Schizophrenia with the 22q11.2 deletion and additional genetic defects: case history

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Summary
The 22q11.2 deletion is the most prominent known genetic risk factor for schizophrenia, but its penetrance is at most approximately 50% suggesting that additional risk factors are required for disease progression. We examined a woman with schizophrenia with this deletion for such risk factors. She had high plasma pentosidine levels (‘carbonyl stress’) and a frameshift mutation in the responsible gene, GLO1. She also had a constant exotropia, so we examined the PHOX2B gene associated with both schizophrenia and strabismus, and detected a 5-alanine deletion. We propose that the combination of these genetic defects may have exceeded the threshold for the manifestation of schizophrenia.

Declaration of interest
None.

Method
Previously, we screened 300 unrelated Japanese individuals with schizophrenia who met the DSM-III-R® diagnostic criteria for schizophrenia, by genotyping of markers D22S941, D22S944, D22S264 and D22S311. Concomitantly, we detected one patient with only one allele for each of these polymorphic markers. This individual was a female high-school graduate then aged 28 years. She was born by normal delivery without any obstetric complications. She was admitted to hospital at the age of 18 years for 2 months and again at the age of 25 years for 1 month. Her IQ, which was measured during her first hospital admission, was 61. We decided to re-evaluate her in August 2010. At this time, she was 37 years old and being treated with antipsychotics (risperidone 5 mg/day and quetiapine 95 mg/day), but still suffered from persistent auditory hallucinations. Her only physical symptom was a 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. We re-examined her 22q11.2 microdeletion by fluorescence in situ hybridisation (FISH) with the TUBEL1 probe, and using the NimbleGen Human CNV 3x720K v1.0 Array (Roche Applied Science, Tokyo, Japan).

We recently reported that a subpopulation of individuals with schizophrenia have high plasma pentosidine levels (i.e. ‘carbonyl stress’) and in 2 such individuals out of 3682 participants, we detected frameshift mutations (P122LfsX27 and T27NfsX15) in the glyoxalase 1 (GLO1) gene, a zinc metalloenzyme, providing a possible explanation for the high plasma pentosidine levels. Therefore, we set out to measure pentosidine levels in our patient by high-performance liquid chromatography assay and perform a re-sequencing analysis of GLO1, as described previously.

In another previous report, we showed an association between polyalanine stretch length polymorphisms of the paired-like homeobox 2b (PMX2B or PHOX2B) gene encoding a transcription factor, with people who have schizophrenia, in particular those with strabismus. As our patient has constant exotropia in her right eye, we examined this gene: briefly, the genomic region encoding the 20-alanine tract was amplified using fluorescently labelled forward and reverse primers. Polymerase chain reaction products were run on an ABI 3130xl genetic analyser and the resulting data analysed using the Gene Mapper and Peak Scanner software (Applied Biosystems, Foster City, California, USA).

Results
The FISH data (online Fig. DS1(a)) and the microarray-based CNV analysis (not shown) revealed that the woman has a 3 Mb hemizygous genomic deletion on chromosome 22q11.2, confirming that she has 22q11.2DS. An echocardiogram examination showed no abnormalities in the cardiovascular system (online Fig. DS2). We measured her plasma pentosidine levels twice, 1 week apart, and both values exceeded the mean ±2 standard deviation levels of healthy controls (n = 61) (online Fig. DS1(b)). Then, we examined the GLO1 gene and identified a frameshift mutation...
Discussion

The frequencies of schizophrenia-associated CNVs are rare but generally have large effects on susceptibility to the disease compared with risk-conferring single nucleotide polymorphisms. Among such CNVs, the 22q11.2 deletion has the deepest penetrance of 0.553 (95% CI 0.18–0.97). The penetrance of other large effect size CNVs, for example the 15q13.3 deletion and 16p11.2 duplication are 0.074 (95% CI 0.03–0.16) and 0.069 (95% CI 0.03–0.14) respectively. Nonetheless, the 22q11.2 deletion is not sufficient to develop schizophrenia, and other risk factors are required. This case demonstrates for the first time to our knowledge that concrete additional risk variants could contribute to the manifestation of schizophrenia in combination with 22q11.2DS. In view of the penetrance of these mutations, this view has to be qualified one as other causative mutations may contribute to this individual’s developmental phenotypes.

We showed that one contributing factor is the accumulation of pentosidine, an advanced glycation end-product resulting from carbonyl stress, a state featuring an increase in reactive carbonyl compounds and their attendant protein modifications. Our prior study demonstrated that people with schizophrenia have significantly higher plasma pentosidine levels than controls, and revealed that some participants with high pentosidine levels have impaired GLO1 activity, which is caused by a Glu111→Ala amino acid change and frameshift mutations (see below). Cellular removal of reactive carbonyl compounds, including pentosidine, hinges largely on the activity of GLO1. The identified frameshift mutations were P122LfsX27 and T277NfsX15 (one each out of 1761 participants with schizophrenia), both of which elicit nonsense-mediated mRNA decay resulting in a reduction (to 40–50%) of GLO1 protein expression and activity. The current patient carries the P122LfsX27 frameshift mutation, coincidentally identical to one of our previously detected mutations, further confirming its relationship to disease.

The other genetic defect that we identified in this individual is a polyalanine length variation in the PHOX2B transcription. We previously reported that constant exotropia displays a marked association with schizophrenia (P = 0.00000000906, odds ratio (OR) = 20.6, 95% CI 4.83–87.8), and that deletion/insertion polymorphisms in the 20-alanine homopolymer stretch of PHOX2B disturbs its transcription. We detected a modest association between these functional polymorphisms and all those with schizophrenia (P = 0.012, OR = 1.59, 95% CI 1.01–2.50) and those with schizophrenia and constant exotropia (P = 0.004, OR = 4.52, 95% CI 1.78–11.5).

In summary, we propose a combinatorial model for the genetic risk of schizophrenia where the combination of a CNV, in this case 22q11.2DS, with a large effect; a GLO1 frameshift mutation with a probable moderate to large effect; and a 5-alanine deletion with a modest effect, collaborate to exceed a developmental threshold for the onset of schizophrenia. This proposal is the first discrete evidence for a multiple-hit hypothesis in schizophrenia, and although the current findings are fortuitous, the emerging availability of whole genome sequencing methodologies in the near future should help to systematically decipher the detailed genetic mechanisms that determine which individuals who are schizophrenia-associated CNV carriers go on to develop the disease, and to define its precise genetic spectrum.

References

**Data supplement**

**Fig. DS1** (a) Fluorescence in situ hybridisation; arrows mark both chromosomes 21 with the deleted 22q11.2 labelled. (b) Measurement of plasma pentosidine levels. The dotted horizontal line shows the mean + 2 standard deviation level of healthy controls. The pentosidine levels of our patient were measured twice, 1 week apart. (c) Sequencing results of GLO1. This shows a c.365delC in exon 4, resulting in a frameshift, and a silent polymorphism (rs1130534). The amino acid sequences of GLO1 for both the control and the patient are shown to the right. (d) Structure of PHOX2B (upper panel) and results of fragment length analysis of a 20-alanine stretch portion in the patient, by ABI Gene Mapper.
Fig. DS2  Echocardiograms: (a) abdominal aorta; (b) blood stream from aortic arc to descending aorta; (c) inferior vena cava; (d) aortic arc. There are no abnormalities in the cardiovascular system.
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