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Possible role of rare variants in Trace amine associated receptor 1 in schizophrenia

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Abstract

Schizophrenia (SZ) is a chronic mental illness with behavioral abnormalities. Recent common variant based genome wide association studies and rare variant detection using next generation sequencing approaches have identified numerous variants that confer risk for SZ, but etiology remains unclear propelling continuing investigations. Using whole exome sequencing, we identified a rare heterozygous variant (c.545G > T; p.Cys182Phe) in Trace amine associated receptor 1 gene (*TAAR1* 6q23.2) in three affected members in a small SZ family. The variant predicted to be damaging by 15 prediction tools, causes breakage of a conserved disulfide bond in this G-protein-coupled receptor. On screening this intronless gene for additional variant(s) in ~800 sporadic SZ patients, we identified six rare protein altering variants (MAF < 0.001) namely p.Ser47Cys, p.Phe51Leu, p.Tyr294Ter, p.Leu295Ser in four unrelated north Indian cases (n = 475); p.Ala109Thr and p.Val250Ala in two independent Caucasian/African-American patients (n = 310). Five of these variants were also predicted to be damaging. Besides, a rare synonymous variant was observed in SZ patients. These rare variants were absent in north Indian healthy controls (n = 410) but significantly enriched in patients (p = 0.036). Conversely, three common coding SNPs (rs8192621, rs8192620 and rs8192619) and a promoter SNP (rs60266355) tested for association with SZ in the north Indian cohort were not significant (P > 0.05).

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Contributions

Prof. B.K. Thelma, Prof. S. N. Deshpande and Prof. V. L. Nimgaonkar designed the study; Prof. S. N. Deshpande and Prof. V. L. Nimgaonkar provided the research samples; Mr. Jibin John contributed to study design, data generation, analysed the exome data and performed mutation screening; Jibin John, B.K. Thelma, & V.L. Nimgaonkar wrote the first draft of manuscript. Dr. Triptish Bhatia contributed to sample recruitment and phenotype data documentation; Dr. Prachi Kaushal performed SNP genotyping by PCR-RFLP method and contributed to mutation screening; Dr. K. V. Chowdari assisted with mutation screening in American samples. All authors contributed and have approved the final manuscript.

Conflict of interest

The authors declare that there are no conflicts of interest in relation to the subject of this study.

TAAR1 is a modulator of monoaminergic pathways and interacts with AKT signaling pathways. Substantial animal model based pharmacological and functional data implying its relevance in SZ are also available. However, this is the first report suggestive of the likely contribution of rare variants in this gene to SZ.

Keywords

TAAR1; Psychiatry; Exome sequencing; Rare variants; SNPs; Association

1. Introduction

Schizophrenia (SZ) is a common neuropsychiatric disorder with ~60–83% heritability (Cannon et al., 1998; Lichtenstein et al., 2009) and about 1% life time morbid risk. Evidence from twin, adoption and familial studies supports the contribution of both genetic and environmental factors (Sullivan et al., 2003), but the etiology of this complex disorder remains elusive. Positive symptoms in SZ characterized by hallucination, delusion and disorganized speech and behaviour are believed to be caused by hyperactivity in dopaminergic neurons of subcortical mesolimbic area (Abi-Dargham and Moore, 2003; Heinz et al., 2003), whereas negative symptoms (emotionless, flattened affect and apathetic behaviour/attitude) and cognitive symptoms (impaired executive functions, attention and memory) are ascribed to dopamine deficiency in the prefrontal cortex (PFC) (Abi-Dargham and Laruelle, 2005; Abi-Dargham and Moore, 2003; Heinz et al., 2003) and to some extent *N*-methyl-D-aspartate receptor (NMDAR) hypofunction (Coyle and Tsai, 2004). Though most of the cases of SZ are sporadic in occurrence, a few familial forms suggestive of major genetic component(s) are also documented. Early genome wide linkage scans had identified a number of loci on chr2q,3q,4q,5q,6q,8p,10p,10q and 13q (Riley and Kendler, 2006) linked to the disease. Subsequently, over the last two decades candidate gene based association studies have identified a large number of SNPs associated with this complex phenotype. However, difficulty in replicating these findings within and across populations has been a major limitation in the understanding of genetics and biology of SZ. Recently genome wide association studies (GWAS) have identified, over 100 risk loci (Ripke et al., 2013; Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014). With the advent of next generation sequencing (NGS) and whole exome sequencing (WES), several studies have identified *de novo* rare protein coding variants in SZ (Fromer et al., 2014; Girard et al., 2011; Guipponi et al., 2014; Gulsuner et al., 2013; McCarthy et al., 2014; Takata et al., 2014; Xu et al., 2011). However, most of these variants though provisionally functional are private and establishing their causative role in SZ has been challenging. On the other hand, a recurrent mutation or an independent mutation in phenotypically similar individuals but absent in healthy controls and/or highly penetrant rare variant(s) segregating with disease in a family may provide a strong evidence of causality.

