Investigation of Submicroscopic Chromosomal Anomalies on Patients with Unexplained Intellectual Disabilities with Molecular Karyotyping

Nedeni Açıklanamayan Zihinsel Yetersizliği Olan Hastalarda Submikroskopik Kromozomal Anomalilerin Moleküler Karyotipleme ile Araştırılması

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ABSTRACT

Backround: Intellectual disabilities, developmental delay and accompanying congenital anomalies are rarely seen on general population, but have a large etiologic spectrum. Chromosomal abnormalities are one of the significant reasons of intellectual disabilities, dysmorphic appearance and various congenital anomalies. Conventional cytogenetic techniques can only detect abnormalities greater than 5 Mb. Array based methods can be useful to detect anomalies smaller than 3 Mb to kilobase levels with a ratio of 12-14%. As a result, small interstitial deletions and duplications could be detected and new genes could be discovered with microarray techniques.
Methods: In this study, 29 patients with idiopathic intellectual disability, developmental delay and/or congenital anomaly, had been investigated for small deletions or duplications with "Array CGH 8x60K ISCA".
Results: Causative/ probably causative pathology was detected in 6 patients and the diagnostic power of these systems was confirmed, and we obtained a yield of 20.6%. Feingold syndrome 1, Williams syndrome with atypical findings, 14q11.2 deletion syndrome and 1p36 deletion syndrome, 13q14.3-q21.1 duplication spanning PCDH17 gene and a duplication in Xp11.4 chromosomal region containing ATP6AP2 gene were detected. We reported second Feingold syndrome with renal agenesis and first case of 14q11.2 deletion syndrome with episodic vomitting attacks.
Conclusion: Microarray technology is the first-tier diagnostic method in patients with intellectual disability with multiple congenital anomalies. The genotype-phenotype correlation studies provide explaining the etiology and molecular mechanism of intellectual disability and developmental delay.

Key Words: Microarray, Intellectuel disability, Feingold syndrome, 14q11.2 deletion syndrome.

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ÖZET

Amaç: Zihinsel yetersizlikler, gelişme geriliği ve eşlik eden konjenital anomaliler genel popülasyonda nadiren görülmekle birlikte geniş bir etiyolojik spektruma sahiptir. Kromozomal anormallikler, zihinsel yetersizlik, dismorfik görünüm ve çeşitli konjenital anomalilerin önemli nedenlerinden biridir. Geleneksel sitogenetik yöntemler yalnızca 5 Mb'den büyük anormallikleri tespit edebilir. Dizi tabanlı yöntemler, 3 Mb'den kilobaz seviyelerine kadar olan anomalileri yaklaşık %12-14 oranında tespit etmek için faydalı olabilir. Sonuç olarak, mikroarray teknikleri ile küçük interstisyel delesyonlar ve duplikasyonlar tespit edilebilir ve yeni genler keşfedilebilir.
Yöntem: Bu çalışmada, nedeni açıklanamayan zihinsel yetersizlik, gelişimsel gecikme ve/veya doğuştan anomalisi olan 29 hastada "Array CGH 8x60K ISCA" ile küçük delesyonlar veya duplikasyonlar araştırıldı.
Bulgular: 6 hastada muhtemel genetik patoloji saptandı ve bu sistemlerin tanısal gücü doğrulanarak %20,6 verim elde ettik. Feingold sendromu 1, atipik bulguları olan Williams sendromu, 14q11.2 delesyon sendromu ve 1p36 delesyon sendromu, PCDH17 genini kapsayan 13q14.3-q21.1 duplikasyonu ve ATP6AP2 geni içeren Xp11.4 kromozomal bölgesinde duplikasyon tespit edildi. Çalışmada renal agenezili ikinci Feingold sendromunu ve epizodik kusma atakları olan ilk 14q11.2 delesyon sendromu vakasını bildirdik.
Sonuç: Mikroarray teknolojisi, çoklu konjenital anomalileri olan zihinsel yetersizliği olan hastalarda ilk basamak tanı yöntemidir. Genotip-fenotip korelasyon çalışmaları, zihinsel yetersizlik ve gelişimsel gecikmenin etiyolojisi ve moleküler mekanizmasının açıklanmasını sağlar.

