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Copy Number Variation Analysis in Turkish Patients with Congenital Bilateral Absence of Vas Deferens

Konjenital Bilateral Vas Deferens Yokluğu Olan Türk Hastalarda Genomik Kopya Sayısı Varyasyonları Analizi

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ABSTRACT

Objective: Congenital Bilateral Absence of the Vas Deferens (CBAVD) is a developmental abnormality that causes infertility in males. According to the literature, up to 88% of CBAVD cases have at least one pathogenic Cystic Fibrosis Transmembrane Conductance Regulator gene (*CFTR*) mutation. However, based on our previous data, this rate was 15.90% in Turkish patients with CBAVD. We aimed to identify genomic copy number variations (CNV) and candidate genomic regions which could related to the CBAVD in Turkish population.

Methods: CNV analysis was performed in 19 Turkish CBAVD patients normal karyotypes and a wild type *CFTR* genotype. We suggested that the *DAD1* gene may be a candidate gene related to CBAVD by reviewing online databases and analyzing CNV findings. Sanger sequencing of the *DAD1* gene exons was performed in 22 patients.

Results: We identified 11 CNVs that most likely related with the disease in nine of 19 (47.3%) patients. As the most common CNV, 14q11.2 deletions were detected in there (15.79%) of the patients. There was only *DAD1* gene in the sharing genomic region of two of the 14q11.2 deletions. No sequence variation was detected in the *DAD1* gene of the patients.

Conclusion: The 14q11.2 chromosomal region and the *DAD1* gene may be associated with CBAVD. Further studies are needed to indentify the contribution of CNVs and *DAD1* gene to CBAVD etiology.

Keywords: copy number variations; CBAVD; male infertility; *DAD1*

ÖZ

Amaç: Konjenital Bilateral Vas Deferens Yokluğu (CBAVD), erkeklerde infertiliteye yol açan gelişimsel bir anomalidir. Literatüre göre CBAVD'li olguların %88'e kadarı en az bir Kistik Fibrozis Transmembran Regülatör geni (*CFTR*) mutasyonuna sahiptir. Ancak, daha önceki verilerimize göre CBAVD'li Türk hastalarda bu oran %15.90'dır. Çalışmamız kapsamında Türk popülasyonundaki CBAVD ile ilişkili olabilecek genomik kopya sayısı varyasyonları (CNV) ve aday genomik bölgelerin tanımlanması amaçlanmıştır.

Yöntemler: Normal karyotipe ve yabanıl tip *CFTR* genotipine sahip 19 Türk CBAVD'li hastanın CNV analizi gerçekleştirildi. CNV bulgularımızın analizi ve veritabanlarının taranması ile *DAD1* geni CBAVD ile ilişkili olabilecek potansiyel aday gen olarak tanımlandı. 22 CBAVD'li hastada da *DAD1* geni ekzonlarının dizi analizi Sanger dizileme metoduyla yapıldı.

Bulgular: 19 hastanın dokuzunda (%47.3) hastalıkla ilişkili olabilecek 11 adet CNV saptandı. En sık saptanan CNV olarak 14q11.2 delesyonu, hastaların üçünde (%15.79) belirlendi. 14q11.2 delesyonlarından ikisinin ortak varyant bölgesinde yalnızca *DAD1* geni lokalize idi. Dizi analizi ile, hastaların *DAD1* geninde herhangi bir dizi varyasyonu saptanmadı.

Sonuç: 14q11.2 kromozomal bölgesi ve *DAD1* geni CBAVD ile ilişkili olabilir. CNVlerin ve *DAD1* geninin CBAVD etiyolojisine katkısını tanımlamak için ileri çalışmalar gerekmektedir.

Anahtar Kelimeler: kopya sayısı varyasyonları; CBAVD; erkek infertilitesi, *DAD1*

