Short Report

Identification and molecular characterization of two novel chromosomal deletions associated with autism


Autism is a childhood-onset neurodevelopmental disorder with a strong genetic basis in its etiology. Conventional karyotype analysis has revealed that chromosomal structural aberrations such as translocation, inversion, deletion, and duplication play a role in causing autism spectrum disorders (ASD). In addition, recent array-based comparative genomic hybridization (array CGH) studies discovered that submicroscopic deletion and duplication of DNA segments also contributed significantly to the genetic etiology of ASD. Together, these studies indicate that genomic rearrangement is an important genetic mechanism of ASD. Using karyotyping analysis and array CGH technology, we identified a subtelomeric deletion of approximately 6.8 Mb at 4q35.1-35.2 and a terminal deletion of approximately 2.4 Mb at 8p23.2-pter in two autistic boys, respectively. These two deletions were further validated using fluorescent in situ hybridization and real-time quantitative polymerase chain reaction, and their breakpoints were delineated using high-resolution array CGH. The 4q deletion is a rare de novo mutation, while the transmission of 8p deletion is unknown, because the father of the patient was unavailable for study. These two deletions are rare mutations and were not found in the additional 282 patients with ASD and in the 300 control subjects in our population. The identification of these two chromosomal deletions contribute to our understanding of the genetic basis of ASD, and the haploinsufficiency of several genes located at the deleted regions of chromosome 8p and 4q may contribute to the clinical phenotypes of autism.

Key words: array-based comparative genome hybridization – autism – copy number variation – deletion

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Autism is a childhood-onset neurodevelopmental disorder characterized by the impairment of reciprocal social interaction, deficit in verbal communication ability, and the presence of restricted interest and stereotyped behavior. The prevalence of autism spectrum disorders (ASD) in the general population is approximately six per thousand, and males are more often affected than females (1, 2). The genetic mechanism underlying the ASD is heterogeneous and complex, and several approaches have been used to investigate the genetic underpinnings of ASD, such as karyotype analysis, linkage analysis, candidate gene analysis, and genome-wide association study (3, 4).

Conventional karyotype analyses of ASD show that approximately 5–12% of ASD patients are associated with chromosomal structural aberrations such as translocation, inversion, deletion, and duplication (5–8), suggesting genomic rearrangement plays an important role in the genetic mechanism of ASD (9, 10). Conventional karyotype method, however, has limited resolution of 5–10 Mb in detecting chromosomal deletion and duplication. The recent advent of the array-based comparative genomic hybridization (array CGH) technology has greatly enhanced the detection rate of submicroscopic deletion and duplication, collectively known as copy number variations (CNVs) (11). As a result, several research groups recently discovered that submicroscopic CNV also played a significant role in the genetic etiology of ASD. The prevalence of submicroscopic de novo CNV in ASD varies from 7% to 28% in different studies (12–18). Together, these data indicate that genomic rearrangement is an important genetic mechanism of ASD, and the prevalence of chromosomal structural aberrations in ASD was estimated up to 10–20% when combining the conventional karyotype and array CGH analysis (10).

As part of a series of genetic studies of autism, we conducted karyotyping and array CGH analysis in patients consecutively enrolled in our study. Here, we report the identification of two chromosomal deletions in two patients.

Materials and methods

Subjects

We recruited patients, aged 3–17, who met the diagnostic criteria of autistic disorder defined by the Diagnostic and Statistical Manual of Mental Disorders-IV from the Department of Psychiatry, Chang Gung Memorial Hospital, Kwei-Shan, Taiwan, and the Children Mental Health Center, Department of Psychiatry, National Taiwan University Hospital, Taipei, Taiwan. The clinical diagnosis of autism was confirmed using the Chinese version of the Autism Diagnostic Interview-Revised (ADI-R) by qualified child psychiatrists. The study protocol was approved by the Research Ethics Committee of both hospitals, and written informed consents were obtained from the parents of patients and normal individuals after the procedures were fully explained.

