Balan et al. 22q11.2 deletion carriers and schizophrenia-associated novel variants. *Br J Psychiatry*, doi: 10.1192/bjp.bp.113.138420

Online supplement

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/) using Burrows–Wheeler Aligner (BWA) (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). Ensembl Variant Effect Predictor (VEP) (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/).

URLs of databases and software used

- 1000 Genomes Project (www.1000genomes.org/)
- Exome Variant Server, NHLBI GO Exome Sequencing Project (http://evs.gs.washington.edu/EVS/)

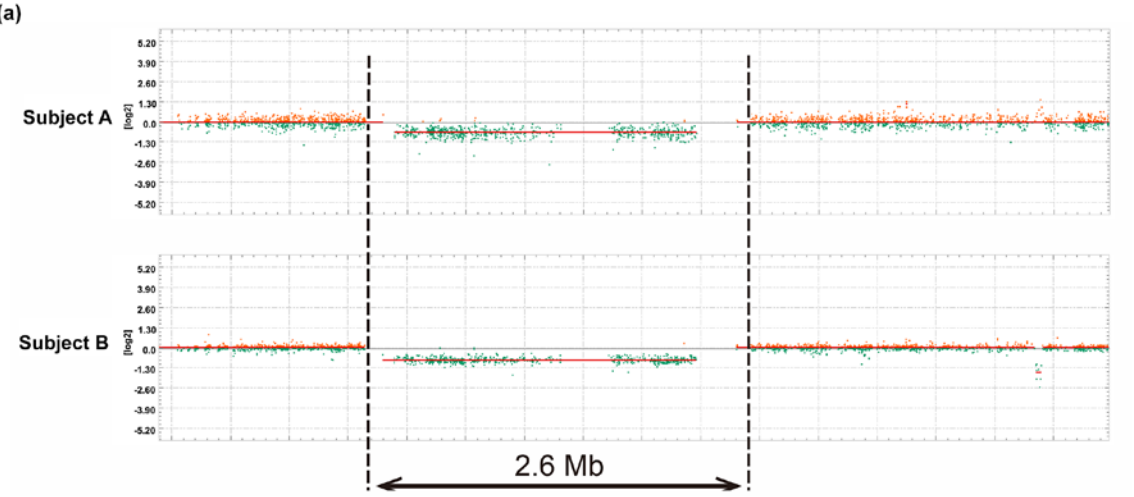
- GERP (Genomic Evolutionary Rate Profiling) (http://mendel.stanford.edu/SidowLab/downloads/gerp/)
- PROVEAN (http://provean.jcvi.org/index.php)
- SIFT (http://sift.jcvi.org/)

Table DS1 Summary of variants called by exome sequencing

Variant calls (effect)	Person A		Person B	
	Homozygous	Heterozygous	Homozygous	Heterozygous
Frameshift coding	103	104	95	117
Stop-gain	33	79	29	71
Stop-loss	14	21	17	16
Non-synonymous coding	4800	6486	4698	6366
Splice site	1008	1084	950	1136
Synonymous coding	4950	6336	4763	6306
Regulatory region	6345	7554	6057	7683
Complex insertion/deletion	63	15	51	17
5'UTR	1158	1249	1124	1234
3'UTR	2379	2665	2256	2770
Upstream	2645	3757	2705	3803
Downstream	1854	2707	1904	2565
Intergenic	2772	3423	2533	3808
Within non-coding gene	3434	5394	3524	5221
Intronic	31621	35636	31186	35748

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.

(a)



(b)

