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Case report

GATA-4 Variants in Two Unrelated Cases with 46, XY Disorder of Sex Development; Review of The Literature

Çelik N et al. GATA-4 Variant as a Cause of Disorder of Sex Development

Nurullah Çelik[1], Hande Küçük Kurtulgan[2], Fatih Kılıçbay[3], Gaffari Tunç[3],Ayça Kömürlüoğlu[4], Onur Taşcı[5], Cemile Ece Çağlar Şimşek[4], Taha Çınar[4], Yeşim Sıdar Duman[2]
[1]Sivas Cumhuriyet University Faculty of Medicine, Department of Pediatric Endocrinology, Sivas, Turkey
[2]Sivas Cumhuriyet University Faculty of Medicine, Department of Genetics, Sivas, Turkey
[3]Sivas Cumhuriyet University Faculty of Medicine, Department of Neonatology, Sivas, Turkey
[4]Sivas Cumhuriyet University Faculty of Medicine, Department of Child Health and Diseases, Sivas, Turkey
[5]Sivas Numune Hospital Clinic of Cardiology, Sivas, Turkey

What is already known on this topic?
The genetic cause of 46, XY DSD still cannot be determined in about half of the cases. GATA-4 haploinsufficiency is one of the rare causes of DSD in genetic males (46, XY).

What this study adds?
Twenty-two cases with 46, XY DSD due to GATA-4 haploinsufficiency (nine missense variant, two copy number variation) have been reported in the literature. Phenotype varied from a mild insufficient virilization to complete female appearance. There is a remarkable phenotype-genotype variation in the GATA-4 related conditions, associated with incomplete penetrance or variable expressivity.

Abstract
The genetic cause of 46, XY Disorder of Sex Development(DSD) still cannot be determined in about half of the cases. *GATA-4* haploinsufficiency is one of the rare causes of DSD in genetic males (46, XY). Twenty-two cases with 46, XY DSD due to *GATA-4* haploinsufficiency (nine missense variant, two copy number variation) have been reported in the literature. In these cases, the phenotype may range from a mild undervirilization to complete female external genitalia. The haploinsufficiency may be caused by a sequence variant or copy number variation (8p23 deletion). The study aimed to present two unrelated patients with DSD due to *GATA-4* variants and to review the phenotypic and genotypic characteristics of DSD cases related to GATA-4 deficiency.
Keywords: Disorder of sex development, GATA-4, Gonad, heart

Nurullah Çelik MD, Sivas Cumhuriyet University Faculty of Medicine, Department of Pediatric Endocrinology, Sivas, Turkey
+905056736145
celiknurullah@hotmail.com, ncelik@cumhuriyet.edu.tr
0000-0003-1583-6807
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Introduction

Disorder of Sex Development(DSD) is defined as atypical development of gonadal, chromosomal, or anatomical sex [1]. It may be related to aneuploidy, copy number variations, or single nükleotid variants causing defect of sex hormone biosynthesis/action, and/or gonadal differentiation/development[2, 3]. The genetic cause of 46, XY DSD still cannot be determined in about half of the cases. *GATA-4* haploinsufficiency is one of the rare causes of DSD in genetic males (46, XY).

GATA-4 gene located on chromosome 8p23.1 encodes GATA-binding protein 4 (GATA-4), a transcription factor that is essential for cardiac and gonadal development[4–6]. By interacting with NR5A1, FOG-2, and WT1, GATA-4 protein regulates the expression of sex-determining genes, SRY, SOX-9, and Anti Müllerian Hormone (AMH)[7]. It has also been shown that the protein modulates a couple of steroidogenic genes that are essential for sexual differentiation[7, 8].

GATA-4 haploinsufficiency as a cause of congenital heart disease is a well-known association, nearly 200 variants have been reported to date. However, to our knowledge, it has been reported only twenty-two cases of *GATA-4* related DSD in the literature[7, 9–14]. In these cases, the phenotype may range from a mild undervirilization to complete female external genitalia. The haploinsufficiency may be caused by a sequence variant or copy number variation (8p23 deletion). Based on a large international cohort study, only 1-2 % of 46, XY DSD cases may be related to *GATA-4* gene[12, 15].

The study aimed to present two unrelated patients with DSD due to *GATA-4* variants and to review the phenotypic and genotypic characteristics of DSD cases related to GATA-4 deficiency.

Case Reports

DNA extracted from peripheral blood sample by using QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany) according to manufacturers instructions. Targeted gene panel* for 46, XY DSD were sequenced by next-generation sequencing technique using custom panel kit (Twist Bioscience, San Francisco, CA, USA). Genemaster analysis program was used for the analysis of the obtained data. Detected changes were analyzed using genomAD (https://gnomad.broadinstitute.org.), dbSNP [16], varsome [17], clinvar [18] databases and interpreted according to ACGM [19] criteria. Written consent was obtained from parents of the probands.

