A de novo Marker Chromosome derived from 9p in a Patient with Autism: Genotype-Phenotype Correlation

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Abstract

We describe a 17-year-old girl with autism, severe mental retardation, and epilepsy in whom GTG-banded chromosome analysis revealed a female karyotype with a marker chromosome in 69% of the analyzed metaphases. Array CGH analysis showed that the marker chromosome originated from 9p with a gain of 38.9 Mb. This 9p duplication was detected in mosaic form only in the proband and not in the parents, her unaffected siblings, or 258 ethnic controls. A careful analysis of the duplicated area identified a number of potentially important genes after excluding genes coding for hypothetical proteins, pseudogenes, or genes deleted or duplicated in individuals with normal phenotype. The current report describes a mosaic duplication that is novel because it includes almost the entire chromosome 9p and because it is associated with autistic features in this patient. We believe that the degree of mosaicism (69%) implies that the increase in gene dosage on 9p is likely a factor contributing to autistic features.

KEYWORDS: 9p duplication; array CGH; autism; marker chromosome
Background

Autism spectrum disorders (ASD), including autism, are neuro-developmental disorders characterized by impairment in social and communication skills together with stereotyped and repetitive behavior and/or a restricted range of interests. Current prevalence estimates in the United States are 0.1-0.2% of live births for autism and 0.6% for ASD [1]. The exact prevalence of ASD in Saudi Arabia is still undetermined, but one rough estimate is that the prevalence of autism in Saudi Arabia is 18 per 10,000, slightly higher than the 13 per 10,000 reported in developed countries [2].

Previous studies focusing on candidate genes and chromosomal regions have identified several copy number variations (CNVs) associated with increased risk of ASD [3-9]. Chromosomal regions implicated by these studies include 1q, 1p, 5q, 7q, 15q, 16p, 17p, 20p, 3p, 10q, 15q, 20p, 22q and Xq. Autistic patients with CNVs show variable expressivity with associated phenotypes such as schizophrenia, mental retardation, developmental delay, and epilepsy [10]. We describe a 17-year-old girl with autism, severe mental retardation, and epilepsy who has a marker chromosome derived from 9p.

Patient and Methods

Patient

The proband (II-3, Fig. 1A) was first examined at age 15 months because of seizures and developmental delay and has been followed since then by MAS and MZS. She has been institutionalized for most of her life. Her parents were unrelated Saudi Arabs, but the family history includes epilepsy and cerebral palsy in maternal cousins. Pregnancy was unremarkable, delivery was normal at term, and she had no neonatal problems.

At the age of 10 months, she developed flexion myoclonic jerks with hypsarrhythmia on EEG typical of infantile spasms, and she was treated with a course of prednisone (2
mg/kg) and clonazepam. A repeat EEG was moderately abnormal with multifocal epileptic discharges consistent with secondary symptomatic epilepsy, and she was started on the anticonvulsants vigabatrin and clonazepam and on risperidone for autistic behavior. Vigabatrin was successfully withdrawn and replaced by lamotrigine (lamictal) with no seizures after the age of 3 ½ years. She was delayed in motor and cognitive functions, sitting at 13 months and walking at 2 years. She never developed speech, and she was diagnosed as severely mentally retarded with autistic features at age 6 years with a Vineland social maturity scale score of 31. She was unable to feed herself and was incontinent of both stool and urine.

Laboratory investigation revealed normal hematologic indices, liver function, and electrolytes. Tandem mass spectrometry (TMS) for metabolic disorders was unremarkable. MRI of the brain and brainstem auditory evoked responses were both normal.

On examination, she had growth retardation with reduced weight (28.3 kg; <2 SD below the mean), height (137 cm; < 2 SD), and head circumference (49 cm; < 3 SD). She had subtle dysmorphic features in the form of deep set eyes, small upper lip, webbing of the neck (Fig. 1B) and clinodactyly (Fig. 1C). X-rays of hands and wrists revealed normal bone age with osteoporosis and confirmed clinodactyly of the fifth finger (Fig. 1D). She had no skin hypopigmentation or other stigmata of neurocutaneous disorders, but the dorsum of the left hand and the bridge of her nose were hyperpigmented because of self injury.

