Disruption of the neuronal PAS3 gene in a family affected with schizophrenia

D Kamnasaran, W J Muir, M A Ferguson-Smith, D W Cox

Schizophrenia is a complex disease, manifesting symptoms which include changes in perception (hallucinations), inferential thinking (delusions), motivation (avolition), and thought and speech (alogia). These clinical signs are also compounded by a spectrum of negative symptoms that include decrease in social interaction, cognitive impairment, and attentional impairment. The diagnostic criteria for this disease have been established by the International Classification of Diseases, 10th edition,1 and the Diagnostic and Statistical Manual of Mental Disorders, 4th edition.1 Many subtypes of schizophrenia have been proposed. Those affected with schizophreniform psychosis exhibit the psychotic symptoms but no deteriorating course. The population prevalence of schizophrenia is estimated to be 1-2%, with an age of onset that is usually in the late teens to early 20s in males and with a five year lag in onset in females.2 Late onset schizophrenia occurs after 40 years of age in 10% of diagnosed cases and is more prevalent in females. Schizophrenia is also diagnosed during early childhood. In general, patients display common clinical and neurophysiological findings at variable ages of onset.

The genetic basis of schizophrenia is suggested by the observation of a higher risk among family members of patients than those in the general population.3 Evidence from adoption and twin studies also showed a higher risk among relatives of affected families than in the general population.4 Owing to the complex nature of this disease, no specific mode of inheritance can be established. Mathematical modelling has, however, favoured polygenic models of inheritance involving several susceptibility genes acting additively, and possibly in conjunction with environmental factors, to result in schizophrenia. Evidence for genetic heterogeneity is supported by several genome wide screens for causative loci, by linkage analysis and association studies.5-7 These studies suggested that several chromosomes including 1q, 4q, 5p, 6p, 6q, 8p, 9q, 10p, 13q, 14q, 15q, 22q, and Xp contain major, or susceptibility, genes for schizophrenia.8-11 Many of these locations have not been supported by independent studies. We report here a family with schizophrenia and a translocation (9;14) chromosome with a breakpoint junction in a novel BHLH-PAS transcription factor on chromosome 14.

MATERIALS AND METHODS

Subjects

Diagnoses were established by an experienced psychiatrist (WJM) using SADS-L, PAS-ADD structured assessment, and hospital case note review. The proband (cell line L6874) has severe learning disability and a clinical diagnosis of schizophreniform psychosis, established in her late teens, according to DSM-IV criteria. She shows periods of almost continuous agitation and blunted speech and stereotypes with no other apparent internal or external causes. The phenomena were controlled with a combination of neuroleptic medications (depot pipritil and chlorpromazine). Repeated attempts at medication reduction have resulted in no improvement in symptoms. A family history of schizophrenia was confirmed in the proband’s father and maternal grandfather. Schizophrenia and its subtypes are part of a complex brain disorder with multiple postulated aetiologies. There is evidence that this common disease is genetically heterogeneous, with many loci involved. In this report, we describe a mother and daughter affected with schizophrenia, who are carriers of a t(9;14)(q34;q13) chromosome. By mapping on flow sorted aberrant chromosomes isolated from lymphoblast cell lines, both subjects were found to have a translocation breakpoint junction between the markers D14S730 and D14S70, a 683 kb interval on chromosome 14q13. This interval was found to contain the neuronal PAS3 gene (NPAS3), by annotating the genomic sequence for ESTs and performing RACE and cDNA library screenings. The NPAS3 gene was characterised with respect to the genomic structure, human expression profile, and protein cellular localisation to gain insight into gene function. The translocation breakpoint junction lies within the third intron of NPAS3, resulting in the disruption of the coding potential. The fact that the BHLH and PAS domains are disrupted from the remaining parts of the encoded protein suggests that the DNA binding and dimerisation functions of this protein are destroyed. The daughter (proband), who is more severely affected, has an additional microdeletion in the second intron of NPAS3. On chromosome 9q34, the translocation breakpoint junction was defined between D9S752 and D9S7972 and no genes were found to be disrupted. We propose that haploinsufficiency of NPAS3 contributes to the cause of mental illness in this family.
intron of the chromosomes. The marker D14S49 mapped within the second included 40 ng human genomic DNA or flow sorted aberrant were used for mapping in standard 20

