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ORIGINAL PAPER

Chromosomal microarray analysis of patients with Duane retraction syndrome

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Abstract
Purpose Duane retraction syndrome (DS) is a rare congenital strabismus with genetic heterogeneity. The genetic causes of DS are not always of monogenic origin; various chromosomal copy number variations (CNVs) have also been reported. The objective of our study was to characterize the CNVs, including gains and losses detected by high-resolution chromosomal microarray in patients with DS.
Methods Twenty patients with DS were investigated using high-resolution chromosomal microarray analysis (CMA) (Affymetrix CytoScan Array 750 K). Conventional cytogenetic analysis was also performed.
Results All samples revealed normal karyotype by cytogenetic analysis. However, in all our patients, multiple CNVs, including gains and losses, were detected using the high-resolution CMA method. Chromosomal loci 1q21.2, 2p11.2–q11.1, 2q21.1–q21.2, 4p16.1, 7p11.2–q11.21, 14q32.33, 17p11.2–q11.1 and 20p11.1–q11.21 were the most frequently affected regions.
Conclusions This study emphasized that CNVs in several chromosomal regions are known to be involved in DS. We also underscore the genetic heterogeneity of DS. Our suggestion is that genes located in the most frequently affected regions should be focused on in the following candidate gene studies.

Keywords Duane retraction syndrome · Chromosomal microarray (CMA) · Copy number variation (CNV)

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Introduction

Duane syndrome (DS) refers to a spectrum of congenital ocular motility disorders associated with the abnormal innervation of extraocular muscles. There are three types of Duane syndrome that differ in patterns of dysinnervation and clinical manifestations. Type I is the most common and is characterized by esotropia and complete limitation of abduction with little or no limitation of adduction, globe retraction as a result of medial and lateral rectus muscle co-contraction on attempted adduction. Type II is the least common and is characterized by exotropia in primary gaze, and complete limitation of adduction with little or no limitation of abduction. Type III involves marked or complete limitation of both adduction and abduction [1–4]. In most cases, DS is isolated and sporadic. However, up to 22% of patients

have a family history of oculomotor disorder and about 10% are familial [5, 6]. In familial cases, inheritance usually follows an autosomal-dominant pattern, and bilateral involvement is more likely than in sporadic cases. However, as Duane syndrome has incomplete penetrance and variable expressivity, some family members may not be affected and familial cases may be unilateral and of varying severity. The syndrome may be also isolated or can be a part of other systemic abnormalities like Okihiro, Rubinstein–Taybi or Holt–Oram, and Wildervanck syndromes [1]. The pathophysiology of DS involves various factors. Multiple genes may be involved in the expression of the trait, and there may be interactions with environmental factors. The genetics of Duane syndrome are complex, and although two genes (*CHN1* and *SALL4*) have been associated with particular complex forms of Duane syndrome, previous studies suggest different chromosomes and gene defects contribute to DS [4, 7–9]. The etiology in most patients remains unclear [10, 11]. Apart from case reports, there are limited data and research on genetics in DS in the literature.

Based on these findings, we aimed to analyze and report the detailed genetic structures in familial and sporadic DS. We performed current worldwide technology high-resolution array comparative genomic hybridization (array CGH) beside conventional chromosomal analysis. Genomic microarray also known as chromosomal microarray analysis (CMA) can detect CNVs within the genomes, which are undetectable using conventional karyotyping.

Materials and methods

Patient information

This study included twenty Duane syndrome patients recruited from the Department of Ophthalmology at Ondokuz Mayis University Faculty of Medicine (Samsun, Turkey). The DS diagnosis was based on clinical findings, which included the limitation of abduction or adduction and globe retraction on attempted adduction either with or without up/down-shoot. Both sporadic and familial cases were included in the study. After the procedures were explained, signed informed consent for participation in this research was obtained from all patients and/or parents. Patients who did not want to participate were excluded from the study. The research followed the tenets of the Declaration of Helsinki, and was approved by the Ondokuz Mayis University Clinical Research Ethics Committee (B.30.2.ODM.0.20.08).

Sample collection and DNA preparation

DNA was extracted from 2 mL venous blood according to the kit procedure (Invitrogen Corporation, Carlsbad, CA, USA) and stored at − 20 °C. The concentration and quality of genomic DNA were analyzed using Nanodrop ND-1000 (Thermo Scientific, Wilmington, DE, USA).

