Molecular Biology Reports (2019) 46:2971–2978
https://doi.org/10.1007/s11033-019-04758-y

ORIGINAL ARTICLE

Gene dosage of *DAX-1*, determining in sexual differentiation: duplication of *DAX-1* in two sisters with gonadal dysgenesis

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Received: 1 December 2018 / Accepted: 12 March 2019 / Published online: 16 March 2019
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Abstract

Two sisters phenotypically normal females, presenting with tumor abdominal mass with histopathological findings of teratoma and gonadoblastoma associated to 46,XY male-to-female sex reversal syndrome, secondary to a duplication in DAX-1, possibly inherited of maternal gonadal mosaicism. Copy number variation and functional effects of the duplication were done by MLPA multiplex ligation-dependent probe amplification and real time PCR. DAX-1, also known as dosage sensitive sex reversal gene (DSS), is considered the most likely candidate gene involved in XY gonadal dysgenesis when overexpressed. The excess of DAX-1 gene disturbs testicular development by down regulation of SF-1, WT1, and SOX9. This is the first report of 46,XY sex reversal in two siblings who have a maternally inherited duplication of DAX-1 associated with reduced levels of expression of downstream genes as SOX9–SF1.

Keywords Sex reversal syndrome · Simple gonadal dysgenesis · Gonadoblastoma · Disorders of sex development · DSD · DAX-1

Introduction

Sex determination refers to the sexual characteristics of an organism, male or female, based on the chromosomal and genetic determinants. Sexual differentiation, is the physiologic and anatomic process that results in the development of a male or female phenotype. This process is directed by hormones, secreted by gonads, that influence the differentiation of external and internal genital structures. Therefore, sexual determination is contingent on a genetic regulation, in which *SRY* is not the only gene necessary for testicular differentiation (some other genes include *SOX9*) [1–3]; like so a gonadal and a hormonal regulation stage [4].

A genetic decontrol of genes such as *SRY, SOX9, DAX-1, WNT4, WT1, DHH, and SF-1* can result in disorders of sex development (DSD), which refer to congenital conditions of atypical development of the chromosomal, gonadal or anatomical sex. During this stage, mutations, deletions or duplications in several of these genes have been identified, and affect the sexual differentiation and final phenotype of the individual. As in the case of gonadal dysgenesis in 46,XY individuals with *DAX-1* duplication [4–6].

DAX-1 (dosage-sensitive sex reversal (*DSS*), adrenal hypoplasia congenital critical region on the X chromosome, gene 1), also known as NR0B1 (Nuclear Receptor Subfamily 0 Group B Member 1), is a member of superfamily of nuclear receptors. This gene is located in short arm of Xp21, contains 2 exons [7] and encodes a protein of 470 amino acids, a potential transcription factor expressed in the adrenal glands, but also in the hypothalamus, pituitary gonadotropic cells and gonads [8–11]. DAX-1 acts as a dominant-negative coregulatory protein that inhibits the transcriptional activity of other nuclear receptors SF-1 [12], estrogen receptor [13] and androgen receptor [14] mediated by the retinoic acid receptor [15, 16]. It has DNA-binding

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domain at the N terminus and the C terminus with characteristics of a nuclear hormone receptor ligand-binding domain.

The existence of a gene in the X-chromosome involved in human sex determination was reported by German et al. [17] assumed that Xp duplication was associate to sex reversal due to double dosage of an X-linked gene which is normally subject to X-inactivation, therefore this locus has previously named *DSS* [18]. It has been postulated as an "anti-testis" gene based on the finding of XY patients with duplication in Xp21 with sexual reversion and dimorphic expression between ovary and testis [16, 18–21]. Hypotheses about the etiology indicate that excess of DAX-1 protein reduces activation of the SOX9 enhancer by inhibiting the interaction of SF1 on WT1, antagonizing steroidogenesis and the production of anti-Müllerian hormone (AMH), it was validated in transgenic mice by Ludbrook et al. [22]. Although the molecular mechanisms by which *SRY* acts are not precisely known, there is experimental evidence that *SRY* and *DAX-1*, interact in early periods of development of the gonadal ridges, expressing both in testicular and ovarian tissue [23, 24]. The exactly time of expression and function of *SRY* and *DAX-1* is important due to a delay in *SRY* expression that would allow an anti-testicular action of *DAX-1*, resulting in the formation of ovotestes or dysgenetic gonads [24]. Disorder of sex development in the absence of *DAX-1* occurred after normal expression of *SRY*, which evidence that both are required for normal testis determination.

Furthermore, point mutations and deletions in the *DAX-1* gene cause congenital adrenal hypoplasia (AHC) and hypogonadotropic hypogonadism (HH) [25], secondary to not differentiation of adrenal gland beyond the fetal stage with disturbance in steroidogenesis and defective development of testis cords [20]. Unlike duplications in *DAX-1* in XY individuals undergo sex reversal and develop as females, XY transgene mice carrying extra copies of *Dax-1* had delayed testis development and reduced expression of *Sry* and *Sox9*, suggesting a *Dax1*-*Sox9* antagonism, but do not normally show sex reversal, the complete sex reversal occurred, when the transgene mice were tested against weak alleles of *Sry* gene [23]. In the study of Ludbrook et al. in mouse model using reporter gene evidenced that *Dax1* overexpression reduced activation of TES, the testis enhancer of *Sox9*, indicating that *Dax1* might repress *Sox9* expression via TES [22].