Cytogenetic investigation and fluorescent in situ hybridization

Cytogenetic analysis was performed on peripheral blood lymphocytes using GTW-banding method according to the standard protocol. Fluorescent in situ hybridization (FISH) analysis was also performed on the lymphocytes from the subject according to the standard protocol. Briefly, for 4q deletion analysis, BAC clones of RP11-33M11 at 4q35.2 and RP11-69L7 at 4p16.3 (as an internal control) were grown and BAC DNA was isolated using a Qiagen plasmid isolation kit (Qiagen Inc., Valencia, CA). The DNA of RP11-33M11 and RP11-69L7 were labeled by nick translation with d-UTP-FITC and d-UTP-Texas Red, respectively. After hybridization in a humidified chamber at 37°C for at least 16 h, the slides were washed and counterstained with 4′,6-diamidino-2-phenylindole (Kirkegaard & Perry Laboratories Inc., Gaithersburg, MD). Cells were observed under the Zeiss fluorescence microscope (Carl Zeiss, Goettingen, Germany) and images were captured and analyzed using the MetaSystems ISIS workstation (MetaSystems, Altlussheim).

Array-based CGH

Oligonucleotide-based 385K whole-genome CGH arrays (with a median interprobe spacing of 6270
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Real-time quantitative polymerase chain reaction

Real-time quantitative polymerase chain reaction (RT-qPCR) was performed using the SYBR Green method and implemented in the ABI Prism 7900HT Sequence Detection System, following the manufacturer’s protocol (Applied Biosystems, Foster City, CA). A comparative ddCt method was used to validate the CNV in this study. For quantification of the 4q deletion, fragments of MLF1 interacting protein gene (MLF1IP, GeneID: 79682) at 4q35.1 and brain-specific angiogenesis inhibitor 3 (BAI3, GeneID: 577) at 6q12 were PCR amplified from the genomic DNA of each individual. The ddCt of each subject was first obtained by subtracting the Ct of MLF1IP by the Ct of BAI3 of his own DNA, and then by dCt of a normal control subject. The relative fold change to the normal subject was determined as $2^{-\Delta\Delta Ct}$. Similarly, for quantification of the 8p deletion, fragments of discs, large (Drosophila) homolog-associated protein 2 gene (DLGAP2, GeneID: 9228) at 8p23 and metaxin 2 (MTX2, GeneID: 10651) at 2q31.1 were PCR amplified from the genomic DNA of each individual. The ddCt of each subject was first obtained by subtracting the Ct of DLGAP2 by the Ct of MTX2 of his own DNA, and then by dCt of a normal control subject. The relative fold change to the normal subject was determined as $2^{-\Delta\Delta Ct}$. The PCR was carried out in triplicates. The primer sequences, optimal annealing temperature and the size of amplicon are listed in the supporting information Table S1.

Sequencing of the CLN8 gene

The CLN8 gene contains three exons; the exons 2 and 3 are protein-coding exons. Two sets of primer pairs were used to PCR amplify the exon 2 (5′-TTGGCGTACTGAGGTAATGA-3′ and 5′-ATCCAGCCTGCTGTAGT-3′) and exon 3 (5′-TTTTGTGTTGGAATAGTATGCA-3′ and 5′-AATTCAAAAGCCATCATTCCG-3′) of the CLN8 gene. After PCR amplification, aliquots of PCR products were processed using a PCR Pre-Sequencing Kit (USB Corp., Cleveland, OH), and then subjected to direct sequencing using an ABI PRISM® BigDye® Terminator Cycle Sequencing Ready Reaction Kit Version 3.1, and an ABI autosequencer 3730 (Perkin Elmer Applied Biosystems), according to the manufacturer’s protocol.

