Original Article

Case-control association study of human netrin G1 gene in Japanese schizophrenia

Masayuki Fukasawa1,2, Mika Aoki1,2, Kazuo Yamada1, Yoshimi Iwayama-Shigeno1, Hitomi Takao1, Joanne Meerabux1, Tomoko Toyota1,2, Toru Nishikawa2 and Takeo Yoshikawa1

1) Laboratory for Molecular Psychiatry, RIKEN Brain Science Institute, Saitama, Japan
2) Section of Psychiatry and Behavioral Sciences, Tokyo Medical and Dental University Graduate School, Tokyo, Japan

The exact etiology of schizophrenia remains undetermined but accumulating evidence suggests that disturbances in neurodevelopment may represent one contributory factor. Netrin G1, a recently cloned gene from the mouse, has been shown to play a potential role in the formation of neural circuitry. To determine whether this gene is involved in the development of psychosis, we performed a genetic association study of human netrin G1 gene in schizophrenia. First, we determined the human genomic structure of netrin G1 by direct comparisons between cDNA and genome sequences, and by database searches. For the subsequent examination of heterozygosity, we selected 10 single nucleotide polymorphisms (SNPs) for an association test in case (n = 180) and control (n = 180) samples. Among these SNPs, IVS8-1467C>T showed significant allelic association (nominal P = 0.020) with disease. This SNP is located in a haplotype block of ~40 kb and haplotypes in this block also displayed significant association (most significant P = 0.017). These findings suggest that netrin G1 or a nearby gene may contribute to the overall genetic risk for schizophrenia.

Key words: netrin family, laminet 1, axon guidance, haplotype, linkage disequilibrium

Introduction

Schizophrenia is a common and devastating mental disorder of unknown etiology. Multiple factors including risk-conferring genes and undefined environmental variables are thought to contribute to overall susceptibility.1 One etiological hypothesis is that neurodevelopmental abnormalities are at least partially involved in the manifestation of schizophrenia. This assertion is supported by a range of epidemiological, clinical and neurobiological evidence.2 The developing nervous system is dependent on the actions of various secreted factors and membrane proteins that allow neuronal axons to find their correct targets. The proteins that provide these cues include netrins, ephrins, semaphorins and slits.3 Classical netrins identified as laminin like molecules that direct migration in Caenorhabditis elegans are soluble secreted proteins that provide bifunctional axon guidance signals that can mediate either attraction or repulsion.4 Three classical netrin molecules (1, 2 and 3) have been characterized in vertebrates.5 The gene family is structurally related to the short arms of the laminin γ chain, comprising a laminin VI domain, three LE repeats, similar to the laminin V domain and a positively charged heparin-binding carboxyl domain.6 Recently, netrin G1 (also called laminet 1) has been identified in the mouse.7 Its predicted domain structure resembles that of the laminin β chain and the protein is...
linked to the plasma membrane by a glycosyl phosphatidyl-inositol (GPI) lipid anchor, an important feature that distinguishes them from classical netrins.\(^7\,8\) Additionally, netrin G1 does not bind to receptors for classical netrins, nor does it attract circumferentially growing axons from the cerebellar plate in explant extracts.\(^7\) No orthologues for netrin G1 gene have been found in the \textit{C. elegans} or \textit{D. melanogaster}. These observations suggest that netrin G1 may play an as yet, undetermined role in cell architecture that is unique to vertebrates.

Based on the potential relevance of netrin G1 to neurodevelopment, we performed a genetic analysis of this gene in schizophrenia.

**Material and Methods**

**Subjects**

Schizophrenic samples were composed of 90 males (mean age, 40.3±8.6 years) and 90 females (mean age, 47.1±13.0 years). All patients were diagnosed according to the criteria of the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) for schizophrenia, to give a best-estimate lifetime diagnosis with consensus from at least two experienced psychiatrists (Yamada K, Toyota T and Yoshikawa T). The interview parameters included those described in the Structured Clinical Interview For DSM-IV Axis I Disorders. All available medical records and family informant reports were also taken into consideration. Controls comprising 90 males (mean age, 39.3±11.5 years) and 90 females (mean age, 46.9±11.9 years), were recruited from hospital staff and company employees documented to be free from psychoses. All of our samples were collected from central Japan.