Anahtar Sözcükler: Mikroarray, Zihinsel yetersizlik, Feingold Sendromu, 14q11.2 delesyon sendromu

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INTRODUCTION

Intellectual disability (ID) causes limitations in the mental activities and cognitive behaviors of the individuals (1). Incidence of disorder has been reported to be 2-3% in the world. It is more common in men than in women. Because, X-linked intellectual disability group diseases mostly affect males (2,3).

Motor development delay, cognitive impairment and speech delay are seen with ID in many cases (4). Causes of ID and accompanying anomalies are investigated in a wide spectrum. Genetic diseases are the most common causes of ID that are detected in approximately 40-50% of cases (5). The frequency of numerical or structural chromosomal anomalies, microdeletion syndromes and single gene diseasesdetected in patients withintellectual disability is quietly high (6). Patients who have not detected any chromosomal changes or cannot be associated with any of the genetic syndromes with karyotype analysis and fluorescent in situ hybridization method (FISH), are definied as unexplained intellectual disability groups. High resolution molecular cytogenetic methods should be used to detect smaller deletions and duplications that cannot be detected by conventional methods (7).

Microarray technology is a successful method in patients with ID whose cause has not been determined, and the rate of diagnosis varies approximately between 15-24%. Identification of specific copy number variation (CNV) in affected cases has enabled the discovery of new microdeletion and microduplication syndromes (8).

In this study, the array CGH results of 29 developmental delay or idiopathic ID patients with dysmorphic features and / or congenital anomalies that could not be diagnosed by conventional methods were analyzed.

MATERIALS and METHODS

Patients

Twenty nine patients who were referred to Medical Genetics Department of Gazi University Faculty of Medicine between 2013 and 2014 were included in the study. The patients were selected from the group of cases with developmental delay or idiopathic ID with dysmorphic findings and / or congenital anomalies, could not be clinically associated with a specific syndrome. The clinical featuresand laboratory findings of patients with chromosomal changes detected by array CGH analyses were summarized in Table 1.

Table 1: The clinical features and laboratory findings

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Sex	M	M	M	F	F	F
Age	2 months old	9 years old	18 years old	5 years old	11 years old	3 years old
Perinatal Information	-	Icterus	Respiratory Failure	IUGR	Placenta previa	Hypotonia
Parental Consanguinitiy	-	-	+	+	+	-
Weight	<3p	25-50p	25-50p	<3p	10-25 p	50-75 p
Length	3-10p	10-25p	<3p	<3p	<3p	25-50p
Head circumference	3p	75-90p	10-25p	3p	<3p	97p
Neurodevelopmental findings	Developmental delay	Intellectual disability Speech delay Stereotype Behavioral abnormality	Intellectual disability	Intellectual disability Developmental delay Speech delay	Intellectual disability Developmental delay Speech delay Epilepsy	Intellectual disability Epilepsy
Dysmorphic Findings	Epicantic folds Wide nose Long philtrum Retrognathia High palate Thumb anomaly Simian line Bilateral clinodactyly Scrotal hypoplasia	Triangular face Wide forehead Irregular teeth	Brachycephaly Coarse face Prominent nose Short philtrum *Carp-shaped mouth* Full lips Wide and open mouth Loose skin Low hairline Contractures at elbows Brachydactyly Camptodactyly	Prominent columella Small and irregular teeth Simple ears Joint hypermobility	Upslanted palpebral fissures Strabismus Hammer toe deformity	Middle face hypoplasia Short philtrum Irregular teeth Small and dysmorphic ears
Other findings	Duodenal atresia		Scoliosis	Episodic vomiting attacks	PDA, VUR	Rickets
Imaging Findings	ECHO: VSD Renal USG: Left renal agenesis	Cranial MRI: Cortical atrophy	Vertebral graphy: Scoliosis	Cranial MRI: NCranial Diffusion MRI: N	Cranial MRI: Syringomyelia EEG abnormality	EEG abnormality