Contrary to the common sporadic forms of SZ, a small proportion of multi member affected familial forms of the disease across ethnic groups have been observed and have been commonly used in the early genome wide linkage analysis. The powerful NGS tools have now enabled reinvestigating such families and interestingly rare variant(s) in genes such as

Glutamate Receptor, Metabotropic 5 (*GRM5*) and Low Density Lipoprotein Receptor-Related Protein 1B (*LRP1B*) (Timms et al., 2013), *unc-13* homolog B (*UNC13B*) (Egawa et al., 2016), SH3 and Multiple Ankyrin Repeat Domains 2 (*SHANK2*) and SWI/SNF Related, Matrix Associated, Actin Dependent Regulator of Chromatin (*SMARCA1*) (Homann et al., 2016) have been reported, albeit with incomplete segregation. Accordingly, in the present study, we analysed one small SZ family using the WES approach, followed by focused sequencing in two independent SZ cohorts, one of Indian and another of African-American/Caucasian ancestry. We report seven rare protein disturbing variants (MAF < 0.001) in *TAAR1*, a gene extensively investigated for pharmacological attributes in animal models (Revel et al., 2013), that could elevate risk for SZ.

2. Materials and methods

2.1. Sample recruitment

Patients with family history and receiving treatment for psychotic illnesses at Dr. RML Hospital, New Delhi were assessed using the Hindi version of the Diagnostic Interview for Genetic Studies (DIGS) and the Family Interview for Genetic Studies (FIGS) (Deshpande et al., 1998; Kukshal et al., 2013a, 2013b). Additional information was obtained from medical records and consensus diagnoses were assigned using DSM IV criteria. Unaffected members from the multimembers affected family all of north Indian origin were also recruited alongside. In addition, ~1000 SZ cases and ~1050 ethnically matched controls of north Indian origin available in the laboratory and used in a few previous genetic association studies (John et al., 2016; Kukshal et al., 2013a, 2013b) were also included in the study. Further, ~310 sporadic SZ cases mostly of African-American/Caucasian origin (henceforth referred to as American samples) recruited at Western Psychiatric Institute and Clinic (WPIC), University of Pittsburgh School of Medicine, USA were used in this study. Written informed consent was obtained from the participants and the study was approved by the institutional ethical committees of the respective institutions. Venous blood was drawn from each of the individuals for DNA isolation by the phenol chloroform method and used for genetic analysis.

2.2. Whole exome sequencing

A family comprising an affected mother with two affected and two unaffected children (Fig. 1) was analysed in this study. WES of four out of five members was performed.

2.3. Exome capture and sequencing

Exome library preparations of DNA from the selected family members were performed using Agilent SureSelect Human All Exon V5 + UTR kit. Sequencing was performed in Illumina HiSeq2000, using paired-end module for 101-bp reads at Axeq Technologies, USA (<http://www.axeq.com>).

2.4. NGS data processing and variant calling

These were performed using standard tools and software, which are detailed in Supplementary Material under Materials and Methods.

2.5. Variant prioritization

Variants generated by WES were prioritized following the recent guidelines for identifying disease causing variants (MacArthur et al., 2014). Consistent with the goals of the study, common variants (MAF $\geq$ 0.001) present in public databases such as dbSNPs, 1000 genomes, ExAC browser, CG40, were removed initially. In the next step all the non-protein disturbing variants (introns, UTRs, intergenic, synonymous etc.) were removed. Keeping in mind the suggestive autosomal dominant mode of inheritance in the study family, all the homozygous variants were removed and only the heterozygous variants present in the all three affected individuals and absent in the sequenced unaffected sib in the pedigree were retained. Variants from regions with segmental duplication were also removed (Peng et al., 2013; Wang et al., 2010). Further all variants present in whole exome data of 153 control individuals of Indian ethnicity available in the laboratory were removed. In parallel, KGGSeq (Li et al., 2012) was used for prioritizing the variants as detailed above.

