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Investigation of genetic variants in ubiquitin enzyme genes involved in the modulation of neurodevelopmental processes: a role in schizophrenia susceptibility?

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Abstract

Despite extensive research during the last few decades, the etiology of schizophrenia remains unclear. Evidence of both genetic and environmental influences in the developmental profile of schizophrenia has grown, and due to the complexity of this disorder, a polygenic aspect has been associated with this neuropsychiatric pathology. Unfortunately, no diagnostic strategies based on biological measurement or genetic testing is currently available for schizophrenia. Gene-expression profiling and recent protein studies have shown a decrease in the expression of ubiquitin pathway proteins in the prefrontal cortex of schizophrenia patients. We have examined single nucleotide polymorphisms (or SNPs) within three genes from the ubiquitin protein system: the ubiquitin conjugating enzyme E2D1 (UBE2D1) gene, the E3 SUMO-protein ligase protein inhibitor of activated STAT 2 (PIAS2) gene, and the E3 ubiquitin ligase F-box and leucine-rich repeat protein 21 (FBXL21) gene, in a Caucasian case-control population for schizophrenia. After Bonferroni correction for multiple testing was applied, no significant associations were reported for any of the tested SNPs. Additional genetic analyses will be necessary to fully explore the role of these three genes in schizophrenia. Regarding the rising interest in ubiquitin-related proteins as a therapeutic target in other pathologies such as cancer, further research into the role of ubiquitin pathways in schizophrenia seems topical and timely.

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Investigation of genetic variants in ubiquitin enzyme genes involved in the modulation of neurodevelopmental processes: a role in schizophrenia susceptibility?

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Summary

Despite extensive research during the last decades, the etiology of schizophrenia remains unclear. Evidence of both genetic and environmental influences in the developmental profile of schizophrenia has grown, and due to the complexity of this disorder, a polygenic aspect has been associated with this neuropsychiatric pathology. Unfortunately, no diagnostic strategies based on biological measurement or genetic testing is currently available for schizophrenia. Gene expression profiling and recent protein studies have shown a decrease in the expression of ubiquitin pathway proteins in the prefrontal cortex of schizophrenia patients. We have examined Single Nucleotide Polymorphisms (or SNPs) within three genes from the ubiquitin protein system: the ubiquitin conjugating enzyme *E2D1 (UBE2D1)* gene, the E3 SUMO-protein ligase *protein inhibitor of activated STAT 2 (PIAS2)* gene, and the E3 ubiquitin ligase *F-box and leucine-rich repeat protein 21 (FBXL21)* gene, in a Caucasian case-control population for schizophrenia. After Bonferroni correction for multiple testing was applied, no significant associations were reported for any of the tested SNPs. Additional genetic analyses will be necessary to fully explore the role of these three genes in schizophrenia. Regarding the rising interest of ubiquitin related proteins as a therapeutic target in other pathologies such as cancer, further research into the role of ubiquitin pathways in schizophrenia seem topical and timely.

Keywords: Case-control association; Schizophrenia; Single Nucleotide Polymorphisms; Ubiquitin related genes

1. Introduction

Episodes of mental illness may appear and disappear throughout a person's life, affecting around one in five individuals. Schizophrenia is a mental disorder affecting approximately one percent of the general population and is characterized by symptoms such as hallucinations, delusions, disorganized communication, poor planning, reduced motivation, and blunted affect (Lewis and Lieberman, 2000; Lewis and Levitt, 2002). Despite 50 years of research in schizophrenia, no effective approach for prevention or cure has been produced. The etiology of this disorder remains unclear, although research strongly points towards the interaction between genetic and environmental influences (Tsuang, 2000; Aukes et al., 2008). Recent gene expression and protein studies have reported an alteration of ubiquitin pathways in the brains from schizophrenia sufferers compared to controls (Bousman et al., 2010; Rubio et al., 2013). The ubiquitin protein system plays an essential role in the regulation of membrane and cellular proteins, and has often been referred to as the "kiss of death" due to its labeling of proteins for degradation by proteases (Petroski, 2008; Tai and Schuman, 2008). The major function of the ubiquitin protein complex is to assure intracellular protein degradation by ubiquitination; a highly complex process involving a set of successive enzymes: E1 (ubiquitin activating enzymes), E2 (ubiquitin-conjugating enzymes), E3 (ubiquitin protein ligases), the 20S proteasome, and deubiquitinating enzymes (Yi and Ehlers, 2007). Ubiquitin is first activated by E1 ubiquitin-activating enzymes, before being transferred to its active site, the amino acid cysteine. This transfer requires ATP, making the process energy-dependent. The ubiquitin molecule is then passed on to the second enzymes within the complex, E2 (ubiquitin-conjugating enzymes), before reaching the final group of enzymes, the E3 ubiquitin protein ligases, which recognize and bind the target substrate and labels it with the ubiquitin. This process can be

