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Short report

22q11.2 deletion carriers and schizophrenia-associated novel variants

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Summary
The penetrance of schizophrenia risk in carriers of the 22q11.2 deletion is high but incomplete, suggesting the possibility of additional genetic defects. We performed whole exome sequencing on two individuals with 22q11.2 deletion, one with schizophrenia and the other who was psychosis-free. The results revealed novel genetic variants related to neuronal function exclusively in the person with schizophrenia (frameshift: *KAT8, APOH* and *SNX31;* nonsense: *EFCAB11* and *CLVS2*). This study paves the way towards a more complete understanding of variant dose and genetic architecture in schizophrenia.

Declaration of interest
None.

Among structural variants, 22q11.2 deletion is one of the highest risk factors for developing schizophrenia. Caused by hemizygous microdeletions at chromosome 22q11.21, it has a population prevalence of about 1 in 2000–4000 live births and about a fourth of the carriers develop schizophrenia.[1] This chromosomal region is considered to be one of the main schizophrenia susceptibility loci, harbouring several candidate genes for disease pathogenesis. The incomplete penetrance of schizophrenia in 22q11.2 deletion suggests polygenic mechanisms that require additional genomic variants outside of the deleted region.[2] Evidence for this notion was first reported by our group, where we detected two additional, rare schizophrenia-associated genetic defects outside of the deletion region,[3] which was followed by a report on White patients.[4] Both studies highlighted the role of multiple hit mutations in conferring additional risk for psychosis and emphasised the importance of identifying additional variants by closely examining 22q11.2 deletion syndrome. This study aimed to decipher the role of genetic defects outside of the 22q11.2 region in increasing the risk for schizophrenia. We performed whole exome sequencing on two individuals with 22q11.2 deletion; one carrier with schizophrenia and the other who was psychosis-free. This analysis provides a way to search for additional candidate genes responsible for schizophrenia pathogenesis.

Method

Person A (22q11.2 deletion with schizophrenia) was a 37-year-old Japanese female high-school graduate and Person B (22q11.2 deletion without schizophrenia) was a 25-year-old Japanese male who was born with Tetralogy of Fallot. The detailed clinical history of the participants is provided in the online supplement, Method DS1. The current study was approved by the ethics committee of RIKEN, and informed, written consent to participate in the study was provided by both participants and family members, after receiving a full explanation of the study protocols and objectives. To confirm the 22q11.2 microdeletion, fluorescence *in situ* hybridisation (FISH) with the TUBLE1 probe and array comparative genomic hybridization (aCGH) using NimbleGen Human CGH 3x1.4M Whole-Genome Tiling v1.0 array (Roche NimbleGen, Wisconsin, USA) were performed. Target enrichment for whole exome sequencing was performed using Agilent's SureSelect All Exon hs50Mb.h19 (Agilent Technologies, California, USA) and samples were sequenced using the Illumina HiSeq 2000 platform (Illumina, California, USA) generating paired-end 100 base pair reads. The analysis of the primary data and variant filtering were performed as outlined in the online supplement, Method DS2.

The variants were prioritised based on the following criteria: (a) they are present only in Person A, a 22q11.2 deletion carrier manifesting schizophrenia; (b) they are novel, therefore not present in the National Center for Biotechnology Information dbSNP database (Build 137), 1000 Genomes Project or the Exome Variant Server of NHLBI GO Exome Sequencing Project (ESP6500SI-V2); (c) they are deemed functional, such as frameshift, stop-gain or non-synonymous mutations; (d) they are conserved on the basis of GERP (Genomic Evolutionary Rate Profiling) scores (>5); and (e) they are predicted to be deleterious and damaging by PROVEAN (Protein Variation Effect Analyzer) and SIFT software. The identified variants were further validated and reconfirmed by Sanger sequencing.

Results

The FISH analysis and aCGH confirmed the 22q11.2 microdeletions, showing a 2.6 Mb hemizygous genomic deletion in both participants (online Fig. DS1). The exome sequencing yielded a large number of variants in both participants including the previously reported frameshift mutation in *GLO1* in Person A.[3] The exome sequencing coverage statistics and summary of called variants is provided in the online supplement, Table DS1. Based on the specified criteria for variant prioritisation, we identified five heterozygous variants (three frameshift and two nonsense variants) in Person A) (Table 1), which were validated by Sanger sequencing (online Fig. DS2). Interestingly, none of the genes harboring these variants was previously reported to be associated with any neurological or psychiatric phenotypes, and therefore the roles of these genes in manifesting or modulating psychiatric phenotypes warrant future examination.

