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Clinical research

Duplication of 8q12 encompassing *CHD7* is associated with a distinct phenotype but without duane anomaly

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ABSTRACT

Interstitial duplications of 8q12 encompassing *CHD7* have recently been described as a new microduplication syndrome. Three 8q12 duplications have been reported with shared recognizable phenotype: Duane anomaly, developmental delay and dysmorphic facial features. We identified a 2.7 Mb duplication on chromosome 8q12 with SNP-array in a patient with growth delay, congenital heart defects, ear anomalies and torticollis. To our knowledge, this is the smallest duplication reported to date. Our findings support the notion that increased copy number of *CHD7* may underlie the phenotype of the 8q12 duplication. Our study together with previous studies suggest that the 8q12 duplication could be defined as a novel syndrome.

1. Introduction

Haploinsufficiency of the *CHD7* gene, either by microdeletions containing *CHD7* or mutations within this gene, have been identified as the molecular basis of CHARGE syndrome [1,2]. CHARGE syndrome (OMIM# 214800) is a highly variable syndrome with multiple congenital malformations characterized by ocular coloboma, heart defects, atresia of the choanse, retardation of growth and/or development, genital anomalies and ear anomalies [1,3–5]. CHARGE syndrome has a prevalence of one per 10,000 newborns [6]. In contrast, duplications of 8q12 encompassing *CHD7* have been rarely reported: to date, only three 8q12 duplications have been reported.

In 2008, Monfort et al. reported a 3 Mb duplication of 8q12 in a boy with moderate mental retardation, Duane syndrome and congenital heart defects (CHD, ventricular septal defect and pulmonary stenosis) [7]. In 2009, Lehman et al. described a 6.9 Mb duplication in a 4-year-old girl with multiple congenital malformations, which included Duane syndrome, developmental delay, external ear malformation with deafness and CHD (atrial and ventricular septal defects) [8]. Amouroux et al. recently reported a 3.9 Mb duplication in a patient with Duane retraction syndrome and developmental delay, but without CHD and deafness/external ear malformation [9].

High-resolution Single Nucleotide Polymorphism (SNP) arrays and oligo arrays have made the detection of submicroscopic chromosome rearrangements (smaller than 5 Mb in size) possible [10,11]. We have identified pathogenic CNVs that are associated with cardiac defects in a cohort of syndromic CHD patients [12,13]. Here, we present a detailed study of a patient who carries a de novo 2.7 Mb duplication on chromosome 8q12 with a congenital heart defect, ear anomalies and torticollis.

2. Methods and detection

The Review Board of the Second Xiangya Hospital of the Central South University approved this study. All subjects consented to this study.

2.1. Cytogenetic analysis

Chromosome analysis was performed on peripheral blood of the patient and her parents by conventional G-banded techniques (550

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bands resolution). 5 ml peripheral blood was collected for each individual. All samples were subjected to lymphocyte culture according to standard cytogenetic protocol.

2.2. DNA extraction

The genomic DNA was prepared from peripheral blood of the patient and her parents. Genomic DNA was prepared using a DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA) on the QIAcube automated DNA extraction robot (Qiagen, Hiden, Germany).

2.3. Mutation analysis

Our patient showed some phenotypic features that also occur in CHARGE syndrome (CHD and ear anomaly). Therefore, the flanking intronic sequences of *CHD7* (Ref seq.: NM_017780) were amplified with polymerase chain reaction (PCR). Sequences of the PCR products were determined using the ABI 3100 Genetic Analyzer (ABI, Foster City, CA) as previously reported [5].

2.4. SNP-array analysis

Genomic DNA samples of the patient and her parents were adjusted to a final concentration (50 ng/μl). The HumanOmni1-Quad Chip (Illumina Inc., San Diego, USA) and the Illumina BeadScan genotyping system (Beadstation Scanner) were employed to obtain the signal intensities of SNP probes. HumanOmni1-Quad Beadchip contains over 1.1 million loci across the human genome, including markers derived from the three HapMap, the 1000 Genomes Project and recently published studies [11]. The GenomeStudio V2011 software was used to analyze the genotypes (human genome build 37/Hg19 for analysis) and evaluate the experimental quality. The call rates of the samples are greater than 99.0%.