repeated until a short chain is formed, with three or more ubiquitin molecules usually targeting the protein to the proteasome, where the degradation occurs. Both E2 and E3 proteins exist as large families: more than 35 E2s and 600 E3s have been identified so far, resulting in highly complex combinations of E2s with different E3 proteins defining the substrate specificity. Defects in this ubiquitin-dependent protein degradation have been implicated in the etiology of neurodegenerative diseases, metabolic disorders, cancer (Weathington and Mallampalli, 2014), developmental deficiency, immunity pathologies (Sakamoto, 2002; Pagano and Benmaamar, 2003) and schizophrenia.

We have focused our analysis on three genes coding for ubiquitin related proteins (the ubiquitin conjugating enzyme E2D1 (*UBE2D1*), the E3 SUMO-protein ligase protein inhibitor of activated STAT 2 (*PIAS2*) and the E3 ubiquitin ligase F-box and leucine-rich repeat protein 21 (*FBXL21*), which is a SKP1-cullin-F-box (SCF) protein, implicated in the regulation of the p53 pathway (Figure 1). The p53 pathway plays an essential role in the modulation of neurodevelopmental processes (including cerebral vascularization and neurogenesis) and/or to neurodevelopmental disorders such as schizophrenia. Due to their previous association in different reports and populations (Chen et al., 2008; Middleton et al., 2002), we analyzed a set of potential Single Nucleotide Polymorphisms (SNPs) in the *UBE2D1* (coding for E2D1 protein), *PIAS2* (coding for the protein inhibitor of activated STAT 2) and *FBXL21* gene (coding for F-box and leucine-rich repeat protein 21) in the largest schizophrenia case-control Caucasian population collected in Australia to examine their potential associations with this devastating neurodevelopmental disorder.

2. Materials and methods

(i) DNA samples

DNA samples were obtained from the Australian Schizophrenia Research Bank (ASRB). Subjects with schizophrenia were identified using the *Diagnostic and Statistical Manual of Mental Disorders IV* criteria. All samples were from Caucasian volunteers. Subjects were matched for gender and age. The complete sample consisted of 268 schizophrenia cases, comprised of 186 males and 82 females, with an average age of 38.86 ± 11.01 years; and 268 matched controls, comprised of 169 males and 99 females, with an average age of 38.56 ± 12.57 years, with no prior history of mental disorders. After a complete description of the study to the subjects, written informed consent was obtained. This study was approved by, and conducted according to the guidelines of the University of Wollongong Human Research Ethics Committee (HE10/161).

(ii) SNP genotyping

SNPs within the *UBE2D1* (rs11006122 and rs1905455), *PIAS2* (rs8094449, rs10502878, rs11876274, and rs56352844) and *FBXL21* (rs1859427 and rs6861170) genes were tested in our Caucasian schizophrenia case-control population. The selection of these SNPs was based on their previous associations with schizophrenia and/or other disorders, and on their Minor Allele Frequencies (MAF) reported in Caucasian populations (MAF > 15%) using HapMap data (<http://hapmap.ncbi.nlm.nih.gov>). High-throughput SNP genotyping was performed using the MassARRAY® genotyping assay (Sequenom, Inc., San Diego, CA, USA), with the analysis performed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). PCR

and extension primer design, selection and multiplexing were performed using MassARRAY® Designer Software (Sequenom, Inc., San Diego, CA, USA).

