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ORIGINAL ARTICLE – OPEN ACCESS



# Molecular Karyotyping in Anorectal Malformations: Could *DGCR6* Gene Haploinsufficiency Cause Anal Atresia in 22q11 Deletion Syndrome?

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ABSTRACT

**Objective:** Anorectal malformations (ARM) are classified as a multifactorial disease. The etiology of ARM is still not clear due to the complexity of the pathological anomalies.

**Materials and Methods:** The microarray-based comparative genomic hybridization (array CGH) results of 10 patients with ARM not associated with a specific syndrome were analyzed using the 8x60K ISCA Agilent microarray platform (Human Genome CGH Microarray; Agilent Technologies, Inc., Santa Clara, CA, USA). Pathogenic copy number variants were further confirmed using fluorescence in situ hybridization or quantitative real-time polymerase chain reaction testing.

**Results:** Chromosome 22q11.2 deletion was detected in 2 patients. One of these patients had anal stenosis, minor cardiac abnormalities, and a small 0.89-Mb deletion. The second patient had anal atresia, immune deficiency, inguinal hernia, and a 2.7-Mb cryptic deletion. The overlapping genes in the deletion regions of the 2 patients were the *DGCR5, DGCR6*, and *PRODH* genes.

**Conclusion:** *DGCR6* alters the expression of important genes such as *TBX1* and affects neural crest migration. Given that ARM are caused by abnormalities in neural crest cell migration, it may be that these genes play a role in the etiology. To our knowledge, this is one of the smallest interstitial deletions in the chromosome 22q11.2 region to be published to date. Further research on the *DGCR6* gene, which may be a candidate gene responsible for anal atresia, will clarify this point.

**Keywords:** 22q11 deletion syndrome, anal atresia, anorectal malformation, *DGCR5, DGCR6*, microarray, *PRODH*

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## INTRODUCTION

Anorectal malformations (ARM) are among the most common congenital defects to be surgically repaired (1). The prevalence has been reported to be 3.6 per 10,000 births (2). ARM are classified as a multifactorial disease due to the coexistence and complex interactions of several factors. ARM vary widely, ranging from an anteriorly displaced anus, which can be easily corrected surgically, to a cloaca anomaly, which requires much more complex and repetitive surgeries (3). The prevalence is higher in boys than in girls; the male/female ratio is reported to be about 1.2:1.6 (4). ARM can occur in family members and twins; genetic as well as environmental factors play a prominent role in the development of ARM (5). Environmental factors associated with ARM include drug use during pregnancy, smoking, maternal diabetes mellitus, and retinoic acid embryopathy (6, 7).

An additional congenital anomaly is seen in 40% to 70% of children who are born with ARM (8). VACTERL association (vertebral defects, anal atresia, cardiac defects, tracheo-esophageal fistula, renal anomalies, and limb abnormalities) (MIM #192350) and CHARGE syndrome (coloboma, heart defect, atresia choanae, retarded growth and development, genital hypoplasia, ear anomalies/deafness) (MIM #214800) should be considered in the genetic approach to ARM in the presence of additional major components (9). Approximately 50% of all ARM are associated with a syndrome, complex multiple anomalies, or a chromosomal disorder (10). Although the genetic etiology of ARM has not been fully elucidated, chromosomal diseases, such as Down syndrome (MIM #190685), 22q11 deletion syndrome (MIM #611867), 22q11 duplication syndrome (MIM #608363), and cat eye syndrome (MIM #115470), as well as single-gene disorders, such as Currarino syndrome (MIM #176450) and FG syndrome (MIM #145410) are well-known causes of ARM (9).

Submicroscopic deletions and duplications have frequently been reported in patients with ARM (11). Molecular karyotyping is important in determining the underlying cause in patients diagnosed with ARM because of this common association, especially if there are accompanying additional findings and/or dysmorphic features of other systems. Microarray-based comparative genomic hybridization (array CGH) allows for examination of the entire genome at one time and enables determination of submicroscopic microdeletions and microduplications in patients with ARM (12).

The objective of this study was to evaluate the molecular cytogenetic analysis results of 10 patients with ARM using related databases and literature data.

## MATERIALS and METHODS

The ethics committee of Keçiören Training and Research Hospital granted approval for this study on January 13, 2016 (no: 15/1056). Written, informed consent was obtained from the patients or guardians for the publication of this article and the accompanying images. This study was performed in accordance with the Declaration of Helsinki.