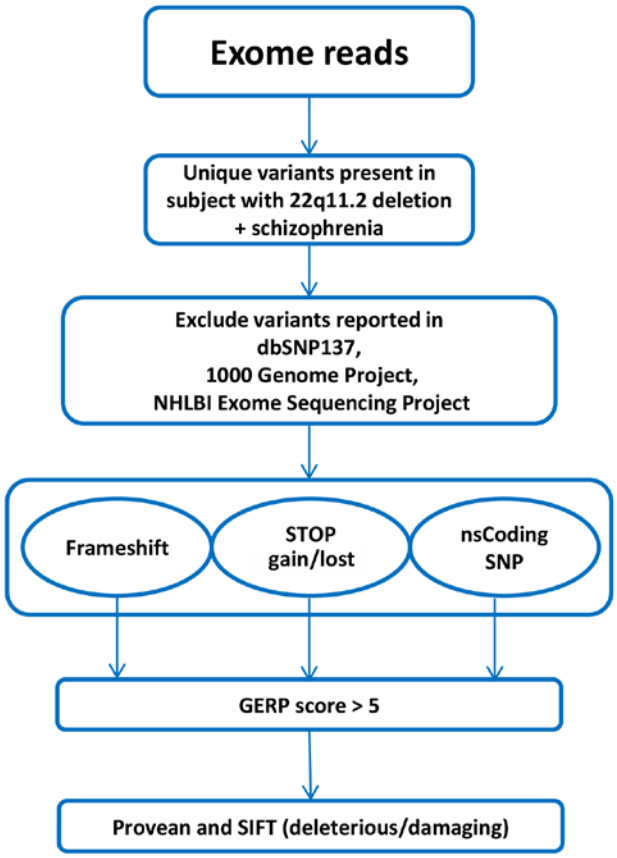
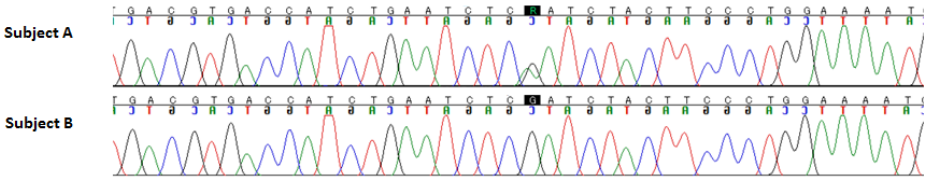
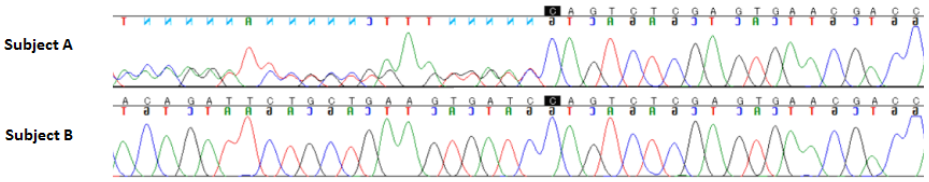


Figure DS1: (a) CGH array analysis of chromosome 22; Both subjects show a 2.6 Mb hemizygous deletion at chromosome (b) Variant-filtering work flow; ns, non-synonymous; SNP, single nucleotide polymorphism

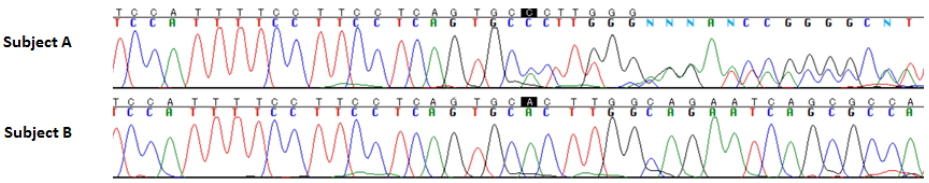
Chromosome	Position	Allele1	Allele2	Effect (PROVEAN)	Gene
14	90,263,658	G	A	Nonsense	*EFCAB11*



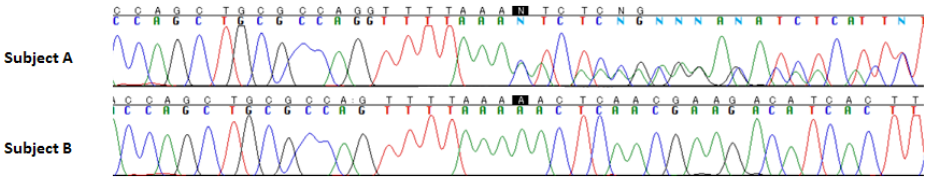
Chromosome	Position	Allele1	Allele2	Effect (PROVEAN)	Gene
16	31,131,524	C	-	Frameshift	*KAT8*



Chromosome	Position	Allele1	Allele2	Effect (PROVEAN)	Gene
17	64,219,861	A	-	Frameshift	*APOH*



Chromosome	Position	Allele1	Allele2	Effect (PROVEAN)	Gene
8	101,642,574	AA	-	Frameshift	*SNX31*



Chromosome	Position	Allele1	Allele2	Effect (PROVEAN)	Gene
6	123,319,142	C	T	Nonsense	*CLVS2*

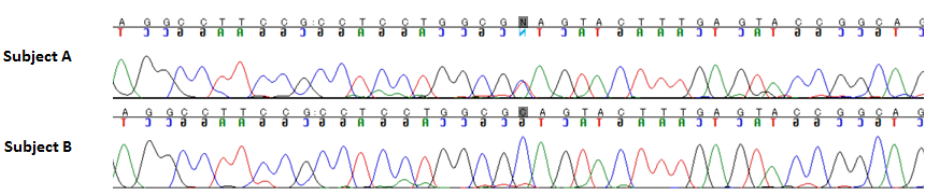


Figure DS2: Sanger sequencing results of identified variants in exome sequencing. The electropherograms show verification of the five mutations detected from five genes by whole exome sequencing. Allele 1 is the major allele while Allele 2 is minor. "-" denotes a deletion.