(iii) *Statistical analysis*

Power calculations indicated that for the smaller of the two cohorts, a sample size of 204 cases (408 alleles) with a minor allele frequency of 0.2; has >90% a priori power to detect a significant allelic association conferring an odds ratio of 1.5 or greater. The distribution of all tested SNPs did not deviate significantly from Hardy Weinberg Equilibrium (HWE) ($p > 0.05$), except for *UBE2D1* (rs1905455, $p = 1.88 \times 10^{-17}$) which was then excluded from further analysis. To detect associations between each SNP and schizophrenia, chi-square (χ^2) analysis was performed to test for significant differences in allele and genotype frequencies between the case and control groups. The significance for all statistical tests was set to $p < 0.05$ and values $p < 0.10$ were described as trends. Data are expressed as specific counts for alleles and genotypes. Due to multiple testing in the SNP analysis, a standard Bonferroni-corrected p-value of 0.007 (0.05/7) was required to give a 95% probability of correctly concluding not to reject the null hypothesis in the χ^2 test.

3. Results

Two SNPs within *UBE2D1*, four SNPs in *PIAS2* and two SNPs within *FBXL21* were analyzed for association with schizophrenia in a large Australian Caucasian population (268 schizophrenia patients *versus* 268 matched controls with no prior history of mental disorders).

Following Bonferroni correction for multiple comparisons there were no significant associations between the allelic frequency of any of the tested SNPs (*UBE2D1*

rs11006122, *PIAS2* rs8094449, rs10502878, rs11876274, and rs56352844; *FBXL21* rs1859427 and rs6861170) and schizophrenia ($0.08 \leq p \leq 0.92$; Tables 2, 3 and 4 respectively). In addition there were no genotypic associations between any of the tested SNPs and schizophrenia following Bonferroni correction ($0.17 \leq p \leq 0.71$; Tables 2, 3 and 4). Further, analysis of each of the genetic markers by gender revealed no significant allelic or genotypic associations with any of these genetic markers and either gender ($0.08 \leq p \leq 1.00$; Tables 2, 3 and 4). Interestingly, the demographics of our tested population (Table 1) revealed that a large percentage of our schizophrenia subjects had a family history of mental disorders, twice as many as the control group. χ^2 analysis revealed that both of the *FBXL21* genetic markers rs1859427 and rs6861170 had a significant genotypic association with schizophrenia in the subjects whose mother (rs1859427: $\chi^2=8.35$, $p=0.01$; rs6861170: $\chi^2=8.20$, $p=0.01$) and/or father (rs1859427: $\chi^2=16.80$, $p<0.001$; rs6861170: $\chi^2=15.54$, $p<0.001$) had a history of mental disorders. None of the other SNPs from any of the other genes studied had any significant associations with schizophrenia in subjects who had a family history of mental disorders ($0.10 \leq \chi^2 \leq 4.17$, $0.12 \leq p \leq 0.95$). In addition, a large percentage of subjects from both tested groups experienced some form of self-reported childhood trauma, including but not limited to neglect, physical abuse, sexual abuse, and post-traumatic stress, with a larger number of schizophrenia subjects (41.0%) having a traumatic childhood compared to the control group (22.4%). Again, χ^2 analysis revealed that both of the *FBXL21* genetic markers rs1859427 and rs6861170 had a significant genotypic association with schizophrenia in the subjects who experienced childhood trauma (rs1859427: $\chi^2=9.67$, $p=0.007$; rs6861170: $\chi^2=7.75$, $p=0.02$). Again, none of the other SNPs analyzed had any significant associations with schizophrenia in subjects who experienced trauma during childhood ($1.49 \leq \chi^2 \leq 4.34$, $0.11 \leq p \leq 0.47$).

4. Discussion

We have investigated the association of three genes from the ubiquitin protein system involved in the regulation of the p53 pathway in a large Australian Caucasian case-control schizophrenia population. We did not report any significant associations with schizophrenia for any of the tested SNPs in the *UBE2D1*, *PIAS2* and *FBXL21* genes in our population.

