Molecular Genetic Approach to Craniosynostosis Patients and Whole Genome Array CGH Analysis of Nonsyndromic Cases

Kraniosinositozlu Hastalara Moleküler Genetik Yaklaşım ve Nonsendromik Vakalarda Array CGH ile Tüm Genom Boyu Analiz

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ABSTRACT

Craniosynostosis is a craniofacial malformation in which one or more sutures of the cranial vault are fused prematurely. It is estimated that craniosynostosis affects 1 in 2,000 to 2,500 live births worldwide. Early and accurate diagnosis of craniosynostosis is very important since premature suture closure causes not only a deformity of skull but also can directly affect the development of brain. Craniosynostosis occurs in all racial groups and more than 70% of all cases are non-syndromic. Until now, mutations in genes such as *TWIST*, *EFNB1*, *FGFR1*, *FGFR2* and *FGFR3* have been shown to play a role in craniosynostosis, whereas genetic factors of non-syndromic cases have not been fully identified yet.The aim of this study is to identify novel genes and gene regions for non-syndromic craniosynostosis cases by utilizing a genetic approach and a high resolution aCGH. Out of 10 patients included in this study, two patients were diagnosed with Craniofrontonasal syndrome and Apert syndrome, respectively. For these two patients, molecular analysis of *EFNB1* and *FGFR2* genes were carried out using Sanger sequencing. The patient diagnosed with Craniosynostosis syndrome has been shown to carry a novel mutation. The other patient carried one of the most common mutations of *FGFR2*; c.755 C>G. For the remaining 8 non-syndromic cases, aCGH analysis was performed by using microarray chips at 5.3KB resolution (SurePrint G3 Human CGH Microarray Kit, 2x400K, Agilent Technologies), and various deletions and duplications were detected. Overall, this study provides insights into the genetic determinants of craniosynostosis, which will contribute to the future studies in the field.

Key Words: Craniosynostosis, nonsyndromic, comparative genomic hybridization

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ÖZET

Kraniosinositoz; kafatası üzerindeki bir veya daha fazla sütürün erken füzyonuyla karakterize bir kraniofasyal malformasyondur. Dünya genelinde canlı doğumların yaklaşık olarak 1/2000-2500'ü kraniosinositozdan etkilenir. Sütürlerin erken kapanması, şekil açısından bozukluk ortaya koyabildiği gibi, beyin gelişimini de doğrudan etkileyebildiği için, hastalığın erken ve doğru tanısı çok önemlidir. Kraniosinositoz tüm ırksal gruplarda görülür ve tüm vakaların %70'inden fazlası non-sendromiktir. Bugüne kadar TWIST, EFNB1, FGFR1, FGFR2 ve FGFR3 gibi genlerindeki mutasyonların kraniosinositozda rol oynadıkları gösterilmişken, non-sendromik vakalardaki genetik etkenler ise hala daha tam olarak çözüme kavuşturulabilmiş değildir.Bu çalışmanın amacı, kraniosinositoz vakalarına genetik yaklaşım ve yüksek çözünürlüklü aCGH yöntemi kullanılarak nonsendromik kraniosinositoz izole vakalarda yeni gen ve gen bölgeleri tespit edebilmektir. Çalışmamıza dahil olan 10 hastadan 2'si kraniosinositoz sendromlarından sırasıyla Craniofrontonasal sendrom ve Apert sendromu tanısı aldı. Bu hastalarımıza sendromlarla ilgili olarak sırasıyla EFNB1 ve FGFR2 gen dizi analizleri Sanger sekans yöntemi ile uygulandı. Bu hastalardan ilkinde EFNB1 geninde literatürde daha önceden tanımlanmamış bir mutasyon tespit edildi. Diğer hastamızda ise FGFR2 geninde sık görülen c.755 C>G mutasyonu tespit edildi. Geriye kalan 8 nonsendromik vakada ise 60 mer, toplamda 411.056 prob içeren, çözünürlüğü 5.3 KB olan "SurePrint G3 Human CGH Microarray Kit, 2x400K (Agilent Technologies)" çipi kullanılarak aCGH uygulandı ve çeşitli bölgelerde delesyon ve duplikasyonlar tespit edildi. Elde edilen verilerin, kraniosinositoza yol açan yolakları aydınlatabilmek adına ön bilgiler sağlayarak kraniosinositozla ilgili yapılacak olan gelecekteki çalışmalara katkı yapacağı öngörüldü.

