Partial Deletion of the *NR5A1* (*SF1*) Gene Detected by Synthetic Probe MLPA in a Patient with XY Gonadal Disorder of Sex Development

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Key Words

Disorders of sex development • MLPA • *NR5A1* • Steroidogenic factor 1

Abstract

Steroidogenic factor 1 (SF1, officially *NR5A1*) is a nuclear receptor involved in adrenal and gonadal development. *NR5A1* mutations have been identified in patients with various forms of 46,XY disorders of sex development (DSD), including complete gonadal dysgenesis with or without adrenal insufficiency, mild testicular dysgenesis with ambiguous external genitalia or female external genitalia with clitoromegaly, and penoscrotal hypospadias. We developed a synthetic probe set for MLPA analysis of the *NR5A1* gene covering its 7 exons and analyzed 20 patients with 46,XY gonadal DSD in whom analyses failed to identify a genetic cause. We identified a partial *NR5A1* deletion affecting exons 2 and 3, leading to *NR5A1* haploinsufficiency in 1 patient presenting with female external genitalia with clitoromegaly, absence of a uterus, and mildly dysgenetic testes. This is the first partial *NR5A1* gene deletion identified by MLPA in a patient with

46,XY gonadal DSD. This finding stresses the importance of investigating copy number changes, even at the exon level, in genes involved in gonadal DSD. As *NR5A1* mutations can cause a wide spectrum of DSD with relatively high frequency, the analysis of the *NR5A1* gene by MLPA is quite important and should be extended to larger groups of patients.

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Steroidogenic factor 1 (SF1, officially named *NR5A1*) is a nuclear receptor that was initially identified as a regulator of steroidogenesis [Lala et al., 1992]. In mice and humans its expression is initiated in the urogenital ridge and continues in the adrenal glands, gonads, hypothalamus, and pituitary gland [Ingraham et al., 1994; Hanley et al., 1999]. In the developing gonad, *NR5A1*, together with other transcription factors (i.e. *SRY*, *DAX1*, *SOX9*, and *WT1*), is involved in gonad determination and differentiation. In testis Leydig cells, *NR5A1* activates the expression of several enzymes involved in steroidogenesis, thus leading to the synthesis of testosterone required for Wolffian duct development and the formation of ex-

ternal genitalia in a male embryo [Ozisik et al., 2003]. In Sertoli cells, NR5A1 activates the expression of AMH, leading to the regression of Müllerian structures. In females, NR5A1 is expressed in the granulosa and theca cells where it regulates genes required for ovarian steroidogenesis and follicle growth maturation [Lourenco et al., 2009].

Sf1 null mice lack both adrenals and gonads [Luo et al., 1994], while mice heterozygous (+/–) for a *Sf1* deletion present a milder phenotype with adrenal defects and reduced testicular size [Bland et al., 2004].

NR5A1 mutations in humans were initially identified in patients with 46,XY gonadal dysgenesis (GD) and adrenal insufficiency [Achermann et al., 1999, 2002]. However, after the description of heterozygous mutations in some patients with 46,XY gonadal disorders of sex development (DSD) but without adrenal failure [Correa et al., 2004; Mallet et al., 2004], several mutations have been identified in patients with varying degrees of 46,XY gonadal DSD and apparently normal adrenal function [Kohler et al., 2007; Lin et al., 2007].

Patients with complete 46,XY GD are usually identified at puberty when they present as girls with primary amenorrhea and a 46,XY karyotype or at birth when a girl is born despite a 46,XY karyotype at prenatal testing. In these patients, the absence of testosterone production during embryonic development due to testis dysgenesis leads to regression of the Wolffian structures and to the development of female external genitalia, while the absent production of AMH allows the development of Müllerian structures (Fallopian tubes and uterus). Complete XY GD can generally be discriminated from an XY defect due to impaired androgen action or synthesis, by high gonadotropin values postpubertally, low testosterone levels, and by the presence of a uterus and other Müllerian structures. However, partial forms of GD presenting with ambiguous external genitalia at birth are not always easy to discriminate from other partial defects in androgen production or response.