There is little information in the literature regarding association studies for our tested genes. Only the *FBXL21* gene has been previously studied in two independent Irish populations, one corresponding to a high density of schizophrenia in Irish families (1,350 subjects from 273 families) and the other was a large Irish case-control population (814 cases *versus*. 625 controls) (Chen et al., 2008). Chen *et. al.* found rs1859427 and rs6861170 to be significantly associated with schizophrenia within their case-control population, and significance was maintained even after correction for multiple testing ($p=0.01967$ for both markers) (Chen et al., 2008). The MAF for both rs1859427 and rs6861170 *FBXL21* markers were very similar in the schizophrenia group for both the present study (MAF=0.32 for both markers) and in the Chen *et. al.* study (MAF=0.27). This suggests that there is likely to be a difference between the genotyping frequencies in the schizophrenia groups and/or frequencies in the control group between our study and the Chen *et. al.* study.

Interestingly, when we factor into our analysis the subjects who experienced some sort of trauma during childhood, the *FBXL21* SNPs showed a significant association with schizophrenia among the tested SNPs (Table 5), however none of the other tested SNPs in any of the other tested genes had any significant associations in schizophrenia subjects who self-reported any traumatic childhood experiences. Differential epigenetic

gene regulation in relation to traumatic childhood experiences has only ever been reported for one ubiquitin E3 ligase (*Mahogunin Ring Finger 1* or *MGRN1*) in women with fibromyalgia who had experienced traumatic childhood events (Menziez et al., 2013). This suggests that adverse childhood experiences are able to induce genetic and epigenetic variations in ubiquitin protein genes responding to stressful conditions such as Ring Type E3 ubiquitin ligase and F-box proteins (Hermand, 2006; Hua and Vierstra, 2011). A number of studies have shown strong associations between negative childhood experiences and adult psychiatric illnesses, in particular depression, psychosis and schizophrenia (Edwards et al., 2003; Kelleher et al., 2008; Lu et al., 2008). When we accounted for a family history of mental illness from either the mother and/or father within our tested population, we found a positive association between the *FBXL21* SNPs and schizophrenia (Table 5); but again there were no significant associations with any of the other tested SNPs in the tested genes in schizophrenia subjects who had a family history of mental illness, suggesting an inheritance for the *FBXL21* genetic markers in the context of psychiatric disorders. This is in line with the Chen *et. al* study which showed that the haplotype of *FBXL21* markers rs1859472 and rs6861170 were over-transmitted in the Irish study of high density schizophrenia families.

As mentioned above, the *FBXL21* gene encodes for an E3 ubiquitin ligase F-box protein in the SKP1-cullin-F-box (SCF) complex. The *FBXL21* protein is known to be able to stabilize cryptochrome (CRY) proteins CRY1 and CRY2, which are implicated in regulating mammalian circadian rhythms (Hirano et al., 2013). In fact, the *FBXL21* protein was reported to antagonize the *FBXL3* protein, another F-box-type E3 ligase, which ubiquitinates CRY proteins and mediates their degradation (Hirano et al., 2013). By attenuating the destabilizing action of *FBXL3* on CRY proteins, *FBXL21* expression allows for an adapted regulation of circadian rhythm gene transcription by CRY

proteins according to the circadian cycle. Furthermore, the circadian pattern of *FBXL21* expression in the mouse suprachiasmatic nucleus (region responsible for controlling circadian rhythm) is reminiscent of the expression pattern seen for other circadian pacemaker genes such as *Period 1 (PER1)* (Dardente et al., 2008). Mutations in either the *FBXL21* or *FBXL3* genes can lead to a dysfunction of circadian rhythm oscillations and lead to significant behavioral disturbances in individuals and alterations in their sleeping patterns; moreover the absence of *FBXL21* causes a short-period phenotype in both mice and cells (Hirano et al., 2013; Yoo et al., 2013). Interestingly, schizophrenia patients have been reported to have intrinsically unstable circadian oscillators. A study by Bromundt *et. al.* recently provided important new information concerning the link between impairments in neuropsychological function and disrupted circadian rhythms in schizophrenia. (Bromundt et al., 2011); which is further supported by a microarray study which found a significant downregulation of the circadian pacemaker gene *PER1* in the postmortem temporal cortex of schizophrenia patients compared to healthy controls (Aston et al., 2004). Furthermore, an animal model study has shown dysfunction in the synchronization of circadian rhythms between brain cell networks involved in sleep–wake regulation and cognition (Dudley et al., 2003). Overall this suggests that alterations in circadian rhythms are present in schizophrenia, and that polymorphisms in the *FBXL21* gene in addition to the *FBX3* gene, could be involved in the circadian cycle disturbances that have been observed in schizophrenia (Mansour et al., 2009).

