Published in final edited form as:

Am J Med Genet A. 2016 May ; 170A(5): 1148–1154. doi:10.1002/ajmg.a.37592.

Duplication 2p25 in a Child with Clinical Features of CHARGE Syndrome

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Abstract

CHARGE syndrome is a dominant disorder characterized by ocular colobomata, heart defects, choanal atresia, retardation of growth and development, genital hypoplasia, and ear abnormalities including deafness and vestibular disorders. The majority of individuals with CHARGE have pathogenic variants in the gene encoding CHD7, a chromatin remodeling protein. Here we present a 15-year-old girl with clinical features of CHARGE syndrome and a *de novo* 6.5 Mb gain of genomic material at 2p25.3-p25.2. The duplicated region contained 24 genes, including the early and broadly expressed transcription factor gene *SOX11*. Analysis of 28 other patients with CHARGE showed no *SOX11* copy number changes or pathogenic sequence variants. To our knowledge, this child's chromosomal abnormality is unique and represents the first co-occurrence of duplication 2p25 and clinical features of CHARGE syndrome. We compare our patient's phenotype to ten previously published patients with isolated terminal duplication 2p, and elaborate on the clinical diagnosis of CHARGE in the context of atypical genetic findings.

Keywords

duplication 2p25; CHARGE syndrome; trisomy 2p; gene duplication

INTRODUCTION

CHARGE syndrome is a multiple anomaly disorder that presents with ocular colobomata, heart defects, choanal atresia, growth retardation, genital anomalies, and ear malformations [Verloes 2005]. Heterozygous loss-of-function mutations in *CHD7*, an ATP-dependent

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

chromatin remodeling protein, are present in 70% of individuals with CHARGE [Visser et al., 2004]. In addition, chromosomal aberrations have been reported in association with CHARGE features [Lev et al., 2000; North et al., 1995; Wiczyński et al., 1997].

Duplication 2p25 syndrome is a rare and clinically heterogeneous disorder manifest by extra genetic material in variably sized portions of the short arm of chromosome 2. Common features include developmental delay, craniofacial dysmorphic features (including hypertelorism and prominent forehead), and cardiovascular malformations. Duplication 2p25 has thus far has been reported in only ten patients with an isolated aberration of the terminal region of 2p [Al-Saffar et al., 2000; Aviram-Goldring et al., 2000; Engels et al., 1999; Fineman et al., 1983; Ishikawa et al., 2002; Martinez-Juarez et al., 2014; Stoll et al., 1974; Wakita et al., 1985].

Here, we report a child with a complex duplication-triplication of 2p25 and clinical features of CHARGE syndrome. The aberrant region (2p25.3-p25.2) was 6.5 Mb in length, consisting of a duplication of the distal 3.0 Mb and a triplication of the proximal 3.5 Mb. We compare our patient's phenotype to ten previously published patients with isolated terminal duplication 2p, and elaborate on the diagnosis of CHARGE in the context of atypical genetic findings [Al-Saffar et al., 2000; Aviram-Goldring et al., 2000; Engels et al., 1999; Fineman et al., 1983; Ishikawa et al., 2002; Martinez-Juarez et al., 2014; Stoll et al., 1974; Wakita et al., 1985].

MATERIALS AND METHODS

Clinical evaluation

Clinical evaluation and photographs of the proband were performed by DMM with written consent from the patients' parents. This research was approved by the University of Michigan Medical School IRB.

Chromosomal microarray (CMA)

CMA was performed on genomic DNA extracted from peripheral blood lymphocytes using the Illumina CytoSNP-850K array platform (Illumina, San Diego, CA, USA). Patient DNA was isolated, amplified, and enzymatically fragmented. DNA fragments were hybridized to array probes and extended with tagged terminating nucleotides. Aberrations were confirmed by relative-quantitative PCR (Applied Biosystems, Carlsbad, CA, USA).

