcript levels in this region. The positive correlation of *NEUROD1* mRNA transcript levels with storage days on the other hand, was dismissed on the basis of theoretical invalidity

Of the dichotomous variables, only psychosis was found to exert a modest effect on *NEUROD1* mRNA transcript levels in the non-neurogenic HF. Subjects with an episode of psychosis in their medical history exhibited a trend towards higher APh mRNA transcript levels (*Mdn* = .098), when compared to subjects without an episode (*Mdn* = .032), U = 11.0, p = .059. Psychosis was

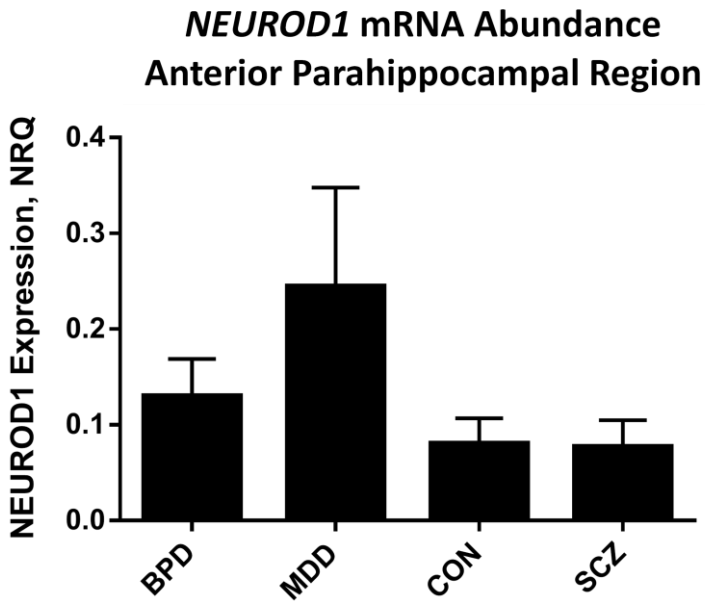
also adopted as a significant confounding factor in the analysis of *NEUROD1* mRNA transcript levels in the non-neurogenic HF.

5.2.2.2 Effect of Diagnosis

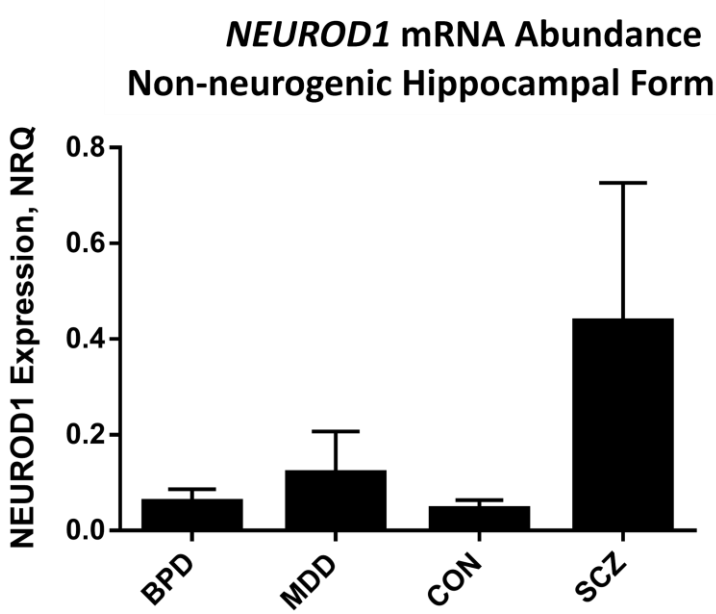
When *NEUROD1* mRNA abundance was examined in the APh region, The Levene's Test of Equality of Error Variances was violated, $F(3,27) = 3.83$, $p = .021$. When *NEUROD1* mRNA abundance was examined in the non-neurogenic HF, The Levene's Test was again violated, $F(3,11) = 4.98$, $p = .020$. Under nonparametric conditions, Levene's testing detected homogenous variances for both regions, but Kruskal-Wallis testing failed to detect any significant effect of diagnosis on *NEUROD1* mRNA transcript levels in either the non-neurogenic HF or APh region.

When attempting to control for the effects of the identified confounding factors for *NEUROD1* mRNA transcript levels, the significant variance heterogeneity observed in the parametric a priori hypotheses tests sustained; except for in the case of alcohol abuse severity in the APh region and qPCR analysis plate in the non-neurogenic HF. This largely precluded confident reporting of ANCOVA test results.

When controlling for the effects of alcohol abuse severity in the APh region, variance heterogeneity was detected as a trend, $F(3,27) = 2.75$, $p = .062$. Notwithstanding this, no main effect of diagnosis on *NEUROD1* mRNA abundance was in the APh region was observed (Fig **5**-**21**). In the non-neurogenic HF, when controlling for the effects of qPCR analysis plate, marginal variance heterogeneity was detected, $F(3,11) = 2.73$, $p = .095$. Notwithstanding this, no main effect of diagnosis on *NEUROD1* mRNA abundance in the non-neurogenic HF was observed (Fig **5**-**22**).



5-21 Bar graph showing *NEUROD1* mRNA transcript levels across diagnosis in the anterior parahippocampal region. BPD = Bipolar Disorder (*N* = 10), MDD = Major Depressive Disorder (*N* = 6), SCZ = Schizophrenia (*N* = 7), CON = controls (*N* = 8).

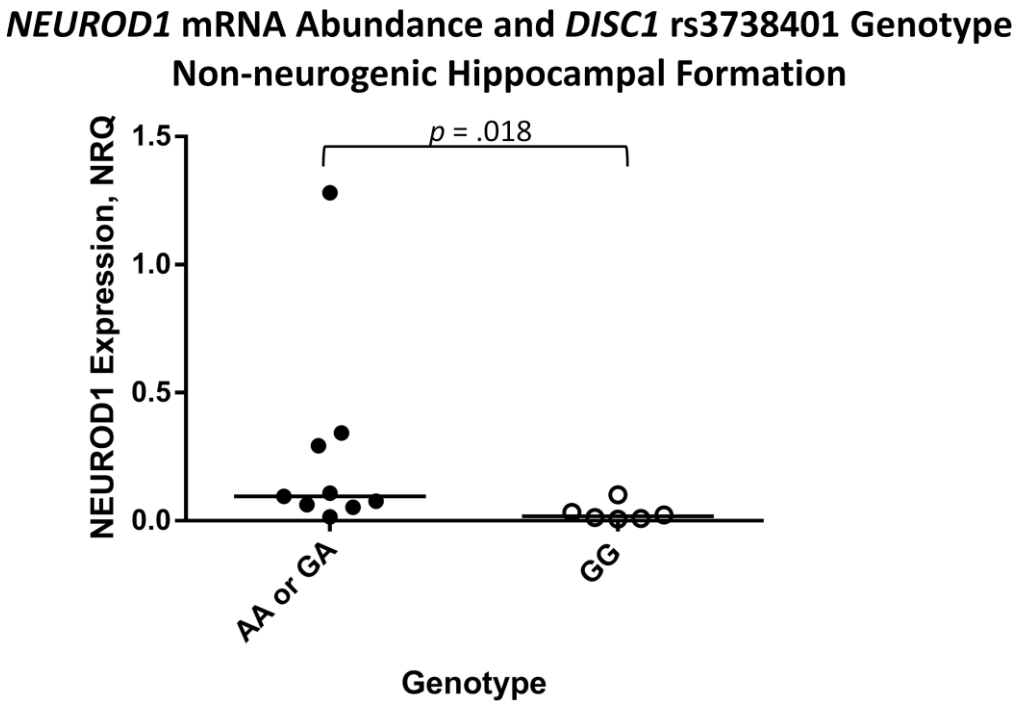


5-22 Bar graph showing *NEUROD1* mRNA transcript levels across diagnosis in the non-neurogenic hippocampal formation. BPD = Bipolar Disorder (*N* = 3), MDD = Major Depressive Disorder (*N* = 3), SCZ = Schizophrenia (*N* = 4), CON = controls (*N* = 5).

5.2.2.3 Genotype Effects

In the APh region, no significant effects of minor alleles were observed when the dominant effect model was assessed via Mann-Whitney U testing. In the non-neurogenic HF, results of Mann-Whitney *U* testing of this dominant effect model suggested a significant effect of the *DISC1* rs3738401 minor allele. Minor allele carriers presented significantly higher *NEUROD1* mRNA transcript levels in the non-neurogenic HF (*Mdn* = .095) when compared to non-carriers (*Mdn* = .018), *U* = 7.00, *p* = .018 (Fig 5-23). As no effect of diagnosis was detected in this region, the rs3738401 minor allele was re-analysed under parametric conditions. Upon *t*-testing, no significant difference in *NEUROD1* mRNA transcript levels was detected between *DISC1* rs3738401 minor allele carriers (*M* = .26, *SD* = .40, *N* = 9) and non-carriers (*M* = .56, *SD* = .49, *N* = 9), *t*(13) = 1.37, *p* = .193.

In order to protect against any artefacts of multiple comparisons, a 4×2 ANOVA analysis was attempted, However due to inadequate sample size in the non-neurogenic HF, the results of the 4×2 ANOVA, with diagnosis and *DISC1* rs3738401 as between-subjects factors could not be reported; the principal results of the nonparametric tests were therefore adopted. Mann-Whitney *U* testing detected no other significant effects of minor alleles in this region.



5-23 Scatter plot showing the *NEUROD1* mRNA transcript levels of rs3738401 minor allele carriers in the non-neurogenic hippocampal formation

5.3 Regional Analysis

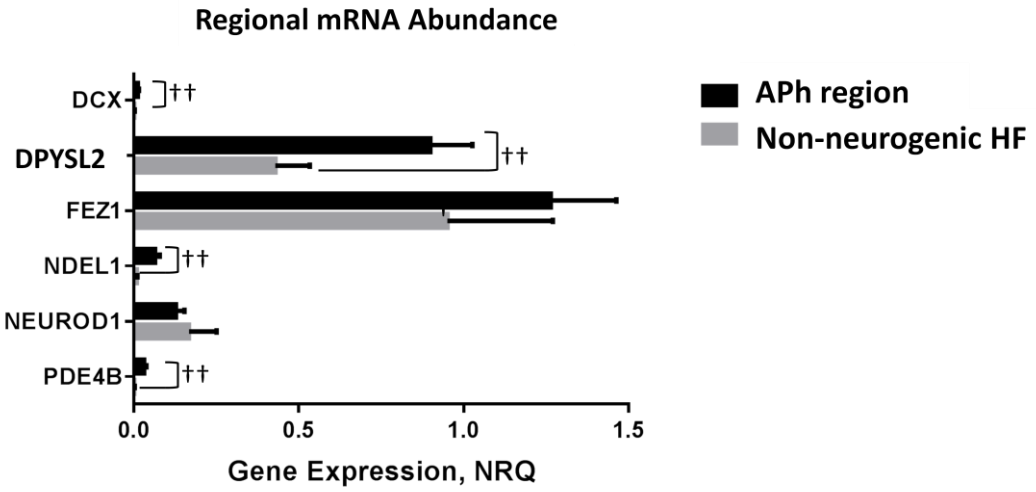
Comparisons of mRNA transcript levels between the non-neurogenic HF and APh region were conducted via non-parametric independent samples testing. Initial Mann-Whitney *U* tests detected regional mRNA transcript levels differences across all genes except *NEUROD1*.

PDE4B mRNA abundance was found to be significantly lower in the non-neurogenic HF (*Mdn* = .0005) when compared to levels in the APh region (*Mdn* = .007; *U* = 60.0, $p < .001$). *DCX* mRNA transcript levels in the non-neurogenic HF (*Mdn* = .0009) was also found to be significantly lower than in the APh region (*Mdn* = .004; *U* = 131.0, *p* = .032). Non-neurogenic HF mRNA transcript levels of *DPYSL2* (*Mdn* = .230) was also found to be significantly lower than in the APh region (*Mdn* = .761; *U* = 147.0, *p* = .009).

Results from these tests were accepted if the non-parametric equivalent of the Levene's Test for Equal variances was satisfied, and no correlation between APh region and non-neurogenic HF mRNA abundance was detected.

These conditions were not satisfied for *NDEL11* and *FEZ1*. The homogeneity of variances condition was violated for *NDEL1*, $F(1,45) = 6.69$, $p = .013$. In the case of *FEZ1*, a trend towards heterogeneous variances was detected, $F(1,44) = 3.577$, $p = .065$; a correlation between APh region and non-neurogenic HF gene mRNA abundance was also detected, $r_s = -.608$, $p = .036$.

In both instances, mRNA transcript levels differences across region was reanalysed via paired-Wilcoxon rank testing. For *NDEL1*, non-neurogenic HF mRNA transcript levels (*Mdn* = .008) was found to be significantly lower than APh region mRNA transcript levels (*Mdn* = .027; $Z = -2.20$, $p = .028$). For *FEZ1*, no significant difference between regions was observed.



5-24 Graph showing mRNA transcript levels of *DCX, DPYSL2, FEZ1, NDEL1 NEUROD1* and *PDE4B* mRNA in the anterior parahippocampal (APh) region and the non-neurogenic hippocampal formation (HF). †† denotes $p < .05$, NRQ = normalised relative quantity

6 QUANTITATIVE IMMUNOHISTOCHEMISTRY

In the assessment of protein density, the effects of diagnosis and minor allele carriers status was examined in each region of interest (ROI) separately. The demographic and clinical variables were also examined separately for each ROI. Due to experimental constraints, no technical replicates were performed during the examination of NEUROD1 protein density. When analysing FEZ1 and NEUROD1 protein density in the APh grey and white matter, signal saturation forced the comparisons of different areas within these broadly defined ROIs. This was not a factor in the analysis dentate gyrus protein density.

6-A Table of the anterior hippocampus protein density levels from each diagnostic category. Asterisk indicates where the Levene's Test of Equality of Error Variances was violated (p < 05), double asterisk indicates, p < 05 when controlling for demographic and clinical variables.; bpd = bipolar disorder, mdd = major depressive disorder, con = controls, scz = schizophrenia

IHC Protein Density Data

Diagnosis			Absolute NEUROD1 (APh Grey Matter)	Absolute NEUROD1 (APh White Matter)	Absolute NEUROD1 (Dentate Gyrus)	Relative FEZ1 (APh Grey Matter)	Relative FEZ1 (APh White Matter)	Relative FEZ1 (Dentate Gyrus)
bpd	*N*	Original	15	15	15	15	15	15
		Received	15	15	15	15	15	15
		Pre-outlier	12	12	11	15	15	14
		No. of Outliers	0	0	0	0	0	0
	N	Valid	12	12	11	15	15	14
	Mean		755.6421	471.4695	2035.272	1.0734	1.3395	1.1192
	Median		760.0842	500.2249	1748.306	1.031	1.2309	1.0537
	Std. Deviation		238.9986	147.9751	965.1086	0.21725	0.26775	0.18796
post-hoc to controls (Bonferroni) p			0.865	1	1	0.846	1	1
mdd	*N*	Original	15	15	15	15	15	15
		Received	10	10	10	10	10	10
		Pre-outlier	8	8	7	10	10	10
		No. of Outliers	1	1	0	0	0	0

IHC Protein Density Data

Diagnosis			Absolute NEUROD1 (APh Grey Matter)	Absolute NEUROD1 (APh White Matter)	Absolute NEUROD1 (Dentate Gyrus)	Relative FEZ1 (APh Grey Matter)	Relative FEZ1 (APh White Matter)	Relative FEZ1 (Dentate Gyrus)
	N	Valid	7	7	7	10	10	10
	Mean		1030.133	515.5843	2439.039	1.2049	1.4116	1.15
	Median		932.6944	483.5322	2573.919	1.2178	1.36	1.136
	Std. Deviation		228.1784	96.03043	945.5413	0.21904	0.22344	0.23372
post-hoc to controls (Bonferroni) p			1	1	1	1	1	1
con	*N*	Original	15	15	15	15	15	15
		Received	13	13	13	13	13	13
		Pre-outlier	11	11	9	12	12	12
		No. of Outliers	1	0	0	0	0	0
	N	Valid	10	11	9	12	12	12
	Mean		985.263	569.7127	2250.039	1.2035	1.3662	1.2182
	Median		1138.16	460.4171	2147.771	1.1789	1.3867	1.1989
	Std. Deviation		376.2874	260.1768	996.3947	0.19968	0.19923	0.21298
post-hoc to controls (Bonferroni) p			-	-	-	-	-	-
scz	*N*	Original	15	15	15	15	15	15
		Received	13	13	13	13	13	13
		Pre-outlier	12	12	12	13	13	13
		No. of Outliers	0	0	0	0	1	0
	N	Valid	12	12	12	13	12	13
	Mean		1002.904	500.6704	2279.839	1.1765	1.4535	1.2397
	Median		1036.032	448.3012	2110.524	1.1288	1.4201	1.1656
	Std. Deviation		482.9859	240.1595	932.1107	0.2558	0.22017	0.18903
post-hoc to controls (Bonferroni) p			1	1	1	1	1	1
Diagnosis Kruskal-Wallis	Pre-outlier	*H*	5.177	0.38	0.896	2.818	2.01	3.202
		p	0.159	0.944	0.827	0.421	0.57	0.362
	Valid	*H*	3.883	0.787	0.896	2.818	1.264	3.202
		p	0.274	0.853	0.827	0.421	0.738	0.362
ANOVA	Valid	*F*	1.365	0.457	0.274	1.051	0.606	0.994
		p	0.268	0.714	0.844	0.379	0.614	0.404

6-B Table showing the results of the non-parametric testing of minor carrier allele status effects on protein density levels in sections of anterior hippocampus

IHC Minor Allele Carrier Status Data

		Relative FEZ1 (APh Grey Matter)	Relative FEZ1 (APh White Matter)	Relative FEZ1 (Dentate Gyrus)	Absolute NEUROD1 (APh Grey Matter)	Absolute NEUROD1 (APh White Matter)	Absolute NEUROD1 (Dentate Gyrus)
DISC1 rs3738401 minor allele carriers	*N*	32	32	31	24	26	24
	Mean Rank	23.13	24.13	22.90	18.58	23.00	19.54
	Sum of Ranks	694.00	724.00	687.00	446.00	598.00	469.00
Non-carriers	*N*	18	17	18	17	16	15
	Mean Rank	25.53	23.76	25.94	24.41	19.06	20.73
	Sum of Ranks	434.00	404.00	441.00	415.00	305.00	311.00
Total	*N*	50	49	49	41	42	39
	Mann-Whitney U	229.000	251.000	222.000	146.000	169.000	169.000
	Wilcoxon W	694.000	404.000	687.000	446.000	305.000	469.000
	Z	-.576	-.089	-.731	-1.535	-1.010	-.318
	p (2-tailed)	.565	.929	.465	.125	.312	.751
DISC1 rs6675281 minor allele carriers	*N*	14	14	14	13	13	12
	Mean Rank	24.00	25.36	24.14	26.69	21.77	22.25
	Sum of Ranks	336.00	355.00	338.00	347.00	283.00	267.00
Non-carriers	*N*	36	35	35	28	29	27
	Mean Rank	24.00	23.42	23.94	18.36	21.38	19.00
	Sum of Ranks	792.00	773.00	790.00	514.00	620.00	513.00
Total	*N*	50	49	49	41	42	39
	Mann-Whitney U	231.000	212.000	229.000	108.000	185.000	135.000
	Wilcoxon W	792.000	773.000	790.000	514.000	620.000	513.000
	Z	0.000	-.442	-.047	-2.073	-.095	-.822
	p (2-tailed)	1.000	.659	.963	.038	.924	.411
DISC1	*N*	22	22	21	18	18	17