The *FBXL21* gene is located on Chromosome 5q31, a region that has previously been shown to contribute to the susceptibility for schizophrenia in both German and Israeli pedigree families (lod score 1.8) (Schwab et al., 1997). Putative loci associated with psychosis in bipolar disorder pedigrees were characterized in the chromosomal region

18q21, which includes the locus for the *PIAS2* gene (Park et al., 1995). This region has also been suggested to be an influential genetic loci, common to both schizophrenia and bipolar disorders, depending on polygenic influence and critical environmental factors (Mors et al., 1997). Unfortunately, we did not report any significant associations between the tested SNPs (promoter and intron variants) in the *PIAS2* gene with schizophrenia, suggesting that other genetic markers within this gene may be involved. Similarly, the genetic polymorphisms analyzed within the *UBE2D1* gene (located in the promoter region) were not associated with schizophrenia in our tested population. The *UBE2D1* gene is located at the chromosomal region 10q21.1 (<http://www.ncbi.nlm.nih.gov/gene/7321>). This region includes the *Ankyrin 3 (ANK3)* gene, previously associated with schizophrenia by a number of studies that found the rs10761482 SNP to be associated with schizophrenia in a large European population as well as Han Chinese populations (Gella et al., 2011; Yuan et al., 2012).

Due to its early expression in brain development and its key role in genomic stability as well as apoptotic process in brain cells, the p53 pathway plays an essential role in the modulation of neurodevelopmental processes, including those within the schizophrenia pathophysiology. Interestingly a reduced risk of cancer has been observed in individuals with schizophrenia during the last decade; considering the significant role p53 plays in the progression of cancer, this suggests a significant role of p53 in schizophrenia. Previous *p53* polymorphisms were found to be associated with schizophrenia in both Chinese and Caucasian case-control populations and in family studies (Yang et al., 2004; Ni et al., 2005). It is thought that the regulation of p53 expression by ubiquitin degradation may play an important role in schizophrenia pathophysiology. Although the present study did not examine polymorphisms within *p53*, all of the genes examined are involved in p53 signaling. While we did not find any significant associations between

any of the tested polymorphisms and schizophrenia, replicating our study in a larger population and/or testing additional polymorphisms within the same genes will add to, and allow for further exploration of the role of the tested ubiquitin related genes in the genetic vulnerability of schizophrenia.

Our study reports the analysis of potential SNPs in three candidate genes from the ubiquitin protein system in a large Australian case-control schizophrenia population. Due to the limited information available on the role of E2 ubiquitin conjugating enzymes and E3 ubiquitin ligases in schizophrenia, in addition to the increasing variety of these groups of proteins, additional studies will be necessary to further examine the role of these ubiquitin proteins in the genetics of schizophrenia. A growing interest has recently emerged which is targeting ubiquitin related proteins in the treatment of cancer and inflammatory related diseases. Keeping in mind the paradoxical relationship between cancer and schizophrenia, the ubiquitin protein systems seem to be a good candidate to further analyze in the genetics and therapy for schizophrenia.

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Declaration of Interest

None

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Table 1. Subject Demographics for Control (n = 268) and Schizophrenia Subjects (n = 268).

	Control subjects n (%)	Schizophrenia subjects n (%)
Gender		
<i>Female</i>	99 (37%)	82 (30.6%)
<i>Male</i>	169 (63%)	186 (69.4%)
Age at assessment (years)		
<i>Female</i>	32.19	40.48
<i>Male</i>	42.22	38.14
Family history of mental disorders		
<i>Mother</i>	41 (15.3%)	95 (35.5%)
<i>Father</i>	49 (18.3%)	106 (39.6%)
Traumatic childhood experience	60 (22.4%)	110 (41.0%)

Table 2. Allelic and genotypic distributions for *UBE2D1* genetic marker in schizophrenia subjects and controls.

