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Analysis of protocadherin alpha gene enhancer polymorphism in bipolar disorder and schizophrenia

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Abstract

Cadherins and protocadherins are cell adhesion proteins that play an important role in neuronal migration, differentiation and synaptogenesis, properties that make them targets to consider in schizophrenia (SZ) and bipolar disorder (BD) pathogenesis. Consequently, allelic variation occurring in protocadherin and cadherin encoding genes that map to regions of the genome mapped in SZ and BD linkage studies are particularly strong candidates to consider. One such set of candidate genes is the 5q31-linked *PCDH* family, which consists of more than 50 exons encoding three related, though distinct family members – α, β, and γ – which can generate thousands of different protocadherin proteins through alternative promoter usage and cis-alternative splicing. In this study, we focused on a SNP, rs31745, which is located in a putative *PCDHα* enhancer mapped by ChIP-chip using antibodies to covalently modified histone H3. A striking increase in homozygotes for the minor allele at this locus was detected in patients with BD. Molecular analysis revealed that the SNP causes allele-specific changes in binding to a brain protein. The findings suggest that the 5q31-linked *PCDH* locus should be more thoroughly considered as a disease-susceptibility locus in psychiatric disorders.

1. Introduction

Cadherins are transmembrane proteins with extensive extracellular domains that exhibit adhesion properties by homophilic and heterophilic protein-protein interactions, through which they guide neuronal migration and positioning during development (reviewed by Yagi and Takeichi, 2007). They also play a role in neuronal differentiation and synaptogenesis, processes that are believed to underlie the development of schizophrenia (SZ) and bipolar disorder (BD). Thus, genetic variation occurring in cadherin-encoding genes, especially those that map to regions of the genome implicated in SZ and BD by linkage analysis, should be viewed as candidates underlying disease susceptibility.

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The cadherin family consists of nearly 100 different genes scattered throughout the genome either as separate entities or as members of tandem clusters that arose through gene duplication. The largest such cluster is the *PCDHαβγ* multigene family of protocadherins on chromosome 5q31 (Sano et al., 1993; Wu and Maniatis, 1999; Frank and Kemler, 2002; Tasic et al., 2002; Hirayama and Yagi, 2006; Zou et al., 2007). The organization and regulation of the *PCDHαβγ* cluster is consistent with an underlying innate mechanism for generating protein diversity. An array of PCDHα, and PCDHγ proteins is generated from a series of N-terminal encoding variable exons, which are transcribed via alternative promoter usage, and cis-alternative splicing to one of several different genes coding for C-terminal constant domains (Wu and Maniatis, 1999; Tasic et al., 2002; Wang et al., 2002; Hirayama and Yagi, 2006; Kanecko et al., 2006). There are 13 variable *PCDHα* exon domains, which are paired with one of two constant genes encoding class-specific C-termini. *PCDHγ* is configured in a similar manner with 19 variable exons and 3 constant genes. The *PCDHβ* gene locus contains 18 variable exons, but lacks a constant region. The repertoire of diverse isoforms encoded by this locus is increased by the presence of an unusually large number of polymorphic, nonsynonymous SNPs. With respect to the genetic diversity generated from a relatively small number of subunits, the 5q31-linked *PCDH* locus displays characteristics similar to the immunoglobulin and T-cell receptor loci, except that genetic diversity in B and T-lymphocytes is generated by somatic rearrangement.

PCDHα is expressed in the central nervous system during development, primarily in the postsynaptic density fractions, whereas *PCDHγ* is more ubiquitous (Bonn et al., 2007). Interestingly, RT-PCR analysis and DNA sequencing carried out in single Purkinje cells indicate that only one or two *PCDHα* and γ variable exons are expressed in individual cells, and that expression occurs in an allele-specific manner (Kohmura et al., 1998; Esumi et al, 2005; Kaneko et al., 2006). Restricting protocadherin expression irrespective of the isoform diversity capable of being generated is consistent with the notion that these proteins provide specific instructions and addresses to individual cells in their migration paths during development. Cell-cell contact is accomplished through homophilic interaction of protocadherin variable subunits. However, protocadherins contain specific disulfide-bonded Cys-X(5)-Cys motifs not found in classical cadherins, which suggest heterophilic cell adhesion properties as well, possibly through beta1 integrin or between PCDH α and γ proteins (Morishita et al., 2006; Bonn et al., 2007).

