MLPA Method does not Always Confirm the Results of aCGH: A Study of *KANSL1* Gene Deletion Patients

MLPA Yöntemi Her Zaman aCGH Sonuçlarını Doğrulamaz: *KANSL1* Gen Delesyon Hastaları Üzerine Bir Çalışma

Selin Akad Dinçer[1], Zerrin Yılmaz Çelik[1], İlknur Erol[2], Feride İffet Şahin[1]

[1]Baskent University Faculty of Medicine Department of Medical Genetics, Ankara, Turkey
[2] Baskent University Faculty of Medicine Department of Pediatrics, Ankara, Turkey

ABSTRACT

Background: Microdeletion and microduplications are detected on chromosomes as a pathological subgroup of copy number variants of DNA. It has become easier to identify such chromosomal syndromes after use of array-based comparative genomic hybridization technology. One of them is the 17q21.31 microdeletion and microduplication syndrome. A 500-650 kb sized copy loss on 17q21.31 results in a phenotype which was described as Koolen-de Vries Syndrome including mental retardation, epilepsia, hypotonia and characteristic facial features. Today, we know that haplo-insufficiency of *KANSL1* gene located in this region is responsible for these findings.
A total of 30 patients with *KANSL1* deletion detected during aCGH analyses were enrolled in the current study. All patients were analyzed by Multiplex Ligation-Dependent Probe Amplication (MLPA) method in order to confirm the results.
Results: Three of the 30 patients had *KANSL1* gene deletion detected by both methods and duplication was found in one patient.
Conclusion: As a result of the study, we concluded that although new generation molecular methods enable us to obtain big and valuable data, each method has its own limitations and confirming the reults with another method increases test reliability. Using together of these methods are useful for the geneticists during diagnosis, clinical assessment and genetic counseling of patients.

Keywords: aCGH, CNV, *KANSL1*, KdVs, MLPA, 17q21.31

Received: 10.12.2021 **Accepted:** 11.11.2021

ÖZET

Arka plan: Mikrodelesyon ve mikroduplikasyonlar, DNA'nın kopya sayısı varyantlarının patolojik bir alt grubu olarak kromozomlar üzerinde tespit edilir. Dizi tabanlı karşılaştırmalı genomik hibridizasyon (aCGH) teknolojisinin kullanımından sonra bu tür kromozomal sendromları tanımlamak daha kolay hale gelmiştir. Bunlardan biri de 17q21.31 mikrodelesyon ve mikroduplikasyon sendromudur. 17q21.31'de 500-650 kb boyutunda bir kopya kaybı, zihinsel gerilik, epilepsi, hipotoni ve karakteristik yüz özelliklerini içeren Koolen-de Vries Sendromu olarak tanımlanan bir fenotip ile sonuçlanır. Günümüzde bu bulgulardan aynı bölgede yer alan KANSL1 geninin haployetersizliğinin sorumlu olduğunu bilinmektedir.
Yöntem: Bu çalışmaya aCGH analizleri sırasında KANSL1 delesyonu saptanan toplam 30 hasta dahil edildi. Sonuçları doğrulamak için tüm hastalar ek olarak "Multiplex Ligation-Dependent Probe Ampplication" (MLPA) yöntemi ile analiz edildi.
Bulgular: 30 hastanın üçünde her iki yöntemle de KANSL1 geni ekzonik delesyonu saptanırken bir hastada duplikasyon saptandı.
Sonuç: Çalışma sonucunda, yeni nesil moleküler yöntemlerin büyük ve değerli veriler elde etmemizi sağlasa da, her yöntemin kendi sınırlamaları olduğu ve sonuçların başka bir yöntemle teyit edilmesinin test güvenilirliğini artırdığı sonucuna vardık. Bu yöntemlerin bir arada kullanılması, genetik uzmanlarına tanı, klinik değerlendirme ve hastaların genetik danışmanlığı sırasında yararlı olacaktır.

Anahtar Sözcükler: : aCGH, CNV, KANSL1, KdVs, MLPA, 17q21.31

Geliş Tarihi: 12.10.2021 **Kabul Tarihi:** 11.11.2021

This study (Project No: KA 17/01) was approved by Baskent University Medical and Health Sciences Research Board and Ethics Committee, and supported by Baskent University Research Fund.Also, was presented as a poster at the "Erciyes Tıp Genetik Günleri 2018" Congress, Kayseri, Türkiye, March 7-10, 2018.
ORCID IDs: S.A.D.0000-0002-3808-7004, Z.Y.Ç.0000-0001-9158-220X, İ.E.0000-0002-3530-0463, F.İ.Ş.0000-0001-7308-9673