Cytogenetic analysis

Cytogenetic analysis was performed using phytohemagglutinin-stimulated peripheral blood lymphocyte cultures [12, 13]. Metaphase chromosomes were banded using the Giemsa banding (GTG) technique, and a minimum of 20 metaphase plaques were analyzed for each case. The karyotypic analysis was performed using an Olympus Bx51 microscope (Olympus, Tokyo, Japan), and images were captured with a CCD camera using an image analysis system (CytoVision). The karyotypes were described according to the International System for Cytogenetic Nomenclature (ISCN 2009) [14].

Analysis of array comparative genomic hybridization (CGH) data

Chromosomal microarray (CMA) was performed using a CytoScan 750K array (Affymetrix, Santa Clara, CA, USA) according to the manufacturer's recommendations. The array allows the detection of known or novel chromosomal aberrations with high-density coverage across the entire genome. The platform is composed of 550,000 non-polymorphic CNV probes and over 200,000 SNP probes with an average resolution of 100 kb. The data were analyzed by using Chromosome Analysis Suite v2.1 (Affymetrix) and Nexus Copy Number v.7.5 software (BioDiscovery, El Segundo, CA, USA).

Results

Of the 20 patients analyzed, 6 (30%) were male and 14 (70%) were female. The patients' mean age was 14.3 ± 13.0 years (range 1–49). Nineteen patients had DS type 1, and only one patient had DS type 3. The right eye was affected in five (25%), the left eye in 12 (60%) cases, and three (15%) had bilateral DS. Ophthalmological examination findings and clinical features of the patients are given in Table 1. Positive family history was detected in 11 (55%) patients for strabismus. Seven of those cases (35%) were the members of three families and were confirmed to have DRS. In the first family DRS was present in father and daughter; in the second family it was present in two siblings (brother and sister) and their maternal aunt; and in the third family, the father and son had DRS (Figs. 1, 2 and 3). Copy number variations including losses and gains were found in all patients (100%), ranging from 7 to 23 CNVs per patient (Table 2). Over 50% of patients had duplications on chromosomes 1, 2, 4, 7, 14, 17, 20. Genes located on these loci are given in Table 3. The size of chromosomal imbalances ranged from 402.12 Kb to 32.72 Mb. All (100%) familial cases had involvement of the loci 1q21.2, 2p11.2–q11.1, 7p11.2–q11.21, 20p11.1–q11.21; however, the involvement of these loci in the whole study group was 75%, 100%, 85%, and 85%, respectively. 17p11.2–q11.1 and 14q32.33 were affected in 6 out of 7 (85%) familial cases, though 55% and 80% were affected in the whole group, respectively (see figures and Table 3).

Discussion

Herein, in a cohort of patients with Duane retraction syndrome we performed a high-resolution technology array CGH (CytoScan 750K) besides conventional karyotype analysis. Cytogenetic analysis was the first step of karyotyping which evaluates all chromosomes. However, nowadays, CMA is a relatively recent addition to cytogenetic technologies [15].

In the literature, Hochstenbach et al. performed the same CMA methodology (CytoScan 750K) in patients with developmental delay and mental retardation and reported a > 99% detection of chromosomal abnormalities [16]. CMA was recommended as a first-line testing method for multiple congenital anomalies [17]. The advantages of CMA include the high genomic resolution of > 250 thousand base pairs (kilobases) compared to standard karyotyping, which has a resolution of > 7–10 million bases (megabases). Another benefit of CMA is its ability to precisely define a region of imbalance. The disadvantage of CMA is that it detects differences reflected by imbalances in patients compared to controls and the balanced rearrangements may escape detection [18].

We used CMA in DS patients to reveal the genome-wide CNV findings. Conventional cytogenetic analysis revealed no chromosomal anomaly in DS patients, but we detected several submicroscopic chromosomal variations and changes by CMA in all patients.