The 5q31-linked *PCDH* family is also a positional candidate locus in SZ and BD since linkage to the region has been reported in several studies in both conditions, although the most positive findings are somewhat telomeric (Schwab et al., 1997; Straub et al., 1997,2002; Kendler et al., 2000; Gurling et al., 2001; Paunio et al., 2001; DeLisi et al., 2002; Devlin et al., 2002; Lewis et al., 2003; Sklar et al., 2004; Hong et al., 2004; Herzberg et al., 2006; Kerner et al., 2007).

Several candidate genes in the region have been considered including epsin 4 (*CLINT1*), and the *GABAA* locus between 160–170Mb, although the results are equivocal (Ikeda et al., 2005; Liu et al., 2005; Petryshen 2005; Pimm et al., 2005; Tang et al., 2006; Jamra et al., 2007; Lo et al., 2007). Recently, Fanous et al., (2007) presented data showing a modest association in SZ to a haplotype in the neurogenin 1 gene, which maps to ~134.9Mb. Kirov et al (2003) found a significant difference in the distribution of a frameshift mutation in the *PCDHγA8* gene in SZ and controls. However, the frequency of the variant was higher in controls than patients and no selective transmission of the allele was found in family triads. In a previous study, we analyzed a polymorphic copy number variation (CNV), a16.7 kilobase (kb) deletion affecting *PCDH* exons α8–10, initially characterized by Noonan, et al., (2003). However, no differences were detected in patients with BD and SZ compared with controls (in press).

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We have now extended our analysis of this interesting locus by examining a SNP located in an enhancer element located 3’ to the *PCDHα* locus. A significant increase in homozygosity for the minor allele was found in patients with BD.

2. Methods and Materials

2.1. Subjects

Individuals with BD from the Czech Republic were unrelated subjects recruited from in-patient and out-patient units at the Prague Psychiatric Center, Psychiatric Hospital Bohnice, Psychiatric Clinic (n=167). Diagnosis was made on the basis of either a Schedule for Affective Disorders and Schizophrenia-Lifetime (SADS-L; Endicott and Spitzer, 1978) interview (N=68) or by unstructured clinical interview modified from SADS-L using Research Diagnostic Criteria (RDC) criteria for diagnosis of either bipolar disorder I or bipolar disorder II (Spitzer et al, 1978) (N=99). Control subjects from the Czech Republic were blood-bank donors and individuals hospitalized for medical reasons (n=211). Seventy-one control subjects did not have underlying psychiatric illness based on a brief psychiatric clinical interview. In the remaining controls, all from blood bank donors, no formal testing procedure was used to screen for personal history of mental illness. However, the blood bank only accepted subjects who were not being treated for a psychiatric illness and had no family history of mental illness.

Individuals with SZ (n=176) were recruited from Rockland State Hospital. Diagnosis was established by Research Diagnostic Criteria (RDC) using SCID or clinical interview. U.S. controls (n=175) were blood bank donors. No formal testing procedure was used to screen these subjects to rule out individuals who had a personal or family history of mental illness, although the frequency of BD and SZ in a population of blood donors would be expected to be 1% or less for each. All subjects were Caucasians. They each signed an informed consent approved by the Ethical Committee on Clinical Investigation (Czech samples) and the AECOM IRB (U.S. samples).