Anahtar Sözcükler: Kraniosinositoz, nonsendromik, karşılaştırmalı genomik hibridizasyon

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INTRODUCTION

Craniosynostosis is a craniofacial malformation characterized by premature fusion of one or more sutures on the skull. Its clinical severity depends on the time of the anomaly, and anomalies occurring in the early period of development are generally associated with more severe clinical findings. The sutures are thought to allow secondary growth areas against intracranial tissue pressure, and the pressure within the skull separates the cranial bones in the sutures where new bones are deposited for adaptation of the skull and the brain.

Approximately 1 out of 2000-2500 live births worldwide are affected by craniosynostosis. Craniosynostosis occurs in all racial groups, and more than 70% of all cases are non-syndromic. Sagittal synostosis is the most common phenotype, responsible for 40-55% of non-syndromic cases. The second most common type is coronal synostosis with a frequency of 20-25%. Metopic synostosis is the third one with 5-15%, and lambdoid synostosis is responsible for only 0-5% of nonsyndromic cases. Instead of identifying structure and form anomalies, research today is more focused on solving the underlying molecular mechanisms. Despite all the advances, the pathways that cause craniosynostosis are not fully understood, although the knowledge on suture biology is well established (1, 2).

Genetic tests have become an integral part of the diagnostic examination process of suspected genetic diseases. Like many other conditions, craniosynostosis is highly heterogeneous etiologically and can be regarded as a non-genetic or part of a more complex genetic disease. It may manifest as isolated craniosynostosis due to environmental factors and / or genetic changes, or it can be classified as idiopathic due to limited information and impossibilities. Identifying the genetic changes illuminates the differential diagnosis for the patient via assessment of the underlying genetic cause and different clinical situations. Clarification of the diagnosis provides an opportunity for a more precise prediction of the clinical course and correct genetic counseling.

In addition, the risk of recurrence for the affected families, the risk of being seen in their relatives, as well as prenatal diagnosis approach in future pregnancies, even preimplantation genetic diagnosis provides the opportunity. Genetic test results in patients with craniosynostosis can directly affect the patient's follow-up intervals and even treatment decisions.

The first step is clinical evaluation. A patient with craniosynostosis should be evaluated in a multidisciplinary manner by a good cranio surgeon and clinical dysmorphologist. Important clinical information to consider is:

- Which cranial sutures are affected and the shape of the head
- Associated craniofacial abnormalities and dysmorphism
- Brain malformations and / or anomalies
- Extracranial malformations / anomalies (such as skeletal anomalies)
- Findings suggestive of underlying Mendelian inheritance (such as presence of positive family history for craniosynostosis, developmental disorder, seizures).

In the presence of positive family history, motor-mental retardation and / or additional extracranial malformations, karyotype analysis or aCGH may be the first test option to investigate the potential genomic changes. Some craniosynostosis syndromes (such as Apert, Craniofrontonasal syndrome) can be easily recognized by their distinctive clinical findings and the diagnosis can be confirmed with a syndrome-specific genetic test.

In cases where there is absence of symptomatic general appearance and an obvious syndrome, and in the presence of extracranial malformation, a genetic test should be planned according to the affected suture type and the shape of the head.

In the second step, a genetic testing is planned.In the absence of the original syndrome, specific general appearance and extracranial malformations, genetic tests based on the type of the relevant suture and the craniofacial morphology should be planned. The well-defined common craniosynostosis syndromes, genetic etiology, inheritance patterns, clinical and neurological findings are summarized in Table-1.

