Unveiling the 46,XY disorder of sex development patients family with a novel mutation in the GATA4 gene

Liwei Li
Xingtai people's hospital
Hui Yang
The Affiliated Hospital of Guizhou Medical University
Junhong Zhang
Hebei Eye Hospital
Huijing Lv
Xingtai Maternal and Child Health centre
Qing Li
The Affiliated Hospital of Guizhou Medical University
Yi Cui
The Affiliated Hospital of Guizhou Medical University
Tongqian Wu
The Affiliated Hospital of Guizhou Medical University
Yuan Tian
The Affiliated Hospital of Guizhou Medical University
Yajun Mou
The Affiliated Hospital of Guizhou Medical University
Xinxin Chen
Xingtai people's Hospital
Shuping Wang
Maternity and Child Health Care Hospital of Ningxia
Yanli Feng
Xingtai people's hospital
Shujun Li
Xingtai people's Hospital
Shuping Li
Xingtai people's Hospital
Pingsheng Hu
The Affiliated Hospital of Guizhou Medical University
Shirui Hao

Xingtai people's hospital

Xiaowei Dou (douxw@gmc.edu.cn)

the Affiliated Hospital of Guizhou Medical University https://orcid.org/0000-0002-9601-7047

Research

Keywords: 46,XY disorder of sex development, GATA4, mutation

Posted Date: January 29th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-154837/v1

Abstract

Background

GATA-binding protein 4 (GATA4) is the critical regulator in gonadal development and its mutation has been reported related with 46,XY disorder of sex development (DSD). Here, we found the two Chinese cases with 46,XY DSD carried the GATA4 mutation. Physical examinations, B-ultrasound and Karyotype analysis were performed and confirmed the two patients with 46,XY DSD.

Results

Sequencing were performed and the heterozygous mutation p.Gly375Arg in GATA4 gene was identified in the 2 cases with 46,XY DSD. Their mother was identified carrying the p.Gly375Arg mutation in GATA4 protein. However, their father and litter sister without 46,XY DSD didn't be found carrying the p.Gly375Arg mutation in GATA4 gene.

Conclusion

This is the first report that the case with 46,XY DSD carried the mutation Gly375Arg in the GATA4 gene. Our

Introduction

The phenotype of 46,XY DSD is well characterized by normal 46, XY karyotype, testes, male accessory ducts and predominantly female external genitalia[1–3]. At present, the gonadal anomalies occur about 1 in 4500 infants[4]. A variety of genetic factors that affect sex development during gonadal differentiation have been identified and the mutation of these genetic factors will block gonadal differentiation and lead to DSD occurrence[5, 6]. Despite the considerable advances in our learning of the genetic mechanisms of gonad development, the underlying etiological factor in the majority of 46,XY DSD cases still cannot be explained.

To date, an increasing number of causative genes have been identified for 46,XY DSD, including GATA-binding protein 4 (GATA4)[7–11]. GATA4 is an evolutionarily conserved zinc finger transcription factor gene that plays an essential role in early development of many organs in the embryonic stage, including the skin, brain, gonads, liver, hematopoietic, cardiovascular and urogenital systems [12, 13]. GATA4 variants have been identified not only in patients with congenital heart disease[14] but also in those with 46,XY DSD due to impaired testis formation [4, 7, 11, 15].

Here, we report a heterozygous GATA4 mutation p.Gly375Arg (c.1123G > A) in a 7yearold proband and her 5-year-old sister with 46,XY DSD. The heterozygous p.Gly375Arg mutation in GATA4 gene is inherited from their mother. The p.Gly375Arg mutation in GATA4 gene has not been reported in previous research.

Our identification of the p.Gly375Arg mutation in GATA4 gene from 46,XY DSD cases may provide insight of the domain function of GATA4 gene into gonadal development and DSD occurrence.

Results

Clinical characteristics of the family

The proband II-1 was treated for a right inguinal hernia and found a testicular-like tissue. Thence, she was referred to our hospital. Physical examination revealed a female vulva with a blind-ending vagina. Color Doppler Ultrasound (Voluson E8, GE, Salzburg, Austria) showed that she had no a uterus and ovaries, but detected testicular tissues in the right groin and in the left abdominal cavity (Figure.1a). Ultrasonic examination found no cardiac abnormalities. Her karyotype was a 46, XY with no apparent anomalies in the chromosome number or structure (Figure.1b).