IHC Minor Allele Carrier Status Data

		Relative FEZ1 (APh Grey Matter)	Relative FEZ1 (APh White Matter)	Relative FEZ1 (Dentate Gyrus)	Absolute NEUROD1 (APh Grey Matter)	Absolute NEUROD1 (APh White Matter)	Absolute NEUROD1 (Dentate Gyrus)
rs821616 minor allele carriers	Mean Rank	18.82	23.14	20.19	15.78	15.61	18.00
	Sum of Ranks	414.00	509.00	424.00	284.00	281.00	306.00
Non-carriers	*N*	28	27	28	23	24	22
	Mean Rank	30.75	26.52	28.61	25.09	25.92	21.55
	Sum of Ranks	861.00	716.00	801.00	577.00	622.00	474.00
Total	*N*	50	49	49	41	42	39
	Mann-Whitney U	161.000	256.000	193.000	113.000	110.000	153.000
	Wilcoxon W	414.000	509.000	424.000	284.000	281.000	306.000
	Z	-2.873	-.824	-2.041	-2.469	-2.694	-.963
	p (2-tailed)	.004	.410	.041	.014	.007	.336
DISC1 rs3737597 minor allele carriers	*N*	2	2	2	2	2	2
	Mean Rank	25.50	26.50	26.00	9.50	5.50	18.50
	Sum of Ranks	51.00	53.00	52.00	19.00	11.00	37.00
Non-carriers	*N*	48	47	47	39	40	37
	Mean Rank	23.93	23.89	23.91	21.59	22.30	20.08
	Sum of Ranks	1077.00	1075.00	1076.00	842.00	892.00	743.00
Total	*N*	50	49	49	41	42	39
	Mann-Whitney U	42.000	40.000	41.000	16.000	8.000	34.000
	Wilcoxon W	1077.000	1075.000	1076.000	19.000	11.000	37.000
	Z	-.158	-.264	-.211	-1.392	-1.890	-.191
	p (2-tailed)	.874	.792	.833	.164	.059	.849
BDNF rs6265 minor allele carriers	*N*	14	13	14	11	12	11
	Mean Rank	31.00	27.77	30.36	24.91	27.67	20.91
	Sum of Ranks	434.00	361.00	425.00	274.00	332.00	230.00
Non-	*N*	36	36	35	30	30	28

IHC Minor Allele Carrier Status Data

		Relative FEZ1 (APh Grey Matter)	Relative FEZ1 (APh White Matter)	Relative FEZ1 (Dentate Gyrus)	Absolute NEUROD1 (APh Grey Matter)	Absolute NEUROD1 (APh White Matter)	Absolute NEUROD1 (Dentate Gyrus)
carriers	Mean Rank	23.36	24.00	22.86	19.57	19.03	19.64
	Sum of Ranks	841.00	864.00	800.00	587.00	571.00	550.00
Total	*N*	50	49	49	41	42	39
	Mann-Whitney U	175.000	198.000	170.000	122.000	106.000	144.000
	Wilcoxon W	841.000	864.000	800.000	587.000	571.000	550.000
	Z	-1.664	-.815	-1.660	-1.265	-2.060	-.312
	p (2-tailed)	.096	.415	.097	.206	.039	.755
RAPTOR rs2289759 minor allele carriers	*N*	26	26	26	21	21	20
	Mean Rank	24.42	25.04	26.08	18.57	18.76	18.25
	Sum of Ranks	586.00	601.00	652.00	390.00	394.00	365.00
Non-carriers	*N*	24	23	23	20	21	19
	Mean Rank	23.57	22.91	21.64	23.55	24.24	21.84
	Sum of Ranks	542.00	527.00	476.00	471.00	509.00	415.00
Total	*N*	50	49	49	41	42	39
	Mann-Whitney U	266.000	251.000	223.000	159.000	163.000	155.000
	Wilcoxon W	542.000	527.000	476.000	390.000	394.000	365.000
	Z	-.213	-.532	-1.109	-1.330	-1.446	-.983
	p (2-tailed)	.831	.595	.268	.183	.148	.325

6.1 FEZ1 Protein Density

In the APh grey matter, none of demographic and clinical variables were adopted as significant confounding variables for FEZ1 protein density. Furthermore, ANOVA testing of the mean relative FEZ1 protein densities in each of the ROIs revealed no significant main effect of diagnosis. Mann-

Whitney testing of minor allele carrier statuses suggested an effect of the *DISC1* rs821616 SNP. *DISC1* rs821616 minor allele carriers (*Mdn* = 1.23) exhibited lower levels of FEZ1 protein density in the grey matter when compare to major allele homozygotes (*Mdn* = 1.41, *U* = 160, *p* = .004).

As no significant main effect of diagnosis was detected in this region, the rs821616 minor allele was re-analysed under parametric conditions. Upon *t*-testing, the relative FEZ1 protein density of *DISC1* rs821616 minor allele carriers (*M* = 1.06, *SD* = .20, *N* = 22) was found to be significantly reduced when compared to non-carriers (*M* = 1.23, *SD* = .21, *N* = 28), *t*(48) = -2.933, *p* = .005. Mann-Whitney and *t* tests on all other minor alleles revealed no significant effects on FEZ1 protein density were detected in this region.

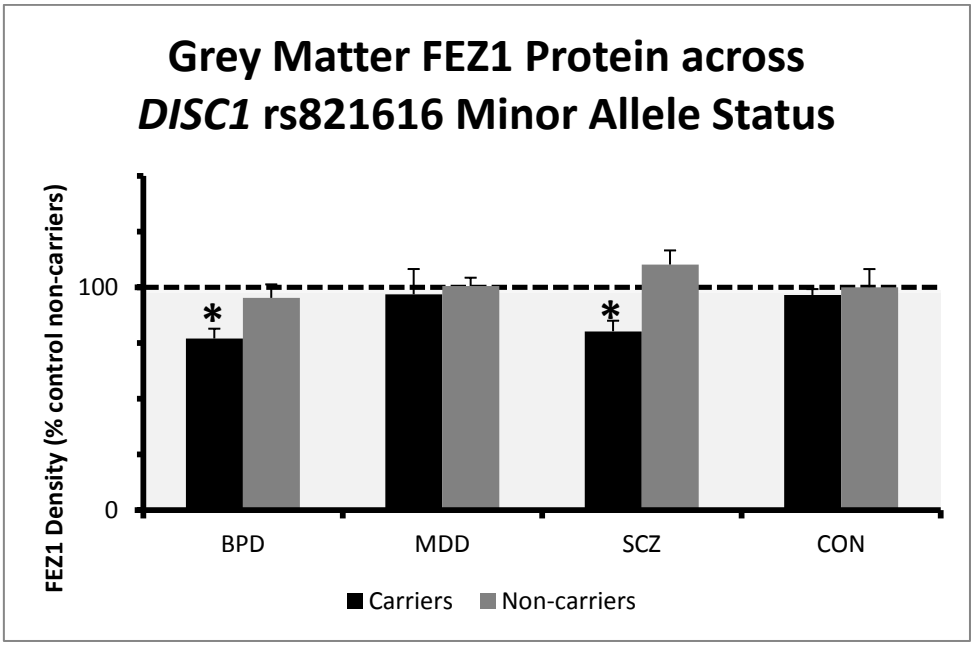
In the dentate gyrus, *DISC1* rs821616 minor allele carriers (*Mdn* = 1.22) exhibited lower levels of FEZ1 protein density in the grey matter when compare to major allele homozygotes (*Mdn* = 1.28, *U* = .193, *p* = .041). Upon *t*-testing, the relative FEZ1 density of *DISC1* rs821616 minor allele carriers (*M* = 1.11, *SD* = .16, *N* = 21), was also detected as significantly lower than in non-carriers (*M* = 1.24, *SD* = .22, *N* = 28), *t*(47) = -2.425, *p* = .019; Levene's test indicated unequal variances (*F* = 4.342, *p* = .043), so degrees of freedom were adjusted from 46.9 to 47. Analysis of the demographic and clinical variables in the dentate gyrus revealed no significant confounding variables for FEZ1 protein density. Mann-Whitney *U* and *t* tests on all other minor alleles revealed no significant effects on FEZ1 protein density were detected in this region.

In the APh white matter, the demographic and clinical variable of age at death (r_s = -.326, *p* = .022) exhibited a significant correlation with FEZ1 protein destiny; duration of disease (r_s = -.270, *p* = .061) and PMI (r_s = .243, *p* = .092) exhibited marginal correlations. These were therefore adopted as significant confounding variables. Nevertheless, no significant main effect of diagnosis was detected under ANOVA testing. There were also no effects of the minor alleles subjected to Mann-Whitney *U* and *t* tests in this ROI.

6.1.1 *DISC1* rs821616

In order to protect against artefacts of multiple comparisons, 4×2 ANOVA analyses with diagnosis and *DISC1* rs821616 minor allele carrier status as between-subjects factors were performed on the results derived from tests in the grey matter and dentate gyrus ROIs. In the grey matter, the 4×2 ANOVA detected no significant main effect of diagnosis, $F(3,42) = 1.67$, $p = .189$, but a significant effect of minor allele carrier status, $F(1,42) = .8,553$ $p = .006$. This effect was not qualified by an interaction between diagnosis and rs821616 minor allele carrier status, $F(3,42) = 1.801$, $p = .162$. The assessment within subjects, based on the estimated marginal means, revealed the significant effect of *DISC1* rs821616 to be specific to both SCZ [$F(1,42) = 10.716$, $p = .002$] and BPD [$F(1,42) = 4.433$, $p = .041$]; minor allele carriers exhibiting significantly lower FEZ1 protein density levels than the non carriers in both groups.

Sufficiently protected from artefacts of multiple comparisons, and the potential for an interaction between diagnosis and *DISC1 rs821616* minor allele status, *t* test were performed separately within each diagnostic group. Upon *t* testing within diagnoses, the FEZ1 density of rs821616 minor allele carriers in the grey matter demonstrated a 27.2% reduction in SCZ ($t(11) = -3.671$, $p = .004$) and a 19.2% reduction in BPD ($t(13) = -2.197$, $p = .047$) (Fig **6-1**). No significant effects were observed in the MDD or CON groups.

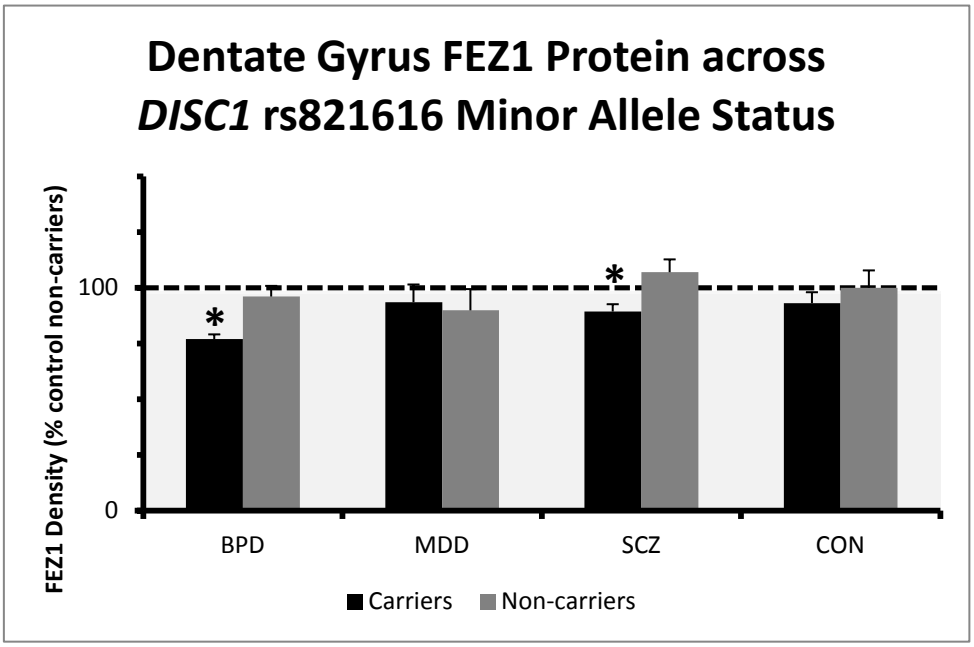


6-1 Bar graph showing the results of the within-group comparisons of grey matter FEZ1 protein density levels across *DISC1* rs821616 minor allele carrier status from slides of anterior hippocampus. * denotes $p < .05$ (*t*-tests within groups); BPD = Bipolar Disorder (*N*, carriers = 6, non-carriers = 9), MDD = Major Depressive Disorder (*N*, carriers = 5, non-carriers = 5), SCZ = Schizophrenia (*N*, carriers = 6, non-carriers = 7), CON = controls (*N*, carriers = 5, non=carriers = 7)

In the dentate gyrus, similar results were observed, The 4×2 ANOVA detected no significant main effect of diagnosis, $F(3,41) = 1.50$, $p = .230$, but a significant effect of minor allele carrier status, $F(1,41) = .5,026$, $p = .030$. This effect was not qualified by an interaction between diagnosis and rs821616 minor allele carrier status, $F(3,41) = 1.801$, $p = .162$. The assessment within subjects, based on the estimated marginal means, revealed the significant effect of *DISC1* rs821616 in the dentate gyrus to be specific to both SCZ [$F(1,42) = 4.309$, $p = .044$] and BPD [$F(1,42) = 5.029$, $p = .030$]; minor allele carriers exhibiting significantly lower FEZ1 protein density levels than the non carriers in both groups.

Upon *t* testing within diagnoses, the FEZ1 density of rs821616 minor allele carriers in the dentate gyrus was reduced by 16.5% in SCZ ($t(11) = -2.540$, $p = .027$) and 19.9% in BPD ($t(11) = -2.845$, $p = .004$) (Fig **6-2**). No significant effects were observed in the MDD or CON groups. Levene's test

indicated unequal variances between minor allele carriers and non-carriers in the BPD group (F = 5.878, p = .032), degrees of freedom were therefore adjusted from 12 to 11.



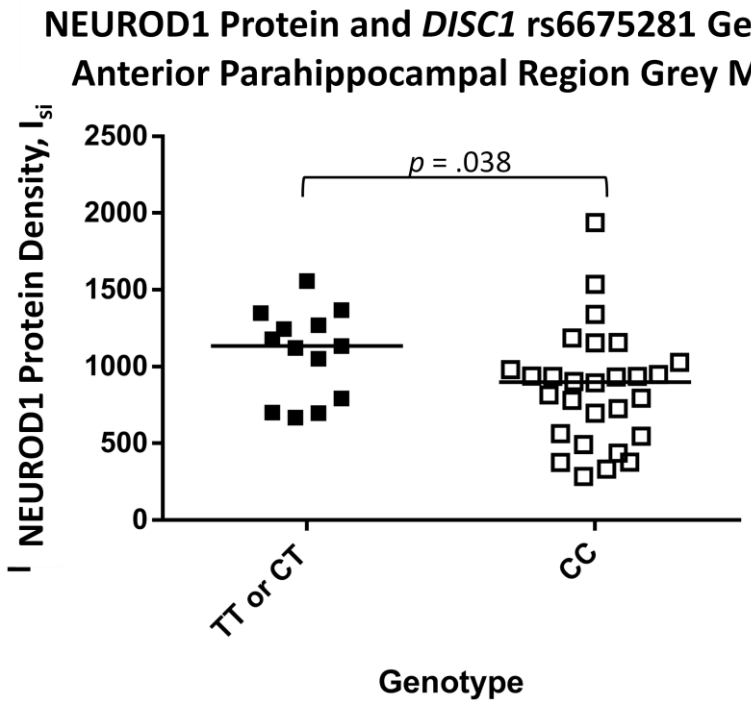
6-2 Bar graph showing the results of the within-group comparisons of dentate gyrus FEZ1 protein density levels across *DISC1* rs821616 minor allele carrier status from slides of anterior hippocampus. * denotes $p < .05$ (*t*-tests within groups); BPD = Bipolar Disorder (*N*, carriers = 5, non-carriers = 9), MDD = Major Depressive Disorder (*N*, carriers = 5, non-carriers = 5), SCZ = Schizophrenia (*N*, carriers = 6, non-carriers = 7), CON = controls (*N*, carriers = 5, non=carriers = 7)

6.2 NEUROD1 Protein Density

In the APh grey matter, the demographic and clinical variable found to exhibit a significant correlation with NEUROD1 protein density was brain pH (r_s = -.32, p = .039). From the dichotomous demographic and clinical variables, suicide was detected to have a significant effect on NEUROD1 protein density; subjects with the reported cause of death as suicide exhibited lower NEUROD1 protein density levels (*Mdn* = 793), when compared to non-suicide counterparts (*Mdn* = 1040, U = 97.0, p = .017). Suicide and brain pH therefore adopted as adopted significant confounding variables for grey matter NEUROD1 protein density. No other significant effects of demographic and clinical variables were

observed in this ROI. Under ANOVA testing, no significant main effect of diagnosis in the APh grey matter was detected.