### Patients

The study patients were children who had been diagnosed with ARM with minor additional anomalies and a normal karyotype at Gazi University Hospital between January 2012 and December 2014. In all, 8 boys and 2 girls aged between 12 days and 9 years were assessed. The clinical diagnosis of ARM was made based on phenotypic and radiographic evaluation by a pediatric surgeon. Anal stenosis was present in 2 of the patients, anal atresia was present in the remaining 8 patients, and anal fistula was present in only 1 patient. All of the patients were referred for an evaluation of genetic etiology. None of the patients had a history of ARM in other family members. Only 1 patient's parents were consanguineous. Dysmorphic and other findings of all of the patients are summarized in Table 1. Although there were some additional findings, 9 of the 10 cases were not associated with either a specific single-gene disease or association following a detailed dysmorphology examination. The coexistence of anal atresia and distal sacral agenesis in patient #4 met 2 elements of the Currarino syndrome triad. Therefore, *HLXB9* gene sequence analysis was planned for this patient if the molecular karyotyping result was normal. Array CGH analysis was performed. Pathogenic copy number variants (CNVs) were further confirmed using fluorescence in situ hybridization (FISH) or quantitative real-time polymerase chain reaction (qPCR) testing.

### Molecular Karyotyping

After the patients were evaluated clinically, blood samples were taken for routine genetic studies. Genomic DNA was isolated from peripheral blood using the salting-out method. Array CGH analysis was performed using the 8×60K ISCA, Agilent microarray platform (Human Genome CGH Microarray, Agilent Technologies Inc., Santa Clara, CA, USA) according to the manufacturer's protocol. Data analysis was performed using CytoGenomics software (v. 2.0.6.0) (Agilent Technologies Inc., Santa Clara, CA, USA).

### Variant Interpretation

The standard probe cut-off levels used in routine diagnostics at the Department of Medical Genetics, Gazi University Hospital were used to interpret CNVs: (i) CNVs of ≥100 kb, (ii) including the gene, (iii) absolute log2-ratios >0.25, (iv) CNVs with a minimum of 3 consecutive probes. CNVs of <100 kb that contained genes associated with ARM were confirmed by a second method and then included in the results.

After analyzing the extracted data according to these criteria, CNVs were compared with variants reported in the Database of Genomic Variants (DGV, http://projects.tcag.ca/variation), the Database of Chromosomal Imbalance and Phenotype in Humans Ensembl Resources (DECIPHER, https://decipher.sanger.ac.uk), ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/), and the in-house database at the Department of Medical Genetics, Gazi University Hospital. CNVs were classified as benign if they overlapped with CNVs reported in the DGV (more than 3 studies or more than 1% reported in the normal population) or if they were frequently observed in the in-house database. CNVs were interpreted as pathogenic if they overlapped with the critical region of a microdeletion or microduplication syndrome or contained a dose-sensitive gene according to the ClinGen Dosing Sensitivity Map (https://dosage.clinicalgenome.org/). CNVs were interpreted as a variant of unknown significance (VOUS) when it was not known whether the genes contained were dosage-sensitive. Various databases have been used to identify CNVs that may be associated with ARM etiology: UCSC Genome Bioinformatics, PubMed (U.S. National Library of Medicine), OMIM (Online Mendelian Inheritance in Man), Uniprot, Genecards.

### Fluorescence in situ Hybridization

FISH analysis was performed from peripheral blood samples using a Vysis DiGeorge region probe (LSI TUPLE1 SpectrumOrange/LSI ARSA SpectrumGreen probe; Abbot Laboratories Inc., Abbott Park, IL, USA) to confirm the 22q11 deletion, according to the manufacturer's instructions.

### Quantitative Polymerase Chain Reaction

qPCR was performed using EvaGreen dye (Biotium Inc., Fremont, CA, USA) on a BIO-RAD CFX96 Touch real-time PCR system (Bio-Rad Laboratories, Inc., USA) with the recommended PCR conditions. All of the experiments were repeated 3 times. The primers were 5' GATGGCTTTTCCTGGCTGTA 3' and 3' ACCTTATGCTGACAGAGATGTGA 5'. The cycle of threshold values of patient #1 are available on request.

## RESULTS

Demographic characteristics and examination findings of the patients with ARM are summarized in Table 1. Five of 10 patients' results did not include a CNV that met the evaluation criteria. An ARM-related CNV was found in 2 patients (patient #1 and patient #8). A variant of uncertain significance (VOUS) was found in 2 patients (patient #1 and patient #2) and likely benign changes were determined in 2 patients (patient #7 and patient #9). The molecular karyotyping results of the patients are shown in Table 2.