M:Male, F:Female, IUGR:Intra uterine growth retardation, PDA:Patent ductus arteriozus, VUR: Vesicoureteral reflux, VSD:Ventricular septal defect

This study was approved by Kecioren Research and Training Hospital Ethics Committee in January 13, 2016 with the decision number 1057.

DNA extraction and molecular analysis

Genomic DNA was extracted from peripheral blood, using a salting-out isolation method according to standard protocols.

Array-CGH analysis was performed using the 8x60K ISCA, Agilent® microarray platform (Human Genome CGH Microarray, Agilent Technologies Inc., Santa Clara, CA, USA) according to the manufacturer's protocol. Due to lack of funds reasons, parental studies have not been performed.

Variant Interpretation

For interpretation of CNVs the standard probe cut off levels used in routine diagnostics at the Department of Medical Genetics, Gazi University Hospital; (i) CNVs of 100 kb and over, (ii) including the gene, (iii) absolute log2-ratios above 0.25, (iv) CNVs with a minimum of three consecutive probes. The obtained data were analysed according to human genome version hg19 (GRCh37) using Agilent CytoGenomics software (v.2.0.6.0).

CNVs were interpreted as benign listed in Database of Genomic Variants (DGV) database (n > 3 studies or documented in >1% of the normal population) or no gene included (not close to known Intellectual disability genes within 1 Mb). CNVs was classified as pathogenic copy number variations if these CNVs overlapped completely with the minimal critical region of a wellknown microdeletion or microduplication syndrome, or if the CNV comprised a dosage-sensitive gene known to cause a similar phenotype, referring to ClinGen Dosage Sensitivity Map. Rare CNVs (<1% in normal population and without OMIM genes) that do not meet the criteria above, should be considered as variant of uncertain clinical significance (VOUS) (9). For interpretation of these results, our in-house database and the following public database were used: DGV (http://projects.tcag.ca), Cytogenomics Array Group CNV Database (https://www.cagdb.org), Database of Chromosomal Imbalance and Phenotype in Humans using Ensembl Resources database (DECIPHER, http://decipher.sanger.ac.uk), University of California Santa Cruz (UCSC, http://genome.ucsc.edu/, hg19), ClinVar (http://www.clinvar.com), Online Mendelian Inheritance in Man (OMIM, http://www.omim.org) and PubMed (The U.S. National Library of Medicine) (https://www.ncbi.nlm.nih.gov/pubmed)

RESULTS

The chromosomal changes that could explain the phenotype or be related to the patient's findings were detected in 6 of 29 patients. Rest of the patient results did not have a CNV that meets the evaluation criteria. The CNV detection rates were 20.6%. Table 2 shows the summary CNV findings of the changes that explain the patient's clinic were detected. Of the 6 CNVs detected, 4 were classified as pathogenic(Cases: 1, 3, 4, 5). Pathogen CNVs, which were 2p24.3-p23.3deletion in 10.5 Mb size [arr(hg19) 2p24.3-p23.3 (13,664,684-24173207)x1], 7q11.23 deletion in 1.3 Mb size [arr(hg19) 7q11.23 (72,766,313-74,133,332)x1], 14q11.2 deletion in 1 Mb size [arr(hg19)14q11.2 (21,892,210-22,897,089)x1], and 1p36.33-p36.31 deletion in 5 Mb size [arr(hg19) 1p36.33-p36.31 (1,109,858-6,147,900)x1] (Table 2). In 2 cases (Case 2 and 6), VOUS were detected which were 13q14.3-q21.1 duplication in 6.1 Mb size [arr(hg19) 13q14.3-q21.1 (53,402,739-59,547,533)x3] and Xp11.4 duplication in 543 kb size [arr(hg19) Xp11.4 (39,953,078-40,496,404)x3]. Pathogenic CNV was further confirmed by FISH technique in Case 3.