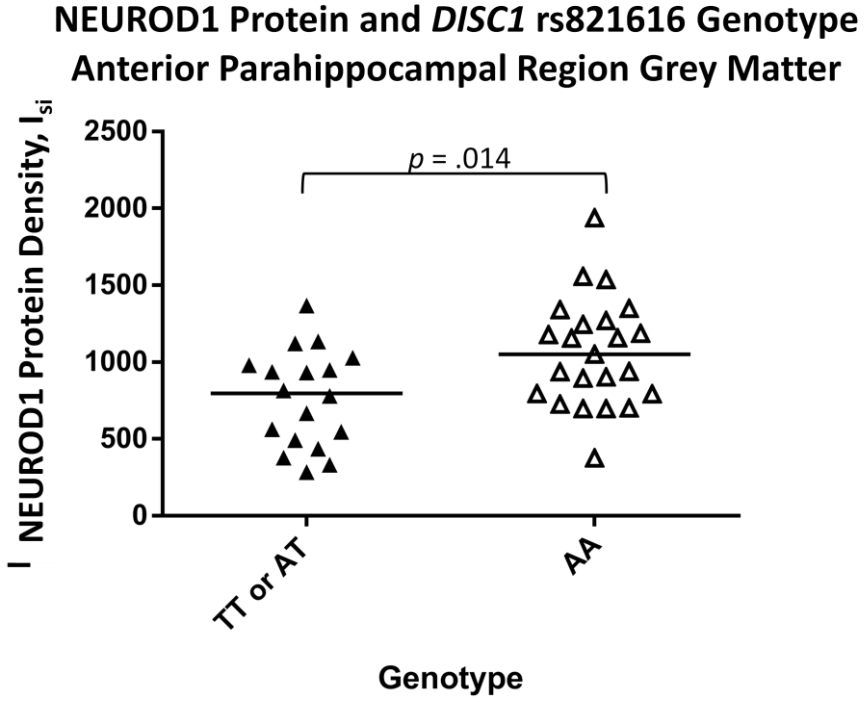
Mann-Whitney *U* testing of minor allele carrier statuses suggested an effect of two *DISC1* SNPs. Minor allele carriers of the rs6675281 SNP (*Mdn* = 1135) exhibited significantly higher absolute NEUROD1 protein density levels when compared to non-carriers (*Mdn* = 899, *U* = 108, *p* = .038). As no significant main effect of diagnosis was detected in this region, the rs6675281 minor allele was re-analysed under parametric conditions. Upon *t*-testing, minor allele carriers (*M* = 1087, *SD* = 289 *N* = 13) exhibited marginally higher absolute NEUROD1 protein density levels in the grey matter when compared to non-carriers carriers (*M* = 858, *SD* = 377, *N* = 28), *t*(39) = 1.93, *p* = .061 (Fig **6-3**).



6-3 Scatter plot showing the NEUROD1 protein density levels in the anterior parahippocampal grey matter of *DISC1* rs6675281 minor allele carriers from slides of anterior hippocampus

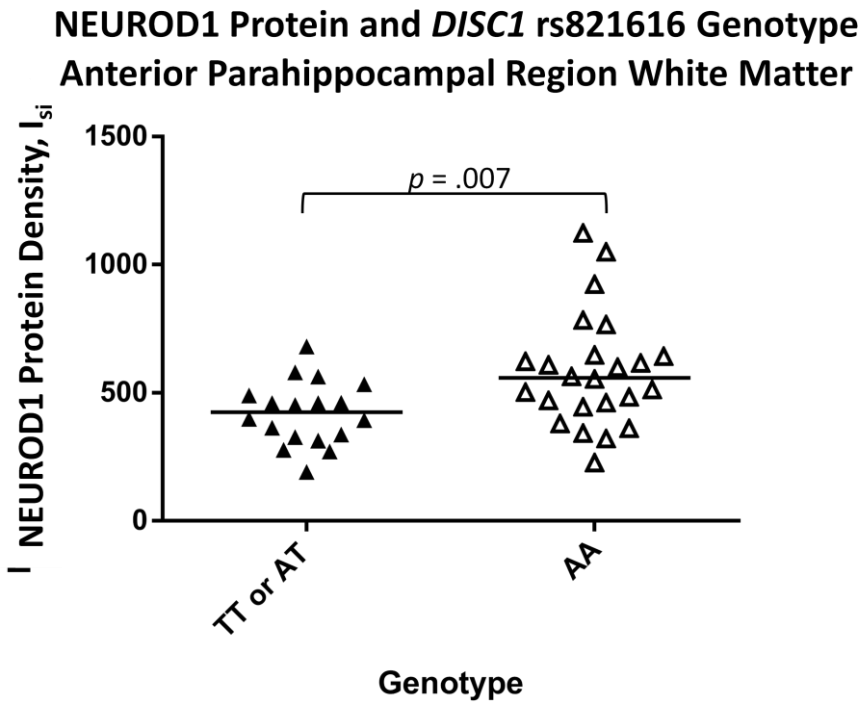
Minor allele carriers of the *DISC1* rs821616 SNP (*Mdn* = 797) exhibited significantly lower absolute NEUROD1 protein density levels when compared to non-carriers (*Mdn* = 1051, *U* = 113.0, *p* = .014). As no significant main effect of diagnosis was detected in this region, the rs821616 minor allele was re-

analysed under parametric conditions. Upon *t*-testing, minor allele carriers (*M* = 764, *SD* = 315 *N* = 18) exhibited significantly lower absolute NEUROD1 protein density levels in the grey matter when compared to non-carriers carriers (*M* = 1061, *SD* = 352, *N* = 23), *t*(39) = -2.81, *p* = .008 (Fig **6-4**). No other significant effects of minor alleles were detected in this region.



6-4 Scatter plot showing the NEUROD1 protein density levels in the anterior parahippocampal grey matter of *DISC1* rs821616 minor allele carriers from slides of anterior hippocampus

In the APh white matter, no demographic or clinical variables were adopted as significant confounding factors for NEUROD1 protein density. Additionally, ANOVA testing revealed no significant main effect of diagnosis in this region. When the effect of minor allele carrier statuses were assessed in the white matter, *DISC1* rs821616 minor allele carriers were found to exhibit significantly lower absolute NEUROD1 protein density levels (*Mdn* = 425) when compared non-carriers carriers (*Mdn* = 558, *U* = 110.0, *p* = .007).



6-5 Scatter plot showing the NEUROD1 protein density levels in the anterior parahippocampal white matter of *DISC1* rs821616 minor allele carriers from slides of anterior hippocampus

As no significant main effect of diagnosis was detected in this region, the rs821616 minor allele was re-analysed under parametric conditions. Upon *t*-testing, minor allele carriers (*M* = 419, *SD* = 125, *N* = 18) exhibited significantly lower absolute NEUROD1 protein density levels in the grey matter when compared to non-carriers carriers (*M* = 583, *SD* = 221, *N* = 24), *t*(39) = -2.82, *p* = .007. No other significant effects of minor alleles were detected in this region. Marginal reductions in NEUROD1 protein density were predicted by the rare rs3737597 *DISC1* SNP; minor allele carriers exhibiting significantly lower absolute NEUROD1 protein density levels when compared to non-carriers (*U* = 8.00, *p* = .058). However, this was based on only 2 minor allele carriers. Consequently, upon *t* testing this effect was no longer detected as significant, *t*(40) = -1.53, *p* = .134.

In the dentate gyrus, no demographic or clinical variables were adopted as significant confounding factors for NEUROD1 protein density. Nevertheless, ANOVA testing revealed no significant main effect of diagnosis. There was also

no significant effect of minor allele carrier status detected in this ROI under *t* testing or Mann-Whitney *U* conditions.

7 DISCUSSION

The noteworthy findings of this report include; significant reductions of APh *FEZ1* mRNA transcript levels in SCZ and BPD, significant reductions of FEZ1 protein density in the APh grey matter and dentate gyrus of *DISC1* rs821616 minor allele carriers diagnosed with SCZ and BPD, increased APh *DCX* mRNA abundance in SCZ, and increased *NEUROD1* mRNA transcript levels in the non-neurogenic HF of *DISC1* rs3738401 minor allele carriers. The findings of significantly reduced NEUROD1 protein density levels associated with the DISC1 rs821616 minor allele in both the APh grey and white matter, were not based on technical replicates. These were therefore not a focus of these discussions.

7.1 Genotype Analysis

The results show that at each non-synonymous SNP, the Stanley Consortium sample is in Hardy-Weinberg Equilibrium (HWE; χ^2, $p \geq 0.05$) with both the Stanley Consortium and HapMap allele frequencies. This is an indication of genotype analysis authenticity and, notwithstanding the mixed diagnostic composition of the sample, also suggests the absence of any genotype frequency distortions in our sample[251].

DISC1 SNPs have exhibited associations to schizophrenia and BPD (see 1.8), though there is less evidence of a *DISC1* association to MDD. The association between the DISC1 locus and schizophrenia is likely to be a complex interplay between SNPs[252]. Therefore the absence of any genotype frequency distortions was not necessarily unexpected for a sample of this size.

Though all allele distributions were all found to be in HWE with HapMap allele frequencies, the. rs821616 SNP exhibited a χ^2 alpha value of $p = .012$. This is intriguing as the rs821616 allele has been demonstrated to predict lower expression of DISC1 binding partners FEZ1 and NDEL1 in homozygotes[204]. It lies proximal to the predicted binding region of NDEL1 and has also been shown to exhibit epistatic interactions with the G allele of the rs1361768 SNP in this gene.

This risk allele has also been associated in with reduced hippocampal grey matter volume, and deficits in episodic and working memory[253]. A larger sample size would offer the statistical power necessary to further interrogate the existence of genotype distortions at this locus in relation to mental illness. This may contribute to the evidence suggesting that this locus mediates risk for psychiatric phenotypes.

7.2 Gene Expression

7.2.1 DCX

7.2.1.1 DCX and schizophrenia

The results reported here are suggestive of an increase in the transcription of the *DCX* gene in the APh region of subjects diagnosed with schizophrenia. *DCX* is a gene with well characterized roles in the migration and neurite outgrowth of newborn neurons[141; 254]. However, evidence is beginning emerge that, quite apart from its neurogenic roles, *DCX* also functions in mature neurons[255]. Despite this, a scant number of studies have directly investigated *DCX* function in schizophrenia. *DCX* has primarily been utilised in psychiatric research as a marker for neurogenesis in tissues, distinguished from the BrdU-labelling which is used to measure neural progenitor proliferation. Accordingly, there are limitations in the ability to parallel these results with previous research.

For example, a role for *DCX* in the subtle migration dynamics of newborn neurons has been forwarded by the animal studies of Kvajo and colleague[256]. In a mutant mouse model of the DISC1 t(1:11) translocation, though they detected lower overall numbers of DCX-positive neurons, the proportion of these neurons in the outer granule cell layer was increased in the mutant mice. The outer region of the granule cell layer is the destination of tangentially migrating neurons generated from the inner layer. Interestingly, Kvajo and colleagues[256] were unable to detect concomitant increases in NeuN, a mature neuron marker. Taken with the SCZ-like behavioural deficits observed, this indicates a role for DCX as an indicator of uncoordinated migration (i.e. occurring without neuronal maturation) in SCZ neurobiology.

However, there are limitations to the extent to which this data can be interpreted. Firstly, the mutant mice expressed a truncated form of the mouse Disc1. This truncation was intended to mimic the genomic disruption present in the original DISC1 t(1:11) translocation, however their deletion variant introduced a premature stop codon in exon 7 whereas the original truncation point has been demonstrated to reside between exons 8 and 9. Secondly, Kvajo and colleagues detected these results in the dentate, a neurogenic subfield of the mammalian hippocampus. The results report in the present study relate to the APh region which does not host neurogenesis.

Recent work has illustrated this duality of *DCX* expression in the mammalian brain. Kremer and colleagues[255] report a constitutive expression of *DCX* in non-neurogenic regions of the brain. However, they did not find this non-neurogenic expression to be significantly altered upon environmental stimulation of neurogenesis in adult rodents. This was in direct contrast to the increase in *DCX* expression observed in the neurogenic niche of the dentate. This demonstrates that, in non-neurogenic regions, *DCX* expression may operate independently of adult neurogenesis.

It therefore follows that the SCZ-associated increase in DCX mRNA transcript levels observed in the APh region reflects an increased burden on the DCX-dependent processes occurring in mature neurons. Extrapolating from the neurodevelopment roles of DCX, this would implicate mature neuronal migration and axon growth. This would speculatively associate schizophrenia with disturbances in the neuroplasticity of the adult brain. It would also be consistent with some of the SCZ cytoarchitectural findings such as abnormal neuronal clustering in the entorhinal cortex[29; 30], and reduced synaptic marker expression in the temporal lobe[35; 39]. However, such distinct findings are likely the result of changes actuated early in development, which would be relevant if the abnormal expression of DCX detected in this study could be confidently reported for a neurogenic region of the brain. Unfortunately, due to sample sizes in the neurogenic HF dissections, the data does not support such an interpretation.

This also limits the correlations that can be made with the post-mortem findings reported by Fung and colleagues[257] . In their study, they were unable to detect differences in *DCX* expression levels between subjects with schizophrenia/schizoaffective disorder and normal controls in the dorsolateral prefrontal cortex (DLPFC). However, in the schizophrenia group they were able to detect evidence of a negative correlation between numbers of DLPFC interstitial white matter neurons (IWMNs) and *DCX* expression levels. Having previously reported a significant increase in IWMN density in SCZ[258], and a negative correlation of grey matter somatostatin expression with IWMN density, they understood this to reflect a failure of interneurons to migrate from white matter tracts into the cortex during development.

That the results indicate defects in neuro-plasticity is further evidenced by the finding that the *RPTOR* rs2289759 minor allele is associated with increased hippocampal *DCX* transcript levels. The RPTOR protein is a major constituent of the mTORC1 complex which regulates synaptic protein translation[259]. It acts downstream of the neurotrophin BDNF in the PI3K-Akt signalling cascade[260]; important in the formation of memories[261]. There is evidence that the minor G allele of the rs2289759 *RPTOR* SNP promotes the expression of a *RPTOR* splice variant with limited mTOR substrate binding capacity[262]. Substrate binding is critical to mTORC1 function, the inhibition of which has been demonstrated to result in impairments to long-term potentiation[263].

Our additional finding in the schizophrenia cohort, of an increased level of parahippocampal *DCX* transcript in *BDNF* rs6265 minor allele carriers, further implicates the BDNF signalling pathway. BDNF serves as a major regulator of synaptic transmission and plasticity in numerous brain regions[264]. The minor A allele of the *BDNF* rs6265 SNP has been associated to deficits in episodic memory and reduced hippocampal activation in humans[265]. This demonstrates that, as with the *RPTOR* rs2289759 SNP in the hippocampus, the minor allele of a polymorphism previously associated to memory processing defects, may also be associated to an increase in DCX expression. It is interesting that both

these genotype-mediated effects involve genes in the BDNF-mTORC1 signalling pathway but manifest in separate brain regions. As Kendler[266] has recently argued, this may reflect the different levels at which a molecular pathway may impact on disease risk..

7.2.1.2 Implications for *DCX* as a Neurogenesis Marker

As a putative marker for neurogenesis, these results, along with the work of Kremer and Colleagues[255], raise important questions regarding the interpretation of *DCX* expression in post-mortem studies. Couillard-Despres and colleagues[267] demonstrated that *DCX* expression in adult neurogenic regions does not overlap with either nestin; a marker for pluripotent neural stem cells, or GFAP; a marker for astrocytes. This was taken as strong evidence that *DCX* expression is specific to new-born neurons. However, there is now a growing appreciation that DCX is expressed in non-neurogenic pools, operating independent of neurogenesis. This behoves caution when interpreting DCX expression data from post-mortem tissues and also explains the apparent disparity between the increase of DCX expression, detected in this study, and previous reports of reduced adult neurogenesis in SCZ (see 1.4.4).

In addition to this, the extent to which *DCX* expression accurately reflects neurogenesis is also under scrutiny. Merz and colleagues[268] have recently demonstrated that the absence of *Dcx* expression in migrating neurons does not necessarily result in 'heterotopic' positioning of neurons within the mammalian adult brain. Their results, when taken with the findings of previous reports[254; 269], indicate that *DCX* plays a critical role in the early stages of migration; but a 'dispensable' one in the latter stages.

Accordingly, researchers have argued against the use of solitary markers for neurogenesis in immunohistological studies[137]. However, for quantitative studies, the accurate targeting of specific brain regions and subfields will aid the accurate interpretation of results. This approach has previously been explored by Altar and colleagues[150] who used laser-capture micro-dissection (LCM) to profile neurogenic gene expression.

7.2.2 FEZ1

7.2.2.1 *FEZ1* and Psychosis

The data reported in this study strongly suggest that expression of the *FEZ1* gene is reduced in the major psychoses. Not only were *FEZ1* mRNA transcript levels in the APh region found to be significantly reduced in both SCZ and BPD, but the FEZ1 protein reductions, found existing in the brains of minor allele carriers of the *DISC1* rs821616 SNP, were revealed to be specific to the SCZ and BPD groups. This is consistent with the findings of Lipska and colleagues[204] which demonstrated FEZ mRNA reductions in the hippocampus and DLPFC of SCZ sufferers. Lipska and colleagues[204] also reported evidence that the *DISC1* rs821616 minor allele predicts reductions in *FEZ1* mRNA in the context of a SCZ diagnosis. This is consistent with the findings of reduced FEZ1 protein density in rs821616 minor allele carriers reported in this study. Genetic association of the *FEZ1* gene has identified two adjacent loci exhibiting nominally significant associations to SCZ, but not BPD, in the Japanese population[222]. Further studies have yet to replicate this finding[223; 270], including a second study in a Japanese cohort[224].

7.2.2.2 Impact of Reduced *FEZ1* Expression

There have been previously reported examples of *FEZ1* insufficiency correlating to indicators of psychosis. *Fez1* knockdown mice, when subjected to behavioural tests, have been shown to exhibit hyperactivity traits and demonstrate an increased sensitivity to psycho-stimulation of the mesolimbic dopaminergic system[271]. Researchers have postulated that these behavioural abnormalities correspond to psychiatric endophenotypes[271]. Hyperactivity is a proposed endophenotype in psychiatric conditions, including SCZ[272], and increased dopaminergic sensitivity directly correlates to the hyper-functioning dopamine system observed in SCZ and BPD patients. These findings therefore implicate *FEZ1* expression in behavioural and neurochemical correlates of psychosis.

Previously demonstrated roles of *FEZ1* include a participation in dendritic outgrowth[218] and microtubule transport[219]. It would be reasonable to expect that

reduced *FEZ1* expression might correspond to deficits in these aspects of cell biology. Accordingly, Fujita and colleagues report that *FEZ1*-deficient PC12 cells show reduced levels of NGF-induced neurite extension. This is somewhat consistent with the decreased neuronal soma size and reduced synaptic marker density observed in SCZ. They also found that these cells exhibited defects in the anterograde transport of mitochondria along axons[218]. Mitochondrial transport is thought to be critical in neural differentiation, providing energy for neurite development.

Abnormalities in mitochondrial morphology have also been detected in *FEZ1*-deficient PC12 cells[219], and there is evidence to suggest that mitochondrial changes operate as a neurobiological correlate of both BPD and SCZ[273]. This implicates *FEZ1* reductions in the mitochondrial disturbances associated with these disorders. Interestingly, mitochondria-related disturbances are gaining increasing relevance in the molecular basis of psychiatric disorders. Mitochondrial DNA (mtDNA) mutations have been linked to BPD and SCZ, as have mtDNA polymorphisms[274-276] and subjects with mitochondrial disorders have been reported to present psychotic symptoms[277].