Several families of 46,XY individuals with female phenotype and gonadal finding of gonadal streaks or gonadoblastoma have been reported [8–11, 15, 17–19, 26, 27]; suspicion of "sex reversal" in families without parental consanguinity and the characteristic distribution of affected individuals suggest X-linked recessive inheritance. Bardoni et al. identified a region of approximately 20-Mb on Xp21.2–p22.1 that was duplicated only in the 46,XY females [18]. The similarities in the phenotype of the 46,XY females with different Y chromosome (from different fathers) is the best evidence to explain that the Y is not enough to differentiation of the testis, and is the interaction between genes of X and Y chromosomes that defines gonadal differentiation.

46,XY sex reversal occur in about 1 in 3000 births [21], in 15% of the patients it is secondary to *SRY* deletions or mutations [28] in the other percentage of patients, mutations in other genes have been identified [29]. We report the case of two sisters with duplication of the Xp chromosome segment within the region of Xp21.1–22.2 (*DAX1*) resulting in 46,XY sex reversal.

Materials and methods

Patients

We studied two Colombian sisters born of non-consanguineous parents (Pedigree—Fig. 1), at birth and throughout childhood each had appeared to be a female phenotypically. In one of them at the time expected for the puberty, axillary and pubic hair developed; breast development and menstruation failed to occur. The physical exam of both without major anomalies, adequate height for age and with female external genitalia (normal appearing clitoris, vaginal opening, labia majora and minora). Each one had a hypoplasic uterus and fallopian tubes.

The older sister (sister 1) presented at 15 years an abdominal mass so she was taken to laparoscopic surgery, and the freezing biopsy analysis was documented a teratoma in right gonad, subsequent studies confirmed the presence of a gonadoblastoma at left side.

The youngest sister (sister 2) at the age of 10 years, due to her sister's antecedents, was evaluated through cytogenetic study with finding of 46,XY karyotype; therefore, a laparoscopic gonadal biopsy was performed, histopathological studies described a gonadoblastoma and, consequently, a gonadectomy was performed.

Methods

Cytogenetic

Cytogenetic analyses were carried out based on phytohaemaglutinin-stimulated peripheral blood lymphocyte cultures, of the patients and parents, according to standard laboratory protocols [30].Chromosome preparations were treated with trypsin and stained with Giemsa. A total of 50 metaphase cells were analyzed at the 550-band resolution level.

Molecular cytogenetics using fluorescence in situ hybridization (FISH) using the following probes and probe sets was subsequently performed following the manufacturer's instructions: SRY (in Yp11.31; Cytocell—Aquarius,

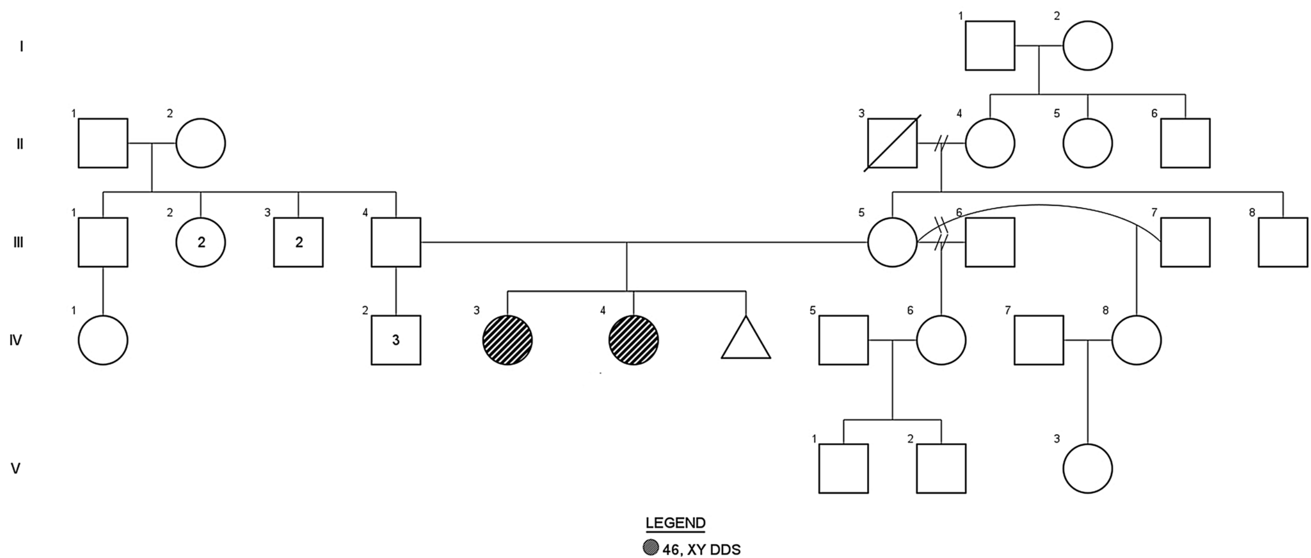

Fig. 1 Pedigree of sisters with DSD 46,XY. Vertical roman numbers refer to each generation. Vertical Arabic numbers below each symbol refer to the individual of each generation. Numbers within the symbols refers to the number of individuals of that sex