The present study was approved by the Ethics Committees of RIKEN and Tokyo Medical and Dental University, and all participants provided written informed consent.

**Determination of genomic organization of netrin G1 gene**

A mouse netrin G1 cDNA sequence NM_030699 and a human EST (expressed sequence tag) clone KIAA0976 sequence (NM_014917) were compared to human BAC clones forming the contig NT_029860 using BLAST, to determine the intron/exon structure of the human netrin G1 gene. This led to the identification of 10 exons, with translation starting within exon 2 (Fig. 1). The UCSC April 2003 draft assembly of the human

![Fig. 1. Genomic structure and locations of polymorphic sites for the human netrin G1 gene. Exons are denoted by boxes, with untranslated regions in open boxes and translated regions in closed boxes. The sizes of exons (bp) and introns (kb) are also shown. The rs number of each SNP is the NCBI SNP cluster ID from the dbSNP database (http://www.ncbi.nlm.nih.gov/SNP/).](image-url)
genome (UCSC Genome Bioinformatics web site, http://genome.ucsc.edu/) included only exons 1 to 5 in its gene prediction program. "A" from the ATG initiation codon was considered at +1.

**Single nucleotide polymorphisms (SNPs) search and genotyping**

We consulted the JSNP database (http://snp.ims.u-tokyo.ac.jp/) and The SNP Consortium Ltd database (http://snp.cshl.org/) to find polymorphisms within the netrin G1 gene, and identified a total of 16 SNPs (Fig. 1). We first genotyped these SNPs using 40 randomly chosen schizophrenic samples, and direct sequencing of PCR products (the SNPs located in exons and nearby introns) or the TaqMan method (Applied Biosystems, Foster City, California, US) (the SNPs located in deep introns). The primers used for PCR amplification are shown in Table 1. Sequencing of PCR products was performed using the BigDye Terminator Cycle Sequencing FS Ready Reaction kit (Applied Biosystems) and the ABI 3700 Genetic Analyzer (Applied Biosystems). The SNPs that were not polymorphic in the 40 samples were excluded from further genetic analyses. These included IVS2-6691A>T (rs4471296, http://www.ncbi.nlm.nih.gov/SNP/), IVS2-48075A>G (rs4550083), IVS2-6059G>A (rs4463701), IVS3-9907G>T (rs1899775), IVS6+1905G>A (rs556157) and 1746+5572C>A (rs2166017) (Fig. 1). The remaining 10 variants were genotyped in all samples using the Taqman method, that utilizes the 5'-exonuclease activity of the Taq polymerase in combination of PCR and competitive hybridization. Probes and primers were designed using the Assays-by-Design File Builder v2.0 software and the Primer Express software (Applied Biosystems) (Table 2). PCR reactions were performed using an ABI 9700 thermocycler, and fluorescence-based genotyping was conducted using an ABI 7900 sequence detection system and SDS v2.0 software (Applied Biosystems). The samples with ambiguous genotypes were not used in statistical analyses.