Of course, *FEZ1* also participates on the microtubule transport of other cargoes, including neurotransmitter vesicles. Therefore, the mechanism by which reduced *FEZ1* expression may mediate risk for SCZ and BPD is open to further speculation. For example, the previous genetic association of *FEZ1* to SCZ was detected in a locus which translates to the N-terminal portion of the protein. This portion of the protein has been reported to be involved in the axonal targeting activity of the UNC76 ortholog, which has been demonstrated to functionally overlap with human *FEZ1*[217]. It is an intriguing possibility that reduced *FEZ1* expression translates to deficits in the axonal targeting activity of *FEZ1* highlighted by the genetic association studies. This would retard anterograde transport of cargoes including mitochondria and neurotransmitters, reducing the capacity of neurons to accurately extend processes and maintain active synaptic contacts. This is one means by which *FEZ1* expression

reductions could contribute to the abnormalities observed in the major psychoses.

Further evidence of *FEZ1*'s role in psychiatric disorders is reported by Yu and colleagues[278]. Their study reported the results of a microarray expression analysis of 11,214 genes in human astrocyte-derived cells treated with the classic mood stabilisers, lithium, valproic acid, carbamazepine, and lamotrigine. The results were then confirmed with follow-up qPCR analysis. Intriguingly, they found *FEZ1* to be the only gene for which expression levels were significantly increased in response to each of the 4 drug regimens. This provides a direct link between the treatment of mood disorders, including BPD, and a regulation of cellular *FEZ1* expression levels. Whereas the previously discussed evidence demonstrates a correlation between *FEZ1* expression and psychotic pathology, these results appear to implicate increased *FEZ1* expression in therapeutic recovery.

BPD and SCZ are distinctly characterised psychotic disorders; however there is a growing appreciation of the extent to which they overlap. Reviews of the genetic association literature highlight many of the most strongly supported SCZ susceptibility genes as being associated to BPD[279]. More recently, The International Schizophrenia Consortium used a novel form of GWAS analyses to corroborate the polygenic theory of SCZ. However, they were also able to demonstrate a substantial overlap between the polygenic component of SCZ and BPD[67].

This suggests that though these disorders are understood to manifest divergently, there is a strong possibility that aspects of the underlying molecular mechanisms are shared. This would explain the findings in this study of reduced *FEZ1* expression in the merged SCZ and BPD psychosis group. It would also be consistent with the results of *FEZ1*-silencing studies, which correlate to both SCZ and BPD neurobiology.

Specifically, acute psychosis; a feature of both SCZ and BPD, is thought to be associated to an elevation in striatal dopamine neurotransmission[280]. *FEZ1* expression is prominent in the mammalian striatum, where it is

predominantly found in inhibitory GABA neurons[271]. Reductions in *FEZ1* expression, and its reported consequences, may induce subtle alterations to the brain's capacity to appropriately regulate neurotransmission. When related to the striatum, this may have substantial implications for our understanding of the acute psychosis phase. Whether any pathological relationship between reduced *FEZ1* expression in the APh region and psychosis is causal or secondary in nature requires further investigation. Genome-wide and animal model studies, incorporating both SCZ- and BPD- related phenotypes, will be invaluable in this regard.

7.2.2.3 FEZ1 and *DISC1* rs821616

The analysis of the data indicates that reductions in the amount of FEZ1 protein exist in the brains of carriers of the minor allele of common *DISC1* variant rs821616. This minor T allele encodes an amino acid change in the gene product from serine, encoded by the major A allele, to cysteine. The expression reduction was detected in the grey matter and dentate gyrus of slides encompassing the anterior hippocampus.

There is previous evidence to suggest that variation in *DISC1* predicts reductions in FEZ1 expression. Lipska and colleagues[204] reported that variation at 3 *DISC1* loci which, in the context of a schizophrenia diagnosis, coincided with reduced *FEZ1* expression. Schizophrenia subjects homozygous for SCZ-associated alleles at 2 silent intronic SNPs (hCV219779 and rs821597), and the non-synonymous SNP highlighted in this study (rs821616), were found to exhibit significantly lower levels of *FEZ1* expression in the hippocampus. In the DLPFC these effects were only associated with rs821597 and rs821616.

This provides evidence that the non-synonymous SNP rs821616 can exert influences on the expression of *FEZ1* in both cortical and sub-cortical regions. However, whereas previous studies have demonstrated an effect on FEZ1 mRNA[204], the results reported here indicate that these effects may manifest at the protein level. This has a further relevance for a SNP which moderates an amino acid substitution.

There is evidence to suggest that this amino acid substitution coincides with a wide array of neurocognitive, neuroanatomical and cytoarchitectural alterations. These may shed light on the nature of dysregulated *FEZ1* mRNA and protein expression visited by this particular *DISC1* variant.

7.2.2.3.1 *DISC1* rs821616 and Neuroanatomy

The reports of an effect of *DISC1* rs821616 on grey matter volume have been mixed. In one of the first studies to analyse the potential for an effect of this SNP on temporal cortical neuroanatomy, Calicott and colleagues[253] were able to highlight significant enlargements in hippocampal grey matter volumes of subjects homozygous for the minor T allele (when compared to the A allele homozygotes). However, in 2008, Di Giorgio and colleagues[281] reported reductions in the grey matter volume of minor T allele carriers when compared to their A allele-homozygous counterparts.

More recent studies concord with the theory that it is the rs821616 major A allele which is associated with reduced temporal region brain volumes. Raznahan and colleagues[282], associate minor T allele carriers with thicker cortices in temporal regions encompassing the inferior, middle and superior temporal gyri. Knickmeyer and colleagues[283], associate T allele carrier status with increased brain volume in the lateral APh region.

These results would appear to contradict the findings reported in this current study. However, the complexity involved in directly translating the effect of genetic variation on brain volume is demonstrated by a recent study by Trost and colleagues[284]. Here, the researchers were able to demonstrate that T allele carriers exhibit both reductions and enlargements in grey matter volume, sometimes in the same brain region.

They report the minor T allele as being associated with reductions in the grey matter volume in the left middle frontal gyrus. However, they also detect grey matter volume reductions in A allele homozygotes in this region. These somewhat contradictory findings were recapitulated in the right middle frontal gyrus. However, the Montreal Neurological Institute (MNI) coordinates of the

left- and right-sided T allele-associated reductions (-33 37 43 and 36 62-4 respectively) differ slightly from those of the left- and right-sided A allele homozygosity-associated reductions (-24 48 1 and 32 34 30).

The results therefore suggest that minor shifts in brain region may profoundly affect the observed influence of *DISC1* rs821616 variation on brain volume. This, to some degree, corresponds with the findings here in this study that *DISC1* rs821616 minor T allele affects FEZ1 protein density in the grey matter of coronal sections of the APh region, but not the white matter.

Brain structure consists of a voluminous array of connections. It is highly likely that that any effects of the rs821616 SNP manifest in multiple forms at the level of brain volume. The observance of these effects may be contingent on brain region and/or level of neural circuitry and much more. It is therefore likely to be useful for researchers to analyse subtler measures of brain function for clues as to the relationship between genetic variation and gene expression.

7.2.2.3.2 *DISC1* rs821616 and Brain Function

In their early study into *DISC1* variation, Callicott and colleagues[253] were able to report the results of a series of cognitive tests. They detected subjects homozygous for the rs821616 A allele as exhibiting significantly different brain activity levels than their minor T allele-carrying counterparts. During the neutral scene encoding of the declarative memory task, A allele homozygosity was associated with bilateral reductions in blood oxygen levels in the hippocampal formation. Interestingly, the A allele was also associated with decreased hippocampal grey matter volume in this study. However, Callicott and colleagues[253] also detected A allele homozygosity to be associated with increased bilateral hippocampal activity during the N-back working memory task.

Conversely in the Di Giorgio study[281], A allele homozygosity was found to coincide with an increase in hippocampal bilateral blood oxygen levels during memory encoding of the declarative memory task. In thus study, the A allele was found to be associated with greater parahippocampal gyrus volume.

This suggests that not only do the effects of *DISC1* variation seemingly manifest divergently at the level of brain volume, but also at the level of brain activity (as measured by blood oxygen levels). This may reflect that the effect of DISC1 variation on brain activity is subject other influences.

For example, in SCZ and BPD patients Prata and colleagues[285] detected no significant difference between A allele homozygotes and minor T allele carriers in prefrontal brain activation levels during verbal fluency testing. This was despite previously detecting decreased prefrontal activation in healthy minor T allele carriers during the same task in a previous study[286]. The researchers speculated that this may reflect the interaction of *DISC1* with other genes, and/or the environmental risk factors, associated with SCZ and BPD.

This may provide clues to the nature of the association between *DISC1* variation and *FEZ1* expression detected in this study. Lipska and colleagues report an effect of *DISC1* rs821616 specific to SCZ subjects, Praha and colleagues[286] also report an effect of rs821616 that is specific to SCZ. In this current study, the effects of rs821616 were more pronounced in psychosis sufferers (SCZ and BPD). This suggests that DISC1 rs821616 may moderate brain activity and FEZ1 expression in combination with one or more of the risk factors associated with SCZ and BPD.

7.2.2.3.3 *FEZ1* and *DISC1* function

In a comprehensive study on *Fez1* and *Disc1* function in adult neurogenesis, Kang and colleagues[287] were able to demonstrate that the consequences of *Fez1* knockdown partially parallel the effects seen in *Disc1* silencing. In both cases, significant increases in neuron soma size and dendritic arborisation are observed. However, in *Disc1* knockdown further effects of aberrant neuron positioning and ectopic neurite extension were also detected.

The alterations observed upon *Fez1* knockdown were susceptible to recovery by wild type FEZ1 rescue vectors. Taken together with the interaction between FEZ1 and DISC1 proteins, and their evidence of functional synergy

between the two, Kang and colleagues[287] proposed that FEZ1 and DISC1 interact to regulate dendritic growth.

The potential for variation at the rs821616 locus to regulate *DISC1*, and thus FEZ1 function, is not entirely without basis. For example, though the rs821616 SNP does not lie within the purported FEZ1 binding site of *DISC1*, it does reside within the oligomerisation site. *DISC1* has been demonstrated to organise into dimers and then oligomers, the 668-747aa region of *DISC1* has been shown to be critical for this assembly[207].

Furthermore, the minor T allele Cys704 protein product, associated with reduced FEZ1 protein expression in this study, was demonstrated to be associated with increased oligomerisation. The researchers posited that this manipulation of oligomerisation dynamics could serve as a regulatory mechanism for *DISC1* function[207].

This may have implications for the function and/or expression of *DISC1* binding partners including FEZ1. In their study, Kamiya and colleagues[227] were able to demonstrate the effect of *DISC1* rs821616-mediated oligomerisation on NDEL1 binding. DISC1 and NDEL1 binding was demonstrated to be critical to neurite outgrowth[227]. The *DISC1* rs821616 SNP was shown to exert a mild influence on the DISC1-NDEL1 interaction[227]. The reported NDEL1 binding region does not overlap with this SNP. It is therefore possible that this effect does not reflect a direct interference with this interaction. However, it may serve as a secondary consequence of altered *DISC1* oligomerisation dynamics.

This would be consistent with the findings of Narayanan and colleagues[288]. In their study they found that DSIC1 rs821616 altered quaternary structural assembly of *DISC1* without significantly affecting NDEL1 binding. They thusly proposed that altered oligomerisation as the primary regulatory mechanism for *DISC1* rs821616-associated phenotypes. This phenomenon is therefore also likely to persist in FEZ1 binding, with potential ramifications for FEZ1 expression. Thus would concord with two independent evidences that *DISC1* rs821616 exhibits epistatic interactions of with *FEZ1* rs12224788[287] and *NDEL1* rs1391768[229] in risk for schizophrenia.

The findings reported in this study of reduced FEZ1 protein expression, in association with genetic variation at the *DISC1* rs821616 SCZ risk locus, is consistent with the literature. This locus has been implicated in numerous neuroanatomical and neurocognitive alterations. Research is continually revealing clues as to the mechanism of these effects. Understanding this mechanism is likely to further our understanding of *DISC1* and *FEZ1* function in psychiatric disorders.

7.2.3 *NEUROD1* and *DISC1*

The findings reported in this study suggest that *NEUROD1* mRNA abundance in the non-neurogenic HF is to some degree contingent on *DISC1* variation at the rs3738401 locus. Carriers of the minor A allele, which encodes an amino acid base change from arginine to glutamine, were found to harbour reduced expression levels of *NEUROD1* transcript when compared to subjects homozygous for the major G allele. Though there is limited evidence to suggest that genetic variation at this locus acts as an independent risk factor for disease[289], the minor A allele has been associated to SCZ as part of a haplotype marker.

There also a paucity of evidence linking *NEUROD1* directly to *DISC1* function or vice versa. NEUROD1 protein does not number amongst the many reported binding partners of DISC1 [213]. Despite this, any link between the molecular pathways in which these genes participate may offer further insights into their functional role in psychiatric disorders. An example of such a potential link is found in *Wnt* signalling. GSK-3β is a prominent signalling molecule in the canonical *Wnt* signalling pathway and is amongst reported binding partners of DISC1. The GSK-3β-DISC1 complex is understood to be critical to neurogenesis[290].This direct interaction between GSK-3β and DISC1 protein has been shown to inhibit GSK-3β activity leading to increased intracellular β-catenin levels[291].

In the canonical WNT pathway, GSK-3B functions as part of the Axin destruction complex which phosphorylates β-catenin signalling, targeting it for ubiquitination. Upon WNT stimulation, members of the axin destruction complex

are recruited to the inner plasmalemmal surface, stabilising intracellular β-catenin. The increased levels of intracellular β-catenin lead directly to the transcription of *NEUROD1* via its TCF/LEF elements[147]. Evidence for the direct interaction of β-catenin to *NEUROD1* regulatory elements has been demonstrated by Gao and colleagues[145].

Interestingly, the Arg264Gln mutation, coded by the *DISC1* rs3738401 SNP, lies proximal to the reported binding region of GSK-3β. It is quite possible that this mutation affects the direct interaction between these two proteins, reducing the *DISC1*-mediated inhibition of GSK-3β. This would reduce intracellular β-catenin levels and cause a decrease in the transcription of *NEUROD1*. This would be consistent with the evidence presented by Singh and colleagues[292]. In their study, Singh and colleagues were able to demonstrate that in all the model systems tested, Zebrafish, Mouse and also in Humans, the rs3738401 minor A allele Gln264 DISC1 variant, produced a negative effect on WNT signalling.

In mouse P19 carcinoma cells, *Disc1* knockdown reduced WNT signalling, an effect rescued by wild type DISC1 but not Gln264 DISC1. In *DISC1*-deficient N2A cells, Gln264 DISC1 did not stimulate cell proliferation, unlike wild-type DISC1. This effect was determined to be dependent on WNT-signalling; when WNT signalling was inhibited downstream (by dominant-negative LEF), none of the DISC1 variants were able to stimulate proliferation. In HEK293 cells, Gln264 DISC1 was unable to potentiate WNT signalling. It also bound GSK-3β to a lesser degree when compared to wild type DISC1

These results translated to their in vivo findings. Singh and colleagues[292] were able to demonstrate that *Disc1* knockdown resulted in reduced neural cell proliferation and early the cell-cycle exit of neurons in mouse brain. These effects were rescued by wild-type DISC1, but not Gln264 DISC1. They also detected evidence of a dominant negative effect of this *DISC1* variant in vivo. Gln264 DISC1 was able to significantly influence progenitor proliferation and cell-cycle exit, even without *Disc1* knockdown.

In order to test the longer term implications of these effects on embryonic development the researchers also investigated the ability of the DISC1 protein to rescue the severe zebrafish *Disc1* knockdown phenotype. When *Disc1* is silenced in zebrafish, they exhibit altered brain morphology, muscular segment defects and axon tract defects. These are characteristics of disturbed WNT signalling[293]. In keeping with their previous observation, whereas wild type DISC1 was able to restore much of the developmental deficits, Gln264 DISC1 was not.

In an attempt to translate their findings to human cells, they then tested human lymphoblast cell lines derived from bipolar patients homozygous for the rs3738401 A or G allele. Cells from subjects expressing Gln264 DISC1 exhibited lower constitutive TCF/LEF reporter activity. This effect was attributed to effectors downstream of WNT signalling as no significant differences in WNT receptor levels were detected. Interestingly, TCF/LEF reporter activity was also found to be reduced in bipolar patients independent of *DISC1* rs3738401 genotype.

In an attempt to further clarify the mechanisms involved in the effects of the DISC1 variation on the tested models, GSK-3β phosphorylation and β-catenin levels were also examined. GSK-3β is regulated by phosphorylation in a site-specific fashion. Phoshporylation at the tyrosine 216 residue stimulates activity; at serine 9 it is inhibited. Cells derived from subjects homozygous for Gln64 DISC1 exhibited higher levels of tyrosine 216 phosphorylation and lower levels of β-catenin. This suggests that *DISC1* variation at the rs3738401 locus regulates WNT signalling via GSK-3β activity.

The implications for our understanding of psychiatric disorders are evident. Members of the WNT signalling pathway have been reported as dysregulated in the hippocampus in psychiatric disorders. TCF4, a downstream effector of WNT signalling has been shown to act in concert with NEUROD1 to impact on higher-order cognitive processing in mice[294]. The evidence presented by Singh and colleagues[292] comprehensively establishes a link between *DISC1* and WNT signalling. Taken together with the role of WNT signalling in

NEUROD1 transcription, this evidences a theory whereby *DISC1* rs3738401 impacts on *NEUROD1* expression; explaining the results reported in this study.

Though no independent association of *DISC1* rs3738401 to BPD or SCZ has been reported, this does not preclude a role in more subtle aspects of the disease state. For example, a strong association with this SNP and resistance to drug treatment has recently been reported in two independent populations [289]. Molecular links, such as those highlighted by this study, will hopefully contribute to directing future research, increasing our understanding of the role this locus plays in psychiatric illness.

7.2.4 Other Effects

The preliminary analysis of the results using Mann-Whitney *U* test also suggested significant effects of dichotomous clinical and demographic variables. For the purposes of the current study, the analysis of these effects was deemed tertiary to the effects of diagnosis and genotype. Accordingly, sample size restricted the ability to fully analyse these effects and sufficiently protect against the artefacts of multiple comparisons (with repeated measures ANOVA). These results must therefore be interpreted with caution.

Nevertheless, noteworthy amongst the findings were significant reductions in right hemisphere non-neurogenic HF *PDE4B* mRNA transcript levels and right hemisphere APh *FEZ1* mRNA transcript levels (N = 8) when compared to the left hemisphere mRNA abundance levels of the same region. Though does not correlate to the perceptions of right-sided hyperactivity in MDD[295], it does however correlate with the suggested lateralisation deficits associated with SCZ[296; 297].