Table-1. Some syndromes accompanied by craniosynostosis

Disease name	Gene	Inheritance	Clinical Features	Neurological Symptoms
Apert	FGFR2	AD	Craniosynostosis (Coronal), Osseous and / or cutaneous syndactyly in hands and feet	Variable degree of mental retardation, hearing loss, central nervous system malformations
Crouzon	FGFR2 FGFR3*	AD	Craniosynostosis (Coronal, sagittal, lambdoid) Proptosis, maxillary hypoplasia, prominent jaw, (* The type accompanied by acanthosis nigrigans is caused by FGFR3 A391E mutation.)	Headache, seizures, hearing loss
Pfeiffer	FGFR1 FGFR2	AD	Turribracycephaly, hypertelorism, large toe and feet, partial syndactyly in hands and feet	Hydrocephalus, Arnold-chiari malformation
Muenke	FGFR3 spesific variant (P250R)	AD	Coronal craniosynostosis (unicoronal or bicoronal), facial midline hyploplasia	Macrocephaly, sensorineural hearing loss, growth retardation, intellectual delay
Saethre-Chotzen	TWIST1 FGFR2	AD	Craniosynostosis (Coronal, lambdoid and / or metopic), mild syndactyly, congenital heart defect	Intracranial hypertension, parietal foramina
Shprintzen-Goldberg	SKI	AD	Joint laxity / contractures, Pectus excavatum / carinatum, scoliosis, camptodactyly, arachnodactyly, umbilical hernia, cardiac anomalies aortic root dilatation, MVP	Hypotonia, growth retardation, mental retardation, hydrocephalus, Arnold chiari malformation
Baller-Gerold	RECQL4	AR	Craniosynostosis (Coronal, metopic, lambdoid), short stature, underdeveloped or undeveloped thumb, cardiac-renal-vertebral anomalies	Intellectual delay
Antley-Bixler	FGFR2 POR	AR	Craniosynostosis (Coronal and lambdoidal), radiohumeral synostosis; narrow chest, choanal atresia, or stenosis	Hydrocephalus, intellectual delay
Greig Cephalopolysyndactyly	GLI3	AD	Polydactyly and syndactyly	Hydrocephalus, corpus callosum agenesis
Craniofrontonasal syndrome	EFNB1	XLD	Coronal craniosynostosis, hypertelorism, scoliosis, wide toe (Girls are more severely affected than boys)	Hypotonia, corpus callosum hypoplasia, growth retardation
Carpenter	RAB23 MEGF8	AR	Craniosynostosis (Coronal, sagittal, lambdoid), obesity, cardiac and renal defects	Growth retardation, Hearing loss, optic atrophy
Beare-Stevenson cutis gyrata	FGFR2	AD	Craniosynostosis, clover leaf skull, respiratory distress, palm and soles wrinkles, cutis girata on the back of the scalp and lumbosacral region, acanthosis nigricans	Growth retardation, hydrocephalus, corpus callosum agenesis
Cranioectodermal dysplasia	IFT122 WDR35 IFT43 WDR19	AR	Heterogeneous ciliopathy, skeletal anomalies (sagittal craniosynostosis, about 50% of patients), Narrow rib cage, short limbs, brachydactyly, ectodermal defects, nephronophyllis, hepatic fibrosis, heart defects, retinitis pigmentosa	Neuromotor growth retardation (in some patients)
Boston type craniosynostosis	MSX2	AD	Turribracycephaly due to coronal craniosynostosis, fronto-orbital recession, frontal dislocation, clover leaf head	Rarely headache, seizures

In this study, the patients with craniosynostosis were classified as syndromic and non-syndromic by applying a molecular genetic approach. Then, known-gene analyses were performed in syndromic patients by Sanger sequencing. In the remaining non-syndromic cases, the *FGFR2* gene, which is the most mutated gene in craniosynostosis cases, was also investigated by Sanger sequencing.

A whole genome analysis with high resolution array-based comparative genome hybridization (aCGH) was performed in order to detect any novel genes to reveal the genetic basis of the patients with no mutations.