Address for Correspondence / Yazışma Adresi: Zerrin Yilmaz Celik, MD Baskent University Faculty of Medicine Department of Medical Genetics Sehit H Temel Kuğuoğlu Cad No:34 Bahçelievler 06490, Ankara, Turkey E-mail: zylmaz@hotmail.com

doi:http://dx.doi.org/10.12996/gmj.2022.37

INTRODUCTION

Copy number variants (CNV) observed during array-based comparative genomic hybridization (aCGH) analyses are defined as an increase or loss of a fragment of the DNA; from a kilobase to a few megabases or even a whole chromosome (trisomies and monosomies) (1). CNVs may contain one or more genes or may contain no genes. Microdeletion and microduplication syndromes can be identified as pathogenic CNVs by molecular genetic methods. These syndromes are caused by homologous chromosome mismatches during meiosis (1). The incidence of microdeletion syndromes is 1.99 and microduplication syndromes is 0.7 in 10,000 births (2). Changes under 5-10 Mb cannot be detected by light microscopy, as a result, conventional karyotype of the patients are usually reported to be normal. aCGH, Fluorescence in Situ Hybridization (FISH) or Multiplex Ligation-Dependent Probe Amplication (MLPA) are the methods used for diagnosis of these small chromosome changes (3).

Koolen-de Vries syndrome (KdVS) (OMIM# 610443), also known as 17q21.31 microdeletion syndrome was first identified in 2006 in patients with dysmorphic features, hypotonia, mental retardation (MR) and organ disorders (4, 5, 6). More than 50% of cases are observed with structural disorders in the brain (Corpus callosum agenesis, ventricular enlargement), epilepsy, cardiac and genitourinary system features (7, 8, 9). Pulmonary stenosis (27%), septal defects (18%), bicuspid aortic valve (18%), patent ductus arteriosus, foramen ovale or aortic dilatation have been reported as congenital cardiac defects. Cryptorchidism, hypospadias and vesicoureteral reflux were observed in 82% of patients with urological anomalies. 58-73% of patients have joint hypermobility during childhood and sometimes hip dislocation (congenital boor acquired) or position disorders can be detected (9, 10). More rarely, hearing loss (26%), ectodermal anomalies, hyperopia, and other eye defects have also been reported (9, 11). KdVS syndrome has an autosomal dominant inheritance pattern. Genetic change usually develops de-novo during formation of reproductive cells or during early fetal development. Affected individuals usually do not have a similar history of illness in their families (12, 13).

KdVS is caused by a copy loss of 440-680 kb in the 17q21.31 region including *KANSL1, CRHR1, MAPT, IMP5* and *STH* genes or an intragenic *KANSL1* mutation (14). Although the exact size of the deletion site varies among the affected individuals, a common deletion region of approximately 500 kb may be seen. However, it has been suggested that individuals with *KANSL1* gene mutation have the same phenotype as microdeletion, so this gene may be responsible for the phenotype (15).

KANSL1 gene is located on the long arm (q) 21.31 [(GRCh38): 46,029,915_46,225,373] of chromosome 17. *KANSL1* gene consists of 15 exons and encodes 1105 amino acids (16). *KANSL1* gene product is a member of the protein complex that interacts with a group called the K (lysine) acetyltransferase 8 (KAT8) regulatory NSL complex. This complex is classified as a histone acetyltransferase (HAT) complex and plays role in chromatin modification (15). The region of the gene including aminoacids 850 to 882 is the region responsible for HAT8 activation. Mutations in this site inhibit KANSL1 protein activity (17). The KANSL1 protein is an evolutionarily conserved regulator of the chromatin modifier KAT8, which affects gene expression through histone H4 lysine 16 (H4K16) acetylation (4, 10, 18, 19). KAT8 is critical for important biological processes, including cell proliferation and conservation of genome stability (15). Specifically, histone H4 lysine 16 has been reported to play role in various biological processes such as acetylation, DNA replication, DNA repair, cell cycle and tumor formation (20, 21, 22, 23).

While the incidence of 17q21.31 microdeletion is 1/16,000 at birth, 17q21.31 microduplication has been reported only in 5 patients (19). 17q21.31 region CNVs can be detected by cytogenetic GTG-banding, MLPA or aCGH (24,25), whereas *KANSL1* mutations can be identified by gene sequence analysis (20).

In this study, we reevaluated 30 patients with 17q21.31 region CNVs including *KANSL1* gene by aCGH analysis. We analyzed *KANSL1* gene exonic deletions or duplications by MLPA to confirm aCGH results. We aimed to compare the results of aCGH and MLPA, and patients' phenotypes to establish a methodologic algorithm for genetic diagnosis of KdVS.

MATERIAL and METHODS

Patients and Samples

Archieved DNA samples of 30 patients detected to have 17q21.31 CNV by aCGH analysis (6x720 K and 12x135K arrays and Roche NimbleGen Human CGH ISCA Plus 3x1.4M Array platform (Roche, NimbleGen, Madison, WI, USA)) in the time period between January 2013 and December 2015 constituted the study group. The patients had their clinical follow up in Pediatric Neurology Department of Başkent University Adana Research Center and all the wet lab procedures were carried on in our department after the study was approved by the Institutional Review Board.