2.2 ChIP-chip

A custom tiled microarray was designed containing the entire *PCDHαβγ* cluster including extensive flanking domains (chromosome 5, 138,700,000–141,500,000). 50-mer oligonucleotide probes were tiled every 38 bp across the region, minus repetitive sequences (NimbleGen Systems [NGS]; Madison WI and Reykjavik, Iceland). The array included a number of other SZ and BD candidate genes, the results of which will be reported elsewhere. Coordinates are from the hg18 assembly, NCBI build 36.1 (genome.ucsc.edu). Chromatin immunoprecipation was carried on brain tissue from a 20-week old aborted fetus using procedures described in Oberley et al., (2004) and Xu et al., (2007). Tissue was obtained from the Human Fetal Tissue Repository at the Albert Einstein College of Medicine under an IRB-approved protocol. IgG antibody to acetylated histone H3K9/14 (H3K9/14Ac) (Upstate Cell Signaling, now part of Millipore, Billerica, MA USA), and monomethylated histone H3K4 (H3K4me1) (Abcam ab8895) were used for the immunoprecipitation. Sheared input chromatin was used as a control. The entire procedure for preparing ligation mediated PCR amplified DNA from immunoprecipitated chromatin can be found in supplementary table 1. H3K4me1 ChIP-chip was carried out twice, while the H3K9/14Ac experiment was performed only once since results from this and other arrays showed that there was excellent overlap with the monomethylation data. Arrays were hybridized with Cy5 and Cy3-labeled DNA. The samples were labeled and hybridized by NGS using their standard in-house protocols. GFF files generated from the hybridization signals were imported for data analysis. Significant differences in the log2 ratio signal between modified histone immunoprecipitates and control DNA and an estimate of the false discovery rate (FDR) were determined using NimbleScan (NimbleScan user’s guide). An FDR <0.05 (based on randomizing the data 20 times) provides

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the highest confidence level that the peak corresponds to a true protein binding site (red peaks). FDR rates between 0.05 and 0.2 are also indicative of a protein-binding site (orange peaks, 0.05–0.1; yellow peaks, 0.1–0.2. Grey peaks (FDR>0.2) were viewed as false positives.

2.2. Genotyping

Genotyping was carried out using the TaqMan® Allelic Discrimination Technique according to the manufacturer's instructions. Samples were amplified by PCR in 384 well plates using an Applied Biosystems Model 7900HT thermal cycler and SDS 2.1 software (Applied Biosystems). A total of six SNPS were analyzed in the *PCDHα* gene locus, which spans ~260kb (140,146,060–140,372,113): rs10036519 (140,131,885 upstream of start site), rs3756337 (140,166,648 in PCDHα4), rs59479 (140,241,887 in PCDHα13), rs6876364 (140,387,178), rs31745 (140,400,408 in PCDHα 3' enhancer) and rs17119385. (140,402,321). All SNPs were available through the ABI pre-designed assays except rs59479, which was designed from a ~600bp sequence using File Builder v3.1 software (Applied Biosystems) (supplementary table 2).

2.3. Electromobility Gel Shift Assay (EMSA)

EMSA was performed according to published procedures (Hope et al., 1994). Briefly, double-stranded oligonucleotide probes containing the polymorphic variants were constructed. These were annealed and end-labeled with ^{32}P deoxynucleotides using Klenow polymerase to fill in 5' overhangs, which generated double stranded probes that were used for protein binding experiments (probe size 32bp after filling in: primers used to generate allele specific probes are shown in supplemental table 2). Nuclear protein extract was isolated from fetal (whole brain) and adult brains (parietal lobe). Protein (10 micrograms) was mixed with probe (1 nanogram, ~10^6 counts) and incubated for 20 minutes at room temperature. Probes containing the different alleles were labeled simultaneously using the same amount of DNA and radioactive nucleotides. After purifying the probes, an aliquot was analyzed by scintillation counting to ensure that the labeling efficiency was greater than 10^8 cpm/μg. DNA-protein complexes were resolved by electrophoresis in a non-denaturing gel system containing 6% acrylamide and 1.6% glycerol. Specificity of the resulting binding activity was demonstrated by competition with non-radioactive probe added in 100-fold excess. Autoradiograms were scanned and quantified by normalizing against unused probe. Differences between the two alleles for each sample were analyzed by using a paired t-test.

2.4. Analysis of copy loss by Quantitative PCR

Quantitative real-time PCR (qPCR) was used to detect copy differences applying the technique described by Meijerink et al (2001), Ponchel et al (2003), and Weksberg et al (2005). The fluorescent signal from the TaqMan® assay was used to assess the test region, while for the control gene, *H6PD* (hexose-6-phosphate dehydrogenase — the autosomal or H-form of glucose 6-phosphate dehydrogenase PCR was carried out in the presence of SYBR® Green 1 (SYBR® Green 1 Master Mix, Applied Biosystems). The complete procedure is shown in supplementary table 3.

2.5. Statistical analysis

Tests for Hardy-Weinberg equilibrium (HWE) were conducted for each of the SNPs separately for controls and cases. The exact p-value for the HWE test was approximated by 10,000 permutations. A statistical program, StatXACT_5 (Cytel Software Corporation, Cambridge MA) was used to compute chi square statistics for differences in allele and genotype frequencies. The level of significance was set at $p<.05$.