MATERIALS and METHODS

The diagnosis of craniosynostosis was made and the fact that the main problem was related to primary craniosynostosis was determined as the inclusion criteria. In our study, craniosynostosis patients were classified as syndromic and non-syndromic, and patients with non-syndromic craniosynostosis whose known genetic causes were excluded were included in our aCGH study. The patients to be included in the study, after detailed history, physical examination, radiological imaging, biochemical tests, metabolic tests and analyses were performed, patients without trauma, infection and hypoxia sequela, and those with normal pre- and perinatal-postnatal histories were determined. As a result of the evaluations, a total of 10 patients were included in our study. Informed consent forms were obtained from the families of all patients.

DNA isolation was performed from blood samples taken from 10 patients included in the study. Two of the patients were diagnosed with syndromic craniosynostosis, and sequencing of these patients was performed using Sanger sequencing for the *EFNB1* and *FGFR2* genes responsible for the syndrome. The other 8 patients were evaluated as non-syndromic craniosynostosis and the aCGH method was used for genetic etiological research. In the aCGH study, the SurePrint G3 Human CGH Microarray Kit, 2x400K (Agilent Technologies) chip was used. Ethical approval was obtained from Cerrahpaşa Faculty of Medicine Clinical Research Ethics Committee for our study. (No: B.30.2.İST.0.30.90.00/29617)

RESULTS

Since the type of affected suture causing craniosynostosis was different for each patient included in the study, detailed anamnesis of each patient was taken one by one, and all other system examinations were performed in terms of possible accompanying anomalies. The clinical diagnoses, physical examination and radiological findings of the patients are summarized in Table-2.

Table-2. Clinical diagnosis, physical examination and radiological findings of the patients

	Case No	Clinical Diagnosis	Physical examination findings	Radiological findings
Syndromic	1	CFNS	Brachycephaly, wide anterior fontanel, hypertelorism, telecanthus, downslanting palpebral fissures, broad nasal root, bifid nasal tip, short neck, right hallux duplication	Bilateral coronal synostosis, wide anterior fontanel and prominent metopic suture
	2	Apert syndrome	Brachycephaly, downslanting palpebral fissures, hypertelorism, bilateral syndactyly between the 3rd and 4th fingers in the hands, total cutaneous syndactyly in the feet	Bilateral coronal synostosis
Non- Syndromic	1	Plagiocephaly	Asymmetry on the head and face, flattened face profile, long philtrum, low set ears	Metopic suture synostosis, coronal suture partially synostosis on right, lambdoid suture upper part synostosis on left
	2	Plagiocephaly, Multiple suture synostosis	Asymmetry on the head and face, scar marks secondary to previous suture surgery, wide forehead, flattened nasal root, bulbous nose	Closed coronal, sagittal and lambdoid sutures.
	3	Isolated Brachycephaly	Abnormal head shape (brachycephaly), broad forehead, flattened nasal root, thin lips	Bilateral coronal synostosis
	4	Isolated Brachycephaly	Abnormal head shape (brachycephaly), broad forehead, flattened nasal root, thin lips	Bilateral coronal synostosis
	5	Anterior Plagiocephaly	Severe swelling in the right frontal region, depression in the left parietal region and left supraorbital region, increased anteroposterior diameter of the head, facial asymmetry, back located ears, micrognathia	Anterior plagiocephaly in which all sutures are closed
	6	Brachycephaly	Microcephaly, strabismus, high narrow palate, mild exophthalmos, long face, persistent milk teeth in the upper jaw, gum hyperplasia, prominent jaw, pectus carinatum	Closed coronal and lambdoid sutures
	7	Isolated Brachycephaly	Wide forehead, prominent philtrum, dimple in chin, reduction in head anterior posterior diameter	Closed both coronal sutures and metopic sutures are
	8	Plagiocephaly	Head deformity, flat occiput, narrow forehead, low forehead line, facial asymmetry, depressed nasal root, nasal bridge collapsed, nasal tip depressed, filterum long, proptosis (more prominent on the left)	Partially closed coronal sutures and right lambdoid suture

In our case with a diagnosis of CFNS, one of our syndromic patients, it was confirmed that the newly unidentified missense mutation we detected in the 2nd exon in the EFNB1 gene changed the protein configuration and was pathological. Additional skeletal anomaly (duplicated hallux) found in our patient was seen as a rare finding accompanying CFNS patients in the literature. (3). Interestingly, when the cases of duplicated hallux accompanying CFNS in the literature were examined, it was seen that mutations in all cases were detected in the first 2 exons of the gene. In our other patient with Apert syndrome, S252W mutation was detected in the *FGFR2* gene. It is known that this mutation is the most common mutation in patients with Apert syndrome.