Cambridge, UK), DYZ1 (in Yq12; Cytocell – Aquarius, Cambridge, UK). Also, a control probe for Xp11.1–q11.1 was hybridized. As counterstain 4′,6-diamidino-2-phenylindole DAPI was used. 200 metaphases and 50 nuclei were analyzed with the ZEISS ZEN microscope software.

Multiplex ligation-dependent probe amplification (MLPA)

Study of deletions or duplications of *SOX9, DAX-1, SF-1, SRY*, and *WNT4* genes was carried out using the MLPA SALSA P185-B2 Intersex (version 08; May 07, 2015) (MRC Holland, Amsterdam, The Netherlands), following the manufacturer's instructions. Information on probe sequences can be freely obtained from the MRC Holland website (http://www.mlpa.com).

After 35 cycles of PCR amplification, PCR products were separated using an ABI 3100 genetic analyzer. Row data was analyzed using the Coffalyser Software (MRC Holland ®). For each sample, the peak areas corresponding to each probe were normalized to the average of the peak areas in three controls. DNA samples showing a reduction or increase in the MLPA peak area values were reanalyzed by the same MLPA procedure and only the samples showing consistent results between the two experiment replicates were considered positive for a copy number alteration.

DNA-RNA extraction from formalin fixed paraffin embedded tissue

Extraction of DNA/RNA from formalin fixed paraffin embedded tissue (FFPE) was following manufacturer's instructions of Quick-DNA/RNA FFPE Kit Zymo (Catalogue No D3067). All DNA samples were treated with RNaseA for 1 h at 45 °C, quantified and assessed for purity by NanoDrop (Thermo Scientific, MA, USA) 260/280 and 260/230 ratio measurements. DNA integrity of samples was checked by electrophoresis in a 1.3% agarose gel.

Quantitative real-time PCR

1 µg of total RNA was reverse transcribed to cDNA with random primers using high capacity cDNA reverse transcription kit (New England, USA) as per manufacturer's protocol in a 20 µL volume. cDNA was diluted 1:10 with nuclease free water to a final concentration of 5 ng/µL and stored at − 20 °C until the next use. All RT-qPCR reactions were performed on ABI7900 HT Fast Real Time PCR Instrument (Thermo Fisher Scientific) in duplicate, except the samples in which the analysis outcome was questionable. If this had happened, another two replicates were analysed. Detection of the amplification product was enabled with SYBR Green dye (Roche—OR, USA), according to the manufacturer's recommendations, in total reaction volume of 10 µl. Expression levels were normalized using *18S* gene (housekeeping gene). Relative expression was calculated with the mathematical model allowing for correction of reaction efficiency and using the Universal Human Reference RNA (Stratagene, La Jolla, CA, USA) as a reference. Primer sequences used in this study are shown in Table 1; detailed PCR protocols are available upon request from the corresponding author.

Table 1 Real-time RT-PCR primers and reaction conditions used for expression analysis of specified genes

Gene name	Gene primers (For/Rev) sequence (5′–3′)	Tm	GC%
SRY	For: CATGAACGCATTCATCGTGTGGTC	62.98	50
	Rev: CTGCGGGAAGCAAACTGCAATTCTT	64.94	48
SOX9	For: AGCACTCCGGGCAATCT	58.14	58.82
	Rev: CGGCAGGTATTGGTCAAACT	58.18	50
DAX1	For: AGGGGACCGTGCTCTTTAAC	59.68	55
	Rev: ATGATGGGCCTGAAGAACAG	57.57	50
WNT4	For: CCTTCGTGTACGCCATCTCT	59.54	55
	Rev: GCCTCATTGTTGTGGAGGTT	58.38	50
SF1	For: GGCATGGACTATTCGGACGACGAGGACCTGG	72.35	61.29
	Rev: CCAGGTCCTCGTCGTCCGAATAGTCCATGCC	72.35	61.29

Data analysis

RT-qPCR data was analyzed by calculating the fold difference respect to the housekeeping gene. Cycle threshold (Ct) is defined as the number of PCR cycles at which the fluorescence signal rises above the threshold value and is inversely proportional to the amount of template present in the reaction. Ct values of genes in gonadal tissue of patients and control samples (normal embryonic tissue of Hs 1.Tes cell line (ATCC® CRL-7002™) and adult testes) were compared and the fold difference calculated by the equation:

Fold difference = $2^{\Delta\Delta Ct}$
where $\Delta Ct = CtGonadal\ tissue\text{-}CtControl$ [31].

Statistical significance was determined by calculating probability values using GraphPad software (CA, USA). *p* values less than 0.05 were considered significant.