Patient #1: A male, aged 3 years, who had anal stenosis and a minor cardiac anomalies (secundum atrial septal defect and patent foramen ovale) and mild spinal enlargement at the 10$^{th}$ thoracic vertebral level in the spinal cord, had 89 kb monoallelic deletion in the 22q11.21 region (arr(hg19) 22q11.21(18,894,835–18,984,519) ×1). It was represented by 3 probes and included 3 genes (*DGCR6, DGCR5* and *PRODH*). Though the deletion was smaller than 100 kb, it was confirmed using qPCR because it contained 22q11 deletion syndrome critical region genes and could be associated with the ARM phenotype. That CNV has been reported in healthy individuals in the DGV database, but the 115 kb deletion (patient #332402) containing *PRODH* and *DGCR6* genes

**Table 1.** Demographic characteristics, examination findings, and test results of the patients

| | Patient 1 | Patient 2 | Patient 3 | Patient 4 | Patient 5 | Patient 6 | Patient 7 | Patient 8 | Patient 9 | Patient 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| Age/Gender | 3,5 y/M | 4 y/M | 4,5 y/M | 7 y/M | 5 m/M | 9,5 y/M | 5,5 y/M | 12 day/M | 7 m/F | 7 y/F |
| Prenatal | Maternal smoking (3 cigarettes/day) | Uneventful | High risk of NTD | Uneventful | Uneventful | Uneventful | Uneventful | Uneventful | Uneventful | Uneventful |
| Natal | C/S, term | NSVD, term | NSVD, term | C/S, 35 weeks | NSVD, term | NSVD, term | NSVD, term | C/S, term | NSVD, term | NSVD, term |
| Postnatal | Anal stenosis | Anal atresia | Anal atresia | Anal atresia | Anal stenosis | Anal atresia | Anal atresia | Anal atresia | Anal atresia | Anal atresia, rectovestibular fistula |
| Consanguinity | No | No | No | No | No | Same village | No | No | No | 3rd degree |
| Dysmorphic features | Prominent forehead, metopic ridge, sparse eyebrows, dysplastic ears | Obesity, fusiform fingers | Broad arched eyebrows, thin upper lip, smooth philtrum hypertrichosis | Synophrys, long eyelashes, uplifted ear lobe | upsweep, sparse eyebrows, upslanting palpebral fissures | Upslanting palpebral fissures | Wide ear lobe | Sparse eyebrows, narrow palpebral fissures, small mouth | Upslanting palpebral fissures, small mouth | Hirsutism, high nasal bridge, posteriorly rotated ears |
| Cardiac anomaly | ASD, PFO | – | – | – | – | – | Secundum ASD | Normal | Secundum ASD | Secundum ASD, PFO |
| Abdomen USG | Normal | Normal | Normal | – | – | – | Normal | Normal | – | Normal |
| Skeletal anomaly | Spinal cord enlargement at T10-11 | Normal | Normal | Distal sacral agenesis, coccygeal agenesis and syringohydromyelia | – | Normal | Normal | Normal | Normal | Normal |
| Genitourinary | – | – | Hypospadias | – | Phimosis | Vesicoureteral reflux | Inguinal hernia | Undescended testicle | – | – |
| Other | – | – | – | – | Atopic dermatitis | – | Amblyopia | Frequent infections | – | Muscle weakness high muscle and liver enzymes |
| Karyotype | 46, XY | 46, XY | 46, XY | 46, XY | 46, XY | 46, XY | 46, XY | 46, XY | 46, XX | 46, XX |

F: Female; M: Male; NTD: Neural tube defect; C/S: Cesarian section; NSVD: Normal spontaneous vaginal delivery; ASD: Atrial septal defect; PFO: Patent foramen ovale; USG: Ultrasonography