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INTRODUCTION

As a global health problem, infertility affects approximately 8-12% of all couples [1]. It has been estimated that 40-50% of all infertility cases is due to 'male factor' infertility and affects about 7% of all men [1]. Congenital structural anomalies of the male genital tract are contributing factors in male infertility. Numerical or structural chromosomal abnormalities, Y chromosome microdeletions, and Cystic Fibrosis Transmembrane Conductance Regulator (*CFTR*, NM_000492.3) gene mutations associated with congenital bilateral absence of vas deferens (CBAVD, OMIM 277180) are the most common genetic reasons of male infertility [2]. CBAVD accounts for 1-2% of all male infertility, which is a condition characterized by the blockade of the transportation of the spermatozoa from testicular or epididymal structures to the distal genital tract, resulting in azoospermia [3]. 88% of Caucasian, 78% of Non-Caucasian patients with CBAVD carry at least one *CFTR* mutation [4]. However, *CFTR* mutations were found to be less common in Turkish CBAVD patients [5-7]. We previously reported that only 15.90% had *CFTR* mutations [7]. The lower frequency of *CFTR* mutations among Turkish CBAVD cases, as well as differential mutation profiles, demonstrated the impact of geographical and ethnic backgrounds on CBAVD etiology [5, 7]. Besides *CFTR*, *ADGRG2* (Adhesion G protein-Coupled Receptor G2) gene variants were identified as a cause of CBAVD with a prevalence of 7.4% and 20% in French and Chinese cases, respectively [8, 9]. Recently, it was shown that, heterozygous *SLC9A3* deletions were found in 37.9% of the Taiwanese patients, and *SCNN1B* and *CA12* genes regulating CFTR protein levels were found to be associated with CBAVD in some patients without *CFTR* mutations [10, 11]. Nevertheless, these gene variations explain the disease in only a limited fraction of all CBAVD patients. The genetic structure of the present day Turkish people may not be similar with Europeans or Asians because of migrations from different geographical areas [12]. We hypothesized novel genes and/or genomic regions may be related to idiopathic CBAVD in Turkish people.

Genomic copy number variations (CNVs) can lead to disease by various molecular mechanisms including gene dosage effect, gene disruption, gene fusion, position effect and other effects on gene function [13]. To date, CNV analyses for idiopathic azoospermia from different ethnic backgrounds or populations have been performed in several studies [14, 15]. We hypothesise that CNVs might explain, at least partially, the unknown genetic etiology of CBAVD. Therefore, we aimed to identify the possible candidate genomic regions and genes which could cause CBAVD in Turkish patients not carrying *CFTR* mutations by performing whole genome CNV analysis. Here, we report that Defender Against Cell Death 1 (*DAD1*) localized at 14q11.2 in our cohort is a gene that may need to be investigated further in association with CBAVD. *DAD1* is known to be a negative regulator of apoptosis [16].

MATERIALS AND METHODS

Ethical approval and patients

This clinico-genetic study was conducted with the collaboration of Akdeniz University Faculty of Medicine, Izmir Katip Celebi University Faculty of Medicine, and Istanbul Sisli Memorial Hospital, Turkey. The study protocol was approved by the Akdeniz University Clinical Research Ethics Committee (approval No: 2014-# 383) under the recent version of the Helsinki Declaration. Informed consent was obtained from all subjects when they were enrolled in the study.

The patients who applied to the urology outpatient clinic due to primary infertility were evaluated. All of the patients had not used any birth control method for at least one year, they had no sexual dysfunction and no comorbidity. Twenty-two unrelated, adult Anatolia-originated Caucasian males with CBAVD were included in the study. All patients were azoospermic. CBAVD patients were determined by using physical examination, scrotal, transrectal ultrasound (USG), semen analysis, and biochemical tests. CBAVD cases with wild-type *CFTR* genotype, normal karyotype, without a Y chromosome microdeletion were included in this study. Renal aplasia was excluded by pelvic USG.

Copy Number Variation Analysis

For molecular genetic analyses, patients' genomic DNA was isolated from peripheral blood by using a non-enzymatic method modified from Lahiri and