The phenotypic presentation of XY patients with NR5A1 mutations has turned out to be quite variable, making the evaluation of 46,XY DSD patients complicated. XY patients with NR5A1 mutations can present not only with female external genitalia due to complete testicular dysgenesis but also with clitoromegaly as the only sign of masculinization despite the presence of only mildly dysgenetic testes, thus showing unexpectedly severe underandrogenization of external genitalia compared to the degree of abnormal testicular differentiation [Lin and Achermann, 2008]. Furthermore, Wolffian as well as

Müllerian structures can be present or absent [Lin and Achermann, 2008]. Other patients present with ambiguous external genitalia, resembling the partial androgen insensitivity syndrome [Coutant et al., 2007]. Lately, NR5A1 mutations have also been described in patients with even milder DSD phenotypes such as severe penoscrotal hypospadias [Kohler et al., 2009] and bilateral anorchia with micropenis [Philibert et al., 2007]. Furthermore, the non-synonymous polymorphism Gly146Ala, that slightly diminishes NR5A1 transactivation activity [WuQiang et al., 2003], has been indicated by association studies as a susceptibility factor for micropenis [Wada et al., 2005] and cryptorchidism [Wada et al., 2006]. Recently, missense mutations altering transcriptional activity have been reported in men with severe spermatogenic failure, representing the mildest form of the phenotype spectrum caused by NR5A1 mutations [Bashamboo et al., 2010].

In 2009, mutations in NR5A1 have also been shown to be associated with ovarian insufficiency in 46,XX women presenting with primary amenorrhea or premature ovarian failure [Lourenco et al., 2009].

The human NR5A1 (*SF1*) gene on chromosome 9q33.3 is composed of 7 exons, with exons 2–7 being coding while the first exon is non-coding [Oba et al., 1996]. Considering all phenotypes described, more than 30 NR5A1 mutations have been described so far. The mutation type ranges from missense, nonsense, splicing, small deletions, and insertions to entire gene deletions. No clear genotype-phenotype relationship has been documented. Most patients are heterozygous for a mutation, but some patients carrying homozygous mutations with at least 50% residual activity have been described [Achermann et al., 2002; Lourenco et al., 2009]. In 46,XY DSD cases, mutations have been de novo or inherited from apparently healthy mothers [Lin et al., 2007]; however, in some families, premature ovarian failure can eventually develop in carrier mothers [Lourenco et al., 2009]. Thus, gene dosage and degree of residual NR5A1 activity are both critical for disease presentation. In addition, the penetrance of a mutation is likely affected by modifier genes and environmental factors, as indicated for example by the different phenotypes described in a pair of siblings [Coutant et al., 2007] and in 2 dizygotic twins with the same NR5A1 mutation [Philibert et al., 2007].

Based on these facts, availability of highly informative mutational screening methods for NR5A1 is important, both from a clinical as well as a biological point of view. We therefore developed a synthetic probe set for MLPA analysis of the NR5A1 gene, covering each of the 7 exons.

Using this approach, 20 46,XY gonadal DSD patients from Sweden, the Netherlands, and Belgium were investigated in whom routine analysis failed to identify a genetic cause.

Patients and Methods

Patients

Eleven patients with 46,XY GD in whom routine analysis failed to identify a genetic cause were selected among the 46,XY gonadal DSD patients referred to the Clinical Genetic Laboratory of Karolinska University Hospital Stockholm, Sweden. GD was diagnosed by the presence of female or undervirilized external genitalia, menopausal levels of FSH, and gonad biopsies when available. A group of 9 patients with 46,XY gonadal DSD was selected among the 46,XY DSD patients of the Netherlands and Belgium DSD group. The presence of a uterus was not a selection criterion; sometimes this information has been missing in the first patient referral to the genetic clinic, furthermore some patients with *NR5A1* mutations have been reported with an absent uterus.

One of the patients presented with adrenal failure in addition to GD. In this case, point mutations in the *CYP11A1* gene were excluded. Point mutations in the *SRY* and *NR5A1* genes were excluded in all 20 patients. Karyotype analysis on blood cells did not detect any mosaicism, however, a mosaicism in the gonadal tissue could not be ruled out in all cases because tissue was not available.

All patients were also analyzed by MLPA with a DSD probe set [Barbaro et al., 2008] designed with the aim to identify entire deletions/duplications of genes involved in DSD, especially XY gonadal DSD, and with a 9p24 probe set [Barbaro et al., 2009] to exclude deletions in this region containing several genes, including the *DMRT* genes 1, 2, and 3. No entire gene deletions or duplications were identified. However, in one patient (patient A) a possible *NR5A1* partial deletion was identified (see results section for more details). The study was approved by the regional Ethics Committee at the Karolinska Institutet.