**Statistical analysis**

Deviation from Hardy-Weinberg equilibrium was

---

**Table 1.** PCR Primers used to examine nucleotide variants in the *NTNG1*

<table>
<thead>
<tr>
<th>Region</th>
<th>Primers (F, forward; R, reverse)</th>
<th>Product size (bp)</th>
<th>3' end of primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exon 2</td>
<td>(F) 5'-TGCTTTATATTGCCATTACGACCTC-3'</td>
<td>480</td>
<td>-603 (Intron 1)</td>
</tr>
<tr>
<td></td>
<td>(R) 5'-GACCTCAAACGACATCCCCAAA-3'</td>
<td></td>
<td>-146  (exon 2)</td>
</tr>
<tr>
<td></td>
<td>(F) 5'-AGTATGTTAGCTTTCCAGCA-3'</td>
<td>566</td>
<td>-218  (exon 2)</td>
</tr>
<tr>
<td></td>
<td>(R) 5'-GCTTTCAGCTCACCCACATC-3'</td>
<td></td>
<td>+328  (exon 2)</td>
</tr>
<tr>
<td>Exon 3</td>
<td>(F) 5'-TAGGGCAATAAAAAATTGC-3'</td>
<td>584</td>
<td>+175568 (Intron 2)</td>
</tr>
<tr>
<td></td>
<td>(R) 5'-AAAAGGAGGAACCTGC-3'</td>
<td></td>
<td>+176134 (exon 3)</td>
</tr>
<tr>
<td></td>
<td>(F) 5'-AGCAAGATGCGAGGCTATCAG-3'</td>
<td>529</td>
<td>+175966 (exon 3)</td>
</tr>
<tr>
<td></td>
<td>(R) 5'-ATGCTTACGAAACCACT-3'</td>
<td></td>
<td>+176473 (exon 3)</td>
</tr>
<tr>
<td>Exon 4</td>
<td>(F) 5'-GGCTTGGAAAATCTATTCTTTACTA-3'</td>
<td>511</td>
<td>+246439 (Intron 3)</td>
</tr>
<tr>
<td></td>
<td>(R) 5'-GATGACGTATGATTTTACTGAT-3'</td>
<td></td>
<td>+246877 (Intron 4)</td>
</tr>
<tr>
<td>Exon 5</td>
<td>(F) 5'-TGCCTGTATTTTGTGTGTCG-3'</td>
<td>283</td>
<td>+258662 (Intron 4)</td>
</tr>
<tr>
<td></td>
<td>(R) 5'-CCTATTAGCATGAAATGGGACAT-3'</td>
<td></td>
<td>+259221 (Intron 5)</td>
</tr>
<tr>
<td>Exon 6</td>
<td>(F) 5'-AATTGCTTGGCTTTTGT-3'</td>
<td>347</td>
<td>+269832 (Intron 5)</td>
</tr>
<tr>
<td></td>
<td>(R) 5'-TTTCAAGAGCATAGCATGATT-3'</td>
<td></td>
<td>+270162 (Intron 6)</td>
</tr>
<tr>
<td>Exon 7</td>
<td>(F) 5'-CTTAATTAGGGCTACTTCCA-3'</td>
<td>254</td>
<td>+272404 (Intron 6)</td>
</tr>
<tr>
<td></td>
<td>(R) 5'-TCATGTTCCAGATATCAGATT-3'</td>
<td></td>
<td>+2727636 (Intron 7)</td>
</tr>
<tr>
<td>Exon 8</td>
<td>(F) 5'-ATGCGATTCCAGCTTTT-3'</td>
<td>406</td>
<td>+282063 (Intron 7)</td>
</tr>
<tr>
<td></td>
<td>(R) 5'-AGGATTTTTCTCAGATGAG-3'</td>
<td></td>
<td>+282450 (Intron 8)</td>
</tr>
<tr>
<td>Exon 9</td>
<td>(F) 5'-TCATTAATGGACATCTTT-3'</td>
<td>352</td>
<td>+287966 (Intron 8)</td>
</tr>
<tr>
<td></td>
<td>(R) 5'-GGACTTTTCTGCTGTA-3'</td>
<td></td>
<td>+288300 (Intron 9)</td>
</tr>
<tr>
<td>Exon 10</td>
<td>(F) 5'-GGTGAAAAACATGATACGAGTTG-3'</td>
<td>453</td>
<td>+331947 (Intron 9)</td>
</tr>
<tr>
<td></td>
<td>(R) 5'-AGGCTTCTTTGTTAGCAGTTC-3'</td>
<td></td>
<td>+332375 ( exon 10)</td>
</tr>
</tbody>
</table>

Nucleotide positions are counted from A of the start codons on the genomic stretches of *NTNG1* (GenBank accession No. NM_014917).
examined using the $\chi^2$ test. Differences in genotype and allele frequency were evaluated using Fisher’s exact test. Linkage disequilibrium (LD) statistics were calculated using COCAPHASE\textsuperscript{10} (http://www.hgmp.mrc.ac.uk/~fdudbrid/software/). Estimation and comparison of haplotype frequencies were also made using COCAPHASE. Graphical overview of pair-wise LD strength between markers was made using GOLD software\textsuperscript{11} (http://www.well.ox.ac.uk/asthma/GOLD/).

