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Rare variant based evidence for oligogenic contribution of neurodevelopmental pathway genes to schizophrenia

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Dear editors,

Family and twin studies suggest a multi-factorial polygenic thresh-old model (MFPT) in schizophrenia (SZ) (Gottesman, 1991). This is supported by genome-wide association studies (GWASs) (Ripke et al., 2014) and also polygenic burden of rare variants reported in individuals with SZ (Purcell et al., 2014). However, this model focusing on rare variants has been rarely checked in families and whole exome sequencing (WES) of multiply affected families and findings thereof, may facilitate testing the model. Two multigenerational families of north Indian ancestry with multiple affected and unaffected members (Fig. 1a,b) were recruited as previously described (John et al., 2018). WES was performed on three affected members each from the two families, using Agilent SureSelectXT Human All Exon V5 + UTR kit for library preparation and sequenced (101 bp paired end mode) on Illumina HiSeq 2000 sequencer, using a commercial facility (Medgenome Labs Pvt. Ltd., Bangalore,

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Contributions

Prof. B.K. Thelma, Prof. S. N. Deshpande and Prof. V. L. Nimgaonkar designed the study and obtained research funding; Prof. S. N. Deshpande diagnosed and recruited the study samples; Dr. Jibin John performed all the WES data analysis, interpretation, and confirmation of variants by Sanger sequencing. Dr. Triptish Bhatia contributed to sample recruitment and phenotype data documentation; Dr. Prachi Kukshal maintained the DNA repository and contributed to data analysis; Jibin John, B.K. Thelma and V. L. Nimgaonkar wrote the first draft of manuscript; all authors contributed to 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.

India). GATK Best Practices for germline variant discovery was used for variant calling and the variants were annotated and prioritised as described earlier (John et al., 2018) using KGGSeq and checked in the remaining members by Sanger sequencing.

On an average, >97% of the target regions were covered with >10× and had a mean on target depth of 54× across the samples. In family #1 a total 45 protein sequence altering rare variants (MAF ≤0.01) were seen to be shared across three affected siblings and were predicted to be damaging by Polyphen2/SIFT and with CADD score >15. Contrary to the expectation, none of these were homozygous/compound heterozygous. We then explored for the possibility of multiple heterozygous variants together contributing to the disease. To check the hypothesis we focused on heterozygous variants in known candidate genes as previously described (John et al., 2018; Purcell et al., 2014). This resulted in, three promising rare heterozygous missense variants in three different genes namely *FGFR3* (NM_022965:c.1412A>T:p.K471M); *FGF4* (NM_002007:c.380G>A:p.S127N); and *RERE* (NM_012102:c.4054G>A:p.A1352T) (Fig. 1a). Variant in *FGFR3* is inherited from unaffected father and other two are inherited from unaffected mother. In family #2 (Fig. 1b) we identified a total of 41 rare heterozygous variants (MAF ≤0.01) shared among the three SZ affected individuals, and absent in the unaffected mother. These were predicted to be damaging by Polyphen2/SIFT and CADD scores were >15. Further prioritisation of the 41 variants done as mentioned above, resulted in identification of three most promising missense variants in three different genes namely *EGFR* (NM_005228:c.3250G>A: p.D1084N;); *FGFR1* (NM_023105:c.119A>C: p.D40A); and *MDGA1* (NM_153487: c. 2324G>A: p.R775Q) (Fig. 1b). Variant in *MDGA1* was absent in the individual with psychosis.

On screening of whole exome data of 357 unrelated but ethnicity matched SZ individuals and 250 exomes of unrelated and non-psychiatric individuals available and used previously (John et al., 2018), we identified multiple protein sequence altering rare (MAF 0.000009 to 0.006) variants including nine in *RERE*, eight in *FGFR3*, 13 in *MDGA1*, four in *EGFR*, four in *FGFR1* and two in *FGF4*. Furthermore, five variants among these were observed in two or more affected individuals. Interestingly, one missense variant (NM_153487:c.2803C>T:p. P935S) in MDGA1 was observed in nine of 357 SZ exomes screened, but was notably absent in the 250 exomes of unrelated and non-psychiatric individuals.