The case II-2 was referred to our hospital due to her sister's symptom. Physical examination found that she had a female vulva but with a blind-ending vagina. Ultrasound found no a uterus and ovaries, but detected testicular echo in the right groin and in the left abdominal cavity (Figure.2a). Ultrasonic examination of the heart was normal. Cytogenetic analysis showed that the case had a 46, XY karyotype with no apparent anomalies in the chromosome number or structure (Figure.2b).

The proband's parents and her little sister II-3 was also referred to our hospital due to the proband's symptom. Physical examination found no abnormity of the external genitals. Ultrasound found no abnormity of the sex organ. Their chromosome karyotypes were normal. From these clinical features, family pedigree of the proband can be obtained (Figure.2c).

p.Gly375Arg (c.1123G > A) mutation identified in the GATA4 gene

The genomic DNA was extracted from the peripheral blood sample of the proband. Chip capture high-throughput sequencing by BGI corporation and alignment to the ESP data, QIANREN data and EXAC data found that the proband II-1 carried a heterozygous missense mutations (C.1123G > A; p.Gly375Arg) in GATA4 gene. Then, we extracted the genomic DNA from the peripheral blood samples of the other family members and amplified the GATA4 gene by PCR. Sanger sequencing was performed for the family members by Fulgent Technologies Inc.. We confirmed the heterozygous missense mutations (C.1123G > A; p.Gly375Arg) in GATA4 gene of the proband II-1(Fig. 3a) and found that the case II-2(Fig. 3b) and her mother I-2 (Fig. 4b) also carried the heterozygous missense mutations (C.1123G > A; p.Gly375Arg) in GATA4 gene. The father I-1 (Fig. 4a) and the proband's little sister II-3 (Fig. 4c) didn't be found carrying the heterozygous missense mutations (C.1123G > A; p.Gly375Arg) in GATA4 gene. This mutation has not been previously found in the databases of the human genome, including EPS6500, 1000 genomes, GnomAD, ExAC, and their own sequencing databases of BGI corporation. These results indicated that the heterozygous missense mutations (C.1123G > A; p.Gly375Arg) in GATA4 gene may lead to 46,XY DSD occurrence.

Discussion

We identified a new heterogeneous GATA4 variant (p.Gly375Arg) in the two Chinese cases with 46, XY DSD. No clinically recognizable cardiac feature was found in the GATA4 (p.Gly375Arg) mutation cases. Their father without 46, XY DSD symptom didn't carry the heterogeneous p.Gly375Arg in GATA4 gene. The heterogeneous p.Gly375Arg in GATA4 gene of the two DSD cases was inherited from their mother. This is the first time to report the p.Gly375Arg mutation in GATA4 gene identified in the DSD cases.

GATA4 is a member of the GATA proteins family containing 442 amino acids and 7 exons, which is a tissue- and organ-specific transcriptional regulator implicated in development and differentiation of endoderm- and mesoderm-derived tissues [12, 13]. GATA4 have two zinc finger domains (ZNI and ZNII). The zinc finger ZNII region at the C-terminal requiring for the DNA recognition and binding[16], and the zinc finger ZNI region at the N-terminal contributing to the stability. Both the zinc fingers are indispensable for protein-protein interactions with other transcription factors [17–19]. GATA4 is expressed in adult vertebrate heart, gut epithelium, and gonads. The mutation of GATA4 gene have been identified prevailingly in patients with ventricular septal defect, tetralogy of Fallot, or other cardiac malformations [20–23].

GATA4 gene mutation lead to 46,XY DSD with or without the congenital heart diseases. A family with 46,XY DSD and congenital heart disease was identified of p.Gly221Arg mutation in the conserved N-terminal zinc finger of GATA4 since the mutation compromised the ability of the protein trans-activating the anti-Müllerian hormone (AMH) promoter and disrupted synergistic activation of the AMH promoter by GATA4 and NR5A1[11]. The inactivation of GATA-binding motif of the AMH promoter failed to upregulate AMH expression in the developing fetal and neonate testis, suggesting that GATA4 is a positive modulator of AMH expression [24]. The p.Pro407Gln mutation in GATA4 gene were identified in four patients with 46,XY DSD but no heart anomaly because p.Pro407Gln mutation in GATA4 gene had markedly decreased transactivation activity of AMH promoter similar to p.Gly221Arg [7]. The p.Glu359X mutation in GATA4 gene reduced GATA4 transcriptional activity on the different gonadal promoters and have deleterious effects on gonadal development [15]. Although GATA4 gene p.Pro394Thr (c.1180C > A) [25] was detected in 46,XY DSD, there was no evidence to prove that this mutation would cause 46,XY DSD. The variant p.Gly375Arg in GATA4 gene, like p.Glu359X and p.Pro407Gln mutation locating at C-terminal far away from zinc finger ZNI and ZNII region, was identified with the 46,XY DSD cases. Both men and women with p.Gly375Arg mutation in GATA4 gene had no congenital heart disease, so this mutational site in GATA4 did not cause CHD.

