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Rare truncating variations and risk of schizophrenia: Whole-exome sequencing in three families with affected siblings and a three-stage follow-up study in a Japanese population

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ABSTRACT

Rare inherited variations in multiplex families with schizophrenia are suggested to play a role in the genetic etiology of schizophrenia. To further investigate the role of rare inherited variations, we performed whole-exome sequencing (WES) in three families, each with two affected siblings. We also performed a three-stage follow-up case-control study in a Japanese population with a total of 2617 patients and 2396 controls. WES identified 15 rare truncating variations that were variously present in the two affected siblings in each family. These variations did not necessarily segregate with schizophrenia within families, and they were different in each family. In the follow-up study, four variations (*NWD1* W169X, *LCORL* R7fsX53, *CAMK2B* L497fsX497, and *C9orf89* Q102X) had a higher mutant allele frequency in patients compared with controls, although these associations were not significant in the combined population, which comprised the first-, second- and third-stage populations. These results do not support a contribution of the rare truncating variations identified in the three families to the genetic etiology of schizophrenia.

1. Introduction

Genetic risk for schizophrenia has been suggested to involve the combined effects of many common variations of small effect, as well as rare variations of large effect (Gratten et al., 2014). Recently, 108 common loci were associated with schizophrenia by a multi-stage genome-wide association study of up to 36,989 patients and 113,075 controls (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014). Meanwhile, whole-exome sequencing (WES) studies have revealed that rare variations contribute substantially to schizophrenia liability (Kato, 2014). WES studies of proband-parent trios show that rare de novo mutations play an important role in the genetic etiology of schizophrenia (Girard et al., 2011; Xu et al., 2012; Gulsuner et al., 2013; Fromer et al., 2014; Guipponi et al., 2014; McCarthy et al., 2014). Rare inherited variations also contribute to the genetic architecture of schizophrenia. In 231 schizophrenia and 34 control trios, rare loss-of-function (nonsense, splice site and frameshift) variations were more significantly transmitted to affected probands than controls (Takata et al., 2014).

WES studies of multiplex families may be fruitful for identifying rare variations with notable effects on the risk of developing common diseases (Cirulli and Goldstein, 2010). A WES study of 14 multiplex families with late-onset Alzheimer's disease identified a rare missense variation (V232M) in the phospholipase D3 (*PLD3*) gene that segregated with disease status in two independent families, and a follow-up study showed that *PLD3* V232M was associated with the disease in seven independent case-control populations (4998 patients and 6356 controls; Cruchaga et al.

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(2014)). Timms et al. (2013) carried out a WES study in five multiplex families with schizophrenia and identified 22 rare inherited protein-altering (missense, nonsense, frameshift, and disrupted splicing) variations. All affected individuals in each family carried at least one of these variations.

To further investigate the role of rare inherited variations in the genetic etiology of schizophrenia, we performed WES in three families, each with two affected siblings. To determine if rare truncating variations identified in the three families by WES are associated with increased risk for schizophrenia, we performed a three-stage follow-up study in a Japanese population with a total of 2617 patients and 2396 controls. We chose a multi-stage follow-up design to enable the efficient detection of associations (Yang and Thomas, 2011; Jin et al., 2015).

2. Materials and methods

2.1. Participants

This study was approved by the Ethics Committee of each participating institute, and written informed consent was obtained from all participants. All participants were of Japanese descent.

We included three families, each with two schizophrenia siblings, in a WES study. In family #1 (Fig. 1A), the proband (II-2) was diagnosed with schizophrenia. Her brother (II-3) also had schizophrenia and moderate mental retardation (MR) with a full-scale intelligence quotient (IQ) of 54. Their parents (I-1 and I-2) and sister (II-1) were not diagnosed with any psychiatric disorder. In family #2 (Fig. 1B), the proband (II-1) and his brother (II-3) were diagnosed with schizophrenia. Their brother (II-2), of unknown disease status, committed suicide. Their parents (I-1 and I-2) were not diagnosed with any psychiatric disorder. In family #3 (Fig. 1C), the proband (II-2) was diagnosed with schizophrenia. Her sister (II-3) also had schizophrenia and mild MR with a full-scale IQ of 60. Her brother (II-1) had epilepsy and was suspected of having MR, although IQ data were unavailable. Their father (I-1) was not diagnosed with any psychiatric disorder, whereas their mother (I-2) was suspected of having bipolar disorder. Participants were diagnosed according to Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV) criteria, as previously described (Arinami et al., 2005).

Three independent case-control populations comprising a total of 2617 patients and 2396 controls were used for the follow-up study. The first-stage population comprised 674 patients with schizophrenia (361 men and 313 women; mean age 39.9 ± 13.8 years) and 667 control individuals (341 men and 326 women; mean age 38.3 ± 10.8 years), who were recruited from Niigata University (Watanabe et al., 2006). The second-stage population comprised 731 patients with schizophrenia (377 men and 354 women; mean age 53.0 ± 14.9 years) and 777 control individuals (364 men and 413 women; mean age 53.9 ± 18.5 years), who were recruited from Kobe University (Okazaki et al., 2014). The third-stage population comprised 1212 patients with schizophrenia (627 men and 585 women; mean age 46.0 ± 14.8 years) and 952 control individuals (485 men and 467 women; mean age 42.1 ± 12.9 years), who were recruited from Fujita Health University (Ikeda et al., 2011, 2013). Psychiatric assessment of each participant was conducted, as previously described (Watanabe et al., 2006; Ikeda et al., 2011, 2013; Okazaki et al., 2014). In brief, the patients were diagnosed according to DSM-IV criteria by at least two experienced psychiatrists. Controls were mentally healthy individuals, with no personal or family history (within first-degree relatives) of psychiatric disorders.