Patient groups were divided into three groups according to 17q21.31 CNV gene content (Table1-3). The first group consisted of three patients with *KANSL1* gene copy deletion only. The second group of 16 patients had *KANSL1* and *KANSL1-AS1* genes copy deletions. The third group included 11 patients with copy deletions of *KANSL1, KANSL1-AS1* and several other genes.

Table 1. Clinical features of patients in group 1

.

Patient No	Sex	Age	Findings	a-CGH start-stop	Deletion size (kb)	Probe number	İncluding genes
1	M	3	Abnormal face	44,209,537_ 44,270,054	60,518	13	KANSL1
2	F	11	Epilepsy	41,571,777_ 41,631,306	59,53	11	KANSL1
3	M	10	Mental Retardation (MR), Abnormal face	44,209,537-44,264,622	55,09	10	KANSL1

Table 2. Clinical features of patients in group 2.

Patient No	Sex	Age	Findings	a-CGH start-stop	Deletion size (kb)	Probe number	İncluding genes
4	M	15	MR, Minor Malformations	41,541,432-41,631,306	89,87	15	*KANSL1, KANSL1-AS1*
5	F	8	MMR, Abnormal face	41,541,432-41,631,306	89,87	15	*KANSL1, KANSL1-AS1*
6	M	4	Abnormal face	41,541,432-41,631,306	89.87	15	*KANSL1, KANSL1-AS1*
7	F	6	Abnormal face, Epilepsy	41,571,777-41,631,306	59,53	11	*KANSL1, KANSL1-AS1*
8	F	6	Mild developmental delay	41,571,777-41,631,306	89,87	15	*KANSL1, KANSL1-AS1*
9	F	9	Abnormal face, Developmental delay	41,571,777-41,631,306	59,53	11	*KANSL1, KANSL1-AS1*
10	F	16	Epilepsy, MMR, Microcephaly	41,541,432-41,631,306	89,87	15	*KANSL1, KANSL1-AS1*
11	M	5	Severe developmental delay, Polydactyly	41,571,777-41,631,306	59,53	11	*KANSL1, KANSL1-AS1*
12	F	7	Epilepsy	41,571,777-41,631,306	59,53	11	*KANSL1, KANSL1-AS1*
13	F	8	Mild mental retardation, Abnormal face	41,571,777-41,631,306	89,87	15	*KANSL1, KANSL1-AS1*
14	M	3	Developmental delay, Epilepsy	44,216,580-44,348,898	132,319	30	*KANSL1, KANSL1-AS1*
15	M	18	Abnormal face, Epilepsy, MMR	44,205,685-44,301,891	96,207	23	*KANSL1, KANSL1-AS1*
16	M	9	MMR, Multiple Minor Malformation	44,240,510-44,361,066	120,557	29	*KANSL1, KANSL1-AS1*
17	M	18	Abnormal face, Hypotonia	44,184,364-44,301,891	117,528	29	*KANSL1, KANSL1-AS1*
18	M	12	MR, Abnormal face, Hypotonia	44,198,755-44,301,891	103,14	25	*KANSL1, KANSL1-AS1*
19	M	9	Epilepsy, Aorta Coarctation	44,184,364-44,348,898	164,535	38	*KANSL1, KANSL1-AS1*

MLPA Method

Salsa MLPA probemix P443-A1 *KANSL1* kit was used. The Salsa MLPA P443-A1 *KANSL1* kit contains 33 MLPA probes containing amplification products ranging from 128 to 373 nucleotides. This kit has also two probes for the *MAPT* gene and two probes for the *CRHCR1* gene. MLPA reactions were performed as described by the manufacturer. Polymerase chain reaction (PCR) products were denatured and loaded onto ABI 310 capillary electrophoresis system (Applied Biosystems, Foster City, CA). Fragment analysis and peak area and product size determination was performed by GeneScan software (Applied Biosystems). The results were analyzed by Coffalyser program database.

Statistical Analysis

In this study, IBM SPSS Statistics 25 was used for statistical analysis. Kolmogorov Smirnov test statistics were used for testing the normality of the data. Descriptive statistics for the categorical data are given with the frequency and percentage, while the statistics for the continuous data are given with mean and standard deviation. ANOVA test was used for group comparisons of the continuous data showing normal distribution, while chi-square test was used for the comparison of the categorical data. The confidence level was accepted as 0.95 and hypothesis was rejected if p value less than 0.05.

This study (Project No: KA17/01) was approved by Başkent University Medical and Health Sciences Research Board and Ethics Committee.

Table 3. Clinical features of patients in group 3.