On neurologic examination, she was awake and alert but unable to follow commands or speak other than some echolalia and repetitive non-specific sounds. She moved all four extremities with normal tone; reflexes were brisk, but toes were down going. Ophthalmologic exam and ocular motility were grossly normal, but she did not make eye contact. She responded appropriately to visual threat and loud sounds. She was generally quiet and motionless, typically leaning to one side in an awkward posture,
making stereotyped movements of her hands and trunk and frequently hitting her nose with her hand and grinding her teeth. Social interaction was minimal with no smile, response to commands, or copying of examiner’s movements. Applying DSM4-R criteria and PDD assessment scale/screening questionnaire, a psychiatric assessment yielded the diagnoses of (Axis I) pervasive developmental disorder (autistic type) and (Axis II) severe mental retardation.

**Genetic Analysis**

Conventional cytogenetic analysis on GTG-banded chromosomes was performed according to the standard technique on cultured lymphocytes from the father (I-1), the mother (I-2), the proband (II-3), and her unaffected brother (II-1) after obtaining informed consent. Agilent human CGH microarrays (Agilent Technologies, Santa Clara, CA, USA) were employed for array CGH experiments using chips containing unique oligonucleotides for 244,000 probes (244K) with average probe spacing across the human genome of 6.4 Kb. Labeling reactions were performed with 1 µg genomic DNA with Agilent Genomic DNA Labeling Kit PLUS (Agilent Technologies) according to manufacturer’s protocol, and the microarray chip was then scanned by the Agilent Microarray Scanner. Data analysis was performed by Agilent Feature Extraction 9.1 and CGH Analytics 3.4. In brief, log2 expression ratios were computed and normalized for forward and reverse fluor (i.e. dye-swap) experiments using the CGH Analytics 3.4 software. Putative chromosome copy number changes were defined by intervals of three or more adjacent probes with log2 ratios suggestive of a deletion or duplication when compared with the log2 ratios of adjacent probes. The quality-weighted interval score algorithm (ADM2) was used to compute and assist in the identification of aberrations for a given sample. Controls were 258 normal individuals of similar ethnic background.
Results

Conventional cytogenetic analysis on GTG-banded chromosomes revealed a female karyotype in the proband with a marker chromosome (Fig. 2A) in 69% of the analyzed metaphases after counting more than 150 metaphase spreads; thus, her karyotype was 47,XX,+mar[69]/46,XX[31]. The marker chromosome was relatively small, with unknown source, and was seen in the proband but not in her parents or unaffected siblings, all of whom had a normal karyotype.

Oligonucleotide whole genome array CGH analysis showed that the marker chromosome originated from 9p. The duplicated area started from the region surrounding the CBWD1 locus in 9p24.3 → 9p13.1 (from 153131 bp to 39131894 bp on chromosome 9) with a size of 38.9 Mb (Fig. 2B). This gain of chromosome 9p was detected in mosaic form only in the proband and not in parents, unaffected siblings (Fig. 2C), or controls. Assessment of array-CGH results utilizing the NCBI Map Viewer revealed that the duplicated area of 9p encompassed 381 genes (see Online Table).

Discussion

We describe a 17-year-old Saudi girl with autism, severe mental retardation, and epilepsy who had a marker chromosome derived from 9p. To our knowledge, this is the first report of an autistic patient with an isolated mosaic de novo 9p duplication. By analyzing the genes encompassed in the duplicated area of 9p, we found that 97 genes could be excluded because they were reported in the database of genomic variants (http://projects.tcag.ca/variation/) to be deleted or duplicated in individuals with normal phenotypes. We also excluded 38 genes coding for hypothetical proteins, 135 pseudogenes, and 21 genes coding for chromosome 9 open reading frames. The remaining 90 genes, which might be relevant to the phenotype of the proband, primarily coded for proteins related to spermatogenesis, oogenesis, apoptosis, cell
communication, phospholipids metabolism, purine metabolism, cation transport, and cell cycle control.