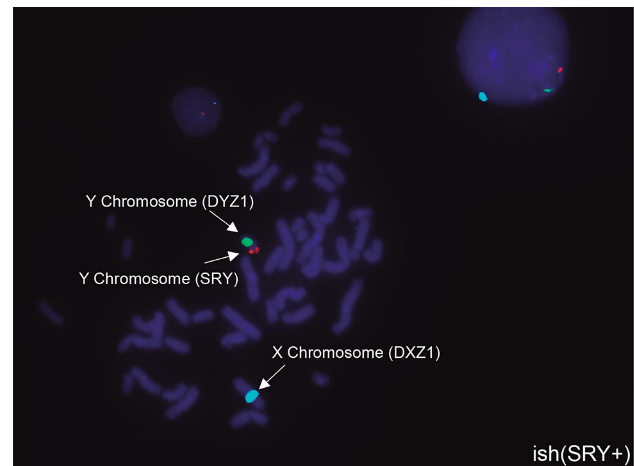

Fig. 2 FISH SRY analysis of one of the sisters 46,XY showing hybridization of the three probes of Cytocell Kit, specific to X and Y Chromosomes, discard the deletion of SRY

Results

The karyotype of the patients was 46,XY analyzed in 50 metaphases, with complementary FISH test that indicated positive expression of *SRY* gene. These results constitute strong evidence against the existence of a mosaicism, and discard the deletion of *SRY* as etiology of the clinical findings (Fig. 2).

The family karyotype analysis showed normal chromosome complements, the father was 46,XY and that the mother as well as the maternal half-sisters analyzed were 46,XX. Confirming no other individuals carried this type of DSD in the family.

MLPA analysis with Intersex kit (MRC Holland ®) in both sisters XY showed duplication of probes that hybridize exons of the *DAX-1* gene in peripheral blood and gonadal tissue (Fig. 3). Analysis in the mother and maternal half-sisters did not show the same results, as the genetic dose of all genes evaluated (*SOX9, DAX-1, SF-1, SRY*, and *WNT4)* was normal.

To evaluate mRNA expression levels of *SOX9, DAX-1, SF-1, SRY*, and *WNT4*, quantitative real-time PCR (qPCR) analysis were performed. qPCR revealed an increase of mRNA levels of *DAX-1* in both patients, compared to the expression of a 46,XY control tissues. Interestingly, we detected a decrease in the expression level of *SOX9*, and *SF-1* (Fig. 4), genes downstream of *DAX-1*.

Discussion

Generally, sex determination in mammals begins during embryogenesis, with the bipotential embryonic gonad either committing to male or female differentiation. The onset of this process involves a complex network of genes. An adequate regulation of these is required for the proper