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3. Results

3.1. ChIP-chip

The <50 different *PCDHαβγ* exons are heterogeneously expressed throughout the central nervous system. We reasoned, therefore, that if this locus is involved in SZ or BD pathogenesis, which appear to affect a variety of cortical and subcortical regions, regulatory factors that influence the expression of many components, such as an enhancer element, would be the most reasonable targets to analyze for disease involvement. To identify putative regulatory domains in the *PCDH* locus in human tissue, ChIP-chip was used. Chromatin from fetal brain was immunoprecipitated with antibodies to histone H3 monomethylated at lysine 4 (H3K4me1), which is associated with expressed genes, active promoters and some enhancers, and histone H3 acetylated at lysines 9 and 14 (H3K9/14Ac), which is enriched in promoters, enhancers and putative regulatory domains of unknown function (Bernstein 2005; Roh et al., 2005, 2007; Heintzman et al., 2007). Immunoprecipitates were hybridized to tiled microarrays containing the entire *PCDHαβγ* locus. Hybridization peaks from representative H3K9/14Ac and H3K4me1 ChIP-chip experiments, indicative of enrichment of DNA in the chromatin immunoprecipitates generated using antibodies to modified histones compared to control chromatin, are shown in supplementary figure 1 and supplementary figure 2; a summary of the findings is shown on table 1. A total of 13 peaks or peak clusters were found in the H3K9/14Ac ChIP-chip, and 12 were found in the H3K4me1 ChIP-chip experiments. Of these, 11 overlapped. A number of peaks are near the transcription start sites (TSS) for several genes, corresponding to enrichment of their respective promoter regions in the immunoprecipitates, including *PCDHαC1, PCDHαC2, PCDHγA1, PCDHγC3, PCDHγC4, PCDHγC5*, and *TAF7*, a closely linked gene. The ability to detect promoters with the ChIP-chip strategy we used is consistent with previous observations (Bernstein et al., 2005), Roh et al., 2005) and Heintzman et al., 2007; Lachman et al, submitted). Of note, however, is the absence of significant peaks at all but one of the *PCDHαβγ* variable exon promoters; the exception is *PCDHγA1* (H3K9/14Ac peak 6). The absence of other variable exon promoter peaks is most likely due to their heterogeneous activation throughout the CNS. This is consistent with data reported showing DNA hypomethylation at an active *PCDHα* promoter in a cell line in contrast to the heterogeneous DNA methylation pattern found in brain tissue (Kawaguchi et al., 2008).

ChIP-chip peaks were also found near constant gene exons (H3K4me1 peaks 8–10; H3K9/14Ac peaks 9 and 10), suggestive of a role in alternative splicing. Finally, most relevant to this study, two peaks (peaks 3 and 4 in both ChIP experiments) were detected in regions corresponding to two *PCDH* enhancers identified in the murine *PCDHα* locus by Ribich et al., referred to as HS7 and HS5–1, respectively. Of these, HS5–1 (peak 4) was found to increase the expression of *PCDHαC1*, which codes for one of the constant genes, and all *PCDHα* variable exons, in the CNS of transgenic mice (Ribich et al., 2006). Based on our hypothesis that involvement of PCDHαβγ in SZ and BD would more likely involve multiple genes in the locus, we decided to focus on this region for further investigation.

3.2. Genetic analysis of rs31745

The region defined by peak 4 is highly conserved, contains two transcription factor binding sites (TFBS track, UCSC Browser), and has been immunoprecipitated with antibodies to H3K4me3 and c-myc in hes-3 ES cells and P493 cells, respectively, in other ChIP experiments (ChIP-PET track from Genome Institute of Singapore, UCSC Browser). These are features characteristic of enhancers. The two TFBS within the peak are LHX3, a LIM homeobox transcription factor, and the pro-apoptotic transcription factor CHOP/GADD153, which is a C/EBP homologue, a transcription factor targeted by the GSK3β signaling pathway, a target of lithium salts (Gould et al., 2007; Mennen et al., 2007; Mullen et al., 2007).