Research STS YAC physical map (www-genome.wi.mit.edu), markers, selected from the Whitehead Institute for Genome proximal chromosome 14q and 16 chromosome 9q specific template for mapping, as previously described. 300-500 copies of the normal or aberrant sorted chromosome 14 were subjected to DOP-PCR amplification before use as a

3

A panel of 16

5

9

A total of 300-500 copies of the normal or aberrant sorted chromosome 14 were subjected to DOP-PCR amplification before use as a template for mapping, as previously described. A panel of 16 proximal chromosome 14q and 16 chromosome 9q34 specific markers, selected from the Whitehead Institute for Genome Research STS YAC physical map (www-genome.wi.mit.edu), were used for mapping in standard 20 µl PCR reactions that included 40 ng human genomic DNA or flow sorted aberrant chromosomes. The marker D14S49 mapped within the second intron of the NPAS3 gene. The marker D14S1014 mapped within the third intron of the KIAA0391 gene. The PCR primers, conditions, and cycles for these markers are reported by Research Genetics. Primer pairs, listed in table 1, were designed from the sequences of BAC clones R1075M22 (Genbank accession number AL157689), R107814 (Genbank accession number AL161851), and R66M11 (Genbank accession number AL133305), using primer premiere 3 (www-genome.wi.mit.edu/cgi-bin/primer/primer3.cgi), to map precisely the chromosome 14q breakpoint junction within NPAS3. The genomic sequence of BAC clone R173D09 (Genbank accession number AL121594) was used to design primer pairs for mapping within the KIAA0391 gene (Genbank accession number NM_014672).

### Table 1 Primmers, PCR conditions, and cycles used for mapping within the NPAS3 and KIAA0391 genes

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<th>Marker</th>
<th>Map position</th>
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<th>PCR product size (bp)</th>
<th>MgCl2 (mmol/l)</th>
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The PCR cycle is 94°C-four minutes, 30 cycles of 94°C-30 seconds, 57°C-30 seconds, followed by 72°C-five minutes final extension.

father, who had died, was diagnosed with DSM-IV bipolar I disorder, with an onset in his twenties. The disorder followed a classical pattern with recurrent episodes of mania alternating with major depressive disorder that required hospitalisation. Cranial imaging on these subjects could not be obtained. Both proband and mother had a 46,XX, t(9;14)(q34;q13) karyotype. The father had a normal karyotype. The cytogenetic findings on this family have previously reported. An older sib of the proband, with severe mental delay, was known to carry the translocation. However, this sib was separated from the family early in life, and no cell line or information on mental status is available.

### Mapping on flow sorted chromosomes

Transformed lymphoblast cell lines were flow sorted for the aberrant chromosomes as previously described. A total of 300-500 copies of the normal or aberrant sorted chromosome 14 were subjected to DOP-PCR amplification before use as a template for mapping, as previously described. A panel of 16 proximal chromosome 14q and 16 chromosome 9q34 specific markers, selected from the Whitehead Institute for Genome Research STS YAC physical map (www-genome.wi.mit.edu), were used for mapping in standard 20 µl PCR reactions that included 40 ng human genomic DNA or flow sorted aberrant chromosomes. The marker D14S49 mapped within the second intron of the NPAS3 gene. The marker D14S1014 mapped within the third intron of the KIAA0391 gene. The PCR primers, conditions, and cycles for these markers are reported by Research Genetics. Primer pairs, listed in table 1, were designed from the sequences of BAC clones R1075M22 (Genbank accession number AL157689), R107814 (Genbank accession number AL161851), and R66M11 (Genbank accession number AL133305), using primer premiere 3 (www-genome.wi.mit.edu/cgi-bin/primer/primer3.cgi), to map precisely the chromosome 14q breakpoint junction within NPAS3. The genomic sequence of BAC clone R173D09 (Genbank accession number AL121594) was used to design primer pairs for mapping within the KIAA0391 gene (Genbank accession number NM_014672).