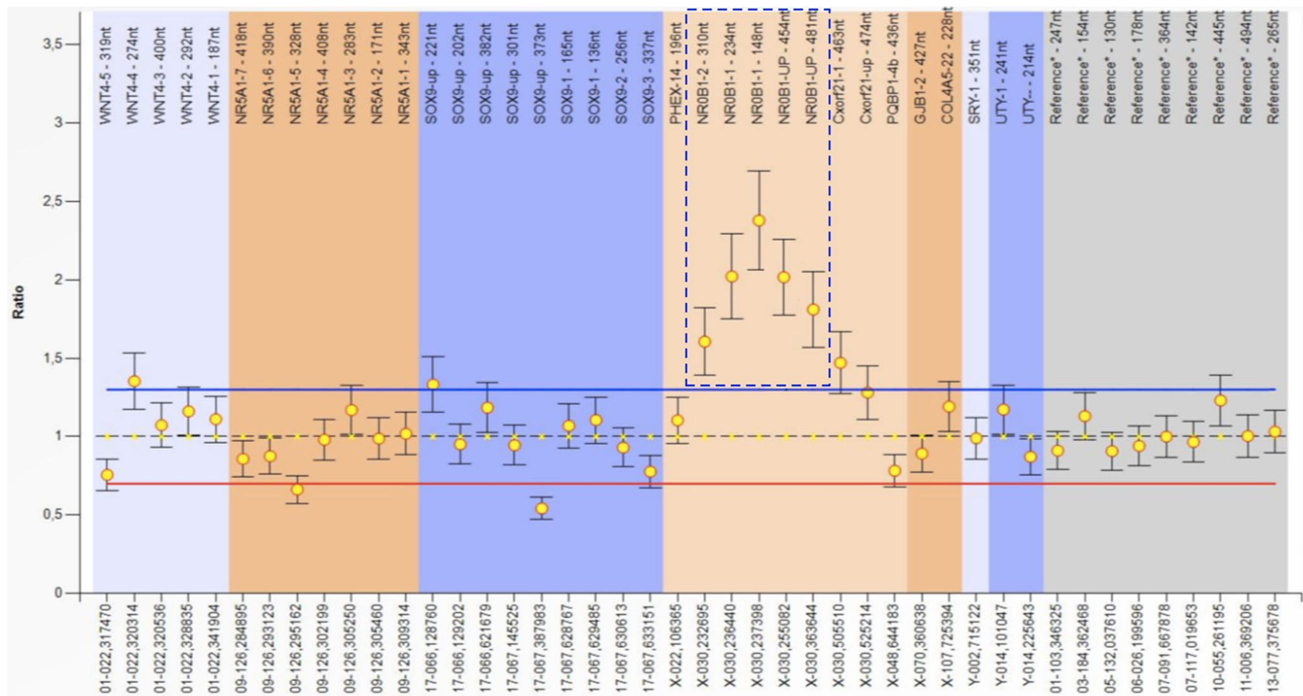

Fig. 3 MLPA analysis of one of the sisters 46,XY showing increase in the peak area in NR0B1 (DAX1) region. Points outside the normal range (between red and blue lines) suggest duplication of gene with this specific probe. The duplication was confirmed in peripheral blood and gonadal tissue. (Color figure online)

development of the gonads and other sex-specific anatomical structures and external genitalia of the individual [3, 32, 33].

Mutations in *SRY* and *SOX9* account for about 20% of 46,XY complete gonadal dysgenesis patients. Causative mutations involving several other genes (including *DAX-1, SF-1, WNT4, DHH* and *MAP3K1*) have been identified in approximately 30% of cases, though little is known about the underlying genetic cause of the remaining 50% of cases [32]. In the present study, we determined the duplication of *DAX-1* by MLPA and validated the effects of it by mRNA expression levels in two 46,XY *SRY*-positive sisters with gonadal dysgenesis.

DAX-1 acts as a mediator of sexual differentiation. In normal 46,XY males, the *DAX-1* gene is held inactive by *SRY*, and thus, a male gonadal differentiation pathway is followed. If an extra copy arises on an active X chromosome, as in Xp duplication, there is not enough *SRY* to overcome the amount of *DAX-1* and a female pathway is followed. Subsequently, excesses *DAX-1* dosage represses TES, and thus, *SOX9* is inactivated and testicular formation is blocked [22]. In the absence of testosterone produced by fetal Leydig cells of a normal testis, no virilization from external genitalia occurs. Equally AMH is not produced and Müllerian duct regression is repressed, this correlates with the findings of a hypoplasic uterus in 46,XY female individuals as in the patients evaluate in this study. Proposed model of genetic regulation of sexual determination in individuals 46,XX and 46,XY with concordant phenotype are shown in Fig. 5.

DAX-1 overexpression may alter other developmental pathways that interfere with gonadal development and maintenance of the ovarian follicles consequently generating a dysgenetic gonad (Fig. 5). In addition, genes on the Y-chromosome in 46,XY female does not allow oogenesis and there is degeneration, concluding in gonadal dysgenesis.

Additionally, qPCR also showed evidence of decreased *SF-1* and *SOX9* expression in both sisters in reference to control tissue, antagonistically to the typical process of male differentiation in which *SOX9* is known to play a pivotal role in testis development, and chondriogenic tissue and thus inhibition of this gene further blocks testis development. Moreover, *SOX9* activates *SF-1* and both *SOX9* and *SF-1* activate anti-Müllerian hormone gene, *AMH* [4, 34]. Interestingly, it has also been reported that *DAX-1* inhibits *SF-1* [34], and may have an antagonistic effect over *SOX9* by inhibiting SF-1 activation of *SOX9* [4, 34]. The expression profile of the genes evaluated of both sisters seem to be collaborating in a 46,XY male-to-female reversal as seen in Fig. 3.

Phenotypic females with 46,XY chromosome complement usually refer to medical attention at a later age, because of primary amenorrhea or infertility. The patients with gonadal dysgenesis due to duplication of *DAX-1* do not have short height because they have the homologous