Prioritized variants were then checked for segregation with SZ in the unaffected member (who was not exome sequenced) by targeted sequencing. Custom-made Haloplex enrichment kit (Agilent, USA) used for the library preparation and sequencing performed on Illumina Hiseq2500 at Strand Life Sciences Pvt. Ltd., India (<http://www.strandls.com>). Segregating variants were further prioritized based on their functional/biological relevance or their prior implication for SZ, based on association, linkage, exome sequencing and animal studies reported in literature. All variant(s) thus identified were confirmed by Sanger sequencing at SciGenom Labs, India (<http://www.scigenom.com>).

2.6. In silico functional analysis

In silico functional effect of the validated variants was conducted using 10 *in silico* tools available in dbNSFP2.9 (Liu et al., 2013), SNP&GO (<http://snps-and-go.biocomp.unibo.it/snps-and-go/index.html>), SNAP (Bromberg et al., 2008) (<https://roslab.org/services/snap>), PhD-SNP (Capriotti et al., 2006) (<http://snps.biofold.org/phd-snp/phd-snp.html>), Combined Annotation Dependent Depletion (CADD) (Kircher et al., 2014) and consensus deleteriousness score of missense mutations (Condel) (González-Pérez and López-Bigas, 2011). For gene level analysis, Residual Variation Intolerance Score (RVIS) (<http://chgv.org/GenicIntolerance>) was used. Briefly, RVIS identifies a gene as 'intolerant' based on a score that it generates by comparing a gene containing a higher number of rare mutations with another gene with similar number of variants. We also examined the evolutionary conservation of the validated variants using GERP, PhyloP, phastCons7way and SiPhy scores.

2.7. Screening for variants in two independent SZ cohorts

Complete coding sequence of gene(s) encompassing validated variant(s) was screened for additional variants, if any, in two independent sporadic SZ cohorts comprising 475 north Indian SZ cases and 410 healthy controls; and 310 American SZ cases. Of the 410 north Indian control samples, exome data was available for 153 samples and all the remaining samples were screened by Sanger sequencing (Primer details in Supplementary Table 1). Burden of rare variants in the gene(s) thus identified was tested by Combined Multivariate and Collapsing (CMC) method (Li and Leal, 2008) available in RVTESTS software package

(Zhan et al., 2016). All coding rare variants (MAF < 0.001) barring synonymous changes in the putative gene(s) were included for analysis using north Indian SZ patients (n = 475) and healthy controls (n = 410).

3. Results

3.1. Whole exome sequencing in the index family

Mean target depth ranging from 61× to 103× (average 75×) was obtained for the four individuals exome sequenced in the study family (Fig. 1). On an average, 80% of the target region was covered with >20× depth (Supplementary Table 2). Variant prioritization performed as detailed in the methodology yielded 13 variants (Table 1). Following segregation analysis in the family, nine variants were retained (Table 2), which was further prioritized based on functional significance and/or previous reports in SZ.

Based on this strategy, we identified a heterozygous rare missense variant rs367888752 (NC_000006.11:g.132966598C>A; NM_138327.2:c.545G>T; NP_612200.1:p.Cys182Phe) in Trace amine associated receptor (*TAAR1*). The variant was present in the affected mother and two affected children but not in two unaffected siblings as confirmed by Sanger sequencing (primer details in Supplementary Table 1; Supplementary Fig. 1). The wild type allele is highly conserved across species (Supplementary Table 3). *TAAR1* is an intronless gene of 1.02 kb located on chromosome 6q.23.2 and is a G protein coupled-receptor (GPCR). The index variant is present in the extracellular domain of this GPCR and causes a cysteine to phenylalanine change consequently leading to the breakage of a highly conserved disulfide bond of this receptor. This variant was predicted to be damaging by all the 15 *in silico* tools used (Supplementary Table 4). CADD predicts this variant to be among the top 1% most deleterious substitutions in the human genome. Further, RVIS analysis showed that, *TAAR1* is among 34.6% of the most intolerant of human genes.