Nurnberger [17]. The quality of DNA samples was tested by agarose gel electrophoresis and by measuring the A260/A280 ratios on the Nanodrop 8000 Spectrophotometer (Thermoscientific Instruments, CA, USA). Nineteen DNA samples of sufficient quality for microarray were examined for whole-genome CNVs. Nine out of the 19 samples were profiled by using the Affymetrix CytoScan HD Array (Affymetrix, Santa Clara, CA, USA). Affymetrix CytoScan HD Array is a high-density platform containing more than 2.6 million-copy number markers with 750.000 SNP markers. Agilent ISCA 8x60K CGH Array (Agilent Technologies, Santa Clara, CA, USA), comprised of 60.000 oligonucleotide probes, was used for the rest of the samples. After the wet laboratory steps according to the manufacturer's instructions, data were analyzed and visualized with different software (Affymetrix Chromosome Analysis Suite 3.1, Agilent Cytogenomics 4.0.2.21.) depending on the platform utilized. In Affymetrix Chromosome Analysis Suite software to exclude false-positive CNVs, only deletions and duplications more than 100 Kbp in length that involved at least 25 and 50 consecutive probes respectively were considered real alterations. In Agilent Cytogenomics software at least 100 Kbp of DNA amplification or deletions including a minimum of 4 consecutive probes were reported as real copy number variations. Human genome hg19 assembly was used to map genomic coordinates, and CNVs were classified as pathogenic, variants of uncertain significance, likely benign, and benign following the latest ACMG guidelines [18]. To determine the pathogenicity of detected CNV regions; DGV (http://projects.tcag.ca/cgi-bin/variation/gbrowse/hg19/), DECIPHER (https://decipher.sanger.ac.uk/), ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) databases were used. We excluded benign CNVs that had previously been reported to occur in healthy individuals frequently and reported CNVs possibly associated with a pathology.

Selection of the possible candidate gene for CBAVD

Overlapping CNV regions in at least two patients were determined by comparing the length of the shared sequence. The protein-coding gene/genes localized in the overlapping genomic region were hypothesized to be the potential candidate gene for CBAVD. The gene with a possible role in the development of the male genital tract was selected through a review of the literature (https://www.ncbi.nlm.nih.gov/pubmed) as well as databases on gene expression profiles (http://www.proteinatlas.org/), genotype–phenotype correlations (http://omim.org/, https://www.ncbi.nlm.nih.gov/gene), and animal models (http://www.informatics.jax.org/).

Sanger DNA sequencing for *DAD1*

Defender Against Cell Death 1 (*DAD1*) (NM_001344.4) was found to be a potential candidate gene associated with CBAVD by this study because of its localization in the overlapping genomic region of the common CNVs. Sanger sequencing was performed in all 22 CBAVD patients for all three exons and exon-intron boundaries of *DAD1*. The polymerase chain reaction (PCR) oligonucleotide primer sets are shown in Table 1. Amplification conditions consisted of an initial denaturation at 95 ℃ for 8 minutes, followed by 35 cycles at 95℃ for 30 seconds, 55℃ for 30 seconds, 72 ℃ for 30 seconds, and a final extension at 72 ℃ for 5 minutes. Each fragment was resolved by agarose gel electrophoresis. Aliquots of the samples were subjected to dideoxy sequencing on an ABI 3130XL Automated DNA Sequencer (Applied Biosystems, Foster City, CA, USA). NCBI Blast (http://blast.ncbi.nlm.nih.gov/Blast.cgi) was used to analyze obtained sequences.

Table 1: Primers used in Sanger sequencing of *DAD1*

Exon	Forward primer (5'-> 3')	Reverse primer (5'-> 3')	$MgCl_2$ (mM)	Tm ℃
1	GGCGGTGGT-CTGATATAGAGT	AGCTCATTG-ATAGGGCCATT	1,4	55
2	CCAGTCCCAT-CCAATTTCTC	TTTGGGGC-TATCTGGGTATC	1,4	55
3	ACAAGGAGG-CAGGTTCACAG	GATTTGGGG-CCCTGTCTCTA	1,7	55

RESULTS

Patients and clinical findings

The mean age of patients was 43 ± 12 years. All of the patients were primary infertile and azoospermic. On physical examination, the vas deferens of the patients were not palpable (both sides), and unilateral agenesis of vas deferens was confirmed radiologically. Their testicular volume