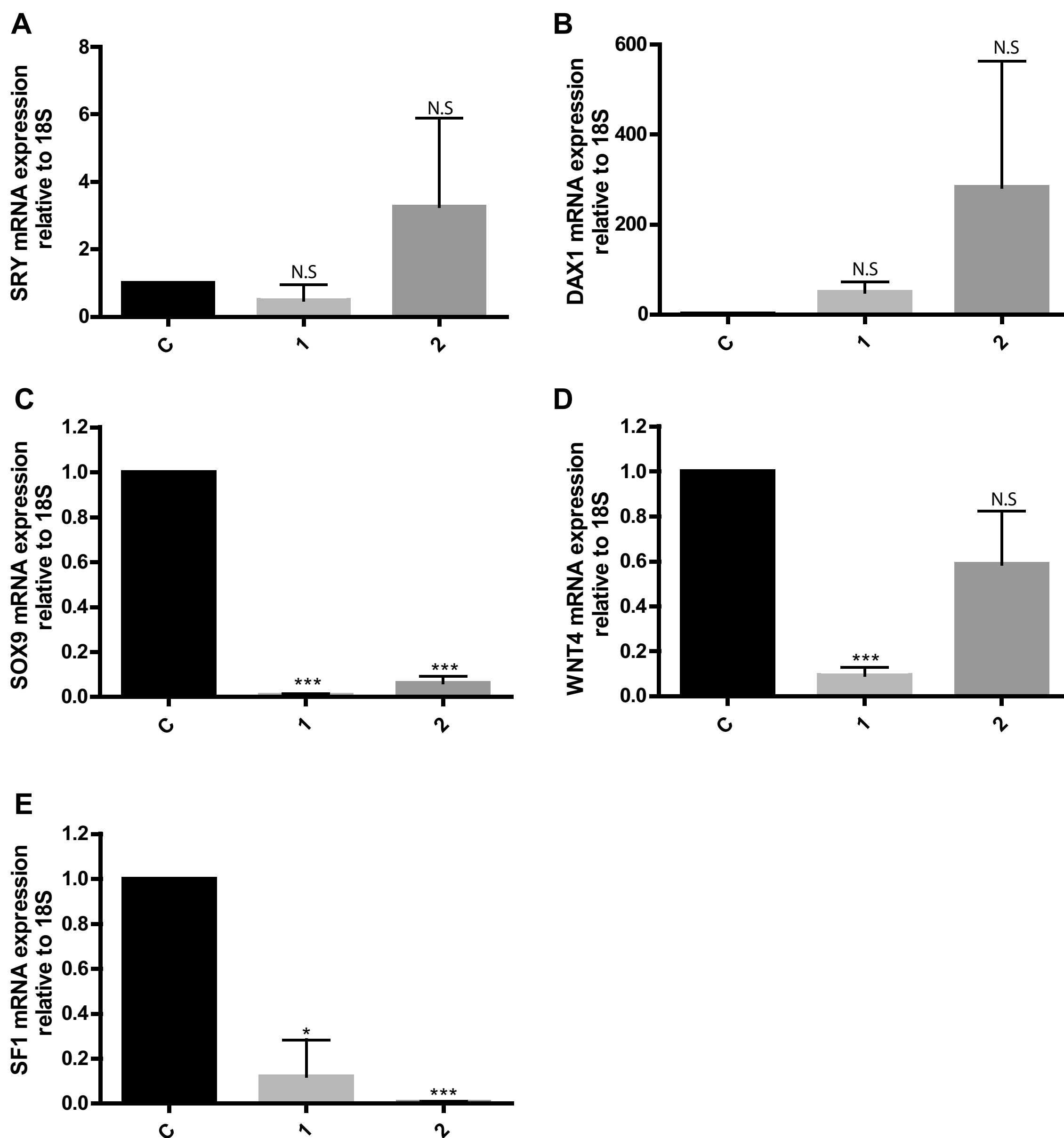

Fig. 4 Real-time PCR analysis of both sisters (1 and 2), in which the control (C) is a 46,XY cell line of testis [Hs1.Tes] **a** DAX1, **b** SOX9, **c** SRY, **d** SF1 and **e** WNT4. Error bars: standard deviation

region of the short arm that does not cause them low height like in Turner syndrome, establishing the precise diagnosis of gonadal dysgenesis is important due to increased risk for gonadoblastoma, management of puberty delay and for genetic counseling of the family about recurrence risk.

Conclusion

We report a duplication on Xp21.2 in two sisters with isolated 46,XY gonadal dysgenesis. The results suggest *DAX-1* duplication as the etiological cause of the gonadal dysgenesis, the analysis of mRNA expression levels validates the effect of duplication by the finding of decreased *SOX9* and *SF-1*. Although MLPA analysis in the mother did not show a duplication on *DAX-1*, we are led to consider the possibility of a gonadal mosaicism of the duplication, which would explain the presence of his other healthy daughters. However, given the impossibility of obtain a sample from this tissue, it was not possible to confirm this hypothesis. Furthermore, we can hypothesize that healthy female carriers are fertile because the ovary can tolerate an extra dose of *DAX1*.