In our study for patients with non-syndromic craniosynostosis, 2x400K Array chips were used for 8 patients. As a result of the analysis of the data with CytoGenomics software, some chromosomal deletions and duplications were detected in the patients.

In case 1, a duplication of 148,729 bp in the 2p11.2 region, a deletion of 584,094 bp in the 8p23.1 region, a duplication of 478,952bp and 369,565bp in the 14q32.33 region, respectively, and a deletion of 2,275,008 bp in the 15q11.1-q11.2 region were detected.

In case 2, 112,809 bp duplication in 1p21.1 region, 97.546 bp deletion in 7p14.1 region, 151.167 bp duplication in 8p11.22 region, duplication in 9p12-p11.2 region, 692.204bp in regions 14q11.2 and 14q32.33, respectively Duplications of 220.129bp, 334.112bp in length and 195,479 bp in length in the 22q11.22 region were detected.

Case 3 was not included in the analysis because the QC (Quality control) assessment could not provide sufficient analysis values.

In case 4, 730.941 bp duplication in the 10q11.22 region, 126.435bp deletion in the 10q26.3 region, 1.044.088 bp, 220.129bp and 334.112bp duplications in the regions 14q11.2 and 14q32.33, respectively, and 189.327 bp duplication in the 22q11.22 region were detected.

In case 5, 176,972 bp duplication in the 2p11.2 region, 96,222 bp duplication in the 4q13.2 region, 77,368 bp deletion in the 7p14.1 region, 1,044,088 bp and 634,499 bp duplication in regions 14q11.2 and 14q32.33 respectively, and, 2,145,780 bp duplication in the 15q11.1-q11.2 region were detected.

In case 6, 148,295 bp deletion in the 3p14.2 region, 316,309 bp deletion in the 5q33.1 region, 514,093 bp deletion in the 8p23.1 region, 628,448 bp deletion in the 10q11.22 region, and 478,952 bp duplication in the 14q32.33 region were detected.

In case 7, 176,972 bp duplication in the 2p11.2 region, 151,167 bp duplication in the 8p11.22 region, 692,209 bp and 634.499 bp duplications in the 14q11.2 and 14q32.33 regions, respectively, and 189.327 bp duplication in the 22q11.22 region were detected.

In case 8, 176.972 bp duplication in 2p11.2 region, 100.502 bp duplication in 3p26.1 region, 96.222 bp duplication in 4q13.2 region, 81.737 bp deletion in 7p14.1 region, 151.167 bp duplication in 8p11.22 region, and 189.327 bp in 22q11.22 region bp duplication were detected. All chromosomal changes are summarized in Table-3.