*panel list: (*AMH, AMHR2, AKR1C2, AR, ARX, ATRX, B3GALTL, CYB5A, CYP11A1, CYP17A1, DHCR7, DHH, GATA4, HCCS, HSD17B3, LHCGR, MAMLD1, MAP3K1, NR5A1, OPHN1, SOX9, SRD5A2, SRY, WT1, ZFPM2).*

Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH), Estradiol, Total testosterone, Anti Mullerian Hormone (AMH), Adrenocorticotrophin (ACTH), Cortisol, and Dehydroepiandrosterone sulfate (DHEA-SO4) levels were measured by an automated Electrochemiluminescence Immunoassay (Roche Cobas 8000) using the standard reagent kits supplied by the instrument manufacturer. Dihydrotestosterone and 17-OH progesterone levels were measured using liquid chromatography-tandem mass spectrometry (LC-MSMS).

Case 1

A neonate was hospitalized to the neonatal intensive care unit due to prematurity, and respiratory distress. The baby was born from a 20-year-old mother at 32 weeks 3 days and 1960 gr as a consanguineous parents first child, with cesarean section, due to loss of doppler activity, and polyhydramnios. Physical examination revealed dysmorphic ear, epicanthus, hypertelorism, umbilical hernia, standing trigger finger, bilateral simian line, central hypotonicity, micropenis (1.5x1cm), scrotal hypoplasia, and bilateral undescended testis. Atrial septal defect (ASD), Patent ductus arteriosus (PDA), and Pulmonary stenosis (PS) were diagnosed with echocardiographic assessment at the fourth month of age. Adrenal gland hormones were within normal limits according to age and gender. Pituitary-gonadal functions were in the normal range, consistent with mini puberty. Gonad and adrenal function tests are presented in Table 1.

The chromosomal analysis revealed a 46, XY karyotype. Targeted gene panel sequencing for 46, XY DSD showed that a heterozygous novel variant in Exon 2 of the *GATA-4* gene, c.337A>C (p.Thr113Pro). This variant has not yet been previously reported. VarSome software classified this substitution as variant of "Uncertain Significance". Computational analysis verdict was eight pathogenic predictions (BayesDel_addAF, DEOGEN2, FATHMM-MKL, M-CAP, MutationTaster, PrimateAI and SIFT) and four benign predictions (DANN, EIGEN, MVP and MutationAssessor). Segregation analysis showed that the variant was de novo. We think that this new variant is compatible in terms of genotype and phenotype correlation.

Case 2

Three days old -patient was referred to our endocrinology clinic due to ambiguous genitalia. He was born as a non-consangiounus parent at 38 gestational weeks and 3185 gr. Microphallus, bifid scrotum, perineo-scrotal hypospadias were obtained on physical examination. Bilateral gonads were palpated in the scrotum. System examination was normal except for ptosis in the left eye. Congenital heart disease was not detected by echocardiography. Adrenal gland hormones were within normal limits according to age and gender. Pituitary-gonadal functions were in the normal range, consistent with mini puberty. Gonad and adrenal function tests are presented in Table 1. Chromosome analysis revealed a 46, XY karyotype. Targeted gene panel sequencing for 46, XY DSD showed that a heterozygous likely pathogenic variant in Exon 2 of the *GATA-4* gene, c.487C> T (p.*pro*163*Ser*). In the segregation analysis, the mother did not carry this variant, The analysis could not be done for father.

Discussion

Twenty-two cases with 46, XY DSD due to GATA-4 haploinsufficiency (nine missense variant, two copy number variation) have been reported in the literatüre (Table 2). Eighteen of these cases (82%) were raised as a male. Only 2 (9%) cases were accompanied by Congenital Heart Disease (CHD), as ASD and Ventricular Septal Defect (VSD). Phenotype varied from mild insufficient virilization to a complete female appearance.

In the literature, firstly, Lourenco et al.[9] defined three DSD cases having the same missense variant in the *GATA-4* gene. This variant is located in the Zinc finger domain, which is responsible for DNA binding and protein interaction of the GATA-4 protein. Moreover, they showed a 50% reduction in AMH activity with expression analysis of this variant. While the index case had only DSD, the brother and one cousin with the same variant of the index case had both DSD and CHD. Also, the mother and aunt of the index case, who carry the same variant, have neither DSD nor CHD.

In another study evaluating 278 cases with 46 XY DSD, 4 different *GATA-4* variants were detected in 7 cases[12]. However, the authors declared that only one of the 4 variants was pathogenic, and the others were benign, in their later work[15]. In particular, they emphasize that the variants in *GATA-4* gene located outside the N-terminal part of the zinc finger domain should be approached with a suspicion that there is a causal relationship with DSD. Although van den Bergen et al.[15] mentioned that p.P407Q variant in the GATA-4 gene, the most reported GATA-4 variant in 46 XY DSD, was benign, it has been shown in experimental studies that the variant causes reducing the expression of AMH and SRY gene[13, 14]. On the other hand, it was found to be associated with CHD in the previous studies[15].