### Rapid amplification of cDNA ends (RACE)

5′ and 3′ RACE were performed on a Marathon ready human fetal brain cDNA library (21-30 weeks' gestation, 10 pooled white male and female) (ClonTech) as specified by the manufacturer. Primers for 5′ (F-5′GGTATGGTGTTAGCCATTGC 3′, F-5′CTGCGGCTAGAAGGCCTCCTG 3′ and 3′ (F-5′CTGCGGCTAGAAGGCCTCCTG 3′) RACE reactions were designed from partial cDNA sequences belonging to NPAS3, with melting temperatures between 65°C and 72°C using Primer Premiere 3. Standard 50 µl PCR reactions were performed with the Advantage 2q polymerase mix (ClonTech) as specified by the manufacturer. The RACE products were cloned with the pCR4-TOPO TA kit

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MgCl2, and with the PCR cycle denaturation for three minutes, followed by 35 cycles of 94°C for 30 seconds, 60°C for 30 seconds, and 72°C for five minutes denaturation, 94°C for 30 seconds, 57°C for 30 seconds, and 72°C for 60 seconds, for 45 cycles. The reactions were labelled with 5α-32P-dCTP (10 mCi/ml) using the REDIPrime labelling kit (Amersham Biosciences). The human multitissue northern blots, 12 multitissue, Blot IV and Blot II (ClonTech), were hybridised with the ExpressHyb solution (ClonTech) as specified by the manufacturer.

Protein cellular localisation studies

The open reading frame of the large NPAS3 protein isoform was cloned by standard PCR using the λ TriplEx Human Fetal Brain 5’ Stretch Plus cDNA library (20 to 25 weeks’ gestation, 10 pooled white male and female) (ClonTech) as specified by the manufacturer. The primer pairs (F-5′-tcttggggagcagaaggtaa 3′, R-5′-agatctgcctgccagcagtaa 3′) was used in standard 20 μl PCR reactions containing 1.5 mmol/l MgCl₂, and with the PCR cycle denaturation for three minutes, followed by 30 cycles of 94°C for 30 seconds, 58°C for 30 seconds, 72°C for 30 seconds, and a final extension at 72°C for five minutes. One hundred ng of the PCR product was labelled with 5α-32P-dCTP (10 mCi/ml) using the REDIPrime kit (Amersham Biosciences). About 1.9 × 10⁶ plaque forming units were screened in total. Hybridisation was done with the ExpressHyb solution (ClonTech) as specified by the manufacturer. Up to 35 positive plaque forming units were picked from each plate and converted to plasmids as specified by the manufacturer. The plasmids were sequenced with the IRD700/800 M13 labelled primers using the Thermo Sequenase fluorescent labelled primer cycle sequencing kit with 7-deaza-dGTP (Amersham Biosciences), then subjected to site directed mutagenesis to introduce restriction sites for cloning into the EGFP-N1 vector (ClonTech). Standard PCR reactions containing about 100 ng of plasmid DNA and Platinum Pfx Taq polymerase (Invitrogen) were set up according to the manufacturer. The PCR cycle was denaturation at 94°C for three minutes, 35 cycles of 94°C for 30 seconds, 60°C for 30 seconds, and 72°C for 150 seconds, followed by 72°C for five minutes. The cloned open reading frame was sequenced with the Thermosequenase fluorescent labelled primer cycle sequencing kit with 7-deaza-dGTP (Amersham Biosciences), then subjected to site directed mutagenesis to introduce restriction sites for cloning into the EGFP-N1 vector (ClonTech). The open reading frame of the large NPAS3 protein isoform was cloned by standard PCR using the λ TriplEx Human Fetal Brain 5’ Stretch Plus cDNA library (20 to 25 weeks’ gestation) (ClonTech) and Marathon ready human fetal brain cDNA library (21-30 weeks’ gestation, 10 pooled white male and female) (ClonTech) as templates. Standard PCR reactions were set up with Platinum Pfx Taq polymerase (Invitrogen) and sequenced with the IRD700/800 M13 labelled primers using the Thermosequenase fluorescent labelled primer cycle sequencing kit with 7-deaza-dGTP (Amersham Biosciences). The reactions were run on a Licor DNA Sequencer Long Reader 4200.