Table 3. Copy number changes and characteristics of the cases

Case No	Chr	Band	Start (bp)	Stop (bp)	Change Size (bp)	Log	Prob Number	Interpretation
	1	1p21.1	104.098.248	104.211.056	112,809	1,09	10	Benign
1	2	2p11.2*	89.163.862	89.312.590	148.729	0.897	22	Benign
	7	7p14.1 *	38.296.176	38.393.721	97,546	-0,616	20	Benign
	8	8p23.1 *	7.169.490	7.753.583	584,094	-1,37	8	Benign
	8	8p11.22 *	39.234.992	39.386.158	151,167	4,018	28	Benign
	14	14q32.33 *	106.331.956	106.810.907	478,952	3.116	61	Benign
	14	14q32.33 *	106.848.573	107.218.137	369,565	0,938	72	Likely Benign
	15	15q11.1-q11.2*	20.481.702	22.756.709	2.275.008	-0,9	62	Likely Benign
2	2	2p11.2*	89.135.619	89.312.590	176.972	1,05	27	Benign
	9	9p12-p11.2	43.590.080	47.212.417	3.622.338	0,716	8	Likely Benign
	9	9p22.2	17.167.590	17.216.453	48,864	-1,026	9	Benign
	10	10q11.22	46.971.947	47.702.587	730,941	0,774	54	Benign
	14	14q11.2 *	19.728.641	20.420.849	692,204	0,938	31	Benign
	14	14q32.33 *	106.331.956	106.552.084	220,129	2.336	30	Benign
	14	14q32.33 *	106.632.343	106.966.454	334,112	0,709	53	Benign
	22	22q11.22 *	23.050.410	23.245.888	195,479	3.026	51	Benign
4	2	2p11.2*	89.135.619	89.312.590	176.972	0,951	27	Benign
	4	4q13.2*	69.387.056	69.483.277	96,222	0,881	12	Benign
	7	7p14.1 *	38.296.176	38.393.721	77,368	-0,739	17	Benign
	10	10q26.3	135.252.327	135.378.761	126,435	-0,907	24	Benign
	14	14q11.2*	19.376.762	20.420.849	1.044.088	0,994	35	Benign
	14	14q32.33*	106.331.956	106.552.084	220,129	2,531	30	Benign
	14	14q32.33 *	106.632.343	106.966.454	334,112	0,782	53	Benign
	22	22q11.22 *	23.056.562	23.245.888	189,327	2,936	50	Benign
5	3	3p14.2	60.367.507	60.515.801	148,295	-0,852	30	Benign
	5	5q33.1	151.231.557	151.547.865	316,309	-1,087	48	VUS/Likely Benign
	14	14q11.2 *	19.376.762	20.420.849	1.044.088	1,175	35	Benign
	14	14q32.33 *	106.331.956	106.966.454	634,499	1,014	86	Benign
	15	15q11.1-q11.2*	20.432.851	22.578.630	2.145.780	1,641	62	Likely Benign
6	2	2p11.2*	89.135.619	89.312.590	176.972	1,123	27	Benign
	8	8p11.22*	39.234.992	39.386.158	151,167	4,005	28	Benign
	8	8p23.1 *	7.239.491	7.753.583	514,093	-1,366	7	Benign
	10	10q11.22	46.968.072	47.596.519	628,448	-1,154	38	Benign
	14	14q32.33*	106.331.956	106.810.907	478,952	3,32	61	Benign
7	2	2p11.2*	89.135.619	89.312.590	176.972	1,357	27	Benign
	3	3p26.1	4.053.328	4.153.829	100,502	0,875	21	Benign
	14	14q11.2 *	19.728.641	20.420.849	692,209	0,967	31	Benign
	14	14q32.33 *	106.331.956	106.966.454	634,499	1,162	86	Benign
	22	22q11.22 *	23.056.562	23.245.888	189,327	3,021	50	Benign
8	4	4q13.2*	69.387.056	69.483.277	96,222	0,942	12	Benign
	7	7p14.1*	38.291.807	38.373.543	81,737	-0,837	18	Benign
	8	8p11.22*	39.234.992	39.386.158	151,167	4,34	28	Benign
	22	22q11.22*	23.056.562	23.245.888	189,327	3,354	50	Benign

*: Changes in these regions were seen in more than one patient.

DISCUSSION

In the literature, there are many studies using aCGH on craniosynostosis. For example, in a study conducted in 2007, genetic investigations were performed in a 6-month-old male patient with complex craniosynostosis (metopic, sagittal and lambdoid sutures), prenatal-onset growth retardation, growth retardation, facial dysmorphism, congenital heart defect and inguinal hernia. In the conventional karyotype examination of the patient, a derivative of 5th chromosome was detected. The karyotypes of both parents were found to be normal. Later, 75Kb resolution aCGH was applied and a duplication was found in the 5q35 region and the region containing the *MSX2* gene was found to have an extra copy in FISH study (4).