In previous studies, some genes and chromosomal loci have been identified to cause DS. Mutation of the *CHN1* gene at the DURS2 (2q31.1) chromosomal locus is shown in isolated autosomal-dominant DS. Syndromic Duane has been associated with the deletion in the *SALL4* gene at the chromosomal locus 20q13.2 or abnormalities at the locus DURS1 (8q12-8q13). It is known that the protein products of the *CHN1* and *SALL4* genes are important regulators of brainstem and oculomotor system development [6]. *CHN1* gene association with DS was examined by Miyake et al., and *CHN1* mutation was detected in 35% of familial DS but none in sporadic cases [8, 9]. In our study, we had familial and sporadic cases of DS and none had anomalies at region 2q31 or 20q13 as in Miyake et al.'s study. Despite that, multiple gains and losses were detected in many other regions. The most frequently affected chromosomal loci were 1q21.2, 2p11.2–q11.1, 2q21.1–q21.2, 4p16.1, 7p11.2–q11.21, 14q32.33, 17p11.2–q11.1 and 20p11.1–q11.21. Duplications at these loci were detected in more than 50% of the patients and even higher (> 80%) in familial cases. These findings support the presence of additional genetic alterations in familial and sporadic DS cases. Another study by Abu-Amero et al. reported a case of syndromic Duane, who did not have a sequence change in *SALL4*, *CHN1*, *HOXA1* or *TUBB3* genes, but showed deletions at the 12q24.31 and 22q13.31 cytogenetic band regions [19]. These regions encompass the *RNF34* and *PPARA* genes. Similarly, chromosomal gain at region 22q13.32–13.33 was seen in one of our patients, which did not contain any gene in this region and the patient was not syndromic. The proximity of affected locus to 22q13.31 may lead to the interaction with nearby genes, causing the

Table 1 Clinical features of the patients

Patient	Karyotype	Visual acuity (Snellen) Right eye Left eye	Eye movements	Other
1	46, XY	4/10	Right eye	
		10/10	-1 abduction	
2	46, XX	10/10	Left eye	
		10/10	-4 abduction	
3	46, XY	Object fixation	Right eye	Epilepsy cleft palate
			-4 abduction	
4	46, XX	10/10	Left eye	
		4/10	-4 abduction	
5	46, XX	Object fixation	Left eye	Left 6 cranial nerve agenesis confirmed on MRI
			-4 abduction	
6	46, XY	10/10	Left eye	
		8/10	-4 abduction	
7	46, XX	10/10	Left eye	
		10/10	-4 abduction	
8	46, XX	2/10	Right eye	
		10/10	-4 abduction	
9	46, XY	10/10	Left eye	
		10/10	-3 abduction	
10	46, XX	10/10	Right eye	
		10/10	-2 abduction	
11	46, XX	10/10	Left eye	Migraine
		10/10	-4 abduction	
12	46, XX	7/10	Bilateral	
		7/10	-1 abduction	
13	46, XY	10/10	Left eye	
		10/10	-4 abduction	
14	46, XX	10/10	Left eye	
		10/10	-3 abduction	
15	46, XX	10/10	Left eye	
		7/10	-4 abduction	
			-2 adduction	
16	46, XX	6/10	Bilateral	Hydrocephaly, growth retardation, pectus excavatum, encephalocele
		7/10	-2 abduction	
17	46, XX	10/10	Left eye	
		10/10	-2 abduction	
18	46, XX	6/10	Right eye	
		10/10	-4 abduction	
19	46, XX	10/10	Left eye	
		10/10	-3 abduction	
20	46, XY	10/10	Bilateral	Torticollis
		8/10	-2 abduction	

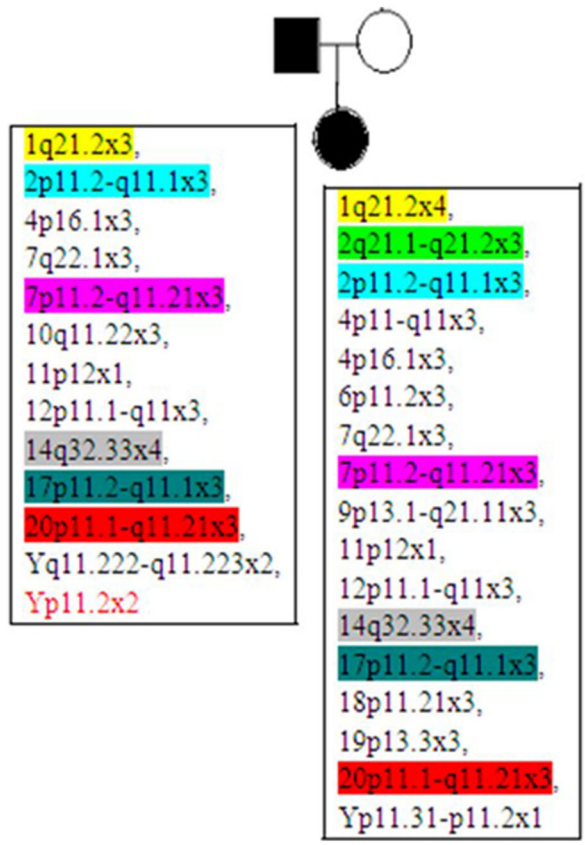

Fig. 1 Affected members and gene regions in the first family

development of DS in our patient without any other systemic pathology.