DNA was obtained from blood samples, Epstein Barr Virus (EBV)-immortalized cell lines, or gonadal fibroblasts when available.

Patient A is a girl, born from consanguineous parents, who first came to medical attention at 13 years of age, because of progressive virilization (facial hair growth, deepening of the voice, muscular body) and lack of breast development. At presentation, Tanner stage was M1P3A2, clitoral length was 40 mm, and gender identity was female. Discrete clitoromegaly had been noticed at birth but was judged insufficient to prompt medical investigations. The testosterone level was 12.4 nmol/l (reference value 0.3–0.9 nmol/l), while LH and FSH levels were 5.3 U/l and 42 U/l, respectively (LH and FSH reference values 2–8 U/l). Cytogenetic investigation revealed a 46,XY karyotype. Gonadectomy was performed at laparoscopy. The left and right gonads were found in the inguinal and abdominal position, respectively. Pathological examination of the gonads revealed bilateral testes with pronounced Leydig cell hyperplasia, normal seminiferous tubules, normal germ cell number with incomplete spermatogenesis

in the left (inguinal) gonad, and scarce germ cells in the right (abdominal) gonad. Both gonads showed the presence of carcinoma in situ, the precursor lesion of malignant germ cell tumors [Oosterhuis and Looijenga, 2005]. The uterus was absent. After gonadectomy, hormonal replacement therapy was initiated which induced normal breast development. An ACTH test performed at the age of 15 years revealed a normal cortisol response.

MLPA

A specific synthetic probe set for MLPA analysis of the *NR5A1* gene, covering each of the 7 exons (table 1) and additional probes for fine-mapping of the encountered deletion (table 2) were designed according to the recommendations by Stern et al. [2004]. The control probes and the pilot probe used have been previously described [Barbaro et al., 2008]. MLPA reactions were performed using the reagents and the recommendations of the EK1 MLPA reagent kit (MRC-Holland, Amsterdam, The Netherlands), starting from 100 ng of genomic DNA. The PCR products were separated by capillary electrophoresis on an ABI 3100 Genetic Analyzer (Applied Biosystems, Warrington, UK). Trace data were analyzed using the Gene Mapper v3.7 software and exported to an Excel spreadsheet (Microsoft). For each sample, the peak heights were first normalized to the average peak height of the control probes followed by normalization to the average peak height of the control samples included in the run. The sample run was considered acceptable if the ratio for the internal control probe was between 0.8 and 1.2. Threshold values for deletion and duplication were set at 0.75 and 1.25, respectively.

Results

MLPA analysis using the DSD kit [Barbaro et al., 2008] in patient A resulted in a ratio value for the probe hybridizing to *NR5A1* exon 2 consistent with a heterozygous deletion, while the probe for exon 6 showed a value consistent with 2 copies. No other probes targeting *NR5A1* are present in the DSD probe set. To exclude the possibility of a false positive result, MLPA analysis was repeated, and exon 2 of *NR5A1* was sequenced again. No mutations or silent variants were identified thus supporting the possibility of a partial gene deletion and pinpointing the necessity to confirm and characterize the deletion. Therefore, we developed the SF1 MLPA probe set.

SF1 Probe Set

The SF1 probe set was first tested in 10 normal controls and in a sample where the entire *NR5A1* gene is deleted. All exon probe pairs showed, after normalization, a standard deviation <10%, thus proving to give reliable and reproducible results (table 1). Furthermore, we tested a sample where one copy of the *NR5A1* gene is entirely deleted, kindly donated by Dr. Ravenswaaij-Arts [van Silf-