**Table 2.** Molecular karyotyping results of the ARM patients

| Patient | Chromosome | Deletion/ Duplication | Genomic coordinates (Grch37/hg19) | Size | Probe | Classification | Genes |
|---|---|---|---|---|---|---|---|
| 1 | 22q11.21 | Deletion | 18894835-18984519 | 89 kb | 3 | VOUS | *DGCR6, DGCR5, PRODH* |
| 1 | 2q13 | Deletion | 110862477-110964737 | 102 kb | 35 | VOUS | *MALL, NPHP1* |
| 2 | 1q31.1 | Duplication | 186762066-187472702 | 710 kb | 14 | VOUS | *PLA2G4A* |
| 7 | 10q26.3 | Duplication | 135243049-135404523 | 161 kb | 30 | Likely benign | *LOC619207, SCART1, CYP2E1, SYCE1, SPRNP1* |
| 8 | 22q11.21 | Deletion | 18628019-21505417 | 2,877 kb | 146 | Pathogenic | *LOC105372871, CA15P2, PPP1R26P2, LOC642643, LOC105372867, LOC105377182, GGT3P, LOC102724123, E2F6P1, LOC101929738, LOC102725072, BCRP7, LOC100287541, LOC100996432,* ***DGCR6, PRODH, DGCR5****, LOC105372855,* ***DGCR9, DGCR10, DGCR2,*** *DGCR11, LOC100129262, TSSK1A,* ***DGCR14****, TSSK2, LOC105377184, GSC2, LINC01311,* ***SLC25A1, CLTCL1****, RPL34P35, DVL1P1, KRT18P62, LOC105372859,* ***HIRA****, MRPL40, C22orf39, LOC105372860,* ***UFD1L, CDC45****, CLDN5, LINC00895, LOC100129254, LOC100420103, SEPT5, SEPT5-GP1BB, GP1BB, LOC105372861,* ***TBX1****, GNB1L, RPL7AP70, C22orf29, TXNRD2, RPL8P5, LOC105377185, COMT, MIR4761, ARVCF, TANGO2, MIR185, DGCR8, MIR3618, MIR1306, TRMT2A, MIR6816, RANBP1, ZDHHC8, CCDC188, LOC284865, LOC105372862, LINC00896, LOC105372863, RTN4R, LOC105372864, MIR1286, LOC440792,* ***DGCR6L****, LOC101927859, LOC105372866, LOC105377189, USP41, ZNF74,* ***SCARF2****, KLHL22, LOC100420177, LOC105372940, RNY1P9, LOC105372939, KRT18P5, LOC101928824, MED15, LOC105372938, CCDC74BP1, SMPD4P1, IGLL4P, LOC100421121, SLC9A3P2, ABHD17AP4, POM121L4P, BCRP5, TMEM191A, PI4KA,* ***SERPIND1****, SNAP29, CRKL, LOC101928891, AIFM3, LZTR1, THAP7, THAP7-AS1, TUBA3FP, P2RX6, SLC7A4, MIR649, P2RX6P, LRRC74B, TUBA3GP, BCRP2, POM121L7, E2F6P2, FAM230B, LOC105372935,* ***GGT2,*** *E2F6P3, POM121L8P, BCRP6, LOC105369215, PPP1R26P5, LOC100996335, LOC105372944, LOC105377190, SUSD2P1, RIMBP3B, HIC2, LOC105377191, TMEM191C, PI4KAP2, LOC105372946* |
| 9 | 5p15.33 | Duplication | 648152-759228 | 111 kb | 3 | Likely benign | CEP72, TPPP |

ARM: Anorectal malformation; VOUS: Variant of unknown significance

was classified as pathogenic in the DECIPHER database. In the ClinVar database, 3 CNVs containing the *PRODH* and *DGCR6* genes, the smallest 30 kb and the largest 109 kb, were interpreted as pathogenic (patient #59070, #4005, and #583475). This CNV was classified as a VOUS according to the recommendation of the American College of Medical Genetics and Genomics and Clinical Genome Resource (ClinGen) (13).

Patient #8: A 12-day-old male with no symptoms other than frequent infection, anal atresia, and a history of an undescended testicle. The molecular karyotyping revealed a 2.7-Mb monoallelic deletion in the 22q11 region, arr(hg19) 22q11.21(18,628,019–21,505,417), which was classified as pathogenic, and the patient was diagnosed as having 22q11 deletion syndrome once it was confirmed using FISH. FISH analysis for 22q11 of his parents was also performed with normal results.

In patient #1, there was also a 102 kb deletion in the 2q13 region containing the *NPHP1* and *MALL* genes. This deletion, which can be found in healthy individuals according to the DGV database, is represented by 35 probes. ARM have not been reported among patients with deletion of a similar region in the DECIPHER database. The *NPHP1* gene encodes a protein involved in cell division, which is mostly expressed in the kidneys, eyes, testes, and ovaries. Homozygous or compound heterozygous missense mutations in the *NPHP1* gene have been associated with Joubert syndrome type 4 (JBTS4, MIM #604583), Senior-Loken syndrome (SLSL1, MIM #266900), and autosomal recessive nephronophthisis 1, juvenile (NPH1, MIM# 256100). The patient was evaluated as a carrier for the related diseases because of the heterozygous deletion of the region. Clinical findings are not expected in heterozygous carriers. Finally, the current duplication was not associated with ARM and was classified as a VOUS.

Patient #2 had a 710 kb duplication in the 1q31.1 region, including the *PLA2G4A* gene (phospholipase A2, group 4A, MIM# 600522). The duplication is not a polymorphic CNV according to the DGV database. According to the UCSC database, the duplication has been classified as a VOUS. There are no patients with anorectal malformation and duplication of the same region in the DECIPHER database. Based on this information, the duplication in patient #2 was classified as a VOUS and it was concluded that it was not associated with ARM.