Results

The alignment of cDNA and EST sequences with genomic sequence revealed that the human netrin G1 gene is comprised of 10 exons (Fig. 1) located on chromosome 1p13.3 (http://genome.ucsc.edu/). A database search for polymorphisms detected only intronic SNPs within the gene, and we selected 16 roughly equidistant SNPs. Then we examined the heterozygosity of each SNP using 40 unrelated DNAs and excluded six SNPs for further analyses based on their low heterozygosity (frequencies of minor alleles $\leq 1\%$). The remaining SNPs were designated SNP1-10 (Fig. 1), and were genotyped in 180 schizophrenics and 180 age- and gender-matched controls.

All genotyped polymorphisms were in Hardy-Weinberg equilibrium in both case and control samples (Table 3). Of the 10 SNPs, SNP8 (IVS8-1476C $\rightarrow$ T) (NCBI dbSNP accession No. rs1373336, http://www.ncbi.nlm.nih.gov/SNP/) displayed a marginally significantly different genotypic distribution between patients with schizophrenia and control subjects ($P = 0.057$; Table 3). Allelic distribution of SNP8 showed a significant deviation in schizophrenics compared to controls: the C allele was over-represented in...

**Table 2.** TaqMan primer and probe sequences used to examine nucleotide variants in the NTNG1

<table>
<thead>
<tr>
<th>Marker $^a$</th>
<th>Primer (F, forward; R, reverse)</th>
<th>3’ end of primer $^b$</th>
<th>Reporter probe sequence (V, VIC label; F, FAM label) $^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>SNP1</td>
<td>(F) CTCTGAACTCTCCCTGACATGGAATTA</td>
<td>-4825 (intron 1)</td>
<td>(V) AAGAAATCTGGAGTTTTTTAA</td>
</tr>
<tr>
<td></td>
<td>(R) GAGGATGGGATATACCCAAAATTTTCA</td>
<td>-4768 (intron 1)</td>
<td>(F) AAGAAATCTGGAGTTTTTTAA</td>
</tr>
<tr>
<td>SNP2</td>
<td>(F) GTTCTGATATTGTTGTTGACATTG</td>
<td>+1944 (intron 2)</td>
<td>(V) CAGAGGAGAGGTGTGGT</td>
</tr>
<tr>
<td></td>
<td>(R) CCTGAGAAAGAAATTTGCTTTTTTACTTAGA</td>
<td>+1985 (intron 2)</td>
<td>(F) CAGAGGAGAGGTGTGGT</td>
</tr>
<tr>
<td>SNP3</td>
<td>(F) CATGCCATTTTTACAGTACTCTATCT</td>
<td>+29765 (intron 2)</td>
<td>(V) ATTTGCCGCTTTCT</td>
</tr>
<tr>
<td></td>
<td>(R) CCTCTAAAAAGATGGCTATAATGAGGA</td>
<td>+29814 (intron 2)</td>
<td>(F) TTTGCCGCTTTCT</td>
</tr>
<tr>
<td>SNP4</td>
<td>(F) GAAAGATGTTTCTAAGCAAGAGGT</td>
<td>+151610 (intron 2)</td>
<td>(V) CACACTGAGCCTGT</td>
</tr>
<tr>
<td></td>
<td>(R) AGCCAAACACTCTATAAGGCAAAT</td>
<td>+151636 (intron 2)</td>
<td>(F) CACACTGACCTGT</td>
</tr>
<tr>
<td>SNP5</td>
<td>(F) CCCACTGACCTTGCTATTCTG</td>
<td>+246328 (intron 3)</td>
<td>(V) CCCTGCCCTGCAAA</td>
</tr>
<tr>
<td></td>
<td>(R) GCCCTCAGAATCTCTCCCCAAGG</td>
<td>+246393 (intron 3)</td>
<td>(F) CCCTGCCCTGCAAA</td>
</tr>
<tr>
<td>SNP6</td>
<td>(F) TTTTAAATATAGTAGGTGAGCTCCTTTG</td>
<td>+256659 (intron 4)</td>
<td>(V) CTTTAGAGTGGGAAATAATCTGGA</td>
</tr>
<tr>
<td></td>
<td>(R) GGAATTTGCTTAAAGAGATTTTAAACTGC</td>
<td>+256999 (intron 4)</td>
<td>(F) TTGAGGGATTTGCAAATCTGGA</td>
</tr>
<tr>
<td>SNP7</td>
<td>(F) AGGCGAAGATTCAACTCAGTACAG</td>
<td>+261371 (intron 5)</td>
<td>(V) CACTGGCTTTCAGC</td>
</tr>
<tr>
<td></td>
<td>(R) GACCTGGAAACACCCATCTG</td>
<td>+261397 (intron 5)</td>
<td>(F) CTTCGGCTTACAGC</td>
</tr>
<tr>
<td>SNP8</td>
<td>(F) CATTTGATTATTTGCTAATCATACAC</td>
<td>+286672 (intron 8)</td>
<td>(V) AATTTGACTTATATCAAGACT</td>
</tr>
<tr>
<td></td>
<td>(R) TTTTTAATTTTCTCTGACATAGGACA</td>
<td>+286624 (intron 8)</td>
<td>(F) TTTGCCTATATCAAGACT</td>
</tr>
<tr>
<td>SNP9</td>
<td>(F) CCCAATAGGACATTAGGCTATGTTA</td>
<td>+298665 (intron 9)</td>
<td>(V) CATGAAATGAAATATATG</td>
</tr>
<tr>
<td></td>
<td>(R) TGCATTACACATCTGCTTGGAACAT</td>
<td>+298684 (intron 9)</td>
<td>(F) TCAGGAAATGAAATATG</td>
</tr>
<tr>
<td>SNP10</td>
<td>(F) GAGATCTGAGATTTTTGGACTATTGT</td>
<td>+327627 (intron 9)</td>
<td>(V) AAGACATAAAAGGATGCT</td>
</tr>
<tr>
<td></td>
<td>(R) CAGCTTTTGACCCATGGAAGAGA</td>
<td>+327641 (intron 9)</td>
<td>(F) ACCATAAAAGGATGCT</td>
</tr>
</tbody>
</table>