Table 1. SF1 probe set

Probe	Size bp	5'-half sequence	3'-half sequence	SD	Deletion control	Patient A	Results
GABRA ^a	84	CAGCCTGTTGTCATAACCATCG	AGCAAAGTGTCCAGGATGCG	nd	nd	nd	nd
RELN2 ^b	87	CAGCATTACGGAATGAAGGTCA	CCACAAGAAGTGGCTTCACAACC	0.06	0.99	0.96	+
SF1ex2	93	GACAAGGTGTCCGGCTACCACTACGGA	CTGCTCACGTGTGAGAGCTGCAAG	0.05	0.56	0.58	-
SF1ex1	96	CCACTGCCACCTCATCCGGTGTGA	GAGCGTGTCTCCGCTTCGTAAGTGA-GGG	0.05	0.61	1.09	+
SF1ex6v2	99	CTGGACCGGCAGAGTTTGTCTGCCTC-AAGTT	CATCATCCTCTTCAGCCTGGGTGAG	0.05	0.50	1.16	+
PCLN1 ^b	105	GACACAAGGGTGTAATATGCACG	TTTCAGGGTGTGTTTGCATATGATTAT-ATCAATCAGTATG	0.06	1.02	1.02	+
SF1ex5	108	GTGGCCGACCAGATGACGCTGCTGCA-GAACT	GCTGGAGCGAGCTGCTGGTGTTCGAC-CACATCTAC	0.05	0.53	1.06	+
SF1ex7	111	CGAGAACCTCCCTTTGCAGATTTGAAG-TTCCTGAA	TAACCACATCCTGGTGAAAGACGCTC-AGGAGAAG	0.04	0.51	1.00	+
SF1ex4	114	CTCCTGTGCAGAATGGCCGACCAGAC-CTTCATC	TCCATCGTGGACTGGGCACGCAGGTG-CATGGTCTTCAAG	0.02	0.56	1.08	+
SF1prom	117	CCCTAAGTGTGCGCTCAGTAAATGTGA-GAC	AAACAGAGGAACACACAAAAGATCCA-GTCACCGTGGGAGCGGCAC	0.03	0.52	1.05	+
SRY	120	GAATGCGAACTCAGAGATCAGCAAG-CAGCTGGGA	TACCATGGAAAAATGCTTACTGAAGC-CGAAAAATGGCCATTTCG	nd	1.05	1.08	+
SF1ex3N2	123	GCAGGGCTTCTTCAAGCGCACGGTGC-AGAACAACAAGCACTAC	ACGTGCACCGAGAGCCAGAGCTGCAA-GATCGACAAGAC	0.04	0.60	0.57	-
RB1ex23 ^b	129	GTCACCAATACCTCACATTCTCGAAG-CCCTTACAAGTTTCCT	AGTTCACCTTACGGATTCTCTGGAGG-GAACATCTATATTTCACC	0.02	0.97	1.04	+
CREBBP ^b	131	CCAGACCCACCCAGGCCTCCTCAATAG-TAACTCTGGCCATAGCTTAAT	TAATCAGGCTTCACAAGGCGCAGGCGC-AAGTCATGAATGGATC	0.02	1.00	1.03	+

The 5'-half probes are preceded by the universal tag sequence GGGTTCCCTAAGGGTTGGA; the 3'-half probes are followed by the universal tag sequence TCTAGATTGGATCTTGCTGGCAC and are phosphorylated at the 5'-end. Values corresponding to a heterozygous deletion are in bold. Plus and minus signs indicate deleted and non-deleted regions, respectively. nd: Not determined because not relevant. ^a Pilot probe. ^b Control probes that hybridize on autosomal chromosomes.

Table 2. Probes used to narrow down the breakpoint in patient A

Probe	Size bp	5'-half sequence	3'-half sequence	Results
SF1ex4	114	CTCCTGTGCAGAATGGCCGACCAGACCTTCATC	TCCATCGTGGACTGGGCACGCAGGTGCATGGTCTTCAAG	+
SF1int3-ex4	99	GATCTGTGGTGGGGCAGCATGGAAGGGGGCTT	AGAGAGGGTGAGTCTGACCCTCTGC	+
SF1int3b	126	GACAGGGCACCTGGGGCAAGGACAGTAGAGC-CCGTTTGCAT	GAATCCAGGAGTGCTTCCTGGAAGTAGTGACATTGAGG-CTGGG	-
SF1int3	108	GGCTCTCTGGTTTATTGGCTCAGCCAGTGAG	ATTCTTCCCGTCATGTTCTTAGCCAGGCCCTGAG	-
SF1ex3N2	123	GCAGGGCTTCTTCAAGCGCACGGTGCAGAACA-ACAAGCACTAC	ACGTGCACCGAGAGCCAGAGCTGCAAGATCGACAAGAC	-
SF1ex2	93	GACAAGGTGTCCGGCTACCACTACGGA	CTGCTCACGTGTGAGAGCTGCAAG	-
SF1int1	90	GGGTTTTGTTCTCAGACAAACGAA	TCCCAATCGAAGCGCATCGAACCC	-
SF1int1c	102	GTCTATCGTTGTGTCTCTGGCTCTGCGTCAG	TCTCTCTTGGGTTTCTCTTCGGGCCTCC	-
SF1int1d	111	GTAATAATGGGATTTCCACGAGTCCCATTTCCA-GCCCAGCGACC	TGAGGCTAGCGAGCAGAAGGGGTTTCG	+
SF1int1e	108	CCTGTGTGTCCAGCTCCAGGTTTCCAGGAGA	CCTCGGGTGAGCTCTACCTTAGAACAGCCAACTGG	+
SF1ex1	96	CCACTGCCACCTCATCCGGTGTGA	GAGCGCTGTCTCCGCTTCGTAAGTGAGGG	+