All the six genes are well documented to be involved in neurodevelopmental processes and more importantly, have been previously implicated in various neuropsychiatric disorders including SZ and/other psychiatric disorders based on GWASs (*FGFR1*, *EGFR* and *RERE*) (Ripke et al., 2014; Sklar et al., 2008); expression studies reporting differential expression of these genes (*EGFR*, *FGFR1*, *FGFR3* and *RERE*) (Gandal et al., 2018) in psychiatric disorders; and psychiatry relevant behavioural abnormalities during inactivation of the gene in rodents (*EGFR*, *MDGA1*, *FGFR1* and *FGFR3*) (Connor et al., 2017; McDonald et al., 2001; Stachowiak et al., 2013; Yokomaku et al., 2005). Based on literature based functional relevance of these genes, their likely dysfunction may be speculated to cause abnormal brain development, this in turn may disrupt well known neurotransmitter signalling pathways leading to disease development. In summary, the observed mode of inheritance of multiple rare variants (Fig. 1a, b) in functionally relevant genes suggests that they may act in an

additive manner and provides elegant genetic evidence for the classical concept of cumulative contribution of genes of minor/moderate effect in manifestation of complex traits.

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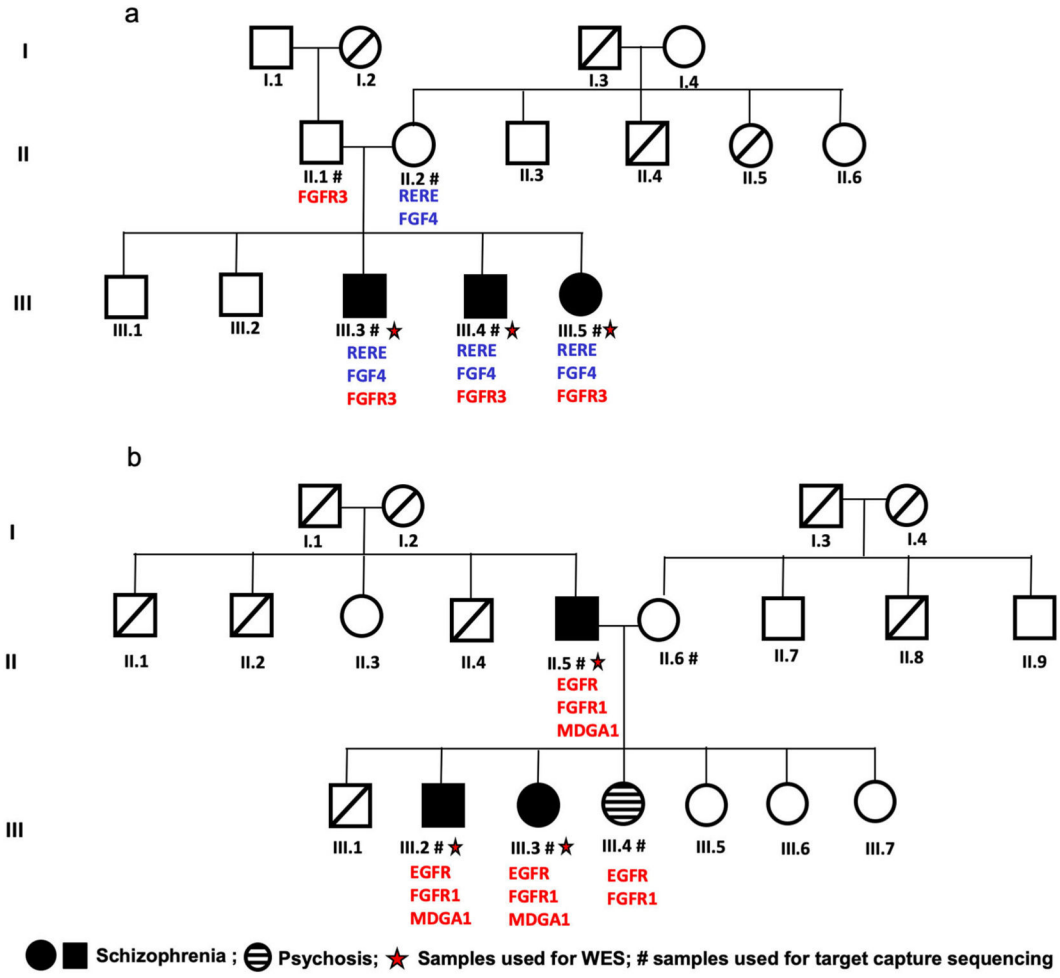

Fig. 1.

Pedigrees of the schizophrenia families used in the study.