Conclusions

Collectively, our study identified the heterogeneous p.Gly375Arg mutation of GATA4 protein in the two 46,XY DSD cases. However, the heterogeneous GATA4 p.Gly375Arg mutation was found in the 46,XY DSD cases without heart anomalies. Further studies are necessary to clarify whether GATA4 p.Gly375Arg variants contribute to the genetic predisposition of 46,XY DSD.

Methods

Cases

The 7-year-old proband was referred to Shahe hospital due to the right inguinal hernia. She was prepared to perform the hernia operation. The surgeon found that the tissue was like a testicular tissue and stop the operation. Then, she was transferred to our hospital. Her 5-year-old sister and 2-year-old sister were also referred to our hospital. Physical examinations were performed to check female external genitalia. B-ultrasound was used to detect male or female internal genital organs, including testis, uterus, and ovaries. Karyotype analysis was performed to test the anomalies in the chromosome number or chromosomal structure.

Written informed consent was obtained from all the family members. The study was approved by the Medical Ethical Committee of the People's Hospital of Xingtai City.

Cytogenetic Analysis

Peripheral blood lymphocytes from the 2 DSD siblings were isolated and cultured in RPMI1640 medium supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, and 10% fetal bovine serum (FBS). Each one's lymphocyte were cultured in duplicate. Phytohemagglutinin was added to promote cell division. Dividing cells were arrested at metaphase stage by Colchicine (20 μg/ml) for two hours and fixed in methanol and acetic acid (3:1). Fixed cells were dropped onto glass slides and placed in an incubator at 75 °C for three hours to air-dry. Giemsa solution was used to stain the G-bands of the chromosomes.

Genetic analysis

The genomic DNA was extracted from the proband according to the manufacturer's instructions using the TIANamp Blood DNA Kit (TIANGEN, China). Chip capture high-throughput sequencing was carried out by BGI Corporation (Shenzhen, China). Sequence data were aligned to the ESP data, QIANREN data and EXAC data. Based on the patients' symptoms, the suspected genes recruited by OMIM data were analyzed. Since the heterozygous missense mutations (C.1123G > A; p.Gly375Arg) in GATA4 gene were found in the proband II-1, the genomic DNA was extracted from the other members of the family and PCR amplified GATA4 gene. Sanger sequencing of GATA4 gene were done for all family members by Fulgent Technologies Inc. (Beijing, China).

Declarations

Acknowledgements: We thank the family and medical staff involved in this study.

Authors' contributions: SL, HY, JZ, PL, QL, YC, SL, QW, SH, SH and PH carried out the molecular genetic studies, participated in the sequence alignment and drafted the manuscript. SL and LL carried out the immunoassay. XD participated in the sequence alignment. SL, LL and XD designed the study. All authors read and approved the final manuscript.

Funding: This work was funded by the National Natural Science Foundation of China (No. 81860516), the Start-up grant from the Affiliated Hospital of Guizhou Medical University (No. I201802), Guizhou science and Technology Department (No. 2016J7234), Guizhou science and Technology Department (No. 2014LH7149), the National Natural Science Foundation of China (No.81460448), the R&D infrastructure and facility Development and Program of Guizhou (No. 20154005), Science and technology foundation of Guizhou Health Committee (gzwjkj2019-1-036).

Availability of data and materials: The data that support the findings of this study are available on request from the corresponding author.

Ethics approval and consent to participate: Experimentation was approved by the Medical Ethical Committee of the People's Hospital of Xingtai City. Parental/guardian consent obtained.

Consent for publication: Not required.