The probes are listed according to their genetic location from the most centromeric to the most telomeric. Probes from the SF1 set that define the initial breakpoint regions are in bold. The 5'-half probes are preceded by the universal tag sequence GGGTTCCCTAAGGGTTGGA; the 3'-half probes are followed by the universal tag sequence TCTAGATTGGATCTTGCTGGCAC and are phosphorylated at the 5'-end. Plus and minus signs indicate non-deleted and deleted regions, respectively.

Fig. 1. SF1 probe set and *SF1* deletion.

a Graph showing the results of the MLPA analysis with the SF1 probe set. Samples are normalized using the average of 2 normal controls; for the SRY probe only the male control was used. Cm = Male control, Cf = female control, Cdel = sample with entire *SF1* gene deletion, Pt = patient A. **b** Representation from the UCSC genome browser (Mar2006, NCBI36, hg18) of the *SF1* gene locus on 9q33.3. Probes included in the SF1 set are represented as vertical red lines. **c** Probes designed to narrow down the breakpoint are represented as vertical green lines. The minimal deletion fragment is represented by a horizontal pink line, with the breakpoint regions delineated by a dotted pink line. The dark green horizontal line indicates the presence of a CpG island. Repeats identified by Repeat Masker are represented by different grey boxes, for the repeats within the breakpoint regions the type is indicated.

hout et al., 2009]. All probes within *NR5A1* had ratio values consistent with a heterozygous deletion, with values ranging from 0.50 to 0.61 (table 1).

Characterization of the Partial *NR5A1* Deletion

In patient A, we could determine that the deletion extended to exon 3 (fig. 1a, b) and almost the entire intron 3. In all the other 19 patients analyzed no copy number changes were identified (data not shown).

Additional synthetic MLPA probes were designed to narrow down the deletion in patient A (table 2). We could establish that the deletion has a size of approximately 3.1–4.8 kb. The telomeric breakpoint lies between the probes SF1intC and SF1int1d, while the centromeric breakpoint lies between probes SF1int3-ex4 and SF1int3 (table 2, fig. 1c).

The program BLAST (bl2seq; http://blast.ncbi.nlm.nih.gov/Blast.cgi?CMD=Web&PAGE_TYPE=BlastHome) was used to identify regions of homology that could be involved in mediating the deletion by aligning the 2 breakpoint regions. When the program was optimized for ‘somewhat similar sequences (blastn)’, a ho-

mology of a stretch of 11 bp was identified between the LINE L2 in the centromeric breakpoint region and the SINE MIRb in intron1.

Discussion

Two entire *NR5A1* gene deletions, identified by array-CGH, have been reported so far. A 3 Mb heterozygous deletion including *NR5A1* and other genes was identified in a 46,XY patient with genitopatellar syndrome, a combination of a XY DSD and nail patella syndrome, the latter most probably caused by haploinsufficiency of the *LMX1B* gene included in the deletion. The XY DSD was characterized by ovotestis, female external genitalia with clitoromegaly, and absence of a uterus [Schlaubitz et al., 2007]. The other *NR5A1* deletion had a size of 970 kb, included several genes, and was originated de novo. The 46,XY patient presented with female external genitalia and clitoromegaly, neither uterus nor other Müllerian structures were present. High testosterone levels initially suggested a diagnosis of partial androgen insensitivity

syndrome [van Silfhout et al., 2009]; no additional syndromic features were described for this patient. Interestingly, although haploinsufficient for the complete *NR5A1* gene, both patients presented with some signs of genital virilization (clitoromegaly) and no uterus, indicating a partial gonadal defect.

The identification of such deletions indicates that *NR5A1* deletions should be investigated in the evaluation of 46,XY DSD patients. While these 2 deletions could be detected by array-CGH as they were quite large, the detection of deletions affecting only the entire *NR5A1* gene, that has a size of approximately 27 kb, by array-CGH is not guaranteed but depends on the practical resolution and the probe distribution of the platform used.