Fluorescence in situ hybridization (FISH)

FISH of interphase and metaphase chromosomes was performed using peripheral blood lymphocytes. Bacterial artificial chromosomes were used as probes for the regions of interest on chromosome 2 (RP11-168K7, RP11-36C8; BlueGnome, UK), chromosome 15 (RP11-475F15; BlueGnome) and subtelomeric regions of chromosomes 2 and 15 (Abbott Molecular, Abbott Park, IL, USA). Probes used for FISH are listed in Supplementary Table I.

Relative-Quantitative PCR (RQ-PCR) and SOX11 gene sequencing

SOX11 RQ-PCR was performed on 28 individuals with clinical features of CHARGE syndrome and negative *CHD7* sequencing (Hs00839199_cn, Applied Biosystems). PCR amplification of the *SOX11* coding DNA sequence was performed using the GC-Rich PCR system (Roche, Indianapolis, IN) followed by Sanger sequencing. Primers used for PCR amplification and sequencing are listed in Supplementary Table II.

RESULTS

Clinical report

Our patient was initially evaluated at 19 months of age in the University of Michigan Pediatrics Genetics clinic for multiple congenital anomalies. She was born at 42 weeks by induced vaginal delivery following an uneventful pregnancy with a birth weight of 3.3 kg (25–50th centile) and length of 51.4 cm (50–75th centile). Parents were healthy and non-consanguineous. Newborn hearing screening was abnormal and she was subsequently diagnosed with right-sided profound sensorineural hearing loss by brainstem auditory evoked response testing. The left ear tested within normal limits.

At 19 months, she exhibited generalized growth delay, including short stature (76 cm, <3rd centile), low weight (9.48 kg, <3rd centile), and microcephaly (45 cm, <3rd centile). She also exhibited developmental delay, and several craniofacial dysmorphic features (Figure 1A–C and Table I). She had a highly-arched palate, bifid uvula, and peg-shaped teeth.

Ophthalmologic exam revealed bilateral colobomata involving the iris, retina, and optic nerves with sensory nystagmus, variable angle esotropia, and ptosis. No corneal opacity, microcornea, lens opacification, or increased intraocular pressures were noted. She had bilateral preauricular pits and posteriorly rotated, simplified external ears (Figure 1D–E). Discontinuous palmar creases and dysmorphic features of the fingers (i.e. hypoplasia of the left thumb and arachnodactyly) and toes (i.e. camptodactyly) with persistent fetal fingertip and toe pads were noted (Figure 1F–G). She had a small thoracic cage with upturned xyphoid process and right-sided spinal muscular prominence in addition to widely-spaced nipples, supernumerary nipples, and hypoplastic labia minora.

Developmental milestones were delayed: she developed a social smile at 4 months, sat at 10 months, crawled at 19 months, pulled to a stand at 21 months, and walked at 4 years. At 21 months she was using three nonspecific consonant-vowel combinations; at 5.5 years she could hum and follow some commands but had no expressive verbal language. By age 11, she could speak in 2–3 word phrases.

Echocardiography at 2 years was normal. A temporal bone CT performed at 43 months showed dysplastic inner ears. The right ear contained a single dysplastic semicircular canal and a severely hypoplastic cochlea while the left inner ear had a Mondini malformation and an expanded endolymphatic duct. Renal ultrasonography revealed bilateral calculi and cysts. She had left-sided facial nerve palsy, night terrors with tongue fasciculations, and partial complex petit mal seizures (onset age 3 years). She developed tonic-clonic seizures at 5.5 years. Brain MRI at 23 months revealed white matter ischemic-hypoxic changes in both

cerebral hemispheres. Biochemical panels for lysosomal enzymes, plasma amino acids, urine organic acids, and carbohydrate deficient transferrins were normal.

Past surgical history was significant for multiple bilateral myringotomies secondary to recurrent otitis media, and strabismus surgery at age 3 years. Preauricular pits were reported in multiple maternal relatives, including an aunt, uncle, grandmother, and cousin. Her mother reported a history of renal calculi.

Our patient met clinical diagnostic criteria for CHARGE syndrome, with two of three major criteria (ocular colobomata and hypoplastic semicircular canals) and three of five minor criteria (cranial nerve dysfunction, abnormal outer/middle ear structure, and intellectual disability) [Hale et al., 2015; Verloes 2005].