Competing interests: The authors declare no competing financial interests.

References

1. Sudhakar DVS,Jaishankar S,Regur P,Kumar U,Singh R,Kabilan U, et al. Novel NR5A1 Pathogenic Variants Cause Phenotypic Heterogeneity in 46,XY Disorders of Sex Development. Sex Dev 2019, 13(4):178-86.
2. Li SP,Li LW,Sun MX,Chen XX,Wang XF,Li ZK, et al. Identification of a novel mutation in the SRD5A2 gene of one patient with 46,XY disorder of sex development. Asian J Androl 2018, 20(5):518-9.
3. Nagaraja MR,Gubbala SP,Delphine Silvia CRW,Amanchy R. Molecular diagnostics of disorders of sexual development: an Indian survey and systems biology perspective. Syst Biol Reprod Med 2019, 65(2):105-20.
4. Ohnesorg T,Vilain E,Sinclair AH. The genetics of disorders of sex development in humans. Sex Dev 2014, 8(5):262-72.
5. Ono M,Harley VR. Disorders of sex development: new genes, new concepts. Nat Rev Endocrinol 2013, 9(2):79-91.
6. Cools M,Nordenstrom A,Robeva R,Hall J,Westerveld P,Fluck C, et al. Caring for individuals with a difference of sex development (DSD): a Consensus Statement. Nat Rev Endocrinol 2018, 14(7):415-29.
7. Igarashi M,Mizuno K,Kon M,Narumi S,Kojima Y,Hayashi Y, et al. GATA4 mutations are uncommon in patients with 46,XY disorders of sex development without heart anomaly. Asian J Androl 2018, 20(6):629-31.
8. Lourenco D,Brauner R,Rybczynska M,Nihoul-Fekete C,McElreavey K,Bashamboo A. Loss-of-function mutation in GATA4 causes anomalies of human testicular development. Proc Natl Acad Sci U S A 2011, 108(4):1597-602.

9. Eggers S,Sadedin S,van den Bergen JA,Robevska G,Ohnesorg T,Hewitt J, et al. Disorders of sex development: insights from targeted gene sequencing of a large international patient cohort. Genome Biol 2016, 17(1):243.
10. Shimizu D,Iwashima S,Sato K,Hayano S,Fukami M,Saitsu H, et al. GATA4 variant identified by whole-exome sequencing in a Japanese family with atrial septal defect: Implications for male sex development. Clin Case Rep 2018, 6(11):2229-33.
11. Martinez de LaPiscina I,de Mingo C,Riedl S,Rodriguez A,Pandey AV,Fernandez-Cancio M, et al. GATA4 Variants in Individuals With a 46,XY Disorder of Sex Development (DSD) May or May Not Be Associated With Cardiac Defects Depending on Second Hits in Other DSD Genes. Front Endocrinol (Lausanne) 2018, 9:142.
12. Lentjes MH,Niessen HE,Akiyama Y,de Bruine AP,Melotte V,van Engeland M. The emerging role of GATA transcription factors in development and disease. Expert Rev Mol Med 2016, 18:e3.
13. Tremblay M,Sanchez-Ferras O,Bouchard M. GATA transcription factors in development and disease. Development 2018, 145(20).
14. Zhao Z,Zhan Y,Chen W,Ma X,Sheng W,Huang G. Functional analysis of rare variants of GATA4 identified in Chinese patients with congenital heart defect. Genesis 2019, 57(11-12):e23333.
15. Bouchard MF,Taniguchi H,Viger RS. The effect of human GATA4 gene mutations on the activity of target gonadal promoters. J Mol Endocrinol 2009, 42(2):149-60.
16. Yang HY,Evans T. Distinct roles for the two cGATA-1 finger domains. Mol Cell Biol 1992, 12(10):4562-70.
17. Bates DL,Chen Y,Kim G,Guo L,Chen L. Crystal structures of multiple GATA zinc fingers bound to DNA reveal new insights into DNA recognition and self-association by GATA. J Mol Biol 2008, 381(5):1292-306.
18. Crispino JD,Lodish MB,Thurberg BL,Litovsky SH,Collins T,Molkentin JD, et al. Proper coronary vascular development and heart morphogenesis depend on interaction of GATA-4 with FOG cofactors. Genes Dev 2001, 15(7):839-44.
19. Wilkinson-White L,Lester KL,Ripin N,Jacques DA,Mitchell Guss J,Matthews JM. GATA1 directly mediates interactions with closely spaced pseudopalindromic but not distantly spaced double GATA sites on DNA. Protein Sci 2015, 24(10):1649-59.
20. Chen Y,Han ZQ,Yan WD,Tang CZ,Xie JY,Chen H, et al. A novel mutation in GATA4 gene associated with dominant inherited familial atrial septal defect. J Thorac Cardiovasc Surg 2010, 140(3):684-7.
21. Chen J,Qi B,Zhao J,Liu W,Duan R,Zhang M. A novel mutation of GATA4 (K300T) associated with familial atrial septal defect. Gene 2016, 575(2 Pt 2):473-7.
22. Xiang R,Fan LL,Huang H,Cao BB,Li XP,Peng DQ, et al. A novel mutation of GATA4 (K319E) is responsible for familial atrial septal defect and pulmonary valve stenosis. Gene 2014, 534(2):320-3.
23. Chen Y,Mao J,Sun Y,Zhang Q,Cheng HB,Yan WH, et al. A novel mutation of GATA4 in a familial atrial septal defect. Clin Chim Acta 2010, 411(21-22):1741-5.