Patient #7 had a 160 kb duplication in the chromosome 10q26.3 region including *LOC619207, SCART1, CYP2E1, SYCE1, SPRNP1* genes, which has been reported as polymorphic CNV according to the DGV database. In the DECIPHER database, similar-sized CNVs have been reported as benign in patients #285904, #286222, and #294706. This CNV detected in patient #7 could not be associated with ARM and was classified as likely benign.

The molecular karyotyping of patient #9 disclosed a 111-kb duplication represented by 3 probes on the short arm of chromosome 5. This duplication, including the *CEP72* and *TPPP* genes, has been reported in healthy individuals in the DGV database. Similar duplications have been reported as benign in the DECIPHER database. Therefore, this CNV, which could not be associated with the clinical findings of the patient, was evaluated as likely benign.

## DISCUSSION

We evaluated the array CGH results of 10 patients in terms of the relationship to ARM by searching databases and the literature. CNVs that could not affect the phenotype were detected in 8 of the 10 patients, and it was thought that the CNVs detected in 2 patients might be related to ARM.

In patient #8, a 2.8-Mb deletion in chromosome 22q11.21 was identified and confirmed using FISH. This region includes the critical region of 22q11 deletion syndrome, which is a well-known microdeletion syndrome. Although 90% of cases of deletion 22q11 syndrome are de novo, in a small portion it is inherited from a parent (14). For this reason, deletion analysis was performed on the parents of patient #8 using FISH and it was determined that they did not carry the deletion. Patient #8 did not show the characteristic findings of 22q11 deletion syndrome, such as a heart anomaly. Of the findings seen in 22q11 deletion syndrome in our patient, there were only anal atresia, undescended testis. and frequent infection. Undescended testis has been reported as a finding of the syndrome in approximately 6% of patients (15).

Immunodeficiency has been defined in 70% of patients with 22q11 deletion syndrome. Pneumonia, especially in the neonatal period, is an important reason for hospitalization. The frequency of infections decreases in late childhood (16). Although chronic constipation is a common feature seen in 35%, ARM is a rare finding of 22q11 deletion syndrome (17). Anal atresia was reported for the first time in DiGeorge syndrome in 1979 (18). Since then, in 1 study, an anteriorly displaced anus has been defined in 1 of 44 patients with 22q11 deletion (19), and in 2 patients in another series of 55 patients (20). Worthington et al. (21) reported 3 patients with ARM without cardiac anomalies in 1997. All 3 patients had hypernasal speech, uvula anomaly, speech delay, and developmental delay. The authors suggested that there might be a common mechanism in the pathogenesis of velocardiofacial syndrome (VCFS) and ARM because both occurred due to abnormal migration of mesenchymal cells (21). In support of this hypothesis, the most common microdeletion syndrome observed in patients with ARM is 22q11 deletion syndrome. In addition, ARM is an important finding in cat eye syndrome caused by overexpression of the 22q region. All of these associations led to the intensification of genetic ARM studies in the long arm of the 22$^{nd}$ chromosome. In genome-wide association studies related to ARM, the most featured region is still the 22q11 region (22).

Deletion of 22q11.21, containing the *DGCR6, DGCR*, and *PRODH* genes, was detected in patient #1 and confirmed using qPCR. To the best of our knowledge, it is the smallest deletion identified in patients with anal atresia in the literature to date. Anal stenosis and minor cardiac anomalies, which were among the phenotypic findings of patient #1, have also been described in 22q11 deletion syndrome. The loss of which of the genes located in the critical region of 22q11 causes ARM is not known. It is possible that one or more of the genes deletions determined in 2 of our patients (patients #1 and #8) may cause the ARM seen in 22q11 deletion syndrome.

In patient #1 and patient #8, different sizes deletions were detected in the 22q11.22 region and the *DGCR6, DGCR5*, and *PRODH* genes overlapped in both patients. When we examined the *DGCR6,*