2.5. Clinical description

The proband, a two-year-old female patient from Central South China, was born at term with a birth weight of 2550 g (<3rd centile), and a length of 40 cm (<3rd centile). She was the only child of non-consanguineous parents. At her birth, her mother was 26 years old and her father was 31 years old, respectively. A pedigree analysis showed no family history of birth defects. She had feeding difficulties, right ear malformation and a heart murmur. Subsequently, a congenital heart defect (tetralogy of Fallot, TOF) was identified by cardiac ultrasound. At the age of two years, the patient attended our Department of Cardiothoracic Surgery for a cardiac procedure. At that time, the patient had moderate growth retardation, she weighted 9000 g (<3rd percentile) with a height of 80 cm (<3rd). She had a round face, upslanting palpebral fissures, deep set eyes, sparse eyebrows and torticollis/congenital wryneck (involved the right side). (Fig. 1). A cranial CT scan excluded malformations in the temporal bone. Ophthalmological investigation excluded the possibility of Duane retraction syndrome (DRS, also known as Duane anomaly). ENT (ear, nose, and throat) investigation revealed normal hearing. She also had normal psychomotor development and there were no additional malformations.

3. Results

The karyotypes of the patient and her parents were normal. Sequence analysis excluded a mutation in *CHD7* of the patient. To explore the presence of genomic imbalances, we employed SNP array system to analyze the whole genome for CNVs (copy number variations). A total of 343 CNVs were discovered. After exclusion of CNVs present in the Database of Genomic Variants (DGV), a de novo 2.7 Mb gain of chromosome 8q12 (Chr8: 60,792,079–63,540,593) was identified. This chromosome region contains about 17 annotated genes, including *CHD7*, *CA8*, *ASPH* (Fig. 2). The parents did not carry this duplication. The 1.6 Mb smallest overlapping region (SRO) was indicated (Fig. 2).

4. Discussion

The duplicated region of chromosome 8q12 in the patient reported in our study spans 2.7 Mb containing genes *CHD7*, *CA8* and others. To the best of our knowledge, this is probably the smallest 8q12 duplication reported to date.

The three previously reported patients shared some phenotypic features, including developmental delay, typical facial features, Duane retraction syndrome (DRS) (Table 1). DRS is defined by a congenital strabismus and divided into two types with abduction paralysis (type1) or adduction paralysis (type 2) [14, 15]. DRS is associated with or without developmental delay and other malformations. However, ophthalmological investigation excluded the possibility of DRS in our patient. Consistent with previous reports, our study suggests that the causative gene for DRS may be mapped outside the duplicated region of our patient. A Duane locus has been mapped to 8q13, about 4 Mb distal to the distal breakpoint of our patient's duplication [8,16].

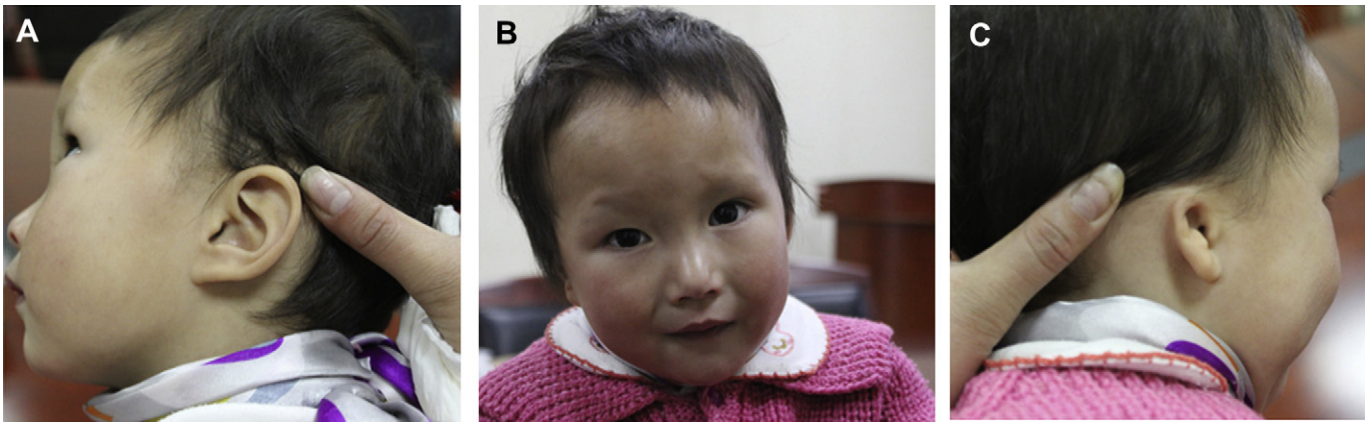

Fig. 1. Facial features of the proband, lateral (A, C) and frontal (B) view of the patient. B: the patient has a round face, upslanting palpebral fissures and telecanthus, deep set eyes, torticollis (involved the right side), A, C: the patient has a malformation of external right ear.