Results

In a total of 44 consecutive patients with autism (40 boys and 4 girls; mean age: 8.0 ± 3.8 years; range: 3–17 years) receiving karyotyping and whole-genome CNV analysis, we identified a subtelomeric deletion at 4q35 and a terminal deletion at 8p in two unrelated patients, respectively.

Patient with 4q35 deletion

The 4q deletion was detected in an 8-year-old boy, who was the only child of unrelated healthy parents (Fig. 1a). Karyotype analysis of the proband showed a terminal deletion at chromosome 4q35 (Fig. 1b), which was not found in his parents, indicating that the 4q35 deletion is a de novo mutation. Whole-genome CGH analysis showed a subtelomeric deletion of approximately 6.8 Mb at chromosome 4q in this patient...
Fig. 1. Genetic analysis of the patient with a 4q35.1-35.2 deletion. (a) The 4q deletion is a de novo mutation occurring in the only child of a family. (b) Karyotype analysis revealed a 4q terminal deletion as indicated by the arrow. The locations of the probes for the fluorescent in situ hybridization (FISH) are also indicated on the ideogram. (c) Whole-genome array-based comparative genomic hybridization analysis showed subtelomeric deletion of approximately 6.8 Mb at 4q. (d) FISH analysis showed an absence of the probe RP11-33M11 (green spots) located at 4q35 in the deleted chromosome of the patient as indicated by the arrow; the red spots are the control probe RP11-69L7 located at 4p terminus. (e) The deletion was also verified by real-time quantitative polymerase chain reaction. The amount of MLF1IP gene at the deleted region in the proband is about half of that in his parents and three other control subjects.
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The chromosome 4q35.2 is prone to genomic rearrangement, and the 4q deletion syndrome is usually associated with a variety of clinical findings.

Discussion

The chromosome 4q35.2 is prone to genomic rearrangement, and the 4q deletion syndrome is usually associated with a variety of clinical findings.
Fig. 2. Genetic analysis of the patient with an 8p23.2-pter deletion. (a) The 8p terminal deletion was detected in the second child who was diagnosed with autism. (b) Whole-genome array-based comparative genomic hybridization analysis showed a terminal deletion of approximately 2.4 Mb at 8p. (c) Fluorescent in situ hybridization analysis showed an absence of the probe 338B22 (green spots) located at 8p23 in the deleted chromosome of the patient as indicated by the arrow; the red spots are the control probe 119A16 located at 8q terminus. (d) The deletion was also verified by real-time quantitative polymerase chain reaction. The amount of DLGAP2 gene at the deleted region in the proband of the patient is approximately half of that in his mother, brother, and three other control subjects.

including growth and mental retardation, physical abnormalities such as craniofacial, digital, skeletal, and cardiac abnormalities (20, 21). The phenotype variability is dependent on the location and size of the deleted region and other factors. The array CGH analysis would help define the genotype–phenotype correlations of 4q deletion syndrome at molecular level (21). The contraction of a polymorphic tandem array of 3.3 Kb repeats (D4Z4) at this region is associated with facioscapulohumeral muscular dystrophy (FSHD) (22). Patients with FSHD can present cognitive dysfunction in addition to their neuromuscular symptoms. The distal breakpoint of the 4q deletion in this patient
was located approximately 100 Kb centromeric to the location of D4Z4; hence, the D4Z4 was not deleted in this patient. There are 59 RefSeq transcripts mapped to the 4q deleted region in this patient according to the Map Viewer of National Center for Biotechnology Information (NCBI; http://www.ncbi.nlm.nih.gov) (Build 37.1 2010), and notably, three genes have been reported to be associated with psychiatric disorders. The MTNR1 gene was believed to participate in the pathophysiology of stress response and depression (23); the leucine-to-proline mutation at codon 98 of the SLC25A4 gene was reported to segregate with bipolar affective disorders in an Italian family (24); and the FAT1 gene has been reported to be associated with bipolar affective disorders in several populations (25). The haploinsufficiency of several other genes in this region that are expressed in the brain, such as ODZ3, CDKN2IP, ING2, RWDD4A, STOX2, ENPP6, CASP3, ACSL1, SNX25, LRP2B, and SORBS2, is also likely to contribute to the clinical phenotypes of this patient.