3.2. Additional variants in TAAR1 identified in independent SZ cohorts

Screening of the complete 1.02 kb coding region of this gene for additional variants, if any, in 475 north Indian sporadic SZ patients by Sanger sequencing (primer details in Supplementary Table 1) identified four rare (MAF < 0.001, two of which were novel) protein disturbing variants namely p.Ser47Cys, p.Phe51Leu, p.Tyr294Ter and p.Leu295Ser in four unrelated SZ patients (Details provided in Supplementary Table 5, sequencing profile not shown). In addition to these rare variants, a rare synonymous variant namely p.Leu253= was also observed in a single SZ patient (Supplementary Table 5). Further, Sanger sequencing of this complete intronless gene in 310 American sporadic SZ patients revealed two more novel rare (MAF = 0.001) missense variants namely, p.Ala109Thr and p.Val250Ala in two unrelated individuals (Details provided in Supplementary Table 6, sequencing profile not shown). These were not observed in the north Indian SZ patients. None of these seven rare variants were observed in 410 healthy controls. This is not unexpected considering that the study is underpowered for detection of rare variants. The rare variant burden analysis performed on this variant data set from *TAAR1* screening (restricted to north Indian case-control cohort, n = 475 sporadic SZ cases; n = 410 controls) showed a significant enrichment (p = 0.036) in case group. Additional relatives of neither

north Indian nor American SZ patients were available for the study. In addition to the above mentioned rare variants, 12 common (MAF > 0.001) variants previously documented in ExAC database were observed among the study samples (Supplementary Table 7, sequencing profile not shown).

3.3. Test of association of common variants in TAAR1 with SZ

Three of the 12 above mentioned common variants namely rs8192621, rs8192620 and rs8192619 had MAF > 0.05. No significant association was observed on test of association with SZ (n = 475 sporadic cases, n = 410 controls) in the north Indian cohort. In addition, to rule out the role of regulatory variant(s) in this intronless gene, one promoter SNP namely rs60266355 selected from dbSNP was also genotyped in an extended north Indian cohort (n = 999 sporadic cases, n = 1021 controls) using PCR-RFLP (primer and restriction enzyme details in Supplementary Table 8) but no significant association was observed.

4. Discussion

Based on a large number of early genome wide linkage scans and candidate gene based association and more recent genome-wide association studies, several genes/loci have been reported to be associated with SZ but well replicated putative risk alleles have been very limited. ~1000 *de novo* variants (Li et al., 2015) in SZ patients have been reported in the recent past, but most of these are private to the individuals tested. In this study, using the WES, we identified a heterozygous missense variant (c.545 G>T, p.Cys182Phe) in 1.02 kb intronless *TAAR1* in three affected individuals across two generations (Fig. 1). Keeping in mind the likely polygenic and complex nature of SZ, the contribution of eight other variants in different genes, which are present in all the three affected individuals in the family (Table 2), cannot be ruled out. However, due to the notable functional relevance of *TAAR1*, this gene was further analysed in the study. The index variant in *TAAR1* was not observed in ~800 unrelated SZ patients of north Indian and American origin and ~400 north Indian controls screened (see Results section). Nevertheless, that this variant in *TAAR1*, a pharmacologically important GPCR (Revel et al., 2013) may be of functional significance in disease etiology, may be inferred from different lines of evidence discussed below.

i). The missense variant (c.545 G>T, p.Cys182Phe) causing a change of cysteine residue to phenylalanine breaks the conserved disulfide bond which is otherwise essential for maintaining intact structure and ligand binding (Cook et al., 1996; Cook and Eidne, 1997; Dohlman et al., 1990a; Karnik et al., 1988; Perlman et al., 1995; Savarese et al., 1992a), G protein activation, protein stability and proper receptor cell surface localization (Davidson et al., 1994; Dohlman et al., 1990; Savarese et al., 1992) in most of the GPCRs; ii) the variant is predicted to be damaging by all the 15 *in silico* tools used (Supplementary Table 4); iii) most importantly, the recent elegant demonstration of altered β PTEA (agonist) induced receptor function due to a non-synonymous change p.C182Y (at the same protein position 182, as in our study but with a cysteine to tyrosine change) using standard cAMP and ligand binding assays (Shi et al., 2016). It may be mentioned that eight SNPs were selected for functional studies by this group based on their location in highly conserved motifs in the transmembrane receptor.