Duane syndrome has been associated with several autosomal chromosomes whereas its association with sex chromosomes is limited. In several of our patients, CMA revealed duplications in Yp11.2, Yq11.222–q11.223, Xp11.21, Xq13.2 and deletions in Yp11.2 and Yp11.31–p11.2 sex chromosomal regions. Weis et al. reported one case with type 1 DS with the 48XXYY karyotype [10]. They proposed that duplication of Y chromosome or duplication of X chromosome in presence of two Y chromosomes may be responsible for the overexpression of a gene responsible for DS. Because DS has not previously been associated with any sex chromosome abnormalities, they drew attention to a possibility of coincidence of DS with 48XXYY. These findings support the idea that some genes on sex chromosomes may be responsible for the development of DS.

We observed 8p11.1–q11.1 duplication in three patients. None of them had any congenital anomaly. Two were siblings of a family whose maternal aunt also had DS. Therefore, besides the syndromic Duane, the genes on chromosome 8 may also be responsible for the familial or sporadic DS without systemic abnormalities. Similar findings were reported in Baris et al.'s study. They showed complex cytogenetic rearrangements at the DURS1 locus in syndromic Duane [20]. They also found a chromosome 8 inversion that transposed highly repetitive centromeric DNA and multiple 8q genes (8p11.1–8q13.2) and a complex mosaic supernumerary marker chromosome containing 8p11.1–8q12.3 material. Additionally, Lehman et al. reported a patient with cognitive impairment, multiple congenital anomalies and DS who had 8q12 duplication shown by array-based CGH [21].

Three cases of DS reported by Smith and Traboulsi had some genetic abnormalities [4]. The first case had