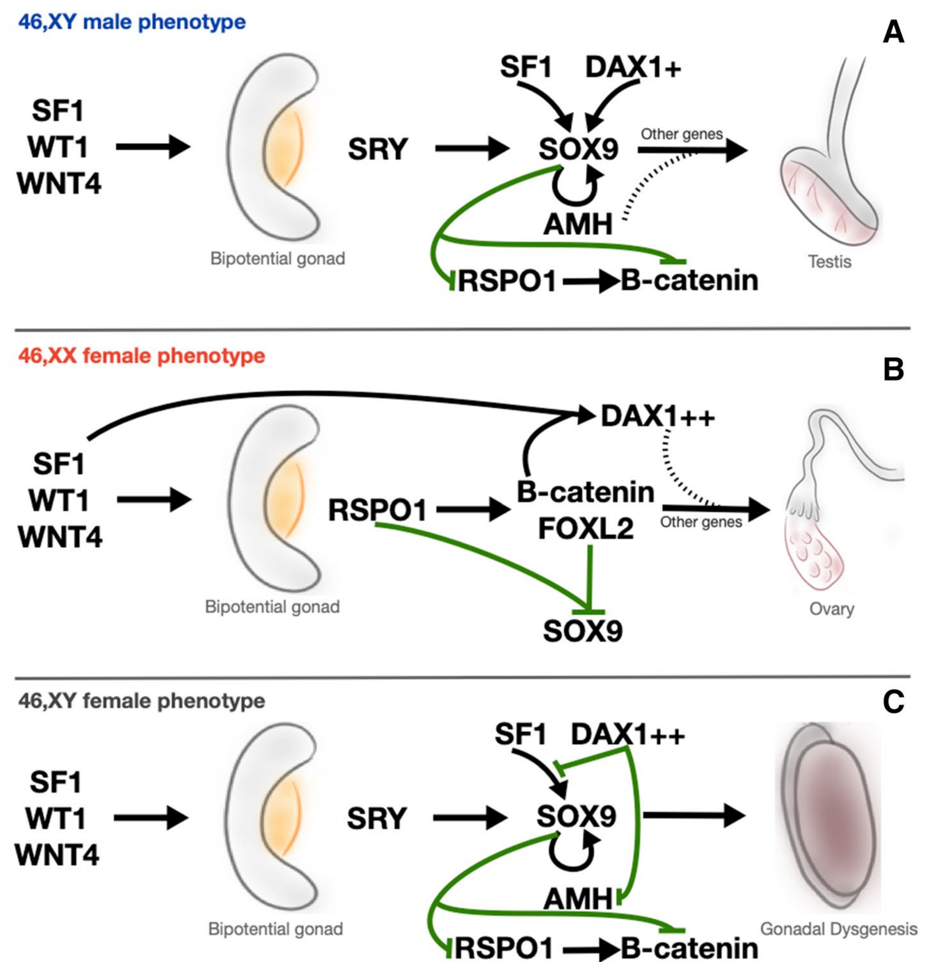

Fig. 5 *Above* DAX-1 acts as a mediator of sexual differentiation. In normal XY males, the DAX-1 gene has a precise amount needed (1 locus) for testicular hormone synthesis. In normal XX females, DAX-1 in double dosage (++) is necessary for ovarian differentiation. *Below* in excessive gene dosage in 46, XY individuals, DAX-1 represses SOX9, antagonizes the synergy between SF-1 and SOX9 in inactivating the promoter of the HAM gene, blocking testicular differentiation, resulting in gonadal dysgenesis

Acknowledgements We thank the family for their cooperation and interest to participate in this study.

Author contributions MGA, AR, AR designed and did the molecular biology studies. MGA, OM and MM worked on the cytogenetic studies. MGA, CF, CC, JCP, JP, FS, clinically evaluated patients and guided clinical management. All authors contributed to interpretation of the findings of the experiments and writing of the paper. All authors read and approved the final manuscript.

Funding This work was funded by COLCIENCIAS [Grant 711/2015—Project Number 120371150037] and MG was beneficiary of the grant "young researcher COLCIENCIAS" 761/2016.

Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was approved by the ethics committee of the Hospital Universitario San Ignacio, Bogotá, Colombia.

Informed consent Informed consent was obtained from all individual participants or patient's legal guardian included in the study in accordance with the guidelines of the Research Ethical Committee of the Hospital Universitario San Ignacio.

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References

1. Biason-Lauber A (2010) Control of sex development. Best Pract Res Endocrinol Metab 24:163–186
2. Houmart B, Small C, Yang L, Cheng E, Hassold T, Griswold M (2009) Global gene expression in human fetal testis or ovary. Biol Reproduct 81:438–443
3. Brennan J, Capel B (2004) One tissue two fates: molecular genetic events that underlie testis versus ovary development. Nat Rev Genet 5:509–521