In addition to the index variant identified in this study, six additional rare protein disturbing variants that we observed in the north Indian and American sporadic SZ cases (Supplementary Tables 5 and 6) warrant discussion. Preliminary evidence for their functionality has been derived from in silico predictions where five have been shown to be damaging by prediction tools used (Supplementary Table 4). Further, prediction of haploinsufficiency in *TAAR1* and RVIS analysis suggesting it to be one of the most intolerant genes may imply their likely contribution. As for the lack of association of the three common coding SNPs (rs8192621, rs8192620 and rs8192619) and the promoter SNP (rs60266355), it is not surprising, in view of the lack of association of *TAAR1* with SZ in the recent GWAS (Ripke et al., 2013, 2014). Taken together, the presence of a large number of rare variants (MAF < 0.001) in *TAAR1* in SZ patient groups in our study (Supplementary Tables 5 and 6) but their absence in controls seem to favour an argument that *TAAR1* is a strong SZ risk conferring gene. Significant enrichment ($p = 0.036$) of rare variants observed in north Indian SZ patients lends support to this. The large number of rare variants observed in this gene is not unlikely considering that it is an intronless gene. Further it may be speculated that *TAAR1* is under selection pressure as evident by the triallelic SNP p.182Y>F, present in this gene. All these observations taken together may indicate that a model of allelic heterogeneity at this pharmacologically relevant gene/locus may provide supportive data for association of this gene with SZ and thereby the first ever genetic evidence for the role of *TAAR1* in SZ etiology. Interestingly, this gene is located at 6q23.2, which is a well-known SZ susceptibility locus (Lerer et al., 2003; Levi et al., 2005) but has never been directly implicated previously. It is relevant to mention here that there are several previous genetic and pharmacological studies suggesting the functional relevance of alteration(s) in this gene in SZ etiology, but all of them are based on over expression/knock out of this gene in animal models (discussed later in this section).

TAAR1, a member of the *TAAR* family, binds to various Trace amines and common biogenic amines and transmits the signals through Gαs heterotrimeric G-protein and raises the intracellular cAMP levels and contributes to phosphorylation of protein kinase A and protein kinase C. Trace amine levels are known to be altered in SZ and other neuropsychiatric disorders (Boulton, 1980; Sotnikova et al., 2009) and this provided the earliest evidence for its role in disease biology. Of note, significant association of SNPs in *TAAR6* (previously described as *TRAR4*) with SZ following the family based association studies in 192 European and African American ancestry samples has been previously reported (Duan et al., 2004). Further efforts to replicate these findings in 265 multiplex families of Irish origin identified additional genotypic and haplotypic association with disease (Vladimirov et al., 2007). This finding was not replicated in an Irish case-control study carried out by the same group (Vladimirov et al., 2009), in Chinese (Duan et al., 2006) and in Japanese populations (Ikeda et al., 2005), but genotypic and haplotypic association of *TAAR6* SNPs with SZ and bipolar disorder were reported in Korean case-control cohorts (Pae et al., 2008). *TAAR1* is a known modulator of dopaminergic (Lindemann et al., 2008), serotonergic, and glutamatergic (Revel et al., 2011) pathways and its role in SZ etiology is amply evidenced in literature. It is known to express in the prefrontal cortex, a well-known brain region implicated in the pathophysiology of SZ (Knable and Weinberger, 1997). It is also known to be expressing in the limbic and monoaminergic systems in the brain, which is

known to influence cognition, mood, motivated behaviors, and movement (Xie and Miller, 2009).

TAAR1 has also been shown to interact with two other strong candidate genes namely dopamine transporter (*DAT*) (Maier et al., 1996; Markota et al., 2014) and *DRD2* (Glatt et al., 2003; Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014) from dopaminergic pathway. In brain it is known to co-express with *DAT* and regulates dopamine transport (Xie and Miller, 2007). It is well known that TAAR1 and *DRD2* can modulate each other's activity. When TAAR1 is activated, increased *DRD2* auto receptor (short form) mediated auto inhibition in dopamine release has been reported in pre-synaptic dopaminergic neurons. It is also known to be co-expressed with the long form of *D2* receptor and functionally and physically interact with each other, this interaction is essential for the TAAR1 mediated modulation of dopaminergic system (Espinoza et al., 2011).

As mentioned earlier, *TAAR1* has been extensively investigated in different animal models. *TAAR1* knock out mice have been reported to manifest schizophrenia like behaviors (Wolinsky et al. 2007). An elevated spontaneous firing rate of dopaminergic neurons, hypersensitivity to amphetamine, increase in the proportion of high affinity *DRD2* receptors in the striatum and displaying a deficit in prepulse inhibition were also shown in *TAAR1* knock-out mice. Following an acute challenge in such mice there was an increase in the locomotion and striatal release of DA, noradrenaline and serotonin (Lindemann et al., 2008; Wolinsky et al., 2007). On the other hand, transgenic mice that over express this protein in brain, showed hyposensitivity to amphetamine and alteration in monoaminergic neurotransmission (Revel et al., 2012). Agonist potency of 5-HT1A autoreceptors has been shown to increase with TAAR1 activation. Activation of TAAR1 reduced the firing frequency of 5-HT neurons in the dorsal raphe nucleus, thus providing a strong evidence of its modulatory role in the serotonergic system (Revel et al., 2011). In brain this protein is constitutively or tonically activated by ambient levels of endogenous agonist(s) in 5-HT and ventral tegmental area DA neurons (Bradaia et al., 2009; Revel et al., 2011).