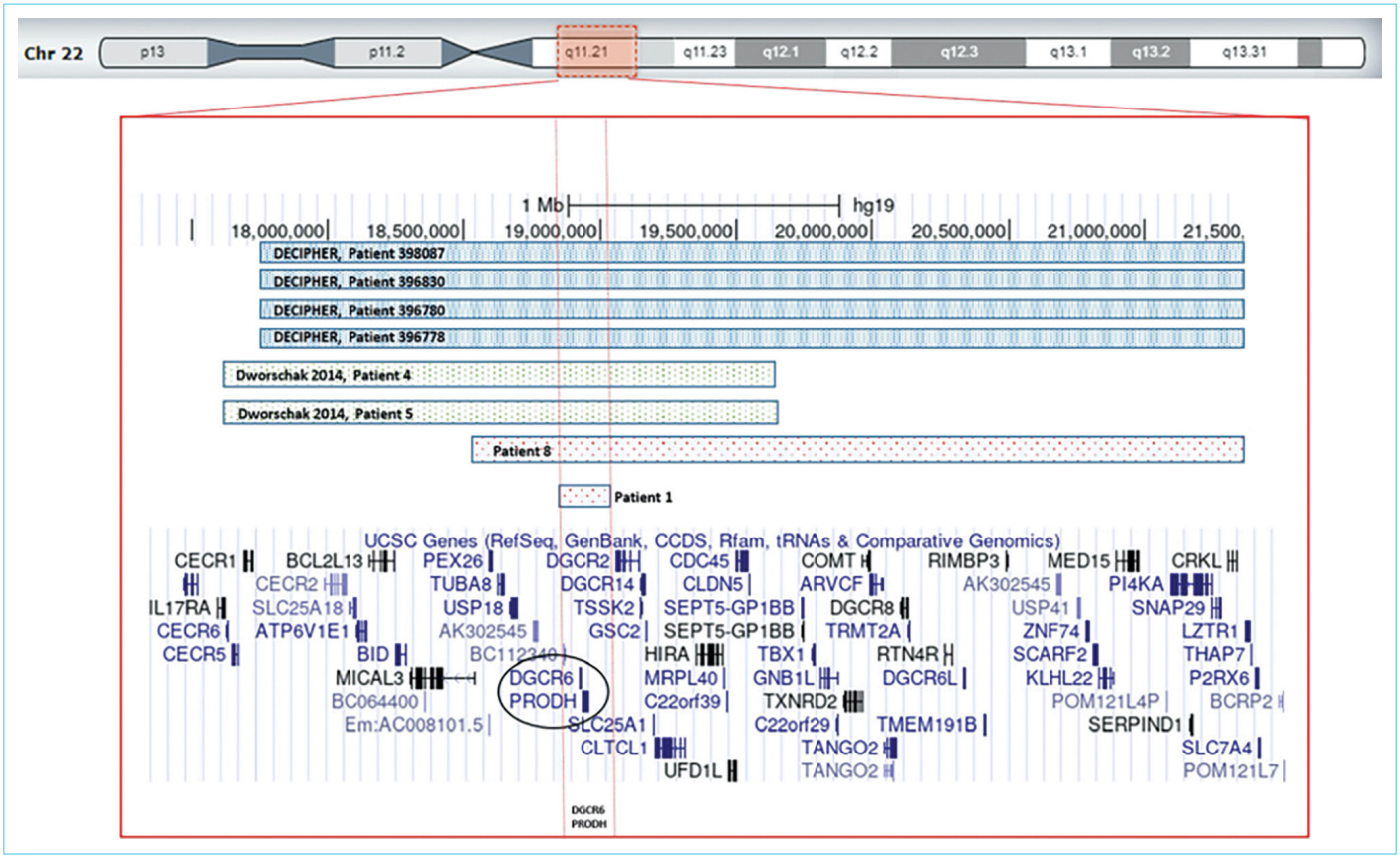


**Figure 1. Schematic representation of patients with anal atresia and 22q11.21 deletion. The *DGCR6* and *PRODH* genes were deleted in all patients**

*DGCR5*, and *PRODH* gene deletions in the DECIPHER database, we found that in 4 patients, much larger deletions, including these genes, had been reported in patients with anal atresia (patients #396778, #396780, #396830, and #398087). Anal atresia and chromosome 22q11.21 deletion were detected in 2 more patients reported by Dworschak et al. (23). In both cases, the deleted region was approximately 2 Mb in size and included the *DGCR6, DGCR5*, and *PRODH* genes (Fig. 1).

The *DGCR6* gene, at the proximal part of the DiGeorge critical region, is one of the candidate genes thought to cause the DiGeorge syndrome phenotype (24). One study that analyzed the gene dosage of DGCR6 in 50 control samples noted no CNV of the gene (25). Heart, liver, and skeletal muscle are the tissues with the highest *DGCR6* protein expression (26). Chicken embryos with reduced *DGCR6* expression have been found to have cardiovascular anomalies similar to DiGeorge syndrome (27).

The probability that the *DGCR6* gene is responsible for ARM is higher than the other 2 deletions detected in patient #1 because the *DGCR6* gene decreases *TBX1* gene expression and regulates neural crest migration. *TBX1* affects neural crest migration in the pharyngeal region (28) and alterations in the levels of *TBX1* impact the severity of the congenital problems in 22q11 deletion syndrome (29).