Several genes in the duplicated area of our patient have been associated previously with autism or ASD. The very low density lipoprotein receptor gene (VLDLR) belongs to a well-known family of similar genes that have the ability to transduce a diversity of extracellular signals across neuronal membranes in the adult central nervous system. Their role in modulating synaptic plasticity and their necessity in hippocampus-specific learning and memory are well documented [11]. Genetic defects in trans-membrane protein 215 (TMEM215) and its related family of trans-membrane proteins involved in calcium channeling can affect a wide variety of mammalian developmental, physiological, and behavioral functions [12]. Similarly, a number of interferon genes were detected in the duplicated area of our patient (IFNA2, IFNA14, IFNA6, IFNA1 and IFNA8). The role of interferons as mediators of apoptosis is well documented [13], and recent studies suggest that apoptotic mechanisms may partially contribute to the pathogenesis of autism [14-16]. Finally, CNVs in ASD patients have been reported in areas surrounding genes involved in the ubiquitin pathway [8]. Among these were the ubiquitin associated protein 1 (UBAP1) gene, which is located within the duplicated area detected in our patient.

The first patient with trisomy 9p was described by Rethore et al. in 1970 [17], and almost 151 patients with partial or complete 9p trisomy have been reported since then [18]. In most patients, the 9p trisomic segment was derived from a parent carrying a reciprocal balanced translocation and was accompanied by a concurrent deletion of another chromosome. Isolated de novo duplications of 9p without a concurrent deletion are infrequent, with fewer than 15 cases reported to date. Typical characteristics include growth and mental retardation, microbrachycephaly, deep and wide-set eyes with down-slanting palpebral fissures, prominent nasal root with a bulbous nasal tip, down-turned corners of the mouth, low-set ears, short fingers and toes with hypoplastic nails, and delayed bone age, some features of which were present in our patient. Clinodactyly
was also present in our patient and has been reported in patients with trisomy 9p and insertion on chromosome 12 [19].

The proband had a relatively typical presentation of severe autism associated with mental retardation, mild dysmorphism, and seizures despite a non-focal neurologic examination and unremarkable neuroimaging [20]. Autism has not been associated previously with isolated 9p duplications [18], but this patient had a unique, large, mosaic isolated 9p duplication that is likely symptomatic given that the duplication was *de novo*, that it segregated with the phenotype in this family, that it was not a polymorphic variant, and that some genes in the duplicated region can be linked to autistic features. We believe that the degree of the mosaicism (69%) implies that the increase in gene dosage on 9p is likely a factor contributing to the patient’s autistic features.

**Conclusion**

Array CGH is a powerful tool which may be used to screen autistic children for possible chromosomal abnormalities. Screening of chromosome 9p for copy number alterations may give further insight into the role of specific genes involved in autism.

**Competing interests**

The author(s) declare that they have no competing interests.

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Figure Legends

**Figure 1**
A) Family pedigree identifying the proband as II-3. Dysmorphic features found in the proband, including B) webbing of the neck and C) fifth finger clinodactyly. D) X-ray of hands and wrists revealed evidence of osteoporosis and clinodactyly.

**Figure 2**
A) GTG-banded karyotype showing the marker chromosome (circled). B) Array CGH demonstrating a chromosome 9p duplicated region in the proband (duplicated area is boxed). C) Normal array CGH in healthy siblings (II-2 and II-4), parents and controls. Array CGH for individual II-2 is shown here.
Additional files provided with this submission:

Additional file 1: Online Table.xls, 116K
http://www.biomedcentral.com/imedia/711727713827925/supp1.xls