Table 2: Results of the microarray analyses

Case	Chromosomal region	Genomic coordinates (GRCh37/hg19)	Genes	Size (kb)	Deletion/ Duplication	Pathogenity	Diagnosis
1	2p24.3-p23.3	13664684-24173207	*NBAS, DDX1, MYCNOS, MYCN, VSNL1, SMC6, GEN1, MSGN1, KCNS3, OSR1, MATN3, SDC1, PUM2, HS1BP3, GDF7, APOB, FAM84A, FAM49A, RAD51AP2, RDH14, NT5C1B, RDH14, NT5C1B, FLJ12334, TTC32, WDR35, LAPTM4A, RHOB, C2orf43, KLHL29, ATAD2B, UBXN2A*	10.508.524	Deletion	Pathogenic	Feingold Syndrome 1 (MIM #164280)
2	13q14.3-q21.1	53402739-59547533	*PCDH8, PCDH17, OLFM4, MIR1297, PRR20B, PRR20C, PRR20D, PRR20E, PRR20A*	6.144.795	Duplication	VOUS	
3	7q11.23	72766313-74133332	*FKBP6, FZD9, BAZ1B, BCL7B, TBL2, MLXIPL, VPS37D, STX1A, WBSCR26, CLDN3, CLDN4, WBSCR27, WBSCR28, ELN, LIMK1, EIF4H, LAT2, RFC2, CLIP2, GTF2IRD1, GTF2I, DNAJC30, WBSCR22, MIR4284, ABHD11, MIR590*	1.367.020	Deletion	Pathogenic	Williams syndrome (MIM #194050)
4	14q11.2	21892210-22897089	*CHD8, RAB2B, METTL3, SALL2, TOX4, OR10G3, OR10G2, OR4E2*	1.004.880	Deletion	Pathogenic	14q11.2 deletion syndrome (MIM #613457)
5	1p36.33-p36.31	*21892210-22897089*	*GABRD, KCNAB2, DVL1, PRDM16, SKI and other 71 genes*	5.038.043	Deletion	Pathogenic	1p36 deletion syndrome (MIM #607872)
6	Xp11.4	39953078-40496404	*BCOR, ATP6AP2, LOC347411, CXorf38*	543.327	Duplication	VOUS	

VOUS: Variant of unknown significance

DISCUSSION

Microarray technology in patients with ID is a successful method and the rate of diagnosis varies approximately between 15-24% (8). Authors suggested that it is the first-tier test that can be applied in cases with idiopathic ID and congenital anomalies (10). Rapid and accurate diagnosis of patients is very important for effective genetic counseling. As widespread studies continue, the etiology of idiopathic ID cases will be elucidated (11). In our study including 29 cases, we obtained a yield of 20.6%. All patients we included in the study was a group that had a detailed clinical examination and had a high probability of submicroscopic deletion. Patients with suspected to have syndromes due to single gene mutations were eliminated. We beleive that these choices affect the rate of diagnosis with array CGH. Different diagnostic rates in several studies are related with the correct selection of the patient groups.

In Case 1,10.5 Mb deletion was detected at 2p24.3-p23.3 chromosomal region which is compatible with Feingold Syndrome 1 (FGLDS1; MIM# 164280) (Table 2). Hemizygous submicroscopic deletion and heterozygous pathogenic variation including *MYCN* gene is cause of FGLDS1. FGLDS1 is characterized by microcephaly, hand and foot anomalies, facial dysmorphism, developmental delay, mild-to-moderate learning disability and gastrointestinal atresias (primarily esophageal and/or duodenal). Cardiac and renal malformations, vertebral anomalies, and deafness have also been described in a minority of patients (12). Our patient had unilateral renal agenesis as well as all the characteristic findings of FGLDS1 syndrome (Table 1). Renal abnormalities which is mostly reported to date include horseshoe kidneys, dysplastic kidneys, hydronephrosis and pelvic dilatation, chronic nephritis, and vesicourethral reflux leading to renal dysplasia and renal failure in FGLDS1 (13). To the best of our knowledge, the agenesis of unilateral kidney seen in our patient is second reported case in the literature (14).