24. Bouchard MF,Bergeron F,Grenier Delaney J,Harvey LM,Viger RS. In Vivo Ablation of the Conserved GATA-Binding Motif in the Amh Promoter Impairs Amh Expression in the Male Mouse. Endocrinology 2019, 160(4):817-26.
25. van den Bergen JA,Robevska G,Eggers S,Riedl S,Grover SR,Bergman PB, et al. Analysis of variants in GATA4 and FOG2/ZFPM2 demonstrates benign contribution to 46,XY disorders of sex development. Mol Genet Genomic Med 2020, 8(3):e1095.

Figures

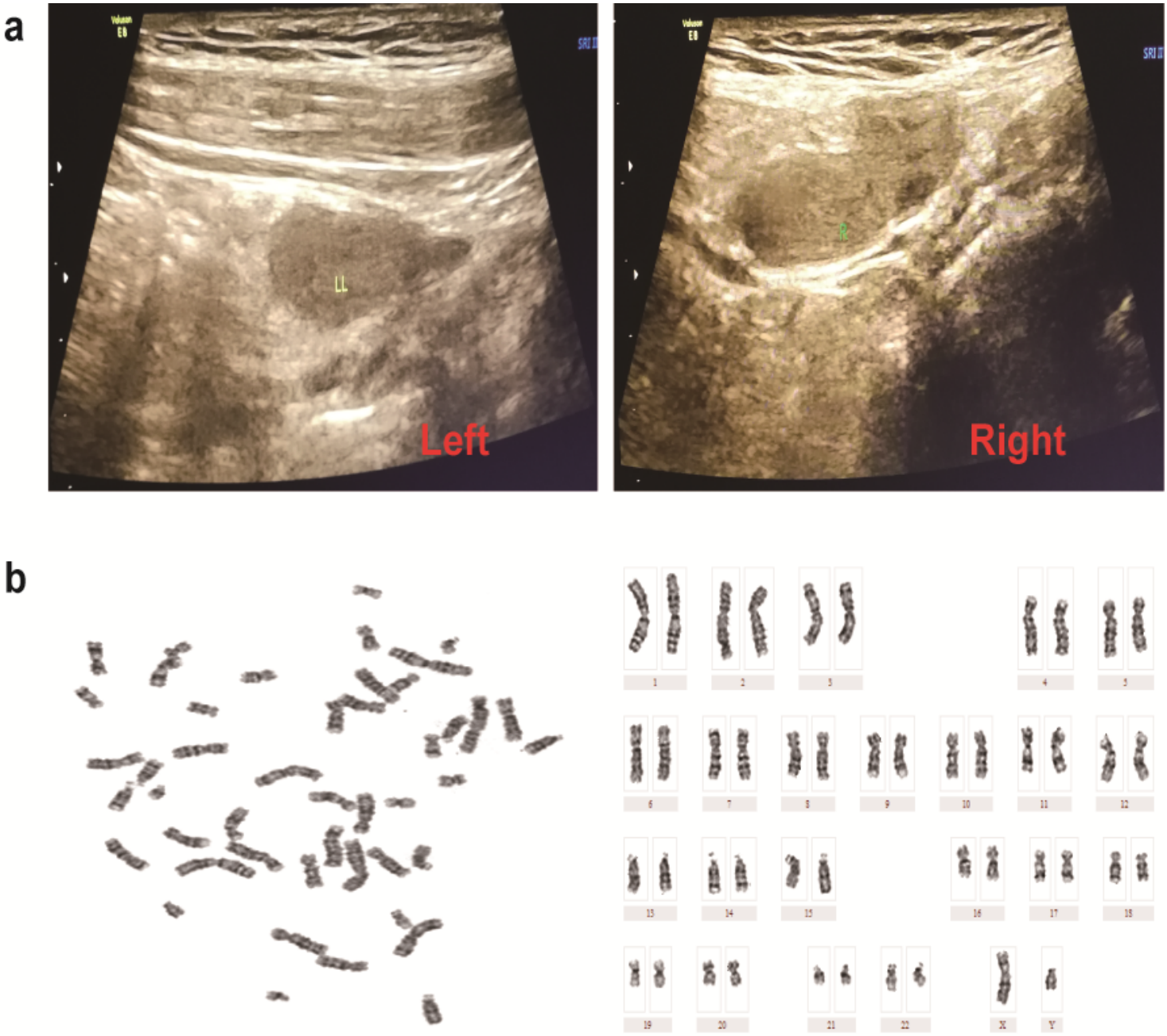

Figure 1

Clinical phenotype of the proband II-1. (a): The left and right testicle of the proband II-1 were examined by type-B ultrasonic. (b): Cytogenetic analysis of the proband II-1 was performed.