Patient No	Sex	Age	Findings	a-CGH start-stop	Deletion size (kb)	Probe number	İncluding genes
20	F	5	CP, Epilepsy, Microcephaly, Abnormal face	44,216,580-44,781,666	517,26	62	*KANSL1, KANSL1-AS1, LOC644172, LRRC37A, ARL17A, ARL17B, LRRC37A,ARL17A, ARL17B, NSFP1, LRRC37A2, ARL17A, NSFP1, NSF*
21	M	3	Abnormal face, Epilepsy	44,240,510-44,781,666	541,157	64	*KANSL1, KANSL1-AS1, LRRC37A, ARL17A, ARL17B, NSFP1, LRRC37A2, ARL17A, NSFP1, NSF*
22	F	8	Mild developmental delay, Multiple minor malformation	44,216,580-44,781,666	565,09	66	*KANSL1, KANSL1-AS1, LRRC37A, ARL17A, ARL17B, NSFP1, LRRC37A2, ARL17A, NSFP1, NSF*
23	M	7	Atypical autism, Cortical dysplasia	44,201,418-44,781,666	580,249	69	*KANSL1, KANSL1-AS1, LRRC37A, ARL17A, ARL17B, NSFP1, LRRC37A2, ARL17A, NSFP1, NSF*
24	M	10	Epilepsy	44,205,685-44,697,027	491,343	61	*KANSL1, KANSL1-AS1, LRRC37A, ARL17A, ARL17B, NSFP1, LRRC37A2, ARL17A, NSFP1, NSF*
25	F	7	Abnormal face, Epilepsy	44,216,580-44,781,666	484.601	52	*KANSL, LRRC37A, ARL17A, ARL17B, NSFP, LRRC37A2, ARL17A, NSFP1, NSF*
26	M	8	Abnormal face, Epilepsy	44,301,891-44,781,666	479.776	45	*KANSL1, LRRC37A, ARL17A, ARL17B, NSFP1, LRRC37A2, ARL17A, NSFP1, NSF*
27	M	13	Abnormal face, Epilepsy, Abnormal behavior	44,301,891-44,697,027	395,137	38	*KANSL1, LRRC37A, ARL17A, ARL17B, NSFP1, LRRC37A2, ARL17A, NSFP1, NSF*
28	F	4	CNS anomaly, Epilepsy	44,301,891-44,781,666	479,776	45	*KANSL1, LRRC37A, ARL17A, ARL17B, NSFP1, LRRC37A2, ARL17A, NSFP1, NSF*
29	M	4	Abnormal face, Developmental delay	44,302,715-44,756,486	453,772	42	*KANSL1, LRRC37A, ARL17A, ARL17B, NSFP1, LRRC37A2, ARL17A, NSFP1, NSF*
30	F	12	MR, Epilepsy	44,301,891-44,781,666	479,777	45	*KANSL1, LRRC37A, ARL17A, ARL17B, LRRC37A, ARL17A, ARL17B, NSFP1, LRRC37A2, ARL17A, NSFP1, NSF*

RESULTS

DNA samples of 16 male and 14 female patients aged between 3-18 years were included in the study. The patients had clinical findings including idiopathic mental retardation, dysmorphic facial appearance, epilepsy and developmental retardation and normal karyotype. One of the patients was excluded from the study because the result could not be evaluated due to low DNA quality.

KANSL1 gene's exonic deletion was found in three (10.3%) patients on group two, whereas *MAPT* and *CRHR1* gene were detected as normal in the all groups. While a patient had copy number deletion on 17q21.32 region by aCGH, exonic duplication of *KANSL1* gene (3.4%) was detected in MLPA. In 25 patients with a copy number deletion according to the aCGH result, there were no abnormal findings with MLPA in the *KANSL1*, *MAPT* and *CRHR1* genes (86.2%).

The copy number deletion size determined by aCGH ranged from 55,09 to 580,249kb in the study group. There was not a statistically significant association between the magnitude of the copy number deletion by aCGH and present of exonic deletion in *KANSL1* gene in MLPA. Clinical findings of patients with deletions and duplication are shown in Table 4.

Table 4. Phenotypic features of the patients with deletion or duplication.

KdVS Findings	Patient 4	Patient 13	Patient 16	Patient 18
Dysmorphic face	Micrognathy, slanting eye, prominent nose tip, strabismus, low-set malformed ears, bifrontal stenosis	Hypertelorism, nasal anomaly	Hypertelorism, prominent jaw, large, low-set malformed ears, prominent forehead	Slanting eyes, fish mouth, large, low-set malformed ears
Hypotonia	+	-	+	+
Epilepsy	+	-	+	-
Mental Disability	+	+	+	+
Congenital Cardiac Defects	-	-	-	-
Structural Defects in the Brain	-	-	-	-
Urological Abnormalities	+	-	-	-
Sympathetic Behavior	+	-	+	-
MLPA result	**Deletion**	**Deletion**	**Deletion**	**Duplication**

The deletion sizes of patient 4 and patient 13 by aCGH were 89.87 kb each. Patient 4 had deletion in 4 exons of *KANSL1* gene (exon 8, 10, 14, 15b) by MLPA (Figure1). Patient 13 had deletion in the upstream region and 5 exons (exon 2b, 3, 10, 14, 15a) of *KANSL1* gene by MLPA. Patient 16 had a deletion size of 120.557 kb by aCGH. We were found deletion four exons (exon 2b, 3, 4, 10) in *KANSL1* gene by MLPA. Facial appearance of the patient 16 is shown in Figure 2. Patient 18 had a deletion of 103.14 kb by aCGH (Figure 3). We were found of duplication in the upstream region and 3 exons (exon 1, 2a, 2b) in *KANSL1* gene by MLPA (Figure 4). This study was repeated twice, and identical results were obtained. MLPA result of patient 18 is shown in Figure 4.