Array CGH revealed6.1 Mb duplication at 13q14.3-q21.1 chrosomal region in Case 2 (Table 2). In DECIPHER, Patient 259961with 4.83 Mb *de novo*heterozygous duplication (13:56731929-61561425) had common clinical findings such as behaviour abnormality, speech delay and stereotype with our Case 2. These cases' duplication location is approximately overlap and contains *PCDH17, PRR20A PRR20B, PRR20C, PRR20D* and*PRR20E* genes (Table 3). Protocadherin 17 (*PCDH17*) a neuronal cell adhesion molecule, expressed at high levels in the frontal, anterior temporal cortex and thalamus. The expression change of the *PCDH17* gene can cause language and speech disorders (15,16).

Table 3: The comparison of Case 2 and Patient 259961 in DECIPHER

	Case 2	**Patient 259961**
Genes	*PCDH8,* ***PCDH17****, OLFM4, MIR1297,* ***PRR20A, PRR20B, PRR20C, PRR20D, PRR20E***	***PCDH17****, PCDH20,* ***PRR20A, PRR20B, PRR20C, PRR20D, PRR20E****, DIAPH3, TDRD3*
Phenotypes	Intellectual disability **Behavioral abnormality** **Delayed speech and language development** **Stereotypy**	**Behavioral abnormality** **Delayed speech and language development,** **Stereotypy** Dysarthria Feeding difficulties Short stature Recurrent infections Strabismus Urinary incontinence

Overlapping genes and common clinical features are shown in bold

Recently in a study consist of single-nucleotide polymorphisms spanning the *PCDH17* region are significantly associated with major mood disorders; subjects carrying the risk allele showed impaired cognitive abilities, increased vulnerable personality features, decreased amygdala volume and altered amygdala function as compared with non-carriers. The risk allele predicted higher transcriptional levels of *PCDH17* mRNA in postmortem brain samples, which is consistent with increased gene expression in patients with bipolar disorder compared with healthy subjects. Authors suggested thattheir results which is revealed brain expressed *PCDH17* as a susceptibility gene for major mood disorders.

Further, overexpression of *PCDH17* in primary cortical neurons revealed significantly decreased spine density and abnormal dendritic morphology compared with control groups, which again is consistent with the clinical observations of reduced numbers of dendritic spines in the brains of patients with major mood disorders (17). Although we do not have the chance to prove this yet, the available data in the literature suggest that it may be related to the dosage increase in the behavioral abnormality, speech delay, stereotype and cortical atrophy *PCDH17* gene in our patient. Despite the fact that new studies are required to support to clarify the data on duplication / dosage increase in the *PCDH17* gene, and the variant we find remains in the VOUS category, it seems to be a valuable data for new researches.