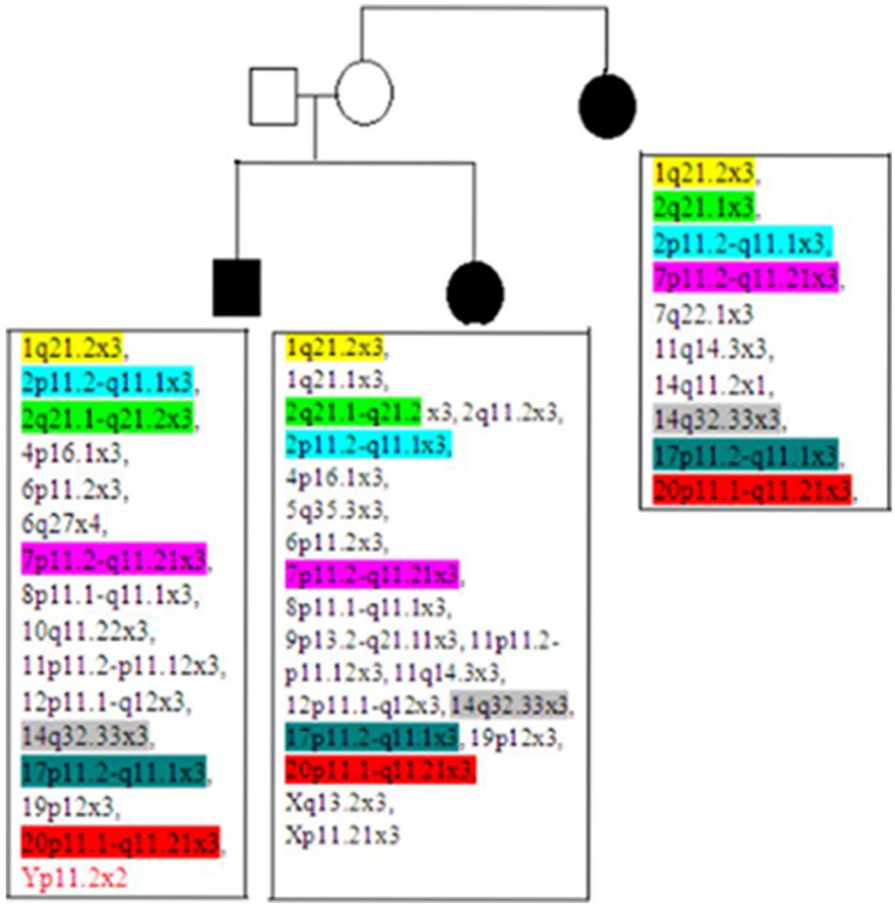

Fig. 2 Affected members and gene regions in the second family

2q13 duplication which did not overlap with the known DS causing locus 2q31. However, they proposed that this duplication may result in overexpression of the gene causing DS. The second patient had trisomy of 10q24.2 and 20q13.12. The third patient had a duplication of chromosome 22q11.1–q11.22 and had findings consistent with cat-eye syndrome. Forbes et al. examined the ocular findings in 19 cases with 22q11.2 duplication and observed a high rate of ocular pathologies including strabismus, congenital cataract, disk drusen, megalocornea and delayed visual maturation in these patients [22]. We had three patients with 2q13 duplication. All were non-syndromic and one had sixth cranial nerve agenesis, which was confirmed by CISS sequence magnetic resonance imaging. Two of our patients had affected regions at chromosome 22. In one patient, CMA revealed 22q11.21 duplication, which included cat-eye and DiGeorge syndrome regions. This patient only had a cleft palate diagnosis when he was included in this study because of DS in his right eye at the age of 9 months. He also had duplications in chromosomes 1, 2, 4, 6, 7, 9, 11, 16, 18, 19, 22 and Y. His father had DS affecting the left eye with no systemic abnormalities. After the results of CMA testing, the patient was referred to clinical genetics for the detailed evaluation. Duplication at the 22q13.32–q13.33 region was observed in one patient, but did not cause a significant

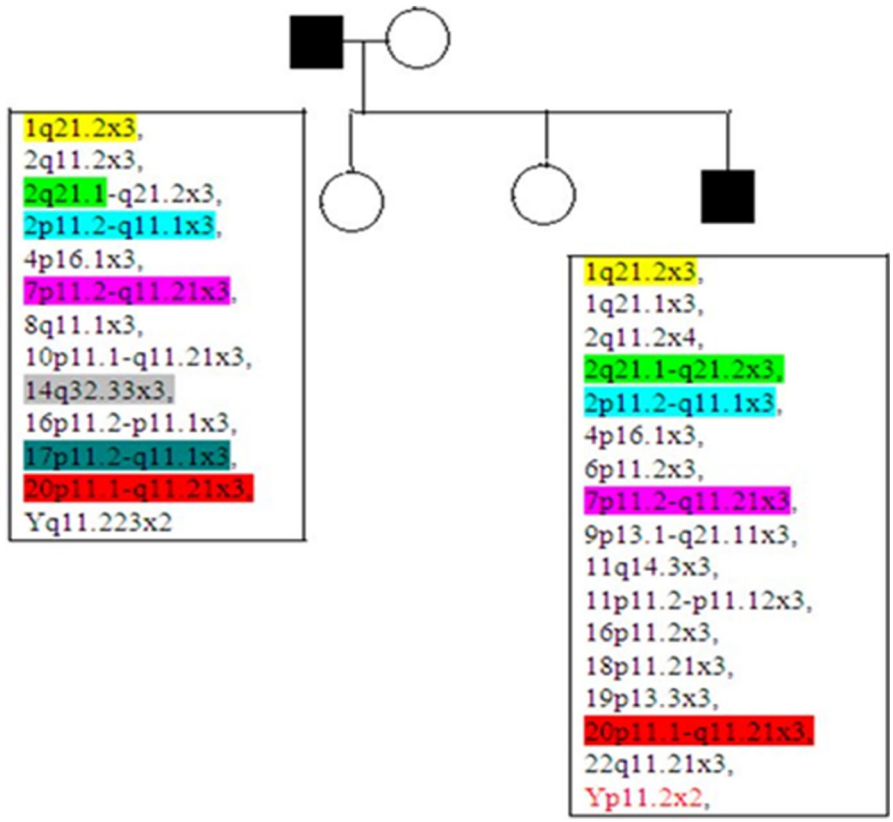

Fig. 3 Affected members and gene regions in the third family

systemic problem except that the patient had Type 1 DS in the left eye.

We evaluated these affected regions in familial cases and observed duplication of 1q21.2, 2p11.2–q11.1, 7p11.2–q11.21, 17p11.2–q11.1, 20p11.1–q11.21 loci in all our cases (7/7) and 14q32.33, 2q21.1–q21.2, 4p16.1 in 6 of 7 cases. All of these chromosomal regions were affected in more than 50% of all cases in the study. We reviewed the genes located in these regions. 1q21.2 and 4p16.1 loci contain genes that are expressed in the brain and are responsible for the normal neuron function [23, 24]. Involvement of these genes in our patients may play a role in the pathophysiology of abnormal neuronal development leading to DS.