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Four SNPs are posted in dbSNP in the region contained within the peak 4. We chose to analyze rs31745, which maps to 140,400,408, because it is found in the region conserved only in higher order mammals (table 2). In addition the major allele (C) appears to be exclusive to humans, while the minor allele (T) is ancestral, being found in chimp, rhesus, dog, mouse, horse and armadillo. Two data sets were analyzed using a case control association design; cohorts of European Caucasian patients with SZ from the U.S. and Caucasian patients with BD from the Czech Republic. As seen in table 3, a significant increase in homozygosity for the T allele was detected in the bipolar patients; 5% had this genotype, but no controls (χ^2 test for independence of genotype distribution, Pearson χ^2 statistic=12.63, exact p=0.001, 2 df, two-tailed). However, the distribution of alleles did not differ between patients and controls because of the smaller number of heterozygotes detected in the bipolar patients (allele frequency Pearson χ^2 statistic=0.32, asymptotic p=0.57, 1df, two-tailed). An increase in homozygotes for the T allele was also seen in SZ patients, but the results were not statistically significant; a larger sample size will be needed to more accurately assess this marker in SZ.

The marked increase in homozygotes, given the allele frequency, and the relative decrease in heterozygotes resulted in strong deviation from HWE (exact p=0.0004). The genotype distribution from the control group for this population, and both the SZ and control samples from the US were, however, in HWE (table 3).

To exclude genotyping error as a cause of HWE deviation in the bipolar subjects, the homozygotes were reanalyzed; no errors were detected. In addition, the entire data set was analyzed for two other markers that are in strong LD with rs31745; rs6876364 (140,387,178) and rs17119385 (140,402,321) (LD between rs31745 and rs6876364 is D'=1.0, r^2=1.0 in HapMap CEPH families, and D'=1.0, r^2=0.849 for rs31745 and rs17119385). Similar results were obtained when the entire data set was genotyped (supplementary table 4). Three other SNPs, rs10036519, rs3756337 and rs59479 located ~160–270kb upstream from the enhancer regions were also analyzed; no significant differences were found in allele or genotype distribution and no deviation from HWE was detected (supplementary table 4). We conclude that the association signal detected using rs31745 is not a genotyping artifact and the deviation from HWE appears to be restricted to SNPs in LD with the *PCDHα* enhancer region.

Another cause for deviation from HWE is copy variation. Several CNVs have been found in the *PCDHαβγ* region, including the 16.7 kb deletion involving *PCDHα* exons 8–10 discovered by Noonan et al., (2003), which we previously analyzed in SZ and BD (in press). An extensive polymorphic copy gain variant involving ~670kb, and a relative rare (<0.045% allele frequency) copy gain variant both appear to affect the 3' enhancer region (variations 9522 and 3578, respectively; see Database of Genomic Variants, http://projects.tcag.ca/variation, based on Wang et al., 2007; Redon et al., 2006). Although copy loss, not gain, would account for the apparent increase in T allele homozygosity, we wanted to determine whether a deletion involving the enhancer region accounted for the increase in the number of apparent homozygotes (i.e, hemizygosity) we detected using rs31745 and neighboring SNPs. No evidence for copy loss was detected in the homozygotes using a variation of the $2^{-\Delta\Delta Ct}$ technique (supplementary table 5).

3.3. EMSA

As a first step towards determining the functional significance of the enhancer polymorphism, EMSA was carried out. Crude nuclear protein extract from fetal brain tissue was used. Protein was annealed to labeled double stranded, allele-specific probes, and the samples were resolved by non-denaturing gel electrophoresis, as described in the methods section. As seen in figure 3, band shifts were detected for both alleles. These were specifically bound DNA-protein complexes as seen by the loss of signal when unlabeled competitor oligonucleotide was added during the annealing reaction (last two lanes). The most striking observation is the loss of signal

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with fetal age. The strongest signal was detected in nine-week old fetuses and the weakest at 20 weeks. Signal was weak in adult brain as well. This is consistent with the higher level of *PCDHα* expression found in developing brain compared with adults. Second, allele specific differences were also detected. Radiolabeled signals generated from the protein-DNA complexes were scanned and normalized. A 49% decrease in signal intensity was detected for the minor allele-containing probe (T) compared with the wild type allele (C) (p=0.044; paired T-test, supplementary table 6). A decrease in the DNA-protein complex generated with the T-allele probe is supported by the EMSA experiment carried out using different concentrations of unlabeled competitor, which shows a more rapid reduction in signal compared with C-allele probe (supplementary figure 3).