$^a$ For SNP numbers, see Fig. 1.

$^b$ Nucleotide positions are counted from A of the start codon on the genomic stretches of NTNG1 (GenBank accession No. NM_014917).

$^c$ Underline shows a polymorphic site.
schizophrenia (nominal $P = 0.020$, odds ratio = 1.44, 95% confidence interval = 1.06–1.96) (Table 3). After Bonferroni correction for the multiple testing of 10 SNPs, the deviation was no longer significant.

Next, we examined pair-wise linkage disequilibrium (LD) between markers. $D'$ (normalized D) and $r^2$ (squared correlation coefficient) values were computed in controls. Both LD measures take values between 0 (lack of LD) and 1 (complete LD). LD relationships between SNPs are shown in Table 4 and Fig. 2. SNPs 5–9 were in the same LD block using the two measures. The polymorphism (SNP8) associated with schizophrenia was located in this LD block. We examined two and three SNP-based haplotypic associations in a sliding manner, using the 10 polymorphisms that spanned netrin G1 gene (Fig. 3). The combinations of SNP7-SNP8 and SNP6-SNP7-SNP8 showed significant associations with schizophrenia (global $P = 0.017$ and 0.021, respectively). For two SNP haplotypes, the haplotype G (SNP7)-C (SNP8) was significantly more frequent in schizophrenia (frequency = 0.461) than in control group (0.362) ($P = 0.007$, odds ratio = 1.51, 95% C.I. = 1.12–2.03). For three SNP haplotypes, the haplotype T (SNP6)-G (SNP7)-C (SNP8) was significantly more over-represented in schizophrenia (0.458) than in controls (0.364) ($P = 0.010$, odds ratio = 1.46, 95% C.I. = 1.08–1.98). The results of these haplotypic associations were consistent with those of gene LD structure and allelic (genotypic) association of SNP8 with schizophrenia.