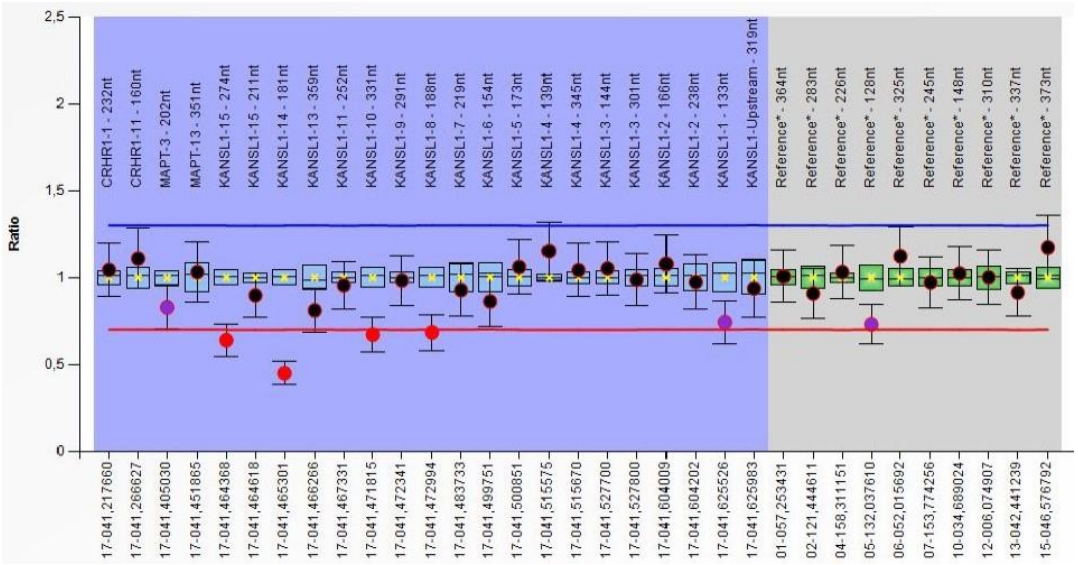

Figure 1: *KANSL1* gene deletion was shown in exons 8, 10, 14 and 15b by MLPA (Patient 4). The red points indicate the presence of deletions in the target probes regions.

Figure 2

Figure 2: Front and side views of the patient with KANSL1 deletion. (Patient 16)

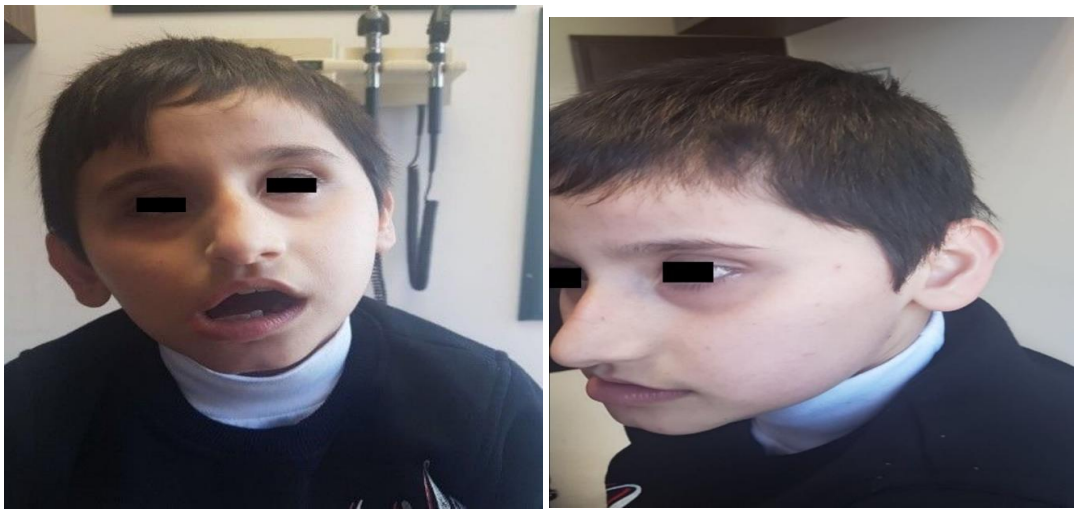

Figure 3: Front and side views of the patient with KANSL1 duplication. (Patient 18)