4. Discussion

SZ is viewed as a neurodevelopmental disorder in which fetal brain development is adversely affected by genetic factors, possibly in combination with an environmental component, such as neonatal hypoxia, maternal infection, malnutrition, and micronutrient deficiency (reviewed by Jarskog et al., 2007). Although prenatal complications have generally not been implicated in BD (Scott et al., 2006), a neurodevelopmental problem may underlie disease pathogenesis in a subgroup of such patients as well, as suggested by the involvement of both SZ and BD by *DISC1*, which codes for various protein isoforms that influence neuronal migration and neurite outgrowth (Kamiya et al., 2005; Maier et al., 2006; Sawamura and Sawa, 2006; Mackie et al., 2007; Roberts, 2007).

Based on the neurodevelopmental model, we and others are beginning to analyze cadherin and protocadherin-encoding genes in SZ and BD. Analysis of nonsynonymous SNPs in *PCDH11X/Y*, a brain expressed, hominoid-specific protocadherin member that maps to the Xq21.3 pseudoautosomal region revealed no significant differences in patients with SZ and controls, although the data sets may have been underpowered (Ross et al., 2003; Giouzeli et al., 2004; Durand et al., 2006). Analysis of a nonsynonymous SNP at *PCDH8* codon 7 showed a trend towards significance for the minor allele in a large case control comparison, but not in a family based association sample (Bray et al., 2002). Another rare nonsynonymous SNP was found in one patient and an affected sibling, but no other patients, so its significance could be not determined. Recently published studies suggest that genetic variation in the cadherin member *FAT* is involved in BD (Blair et al., 2006), and haplotypes within 22q11-linked *ARVCF*, a member of the catenin family that maps immediately 3' to *COMT*, are associated with SZ (Sanders et al., 2005); catenins interact with cadherins to modulate synaptic function (reviewed by Kwiatkowski et al., 2007).

Molecular analysis of protocadherins in SZ and BD has been limited so far. Dean et al., (2007), found an increase in *PCDH17* mRNA in BA 46 in patients with SZ, but only in subjects with a short history of disease. No changes were found in other brain areas. In our previous study of a polymorphic copy deletion variant involving *PCDHα* exons 8–10, the frequency was similar in patients with SZ and BD, as well as controls, although the sample was underpowered to detect a small effect (in press). Similarly, no association was detected in SZ in the analysis of rs31745 in this study, which maps to a putative *PCDHα* enhancer (although there were 2 patients and only 1 control who were homozygous for the minor allele). An underpowered sample size could have resulted in the absence of statistical significance for minor allele homozygosity. Nevertheless, a much more thorough investigation of the *PCDHα* and γ loci is justified in SZ, considering the allele-specific pattern of expression found for PCDH α and γ variable exons; allele-specific expression could explain the MZ concordance rate of ~50% found in SZ and the similar risk of SZ found in the children of discordant and concordant MZ twins (Gottesman and Bertelsen, 1989; Onstad et al., 1991; Tsuang, 2000; Petronis, 2003; Kato et al., 2005; Procopio, 2005).

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In contrast to the negative findings in SZ, however, a strong association was detected in the bipolar cohort from the Czech Republic we analyzed. One caveat to the finding is the significant deviation from HWE in the patient sample. We ruled out several experimental artifacts and genetic phenomena that could account for the finding including genotyping error and copy variation. In addition, the clinical records and demographic profiles for each of the rs31745 homozygotes were examined to determine if population stratification could have artificially caused the deviation from HWE. However, these subjects did not appear to differ from other bipolar subjects analyzed in this study. Nevertheless, more subtle forms of population stratification could exist which would require much more extensive genotyping. Another possibility, of course, is that homozygosity for the minor allele in the enhancer SNP is a risk genotype for BD, and selection for this genotype in the bipolar cohort could have resulted in HWE deviation.