Sometimes the diagnosis may be delayed in cases that have atypical findings of known syndromes. In this study, array CGH results revealed that being Williams syndrome (WS) in a patient (Case 3), 18 years old male, with atypical findings of the syndrome (Table 1-2). WS is a multisystem disorder caused by hemizygous deletion of 1.5 to 1.8 Mb on chromosome 7q11.23, which contains approximately 28 genes. Although several genes of interest (e.g. elastin gene; *ELN*) are within critical region recurrent microdeletion, no single gene in which pathogenic variants are causative of WS has been identified. WS is characterized by cardiovascular disease, distinctive facies, connective tissue abnormalities, intellectual disability, a specific cognitive profile, unique personality characteristics, growth abnormalities, and endocrine abnormalities (18,19). Case 3 had been followed up with a diagnosis of severe ID in various centers for a long time. In our case only a wide mouth and full lips were present among the characteristic facial features of the syndrome, except that the facial findings were different enough to not suggest this syndrome (Table 1). *GTF2IRD1*gene has been implicated in craniofacial features of WS. Although our case had *GTF2IRD1* gene deletion, he did not have the characteristic facial findings of the syndrome (20). Alesi et al. reported three cases who shared *CLIP2*, *GTF2IRD1* and *GTF21* genes with in deletion region with our case. Short philtrum, which is different from the long philtrum seen in WS, and scoliosis, which is a rare finding in WS was present in 2 of 3 cases and in our case. In our case, while there was a more severe ID that can be seen in cases with classical WS, mild ID was defined in 3 cases described by them. This suggests that the ID seen in WS may be caused by the additive effects of more than one gene in the deletion region. Based on the common findings in their cases, they suggested that the *GTF2IRD1* gene might be associated with behavioral and psychiatric manifestations. The presence of this relationship seems suspicious in our opinion, as there were no psychiatric findings and the behavioral pattern was completely different from their cases (21). As a result, it is seen that new studies are needed on the phenotype for which this genes are responsible.

Cardiovascular anomalies and endocrine anomalies, which are among the other characteristic and common findings of the syndrome, were also not found in our case. In our case Array CGH revealed 1.3 Mb deletion at 7q11.23 region which contain *ELN* gene which is confirmed by FISH analyses. Deletion of *ELN* is responsible for the connective tissue abnormalities, including the cardiovascular disease in WS (22). Despite the deletion of the *ELN* gene in our case, our patient did not have a cardiovascular anomaly.

Scoliosis, contracture and loose skin have been in our case are among the rarer findings of the syndrome. Scoliosis was found in 18% and radioulnar synostosis in 10% of cases with WS (23). Although the short structure is a frequently defined finding in WS, brachydactyly has been described in only one case in the literature so far. However that patient who has a large, atypical, visible chromosomal deletion of 7q11.2 and features consistent with, and in addition to, those typically seen in WS (24). Since we did not investigate whether there is a variation in the genes associated with brachydactyly, it is not possible to suggest that the brachydactyly detected in our case is an additional finding of WS.

BAZ1B (bromodomain adjacent to a leucine zipper 1B) gene also in between deleted genes in our case. The BAZ1B protein is part of a chromatin remodeling complex. Because it binds the vitamin D receptor, it has been theorized that it may have a role in hypercalcemia in WS (25). In addition, the *BAZ1B* gene has been associated with hypothyroidism seen in cases with WS (26). However, there was no evidence of hypercalcemia or hypothyroidism in our patient either. Considering all these findings, the etiology of the atypical findings in our case is not fully understood.

We detected a deletion of 14q11.2 in Case 4 with developmental delay, speech delay, episodic vomitting attacks and dysmorphic facial features including prominent nose, irregular teeth and simple ears (Table 1).

The deletion is approximately 1 Mb in size and contains the *CHD8* gene which is a chromodomain helicase DNA binding protein and has been associated with autism spectrum disorders (ASD) (Table 2). It has been reported with a variety of genotypes including chromosomal microdeletions, balanced chromosomal abnormalities, haploinsufficiency of the gene due to a 2.89 Mb deletion, and a recurrent ~100 Kb microdeletion. In addition to cytogeneticstudies, new generation sequencing technologies performed in ASD cohorts have discovered loci associated with an increased risk of ASD. Increasing evidence has indicated that *de novo* loss of function mutations contribute to ASD risk. It has been reported that *CHD8* mutations could result in a behavioral profile consistent with ASD, together with developmental delay, intellectual disability, macrocephaly, distinct facial features, and gastrointestinal complaints includingconstipation, diarrhea and abdominal pain (27). In our case, there were findings other than ASD and macrocephaly among the aforementioned findings associated with variations in the *CHD8* gene. In cases with deletion or microdeletion, on the contrary to macrocephaly and owergrowth findings, microcephaly and poor growth are observed (28). This suggests that other genes in the deletion region have an effect on this situation. Although gastrointestinal system findings such as constipation and diarrhea are common, episodic vomiting episodes found in Case 4 are reported for the first time as far as we know.