<i>UBE2D1</i>	<i>N</i> (alleles) rs11006122	Alleles		Genotypes		
		C	T	CC	CT	TT
Schizophrenia	264	150 (56.8%)	114 (43.2%)	45 (34.1%)	60 (45.5%)	27 (20.4%)
<i>Male</i>	190	106 (55.8%)	84 (44.2%)	30 (31.6%)	46 (48.4%)	19 (20.0%)
<i>Female</i>	74	44 (59.5%)	30 (40.5%)	15 (40.5%)	14 (37.8%)	8 (21.6%)
Control	368	217 (59.0%)	151 (41.0%)	63 (34.2%)	91 (49.5%)	30 (16.3%)
<i>Male</i>	232	134 (57.8%)	98 (42.2%)	40 (34.5%)	54 (46.6%)	22 (18.9%)
<i>Female</i>	136	83 (61.0%)	53 (39.0%)	23 (33.9%)	37 (54.4%)	8 (11.7%)
Total case vs. control		$\chi^2 = 0.29$	$p = 0.58$		$\chi^2 = 0.99$	$p = 0.61$
Male case vs. control		$\chi^2 = 0.17$	$p = 0.68$		$\chi^2 = 0.20$	$p = 0.90$
Female case vs. control		$\chi^2 = 0.05$	$p = 0.82$		$\chi^2 = 3.18$	$p = 0.20$

Table 3. Allelic and genotypic distributions for *PIAS2* genetic markers in schizophrenia subjects and controls.

<i>PIAS2</i>	<i>N</i> (alleles)	Alleles		Genotypes		
		G	A	GG	GA	AA
rs8094449						
Schizophrenia	442	423 (95.7%)	19 (4.3%)	202 (91.4%)	19 (8.6%)	0 (0%)
<i>Male</i>	314	300 (95.5%)	14 (4.5%)	143 (91.1%)	14 (8.9%)	0 (0%)
<i>Female</i>	128	123 (96.1%)	5 (3.9%)	59 (92.2%)	5 (7.8%)	0 (0%)
Control	498	471 (94.6%)	27 (5.4%)	223 (89.6%)	25 (10.0%)	1 (0.4%)
<i>Male</i>	314	300 (95.5%)	14 (4.5%)	143 (91.1%)	14 (8.9%)	0 (0%)
<i>Female</i>	184	171 (92.9%)	13 (7.1%)	80 (87.0%)	11 (11.9%)	1 (1.1%)
Total case vs. control		$\chi^2 = 0.63$	$p = 0.42$		$\chi^2 = 1.19$	$p = 0.55$
Male case vs. control		$\chi^2 = 0.00$	$p = 1.00$		$\chi^2 = 0.00$	$p = 1.00$
Female case vs. control		$\chi^2 = 0.39$	$p = 0.24$		$\chi^2 = 1.44$	$p = 0.48$
rs10502878						
rs11876274						
rs56352844						

Table 4. Allelic and genotypic distributions for *FBXL21* genetic marker in schizophrenia subjects and controls.

<i>FBXL21</i>	<i>N</i> (alleles)	Alleles		Genotypes		
		G	A	GG	GA	AA
	rs1859427					
Schizophrenia	266	179 (67.3%)	87 (32.7%)	64 (48.1%)	51 (38.3%)	18 (13.6%)
<i>Male</i>	192	129 (67.2%)	63 (32.8%)	45 (46.9%)	39 (40.6%)	12 (12.5%)
<i>Female</i>	74	50 (67.6%)	24 (32.4%)	19 (51.4%)	12 (32.4%)	6 (16.2%)
Control	374	264 (70.6%)	110 (29.4%)	98 (52.4%)	68 (36.4%)	21 (11.2%)
<i>Male</i>	234	166 (70.9%)	68 (29.1%)	64 (54.7%)	38 (32.5%)	15 (12.8%)
<i>Female</i>	140	98 (70%)	42 (30%)	34 (48.6%)	30 (42.8%)	6 (8.6%)
Total case vs. control		$\chi^2 = 0.79$	$p = 0.37$		$\chi^2 = 0.70$	$p = 0.70$
Male case vs. control		$\chi^2 = 0.70$	$p = 0.40$		$\chi^2 = 1.60$	$p = 0.44$
Female case vs. control		$\chi^2 = 0.02$	$p = 0.87$		$\chi^2 = 1.97$	$p = 0.37$