4. McClelland K, Bowles J, Koopman P (2012) Male sex determination: insights into molecular mechanisms. Asian J Androl 14:164–171
5. Kun K, Jongwon K (2012) Disorders of sex development. Korean J Urol 53:1–8
6. Barbaro M, Oscarson M, Schoumans J, Staaf J, Ivarsson S, Wedell A (2007) Isolated 46,XY gonadal dysgenesis in two sisters caused by a Xp21.2 interstitial duplication containing the DAX1 gene. J Clin Endocrinol Metab 92(8):3305–3313
7. Guo W, Burris T, Zhang Y, Huang B, Mason J, Copeland K, Kupfer SR, Pagon RA, McCabe ER (1996) Genomic sequence of the DAX1 gene: an orphan nuclear receptor responsible for X-linked adrenal hypoplasia congenita and hypogonadotropic hypogonadism. J Clin Endocr Metab 81:2481–2486
8. Zazopoulos E, Lalii E, Stocco D, Sassone-Corsi P (1977) DNA binding and transcriptional repression by DAX-1 blocks steroidogenesis. Nature 390:311–315
9. Simpson J, Summir R, Merkatz IJG (1976) Etiology of XY gonadal. Gynecol Invest 7:37
10. Sternberg W, Barclay D, Kloepfer H (1968) Familial XY gonadal dysgenesis. N Engl J Med 278:695–700
11. Fechner P (1993) Report of a kindred with X-linked (Or autosomal dominant sex-limited) 46,XY partial gonadal dysgenesis. J Clin Endocrinol Metab 76(5):1248–1253
12. Ito M, Yu R, Jameson J (1997) DAX-1 inhibits SF-1-mediated transactivation via a carboxy-terminal domain that is deleted in adrenal hypoplasia congenita. Mol Cell Biol 17:1476–1483
13. Zhang H, Thomsen J, Johansson L, Gustafsson J, Treuter E (2000) DAX-1 functions as an LXXLL-containing corepressor for activated estrogen receptors. J Biol Chem 275:39855–39859
14. Holter E, Kotaja N, Makela S, Strauss L, Kietz S, Janne O, Gustafsson JA, Palvimo JJ, Treuter E (2002) Inhibition of androgen receptor (AR) function by the reproductive orphan nuclear receptor DAX-1. Mol Endocrinol 16:515–528
15. Niakan K, McCabe E (2005) DAX1 origin, function, and novel role. Molec Genet Metab 86:70–83
16. Zanaria E, Muscatelli F, Bardoni B, Strom T, Guioli S, Guo W, Lalli E, Moser C, Walker A et al (1995) An unusual member of the nuclear hormone receptor superfamily responsible for X-linked adrenal hypoplasia congenita. Nature 372:635–641
17. German H, Simpson J, Chaganti R, Summitt R, L R IRM (1978) Genetically determined sex-reversal in 46,XY humans. Science 202(4363):53–56
18. Bardoni B, Zanaria E, Guioli S, Floridia G, Worley K, Tonini G, Ferrante E, ChiumelloG, McCabe ERB et al (1994) A dosage sensitive locus at chromosome Xp21 is involved in male to female sex reversal. Nat Genet 7(4):497–501
19. Barbaro B, Cook J, Lagerstedt-Robinson K, Wedell A (2012) Multigeneration inheritance through fertile XX carriers of an NR0B1 (DAX1) locus duplication in a kindred of females with isolated XY gonadal dysgenesis. Int J Endocrinol. 2012:7
20. Meeks J, Crawford S, Russell T, Morohashi K, Weiss J, Jameson J (2003) Dax1 regulates testis cord organization during gonadal differentiation. Development 130(5):1029–1036
21. Camerino G, Parma P, Radi O, Valentini S (2006) Sex determination and sex reversal. Curr Opin Genet Dev 16:289–292
22. Ludbrook L, Bernard P, Bagheri-Fam S, Ryan J, Sekido R, Wilhelm D, Lovell-Badge R, Harley VR (2012) Excess DAX1 leads to XY ovotesticular disorder of sex development (DSD) in mice by inhibiting steroidogenic factor-1 (SF1) activation of the testis enhancer of SRY-box-9 (Sox9). Endocrinology 153(4):1948–1958
23. Swain A, Zanaria E, Hacker A, Lovell-Badge R, Camerino G (1996) Mouse Dax1 expression is consistent with a role in sex determination as well as in adrenal and hypothalamus function. Nat Genet 12:404–409
24. Nagamine C, Morohashi K, Carlisle C, Chang D (1999) Sex reversal caused by Mus musculus domesticus Y chromosomes linked to variant expression of the testis-determining gene Sry. Dev Biol 216:182–194
25. Muscatelli F, Strom T, Walker A, Zanaria E, Récan D, Meindl A, Bardoni B, Guioli S, Zehetner G et al (1994) Mutations in the DAX-1 gene give rise to both X-linked adrenal hypoplasia congenita and hypogonadotropic hypogonadism. Nature 372(6507):672–676
26. Stern H, Garrity A, Saal H, Wangsa D, Disteche C (1990) Duplication Xp21 and sex reversal: insight into the mechanism. Am J Hum Genet 47(Suppl):A41
27. Goodfellow P, Camerino G (1999) DAX-1, an 'antitestis' gene. Cell Mol Life Sci 55:857–863
28. Hawkins J, Taylor A, Berta P, Levilliers J, Van der Auwera B (1992) Mutational analysis of SRY: nonsense and missense mutations in XY sex reversal. Hum Genet 88:471–474
29. Scherer G, Held M, Erdel M, Meschede D, Horst J (1998) Three novel SRY mutations in XY gonadal dysgenesis and the enigma of XY gonadal dysgenesis cases without SRY mutations. Cytogenet Cell Genet 80:188–192
30. Moorhead PS, Nowell PC, Mellman WJ, Battips DM, Hubgerford DA (1960) Chromosome preparations of leukocytes cultured from human peripheral blood. Exp Cell Res 20:613–616
31. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔΔCt method. Methods 25:402–408
32. White S, Ohnesorg T, Notini A, Roeszler K, Hewitt J, Daggag H, Smith C, Turbitt E, Gustin S et al (2011) Copy number variation in patients with disorders of sex development due to 46,XY gonadal dysgenesis. PLoS ONE 6(3):e17793
33. Ludbrook L, Harley V (2004) Sex determination: a 'window' of DAX1 activity. TRENDS Endocrinol Metab 15(3):116–121
34. Lalli E (2014) Role of orphan receptor DAX-1/NR0B1 in development, physiology, and disease. Adv Biol. https://doi.org/10.1155/2014/582749

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