It is unclear if the duplication at the loci 2p11.2–q11.1, 14q32.33, 7p11.2–q11.21, 17p11.2–q11.1 and 20p11.1–q11.21 has a significant effect on the development of DS. Normal neuronal development is complex and requires specific interactions of many different genes. The deletion or duplication of some genes might affect nearby genes, leading to multiple developmental or functional abnormalities.

In conclusion, we report several new documented chromosomal changes and variations in DS patients. These chromosomal regions may contain candidate genes that may influence the abnormal neuronal development in DS patients. Further studies are needed to clarify gene products in all these regions

Table 2 Chromosomal microarray findings of patients

Patient ID	Cytogenetic	CMA summary	CMA results
1	46, XY	13 CNVs (1 loss, 12 gains)	arr 1q21.2x3, 10q11.22x3, 11p12x1,12p11.1–q11x3,14q32.33x4, 17p11.2–q11.1x3, 2p11.2–q11.1x3, 20p11.1–q11.21x3, 4p16.1x3, 7q22.1x3, 7p11.2–q11.21x3, Yq11.222–q11.223x2, Yp11.2x2
2	46, XX	14 CNVs (1 loss, 13 gains)	arr 1q21.2x3, 1q21.1x3, 14q32.33x4, 14q11.2x1,16p11.2x3, 17p11.2–q11.1x3, 18p11.21x3, 2p11.2–q11.1x3, 2q21.1x3, 20p11.21–q11.21x3, 4p16.1x3, 6p11.2x3,7p11.2–q11.21x3, Xq13.2x3
3	46, XY	18 CNVs (18 gains)	arr 1q21.2x3, 1q21.1x3, 11q14.3x3, 11p11.2–p11.12x3,16p11.2x3, 18p11.21x3,19p13.3x3, 2q11.2x4,2q21.1–q21.2x3, 2p11.2–q11.1x3, 20p11.1–q11.21x3, 22q11.21x3, 4p16.1x3, 6p11.2x3, 7p11.2–q11.21x3, 9p13.1–q21.11x3, Yp11.2x2, Yq11.223x2
4	46, XX	10 CNVs (10 gains)	arr 1q21.2x3, 11q14.3x3, 14q11.2x1,14q32.33x3, 17p11.2–q11.1x3,2q21.1x3, 2p11.2–q11.1x3, 20p11.1–q11.21x3, 7p11.2–q11.21x3, 7q22.1x3
	46, XX	19 CNVs (19 gains)	arr 1q21.1x3, 1q21.1x3, 1q21.2x3, 10p11.1–q21.21x3, 12p11.1–q12x3, 14q32.33x4, 17p11.2–q11.1x3, 19p13.3x3, 2p11.2–q11.1x3, 2q11.2x3, 2q13x3, 2q21.1–q21.2x3, 20p11.21–q11.21x3, 4p16.1x4, 7p11.2–q11.21x3, 7q22.1x3, 9p13.1–q21.11x3, Xq13.2x3, Yp11.31–p11.2x1
6	46, XY	13 CNVs (13 gains)	arr 1q21.2x3, 2q11.2x3, 2q21.1–q21.2x3, 2p11.2–q11.1x3, 4p16.1x3, 7p11.2–q11.21x3, 8q11.1x3, 10p11.1–q11.21x3, 14q32.33x3, 16p11.2–p11.1x3, 17p11.2–q11.1x3, 20p11.1–q11.21x3, Yq11.223x2