Other genes in the common region were *DGCR5* and *PRODH*. Neither of these genes has been previously associated with ARM. The *DGCR5* does not encode proteins but encodes various RNAs that have important regulatory functions. Biallelic deletions and mutations of the *PRODH* gene cause autosomal recessive hyperprolinemia type 1 disease. Deletions in a single allele have been associated with schizophrenia seen in 22q11 deletion syndrome (30). *PRODH* and *DGCR6* gene deletions have previously been associated with congenital heart disease (29).

Although there have been studies that performed a genotype-phenotype correlation for 22q11 deletion syndrome, it is difficult because it is a contiguous deletion syndrome. It is not known which of the genes lost in 22q11 deletion syndrome causes ARM.

It has been established that there is a common mechanism in the pathogenesis of VCFS and ARM and that both result from abnormal migration of mesenchymal cells, however, to the best of our knowledge we have shown for the first time that *DGCR6* gene dosage alterations might be related to ARM. Our findings may contribute to the understanding of the etiology of ARM. Future studies of patients with ARM and 22q11 deletion syndrome may clarify this candidate gene connection.

## Limitations

The primary limitations of our study are the lack of whole-exome sequencing for the patients, the small number of patients, and the use of a low-resolution array platform. New studies with more patients using higher-resolution array platforms are needed to say with certainty that the change in the *DGCR6* gene is associated with anal atresia.

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**Informed Consent:** Written informed consent was obtained from patients who participated in this study.

**Peer-review:** Externally peer-reviewed.

**Author Contributions:** Concept – EFP; Design – EFP; Supervision – EFP; Resource – EFP; Materials – PÖÇ; Data Collection and/or Processing – PÖÇ; Analysis and/or Interpretation – PÖÇ, GK; Literature Search – PÖÇ; Writing – PÖÇ; Critical Reviews – GK, EFP.