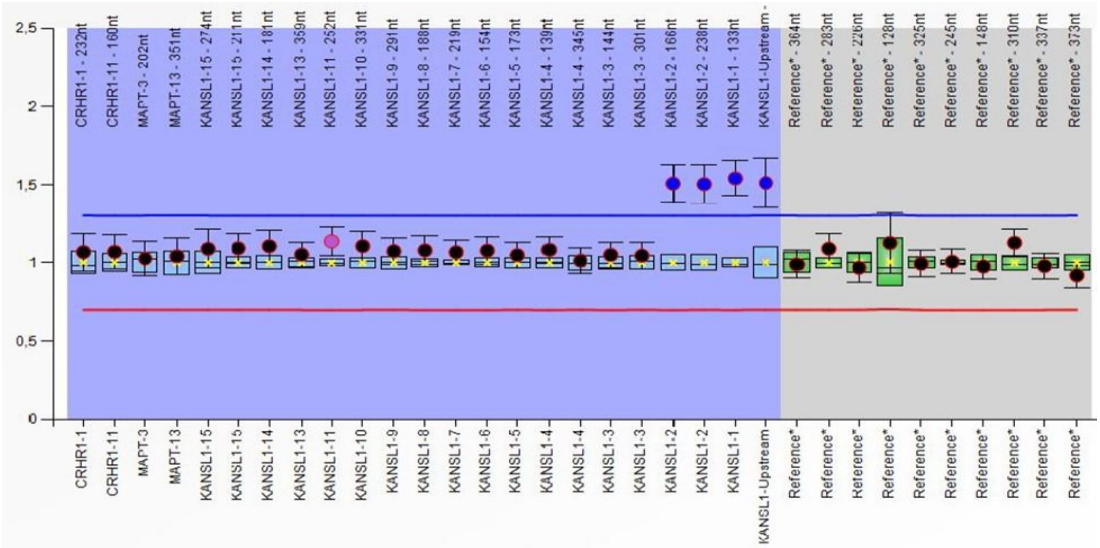

Figure 4: The result of the MLPA study of patient 18. The regions with blue color indicate the presence of probe duplication.

Within the scope of the study, MLPA results were examined in three groups as normal, exonic deletion and duplication for *KANSL1* gene. No statistically significant result was found for aCGH results with normal (37656.2 + 130879.9), deletion (100.1 + 17.7) and duplication (103.1) groups (p> 0.05).

DISCUSSION

Since the introduction of microarray technology, the number of copies variants, microdeletion and microduplication regions have started to be examined at genomic level (16). One of the syndromes that started to be detected with microarray studies is KdVS with 17q21.31 microdeletion. Recent studies have shown that losses or mutations of *KANSL1* gene in this region cause this syndrome (4).

In our study, *KANSL1* gene was examined for the presence of exonic deletion by MLPA method in 30 patients who had been determined to have 17q21.31 region copy number deletion by aCGH analysis. Furthermore, an algorithm is designed for the genetic diagnostic approach to KdVS.

One out of thirty patients were excluded from the study due to low DNA quality. Four of the twenty-nine patients had deletion or duplications within the gene (~13,8%). Three patients had *KANSL1* deletion (10.3%) and one patient had duplication (3.4%). There was no deletion or duplication of targeted *MAPT* and *CRHR1* gene regions.

In the two patients who had *KANSL1* deletion according to the MLPA result, the deletion size was below 100 kb. In our study, the rate of deletion detection with aCGH increased from ~ 3.45 at the limit value of 100 kb and above to 10.34 at the limit value of 50 kb. Therefore, it is seen that the value-dependent threshold used for evaluation is not appropriate for diagnosing these patients. It has been observed that MLPA results were not correlated with the size and gene content of the deletion in result of the aCGH.

Since the DNA quality of the patient who was excluded from the study was low, both deletion and duplication were observed on the *KANSL1* gene during the study. The study was repeated but the result did not change. We concluded that

there could be a problem of probe binding due to the low quality of the DNA sample. Our finding shows also that DNA quality is important for the success of giving results to the patient with MLPA method.

As a result of the MLPA study, the common exon region of the patients with *KANSL1* deletion was exon 10. It is known that *KANSL1* protein is responsible for protein activity in the region between 850-882nd amino acids. When the nucleotide sequence is examined, it is seen that this region corresponds to the 11th exon of the gene. The region in which the *KANSL1* gene protein product is active, covers between 2549 and 2648th nucleotides. The exon 11 probe contains 2656 nucleotides and further. The exon 10 probe contains 2441 nucleotides and further. Since *KANSL1* is in the pre-activated region, it is possible to think about the possibility of covering this area. However, it is not known how much space an area scans after connection. In our study, the deletion in the exon 10 region as a common finding suggests that this region may be important. However, this finding should be evaluated with more studies.