| Table 3. Genotypic and allelic distributions of the netrin G1 gene polymorphisms |
|-------------------------------|----------|-------------|-------------|-------------|-------------|
| Polymorphism | n | Genotype counts (frequency) | HWE | $P$ value | Allele counts (frequency) | $P$ value |
| SNP1: IVS1+2656T/C | T/T | 66 (0.37) | 92 (0.51) | 21 (0.12) | 0.193 | 0.748 | T/C |
| Schizophrenia | 179 | 224 (0.63) | 134 (0.37) | 0.938 | 0.020 | 1.44 | 95% C.I. = 1.06–1.96 |
| Control | 173 | 215 (0.62) | 131 (0.38) | 0.38 |
| SNP2: IVS1+1706T/C | T/T | 78 (0.43) | 88 (0.49) | 14 (0.08) | 0.095 | 0.186 | T/C |
| Schizophrenia | 178 | 240 (0.67) | 116 (0.33) | 0.384 | 0.021 | 1.44 | 95% C.I. = 1.06–1.96 |
| Control | 175 | 225 (0.64) | 123 (0.36) | 0.36 |
| SNP3: IVS2+29544G/A | A/A | 61 (0.34) | 81 (0.45) | 37 (0.21) | 0.294 | 0.431 | G/T |
| Schizophrenia | 180 | 203 (0.57) | 155 (0.43) | 0.764 | 0.021 | 1.44 | 95% C.I. = 1.06–1.96 |
| Control | 179 | 200 (0.56) | 160 (0.44) | 0.44 |
| SNP4: IVS2-24065G/T | G/G | 53 (0.29) | 94 (0.52) | 33 (0.18) | 0.440 | C/T |
| Schizophrenia | 180 | 252 (0.79) | 76 (0.21) | 0.927 | 0.021 | 1.44 | 95% C.I. = 1.06–1.96 |
| Control | 179 | 252 (0.79) | 76 (0.21) | 0.927 |
| SNP5: IVS3-179C/T | C/C | 115 (0.64) | 55 (0.31) | 10 (0.06) | 0.323 | 0.407 | T/T |
| Schizophrenia | 180 | 285 (0.70) | 75 (0.21) | 0.927 | 0.021 | 1.44 | 95% C.I. = 1.06–1.96 |
| Control | 179 | 285 (0.70) | 76 (0.21) | 0.927 |
| SNP6: IVS4-2148T/C | T/T | 115 (0.64) | 54 (0.30) | 10 (0.06) | 0.284 | 0.392 | C/C |
| Schizophrenia | 180 | 284 (0.79) | 74 (0.21) | 0.855 | 0.017 | 1.44 | 95% C.I. = 1.06–1.96 |
| Control | 179 | 284 (0.79) | 77 (0.22) | 0.855 |
| SNP7: IVS5+2275T/G | G/G | 115 (0.64) | 54 (0.30) | 10 (0.06) | 0.284 | 0.392 | T/T |
| Schizophrenia | 180 | 284 (0.79) | 74 (0.21) | 0.855 | 0.017 | 1.44 | 95% C.I. = 1.06–1.96 |
| Control | 179 | 284 (0.79) | 77 (0.22) | 0.855 |
| SNP8: IVS8-16670C/T | C/C | 115 (0.64) | 54 (0.30) | 10 (0.06) | 0.284 | 0.392 | G/G |
| Schizophrenia | 180 | 284 (0.79) | 74 (0.21) | 0.855 | 0.017 | 1.44 | 95% C.I. = 1.06–1.96 |
| Control | 179 | 284 (0.79) | 77 (0.22) | 0.855 |
| SNP9: IVS9+10446A/G | A/A | 79 (0.44) | 73 (0.41) | 28 (0.16) | 0.113 | 0.208 | A/G |
| Schizophrenia | 180 | 231 (0.64) | 129 (0.36) | 0.132 | 0.017 | 1.44 | 95% C.I. = 1.06–1.96 |
| Control | 180 | 231 (0.64) | 130 (0.36) | 0.132 |
| SNP10: IVS9-4386C/T | T/T | 115 (0.64) | 54 (0.30) | 10 (0.06) | 0.284 | 0.392 | C/C |
| Schizophrenia | 180 | 296 (0.82) | 64 (0.18) | 0.344 | 0.017 | 1.44 | 95% C.I. = 1.06–1.96 |
| Control | 179 | 296 (0.82) | 74 (0.21) | 0.344 |

* The samples with ambiguous genotypes were not included.