Discussion

Additive or epistatic gene–gene interactions are known to promote or modify neuropsychiatric phenotypes. An example of this is the 16p12.1 microdeletion, which on its own predisposes carriers to neuropsychiatric phenotypes, but also exacerbates neurodevelopmental phenotypes in association with other large deletions or duplications. These findings support the multiple-hit model for genetically complex diseases, including schizophrenia.[5]

Table 1 List of variants identified solely in 22q11.2 deletion patient with schizophrenia (Person A)

Chromosome	Position (base pair)	Effect	Gene	Gene description	GERP score
14	90,263,658	Nonsense	*EFCAB11*	EF-hand calcium-binding domain 11	6.03
16	31,131,524	Frameshift	*KAT8*	K(lysine) acetyltransferase 8	5.83
17	64,219,861	Frameshift	*APOH*	Apolipoprotein H (beta-2-glycoprotein I)	5.59
8	101,642,574	Frameshift	*SNX31*	Sorting nexin 31	5.44
6	123,319,142	Nonsense	*CLVS2*	Clavesin 2	5.39

GERP, Genomic Evolutionary Rate Profiling.

The specific variants identified in Person A provide putative candidate genes for further analysis in the context of schizophrenia. One is a frameshift mutation in *SNX31* (sorting nexin 31). This gene codes for a family of SNX proteins, which contain a conserved PX (or phagocyte oxidase homology) domain that targets SNX proteins to endosomes.[6] Recent reports have shown that a related protein, SNX27, promotes excitatory synaptic dysfunction by modulating glutamate receptor recycling in Down syndrome, and this process is thought to contribute to pathogenesis.[7] In addition, a nonsense mutation was observed in the *CLV2* gene (clavesin 2). This gene product is thought to modulate neuron-specific regulation of late endosome/lysosome morphology.[8] It is plausible that both of these genes potentially increase schizophrenia risk owing to their roles in neuronal functions.

Another gene identified in Person A carries a nonsense mutation and codes for an EF-hand calcium-binding domain-containing protein 11 (EFCAB11). This protein contains three EF-hand domains and typically, EF-hand motifs constitute calcium-binding proteins which may function as sensor proteins, buffer proteins or calcium-stabilising proteins. Since calcium signalling plays an important role in the regulation of cell metabolism, gene expression, cytoskeleton dynamics, cell cycle, cell death, neurotransmission and signal transduction processes, it is tempting to speculate that EFCAB11 may control psychiatric phenotypes. Two frameshift mutations were detected in the genes for K(lysine) acetyltransferase 8 (*KAT8*) and apolipoprotein H (beta-2-glycoprotein I) (*APOH*). KAT8 is a member of the MYST histone acetylase protein family, which can mediate epigenetic changes through histone modifications, and APOH is a major antigenic target for antiphospholipid antibodies, leading to antiphospholipid syndrome. Memory alterations, cognitive impairment, mood disorders and psychosis are known to precede the onset of primary antiphospholipid syndrome.[9] Therefore, the role of *APOH* mutations in neuropsychiatric phenotypes warrants further study.

No novel mutations in the 22q11.2-hemizygous region were observed in Person A. However, in Person B, a novel, deleterious, non-synonymous mutation (D203N) was observed in the synaptosomal-associated protein, 29kDa (SNAP29). Rare variants of *SNAP29* were recently identified in a series of patients with 22q11.2 deletion syndrome, unmasking an autosomal recessive condition that results in atypical phenotypes such as cerebral dysgenesis, neuropathy, ichthyosis and keratoderma.[10] However, none of these atypical phenotypes was present in Person B.

In summary, we report novel candidate genes that could affect the predisposition to, or modulate the risk of, schizophrenia, although definitive proof of a causal relationship will need to be confirmed in larger sample sizes owing to the interpretative limitation of individual case studies. It would be also possible that the described variants seen in Person A might be associated not only with schizophrenia but also with IQ and social withdrawal.[2] Since we prioritised identified variants based on their novelty, it is possible that other important genes with additive or epistatic interaction with genes located in the 22q11.2 genomic region may have been missed. Nonetheless, we hope that this study provides a platform for the identification of novel genes to complete the landscape underlying the genetic architecture of schizophrenia.

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EXTRA CONTENT ONLINE

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