Also detected were reductions in APh *FEZ1* and non-neurogenic HF *DCX* mRNA transcript levels in cases with a history of psychosis; when compared to those without. The psychosis group was a direct representation of a combined SCZ and BPD group. The prospects of an effect of psychosis on *FEZ1* mRNA in the APh was discussed previously. The effect on DCX however,

may also offer further evidence of the growing appreciation of a shared molecular basis to these two disorders[298].

Another interesting detail from the dichotomous demographic and clinical variable data was the trend toward an effect of gender on *FEZ1* mRNA abundance in the non-neurogenic HF. Of all the trends detected, this effect was also found to be significant under parametric analysis. Males were found to exhibit a significantly higher level of *FEZ1* mRNA abundance than females. This intrigues, because SCZ has a higher prevalence in males[299], but the *FEZ1* expression distortions detected in the current study appear to exhibit a greater association with females. Sex-specific effects have been previously reported in the DISC1 pathway; these include the under-transmission of a DISC1 haplotype in affected females, and 4tag *PDE4B* SNPs associating with SCZ in females. Therefore with potential for a sex-specific effect to moderate *FEZ1* mRNA abundance in females is not without precedence.

7.3 Limitations

There were a number of limitations encountered whilst conducting the current study. Chief amongst these was sample size and thus statistical power. The original sample size was reduced due to the tissue availability. However, these sample sizes were further reduced for the qPCR analysis in an attempt to maintain sufficient RNA quality for accurate quantification. This precluded qPCR analysis of the neurogenic HF dissections. For the APh dissections, diagnostic group sample sizes ranged from 5 to 10. For the non-neurogenic HF dissections, sample group sizes ranged from 3 to 5. For the quantitative IHC assessment sample group sizes ranged from 7 to 15.

Though greater group sample sizes would afford greater statistical power, the group sizes for the APh dissections and quantitative IHC are reasonably informative. The sample sizes in the non-neurogenic HF dissections however, are prohibitive to the drawing of accurate conclusions for highly heterogeneous disorders like SCZ, MDD and BPD. Future studies may yet detect expression abnormalities that have proven beyond data reported here.

Laser capture micro-dissection and RNA extraction strategies, such as RNA amplification, can provide essential relief in this regard.

The reduced statistical power of the qPCR analysis in the APh region may go some way to explain the failure to corroborate some of the Lipska and colleagues study findings. These include reduced *NDEL1* mRNA in SCZ and the rs821616-mediated effects on *FEZ1* expression at the mRNA level[204]. Conversely, it could be argued that this emphasises the significance of the abnormal *FEZ1* expression detected in the current study, as similar results were previously reported by Lipska and colleagues[204].

Statistical power due to sample size is familiar problem for post-mortem studies. However, this has been mitigated by the use of gene expression quality guidelines[300] and the robust protections against Type I error utilised, i.e. Bonferroni-adjusted alpha levels and the requirement for significant main effects to be sustained upon analysis of demographic and clinical variables as covariates. This may have increased the risk of Type II error.

The further reduction in statistical power visited by outlier removal was mitigated by the requirement for outliers to be identified by the Tukey outlier labelling rule and a distribution outside 2.5 standard deviations of the mean (except for the situations where the Tukey labelling rule could not be applied). The proper means of analysing such data whilst maintaining the appropriate balance between risk of Type I and Type II errors is still debated. Therefore, researchers are better advised to rely on the independent corroboration of experimental findings in separate sample sets.

For the assessment of quantitative IHC, a major limitation was spots of signal saturation apparent on scans of brain sections. These reduced the area available for protein density calculations. It also introduced a level of variability between cases due to the different locations within perceived ROIs where signal saturation was detected in the grey and white matter. The dentate gyrus ROI was not affected, due to its relatively small size. The fact that the only significant results detected in the quantitative IHC analysis were directly paralleled in both the dentate gyrus and APh ROIs suggests that the saturation variability did not

affect these specific findings. Despite this, future investigations, using further subdivisions of the ROIs used in the present study, may detect effects not reported from the current set of data (this could be conducted on the scans produced from this present study).

Another limitation was the inability to repeat the quantitative IHC assessment of NEUROD1 protein. The results indicate that both reductions and escalations in NEUROD1 protein density were mediated by *DISC1* genotype. However, due to experimental constraints, these results could not be substantiated in a follow-up experiment. Due to the nature of IHC, large-scale assessments of histological slides are generally thought to be semi-quantitative. This further necessitates the use of technical replicates for the confident reporting of data. Future studies can directly address this limitation, providing further evidence of whether *DISC1* genotype is associated to *NEUROD1* gene expression.

7.4 Conclusions

The results of this study suggest that *FEZ1*, a DISC1 binding partner, is aberrantly expressed in the major psychoses relative to controls. The results also suggest that *DISC1* genotype can exert an influence on the expression of FEZ1 at the protein level. The data reported in this study, when examined in the context of previous evidences, represents a coherent account of reduced *FEZ1* expression, monitored by *DISC1* rs821616 variation, as a feature of psychosis. The role of *FEZ1*, in neuronal migration and microtubule transport, provides clues to the mechanisms by which the *DISC1* molecular pathway contributes to disease.

The results also suggest that the mRNA transcript levels of the neurogenesis marker *DCX*, are increased in schizophrenia; the polymorphisms of two synaptic plasticity genes are implicated in this increase. In the APh region, this is highly suggestive of aberrations in neuroplasticity. However, speculation as to the functional implications of abnormal *DCX* expression may be premature. The same applies to the evidence of abnormal *NEUROD1* expression, detected in relation to *DISC1* genotype, though future studies with

larger sample sizes can help clarify some of these effects. The data reported here illuminate the potential mechanisms by which the *DISC1* molecular pathway may mediate risk for psychiatric illness. This could, in the future, directly assist the development of future diagnostic and treatment strategies.

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9 APPENDICES

Appendix A Dentate Gyrus Gene Expression

9-A Table showing recent investigations into dentate gyrus gene expression (pharmacological investigations are shaded grey).

First Author (Year)	Title (Abbreviation)	Adult-NG Contextualisation	Gene	Expression Findings	Study Design
Namba (2011)	NMDA regulates migration of newly generated neurons.	Manual dissection of DG	Mouse Disc1 NDEL1	Memantine (NMDA antagonist): 50mg/kg Body weight (B.W.) Significant reduction in ratio to GAPDH in dose-dependent manner ($p<0.05$) CPP (NMDA antagonist): 10mg/kg B.W. Significant reduction in ratio to GAPDH ($p<0.05$) Memantine: 50mg/kg	qPCR ($\Delta\Delta Ct$); saline control; mice injected at 3 months, killed 1 day later: methamphetamine 2mg/kg B.W. and D-Cycloserine 30mg/kg B.W. produced no significant reduction

First Author (Year)	Title (Abbreviation)	Adult-NG Contextualisation	Gene	Expression Findings	Study Design
				B.W. no significant effect (very slight increase)	
			Girdin	Memantine: 50mg/kg B.W. no significant effect (very slight increase)	
			LIS1	Memantine: 50mg/kg B.W. no significant effect (very slight increase)	
Ray (2011)	Decreased BDNF, trk-B-TK+ and GAD67 mRNA expression	Auto-radiographic visualisation of DG and CA3 from 14µm mid-hippocampal sections	GAD67	DG: 22% reduction in SZ mRNA levels (p=0.022) CA3: No significant effect on SZ mRNA	In-situ hybridization (ISH) w/ autoradiogram: mean optical density (OD) of 3 ROIs in hippocampal formation subfield; Stanley

First Author (Year)	Title (Abbreviation)	Adult-NG Contextualisation	Gene	Expression Findings	Study Design
				levels (p>0.10) Fluphenazine: mg equivalents exerted no significant effect on mRNA levels	Consortium Study
			BDNF	DG: No significant effect on mRNA levels (p>0.20) CA3: No significant effect on mRNA levels (p>0.10) Fluphenazine: mg equivalents exerted no significant effect on mRNA levels	
			trkB-TK+	DG: No significant	

First Author (Year)	Title (Abbreviation)	Adult-NG Contextualisation	Gene	Expression Findings	Study Design
				effect on mRNA levels (p>0.20) CA3: No significant effect on mRNA levels (p>0.10) Fluphenazine: mg equivalents exerted no significant effect on mRNA levels	
Weickert (2008)	Reduced DTNBP1 mRNA	Medial temporal lobe cryo-sectioning, DG and CA3 region of interest sampling	DTNBP1	DG:25% reduction in SZ mRNA levels (p=0.04) CA3: 39% reduction in SZ mRNA levels (p=0.03) Chlorpromazine: Dysbindin-1 mRNA did not correlate with last, daily or lifetime	ISH diagnostic category comparison of auto-radiographic film images using an average of 3 slides per case/controls; SZ cases matched with controls (n=10)

First Author (Year)	Title (Abbreviation)	Adult-NG Contextualisation	Gene	Expression Findings	Study Design
			Spinophilin	administration (p>0.29) DG: Positive correlation with DTNBP1 mRNA expression in DG(p<0.01); ; Trend towards positive correlation with DTNBP1 in CA4 (p=0.07) CA3: No positive correlation with DTNBP1 mRNA expression after pH correction; Trend towards positive	

First Author (Year)	Title (Abbreviation)	Adult-NG Contextualisation	Gene	Expression Findings	Study Design
				correlation with DTNBP1 in the DG (p=0.06)	
			Synaptophysin	CA3: Trend towards positive correlation with DTNBP1 mRNA expression in CA3 (p=0.08)	
Toro (2007)	The NR1 NMDA subunit and BDNF in temporal lobe epilepsy	Autoradiograph film assessment of 3 layers of the DG	NR1	DG: Molecular layer NR1 protein levels significantly higher in TLE sufferers with interictal dysphoria (p=0.01)	Immunohistochemistry and autoradiography; 54 hippocampal tissue samples from patients suffering intractable TLE divided into 4 groups TLE control, TLE w/ SZ-like symptoms, TLE w/ MDD, TLE w/ dysphoria;

First Author (Year)	Title (Abbreviation)	Adult-NG Contextualisation	Gene	Expression Findings	Study Design
Tian (2007)	Immunoreactivity of 43 kDa growth-associated protein	ROI sampling of CA3 and inner molecular layer of DG	GAP-43	DG: Trend towards correlation with SZ and bipolar disorder diagnosis with reduced GAP-43 levels [F(3,52)=2.290, p=0.089] CA3: No association with SZ diagnosis and GAP-43 levels in CA3 stratum radiatum (p=0.308) and stratum oriens (p=0.816)	Immunohistochemistry OD readings; Stanley Consortium Samples
Chambers (2005)	GAP-43 and synaptophysin alterations in the DG	OD of pre-confirmed antibody IR was measured in the dentate gyrus region of the medial human	GAP-43	DG: Reduced GAP-43 in inner molecular layer (p=0.049) but not outer molecular layer (p>0.05)	35 human brains (Harvard Brain Tissue Resource Centre) 14 SZ-sufferers, 14 matched controls, plus

First Author (Year)	Title (Abbreviation)	Adult-NG Contextualisation	Gene	Expression Findings	Study Design
		hippocampus	Synaptophysin	DG: Reduced synaptophysin in inner molecular layer (p=0.003) and outer molecular layer (p=0.002)	7 similarly matched BPD controls, minimum 4 slides used per case
Rioux and Arnold (2005)	The expression of RAR-alpha is increased	Random sampling and optical fractionation of dentate granule cells, morphologically identified from sections of anterior hippocampus after Nissl staining	RARα	Twofold increase in the proportion of RARα-labelled granule cells in SZ (n=16, Z=2.8, P=0.0045)	Immunohistochemistry OD readings; 10 SZ, 11 matched controls; 1 randomly chosen slide analysed per subject
			RARγ1	No significant change in the proportion of RARγ1-labelled granule cells in SZ (n=18, Z=1.6,	

First Author (Year)	Title (Abbreviation)	Adult-NG Contextualisation	Gene	Expression Findings	Study Design
				P=0.1000)	
			RARγ2	No significant change in the proportion of RARγ2-labelled granule cells in SZ (n=19, Z=1.3, P=0.2006)	
			RXRβ	No significant change in the proportion of RXRβ-labelled granule cells in SZ (n=18, Z=0.8, P=0.4140)	
			RXRγ	No significant change in the proportion of	

First Author (Year)	Title (Abbreviation)	Adult-NG Contextualisation	Gene	Expression Findings	Study Design
				RXRγ-labelled granule cells in SZ (n=19, Z=0.3, P=0.7751)	
Pantazopoulos (2004)	Differences in the cellular distribution of D1 receptor mRNA	ROI sampling of DG (4 boxes for Granule cell layer and 2 for CA3)	D1r	CA3: D1 mRNA was decreased 21% in SZ compared to controls (P=0.029); In this region 56% of cells were D1 mRNA-negative, a significant increase when compared to controls (P=0.0008) and BPD (P=0.009)	ISH, relative OD; Harvard Brain issue Resource Centre 16 controls, 16 SZ, 16 BPD plus a comparison group of 6 1st degree SZ relatives and 6 matched controls; 2 sections per case analyzed
Talbot (2004)	Dysbindin-1 is reduced in intrinsic, glutamatergic terminals	ROI analysis of DG and CA2/3 from coronal slabs of intermediate rostro-caudal levels of the HF	Dysbindin-1	CA3: 30% mean neuropil reduction in CA2/3 stratum oriens Dysbindin-1 immunoreactivity	IHC by net OD (ROI minus background); 17 SZ and 17 matched controls, plus Stanley Consortium Sample;

First Author (Year)	Title (Abbreviation)	Adult-NG Contextualisation	Gene	Expression Findings	Study Design
				(W=49.0, P=0.003); 37% mean neuropil reduction in CA2/3 stratum radiatum dysbindin-1 immunoreactivity (W=51.5, P=0.0001); no dysbindin-1 reduction in parent cells of intrinsic glutamatergic input to DGiml in SZ DG: 42% mean neuropil reduction in DGiml dysbindin-1 immunoreactivity in SZ (W=71.5, P=0.0003); 14% Dysbindin-1 reduction	male C3H control mice subcutaneously implanted with pellet (containing polymer or polymer + haloperidol released as 2mg/kg per day), killed 10 weeks later for IHC

First Author (Year)	Title (Abbreviation)	Adult-NG Contextualisation	Gene	Expression Findings	Study Design
				in parent cells of intrinsic glutamatergic input to DGiml in SZ(W=45.5, P=0.03), correlating with neuropil reductions (Spearman r=0.43, P=0.01); Stanley Consortium SZ cases exhibited a mean reduction in DGiml Dysbindin-1 of 24.9% (W=37.0, P=0.035) Haloperidol: No significant affect of chronic treatment on Dysbindin-1 (P=0.18) immunoreactivity in DGiml	

First Author (Year)	Title (Abbreviation)	Adult-NG Contextualisation	Gene	Expression Findings	Study Design
			VGlut-1	DG: 84% mean increase in SZ (W=47.0, P=0.01); correlating inversely with Dysbindin-1 immunoreactivity (Spearman r=-0.61, P0.0002)	
			Haloperidol	No significant affect of chronic treatment on VGluT-1 (P=0.20) immunoreactivity in DGiml	
Nowakowski (2002)	Reduction of chromogranin B-like immunoreactivity	Immuno-staining density assessment of dentate gyrus and CA3 from coronal sections of	Chromogranin B	DG: Significant reduction in Chromogranin B immunoreactivity	IHC (OD values, 10 3µm sections per brain); 17 SZ, 16 control

First Author (Year)	Title (Abbreviation)	Adult-NG Contextualisation	Gene	Expression Findings	Study Design
		hippocampus	Synapsin I	(inner ml p<0.05; outer ml P<0.05) CA3: Significant reduction in Chromogranin B immunoreactivity (P<0.05) DG: Significant reduction in Synapsin I immunoreactivity (inner ml p<0.05; outer ml P<0.05) CA3: Significant reduction in Synapsin I immunoreactivity	

First Author (Year)	Title (Abbreviation)	Adult-NG Contextualisation	Gene	Expression Findings	Study Design
				(P<0.01)	
Ilia (2002)	Expression of Oct-6, a POU III domain TF	Oct-6 is a POU-III domain transcription factor active developmentally;	Oct-6	DG: immunoreactivity in a subset of neurons in the gcl (granule cell layer) in dramatic contrast with comparison control specimens; high levels of temporal lobe western blot immuno-staining in SZ, compared to very little or no staining in matched controls CA3: clear subset of pyramidal neurons were immunoreactive whereas sections from the hippocampal	IHC: Immuno-staining of sections of hippocampal region, 10 SZ 10 matched controls; WB: Blots of extracts from APh region, WB 3 additional SZ 3 additional matched controls

First Author (Year)	Title (Abbreviation)	Adult-NG Contextualisation	Gene	Expression Findings	Study Design
				region of 10 control comparison subjects failed to show any staining; high levels of temporal lobe western blot immuno-staining in SZ, compared to very little or no staining in matched controls	
Heckers (2002)	Differential hippocampal expression of GAD65 and 67 mRNA	Microscopic image analysis of hippocampal sections, outlining sectors including DG and CA2/3	Glutamic Acid Decarboxylase (GAD)	DG+CA3: No significant decrease in the numerical density of GAD_{65} +ve neurons in SZ ($F_{1,14}$=1.57; P=0.23) No significant decrease in the numerical density of GAD_{67} +ve neurons in	ISH w/ microscopic grain counting, 2 10um sections per subject (inter-slide variability controlled) 15 SZ, 15 BPD, 15 matched con; BPD results proved significant

First Author (Year)	Title (Abbreviation)	Adult-NG Contextualisation	Gene	Expression Findings	Study Design
				SZ ($F_{1,14}$=0.16; P=0.70) No significant decrease in the cellular expression of GAD_{65} or GAD_{67} +ve neurons in SZ ($F_{1,14}$=1.65; P=0.22 and ($F_{1,14}$=0.005; P=0.95 respectively)	
Webster (2002)	Regional specificity of brain glucocorticoid receptor mRNA alterations	Mean optical density with ROIs including DG gcl and CA3 from mid hippocampal sections	Glucocorticoid receptor (GR)	DG: Significant reduction in GR mRNA mean values (P < 0.01, -43%) CA3: Significant reduction in GR mRNA mean values (P < 0.01, -42%)	ISH, calibrated densitometric image analysis of autoradiograms from 14um sections; Stanley Consortium Sample