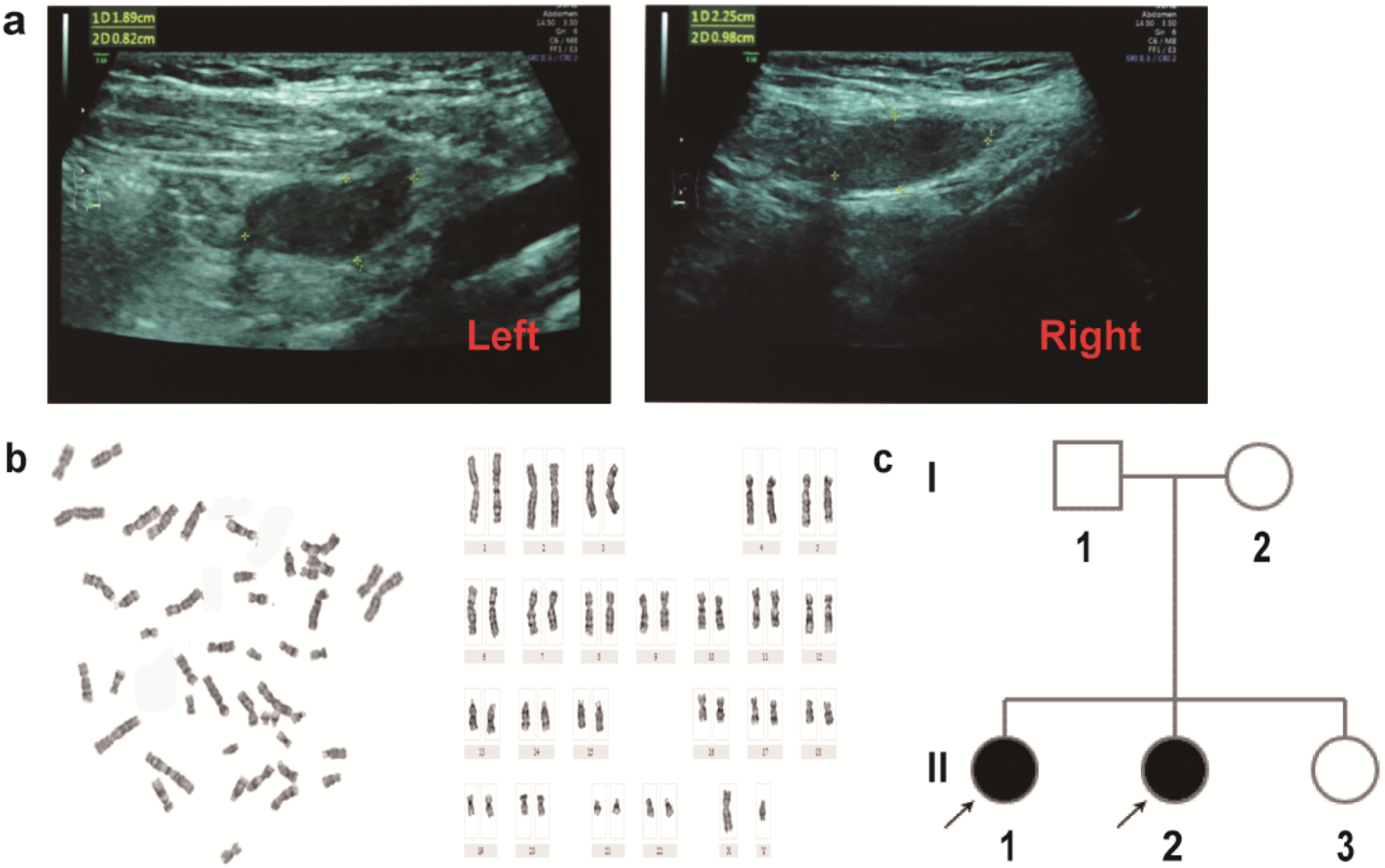

Figure 2

Clinical phenotype of the case II-2. (a): The left and right testicle of the case II-2 were examined by type-B ultrasonic. (b): Cytogenetic analysis of the case II-2 was performed. (c): The family pedigree of the cases. Males and females are indicated by squares and circles, respectively. The cases are indicated by black circles and arrows.