In their study, F S Jehee et al. examined 45 patients with craniosynostosis using various methods such as conventional karyotype, subtelomeric MLPA and whole genome aCGH, and abnormalities were detected in 9 of these patients with conventional karyotype and submicroscopic changes were detected in 10 patients. Array CGH was administered to 28 patients, changes were detected in a total of 12 patients, and two of them had significant changes that could cause craniosynostosis. A duplication of 3.9-4.5Mb in the Xq22.3 region in a patient with metopic suture synostosis and a duplication of 0.9Mb in the Xp22.2 region in another patient with metopic and bilateral coronal synostosis were detected (5).

Heather C. Mefford and colleagues evaluated 187 patients with single-suture craniosynostosis for submicroscopic deletions and duplications using whole-genome aCGH, and detected 1.1Mb duplication involving the *RUNX2* gene in two affected cousins with metopic synostosis and hypodontia (6).

W. Al-Hertani et al. detected 2.68 Mb deletion in the 22q11.21 (chr22:17,256,416-19,941,337, hg18) region using FISH and SNP microarray (Affymetrix SNP 6.0) in a male infant baby with severe multi sutural synostosis and increased intracranial pressure with brain structures displaced and intense skull erosion (7).

In their study, Ariana Kariminejad et al. found 46, XX, add (2) (q37) chromosome establishment in a 1-year-old male patient with metopic and coronal synostosis and dysmorphic findings by conventional karyotype examination. They made 244K oligonucleotide aCGH in order to determine the origin of the chromosomal part added to the patient and as a result; deletion of 2Mb in 2q37 → ter region and 15Mb duplication in 5q34 → ter region were detected, and this duplication includes 70 genes including *MSX2* gene (8).

In another study conducted in 2011, an interstitial deletion of 300 kb was detected in the Xp22.11 region by using microarray-based copy number variation (CNV) analysis (Affymetrix 250 K SNP) in an 11-year-old male patient with periventricular nodular heterotopia, craniosynostosis, growth retardation and multiple congenital anomalies previously followed up with a pre-diagnosis of Fontaine-Farriaux syndrome (9).

In a study published in 2012; array was performed using Affymetrix SNP 6.0 platform in a 13-month-old male patient with skeletal anomalies such as right coronal suture synostosis and accompanying bilateral proximal interphalangeal joints flexion contractures, small feet, lumbosacral dextroscoliosis and bilateral hip dislocation. As a result, 16 different long contiguous stretches of homozygosity (LCSH) regions of 3Mb and larger were found on 12 different chromosomes. However, no study has been conducted on the genes found in these regions (10).

In a study published in 2012, genetic analysis was performed in a boy and a girl with craniosynostosis and additional dysmorphic features. Metopic and sagittal suture synostosis were detected in a male patient, and left coronal and sagittal suture synostosis was observed in a female patient. Patients were analyzed using the Affymetrix 250K SNP array platform. Deletions of 6.9 and 6.8Mb in the chromosome 2p15p16 region were detected in the patients, respectively. Although this region has been previously defined as a microdeletion syndrome in the literature, craniosynostosis has not been described in the patients. This study suggested that complex craniosynostosis should be examined in this microdeletion syndrome (11).

In our study, no pathogenic or likely pathogenic change was detected in our patients within our current knowledge.In recent years, advanced molecular investigations for genetic diagnosis such as whole exome or whole genome sequencing have led to the molecular characterization of craniosynostosis syndromes, emphasizing the importance of genetic heterogeneity of these syndromes and gradually eliminating the ambiguities between syndromic and non-syndromic groups (12). For example, in a study conducted in 2015, individuals with a different phenotype with severe craniosynostosis, especially involving coronal sutures, caused by heterozygous variants in the last exon of the *ZIC1* gene in 5 different families, and with variable learning disabilities were identified by whole genome sequencing (13). In a different study conducted in 2017, whole exome and/ or whole genome sequencing was performed in 40 patients resulting in no mutation in previously known genes, however mutations in 14 novel genes were detected in 15 patients (37.5%) (14). As a result, genetic characterization of patients affected by craniosynostosis suggests a more appropriate genetic counseling and provides information on comorbidity and prognosis.

Conflict of interest

No conflict of interest was declared by the authors.

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