cDNA library screening

The λ TriplEx Human Fetal Brain 5’ Stretch Plus cDNA library (20 to 25 weeks’ gestation, 10 pooled white male and female) (ClonTech) was screened as outlined by the manufacturer. A PCR probe designed with Primer Premiere 3 using partial cDNA sequences obtained from database searches and RACE was used to screen the cDNA library. The primer pair (F-5′-ttctggggagcagaaggtaa 3′, R-5′-agatctgcctgccagcagtaa 3′) was used in standard 20 μl PCR reactions containing 1.5 mmol/l MgCl₂, and with the PCR cycle denaturation for three minutes, followed by 30 cycles of 94°C for 30 seconds, 58°C for 30 seconds, 72°C for 30 seconds, and a final extension at 72°C for five minutes. One hundred ng of the PCR product was labelled with 5α-32P-dCTP (10 mCi/ml) using the REDIPrime kit (Amersham Biosciences). About 1.9 × 10⁶ plaque forming units were screened in total. Hybridisation was done with the ExpressHyb solution (ClonTech) as specified by the manufacturer. Up to 35 positive plaque forming units were picked from each plate and converted to plasmids as specified by the manufacturer. The plasmids were sequenced with the IRD700/800 M13 labelled primers using the Thermosequenase fluorescent labelled primer cycle sequencing kit with 7-deaza-dGTP (Amersham Biosciences). The reactions were run on a Licor DNA Sequencer Long Reader 4200.

Genomic structure determination

Consensus sequences were assembled from partial cDNA sequences obtained from RACE, cDNA library screening, and database searches, using the GeneTool Version 1.0 software. A composite genomic sequence of the interval between the markers D14S70 and D14S730 was made by aligning the scaffolds of genomic sequence data from Celera (Celera.com) and Genbank (www.ncbi.nlm.nih.gov), using BLAST2 (www.ncbi.nlm.nih.gov/blast/b1seq/b1.html). The genomic structure was determined by aligning the assembled cDNA sequences against the composite genomic sequence with BLAST2.

Figure 1  Breakpoint junction analysis of chromosomes 9 and 14 of the proband and mother using analysis of flow sorted chromosomes. (A) chromosome 14q13 analysis. The position of the NPAS3 gene is shown between D14S730 and D14S70. The position of the KIAA0391 gene is shown between D14S988 and D14S888. (B) Chromosome 9q34 analysis. Marker map distances are not drawn to scale. The plus sign indicates the presence of markers on either the derivative 9 or 14 chromosome. The dashed line indicates the translocation breakpoint junction.
Table 2  Genomic structure of NPAS3

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<th>Exon number</th>
<th>Exon position</th>
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<th>Intron size</th>
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<th>5' donor site</th>
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Brackets demarcate a cryptic 3' acceptor splice site at nucleotide position 1502 of exon 2.

RESULTS
Mapping of the translocation breakpoint junctions on chromosomes 9q34 and 14q13

In order to characterise the translocation breakpoint junctions in this family, a panel of 14 proximal chromosome 14q and 16 chromosome 9q34 markers were selected from the Whitehead Institute for Genome Research STS YAC physical maps to define the breakpoint junctions on the flow sorted aberrant chromosomes (fig 1). Both proband and mother had a breakpoint junction defined on chromosome 14 within an estimated 7.5 kb interval between the markers D14S730 and D14S70. On chromosome 9q34, the translocation breakpoint junction was defined between the markers D9S752 and D9S972 in both subjects, an estimated 100 kb interval (fig 1). Both proband and mother had a breakpoint junction defined on chromosome 14 within an estimated 7.9 kb interval within the third intron (fig 4). The translocation breakpoint junction affected both alternative transcripts, which resulted in the first 124 amino acids of the amino terminus of the putative smaller 153 amino acid isoform being displaced. The BHLL domain in the amino terminus of the larger protein was disrupted, preventing the protein from binding to DNA.