Table 2 : CNV profiles of Turkish CBAVD cases

Case ID	CNV Type	Chr. Region	Coordinates (hg19)	Gain/ Loss	Size (Kbp)	Gene Quantity	Gene Involved	# times in DGV	# times in Decipher	Microarray
8	P	15q13.2 q13.3	chr15:30,954,76-32,509,926	Loss	1555	7	*FAN1, MTMR10, TRPM1, MIR211, KLF13, OTUD7A, CHRNA7*	1 (Multiple)	1 (0)	Agilent ISCA 8x60K
4	VUS	11q14.3	chr11:89,474,817-89,895,988	Gain	421	10	*TRIM49, MIR5692A1, TRIM53AP, TRIM64B, TRIM49D2P, TRIM49D1, TRIM64, TRIM49C, UBTFL1, NAALAD2*	4 (Multiple)	0 (0)	Affymetrix CytoScan HD
18	VUS	14q11.2	chr14:22,215,286-24,018,428	Loss	1803	>30	*DAD1, ABHD4, OXA1L, SLC7A7, MRPL52, MMP14, LRP10, REM2, RBM23, PRMT5, HAUS4, JUB, C14orf93, PSMB5, PSMB11, CDH24, ACIN1, 14orf119,CEBPE, SLC7A8...*	0 (Multiple)	0 (0)	Agilent ISCA 8x60K
19	VUS	14q11.2	chr14:22,299,149-23,061,615	Loss	762	1	*DAD1*	7 (Multiple)	0 (0)	Agilent ISCA 8x60K
8	VUS	15q13.3-q14	chr15:32,942,601-34,627,075	Gain	1684	11	*SCG5, GREM1, FMN1, RYR3, AVEN, CHRM5, C15orf24, PGBD4, C15orf29, TMEM85, SLC12A6*	4 (Multiple)	0 (0)	Agilent ISCA 8x60K
15	VUS	17p13.3	chr17:625,416-810,197	Gain	185	5	*FAM57A, GEMIN4, DBIL5P, GLOD4, RNMTL1, NXN*	13 (Multiple)	0 (0)	Agilent ISCA 8x60K
9	VUS	18p11.21	chr18:14,194,813-14,851,736	Gain	657	6	*ANKRD20A5, CYP4F35P, CXADRP3, POTEC, ANKRD30B, MIR3156-2*	2 (Multiple)	0 (0)	Affymetrix CytoScan HD
4	VUS	Xp22.33	chrX:795,717-1,427,556	Gain	632	3	*CRLF2, CSF2RA, MIR3690*	3 (Multiple)	0 (1)	Agilent ISCA 8x60K
6	VUS	Yq11.223	chrY:25,296,950-26,066,972	Gain	770	2	*DAZ1, DAZ2*	3 (66)	0 (3)	Agilent ISCA 8x60K
1	LB	2q21.2	chr2:132,696,181-133,136,747	Gain	441	2	*ANKRD30BL, MIR663B*	13 (Multiple)	0 (0)	Affymetrix CytoScan HD
7	B	14q11.2	chr14:22,598,026-22,897,089	Loss	299	0	-	26 (Multiple)	0 (0)	Agilent ISCA 8x60K

Abbreviations: P, pathogenic; VUS, variant of uncertain clinical significance; LB, likely benign; B, benign; Chr, Chromosome; Kbp, kilobase pair; DGV, Database of Genomic Variants. The number of CNVs in a very similar size is listed for both DGV and Decipher. The number of any size of CNVs overlapped any part of our CNV region is shown in parentheses.

and penises were normal. However all patients are infertile and azoospermic, their ejaculate volumes, semen glucosidase and/or fructose levels and blood follicle stimulating hormone (FSH) levels (1.7-12mIU/ml) were normal. In addition, transrectal ultrasound (TRUS) demonstrated no other obstructive cause for azoospermia. There was no other abnormality accompanying CBAVD.

Genomic Copy Number Variations

In nine of the 19 patients (47.3%), we detected CNVs including a pathogenic (P) deletion, a likely benign (LB) variant of duplication and eight variants of uncertain significance (VUS) consisting of two deletions and six duplications. A benign (B) deletion (299 Kbp) in the 14q11.2 region containing no known genes was also detected in a different patient. We reported this benign variation as an exceptional due to its localization in a recurrent CNV region. The types and localization of these CNVs, minimum-maximum nucleotide numbers that show the borders in chromosomal DNA (based on hg19), length in base pairs, number of genes included in that region were listed in Table 2. All CNVs were detected in a heterozygous state. The average size of the CNVs was 837 Kbp. Duplications were more common than deletions (64% versus 36%).

In Case 8, we identified two CNVs, a pathogenic and a VUS on chromosome 15. Each of the other CNVs was found in different patients. The 15q13.2-q13.3 deletion in Case 8 was defined as pathogenic according to the ACMG criteria. This deletion was 1555 Kbp in length, for which a similar sized CNV present once in both DGV and DECIPHER CNV Syndromes. It overlapped with the genomic region associated with 15q13.3 microdeletion syndrome. Within the 15q13.2-q13.3 deletion, there were seven protein-coding genes (Table 2). We have had no data on whether the case 8 having the the clinical features of 15q13.3 microdeletion syndrome. There was no any other similar sized CNV present in DECIPHER for the other CNVs. When any sized CNV that overlaps our CNVs is considered, this was seen multiple times in DGV for most of all (66 times for the Yq11.223 duplication) and none in DECIPHER except the duplications of Xp22.33 (once) and Yq11.223 (three times) (Table 2).