First Author (Year)	Title (Abbreviation)	Adult-NG Contextualisation	Gene	Expression Findings	Study Design
Law (2002)*	Asymmetrical reductions of hippocampal NMDAI	OD measurements taken over DG and CA3	NMDA receptor subunit 1 (NMDAR1)	DG: Significant left-sided reductions in NMDAR1 mRNA in SZ (-61%; p=0.005) but not in right side (-26%, p=0.16), hypothesis-testing showed SZ diagnosis × side interaction not significant (F=3.59; df=1; p=0.06) and reduction remained significant when expressed as a ratio of the significant poly A mRNA reductions seen in this region (p<0.02) CA3: No significant	ISH, densitometric analysis of autoradiographs; Stanley Consortium Study

First Author (Year)	Title (Abbreviation)	Adult-NG Contextualisation	Gene	Expression Findings	Study Design
				overall reduction in NMDAR1 mRNA in SZ (p=0.07), though significant reductions in left side (p=0.04)	
Fatemi (2000)	Reduction in Reelin immunoreactivity in hippocampus	Immuno-staining of ventral hippocampal sections delineated 5 boundary regions including DG	Reelin	DG: significant reductions in mean reelin positive cell numbers in SZ (-42%, P<0.05) Significant reductions adjusted cell densities (reelin +ve cell counts taking into account changes in areas measured in controls) in SZ (ANOVA, P<0.001)	qIHC, cell counting from 2-4 14um sections per subject; Stanley Consortium Study

First Author (Year)	Title (Abbreviation)	Adult-NG Contextualisation	Gene	Expression Findings	Study Design
				CA3: No significant reduction seen in alveus and whole stratum oriens or whole strata pyramidale, radiatum and lucidum	
Crook (2000)	Decreased muscarinic receptor binding in subjects with SZ	Computerized image analysis of all hippocampal subfields	Muscarinic receptor binding (M1 and M4)	DG: significant reductions in [^{3}H]pirenzepine binding in SZ ($p<0.005$) CA3: significant reductions in [^{3}H]pirenzepine binding in SZ ($p<0.0005$)	q-autoradiography, 4 20um slides per subject from left-side hippocampal formation; 15 SZ, 18 con
:Young (1998)	SNAP-25 deficit and	OD density reading of	SNAP-25	DG: Significant	qIHC, image analysis

First Author (Year)	Title (Abbreviation)	Adult-NG Contextualisation	Gene	Expression Findings	Study Design
	hippocampal connectivity in SZ	hippocampal ROIs incl. DG and CA3	Synaptophysin	reduction in gcl immuno-staining using 10× microscope (percentage area × mean pixel intensity) in SZ (F=14.79, P=0.001) When covaried by synaptophysin immuno-staining, less detailed images showed significant differences between SZ and controls (outer ml: F=14.46, P=0.002) (gcl: F=18.65, P=0.0005) CA3: reductions in synaptophysin	;Frozen samples: 13 SZ 13con, FFPE 12 SZ 12 con

First Author (Year)	Title (Abbreviation)	Adult-NG Contextualisation	Gene	Expression Findings	Study Design
				(P<0.05) did not remain significant after covariance with age and PMI DG: Significant increase in gcl immuno-staining using 10× microscope (percentage area × mean pixel intensity) in SZ (F=7.29, P=0.02)	
Dean (1997)	Changes in PKC and adenylate cyclase in the temporal lobe	Sections taken from hippocampus and DG and CA regions analysed	PKC ([^{3}H]phorbol ester binding)	DG: Trend towards significant decrease in SZ (p=0.06) CA3: no difference in whole CA between SZ	Radioligand assays, total minus non-specific binding; 20 SZ male 20 con male, left brain, 3 20um slides per subject,

First Author (Year)	Title (Abbreviation)	Adult-NG Contextualisation	Gene	Expression Findings	Study Design
				and con (p>0.05)	
			AC ([^{3}H]forskolin binding)	DG: Significant decrease in SZ (p<0.05) CA: no difference in whole CA between SZ and con (p>0.05)	
Porter (1997)	Distribution of kainite receptor subunit mRNAs in human hippocampus	Sections taken from mid-hippocampus, image analysis of regions including DG and CA3	KA2	DG: reduction in SZ mRNA levels (P<0.05) CA3: reduction in SZ mRNA levels (P<0.01)	ISH, densitometric readings from autoradiograms; 11 SZ, 13 con; 3-4 10uM sections per subject
			GluR6	DG: reduction in SZ mRNA levels (P<0.05) CA3: reduction in SZ	

First Author (Year)	Title (Abbreviation)	Adult-NG Contextualisation	Gene	Expression Findings	Study Design
				mRNA levels (P<0.05)	
Eastwood (1995)	Decreased synaptophysin in the medial temporal lobe	Optical density readings from hippocampal subfield regions	Synaptophysin	DG: significant reductions in right side ml of SZs when compared to control right side ml (P<0.05); intra-group asymmetry (greater synaptophysin signal in the right side ml when compared to the left in controls, P=0.034) not seen in SZ	Immuno-autoradiography densitometric quantification of 10um medial temporal lobe sections, SZ=11, matched con=14
Freedman (1995)	Evidence in post-mortem brain tissue for decreased numbers of hippocampal nicotinic receptors	Percentage area labelling analysis in DG and CA3	Nicotinic cholinergic receptors ([^{125}I] -α-bungarotoxin binding)	DG: Significant dentate hilus reductions in SZ (Tukey's HSD, df=42, p<0.01)	Autoradiography, computer algorithm determination of labelling; 8 SZ 8 con, 1 slide per subject

First Author (Year)	Title (Abbreviation)	Adult-NG Contextualisation	Gene	Expression Findings	Study Design
				CA3: Significant reductions in SZ (Tukey's HSD, df=42, $p<0.05$)	
Eastwood (1995)	Decreased expression of mRNAs encoding non-NMDA glutamate receptors	Separate measurements taken over DG and CA3	GluR1	DG:47% (^{35}S nCi/g tissue equivalent) reduction in GluR1 mRNA in SZ ($P<0.01$) CA3: 62% (^{35}S nCi/g tissue equivalent) reduction in GluR1 mRNA in SZ ($P<0.01$) 45% Reduced GluR1 mRNA abundance per neuron ($P<0.01$) DG:50% (^{35}S nCi/g	ISH, film autoradiography OD readings from 18uM slides (3 slides per subject); 9 SZ, 14 matched con

First Author (Year)	Title (Abbreviation)	Adult-NG Contextualisation	Gene	Expression Findings	Study Design
				tissue equivalent) reduction in GluR2 mRNA in SZ (P<0.05)	
			GluR2	CA3: 51% (^{35}S nCi/g tissue equivalent) reduction in GluR2 mRNA in SZ (P<0.02) Approx. 40-45% Reduced GluR2 mRNA abundance per neuron (P<0.01)	
Lexow (1994)*	Alterations in TRH receptors in temporal lobe of schizophrenics	Visualisation of receptor-binding distribution throughout hippocampus	TRH (thyrotrophin-releasing hormone) receptor ([^{3}H]MeTRH binding)	DG: significant increased specific binding in molecular layer between SCZ and their control, HD, ALS and AD	qAutoradiography; 6 SZ, 9 con, 4 HD, 4 AD, 4 ALS; sequential slides were used for each paired total and nonspecific binding

First Author (Year)	Title (Abbreviation)	Adult-NG Contextualisation	Gene	Expression Findings	Study Design
				counterparts (P<0.003 by Dunnett's multiple comparison test) CA3: the density of receptor-binding was not significantly altered	determination
Kerwin (1990)*	Quantitative auto-radiographic analysis of glutamate binding sites	Regional analysis of hippocampal region (incl. DG and CA3)	Kainate-r ([3H]KA) NMDAr	DG: significant bilateral reductions in specific binding seen in SZ (P<0.01) CA3: significant bilateral reductions in specific binding seen in SZ (P<0.01) DG: no significant	qAutoradiography, specific binding film density analysis, 3 slides per subject;7 SZ 8 con

First Author (Year)	Title (Abbreviation)	Adult-NG Contextualisation	Gene	Expression Findings	Study Design
			([3H]glutamate binding) NMDA Quisqualate site ([3H]CNQX binding)	reductions in specific binding seen in SZ CA3: no significant reductions in specific binding seen in SZ DG: no significant reductions in specific binding seen in SZ CA3: significant left-sided reductions in specific binding seen in SZ (P<0.05)	
Kiuchi (1989)*	Benzodiazepine receptors increase in post-mortem brain of SCZ	Analysis of homogenised sections of dentate gyrus and CA1-3	Benzodiazepine receptor ([^{3}H]-FNT binding)	DG: specific binding tended towards an increase but not significant (P>0.05)	Radio-receptor assays of membrane homogenates; 10 con, 13 SZ

First Author (Year)	Title (Abbreviation)	Adult-NG Contextualisation	Gene	Expression Findings	Study Design
				CA3: specific binding in CA1-3 was significantly greater in SZ than in controls	
Scarr (2007)	Altered Hippocampal Muscarinic M4, but not M1, receptor expression	Specific binding readings taken from DG, CA1-3 and Sub (Sub in pirenzepine binding only)	Muscarinic receptor binding (M1 and M4)	DG: decrease in [3H]pirenzepine binding in tissue from subjects with SZ in granular molecular layer (t=2.81, df=35, p=0.008) and polymorphic layer (t=2.69, df=35, p=0.01) CA3: decrease in [3H]pirenzepine binding in tissue from subjects with SZ	qAutoradiography; 20 SZ 20 con, 20um sections from left hemisphere hippocampal blocks, (ISH: 15 SZ/con, 12 10um section)

First Author (Year)	Title (Abbreviation)	Adult-NG Contextualisation	Gene	Expression Findings	Study Design
			Muscarinic receptor mRNA (M1 and M4)	(t=3.13, df=35, p=0.004) DG AND CA3: significant reductions in M4 mRNA seen in across whole sections (F=5.22, df=1,216, p=0.02) but no significant variation in the levels of M4 mRNA in any individual region analysed (p=0.135-0.324)	
Perlman (2005)	Alteration in oestrogen receptor alpha mRNA	OD measurements taken from DG and CA3	ERα	DG: 18.5% reduction in mRNA expression in SZ (p=0.0121) CA3: non-significant	ISH, auto-radiographic calibrated densitometric image analysis; Stanley consortium study, 2 14um sections per

First Author (Year)	Title (Abbreviation)	Adult-NG Contextualisation	Gene	Expression Findings	Study Design
				trend of 9.4% reduction in mRNA expression in SZ Fluphenazine: Significant negative correlation of mRNA density with drug exposure found in CA3 (r=-0.55, p=0.03) but this was recapitulated with duration of illness (r=-0.55, p=0.03)	subject
Altar (2005)	Deficient hippocampal neuron expression	Laser captured dentate granule neurons		DG: decreases in the expression of genes encoding energy metabolism, mitochondrial and neuronal functions and protein	Microarray from qPCR, 2 SZ cohorts (8 SZ 9 con, 14 SZ 15 con)

First Author (Year)	Title (Abbreviation)	Adult-NG Contextualisation	Gene	Expression Findings	Study Design
				processing by ubiquitin and the proteasome	
Zavitsanou (2007)	Effects of typical and atypical antipsychotics on rat brain muscarinic receptors	Regional analysis of 14um brain sections	Muscarinic receptors (M1/M4) ([^{3}H]pirenzepine binding)	Main effect of treatment in DG (F=4.479, df=6, P0.002) Haloperidol (typical antipsychotic): 37% increase in DG [^{3}H]pirenzepine binding in animals sacrificed 2h after LDA (P=0.001) Clozapine (atypical antipsychotic): 29% increase in DG	Autoradiography; Female Sprague Dawley rats given pellets of haloperidol (2mg/kg/day, n=10), clozapine (1.5mg/kg/day, n=10), olanzapine (1.2mg/kg/day, n=10) or vehicle for 36 days and killed (sodium pentobarbitone, 120mk/kg i.p.) 2h or 48h after last drug administration

First Author (Year)	Title (Abbreviation)	Adult-NG Contextualisation	Gene	Expression Findings	Study Design
			M2/M4 ([^{3}H]AF-DX384 binding)	[^{3}H]pirenzepine binding in animals sacrificed 2h after LDA (P=0.022) Olanzapine (atypical antipsychotic): No significant effect in DG (P>0.05) Haloperidol (typical antipsychotic): No significant effect in DG (P>0.05) Clozapine (atypical antipsychotic): No significant effect in	

First Author (Year)	Title (Abbreviation)	Adult-NG Contextualisation	Gene	Expression Findings	Study Design
				DG (P>0.05) Olanzapine (atypical antipsychotic): No significant effect in DG (P>0.05)	
Terry (2006)	Time-dependent effects of haloperidol and ziprasidone	Regional OD analysis of 20um interaural sections	NGF	Haloperidol: Significant increase in NGF immunoreactivity in DG after 7days (p<0.01) and 14days (p<0.01) administration Significant decrease in NGF immunoreactivity in DG after 45days (p<0.05) and 90days (p<0.001)	IHC and qAutoradiography; Male albino Wistar rats treated with Haloperidol (2mg/kg/day), Zip (12mg/kg/day) or vehicle dissolved in 0.1M acetic acid and diluted (1:100) in water for 7, 14, 45 or 90 days then sacrificed 2 weeks after last drug exposure

First Author (Year)	Title (Abbreviation)	Adult-NG Contextualisation	Gene	Expression Findings	Study Design
				administration Significant increase in NGF immunoreactivity in CA3 after 7days ($p<0.01$) and 14days ($p<0.01$) administration Significant decrease in NGF immunoreactivity in CA3 after 45days ($p<0.001$) and 90days ($p<0.001$) administration Zipra: Significant increase in NGF immunoreactivity in DG after 7days	

First Author (Year)	Title (Abbreviation)	Adult-NG Contextualisation	Gene	Expression Findings	Study Design
				(p<0.01) and 14days (p<0.05) administration Significant decrease in NGF immunoreactivity in DG after 90days (p<0.001) administration Significant increase in NGF immunoreactivity in CA3 after 7days (p<0.01) and 14days (p<0.01) administration Significant decrease in NGF immunoreactivity in CA3 after 90days (p<0.001)	

First Author (Year)	Title (Abbreviation)	Adult-NG Contextualisation	Gene	Expression Findings	Study Design
			ChAT	administration Haloperidol: Significant increase in ChAT immunoreactivity in DG after 7days ($p<0.05$) and 14days ($p<0.05$) administration Significant decrease in ChAT immunoreactivity in DG after 45days ($p<0.05$) and 90days ($p<0.001$) administration Significant increase in	

First Author (Year)	Title (Abbreviation)	Adult-NG Contextualisation	Gene	Expression Findings	Study Design
				ChAT immunoreactivity in CA3 after 7days ($p<0.01$) and 14days ($p<0.01$) administration Significant decrease in ChAT immunoreactivity in CA3 after 45days ($p<0.01$) and 90days ($p<0.001$) administration Zpi: Significant increase in ChAT immunoreactivity in DG after 7days ($p<0.05$) and 14days ($p<0.05$)	

First Author (Year)	Title (Abbreviation)	Adult-NG Contextualisation	Gene	Expression Findings	Study Design
				administration Significant decrease in ChAT immunoreactivity in DG after 90days ($p<0.001$) administration Significant increase in ChAT immunoreactivity in CA3 after 7days ($p<0.01$) and 14days ($p<0.05$) administration Significant decrease in ChAT immunoreactivity in CA3 after 90days	

First Author (Year)	Title (Abbreviation)	Adult-NG Contextualisation	Gene	Expression Findings	Study Design
				(p<0.001) administration	
			Nicotinic receptors: α_7 (^{125}I-bungarotoxin) and α_4/β_2 ([^{3}H]epibatidine)	No significant differences in binding associated with different antipsychotic treatments in DG and CA3 (p>0.05)	
			Muscarinic receptors: M_2 receptor ([^{3}H]AF-DX384 binding) and M1/M4 ([^{3}H]pirenzepine binding)	No significant differences in binding associated with different antipsychotic treatments in DG and CA3 (p>0.05)	

First Author (Year)	Title (Abbreviation)	Adult-NG Contextualisation	Gene	Expression Findings	Study Design
Park (2006)	Effects of quetiapine on the BDNF expression	Area of interest OD analysis of 10-20um sections	BDNF	Quetiapine: significant increase in BDNF mRNA levels in DG ($p<0.01$) No significant effect on BDNF mRNA levels in CA3 In both regions immobilization stress produced a decrease ($p<0.01$) which was rescued by drug administration ($p<0.01$)	ISH, 10 section per animal; Male Sprague-Dawley rats treated with vehicle (0.8% glacial acetic acid, 1ml/kg i.p.) or quetiapine (10mg/kg i.p.) both with and without immobilization stress for 3 weeks and sacrificed 24h after last treatment
Terry (2005)	Chronic exposure to typical or atypical antipsychotics in rodents	Regional analysis of 16um sagittal sections DISCREPANCY BETWEEN BINDING TABLE AND WRITTEN	Nicotinic receptors: α_7 (^{125}I-bungarotoxin)	Risperidone: Significant slight reductions in ventral DG binding density after 90 days of drug	qAutoradiography; Male albino Wistar rats treated with Haloperidol (2mg/kg/day), chlorpromazine

First Author (Year)	Title (Abbreviation)	Adult-NG Contextualisation	Gene	Expression Findings	Study Design
		RESULTS SECTION REGARDING 90 DAY RIS TREATMENT (ventral DG or polymorphic DG)		treatment (P<0.01) After 180 days of treatment significant reductions in medial CA2/CA3 (P<0.01) and lateral DG (12%, P<0.01) Olanzapine: Significant slight reductions in ventral DG binding density after 90 days of drug treatment (P<0.01) Other drugs: No significant differences of binding levels in HAL, CPZ or OPZ	(10mg/kg/day), risperidone (2.5mg/kg/day) or olanzapine (10mg/kg/day) orally in drinking water for 90 or 180 days + a 2 week washout period then killed

First Author (Year)	Title (Abbreviation)	Adult-NG Contextualisation	Gene	Expression Findings	Study Design
				treatments groups at either 90 or 180 days of treatment	
Parikh (2004)	Modulation of nerve growth factor and choline acetyltransferase expression	Regional OD analysis of 20um (40um for ChAT IHC) coronal interaural sections	NGF	Haloperidol: Significant reductions in mean OD of stained cells in the DG (1mg/kg/day, -18%, P<0.01; 2mg/kg/day, -32%, P<0.001) and CA3 (1mg/kg/day, -24%, P<0.01; 2mg/kg/day, -39%, P<0.001) Risperidone: No significant effect on mean OD values	qIHC; Male albino Wistar rats treated with Haloperidol (1/2mg/kg/day), Risperidone (1.25/2.5mg/kg/day), Olanzapine (5/10mg/kg/day) or vehicle dissolved in 0.1M acetic acid diluted (1:100) in water for 45 days