Clinical genetic testing

Chromosomal analysis revealed a normal 46,XX karyotype. *CHD7* mutation testing revealed no pathogenic variants, deletions, or duplications. CMA revealed a female chromosomal profile with aberrations on chromosomes 2 and 15.

Chromosome 2 contained two contiguous regions of gain, together spanning 6.5 Mb of genomic material at 2p25.3-p25.2 (chr2:39193-6531231; hg38 (Figure 2A). There were four copies of the proximal 3.5 Mb region (chr2:3126461-6531231) containing 13 RefSeq genes, and three copies of the distal 3 Mb region (chr2:39193-3058475) containing 11 genes (Figure 2E). The 67,986 bp intervening genomic sequence with normal copy number contained no genes (chr2: 3058475-3126461). RQ-PCR confirmed these chromosomal gains in the proband, which were absent in both parents. FISH on interphase chromosomes from 100 cells identified three copies of the distal chromosome 2 gain in 75% of cells examined (Figure 2B), and three or four copies of the proximal genomic gain in 59% and 26% of the cells, respectively (Figure 2C). Importantly, 20% of cells examined by FISH had normal staining patterns, indicating mosaicism for both the distal and proximal gains. We did not assess co-localization of the distal and proximal gains within the same cells. In prophase cells, distal FISH probes bound to the same chromosomal strand (Figure 2D), consistent with a *de novo* interstitial duplication event resulting in mosaicism for three or four copies of the proximal region (2p25.3-p25.2) and three copies of the distal region (2p25.3). There was no evidence of a subtelomeric deletion. Furthermore, FISH performed on cells from each parent revealed no chromosome 2p abnormalities, including inversion or subtelomeric deletion.

The proband had a 602 kb gain of genomic material at 15q11.2 (chr15:22572809-23174546). RQ-PCR confirmed maternal inheritance of this single-copy microduplication. FISH studies were unable to confirm the duplication in either the proband or her mother, likely because it was under the detection threshold of 700 kb. Importantly, there were no supernumerary chromosomes or chromosomal translocations.

RQ-PCR and sequencing for *SOX11* in individuals with CHARGE syndrome

Among the 24 genes in the aberrant 2p region was the triplicated *SOX11*, encoding a SoxC group high mobility group (HMG) box-containing transcription factor. *SOX11* has major

roles in embryonic and adult neurogenesis, craniofacial osteogenesis, ocular morphogenesis, and cardiac development, all specifically disrupted in CHARGE [Gadi et al., 2013; Hargrave et al., 1997; Jankowski et al., 2006; Paul et al., 2014; Pillai-Kastoori et al., 2014; Potzner et al., 2010; Sock et al., 2004; Wang et al., 2013]. Sanger sequencing and RQ-PCR on our patient and on 28 patients with CHARGE phenotypes, negative chromosomal microarray, and negative *CHD7* mutation testing showed no *SOX11* mutations or duplications.

DISCUSSION

Here we report a child with features of CHARGE syndrome and a complex mosaic duplication-triplication 2p25, including triplication of the transcription factor *SOX11*. Given that a minority of cells had two copies of either the distal or proximal gains, and given that neither parent had a detectable chromosome 2p anomaly, it is possible that our patient's 2p anomaly is post-zygotic and thus represents a case of somatic mosaicism. Analysis of 28 other individuals revealed no additional cases of CHARGE and gain of 2p25 or *SOX11* mutations. To our knowledge, our patient represents the first case of 2p25 gain with inner ear anomalies and other CHARGE features.