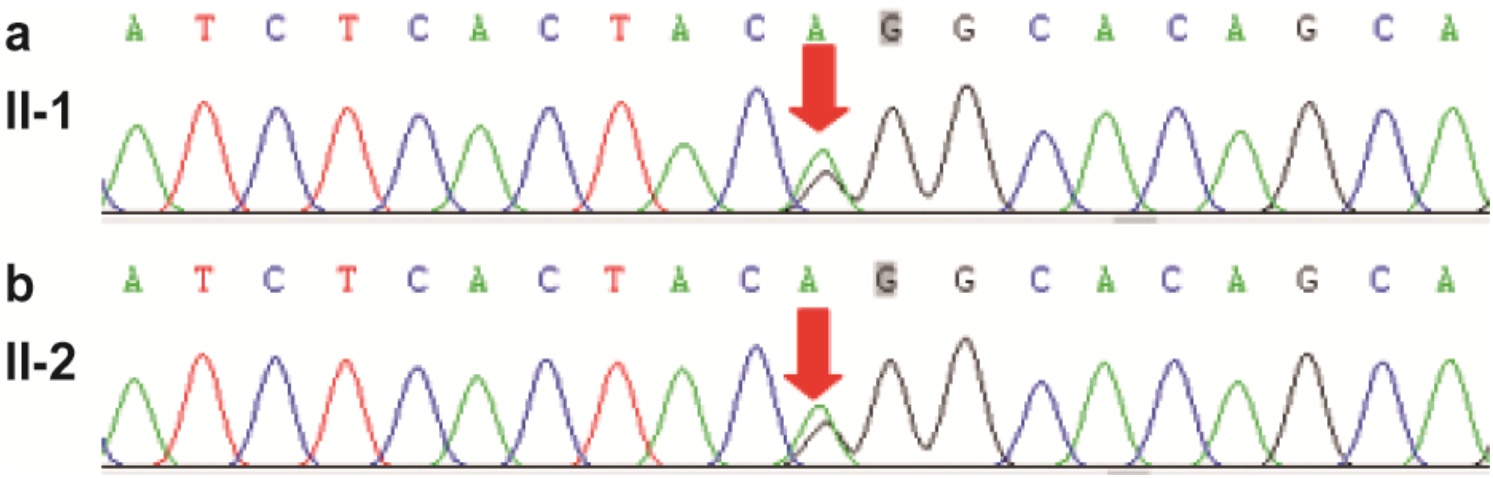

Figure 3

Sequencing analysis of the GATA4 gene in the proband II-1and case II-2. (a): The heterozygous mutation c.1123G>A in GATA4 gene were found in proband II-1, causing amino acid 375 to change from glycine to arginine. (b): The heterozygous mutation c.1123G>A/p.Gly375Arg in GATA4 gene also were found in case II-2.