<i>FBXL21</i>	<i>N</i> (alleles)	Alleles		Genotypes		
		T	G	TT	TG	GG
	rs6861170					
Schizophrenia	268	182 (67.9%)	86 (32.1%)	66 (49.3%)	50 (37.3%)	18 (13.4%)
<i>Male</i>	192	132 (68.8%)	60 (31.2%)	47 (49.0%)	38 (39.6%)	11 (11.4%)
<i>Female</i>	76	50 (65.8%)	26 (34.2%)	19 (50%)	12 (31.6%)	7 (18.4%)
Control	372	264 (71.0%)	108 (29.0%)	98 (52.7%)	68 (36.7%)	20 (10.6%)
<i>Male</i>	234	167(71.4%)	67 (28.6%)	64 (54.7%)	39 (33.3%)	14(12.0%)
<i>Female</i>	138	97 (70.3%)	41 (29.7%)	34 (49.3%)	29 (42.0%)	6 (8.7%)
Total case vs. control		$\chi^2 = 0.69$	$p = 0.41$		$\chi^2 = 0.66$	$p = 0.71$
Male case vs. control		$\chi^2 = 0.35$	$p = 0.55$		$\chi^2 = 0.92$	$p = 0.63$
Female case vs. control		$\chi^2 = 0.46$	$p = 0.49$		$\chi^2 = 4.84$	$p = 0.08$

Table 5. Genotypic distributions for *FBXL21* genetic markers in schizophrenia subjects and controls with respect to parental history of mental health issues and traumatic childhood experiences.

<i>FBXL21</i> rs1859427	Genotypes		
	GG	GA	AA
Schizophrenia	64 (48.1%)	51 (38.3%)	18 (13.6%)
<i>Mother mental history</i>	19 (46.3%)	19 (46.3%)	3 (7.4%)
<i>Father mental history</i>	29 (56.9%)	18 (35.3%)	4 (7.8%)
<i>Traumatic childhood</i>	25 (46.3%)	21 (38.9%)	8 (14.8%)
Control	98 (52.4%)	68 (36.4%)	21 (11.2%)
<i>Mother mental history</i>	8 (29.6%)	14 (51.9%)	5 (18.5%)
<i>Father mental history</i>	18 (52.9%)	13 (38.2%)	3 (8.8%)
<i>Traumatic childhood</i>	13 (37.1%)	19 (54.3%)	3 (8.6%)
Mother mental case vs. control		$\chi^2 = 8.35$	p = 0.01
Father mental case vs. control		$\chi^2 = 16.80$	p < 0.001
Traumatic childhood case vs. control		$\chi^2 = 9.67$	p = 0.007

<i>FBXL21</i> rs6861170	Genotypes		
	TT	TG	GG
Schizophrenia	66 (49.3%)	50 (37.3%)	18 (13.4%)
<i>Mother mental history</i>	20 (47.6%)	18 (42.9%)	4 (9.5%)
<i>Father mental history</i>	29 (54.7%)	18 (34.0%)	6 (11.3%)
<i>Traumatic childhood</i>	25 (44.6%)	21 (37.5%)	10 (17.9%)
Control	98 (52.7%)	68 (36.7%)	20 (10.6%)
<i>Mother mental history</i>	9 (34.6%)	13 (50.0%)	4 (15.4%)
<i>Father mental history</i>	18 (54.5%)	13 (39.4%)	2 (6.1%)
<i>Traumatic childhood</i>	13 (38.2%)	18 (52.9%)	3 (8.8%)
Mother mental case vs. control		$\chi^2 = 8.20$	p = 0.01
Father mental case vs. control		$\chi^2 = 15.54$	p < 0.001
Traumatic childhood case vs. control		$\chi^2 = 7.75$	p = 0.02