Comprehensive review of the literature revealed ten cases presenting with partial trisomy 2p, including our region of interest (Table I) [Al-Saffar et al., 2000; Aviram-Goldring et al., 2000; Engels et al., 1999; Fineman et al., 1983; Ishikawa et al., 2002; Martinez-Juarez et al., 2014; Stoll et al., 1974; Wakita et al., 1985]. Interestingly, our patient had the smallest region of 2p duplication among these ten cases and was the only one to exhibit triplication (Figure 2E). Lack of reported key clinical findings in the three published cases precluded retrospective assignment of a CHARGE syndrome diagnosis; however, they did exhibit some CHARGE features including external ear malformations, hearing loss, cardiac anomalies, craniofacial dysmorphic features, and developmental delay. As with our patient, some of these ten cases also exhibited clinical findings not commonly observed in CHARGE, such as hypertelorism, seizure disorder, cerebral white matter ischemic-hypoxic changes, and arachnodactyly. While this phenotypic cluster could be the result of dysregulation of one gene within the chromosomal region of gain, such as *SOX11*, we cannot rule out other genes within the interval as contributory. Finally, we found 20 individuals in the ClinGen and DECIPHER databases with duplications wholly or partially contained within our patient's duplicated interval. Reported phenotypes are summarized in Supplementary Tables III and IV, respectively. These findings indicate that 2p duplication is associated with developmental delay and craniofacial dysmorphic features.

Our patient's phenotype may be explained in part by duplication of the early and broadly expressed transcription factor *SOX11* which has been implicated in development of CHARGE-relevant structures, such as the eye, ear, and heart [Sock et al., 2004]. Interestingly, a recent report showed that *Sox11* is a direct target of *CHD7* in neural stem cells; however, it is not known whether *CHD7* regulates *Sox11* expression in other developing tissues [Feng et al., 2013]. Of note, loss-of-function mutations in *SOX11* were recently reported in Coffin-Siris syndrome [Tsurusaki et al., 2014]. Our patient does not have clinical features or a genetic diagnosis consistent with Coffin-Siris syndrome. Further, our patient's clinical features overlap with those described in Kabuki syndrome, specifically

her pre-auricular pits, persistent fetal fingertip pads, and craniofacial features. To date, no link between *KMT2D*, the gene most often associated with Kabuki, and any gene in our patient's relevant chromosomal region of gain has been observed. Finally, our patient's duplicated interval contains *PXDN*, a gene in which loss-of-function mutations have been associated with microphthalmia and anterior segment dysgenesis [Choi et al., 2015; Khan et al., 2011]. Duplications of *PXDN* have also been associated with autism without ocular involvement [Meyer et al., 2012]. To date, there are no reports of *PXDN* duplication and coloboma. Taken together, these observations suggest that increased *PXDN* dosage is not sufficient to cause our patient's phenotype.

There are three important caveats to our analysis. First, we cannot rule out noncoding mutations in *CHD7* or elsewhere as contributing to her phenotype. Also, it is not known whether some or all of the duplicated genes in our patient were overexpressed during embryologic development. Finally, while the breakpoints surrounding her duplicated regions were devoid of coding DNA, critical regulatory regions could be disrupted.

In summary, this is the first account of a complex mosaic gain of 2p25 in an individual with features of CHARGE syndrome. This report may prompt detection and surveillance of CHARGE-related features in individuals with duplication 2p25.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank the University of Michigan Medical Genetics Laboratories, the Cytogenetics Laboratory, and the DNA Sequencing Core for clinical testing and sequencing support. EDS is supported by NIH grants 2T32-GM007863 and 5T32-DC000011. DMM is supported by NIH grant 5R01-DC009410 and the Donita B. Sullivan, M.D. Research Professorship.

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Figure 1.

Clinical features of the proband.

Photographs taken at 11 years show a broad, sloping forehead with a slender, prominent nose and flattened nasal tip (A–C). Over-folded helices (D–E), shortened proximal phalanges of the thumbs (F), and elongated fingers and toes (F–G) are noted.

Figure 2.

Chromosomal microarray analysis revealed a gain of genomic material on chromosome 2p25.3-p25.2, confirmed by FISH, and shared with three other cases.

CMA performed on whole blood revealed a *de novo*, distal duplication and proximal triplication of 2p25.3-p25.2 (A, highlighted in red). Interphase FISH confirmed the CMA findings, revealing a distal duplication (B, green probe RP11-168K7) and proximal triplication (C, green probe RP11-36C8). Red label in (B) and (C) corresponds to a 2qter probe, indicating normal dosage of 2q in each case. Visualization of pachytene

chromosomes using prophase FISH (D) confirmed probe arrangement along 2p, confirmed the integrity of the 2p subtelomeric region (2pter red probe), and excluded translocation events. The 24 genes contained within the interval duplicated within our patient are shown (E). In our patient's diploid genome, the 11 distal genes were present in triplicate while the 13 proximal genes were in quadruplicate. Of interest, our patient had four copies of *SOX11*, an SRY-related HMG box-containing SoxC group transcription factor.