Possible candidate gene and direct sequencing

As shown in the Table 2, deletions in the 14q11.2 chromosomal region was detected in 15.79% (n=3) of 19 patients. Software images of the 14q11.2 deletions are shown in Figures 1A, 1B and 1C. The overlapping genomic region of these three was 299 Kbp length where no known protein coding genes were localised. On the other hand, *DAD1* was the only gene localised in the overlapped region of two of the 14q11.2 deletions (Figure 2). DAD1 is a core subunit of the oligosaccharyltransferase (OST) complex catalyzes N-glycosylation of target proteins which is a key modification occurring in eukaryotes. Loss-of-function of the DAD1 protein triggers apoptosis [19]. Homozygous *Dad1* knockout mice models are embryonically lethal revealing essential role of the gene in embryological development [20]. DAD1 protein is expressed as medium level in male reproductive system tissues (https://www.proteinatlas.org/ENSG00000129562-DAD1/tissue). N-glycosylation, a common cotranslational modification, is the attachment of the core oligosaccharide to the asparagine residue of a protein by the OST. N-glycosylation defects cause impairments in the folding and stability of CFTR protein [21]. DAD1 is one of the globular modulators of OST stability thus affects directly OST dependent N-glycosylation [19]. Based on these results, *DAD1* gene coding a protein functioning in the posttranslational modification process of CFTR may lead to CBAVD in cases without *CFTR* mutations. Sequence analysis was performed to investigate if *DAD1* (NM_001344.4) single nucleotide variations contribute to CBAVD etiology. No variation was detected by Sanger sequencing of *DAD1* in our cohort of the study group.

DISCUSSION

If any potentially pathogenic CNV is common in a cohort with a specific phenotype, that CNV region can be thought of as a candidate region for that physiological condition [22]. Within the scope of this study, we aimed to identify the most likely pathogenic CNVs that can cause a CBAVD phenotype. Genetic studies on etiology of CBAVD in Turkish population are very limited. This is the first study investigating CNVs and candidate

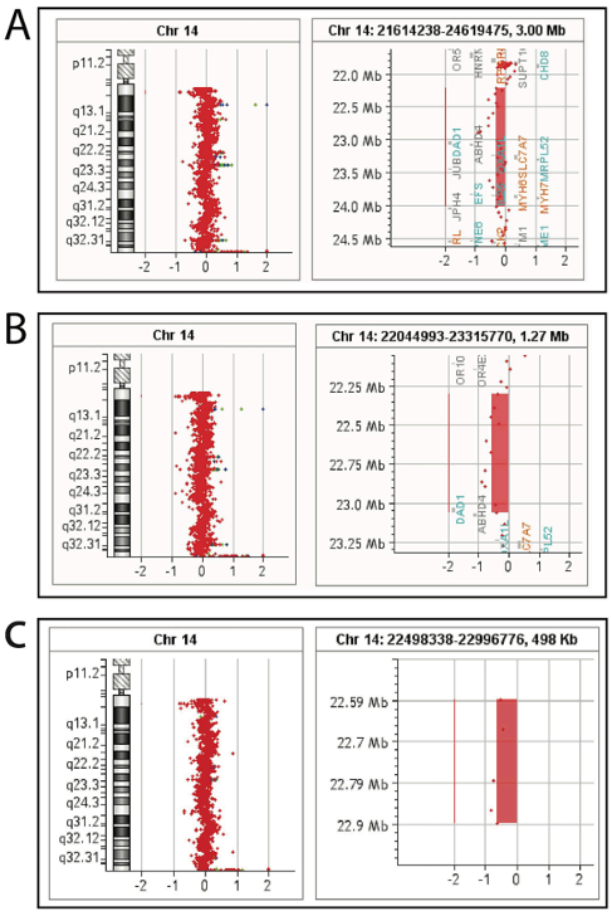

Figure 1: CNV analysis results of heterozygous 14q11.2 deletions. A) 1803 Kbp deleted region. B) 762 Kbp deleted region. C) 299 Kbp deleted region. The images show the log2 ratio of the reference versus patient DNA on the Y-axis and the position of each probe along the chromosome on the X-axis. Vertical red bars indicate the deleted genomic region. Images were produced using Agilent Cytogenomics 4.0.2.21 software.