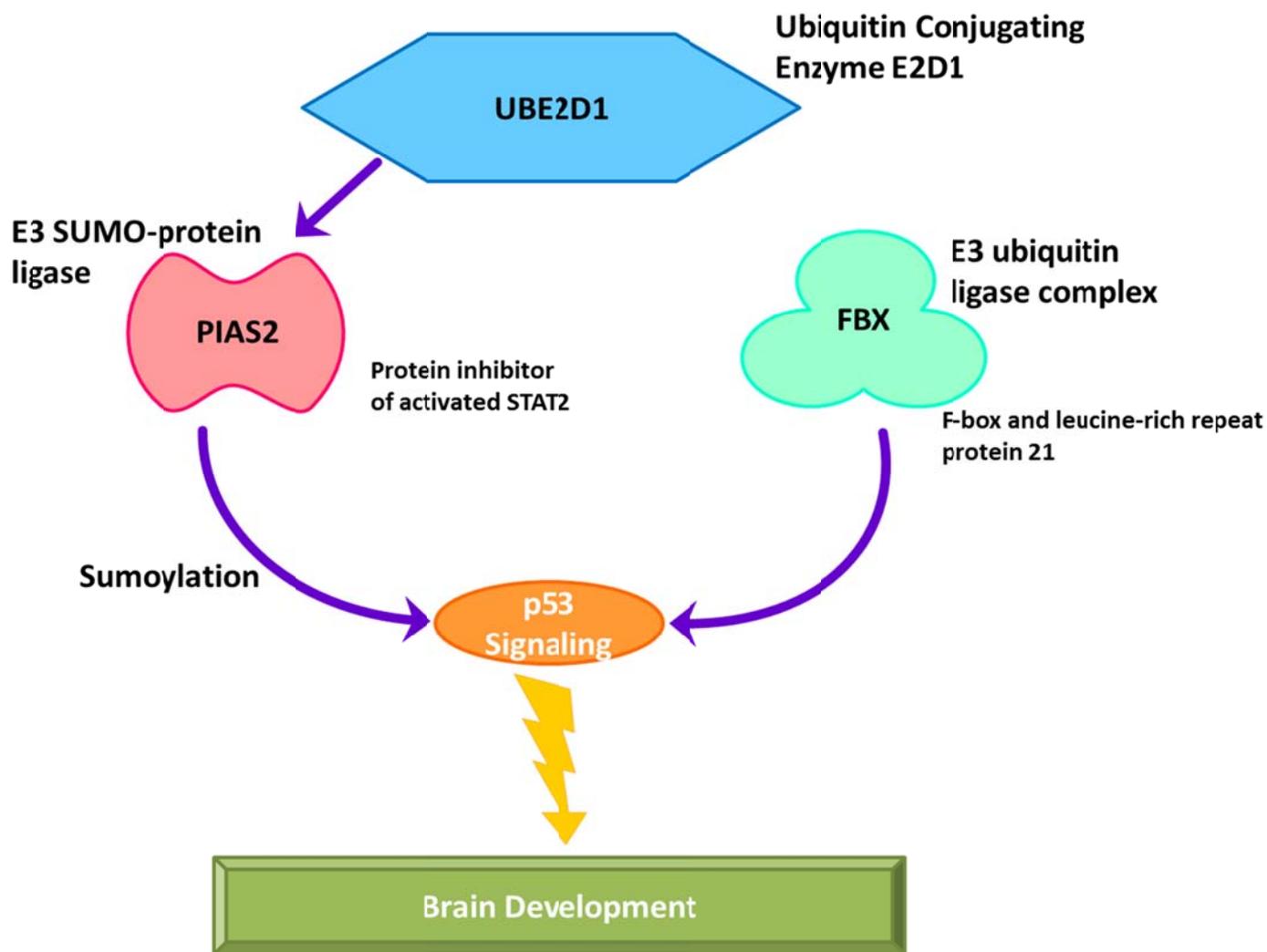


Figure 1. Schematic of the implication of the proteins coded by the genes of interest in brain development.