* $P$ values for Hardy–Weinberg equilibrium are denoted.

* Differences in genotypic and allelic distributions were evaluated by Fisher’s exact test.
Discussion

The mouse orthologue of netrin G1 gene was first cloned by Nakashiba et al. in 2000. They showed that netrin G1 transcripts were first detected at embryonic day 12 in mid and hindbrain regions, reaching peak levels at perinatal stages in various brain regions that include the olfactory bulb mitral cells, thalamus and deep cerebellar nuclei. Expression was primarily restricted to the central nervous system, with most prominent expression in the thalamus. The thalamic neurons relay afferents to the cerebral cortex from various sensory systems, and thus the thalamus is deemed to modulate the motor response to sensory
stimuli or to perform “sensory-motor gating.”

Interestingly, netrin G1 knockout mice exhibited a reduced level of prepulse inhibition (PPI) (Itohara, personal communication). PPI is demonstrated by a reduction in response amplitude to a startle stimulus when this stimulus is immediately preceded by a weaker prestimulus. This sensorimotor phenomenon occurs across-species and it has been investigated as a model to understand the pathophysiology of schizophrenia. Schizophrenic patients often display profound impairments in PPI, raising the possibility that a deficit in the filtering or “gating” of sensory information may explain some of the fundamental symptoms observed in schizophrenia, including an overflow of sensory stimulation and disintegration of cognitive functions. Therefore, netrin G1 is a potentially intriguing target for genetic studies in schizophrenia from both its role in physiological functions relevant to schizophrenia pathology, and its molecular involvement in neuronal circuit development.

Our case-control analysis revealed that the IVS8-1467C>T (SNP8) polymorphism of netrin G1 gene is nominally significantly associated with schizophrenia, with the IVS8-1467C allele overrepresented in schizophrenia (allelic $P=0.020$, and $>0.05$ after Bonferroni correction for multiple testing). Power calculations were performed based on an arbitrary assumption of relative risk and frequency of risk allele. When a relative risk of 2.0 was assumed, the present sample displayed 89% power to detect significant association ($\alpha<0.05$, frequency of risk allele $=0.3$, two-sided). With a relative risk of 1.5, our samples had 45% power to detect significant association ($\alpha<0.05$, frequency of risk allele $=0.3$, two-sided). For LD structure, Abecasis et al. proposed $D^2>0.1$ as a criterion for useful LD. According to their criteria, our LD analysis revealed that SNP8 was located in an LD block spanning from SNP5 to SNP9 with a gap between SNP5 and the neighboring SNP4. Haplotype analysis consistently showed that the haplotypes comprising SNP7-SNP8 and SNP6-SNP7-SNP8 in this LD block were associated with schizophrenia. These results suggest that the real disease-causing variant(s), if one exists, may reside in the 3’ half region of the gene.

We examined sequence variation in the exons and flanking introns using 40 schizophrenics and the primers shown in Table 1, but found no novel polymorphisms. In order to search for candidate functional variant(s), it is necessary to extend polymorphism screening to unscreened genomic regions. The mouse genomic structure of netrin G1 gene is very similar to that of the human ortholog. Equally, we have detected various human netrin G1 transcripts generated by alternative splicing as seen in the mouse. In humans, the splicing involves exons 6, 7, 8 and 9 which code for an unknown domain and two laminin repeat type domains (Meerabux et al. in preparation). These exons are within the same haplotype block. Therefore it is tempting to speculate that dysregulation of transcript processing may have some role in schizophrenia susceptibility. However, the SNPs 5, 6, 7, 8 and 9 are all embedded in introns and distant from branch points or splicing donor and acceptor sites, making them less likely to control the efficiency of alternative splicing. More thorough genomic and genetic analyses are needed to corroborate the contribution of netrin G1 gene to schizophrenia susceptibility and refine the predisposing allele(s). The close parologue, netrin G2 gene, also identified from the mouse quite recently, also
warrants future genetic analysis in schizophrenia based in its complimentary pattern of expression with netrin G1.17

In conclusion, our data suggest the possible involvement of human netrin G1 or a nearby gene in the vulnerability to schizophrenia.

Acknowledgements

We are grateful to all participants in this study.

References