Treatment of mice with EPPTB a selective TAAR1 antagonist increased the firing frequency in dopaminergic neurons and blocked inwardly rectifying K(+) current (Bradaia et al., 2009). On the contrary, in a large study on TAAR1 specific agonist (RO5166017) treatment, it reduced the firing frequency of dopaminergic and serotonergic neurons; altered the desensitization rate and agonist potency at 5-HT1A receptors; prevented stress-induced hyperthermia; blocked dopamine-dependent hyperlocomotion in cocaine-treated and dopamine transporter knockout mice and in NMDA antagonist treated mice it blocked hyperactivity (Revel et al., 2011). Another study using a complete agonist (RO5256390) and a partial agonist (RO5263397) treatment in rodents and primates showed antipsychotic like property by targeting positive and negative symptoms and improvement in cognitive function. However, these agonists did not induce catalepsy or weight gain in various rodent and primate models (Revel et al., 2013b). In other studies on such knock-out mice, NMDA/AMPA ratio is altered as a result NMDA receptor dependent synaptic deficits; GluN1 and GluN2B subunits of NMDA receptors were also shown to be decreased along with behavioral abnormalities (Espinoza et al., 2015b). In yet another study a higher number of high affinity *DRD2* receptors and higher expression of *DRD2* in post-synaptic neurons and

consequently activation of related G protein-independent pathway (AKT/GSK3) in the basal condition were reported (Espinoza et al., 2015a). Interestingly, AKT signaling pathway is known to be associated with SZ (Emamian, 2012; Zheng et al., 2012).

In summary, the novel genetic evidence for the role of *TAAR1* in SZ etiology provided by this study is well supported by a large body of animal model based pharmacological and functional data. Our findings necessitate screening of this gene across ethnic groups to determine its overall contribution to disease etiology.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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*Samples used for exome sequencing; Filled symbols- affected individuals; To protect personal information, sex, age, and order of siblings have been disguised.

Fig. 1.
Shows the pedigree of schizophrenia study family.

Table 1

Prioritization of variants in the whole exome data in the index family.

	Number of variants
Total coding variants (based on wANNOVAR annotation)	31,937
After removal of variants with MAF ≤ 0.001 in 1000G, EXAC, 6500 exomes, CG40, dbSNP	694
Total number of protein disrupting variants (after removal of synonymous variants)	451
Total heterozygous variants present in three affected and absent in one unaffected individual	27
On removal of variants from regions with segmental duplication	24
On removal of variants present in in-house control whole exome data	13
Total number of segregating variants in the index family	9

Table 2

Details of segregating variants in the index family

Variant position (hg19)				Variant allele frequency in databases					
Chromosome	Start	End	Gene name	Ref allele	Alt allele	Variation type	1000 Genome Project Phase 3	ESP6500	ExAC
chr1	55680636	55680636	USP24	C	G	Non synonymous SNV	0	0	0
chr1	150776517	150776517	CTSK	C	T	Non synonymous SNV	0	0	0.00003
chr6	132966598	132966598	TAAR1	C	A	Non synonymous SNV	0	0	0.00004
chr8	37699215	37699215	GPR124	C	A	Non synonymous SNV	0	0	0
chr17	74720007	74720007	JMJD6	T	A	Non synonymous SNV	0	0	0.000008
chr17	79995361	79995361	DCXR	G	A	Non synonymous SNV	0	0	0.00009
chr18	77806147	77806147	RBFA	A	G	Non synonymous SNV	0	0	0
chr20	13756672	13756674	ESF1	ATC	-	Non frameshift deletion	0	0	0.00003
chr22	29876916	29876916	NEFH	C	A	Non synonymous SNV	0	0	0

SNV: single nucleotide variation; ESP6500 - NHLBI Exome Sequencing Project (Version: v0.0.30); ExAC - Exome Aggregation Consortium (Version 0.3).