The function of the enhancer SNP has not yet been determined. However, a significant decrease in binding to an unknown brain protein was detected by EMSA, which is suggestive, but by no means proof of an *in vivo* effect. The SNP is of interest because the major allele appears to be unique to humans and the surrounding 30–35 bases are only conserved in higher animals. The effect of this SNP on enhancer function and the nature of the DNA binding protein detected in the EMSA experiments are currently under investigation. A TRANSFAC search for potential binding sites for transcription factors shows that rs31745 is within a motif for the POU transcription factor Brn2 (POU3F2), which regulates neural development (motif is CATNSRWAATNMR; Core match 0.987, matrix match 0.842 for C allele; Core match 0.987, matrix match 0.790 for T allele). Two NGAAN motifs suggestive of HSF1 (heat shock factor 1) binding sites are also found for the C allele (opposite orientation), while the T allele has only one. However, the random frequency of this motif is very high, so the detection does not have much statistical significance. Also, two motifs are usually insufficient for HSF binding.

The 3’ enhancer in mice referred to as HS5–1, which corresponds to peak 4 in our ChIP-chip experiments, has been shown to affect the expression of all *PCDHα* variable exons, as well as *PCDHαC1*, but not *PCDHαC2* or the adjacent *PCDHβ* locus (Ribich et al., 2006). Whether the same pattern of regulation exists in humans is not known. An effect on an enhancer regulating the expression of each *PCDHα* variable exon and *PCDHαC1*, to the exclusion of an affect on *PCDHαC2*, could influence synaptogenesis in brain development, and in the post-development brain as well, by reducing expression of *PCDHα* variable exons and changing the dynamics of constant region splicing.

Overall, our findings suggest that the *PCDHαβγ* is an interesting gene family to consider in SZ and BD susceptibility, and that rs31745 is a candidate allelic variant.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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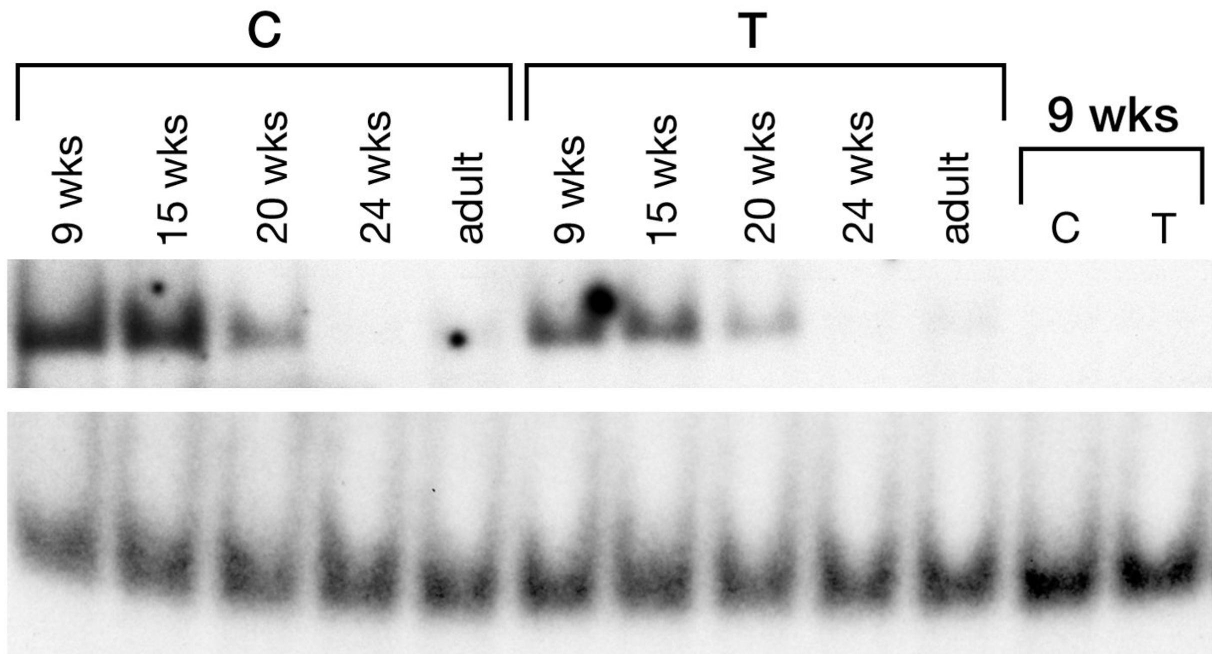

Figure 1.