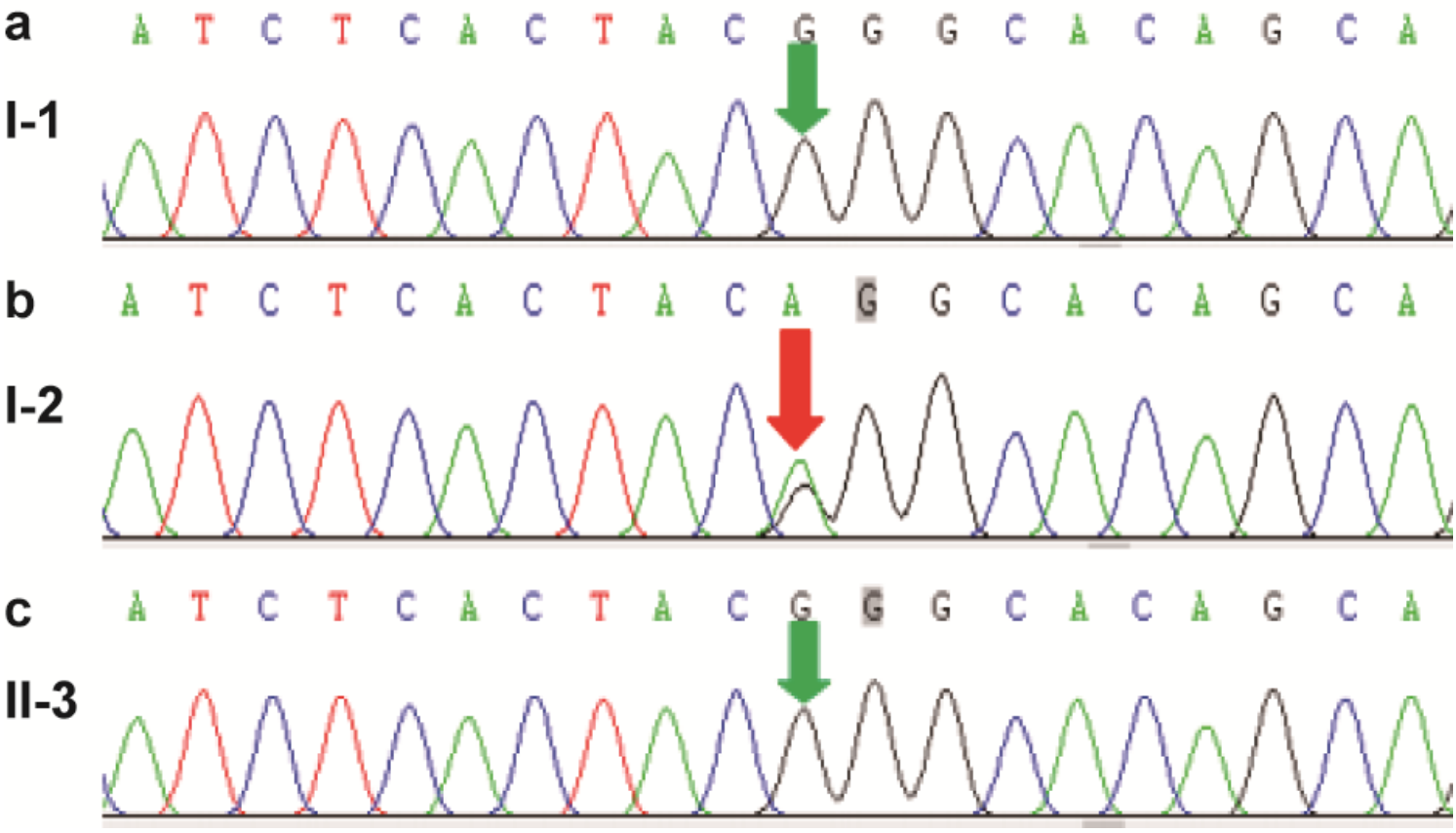

Figure 4

Sequencing analysis of the GATA4 gene in the patient's father (I-1), mother (I-2) and litter sister (II-3). (a) and (c): The heterozygous mutation c.1123G>A in GATA4 gene was not found in the patients' father (I-1)

and litter sister (II-3). (b): The heterozygous mutation c.1123G>A in GATA4 gene was found in the patients' mother (I-2).