Isolation and characterisation of the NPAS3 gene

In order to identify genes within the 683 kb translocation breakpoint interval on chromosome 14q13, the genomic sequence was annotated for EST clusters. These partial cDNA sequences were used to design probes for the screening of a λTripleX 5' stretch human fetal brain cDNA library, and perform 5' and 3' RACE on a Marathon Ready human fetal brain DNA library. In this interval two cDNAs of sizes 2.5 and 3.4 kb (GenBank accession numbers AJ157302, AJ157303) were isolated. These cDNAs were highly similar to the mouse neuronal PAS3 gene (NPAS3) (Genbank accession number AF173871) on mouse chromosome 12, in a region of conserved synteny with genes on human chromosome 14. We were unable to extend beyond the 5' of these two alternative cDNAs using RACE on a Marathon Ready human fetal brain cDNA library; however, the possibility that these are partial cDNAs cannot be excluded. The human neuronal PAS3 gene (NPAS3) is estimated to be about 863 kb in size with 12 exons that encode two alternative transcripts (table 2). Two pseudogenes (Genbank accession numbers AK002000, AK000865), of unknown identity and of sizes 2.2 kb and 2 kb, map within the second and fifth introns of this gene, respectively. These pseudogenes have short open reading frames and contain a 3' poly A tail. A 901 amino acid protein encoded by the 3.4 kb cDNA has a BHLL (basic helix loop helix) dimerisation domain in the amino terminus (amino acids 31 to 72), a PAS (Period, Aryl hydrocarbon receptor, Single minded) domain (amino acids 117 to 183), a PAC (PAS associated carboxyl terminus) motif (amino acids 361 to 404), and a bipartite nuclear localisation signal in the carboxyl terminus (amino acids 568 to 585). This protein is about 90% identical to the mouse NPAS3 protein. The smaller 2.5 kb cDNA encodes a truncated 153 amino acid protein that contains a PAS domain (amino acids 45 to 113). Northern analysis with a 2.6 kb probe containing exons 7 to 12 showed expression of an approximately 7.5 kb transcript only in several human adult brain tissues, including cerebellum, cerebral cortex, medulla, occipital lobe, frontal lobe, temporal lobe, putamen, amygdala, caudate nucleus, corpus callosum, hippocampus, substantia nigra, and thalamus (fig 2). An exon 2 probe specific to the smaller 2.5 kb alternative cDNA showed no expression in human adult tissues. However, both 2.5 and 3.4 kb cDNAs were found to be expressed in human fetal brain (20–30 weeks' gestation). Cellular localisation studies with the 901 amino acid NPAS3 protein isoform, tagged in the carboxyl terminus with enhanced green fluorescent protein, showed that this protein was localised in the nucleus of COS1 and transformed adult human skin fibroblast cell lines (fig 3).

High resolution mapping within the NPAS3 gene

Mapping was performed with an additional 16 amplifiers within the NPAS3 gene, in order to define more precisely the breakpoint junction on chromosome 14q13. Physical mapping with these markers showed that the translocation breakpoint junction of both proband and mother was between 66M11SR and SDR1, an estimated 7.9 kb interval within the third intron (fig 4). The translocation breakpoint junction affected both alternative transcripts, which resulted in the first 124 amino acids of the amino terminus of the larger 901 amino acid isoform, and the first 52 amino acids of the amino terminus of the putative smaller 153 amino acid isoform being displaced. The BHLL domain in the amino terminus of the larger protein was disrupted, preventing the protein from binding to DNA.
For both protein isoforms, the PAS domains that are required for dimerisation were disrupted. The PAC motif and bipartite nuclear localisation signal within the carboxyl terminus of the larger protein isoform remained intact. Interestingly, the proband was found to have deletions of three markers (I3DK, D14S49, I4DK) that mapped within the second intron of NPAS3, suggesting an estimated 94 kb maximum microdeletion within this intron (fig 4). An analysis of this deleted genomic sequence showed several possible transcription factor binding sites. None of these additional micro-rearrangements was found in the mother.