As a result of the microarray analysis performed in Case 5, a deletion of 5 Mb in the chromosome 1p36.33-p36.31 region was detected. 1p36 deletion syndrome is a contiguous gene deletion syndrome with variable phenotype. ID, hypotonia, microcephaly, short stature, deep seated eyes, mid-face hypoplasia, ear anomalies, hearing loss, epileptic seizures, congenital heart defects and genitourinary malformations are common clinical findings of the syndrome (29). The *KCNAB2* gene has been shown as a candidate gene for epileptic seizures seen in the syndrome. Also, the *GABRD* gene is responsible for neuropsychiatric abnormalities and seizures (30,31). Supporting the aforementioned studies, in our Case 5 who has epilepsy, the two mentioned candidate genes were located in the deletion region.

Several genes in this syndrome are shown as candidate genes in congenital heart malformations. The *DVL1, PRDM16* and *SKI* genes being in the deletion region of our case, are mostly associated with cardiac anomalies especially cardiomyopathy in the literature (32,33). Wu et al. presented 4.8 Mb deletion at 1p36.33-p36.31 (chr1:849,466-5,685,789) in child 4 with PDA and leukodystrophy, which contained the *SKI* and *DVL1* genes (34). Based on data from previous studies they suggested that *SKI* and *DVL1* could be the main genes responsible for CHD phenotypes in 1p36 deletion syndrome. The presence of only PDA as a cardiac anomaly in our case supports the view that these two genes may be responsible for PDA.

In Case 6, a duplication of 543 kb in chromosome Xp11.4 region was detected. In the DECIPHER database, two patients whose duplication regions are similar to the our case have been identified. The 514 kb duplication region detected in Patient 289188 in the database contains only the *ATP6AP2* gene, and absence seizures were defined as clinical findings in this case. Another case is Patient 289211, whose clinical finding is only learning disability, and has a duplication region of 88 kb that contains only the *ATP6AP2* gene. Case 6 had ID, speech delay and epilepsy. This suggests that the *ATP6AP2* gene, which is the common gene in the duplication region, may be responsible for the ID and seizures seen in the cases. Point mutations in this gene are associated with X-linked mental retardation is characterized by developmental delay, speech delay and seizures (35,36). Also a duplication of Xp11.4 in a patient with delayed speech development and obesity was reported. Within this region the *ATP6AP2*-gene is located (37). Although it is evaluated as VOUS according to the variant analysis criteria, we assume that the patient's findings were caused by the copy number variation of this gene.

Here, we discussed the patients, who had typical and atypical findings of known syndrome and rare chromosomal changes to contribute ID and / or developmental delay etiology. The results we obtained in this study supported that it would be benefical to choose microarray technology in the first step for diagnosis. The genotype-phenotype correlation studies provide explaining the underlying genetic causes and molecular mechanism. In our study, we made genotype-phenotype correlation in patients who had CNVs both pathogenic and VOUS. Feingold syndrome 1 with unilateral renal agenesis, Williams syndrome with atypical findings, 1p36 deletion syndrome, 14q11.2 deletion syndrome with episodic vomitting attacs are cases with pathogenic CNVs. Also effects of the dosage increase at 13q14.3-q21.1 and Xp11.4 chromosomal duplications containing *ATP6AP2* and *PCDH17* genes, respectively are discussed with literature. We beleive that these cases will contribute to expanding clinic spectrum of well known deletion syndromes with new findings. At the same time of the genes located within duplication regions that we found will be helpfull to have an idea about their role.

Conflict of interest

No conflict of interest was declared by the authors.

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