Our unrelated patients had two different variants in the *GATA-4* gene. Undoubtedly, expression analysis is needed to establish a causal relationship between these variants and DSD, which is the most important limitation of the study. However, although these two variants were not located in the zinc finger domain, they were close to N-terminal part of the domain. On the other hand, the first case (Case 1) with a novel variant of uncertain significance had also CHD, which may be explained with GATA-4 deficiency. We also performed the microarray analysis due to other accompanying syndromic findings in the first case, it was evaluated as normal. Further genetic studies are needed in this case. The GATA-4 variant of the second case (Case 2), which was reported in cases with previous patients with CHD[20-22], classified as likely pathogenic according to The American College of Medical Genetics and Genomics (ACMG) criteria. To our knowledge, a patient with DSD related the latter variant was not mentioned previously. This may be a striking example of the phenotype-genotype mismatch associated with *GATA-4* gene. The phenotype-genotype variation in the *GATA-4* related conditions may be associated with incomplete penetrance or variable expressivity. On the other hand, ıt is not understood that why *GATA-4* variant related CHD is encountered more than DSD. The answer of this question will perhaps enable us to better understand the phenotype-genotype relations.

Conclusion

The role of gonad differentiation of the GATA-4 protein has been shown in experimental studies and expression analysis. The variants of the gene encoding the GATA-4 protein may be responsible for the etiology at a rate of 1-2% in 46, XY DSD. The phenotype may range from a mild undervirilization to complete female external genitalia. The CHD or DSD can be isolated or combined; GATA-4 gene defects should be considered in cases with both CHD and DSD. Different phenotypes have been reported in individuals with the same variant, this phenotypic variability can be explained by variations in incomplete penetrance or variable expressivity.

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Table 1. 15th Day Basal Gonadal and Adrenal Functions of the Cases			
	Case 1	**Case 2**	**References**
FSH, IU/L	4	0,5	0.16-4.1
LH, IU/L	4	3,79	0.02-7
Estradiol, pmol/L	<12	<12	0.3-1
Total Testosterone, nmol/L	15.09	5.24	2.6-13.86
Dihydtestosterone, nmol/L	1.64	1.88	0.4-2.92
AMH*, pmol/L	153.07	772.86	100-3328
ACTH, pmol/L	4.22	3.47	1.32-10.47
Cortisol, nmol/L	105.94	121.39	55-303
DHEA-SO4, µmol/L	8.35	14.32	0.84-11.68
17-OH-Progesterone, nmol/L	14.57	2.88	0.1-6.06

ACTH, Adrenocorticotrophin; **AMH**, Anti Mullerian Hormone; **DHEA-SO4,** Dehydroepiandrosterone sulfate; **FSH**, Follicle Stimulating Hormone; **LH**, Luteinizing Hormone

Table 2. Summary of GATA-4 related Cases with Disorder of Sex Development

Case	Sex of Rearing	Additional findings	CHD	Fenotype	Genotype	References
1	M			fused hypoplastic labioscrotal fold, perineal hypospadias, hypoplasia of corpus cavernosum, bilateral cryptorchidism (Inguinal)	p.G221R (n=3)	9
2	M		ASD	Microphilus, bilateral cryptorchidism (Inguinal)		
3	M			fused labioscrotal folds, hypospadias, bilateral cryptorchidism (Inguinal)		
4	M	Congenital Adrenal Hypoplasia		Complete gonadal dysgenesis, Female external genitalia	8p23 delesyonu	10
5	M			Perineal hypospadias, bifid scrotum, bilateral cryptorchidism, mullerian structures absent	8p23 delesyonu	11
6	M			Micropenis, cryptorchidism	p.W228C	12
7	M			Perineal hypospadias, chordee, and penoscrotal transposition, cryptorchidism	p.A346V	
8	M			Perineal hypospadias, (gonad position is unknown)	p.P394T	
9	F			Female (no virilization), Inguinal bilateral testis, no uterus		
10	M	İmperforate Anüs		Penile hypospadias, cryptorchidism	p.P407Q	
11	M			Scrotal hypospadias, Testes palpable, hypoplastic uterus		
12	M			Perineal Hypospadias cryptorchidism		
13	M			Male type genitalia, cryptorchidism with or without micropenis	p.R265C n=1	13
14-17	M				p.P407Q n=4	
18	F	Autism	VSD	Clitoral hypertrophy, fused labia with posterior raphe, gonads palpable in inguinal canal , rudimentary uterus	p.C238R	7
19	M			Micropenis, hypospadias, bilateral cryptorchidism	p.W228C	
20	M	Severe Obesity		Micropenis , bilateral cryptorchidism (Inguinal)	p.P226L	
21	M			Micropenis, Perineal hypospadias, bilateral cryptorchidism	p.R215G	14
22	F			Complete female genitalia	p.P407Q	
23	M	Dysmorphic ear, Epicanthus Hypertelorism Umbilical hernia	ASD, VSD, PS	Microphilus, scrotal hypoplasia, bilateral cryptorchidism (inguinal)	p.T113P	

24	M	Ptosis		Perineoscrotal hypospadias, microphilus, bifid scrotum	p.p163S
ASD: Atrial Septal Defect; CHD: Congenital Heart Disease; F: Female; M: Male; PS: Pulmonary Stenosis; VSD: Ventricular Septal Defect					

Figure 1. The sequence images of the *GATA-4* gene for the Case 1(a), Case 2 (d) and their parents (b and c for Case 1,e for Case 2)