The chromosome 14q13 region outside NPAS3 was also screened for additional rearrangements in the mother and proband. In the proband, a microdeletion with an estimated maximum size of 22 kb within the third intron of the KIAA0391 gene (Genbank accession number NM_014672) was found. Specifically, markers D14S1014 and K2DK were deleted. This rearrangement was absent in the mother. KIAA0391 has an unknown function, unknown identity, and maps about slightly over 1 Mb distal to NPAS3. Furthermore, the deleted interval contains several possible transcription factor binding sites.

**DISCUSSION**

The proband and mother have a translocation breakpoint junction in the third intron of the NPAS3 gene which disrupts the coding potential of both alternative transcripts. Studies with FISH have confirmed a break within this gene. The NPAS3 gene belongs to the bHLH-PAS (basic Helix Loop Helix, Period, Aryl hydrocarbon receptor, Single minded) superfamily of transcription factors, that are involved in a wide range of functions including circadian oscillations (Npas2, Per, Clock), neurogenesis (Sim), toxin metabolism (Arnt), hypoxia (Hif1a), and tracheal development (Trh). A small protein isoform that may be encoded by the small transcript isoform of NPAS3 was identified. This small protein isoform may form heterodimers via its PAS domain with the larger NPAS3 protein isoform or other proteins containing a PAS domain, to regulate activity. This mode of regulation is seen with the PITX2D protein isoform and other bHLH-PAS transcription factors.

**Figure 2** Northern analysis of the NPAS3 gene showing a single approximately 7.5 kb transcript, ubiquitously expressed in the adult human brain. A 2.6 kb cDNA encompassing exons 7 to 12 of the large transcript isoform was used as a probe to hybridise on ClonTech human adult multitissue northern blots (12 adult multitissue; adult brain blot II and adult brain blot IV). A full length cDNA of the GAPDH gene was used as a control probe.

**Figure 3** Cellular localisation of the large NPAS3 protein isoform. Transient transfections of the NPAS3-EGFP-N1 construct were done in (A) COS1 and (B) transformed adult human skin fibroblast cell lines. DAPI and FITC filters were used to visualise the signals. Images obtained from each filter were merged with the software Spot version 2.2. Blue signals represent the DAPI stained nucleus; green signals represent the EGFP tagged protein.
The NPAS3 protein contains a bHLH domain that is known to bind to DNA, and in this study is found to be localised in the nucleus, suggesting that it may be a transcription factor. Expression of both transcript isoforms was also found in the developing human fetal brain (20 to 30 weeks’ gestation). The murine \textit{Npas3} gene is extensively expressed in the developing central nervous system, but its role is yet to be defined. Expression of the murine \textit{Npas3} gene specifically in the developing nervous system during early embryogenesis suggests that haploinsufficiency could result in central nervous system anomalies causing mental impairment. So far, only the \textit{SIM2} gene of the bHLH-PAS family of transcription factors, mapping on human chromosome 21, has been suggested to be associated with behavioural problems, as seen in Down syndrome patients. No other members of this transcription factor family have yet been associated with schizophrenia, suggesting that \textit{NPAS3} is the first gene of this superfamily to be associated with this disease. The fact that this gene was expressed in 13 adult brain tissues including the hippocampus, thalamus, and cortex supports a possible role in development and/or function of these structures, which may play a role in schizophrenia. The PAS domain of both protein isoforms, required for dimerisation, is disrupted in both proband and mother. Such a disruption is expected to destroy the function of the small NPAS3 protein isoform. In the case of the large protein isoform, the translocation breakpoint junction leaves the bHLH domain intact in the amino terminus, and the PAC and bipartite nuclear localisation motifs intact in the carboxyl terminus. With such disruption of the \textit{NPAS3} gene, the NPAS3 large protein isoform is likely to be non-functional, compatible with a contribution of haploinsufficiency to schizophrenia in mother and proband. Interestingly, the proband was found to have a translocation breakpoint junction in conjunction with a 94 kb microdeletion within \textit{NPAS3} and a 22 kb microdeletion within \textit{KIAA0391}. This finding is not unexpected, since familial translocation chromosomes acquire additional rearrangements owing to unequal recombination during meiosis. The
possibility of minor rearrangements in other genes cannot be excluded. The function of the KIAA0391 gene is currently unknown and is not similar to other genes with known functions. These deleted intervals contain potential transcription factor binding sites. These deletions separate from, or in conjunction with, a genetic background inherited from the father, may explain the more severe phenotype observed in the proband.