First Author (Year)	Title (Abbreviation)	Adult-NG Contextualisation	Gene	Expression Findings	Study Design
			ChAT	Olanzapine: Significant increases in mean OD of stained cells in the DG (5mg/kg/day, 14%, P<0.05; 10mg/kg/day, 18%, P<0.05) and CA3 (5mg/kg/day, 19%, P<0.05; 10mg/kg/day, 22%, P<0.05) Haloperidol: Significant reductions in fibre pixel density of stained neurons in DG (1mg/kg/day, -24%, P<0.05; 2mg/kg/day, -34%, P<0.01) and CA3	

First Author (Year)	Title (Abbreviation)	Adult-NG Contextualisation	Gene	Expression Findings	Study Design
				(1mg/kg/day, -20%, P<0.05; 2mg/kg/day, -28%, P<0.01) Risperidone: No significant effect on fibre pixel densities Olanzapine: Significant increases in fibre pixel density of stained neurons in DG (5mg/kg/day, 16%, P<0.05; 10mg/kg/day, 18%, P<0.05) and CA3 (5mg/kg/day, 17%, P<0.05; 10mg/kg/day, 34%, P<0.01)	

First Author (Year)	Title (Abbreviation)	Adult-NG Contextualisation	Gene	Expression Findings	Study Design
Lipska (2001)	BDNF mRNA expression in rat hippocampus and prefrontal cortex	Regional analysis of DG and CA3 in 20um sections	BDNF	Haloperidol: Acute treatment produced significant reduction in DG (21%, 0.5mg/kg/day) Chronic treatment produced significant reductions in DG (19%, 0.5mg/kg/day; 33%, 1mg/kg/day) Chronic treatment produced significant reductions in CA3 (both 0.5 and 1 mg/kg/day, $P<0.05$), acute treatment produced no significant effect in CA3	ISH and qAutoradiography, Male Sprague-Dawley pups give drug regime for 28 days (Acute = full dose given on 28th day),

First Author (Year)	Title (Abbreviation)	Adult-NG Contextualisation	Gene	Expression Findings	Study Design
				Clozapine (10mg/kg/day): Acute treatment produced significant 55% reduction in DG Chronic treatment produced significant 30% reduction in DG No significant reductions in CA3	
Tarazi (1996)*	Regulation of ionotropic glutamate receptors	OD regional analysis of 16um coronal sections	NMDA-R (^{3}H-MK801 binding) / AMPA-R (^{3}H-CNQX binding) / Kainite-R (^{3}H-kainic acid binding)	SCH23390: sub-chronic treatment showed a trend towards an increase in MK801 binding in the hippocampal formation reaching statistical significance	In vitro qAutoradiography; Male Sprague-Dawley rats treated with haloperidol (1.5mg/kg/day), raclopride (10mg/kg/day), clozapine

First Author (Year)	Title (Abbreviation)	Adult-NG Contextualisation	Gene	Expression Findings	Study Design
				in the DG [+30, F=16.3, P<0.005 (10 df)]; no significant effects on receptor binding in CA3/DG of chronic treatment Clozapine: No significant effects on receptor in CA3/DG of chronic or sub-chronic treatment Raclopride: No significant effects on receptor binding in CA3/DG of chronic or sub-chronic treatment	(25mg/kg/day) or SCH23390 (0.5mg/kg/day) in drinking water (SCH23390 given subcutaneously) for 28 days (sub-chronic) or 8 months (chronic), animals decapitated immediately after LDA

First Author (Year)	Title (Abbreviation)	Adult-NG Contextualisation	Gene	Expression Findings	Study Design
				Haloperidol: No significant effects on receptor binding in CA3/DG of chronic or sub-chronic treatment	
Ossowska (2011)	Chronic treatments with haloperidol and clozapine alter the level of NMDA-R1 mRNA in the rat brain	Regional analysis of hippocampal formation subfields in 10um coronal sections	NMDA-R1 mRNA (radio-labelled oligonucleotide binding)	Haloperidol: elevations in the OD levels were observed in DG, CA3 and CA1; however, only in the CA1 region were these significant Clozapine: No changes in OD levels were observed across any hippocampal formation subfields	Auto-radiographic analysis, 10 Male Wistar rats, treated with either haloperidol (1mg/kg/day), clozapine (30mg/kg/day) or drinking for 3 months then sacrificed 5 days after LDA, a inter-hemispheric mean was used
Abe (2001)	Effects of single and	Regional analysis of	mGluRs 1-5 (radio-	PCP: an almost 20%	Autoradiography, Male

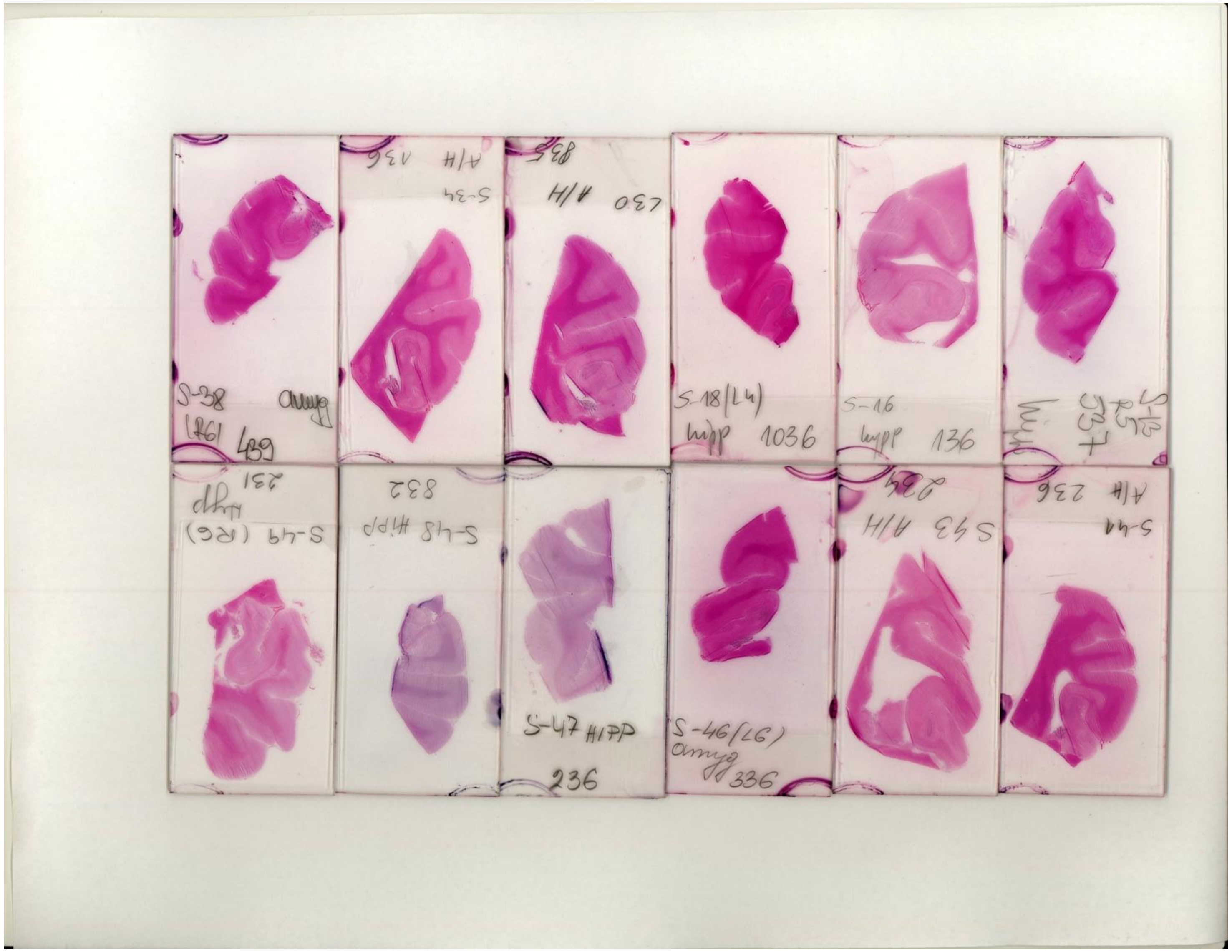
First Author (Year)	Title (Abbreviation)	Adult-NG Contextualisation	Gene	Expression Findings	Study Design
	repeated phencyclidine administration on the expression of metabotropic glutamate receptor	OD fro DG and CA3	labelled oligonucleotide binding)	decrease in mGluR5 mRNA binding observed in DG (p=0.004) and CA3 (p0.002) 1hr after single administration; decreases in mGluR5 and mGluR2 binding 24hr after a single administration and mGluR5 after 14 days administration were not significant	Wistar rats, treated with daily i.p. injections of PCP (7.5mg/kg) for either 1 day and sacrificed 1 hr after LDA or 14 days and sacrificed 24hr after LDA, saline was used as a control
Numachi (2004)	Psychostimulant alters expression of DNA Methyltransferase (Dnmt) mRNA in the Rat Brain	OD readings from 14mm coronal sections	Dnmt2	MAP: 27-39% mRNA-binding reductions seen in DG (P<0.01), CA3 (P<0.0001) and CA1 (P<0.0001) 24hr after drug administration	ISH, Male Wistar Rats (7 wks old)) injected w/ MAP hydrochloride (4mg/kg) and sacrificed 0, 1, 3, 9, and 24hr after drug administration

First Author (Year)	Title (Abbreviation)	Adult-NG Contextualisation	Gene	Expression Findings	Study Design
			Reelin	MAP: significant mRNA reductions were only observed in the frontal cortex 3hr after administration (P<0.03)	
Hammonds (2009)	Effects of 4-week Treatment with Lithium and Olanzapine on Levels of BDNF, Bcl2 and phosphorylated CREB	Manual dissection of DG	BDNF	Lithium: 4-week treatment increased levels in DG (P=0.016) Olanzapine: 4week treatment increased levels in DG (P=0.054)	ELISA, Male Sprague Dawley rats, Li-carbonate pellets (0.24%), olanzapine in tap water (1.5-2mg/kg) or control water and pellets for 4 weeks,
			Bcl-2	Lithium: 4-week treatment increased	

First Author (Year)	Title (Abbreviation)	Adult-NG Contextualisation	Gene	Expression Findings	Study Design
				levels in DG (P<0.001) and CA1 (P=0.025) Olanzapine: 4-week treatment increased levels in DG (P<0.001) and (P<0.001)	
			pCREB	Lithium: 4-week treatment increased levels in DG (P=0.001) and CA1 (P=0.001) Olanzapine: 4-week treatment increased levels in DG (P=0.002) and CA1 (P=0.003)	

Appendix B Anterior Hippocampus Coronal Sections

Scans of haematoxylin and eosin stained slides of anterior hippocampus coronal sections (provided by the Stanley Consortium).

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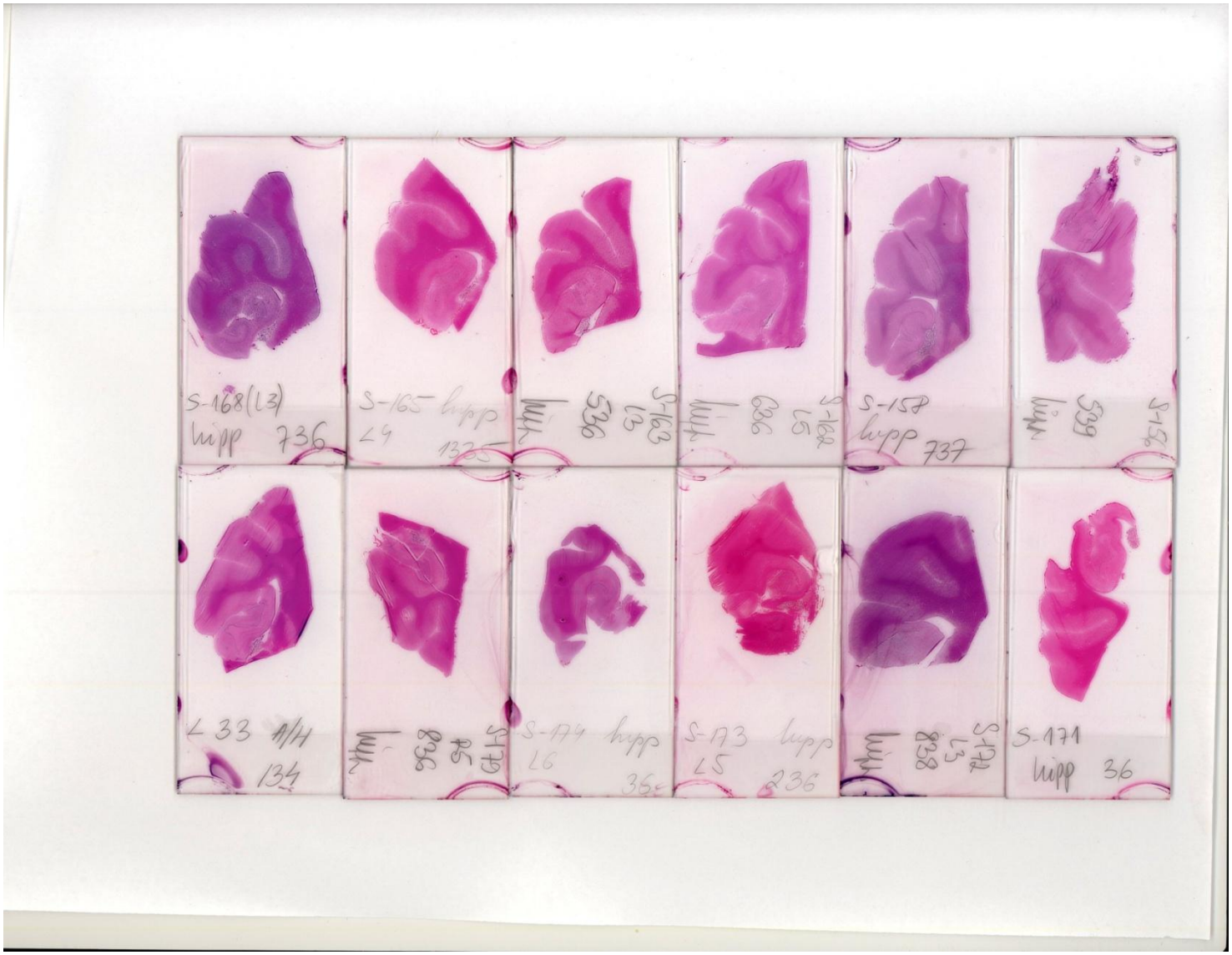
S-64 HIPP 236
S-60(R5) hipp 736
S-59 hipp 739
S-83 HIPP 536
S-82 hipp 134
S-81 hipp 134
S-25 hipp 437
S-73 HIPP 636
S-72 hipp 837


S-93 HMP 137
S-92 hipp 35
S-91 hipp 332
S-89 hipp 633
S-88 hipp 935
S-85 hipp 34
S-116 (RG) hipp 633
S-103 hipp 139
S-101 hipp 336
S-100 hipp 33
S-99 (RG) hipp


S-128 hypp 739
S-123 (RG) hypp 135
S-120 (RG) hypp 336
S-118 hypp 337
S-149 hypp 236
S-147 L5 hypp
S-141 837
S-136 837
S-135 hypp L5 437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S-165 lupp
L4 133
Lun
S-163
L3
536
Lun
S-162
L5
836
S-158
lupp 737
Lun
539
S-156
L 33 11/11
134
Lun
836
S-174 lupp
L6
365
S-173 lupp
L5 236
Lun
838
S-171
lupp 36

Appendix C Parallel Outlier Analyses

9-B Table of the Spearman's rank correlations of demographic and clinical variables with the gene expression normalised relative quantities (italic headings) in the anterior parahippocampal region (APh), the non-neurogenic hippocampal formation (HF) and protein density levels from sections of anterior hippocampus. Asterisk indicates p < 01, double asterisk indicates, p < 05

Continuous Variables: Non-Parametric Correlations (w/ Outliers Incl.)

		DCX (APh)	*DPYSL2 (APh)*	*FEZ1 (APh)*	*NDEL1 (APh)*	*NEUROD1 (APh)*	*PDE4B (APh)*	*DCX (non-neurogenic HF)*	*DPYSL2 (non-neurogenic HF)*	*FEZ1 (non-neurogenic HF)*	*NDEL1 (non-neurogenic HF)*	*NEUROD1 (non-neurogenic HF)*	*PDE4B (non-neurogenic HF)*	Absolute NEUROD1 (APh Grey Matter)	Absolute NEUROD1 (APh White Matter)	Absolute NEUROD1 (Dentate Gyrus)	Relative FEZ1 (APh Grey Matter)	Relative FEZ1 (APh White Matter)	Relative FEZ1 (Dentate Gyrus)
qPCR Plate (APh)	Correlation Coefficient	-.390*	-.359*	-.229	-.166	-.022	-.404*	-.150	-.191	-.368	-.704**	.446	.479	.097	-.202	.280	-.016	.048	.190
	P (2-tailed)	.021	.034	.185	.341	.905	.016	.626	.532	.215	.007	.127	.098	.611	.284	.167	.929	.794	.305
	N	35	35	35	35	32	35	13	13	13	13	13	13	30	30	26	32	32	31
RQI (APh)	Correlation Coefficient	-.508**	-.172	-.357*	-.620**	-.077	-.314	-.278	.179	.110	.168	.085	-.391	.129	-.002	.268	.234	.148	.248
	P (2-tailed)	.002	.331	.038	.000	.682	.071	.358	.559	.720	.584	.782	.187	.506	.990	.195	.205	.426	.186
	N	34	34	34	34	31	34	13	13	13	13	13	13	29	29	25	31	31	30
qPCR Plate (non-neurogenic HF)	Correlation Coefficient	-.280	.361	.671*	-.462	-.265	.711**	.677**	-.119	-.184	-.089	-.612*	.251	-.226	.365	-.378	-.464	.040	-.144

Continuous Variables: Non-Parametric Correlations (w/ Outliers Incl.)