One study reported a 3 Mb deletion at this region in a mild mental retardation patient comorbid with schizoaffective disorder (26). The deleted region in this patient was located telomeric to the deleted region in our patient and also encompassed the FAT1 gene. Another study reported a female patient with childhood autism and high-grade myopia who had an apparently balanced de novo translocation, t(5;18)(q34;q12.2). Further CGH analyses revealed a 3.2 Mb deletion at the 18q breakpoint and an additional deletion of 1.27 Mb on chromosome 4q35 (27). The 4q35 deleted region of this patient was located within the deleted region in our patient that contained MTNR1A and FAT1 genes. Taken together, these results indicate that the FAT1 gene might be an interesting gene associated with autism.

The terminal region of chromosome 8p is also vulnerable to genomic rearrangements (28). Deletions involving the 8p23 to terminal region are associated with a spectrum of anomalies including congenital heart malformations, congenital diaphragmatic hernia, developmental delay, and neuropsychiatric disorders (29). The clinical phenotypes of the 8p terminal deletion are also determined by the size and location of the deleted region and other factors. Array CGH would help define the genotype–phenotype correlations at molecular level. The GATA4 gene is a critical gene that was suggested to be associated with congenital heart disease and diaphragm hernia in patients with 8p terminal deletion (29). The GATA4 gene was not deleted in our patient, which can explain the absence of these abnormalities in our patient.

There are 23 RefSeq transcripts mapped to the 8p deleted region according to the Map Viewer of NCBI (http://www.ncbi.nlm.nih.gov) (Build 37.1 2010), and at least three of them, such as DLGAP2, CLN8, and ARHGEF10, might be associated with the clinical phenotypes of this patient. Marshall et al. reported a duplication with breakpoints intersecting the DLGAP2 gene in an autistic patient (14), while Ozgen et al. also reported a classical inv dup del(8p) in a case of female autism where the DLGAP2 gene was located within the 6.9 Mb terminal deletion of this patient (30). These studies suggest that the DLGAP2 gene is an important candidate gene of autism.

Mutations in the CLN8 gene have been reported to cause a recessive disease of progressive epilepsy with mental retardation (EPMR, MIM600143). We sequenced two protein-coding exons of the CLN8 gene in this patient and did not identify any further mutation of the CLN8 gene. Thus, the patient is unlikely to be a case of EPMR. Nevertheless, the haploinsufficiency of the CLN8 gene may still likely to contribute to the clinical phenotypes of this patient.

The ARHGEF10 is one of the myelin-related genes. A missense mutation of the ARHGEF10 was found to be the contributory factor in a family with a dominant form of slowed nerve conduction velocities and thin myelination in the peripheral nerves (31). Recently, the ARHGEF10 gene was reported to be associated with schizophrenia in an association study of myelin-related genes and schizophrenia (32). Hence, it is conceivable to consider the ARHGEF10 gene as being associated with autism.

In summary, we identified two rare chromosomal deletions in a sample of Han Chinese ASD patients from Taiwan. Our findings not only expand the spectrum of the genetic defects associated with autism, but also suggest that the haploinsufficiency of several genes in the deleted regions may contribute to the clinical phenotypes of autism.

Supporting Information

The following Supporting information is available for this article:

Table S1. The gene symbol, gene ID, chromosome location, sequences of primer sets, optimal annealing temperature, and size of amplicon in real-time quantitative polymerase chain reaction experiments.

Additional Supporting information may be found in the online version of this article.

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Conflicts of interest

The authors declare no conflict of interest whatsoever in this work. Except for financial support, the funding sources play no role in the study design, collection, analysis and interpretation of data, writing of the report and in the decision to submit the report for publication.

References