Table 1

Clinical Features of Pure Trisomy 2p Patients

This comprehensive list of clinical findings reveals significant overlap between our patient, diagnosed with typical CHARGE syndrome, and ten other individuals with the terminal 2p duplication.

Clinical Feature	Our Patient dup(2)(p25.3-p25.2)	Stoll et al., 1974 Case 1 dup(2) (pter- p22)	Stoll et al., 1974 Case 2 dup(2) (pter- p22)	Fineman et al., 1983 dup(2) (pter- p23)	Wakita et al., 1985 dup(2)(p25.3- p25.1)	Engels et al., 1999 dup(2) (pter- p23)	Aviram- Goldring et al., 2000 Case 1 dup(2) (p25.2-p23)	Al-Saffar et al., 2000 dup(2) (pter-p23)	Ishikawa et al., 2002 dup(2)(p25.3- p22.2)	Martinez- Juarez et al., 2014 Case 1 dup(2) (pter- p22.3)	Martinez- Juarez et al., 2014 Case 2 dup(2) (pter- p22.3)
Growth and development											
IUGR (<10%)	-	-	-	-	-	-	-	-	-	-	-
Postnatal growth failure (<3%)	+	+	+	+	+	+	+	+	+	+	+
Developmental delay (motor)	+	+	+	+	-	+	+	+	+	+	+
Intellectual disability	+	+	+	+		+	+	+	+	+	+
Craniofacial dysmorphic features											
High, prominent forehead	+, BC			+	TC		+	+	+	+	+
Hypertelorism	+				+			+	+		
Epicanthal folds	+	+	+			+	+			+	+
Nasal abnormalities	broad, prominent, short philtrum	broad	broad	broad	anteverted nares	short	broad	broad, short		broad, short	broad, short
Ocular abnormalities											
Coloboma	retinal – bilateral										
Myopia	+							+			
Prosis	+										
Ear abnormalities											
External ear anomalies	+				+		+	+	+		
Sensorineural hearing loss	+									+	
Mondini malformation	+										
Semicircular canal dysplasia	+										
Other findings											
WM ischemic-hypoxic change	+							+	+	+	+
Ventricular septal defect	-							+			

Clinical Feature	Our Patient <u>dup(2)(p25.3-p25.2)</u>	Stoll et al., 1974 Case 1 <u>dup(2)(pter- p22)</u>	Stoll et al., 1974 Case 2 <u>dup(2)(pter- p22)</u>	Fineman et al., 1983 <u>dup(2)(pter- p23)</u>	Wakita et al., 1985 <u>dup(2)(p25.3- p25.1)</u>	Engels et al., 1999 <u>dup(2)(pter- p23)</u>	Aviram- Goldring et al., 2000 Case 1 <u>dup(2)(p25.2-p23)</u>	Al-Saffar et al., 2000 <u>dup(2)(pter-p23)</u>	Ishikawa et al., 2002 <u>dup(2)(p25.3- p22.2)</u>	Martinez- Juarez et al., 2014 Case 1 <u>dup(2)(pter- p22.3)</u>	Martinez- Juarez et al., 2014 Case 2 <u>dup(2)(pter- p22.3)</u>
Finger dysmorphic features	hypoplastic left thumb, AD				AD, joint dysplasia	AD			slender	AD	AD
Toe dysmorphic features	CD								slender; clinodactyly	AD	AD
Supernumerary nipples	+						+				
Renal abnormalities	stones						pelvic dilation	VUR			
Hypoplastic external genitalia	+							-		+	+

BC = brachycephaly; TC = trigonocephaly; WM = white matter; AD = arachnodactyly; CD = camptodactyly; VUR = vesico-ureteral reflux