		DCX (APh)	*DPYSL2 (APh)*	*FEZ1 (APh)*	*NDEL1 (APh)*	*NEURO D1 (APh)*	*PDE4B (APh)*	*DCX (non-neurogenic HF)*	*DPYSL2 (non-neurogenic HF)*	*FEZ1 (non-neurogenic HF)*	*NDEL1 (non-neurogenic HF)*	*NEURO D1 (non-neurogenic HF)*	*PDE4B (non-neurogenic HF)*	Absolute NEURO D1 (APh Grey Matter)	Absolute NEURO D1 (APh White Matter)	Absolute NEURO D1 (Dentate Gyrus)	Relative FEZ1 (APh Grey Matter)	Relative FEZ1 (APh White Matter)	Relative FEZ1 (Dentate Gyrus)
	P (2-tailed)	.355	.226	.012	.112	.405	.006	.003	.649	.480	.733	.012	.332	.437	.200	.183	.061	.880	.582
	N	13	13	13	13	12	13	17	17	17	17	16	17	14	14	14	17	17	17
RQI (non-neurogenic HF)	Correlation Coefficient	.052	.041	.044	.122	-.232	.177	-.236	-.303	-.494*	.004	-.033	-.403	.210	.454	-.007	.394	.022	.049
	P (2-tailed)	.865	.893	.886	.692	.467	.563	.361	.237	.044	.989	.905	.109	.470	.103	.982	.118	.933	.851
	N	13	13	13	13	12	13	17	17	17	17	16	17	14	14	14	17	17	17
age at death (years)	Correlation Coefficient	-.001	.161	.128	.128	-.121	.140	-.080	-.075	-.223	-.428	-.028	-.004	.088	.144	-.098	-.015	-.260	-.066
	P (2-tailed)	.995	.355	.462	.465	.510	.422	.761	.775	.389	.087	.918	.989	.576	.357	.551	.920	.068	.654
	N	35	35	35	35	32	35	17	17	17	17	16	17	43	43	39	50	50	49
age of onset of disease (years)	Correlation Coefficient	.154	-.044	-.233	.182	.142	.045	-.585*	-.401	-.348	-.070	.186	-.552*	.034	.020	.111	-.035	.144	-.198
	P (2-tailed)	.378	.802	.177	.294	.437	.796	.014	.111	.171	.791	.491	.022	.829	.899	.501	.810	.317	.173
	N	35	35	35	35	32	35	17	17	17	17	16	17	43	43	39	50	50	49

Continuous Variables: Non-Parametric Correlations (w/ Outliers Incl.)

		DCX (APh)	*DPYSL2 (APh)*	*FEZ1 (APh)*	*NDEL1 (APh)*	*NEUROD1 (APh)*	*PDE4B (APh)*	*DCX (non-neurogenic HF)*	*DPYSL2 (non-neurogenic HF)*	*FEZ1 (non-neurogenic HF)*	*NDEL1 (non-neurogenic HF)*	*NEUROD1 (non-neurogenic HF)*	*PDE4B (non-neurogenic HF)*	Absolute NEUROD1 (APh Grey Matter)	Absolute NEUROD1 (APh White Matter)	Absolute NEUROD1 (Dentate Gyrus)	Relative FEZ1 (APh Grey Matter)	Relative FEZ1 (APh White Matter)	Relative FEZ1 (Dentate Gyrus)
duration of disease (years)	Correlation Coefficient	.028	-.183	-.276	.218	-.066	-.018	-.375	-.129	-.114	.012	.272	-.325	-.075	-.028	-.192	-.150	-.203	-.105
	P (2-tailed)	.873	.292	.109	.208	.721	.918	.138	.622	.663	.962	.309	.203	.631	.861	.242	.298	.158	.471
	N	35	35	35	35	32	35	17	17	17	17	16	17	43	43	39	50	50	49
lifetime quantity of fluphenazine or equivalent (mg)	Correlation Coefficient	.072	-.102	-.449**	.107	.006	-.159	-.472	.261	.206	.130	.477	-.297	-.170	-.137	-.132	-.060	-.038	.108
	P (2-tailed)	.682	.561	.007	.540	.974	.362	.056	.312	.428	.618	.062	.248	.277	.381	.424	.677	.793	.459
	N	35	35	35	35	32	35	17	17	17	17	16	17	43	43	39	50	50	49
severity of substance abuse	Correlation Coefficient	-.134	-.168	-.228	-.105	.107	-.197	-.524*	.110	.237	.162	.178	-.387	-.251	-.025	-.130	-.157	.001	-.162
	P (2-tailed)	.444	.335	.189	.547	.560	.257	.031	.673	.359	.536	.510	.124	.105	.872	.431	.276	.994	.267
	N	35	35	35	35	32	35	17	17	17	17	16	17	43	43	39	50	50	49
severity of alcohol abuse	Correlation Coefficient	-.133	.229	-.039	.005	.331	-.181	-.427	.272	.200	.066	.326	-.167	-.267	-.070	-.030	.020	-.036	-.068
	P (2-tailed)	.447	.186	.823	.977	.064	.299	.088	.291	.441	.800	.217	.523	.084	.654	.856	.892	.803	.641

Continuous Variables: Non-Parametric Correlations (w/ Outliers Incl.)

		DCX (APh)	*DPYSL2 (APh)*	*FEZ1 (APh)*	*NDEL1 (APh)*	*NEUROD1 (APh)*	*PDE4B (APh)*	*DCX (non-neurogenic HF)*	*DPYSL2 (non-neurogenic HF)*	*FEZ1 (non-neurogenic HF)*	*NDEL1 (non-neurogenic HF)*	*NEUROD1 (non-neurogenic HF)*	*PDE4B (non-neurogenic HF)*	Absolute NEUROD1 (APh Grey Matter)	Absolute NEUROD1 (APh White Matter)	Absolute NEUROD1 (Dentate Gyrus)	Relative FEZ1 (APh Grey Matter)	Relative FEZ1 (APh White Matter)	Relative FEZ1 (Dentate Gyrus)
	N	35	35	35	35	32	35	17	17	17	17	16	17	43	43	39	50	50	49
PH	Correlation Coefficient	-.135	.130	-.064	-.359*	-.268	-.122	-.326	-.350	-.115	-.031	-.257	-.332	-.299	-.176	.002	-.040	-.222	.022
	P (2-tailed)	.440	.457	.715	.034	.137	.485	.201	.169	.660	.905	.336	.192	.051	.259	.991	.782	.121	.880
	N	35	35	35	35	32	35	17	17	17	17	16	17	43	43	39	50	50	49
mass of brain (g)	Correlation Coefficient	.131	.095	.129	.014	.346	.027	.028	.158	.128	.247	.041	-.106	-.004	-.208	.103	.002	.080	.047
	P (2-tailed)	.454	.589	.461	.938	.053	.877	.914	.544	.626	.340	.879	.687	.979	.180	.531	.988	.581	.751
	N	35	35	35	35	32	35	17	17	17	17	16	17	43	43	39	50	50	49
post-mortem interval (hours)	Correlation Coefficient	.389*	-.032	.222	.192	-.123	.212	.302	.201	.209	.214	-.018	.292	.080	-.117	.005	-.152	.251	.056
	P (2-tailed)	.021	.855	.199	.269	.502	.222	.238	.438	.421	.410	.948	.255	.610	.456	.976	.292	.079	.704
	N	35	35	35	35	32	35	17	17	17	17	16	17	43	43	39	50	50	49
STORAGE (DAYS)	Correlation Coefficient	.424*	-.192	-.266	.554**	-.191	-.005	-.654**	.140	.194	.110	.612*	-.267	-.106	.024	-.013	.156	-.108	-.111

Continuous Variables: Non-Parametric Correlations (w/ Outliers Incl.)

	DCX (APh)	DPYSL2 (APh)	FEZ1 (APh)	NDEL1 (APh)	NEUROD1 (APh)	PDE4B (APh)	DCX (non-neurogenic HF)	DPYSL2 (non-neurogenic HF)	FEZ1 (non-neurogenic HF)	NDEL1 (non-neurogenic HF)	NEUROD1 (non-neurogenic HF)	PDE4B (non-neurogenic HF)	Absolute NEUROD1 (APh Grey Matter)	Absolute NEUROD1 (APh White Matter)	Absolute NEUROD1 (Dentate Gyrus)	Relative FEZ1 (APh Grey Matter)	Relative FEZ1 (APh White Matter)	Relative FEZ1 (Dentate Gyrus)
P (2-tailed)	.011	.269	.123	.001	.296	.978	.004	.593	.456	.673	.012	.300	.499	.876	.936	.278	.455	.449
N	35	35	35	35	32	35	17	17	17	17	16	17	43	43	39	50	50	49

9-C Table showing the results of Mann-Whitney *U* testing of dichotomous demographic and clinical variables for effects on the gene expression normalised relative quantities (italic headings) in the anterior parahippocampal region (APh), the non-neurogenic hippocampal formation (HF) and protein density levels from section of anterior hippocampus

Dichotomous Variables: Non-Parametric Tests (w/ Outliers Incl.)

		DCX (APh)	DPYSL2 (APh)	FEZ1 (APh)	NDEL1 (APh)	NEUROD1 (APh)	PDE4B (APh)	DCX (non-neurogenic HF)	DPYSL2 (non-neurogenic HF)	FEZ1 (non-neurogenic HF)	NDEL1 (non-neurogenic HF)	NEUROD1 (non-neurogenic HF)	PDE4B (non-neurogenic HF)	Absolute NEUROD1 (APh Grey Matter)	Absolute NEUROD1 (APh White Matter)	Absolute NEUROD1 (Dentate Gyrus)	Relative FEZ1 (APh Grey Matter)	Relative FEZ1 (APh White Matter)	Relative FEZ1 (Dentate Gyrus)
female	*N*	15	15	15	15	14	15	9	9	9	9	9	9	18	18	15	22	22	21
	Mean Rank	18.40	14.33	18.20	17.40	14.71	20.20	10.00	7.44	7.44	6.89	7.78	10.22	23.67	24.06	19.33	23.73	25.23	24.52
	Sum of Ranks	276.00	215.00	273.00	261.00	206.00	303.00	90.00	67.00	67.00	62.00	70.00	92.00	426.00	433.00	290.00	522.00	555.00	515.00
male	*N*	20	20	20	20	18	20	8	8	8	8	7	8	25	25	24	28	28	28
	Mean Rank	17.70	20.75	17.85	18.45	17.89	16.35	7.88	10.75	10.75	11.38	9.43	7.63	20.80	20.52	20.42	26.89	25.71	25.36

Dichotomous Variables: Non-Parametric Tests (w/ Outliers Incl.)

		DCX (APh)	*DPYSL2 (APh)*	*FEZ1 (APh)*	*NDEL1 (APh)*	*NEUROD1 (APh)*	*PDE4B (APh)*	*DCX (non-neurogenic HF)*	*DPYSL2 (non-neurogenic HF)*	*FEZ1 (non-neurogenic HF)*	*NDEL1 (non-neurogenic HF)*	*NEUROD1 (non-neurogenic HF)*	*PDE4B (non-neurogenic HF)*	Absolute NEUROD1 (APh Grey Matter)	Absolute NEUROD1 (APh White Matter)	Absolute NEUROD1 (Dentate Gyrus)	Relative FEZ1 (APh Grey Matter)	Relative FEZ1 (APh White Matter)	Relative FEZ1 (Dentate Gyrus)
	Sum of Ranks	354.00	415.00	357.00	369.00	322.00	327.00	63.00	86.00	86.00	91.00	66.00	61.00	520.00	513.00	490.00	753.00	720.00	710.00
Total	*N*	35	35	35	35	32	35	17	17	17	17	16	17	43	43	39	50	50	49
	Mann-Whitney U	144.000	95.000	147.000	141.000	101.000	117.000	27.000	22.000	22.000	17.000	25.000	25.000	195.000	188.000	170.000	269.000	302.000	284.000
	Wilcoxon W	354.000	215.000	357.000	261.000	206.000	327.000	63.000	67.000	67.000	62.000	70.000	61.000	520.000	513.000	290.000	522.000	555.000	515.000
	Z	-.200	-1.833	-.100	-.300	-.950	-1.100	-.866	-1.347	-1.347	-1.828	-.688	-1.058	-.739	-.911	-.289	-.762	-.117	-.202
	p (2-tailed)	.841	.067	.920	.764	.342	.271	.386	.178	.178	.068	.491	.290	.460	.362	.773	.446	.907	.840
Death by suicide: yes	*N*	9	9	9	9	8	9	6	6	6	6	5	6	13	13	11	18	18	17
	Mean Rank	17.11	12.78	15.33	17.22	14.38	16.22	6.67	8.50	8.17	9.83	9.20	5.50	14.46	18.69	18.64	23.56	25.67	24.53
	Sum of Ranks	154.00	115.00	138.00	155.00	115.00	146.00	40.00	51.00	49.00	59.00	46.00	33.00	188.00	243.00	205.00	424.00	462.00	417.00
no	*N*	26	26	26	26	24	26	11	11	11	11	11	11	30	30	28	32	32	32
	Mean Rank	18.31	19.81	18.92	18.27	17.21	18.62	10.27	9.27	9.45	8.55	8.18	10.91	25.27	23.43	20.54	26.59	25.41	25.25
	Sum of Ranks	476.00	515.00	492.00	475.00	413.00	484.00	113.00	102.00	104.00	94.00	90.00	120.00	758.00	703.00	575.00	851.00	813.00	808.00
Total	*N*	35	35	35	35	32	35	17	17	17	17	16	17	43	43	39	50	50	49
	Mann-Whitney U	109.000	70.000	93.000	110.000	79.000	101.000	19.000	30.000	28.000	28.000	24.000	12.000	97.000	152.000	139.000	253.000	285.000	264.000

Dichotomous Variables: Non-Parametric Tests (w/ Outliers Incl.)

		DCX (APh)	*DPYSL2 (APh)*	*FEZ1 (APh)*	*NDEL1 (APh)*	*NEUROD1 (APh)*	*PDE4B (APh)*	*DCX (non-neurogenic HF)*	*DPYSL2 (non-neurogenic HF)*	*FEZ1 (non-neurogenic HF)*	*NDEL1 (non-neurogenic HF)*	*NEUROD1 (non-neurogenic HF)*	*PDE4B (non-neurogenic HF)*	Absolute NEUROD1 (APh Grey Matter)	Absolute NEUROD1 (APh White Matter)	Absolute NEUROD1 (Dentate Gyrus)	Relative FEZ1 (APh Grey Matter)	Relative FEZ1 (APh White Matter)	Relative FEZ1 (Dentate Gyrus)
	Wilcoxon W	154.000	115.000	138.000	155.000	115.000	146.000	40.000	51.000	49.000	94.000	90.000	33.000	188.000	243.000	205.000	424.000	813.000	417.000
	Z	-.302	-1.774	-.906	-.264	-.740	-.604	-1.407	-.302	-.503	-.503	-.397	-2.111	-2.592	-1.137	-.468	-.707	-.061	-.168
	p (2-tailed)	.763	.076	.365	.792	.459	.546	.159	.763	.615	.615	.692	.035	.010	.255	.640	.479	.952	.867
With Psychosis	*N*	17	17	17	17	15	17	7	7	7	7	7	7	21	21	20	24	24	23
	Mean Rank	17.88	15.59	12.29	18.24	15.47	15.41	5.86	11.14	11.00	10.14	11.43	7.29	18.95	21.14	18.15	24.79	25.38	27.52
	Sum of Ranks	304.00	265.00	209.00	310.00	232.00	262.00	41.00	78.00	77.00	71.00	80.00	51.00	398.00	444.00	363.00	595.00	609.00	633.00
Without Psychosis	*N*	18	18	18	18	17	18	10	10	10	10	9	10	22	22	19	26	26	26
	Mean Rank	18.11	20.28	23.39	17.78	17.41	20.44	11.20	7.50	7.60	8.20	6.22	10.20	24.91	22.82	21.95	26.15	25.62	22.77
	Sum of Ranks	326.00	365.00	421.00	320.00	296.00	368.00	112.00	75.00	76.00	82.00	56.00	102.00	548.00	502.00	417.00	680.00	666.00	592.00
Total	*N*	35	35	35	35	32	35	17	17	17	17	16	17	43	43	39	50	50	49
	Mann-Whitney U	151.000	112.000	56.000	149.000	112.000	109.000	13.000	20.000	21.000	27.000	11.000	23.000	167.000	213.000	153.000	295.000	309.000	241.000
	Wilcoxon W	304.000	265.000	209.000	320.000	232.000	262.000	41.000	75.000	76.000	82.000	56.000	51.000	398.000	444.000	363.000	595.000	609.000	592.000
	Z	-.066	-1.353	-3.201	-.132	-.585	-1.452	-2.147	-1.464	-1.366	-.781	-2.170	-1.171	-1.555	-.437	-1.040	-.330	-.058	-1.162
	p (2-tailed)	.947	.176	.001	.895	.558	.146	.032	.143	.172	.435	.030	.242	.120	.662	.299	.741	.954	.245
Hemisph	*N*	13	13	13	13	12	13	9	9	9	9	8	9	19	19	18	23	23	22

Dichotomous Variables: Non-Parametric Tests (w/ Outliers Incl.)

		DCX (APh)	*DPYSL2 (APh)*	*FEZ1 (APh)*	*NDEL1 (APh)*	*NEUROD1 (APh)*	*PDE4B (APh)*	*DCX (non-neurogenic HF)*	*DPYSL2 (non-neurogenic HF)*	*FEZ1 (non-neurogenic HF)*	*NDEL1 (non-neurogenic HF)*	*NEUROD1 (non-neurogenic HF)*	*PDE4B (non-neurogenic HF)*	Absolute NEUROD1 (APh Grey Matter)	Absolute NEUROD1 (APh White Matter)	Absolute NEUROD1 (Dentate Gyrus)	Relative FEZ1 (APh Grey Matter)	Relative FEZ1 (APh White Matter)	Relative FEZ1 (Dentate Gyrus)
ere: right	Mean Rank	16.46	17.77	13.62	18.08	12.00	13.77	6.44	8.78	9.22	10.11	9.88	5.56	20.16	22.16	21.39	25.48	25.26	21.82
	Sum of Ranks	214.00	231.00	177.00	235.00	144.00	179.00	58.00	79.00	83.00	91.00	79.00	50.00	383.00	421.00	385.00	586.00	581.00	480.00
left	*N*	22	22	22	22	20	22	8	8	8	8	8	8	24	24	21	27	27	27
	Mean Rank	18.91	18.14	20.59	17.95	19.20	20.50	11.88	9.25	8.75	7.75	7.13	12.88	23.46	21.88	18.81	25.52	25.70	27.59
	Sum of Ranks	416.00	399.00	453.00	395.00	384.00	451.00	95.00	74.00	70.00	62.00	57.00	103.00	563.00	525.00	395.00	689.00	694.00	745.00
Total	*N*	35	35	35	35	32	35	17	17	17	17	16	17	43	43	39	50	50	49
	Mann-Whitney U	123.000	140.000	86.000	142.000	66.000	88.000	13.000	34.000	34.000	26.000	21.000	5.000	193.000	225.000	164.000	310.000	305.000	227.000
	Wilcoxon W	214.000	231.000	177.000	395.000	144.000	179.000	58.000	79.000	70.000	62.000	57.000	50.000	383.000	525.000	395.000	586.000	581.000	480.000
	Z	-.683	-.102	-1.946	-.034	-2.102	-1.878	-2.213	-.192	-.192	-.962	-1.155	-2.983	-.856	-.073	-.704	-.010	-.107	-1.407
	p (2-tailed)	.495	.918	.052	.973	.036	.060	.027	.847	.847	.336	.248	.003	.392	.942	.481	.992	.915	.159