MLPA detection kit used in our study does not contain probes for the active site of *KANSL1* gene that is the deficiency of the current method. As every method has its own advantages and disadvantages, in the way to the diagnosis of patients, if *KANSL1* deletion is strongly suspected according to the clinical phenotype, whole gene sequencing of *KANSL1* should be performed.

Patients with KdVS show phenotypic diversity. It is an autosomal dominant syndrome of mental retardation member containing the phenotypic series of 58 genes (OMIM, PS156200). The clinically defined manifestations are developmental retardation, mild to moderate mental retardation, dysmorphic facial features and hypotonia. Dismorphic facial findings typically include long face, broad chin, ptosis, blepharophimosis, large and prominent ears, bolbous nasal tip, high and narrow palate, but these findings vary (OMIM#610443). Facial findings like the KdVS phenotype were found in patients with deletions in patients 4 and 16, but the findings of the 13 patients were lighter than the others. 18 patients with duplication were found to have dysmorphic facial findings consistent with the syndrome but were not diagnosed with KdVS in the clinical evaluation. In all patients, mild or moderate MR and hypotonia are common findings. The presence of dysmorphic facial findings was detected in all patients, but it was observed that the findings were not different because of the different types of findings. Urogenital and other systemic symptoms, the patient is different.

As a result of the comparison of the methods, it is understood that MLPA method is a reliable and practical method in the determination of deletions and duplications of *KANSL1* gene. In patients with a deletion as defined in KdVS phenotypic characteristics are shown as not overlapping with fully. Therefore, it is thought that phenotypic differences may occur due to the interaction of other genomic contents of other genes and patients regulating the *KANSL1* gene. We did not detect deletion or duplication in the *MAPT* and *CRHR1* genes in the 17q21.31 region in any of our patients. Therefore, we concluded that between the number of copies of these genes and our patients' clinical findings was not associated.

As a result of our study, it has been determined that MLPA method can be a reliable method in diagnosis of deletion/ duplication of *KANSL1* gene, and false positivity with aCGH is high in our patient group. It was concluded that obtaining clinical opinion and verifying the result of aCGH by an additional method in the diagnosis stage would be helpful for the patients not to be missed and to reduce false positivity.

Different diagnostic methods can be used in the diagnostic approach to KdVS syndrome. Since the disease has phenotypic similarities with other microdeletion/ duplication syndromes, it would be appropriate to evaluate CNVs by the aCGH method first in patients with normal karyotype. However, our study shows that it is not appropriate to report CNVs under 600kb detected as a result of aCGH, including the *KANSL1* gene, without confirmation by MLPA. In the presence of clinical suspicion, if aCGH or MLPA result is normal, *KANSL1* gene mutation screening should be performed.

As a result of the study, we concluded that although new generation molecular methods enable us to obtain big and valuable data, each method has its own limitations and confirming the reults with another method increases test reliability. Using together of these methods are useful for the geneticists during diagnosis, clinical assessment and genetic counseling of patients.

Conflict of interest

No conflict of interest was declared by the authors.

Acknowledgment

We would like to acknowledge Gözde Kubat, who carried out all statistical analyses of this study.