7	46, XX	20 CNVs (20 gains)	arr 1q21.2x3, 1q21.1x3, 2q21.1–q21.2x3, 2q11.2x3, 2p11.2–q11.1x3, 4p16.1x3, 5q35.3x3, 6p11.2x3, 7p11.2–q11.21x3, 8p11.1–q11.1x3, 9p13.2–q21.11x3, 11p11.2–p11.12x3, 11q14.3x3, 12p11.1–q12x3, 14q32.33x3, 17p11.2–q11.1x3, 19p12x3, 20p11.1–q11.21x3, Xq13.2x3, Xp11.21x3
8	46, XX	16 CNVs (2 losses, 14 gains)	arr 1q21.1x3, 2p11.2–q11.1x3, 2q13x3, 2q21.1–q21.2x3, 6p11.2x3, 7p11.2–q11.21x3, 9p13.2–q21.11x3, 10p14x1, 11p11.2–p11.12x3, 12p11.1–q12x3, 13q12.12x3, 15q11.2x3, 19p13.3x1, 20p11.1–q11.21x3, Yp11.2x1
9	46, XY	9 CNVs (9 gains)	arr 2q21.1x3, 2q13x3, 2p11.2–q11.1x3, 2q11.2x3, 7q11.2–q11.21x3, 16p13.1-12.3x3, 20q11.21x3, Yp11.2x2, Yq11.223x2
10	46, XX	23 CNVs (23 gains)	arr 1q21.1x3, 1q21.1x3, 2q11.2x4, 2p11.2–q11.1x3, 2q21.1–q21.2x3, 2p11.2x3, 4p16.1x3, 4p11–q11x3, 6p11.2x3, 7q22.1x3, 7p11.2–q11.21x3, 8p11.1–q11.1x3, 10p11.1–q11.21x3, 11p11.2–p11.12x3, 11p11.12–q11x3, 12p11.1–q12x3, 13q12.12x3, 14q32.33x3, 17p11.2–q11.1x3, 18p11.21x3, 19p12x3, 19p13.3x3, 20p11.21–q11.21x3
11	46, XX	20 CNVs (2 losses, 18 gains)	arr 1q21.1x3, 1q21.2x3, 2p11.2–q11.1x3, 2q21.1–q21.2x3, 4q35.2x3, 4p16.1x3, 6p11.2x3, 7q22.1x3, 7p11.2–q11.21)x3, 9p13.1–q21.11x3, 11p11.2–p11.12x3, 12p11.1–q12x3, 14q32.33x4, 14q11.2x1, 18p11.21x3, 19q13.33x3, 19p13.3x1, 20p11.21–q11.21x3, 22q13.32–q13.33x3, Xq13.2x3
12	46, XX	17 CNV (1 loss, 16 gains)	arr 1q21.2x4, 2q21.1–q21.2x3, 2p11.2–q11.1x3, 4p11–q11x3, 4p16.1x3, 6p11.2x3, 7q22.1x3, 7p11.2–q11.21x3, 9p13.1–q21.11x3, 11p12x1, 12p11.1–q11x3, 14q32.33x4, 17p11.2–q11.1x3, 18p11.21x3, 19p13.3x3, 20p11.1–q11.21x3, Yp11.31–p11.2x1
13	46, XY	16 CNV (16 gains)	arr 1q21.2x3, 2p11.2–q11.1x3, 2q21.1–q21.2x3, 20p11.1–q11.21x3, 4p16.1x3, 6p11.2x3, 6q27x4, 7p11.2–q11.21x3, 8p11.1–q11.1x3, 10q11.22x3, 11p11.2–p11.12x3, 12p11.1–q12x3, 14q32.33x3, 17p11.2–q11.1x3, 19p12x3, Yp11.2x2
14	46, XX	13 CNV (13 gains)	arr 1q21.2x3, 2p11.2–q11.1x3, 2q21.1x3, 2q11.2x3, 4p16.1x3, 7p11.2–q11.21x3, 8p23.3–p23.2x3, 12p12.1x3, 14q32.33x3, 20p11.1–q11.21x3, Yp11.31–p11.2x1, Yp11.2x1, Yp11.2x1
15	46, XX	13 CNV (10 gains)	arr 1q21.2x3, 2p11.2–q11.1x3, 2q21.1x3, 2q11.2x3, 4p16.1x3, 7p11.2–q11.21x3, 8p23.3–p23.2x3,12p12.1x3, 14q32.33x3, 20p11.1–q11.21x3, Yp11.31–p11.2x1, Yp11.2x1, Yp11.2x1
16	46, XX	10 CNV (10 gains)	arr 1q21.2x3,11q14.3x3, 11p11.2–p11.12x3, 14q32.33x4, 17p11.2–q11.1x3, 2q21.1–q21.2x3,2p11.2–q11.1x3, 20p11.1–q11.21x3, 4p16.1x3, 9p1.1–q21.11x3

