22q11.2 deletion carriers and schizophrenia-associated novel variants
S. Balan, Y. Iwayama, T. Toyota, M. Toyoshima, M. Maekawa and T. Yoshikawa

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: EFCA811 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. 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. 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, which was followed by a report on White patients. 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 hs50Mbh19 (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 GLOI 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
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. 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. 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. 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. 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, neuopathy, ichthyosis and keratoderma. However, none of these atypical phenotypes were 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. 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.

### Table 1

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Position (base pair)</th>
<th>Effect</th>
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<th>Gene description</th>
<th>GERP score</th>
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<td>KAT8</td>
<td>K(lysine) acetyltransferase 8</td>
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<td>17</td>
<td>64,219,861</td>
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<td>APOH</td>
<td>Apolipoprotein H (beta-2-glycoprotein I)</td>
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<td>8</td>
<td>101,642,574</td>
<td>Frameshift</td>
<td>SNX31</td>
<td>Sorting nexin 31</td>
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<td>6</td>
<td>123,319,142</td>
<td>Nonsense</td>
<td>CLV2</td>
<td>Clavesin 2</td>
<td>5.39</td>
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</table>

## References


**Online supplement**

Method DS1: Clinical history of the study participants

Method DS2: Exome analysis

Table DS1: Summary of variants called by exome sequencing

Fig. DS1: CGH array analysis of chromosome 22 and variant-filtering work flow

Fig. DS2: Sanger sequencing results of identified variants in exome sequencing
Method DS1: Clinical history of the study participants

**Person A (22q11.2 deletion with schizophrenia)**

This participant was a 37-year-old Japanese female high-school graduate, born of non-consanguineous parentage, by normal delivery without any obstetric complications. There was no reported family history of psychiatric disease. Developmental milestones were normal. At the age of 15, she developed auditory hallucinations consisting of argumentative and commentary voices, somatic passivity experiences, thought broadcasting delusions, delusional perceptions and delusions of persecution, which exacerbated gradually. Other psychiatric features included depressed mood, social withdrawal, grossly disorganised and unpredictable agitation, angry outbursts and emotional labiality. At the age of 18, she was admitted to the hospital for 2 months. Her IQ was 61 when measured during the first hospital admission. Later, at the age of 25, the symptoms recurred and she was admitted to the hospital for 1 month. She was prescribed bromperidol, which induced a partial improvement in her hallucinations and delusions. At the study evaluation, she was 37 years old and receiving antipsychotic treatment (risperidone 5 mg/day and quetiapine 95 mg/day), but still experienced persistent auditory hallucinations. She had no cardiac, palatal or minor dysmorphic craniofacial anomalies. Slender tapered fingers were the only physical features present, characteristic of 22q11.2 deletion syndrome. She also showed slight hypocalcaemia (8.4 mg/dl in serum, normal range 8.5–10.5 mg/dl). She remains unmarried, lives in a group home and is employed in a semi-sheltered workplace.

**Person B (22q11.2 deletion without schizophrenia)**

This participant was a 25-year-old Japanese male. He was born with Tetralogy of Fallot, a heart disease, and had corrective surgery at the age of 4 months. The operation was successful and he has no need for a cardiac pacemaker. He graduated
from university and worked as a nutrition manager at a hospital when evaluated for this study. To date, he experiences occasional faecal incontinence when under strong psychological pressure, but has never visited a psychiatric clinic or hospital. A magnetic resonance imaging examination revealed the existence of cavum septi pellucidi. He showed a slightly low number of platelets (95,000/µL, normal range 131,000–362,000 per µL), and a low serum concentration of calcitonin (11 pg/mL, normal range 15–89 pg/mL), with a normal calcium concentration (9.2 mg/dL, normal range 8.5–10.2 mg/dL) and a normal parathyroid hormone concentration (16.2 pg/mL, normal range 9–39 pg/mL). He was not taking any therapeutic drugs at the time of evaluation.

Method DS2: Exome analysis

The exome reads were aligned to the hg19 reference genome ([http://hgdownload.cse.ucsc.edu/goldenPath/hg19/bigZips/](http://hgdownload.cse.ucsc.edu/goldenPath/hg19/bigZips/)) using Burrows–Wheeler Aligner (BWA) ([http://bio-bwa.sourceforge.net/](http://bio-bwa.sourceforge.net/)), and sequence-data analysis module CASAVA (Consensus Assessment of Sequence and Variation) v.1.8 (Illumina), which uses the Efficient Large-Scale Alignment of Nucleotide Databases (ELAND) algorithm. In addition, the variants were called using CASAVA v.1.8 and Samtools (v.0.1.17) ([http://samtools.sourceforge.net/](http://samtools.sourceforge.net/)). Ensembl Variant Effect Predictor (VEP) ([www.ensembl.org/info/docs/variation/vep/index.html](http://www.ensembl.org/info/docs/variation/vep/index.html)) was used to annotate the variants by custom PERL scripts. The filtering of variants was performed using VarSifter ([http://research.nhgri.nih.gov/software/VarSifter/](http://research.nhgri.nih.gov/software/VarSifter/)).

**URLs of databases and software used**

- 1000 Genomes Project ([www.1000genomes.org/](http://www.1000genomes.org/))
- GERP (Genomic Evolutionary Rate Profiling)  

**Table DS1 Summary of variants called by exome sequencing**

<table>
<thead>
<tr>
<th>Variant calls (effect)</th>
<th>Person A</th>
<th>Person B</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Homozygous</td>
<td>Heterozygous</td>
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<tr>
<td>Frameshift coding</td>
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<td>Stop-gain</td>
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<td>79</td>
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<tr>
<td>Stop-loss</td>
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<tr>
<td>Non-synonymous coding</td>
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<td>6486</td>
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<td>Splice site</td>
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<td>1084</td>
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<tr>
<td>Synonymous coding</td>
<td>4950</td>
<td>6336</td>
</tr>
<tr>
<td>Regulatory region</td>
<td>6345</td>
<td>7554</td>
</tr>
<tr>
<td>Complex insertion/deletion</td>
<td>63</td>
<td>15</td>
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<tr>
<td>5'UTR</td>
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<td>1249</td>
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<tr>
<td>3'UTR</td>
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<td>2665</td>
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<td>Upstream</td>
<td>2645</td>
<td>3757</td>
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<td>Downstream</td>
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<td>Within non-coding gene</td>
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<tr>
<td>Intronic</td>
<td>31621</td>
<td>35636</td>
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The exome analyses yielded a total of 162,587,314 reads, of which 137,738,230 reads (84.72%) passed quality filters with coverage across the target region of 83.49%, at 50x depth and 91.03% at 25x depth for Person A. For Person B, 180,891,996 reads were obtained, with 152,498,040 reads (84.3%) passing quality filters, with coverage across a target region of 85.51% at 50x and 92.18% at 25x depths.
Figure DS1: (a) CGH array analysis of chromosome 22: Both subjects show a 2.6 Mb hemizygous deletion at chromosome (b) Variant-filtering work flow; ns, non-synonymous; SNP, single nucleotide polymorphism
Figure DS2: Sanger sequencing results of identified variants in exome sequencing. The electropherograms show verification of the five mutations detected from five genes by whole exome sequencing. Allele 1 is the major allele while Allele 2 is minor. "~" denotes a deletion.
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