REFERENCES

1. Watson CT, Marques-Bonet T, Sharp AJ, Mefford HC. The genetics of microdeletion and mikroduplication syndromes: an update. Annu Rev Genomics Hum Genet. 2014; 15:215-244. doi: 10.1146/annurev-genom-091212-153408.
2. Menten B, Maas N, Thienpont B, Buysse K, Vandesompele J, Melotte C, et al. Emerging patterns of cryptic chromosomal imbalances in patients with idiopathic mental retardation and multiple congenital anomalies: a new series of 140 patients and review of published reports. J Med Genet. 2006 Aug;43(8):625-33.
3. Gouas L, Goumy C, Véronèse L, Tchirkov A, Vago P. Gene dosage methods as diagnostic tools for the identification of chromosome abnormalities. Pathol Biol (Paris). 2008 Sep;56(6):345-53.
4. Zollino M, Orteschi D, Murdolo M, Lattante S, Battaglia D, Stefanini C. et al. Mutations in KANSL1 cause the 17q21.31 microdeletion syndrome phenotype. Nat Genet. 2012 Apr 29;44(6):636-8.
5. Koolen DA, Vissers LE, Pfundt R, de Leeuw N, Knight SJ, Regan R, et al. A new chromosome 17q21.31 microdeletion syndrome associated with a common inversion polymorphism. Nat Genet. 2006 Sep;38(9):999-1001.
6. Shaw-Smith C, Pittman AM, Willatt L, Martin H, Rickman L, Gribble S, et al. Microdeletion encompassing MAPT at chromosome 17q21.3 is associated with developmental delay and learning disability. Nat Genet. 2006 Sep;38(9):1032-7.
7. Kitsiou-Tzeli S, Frysira H, Giannikou K, Syrmou A, Kosma K, Kakourou G, et al. Microdeletion and microduplication 17q21.31 plus an additional CNV, in patients with intellectual disability, identified by array-CGH. Gene. 2012 Jan 15;492(1):319-24.
8. Sharp AJ, Hansen S, Selzer RR, Cheng Z, Regan R, Hurst JA, et al. Discovery of previously unidentified genomic disorders from the duplication architecture of the human genome. Nat Genet. 2006 Sep;38(9):1038-42.
9. Terrone G, D'Amico A, Imperati F, Carella M, Palumbo O, Gentile M, et al. A further contribution to the delineation of the 17q21.31 microdeletion syndrome: central nervous involvement in two Italian patients. Eur J Med Genet. 2012 Aug-Sep;55(8-9):466-71.
10. Koolen DA, Pfundt R, Linda K, Beunders G, Veenstra-Knol HE, Conta JH, et al. The Koolen-de Vries syndrome: a phenotypic comparison of patients with a 17q21. 31 microdeletion versus a KANSL1 sequence variant. Eur J Hum Genet. 2016 May;24(5):652-9..
11. Tan TY, Aftimos S, Worgan L, Susman R, Wilson M, Ghedia S, et al. Phenotypic expansion and further characterisation of the 17q21.31 microdeletion syndrome. J Med Genet. 2009 Jul;46(7):480-9.
12. Zollino M, Marangi G, Ponzi E, Orteschi D, Ricciardi S, Lattante S, et al. Intragenic KANSL1 mutations and chromosome 17q21. 31 deletions: Broadening the clinical spectrum and genotype-phenotype correlations in a large cohort of patients. J Med Genet. 2015 Dec;52(12):804-14.
13. Tan TY, Aftimos S, Worgan L, Susman R, Wilson M, Ghedia S et al. Phenotypic expansion and further characterisation of the 17q21.31 microdeletion syndrome. J Med Genet. 2009 Jul;46(7):480-9.
14. Dubourg C, Sanlaville D, Doco-Fenzy M, Le Caignec C, Missirian C, Jaillard S et al. Clinical and molecular characterization of 17q21.31 microdeletion syndrome in 14 French patients with mental retardation. Eur J Med Genet. 2011 Mar-Apr;54(2):144-51.
15. Koolen DA, Morgan A, Bert BA de Vries, et al. Koolen-de Vries Syndrome. GeneReviews [Internet]. Initial Posting: January 26, 2010; Last Update: June 13, 2019. Available from:https://www.ncbi.nlm.nih.gov/books/NBK24676/
16. Coe BP, Ylstra B, Carvalho B, Meijer GA, Macaulay C, Lam WL. Resolving the resolution of array CGH. Genomics. 2007 May;89(5):647-53..
17. Huang J, Wan B, Wu L, Yang Y, Dou Y, Lei M. Structural insight into the regulation of MOF in the male-specific lethal complex and the non-specific lethal complex. Cell Res. 22:1078-1081(2012).

18. Itsara A, Vissers LE, Steinberg KM, Meyer KJ, Zody MC, Koolen DA et al. Resolving the breakpoints of the 17q21.31 microdeletion syndrome with next-generation sequencing. Am J Hum Genet. 2012 Apr 6;90(4):599-613.
19. Koolen DA, Kramer JM, Neveling K, Nillesen WM, Moore-Barton HL, Elmslie FV, et al. Mutations in the chromatin modifier gene KANSL1 cause the 17q21.31 microdeletion syndrome. Nat Genet. 2012 Apr 29;44(6):639-41.
20. Cooper GM, Coe BP, Girirajan S, Rosenfeld JA, Vu TH, Baker C, et al. A copy number variation morbidity map of developmental delay. Nat Genet. 2011 Aug 14;43(9):838-46.
21. Cao Z, Wu R, Gao D, Xu T, Luo L, Li Y, et al. Maternal histone acetyltransferase KAT8 is required for porcine preimplantation embryo development. Oncotarget. 2017 Oct 6;8(52):90250-90261.
22. Taipale M, Rea S, Richter K, Vilar A, Lichter P, Imhof A, Akhtar A. hMOF histone acetyltransferase is required for histone H4 lysine 16 acetylation in mammalian cells. Mol Cell Biol. 2005 Aug;25(15):6798-810.
23. Li X, Corsa CA, Pan PW, Wu L, Ferguson D, Yu X et al. MOF and H4 K16 acetylation play important roles in DNA damage repair by modulating recruitment of DNA damage repair protein Mdc1. Mol Cell Biol. 2010 Nov;30(22):5335-47.
24. Ciaccio C, Dordoni C, Ritelli M, Colombi M. Koolen-de Vries Syndrome: Clinical Report of an Adult and Literature Review. Cytogenet Genome Res. 2016;150(1):40-45.
25. Schluth-Bolard C, Diguet F, Chatron N, et al. Whole genome paired-end sequencing elucidates functional and phenotypic consequences of balanced chromosomal rearrangement in patients with developmental disorders. J Med Genet. 2019;56(8):526-535.