Most studies by linkage and linkage disequilibrium have reported only weak evidence for a locus for schizophrenia on the proximal region of human chromosome 14q. However, with the use of genome screens using non-parametric linkage analyses, two groups have suggested significant linkage to the markers D14S579 (p = 0.01) and D14S306 (p = 0.005) at 14q13,20 suggestive of a susceptibility locus at 14q13. Recently, using the identity by descent datasets of 30 affected sibs in 21 pedigrees from the previous study by Blouin et al.,20 and a modified multilocus non-parametric linkage algorithm,21 evidence for the sharing of two alleles at 8p21 in conjunction with one allele on chromosome 14 between D14S1280 and D14S306 was found among affected sib pairs with the schizophrenia spectrum. These data suggested that a susceptibility locus at 14q13 acted in conjunction with other schizophrenia loci, such as at 8p21.3. Further examination of the NPAS3 gene resides within this interval, and therefore may be a susceptibility gene with a contributory role in the cause of schizophrenia. Proximal chromosome 14q deletion cases reported to date do not describe any symptoms of mental illness.22-24 This may be because these reported patients died neonatally or in early childhood or were too young to manifest such traits. Furthermore, the deletion intervals may not extend into the NPAS3 gene.

Of the diseases localised to date to chromosome 14q13, only Fahr disease (idiopathic basal ganglia calcification) has been associated with neuropsychiatric problems.25 The age of onset for idiopathic basal ganglia calcification is 30 to 60 years and manifests in patients with dystonia, extrapyramidal signs, and ataxia. The central nervous system undergoes calcification in areas such as the globus pallidus, putamen, caudate nucleus, dentate, thalamus, cerebral white matter, and cerebellum, which is postulated to result in progressive dystonia, parkinsonism, and neuropsychiatric manifestations such as schizophrenia or schizophreniaform psychosis. Of the patients who showed significant linkage of disease to chromosome 14q13, only one had schizophreniform psychosis and the Fahr disease spectrum.26 Our subjects have no features in common with the Fahr disease patients showing linkage to chromosome 14q13, except for the schizophrenia spectrum. We were unable to discern whether calcification of the central nervous system was prevalent in our subjects.

On chromosome 9q34, the breakpoint junction was defined within 100 kb. No genes were disrupted within this interval. Although chromosome 9q is speculated to contain susceptibility genes for schizophrenia, none of the reported genes within the breakpoint junction interval at 9q34 in our subjects appeared to be likely candidates for schizophrenia. The possibility of positional effects exerted on the expression of neighbouring genes at the breakpoint junction cannot be excluded. There was evidence for a weak association of the NMDAR1 gene (NM_000832) at chromosome 9q34 with schizophrenia in a South African Bantu speaking tribe.27 The NMDAR1 receptor functions in the glutamnergic pathway and was shown, in mice carrying a hypomorphic allele, to result in schizophrenia-like behaviour that could be treated with haloperidol.28 However, examination of sequences within a 1 Mb interval of the 9q34 breakpoint junction showed that the NMDAR1 gene was not positioned within this interval. There is one case report of a 28 year old man with mental retardation, schizophrenia, short stature, short webbed neck, dysmorphic face, and mild anomalies of the fingers, who had a delt(9)(q32q34).29 The deletion interval in this patient is not yet characterised and the locus for schizophrenia in this patient is as yet unidentified.

In summary, we have found the NPAS3 gene to be disrupted in a two generation family with the schizophrenia spectrum. The NPAS3 gene is proposed to be a susceptibility gene with a contribution to the mental illness observed in this family. Other possibilities cannot be excluded, such as mental illness as a non-specific consequence of mental delay, the translocation breakpoint junction being the result of chance, or the susceptibility locus close to but not at the breakpoint junction.

In order to confirm the role of NPAS3 as a schizophrenia susceptibility gene, association studies in larger case-control samples and mutation analysis are warranted.

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