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## REFERENCES

1. Levitt MA, Peña A. Anorectal malformations. Orphanet J Rare Dis 2007; 2: 33. [CrossRef]
2. Cuschieri A; EUROCAT Working Group. Descriptive epidemiology of isolated anal anomalies: a survey of 4.6 million births in Europe. Am J Med Genet 2001; 103(3): 207–15. [CrossRef]
3. Holschneider AM, Hutson JM. Anorectal malformations in children: embryology, diagnosis, surgical treatment, follow-up. Springer Science & Business Media; 2006.
4. van Rooij IA, Wijers CH, Rieu PN, Hendriks HS, Brouwers MM, Knoers NV, et al. Maternal and paternal risk factors for anorectal malformations: a Dutch case-control study. Birth Defects Res A Clin Mol Teratol 2010; 88(3):152–8. [CrossRef]
5. van de Putte R, van Rooij IALM, Marcelis CLM, Guo M, Brunner HG, Addor MC, et al. Spectrum of congenital anomalies among VACTERL cases: a EUROCAT population-based study. Pediatr Res 2020; 87(3): 541–9. [CrossRef]
6. Vermes G, László D, Mátrai Á, Czeizel AE, Ács N. Maternal factors in the origin of isolated anorectal malformations - a population-based case-control study. J Matern Fetal Neonatal Med 2016; 29(14): 2316–21.
7. Wang Z, Wang Q, Gu C, Zhang J, Wang Y. Abnormal serum vitamin A levels and retinoic acid receptor α expression patterns in children with anorectal malformation. Pediatr Surg Int 2019; 35(8): 903–10. [CrossRef]
8. Wijers CH, van Rooij IA, Marcelis CL, Brunner HG, de Blaauw I, Roeleveld N. Genetic and nongenetic etiology of nonsyndromic anorectal malformations: a systematic review. Birth Defects Res C Embryo Today 2014; 102(4): 382–400. [CrossRef]
9. Khanna K, Sharma S, Pabalan N, Singh N, Gupta DK. A review of genetic factors contributing to the etiopathogenesis of anorectal malformations. Pediatr Surg Int 2018; 34(1): 9–20. [CrossRef]
10. Schramm C, Draaken M, Tewes G, Bartels E, Schmiedeke E, Märzheuser S, et al. Autosomal-dominant non-syndromic anal atresia: sequencing of candidate genes, array-based molecular karyotyping, and review of the literature. Eur J Pediatr 2011; 170(6): 741–6.
11. Marcelis C, de Blaauw I, Brunner H. Chromosomal anomalies in the etiology of anorectal malformations: a review. Am J Med Genet A 2011; 155A(11): 2692–704. [CrossRef]
12. Winberg J, Gustavsson P, Papadogiannakis N, Sahlin E, Bradley F, Nordenskjöld E, et al. Mutation screening and array comparative genomic hybridization using a 180K oligonucleotide array in VACTERL association. PLoS One 2014; 9(1): e85313. [CrossRef]
13. Riggs ER, Andersen EF, Cherry AM, Kantarci S, Kearney H, Patel A, et al. Technical standards for the interpretation and reporting of constitutional copy-number variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics (ACMG) and the Clinical Genome Resource (ClinGen). Genet Med 2020; 22(2): 245–57. [CrossRef]
14. Kates WR, Burnette CP, Bessette BA, Folley BS, Strunge L, Jabs EW, et al. Frontal and caudate alterations in velocardiofacial syndrome (deletion at chromosome 22q11.2). J Child Neurol 2004; 19(5): 337–42.
15. Wu HY, Rusnack SL, Bellah RD, Plachter N, McDonald-McGinn DM, Zackai EH, et al. Genitourinary malformations in chromosome 22q11.2 deletion. J Urol 2002; 168(6): 2564–5. [CrossRef]
16. Cassidy SB, Allanson JE. Management of genetic syndromes: John Wiley & Sons; 2010. [CrossRef]
17. Campbell IM, Sheppard SE, Crowley TB, McGinn DE, Bailey A, McGinn MJ, et al. What is new with 22q? An update from the 22q and you center at the children's hospital of Philadelphia. Am J Med Genet A 2018; 176(10): 2058–69. [CrossRef]
18. Conley ME, Beckwith JB, Mancer JF, Tenckhoff L. The spectrum of the DiGeorge syndrome. J Pediatr 1979; 94(6): 883–90. [CrossRef]
19. Wilson DI, Burn J, Scambler P, Goodship J. DiGeorge syndrome: part of CATCH 22. J Med Genet 1993; 30(10): 852–6. [CrossRef]
20. McDonald-McGinn DM, Driscoll DA, Bason L, Christensen K, Lynch D, Sullivan K, et al. Autosomal dominant "Opitz" GBBB syndrome due to a 22q11.2 deletion. Am J Med Genet 1995; 59(1): 103–13. [CrossRef]
21. Worthington S, Colley A, Fagan K, Dai K, Lipson AH. Anal anomalies: an uncommon feature of velocardiofacial (Shprintzen) syndrome? J Med Genet 1997; 34(1): 79–82. [CrossRef]
22. Wong EH, Cui L, Ng CL, Tang CS, Liu XL, So MT, et al. Genome-wide copy number variation study in anorectal malformations. Hum Mol Genet 2013; 22(3): 621–31. [CrossRef]
23. Dworschak GC, Draaken M, Hilger AC, Schramm C, Bartels E, Schmiedeke E, et al. Genome-wide mapping of copy number variations in patients with both anorectal malformations and central nervous system abnormalities. Birth Defects Res A Clin Mol Teratol 2015; 103(4): 235–42. [CrossRef]
24. Demczuk S, Thomas G, Aurias A. Isolation of a novel gene from the DiGeorge syndrome critical region with homology to Drosophila gdl and to human LAMC1 genes. Hum Mol Genet 1996; 5(5): 633–8.
25. Gao W, Higaki T, Eguchi-Ishimae M, Iwabuki H, Wu Z, Yamamoto E, et al. DGCR6 at the proximal part of the DiGeorge critical region is involved in conotruncal heart defects. Hum Genome Var 2015; 2: 15004. [CrossRef]
26. Pfuhl T, Dürr M, Spurk A, Schwalbert B, Nord R, Mysliwietz J, et al. Biochemical characterisation of the proteins encoded by the DiGeorge critical region 6 (DGCR6) genes. Hum Genet 2005; 117(1): 70–80.
27. Hierck BP, Molin DG, Boot MJ, Poelmann RE, Gittenberger-de Groot AC. A chicken model for DGCR6 as a modifier gene in the DiGeorge critical region. Pediatr Res 2004; 56(3): 440–8. [CrossRef]
28. Das Chakraborty R, Bernal AJ, Schoch K, Howard TD, Ip EH, Hooper SR, et al. Dysregulation of DGCR6 and DGCR6L: psychopathological outcomes in chromosome 22q11.2 deletion syndrome. Transl Psychiatry 2012; 2(4): e105. Erratum in: Transl Psychiatry 2012; 2: e124.
29. Du Q, de la Morena MT, van Oers NSC. The genetics and epigenetics of 22q11.2 Deletion syndrome. Front Genet 2020; 10: 1365. [CrossRef]
30. Pulver AE, Nestadt G, Goldberg R, Shprintzen RJ, Lamacz M, Wolyniec PS, et al. Psychotic illness in patients diagnosed with velo-cardio-facial syndrome and their relatives. J Nerv Ment Dis 1994; 182(8): 476–8.