Figure 2: Identification of reciprocal overlap in size for 14q11.2 deletions. Each colored bar (red, blue, green) represents CNV calls in cases 18, 19, and 7 respectively. Three CNV calls each shares a genomic region of 299 Kbp length at that locus. The overlapping region of two CNV calls is 762 Kbp in length and harbours the DAD1 gene which is marked with a red colored rectangle.

genomic regions or genes associated with CBAVD in Turkish cases. We reported large CNVs by using high-resolution array technologies without any other confirmation from an alternative method in the light of our previous studies [23, 24].

We evaluated the clinical findings of CBAVD patients. Despite the ejaculate volume and the fructose levels are usually low in CBAVD men [25]; we detected normal levels of ejaculate volumes and seminal glucosidase and/or fructose concentrations in our study group as we reported before [7]. Lin et al. also reported a case with CBAVD having normal semen parameters [26].

Additionally, Van der Ven K et al. and reported that *CFTR* mutations were variable and that *CFTR* mutations may cause reduced sperm quality without non-obstructive azoospermia or low fructose volume [27, 28]. Wedenoja et al. agreed with them [28]. Thus, *CFTR* mutations are related to variable semen parameters that may range from normal to subnormal semen parameters. We excluded CBAVD patients having *CFTR* mutations. They had no additional genital abnormalities that were seen on TRUS imaging. Moreover, it has been shown that CBAVD patients with renal aplasia did not have *CFTR* mutations [29]. Besides, the absence of unilateral vas deferens may be part of renal malformations. Gajbhiye et al. published that *CFTR* mutations could be detected with renal malformations [30]. However, we ruled out renal aplasia by using radiological evaluations, and we have not gotten any infertile patients with renal malformations. Nevertheless, we have one of the largest patient populations with *CFTR* mutations in our Turkish community and all genomic mutations were recorded.

The pathophysiology of CBAVD cannot be explained only by *CFTR* mutations, suggesting possible genetic heterogeneity. CNV studies to determine the underlying etiology of idiopathic CBAVD are very limited. To date, a study was done by Lee et al. investigating CNVs by using array CGH in Taiwanese CBAVD patients (n=8) in 2009. The researchers identified CNVs in the 3q26 chromosomal region in five of their cases and proposed that this region can be related to CBAVD. In addition, they also detected CNVs in a region including the *PANK2* gene that plays a role in the development of the urinary system in two of their cases. In our cohort, no CNV was detected in neither the 3q26 nor in the 20p12 region containing the *PANK2* gene. CNVs were found in the Yq11.223 region by both of the studies. While they detected a deletion in the Yq11.223, we determined an amplification [3]. The very low number of CNVs shared between our patients and Taiwanese patients may be due to ethnic and geographic differences between populations.

Despite the presence of apparently benign CNVs in the human genome, a large number of CNVs are involved in the etiology of various human diseases. Therefore, CNVs are an important source of mutation burden associated with human diseases. The disease phenotype occurs depending on the functions of the genes in the CNV region [22]. We searched the literature in terms of the possible relationship of genes localized in our CNV regions with the male reproductive system. However, we could not find a gene known to be directly related to the development of the male reproductive system. CBAVD exhibited variable inheritance patterns like autosomal recessive, autosomal dominant, and X-linked [25]. We found all CNVs present in heterozygous state. The significance of the heterozygous CNVs in the pathogenesis of CBAVD is unknown but suggests a possible inheritance model of autosomal dominant.