Table 2 continued

Patient ID	Cytogenetic	CMA summary	CMA results
17	46, XX	9 CNV (9 gains)	arr 1q21.1x3, 14q32.33x4,17p11.2–q11.1x3, 19p13.3x3, 2p11.2–q11.1x3, 2q21.1–q21.2x3,20p11.1–q11.21x3, 7p11.2–q11.21x3, 7q22.1x3
18	46, XX	8 CNV (8 gains)	arr 1q21.2x3, 10q21.2x3, 14q32.33x3, 2q11.2x3,2p11.2–q11.21x3,4p16.1x3, 7p11.2–q11.21x3
19	46, XX	8 CNV (1 loss, 7 gains)	arr 1q21.2x3,18p11.21x3, 2p11.2–q11.1x3,2q21.1x3, 4p16.1x3,7q22.1x3,8p23.2x1, Xq13.2x3
20	46, XY	7 CNV (7 gains)	arr 1q21.2x3, 14q32.33x3, 2p11.2–q11.1x3, 2q21.1x3, 20p11.1–q11.21x3, 7p11.2–q11.21x3, Yp11.2x3

CMA: chromosomal microarray

CNV: copy number variation

Table 3 Frequently affected chromosomal loci and genes

Chromosomal LOCUS	Frequency (%)	Genes	Names of genes
2p11.2–q11.1	100	*RPIA, ANKRD36BP2, LOC654342, GGT8P, ACTR3BP2, ANKRD20B, TEKT4*	*RPIA*, ribose 5-phosphate isomerase A
			ANKRD36BP2, ankyrin repeat domain 36B pseudogene 2
			LOC, lymphocyte-specific protein 1 pseudogene
			GGT8P, gamma-glutamyltransferase 8 pseudogene
			ACTR3BP2, ACTR3B pseudogene 2
			ANKRD20B, ankyrin repeat domain 20 family, member A8, pseudogene
			TEKT4, tektin 4
7p11.2–q11.21	85	No Gene	–
20p11.1–q11.21	85	*ZNF337, FAM182B, LOC100134868, FAM182A, C20orf191, MIR663, FRG1B, DEFB115, DEFB116, DEFB118, DEFB119*	*ZNF337*, zinc finger protein 337
			FAM, family with sequence similarity, member
			LOC, lymphocyte-specific protein 1 pseudogene
			C20orf191, nuclear receptor corepressor 1 pseudogene 1
			MIR663, microRNA 663a
			FRG1B, FSHD region gene 1 family member B, pseudogene
			DEFB, defensin, beta
14q32.33	80	*ADAM6, NCRNA00226*	*ADAM6*, ADAM metallopeptidase domain 6
			NCRNA00226, long intergenic nonprotein coding RNA 226

Table 3 continued

Chromosomal LOCUS	Frequency (%)	Genes	Names of genes
1q21.2	75	*PPIAL4B, PPIAL4A, NBPF14, PPIAL4F, PPIAL4D, NBPF15, NBPF16, PPIAL4E, LOC645166, LOC388692, FCGR1C, HIST2H2BF, PPIAL4C, LOC728855*	*PPIAL*, peptidylprolyl isomerase A (cyclophilin A)-like *NBPF*, neuroblastoma breakpoint family, member *LOC*, lymphocyte-specific protein 1 pseudogene *FCGR1C*, Fc fragment of IgG, high affinity Ic, receptor (CD64), pseudogene *HIST2H2BF*, histone cluster 2, H2bf
4p16.1	70	*HMX1, LOC650293, USP17, USP17L6P, DEFB131, MIR548I2, DRD5*	*HMX*, H6 family homeobox *LOC*, lymphocyte-specific protein 1 pseudogene *USP*, ubiquitin specific peptidase DEFB131, defensin, beta 131 MIR548I2, microRNA 548i-2 *DRD5*, dopamine receptor D5
2q21.1–q21.2	55	*POTEE, LOC440910, LOC150786, LOC389043, LOC401010, TUBA3D, MZT2A, LOC150776, CCDC74A, POTEKP, C2orf27A, C2orf27B, ANKRD30BL, MIR663B*	*POTEE*, defensin, beta 115 *LOC*, lymphocyte-specific protein 1 pseudogene *TUBA3D*, tubulin, alpha 3d *MZT2A*, mitotic spindle organizing protein 2A *CCDC74A*, coiled-coil domain containing 74A C2orf27, chromosome 2 open reading frame 27 *ANKRD30BL*, ankyrin repeat domain 30B-like *MIR663B*, microRNA 663b
17p11.2–q11.1	55	*FAM27L, MTRNR2L1*	*FAM27L*, family with sequence similarity 27-like *MTRNR2L1*, MTRNR2-like 1

that are responsible for normal cranial nerve growth and differentiation.

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Compliance with ethical standards

Conflict of interest The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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