2.2. WES study

WES was performed on 12 individuals, including from family #1 (I-1, I-2, II-2, and II-3; Fig. 1A), family #2 (I-1, I-2, II-1, and II-3; Fig. 1B) and family #3 (I-1, I-2, II-2, and II-3; Fig. 1C), as previously described (Egawa et al., 2015a, 2015b; Inoue et al., 2015). In brief, exome libraries were prepared using the SureSelect Human All Exon V4 or V5 Kit (Agilent, Santa Clara, CA, USA) and sequenced using the HiSeq2000 system (Illumina, San Diego, CA, USA). Sequencing was performed in one lane of the HiSeq2000 using the paired-end module for 100-bp reads. Paired-end sequence reads were mapped against the reference human genome (UCSC hg19) using the Burrows–Wheeler Aligner v0.5.9 (http://bio-bwa.sourceforge.net/). Polymerase chain reaction duplicates were removed using Picard v1.78 (http://picard.sourceforge.net/). Variation calling was performed using SAMtool v0.1.18 (http://samtools.sourceforge.net/). Variations were annotated using SnpEff v3.0f (http://snpeff.sourceforge.net/).

To prioritize variations, we applied several filtering steps. First, we filtered out variations with less than 10 × coverage. Second, we included variations shared by the two affected siblings in each family. Third, we included variations inherited in a dominant or recessive manner. Fourth, we included truncating variations such as nonsense and frameshift variations. Fifth, we included variations with mutant allele frequency < 0.01 in the Human Genetic Variation Database v1.41 (http://www.genome.med.kyoto-u.ac.jp/SnpDB/) or in Japanese data from the 1000 Genome

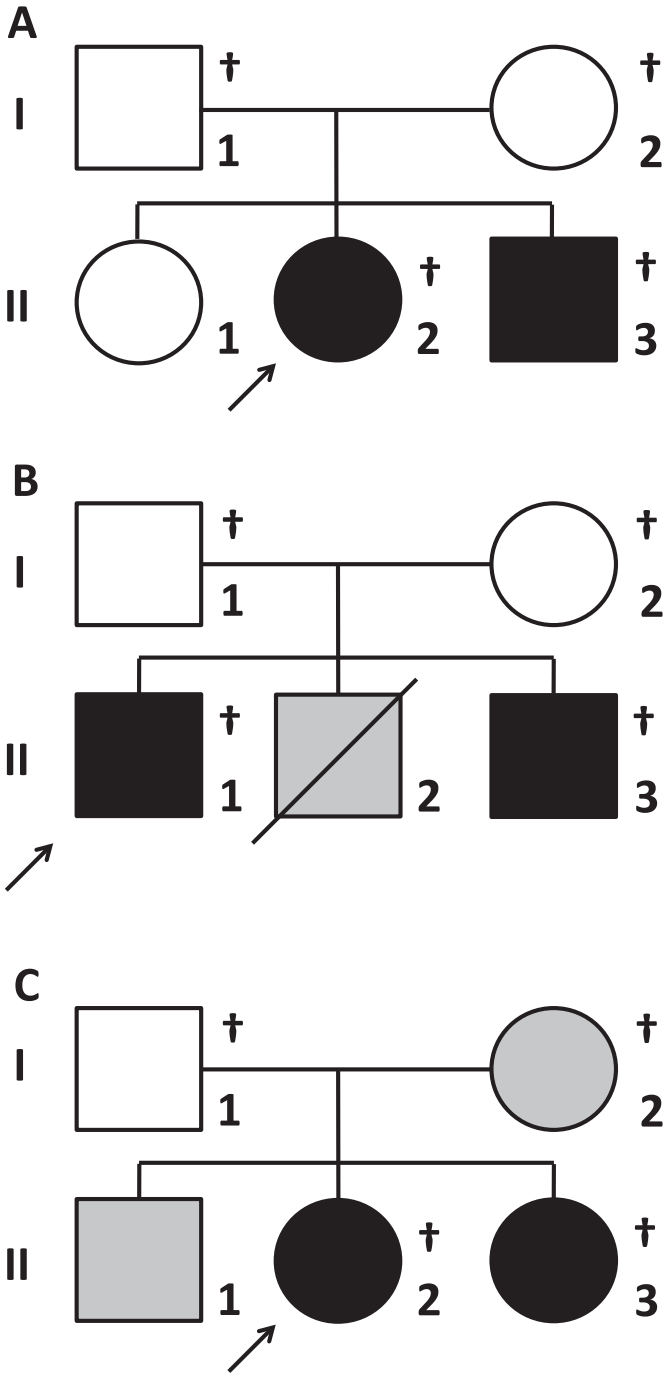

Fig. 1. Pedigrees of three families, each with two schizophrenia siblings. (A) Family #1. The proband (II-2) was diagnosed with schizophrenia. Her brother (II-3) had schizophrenia and moderate mental retardation (MR) with a full-scale intelligence quotient (IQ) of 54. (B) Family #2. The proband (II-1) and his brother (II-3) were diagnosed with schizophrenia. Their brother (II-2), of unknown disease status, committed suicide. (C) Family #3. The proband (II-2) was diagnosed with schizophrenia. Her sister (II-3) had schizophrenia and mild MR with a full-scale IQ of 60. Her brother (II-1) had epilepsy and was suspected of having MR, although data on IQ were unavailable. Their mother (I-2) was suspected of having bipolar disorder. Arrows indicate probands. Black shaded and unshaded symbols indicate affected and unaffected individuals, respectively. Gray shaded symbols indicate individuals of unknown disease status or suspected of having neuropsychiatric disorders. Squares and circles represent males and females, respectively. A diagonal line through a symbol indicates a deceased individual. Crosses represent individuals from whom genomic DNA samples were available.