The most noteworthy findings in our study are the 14q11.2 (762 Kbp, 1803 Kbp, 299 Kbp) deletions in three of the cases. CNVs in the 14q11.2 region have also been identified in infertile men in other studies. Tüttelmann et al. performed CNV analysis in Caucasian patients with idiopathic severe oligozoospermia and Sertoli-cell-only syndrome (SCOS). They determined 14q11.2 deletions and duplications both in their patient and normozoospermic control group. All were 222.3 Kbp length and did not overlap with the ones we detected. In addition, the Yq11.223 duplication that they found in SCOS patients was also detected in our patient group [31]. Halder et al. revealed CNVs in Indian patients with testicular maturation arrest. They also found CNVs (smallest: ~ 207 Kbp, largest: ~ 219 Kbp) in the 14q11.2 region as gain or loss in 10 patients. These CNVs did not overlap with ours, they were located in the upstream region of the 14q11.2del segments we identified [32]. CNVs in Australian cases with azoospermia were investigated in another study. The researchers found heterozygous duplications as pathogenic CNVs in chromosomes 3 and 11 containing various genes that may be associated with male infertility. Incompatible with our findings, they detected a 14q11.2 deletion (~ 37 Kbp) in a case. This deletion overlapped with the 14q11.2 deletions of ours but it was smaller in length, did not contain any gene, thereby considering benign. It was located downstream of the *DAD1* gene [14]. In 2015, Dong et al. reported that three of 33 azoospermia cases had heterozygous CNVs in the 14q11.2 chromosomal region [15]. There was no overlapping region with the CNVs found

in this study and they were located about 2.6-2.7 Mb downstream of the *DAD1* gene. These results from different groups suggest the potential effects of 14q11.2 in male infertility. The 14q11.2 region was also associated with developmental delay, cognitive impairment, and similar minor anomalies [33]. In addition, it should be kept in mind that chromosome 14 has imprinted regions. Disruptions of the 14q11-q13 region may cause low birth weight and delayed growth, according to Kamnasaran and Cox [34]. Additionally, we detected 14q11.2 deletions containing the *DAD1* in two of the cases (Case 18 and Case19). The 14q11.2 deletion in Case 7 was spanning 136.7 Kbp downstream of the *DAD1* gene which coded on the minus strand of DNA. We can not exclude the regulatory role of this region on the *DAD1* gene, it should be further investigated. *DAD1* is expressed both at mRNA and protein levels in the male genital tract. Rockett et al. has published that mRNA levels of the *Dad1* gene can be decreased as a result of heat exposure in a heat-shocked testis mice model [35]. In addition, *DAD1* transcript is found to be down-regulated both in normozoospermic and asthenozoospermic groups when compared with the control group in a sperm transcriptome profiling study. As a result of these findings, it has been offered that apoptotic genes may play an important role in male infertility [36]. Although DAD1 is identified as an anti-apoptotic molecule that regulates programmed cell death negatively, it is one of the subunits of oligosaccharyltransferase (OST) that catalyzes the protein N-glycosylation process in eukaryotes [16, 19]. It has been thought that, as a common post-translational modification, N-glycosylation affects plasma membrane expression of glycoproteins by organizing the folding, targeting, and transportation through the endoplasmic reticulum (ER) [21]. It has been stated, that as a result of Glozman et al.'s study, N-glycans enhanced CFTR folding efficiency and the limited folding efficiency (30–40%) of the wild type CFTR in the ER, conceivably reflect inefficient posttranslational domain assembly [21]. Thus, it has been mentioned that an N-glycosylation defect reduces the cell surface expression of CFTR. According to Li et al., for the sperm capacitation, acrosome reaction, and sperm-oocyte fusion, wild-type CFTR protein is a necessity, and deficient CFTR protein expression may affect the sperm fertilizing capacity [37]. When we consider the relationship between CFTR and CBAVD as a part of OST, defects in the *DAD1* gene may cause an insufficient CFTR via the N-glycosylation process which may result in the CBAVD phenotype. As stated by Flannigan and Schlegel in 2017, there is an urgent need for specific genomic biomarkers in the diagnosis of CBAVD [38].

Limitation of Study: This study was a pilot study aimed to identify novel potential candidate genomic regions and/or genes related to CBAVD. Number of patients included to the study were relatively low. CNV analysis should be done in larger cohorts of CBAVD patients to identfy the candiate genomic regions first, and then the alternative biologic mechanisms associated with the disease pathophysiology.

Conclusıon

Our study was the first study profiling CNVs in Turkish-Caucasian patients with CBAVD. The spectrum and frequency of nuclear gene mutations and copy number variations in Turkish patients with CBAVD are still largely unknown. These patients underscore the importance of comprehensive genomic analysis in Turkish-Caucasian patients. Using additional technologies and functional analyses will help us understand the mechanism of the disease and will highlight the interactions between phenotypes and genotypes. In the light of our data, we suggest that CNV and *DAD1* analysis should be done in a larger cohort of CBAVD patients to determine potential chromosomal regions and genes for CBAVD cases related to male infertility.

Conflict of Interest: The author has no conflict of interest related to this article.

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