EMSA. Double-stranded oligonucleotide probes (32-mers) containing either the C or T alleles for rs31745 were annealed to nuclear brain protein extracts from fetal (different ages shown) and adult tissue. Top panel, DNA-protein complex; bottom panel, unused probe. Last two lanes show loss of signal when 100-fold molar excess of unlabeled probe was added to annealing step (protein from 9 week old fetus used in cold competition).

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TABLE 1
Summary OF ChIP-chip findings in PCDHαβγ locus

Summary of ChIP-chip peaks shown in supplemental figure 1 and supplemental figure 2 for H3K9/14Ac and H3K4me1 immunoprecipitates. Map distances on chromosome 5 are from NCBI build 36.1, hg18.

H3K9/14 Ac Peaks	**H3K4me1 Peaks**	**Notes**
1. 140,286,433–140,287,582	1. 140,287,298–140,287,602	*PCDHαC1* promoter (TSS 140,286,486)
2. 140,326,988–140,327,157	2. 140,326,798–140,328,277	*PCDHαC2* promoter (TSS 140,326,296)
3. 140,361,419–140,362,779	3. 140,361,319–140,362,458	corresponds to HS 7
4. 140,400,198–140,400,868	4. 140,399,758–140,400,086	corresponds to HS 5-1
5. 140,680,813–140,681,002	5. 140,679,488–140,679,732	TAF7 promoter (TSS 140,678,241)
6. 140,686,031–140,686,385		P*CDHγA1* (TSS 140,690,436)
7. 140,836,076–140,838,601	6. 140,835,361–140,839,748	*PCDHγC3* (TSS 140,835,753)
8. 140,844,072--40,845,334	7. 140,843,692–140,847,314	*PCDHγC4* (TSS 140,844,925)
	8. 140,848,685–140,851,154	*PCDHγC5* (TSS 140,848,992) and exon
9. 140,852,520–140,855,580	9. 140,853,196–140,856,220	common constant region exon
10. 140,868,194–140,869,553	10. 140,867,949–140,869,553	~1kb 5' to *PCDHγ* constant region terminal exon
11. 140,872,814–140,874,481	11. 140,872,879–140,874,516	3' to *PCDHγ* locus
12. 140,883,968–140,884,963	12. 140,884,193–140,884,922	3' to *PCDHγ* locus, 5' end of EST AK094264
13. 140,917,578–140,919,962		EST BC041908 at 140,918,062; *DIAPH1* intron

Table 2

Sequence comparison near rs31745

Sequence conservation around rs31745 for seven different species taken from UCSC browser. Major allele (C) highlighted in bold, caps and underlined.

Human	ctctt**C**ttgtctaattagtcgctaagcaactg
Chimp	ctctttttgtctaactagtcgctaggcaactg
Rhesus	ctctttttgtctaactagtcgctaggcaactg
Mouse	tttttgtctaactggtcgctaggcaactg
Dog	ctctttttgtctaatgcattgctaagcaaccg
Horse	ttctttttgtctaatttgttgctaggtaactg
Armadillo	ttctttttgt----------------------------

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TABLE 3

Analysis of rs31745 in BD and SZ

BD vs CONTROLS: allele frequency Pearson χ^2 statistic=0.32, asymptotic p=0.57 (1df, two-tailed); genotype distribution χ^2 test for independence, Pearson χ^2 statistic=12.63, exact p=0.001 (2 df, two-tailed); HWE: CONTROLS, p=0.14; BD, p=0.0004. SZ vs CONTROLS allele frequency Pearson χ^2 statistic=0.45, asymptotic p=0.50 (1df, two-tailed); genotype distribution χ^2 test for independence, Pearson χ^2 statistic=1.28, exact p=0.53 (2 df, two-tailed); HWE (exact p-values): CONTROLS, p=0.70; SZ, p=0.66

	Genotypes			Alleles	
	CC	**CT**	**TT**	**C**	**T**
CONT	166 (.79)	45 (.21)	0 (0)	377 (.89)	45 (.11)
BD	135 (.81)	24 (.14)	8 (.05)	294 (.88)	40 (.12)
CONT	135 (.78)	37 (.21)	1 (.006)	307 (.89)	39 (.11)
SZ	143 (.82)	30 (.17)	2 (.01)	316 (.90)	34 (.10)