For this reason our group has previously developed a DSD probe set for MLPA analysis of patients with XY DSD in order to identify copy number changes for genes involved in 46,XY DSD. The analysis with this probe set of the Dutch patients detected a deletion affecting the *NR5A1* probe in exon 2 but not the *NR5A1* probe in exon 6. The possibility of a partial *NR5A1* deletion lead us to the development of an additional *NR5A1* probe set for MLPA, especially designed to identify *NR5A1* single exon deletions/duplications. In addition to the probes targeting each *NR5A1* exon, coding and non-coding, we included a probe (SF1prom) located approximately 900 bp upstream of the transcription initiation site in order to detect deletions that affect the promoter region and consequently can impair *NR5A1* expression. The basal *NR5A1* transcription has been shown to be mainly regulated by the first 110 bp of the proximal promoter that contains several *cis*-regulatory elements, including a SOX9 binding site (Sox-BS1), an E box, a CCAAT box, and a Sp1 site [Scherrer et al., 2002].

The SF1 probe set was used not only to confirm the partial deletion but also as a diagnostic tool for XY DSD patients where a *NR5A1* defect can be suspected.

By designing more MLPA probes, we could determine that patient A carries a deletion affecting only exon 2 and 3, which are the first 2 coding exons of *NR5A1*, and almost the entire intron 3. This is the first description of a partial *NR5A1* gene deletion. Within the breakpoint regions there are no highly similar repeats, the only sequence homology identified is a 11-bp sequence shared by the LINE L2 in intron 3 and the SINE MIRb within intron1 (fig. 1C). Despite several attempts, we have not been able to amplify and sequence the deletion junction region. This may be due to the difficulty to perform a long PCR for a region containing a CpG island. We can, however, exclude a typical non-allelic homologous recombination

mechanism as the origin of this deletion and can speculate that the deletion has originated by a double strand breakage followed by a non-homologous end joining mechanism.

No functional *NR5A1* can be synthesized from this allele. We actually cannot predict if this allele will even be expressed or not. However, even if mRNA missing exon 2 and 3 is synthesized and initiation of translation occurs at the first in-frame ATG codon (Methionine 88 in exon 4), this would lead to the formation of an N-terminal truncated nuclear receptor missing the DNA binding site which is encoded by exon 2 and 3.

No further deletions have been identified in the other patients analyzed. For the Swedish patient group this can be due to the patients collected, as all patients presented with complete GD, with female external genitalia (without any sign of virilization) and a uterus. Although some *NR5A1* mutations have been identified in patients with complete XY GD, it seems that *NR5A1* mutations are more common in patients showing partial/mild XY GD [Lin et al., 2007]. In this respect, the Netherlands and Belgium DSD group of patients selected presented a broader phenotype.

The *NR5A1* mutation frequency has been reported as 13% in 46,XY patients with GD or impaired androgenization due to impaired Leydig cell function and androgen biosynthesis [Lin et al., 2007], 15% in patients with severe penoscrotal hypospadias and undescended testis [Kohler et al., 2009], and 33% in cases of 46,XY females presenting with primary amenorrhea and low testosterone levels [Philibert et al., 2010]. Interestingly, of the 15 patients analyzed by Philibert et al. [2010], 2 patients carried *SRY* gene mutations that are considered the most common cause of classical XY GD with a frequency of 10–15%, while 5 patients carried mutations in the *NR5A1* gene. Two of these 5 patients presented with complete female external genitalia but 3 with clitoromegaly, showing again a higher frequency of *NR5A1* mutations when including 46,XY GD patients with partial/mild forms. Thus, *NR5A1* mutations are quite frequent in 46,XY DSD. This makes the detection of all types of mutations even more important, as the establishment of a correct genetic diagnosis in XY DSD has several implications for patient management, including sex assignment, treatment choices, gonadal tumor risk evaluation, and accurate genetic counseling to the family.

In conclusion, we describe the first partial *NR5A1* gene deletion identified by MLPA in a patient with 46,XY gonadal DSD. This confirms the importance to use a method that can detect copy number changes, even at the

exon level, in genes involved in gonadal DSD. As *NR5A1* mutations can cause a wide spectrum of 46,XY DSD with relatively high frequency and even XX ovarian insufficiency, the analysis of the *NR5A1* gene by MLPA is quite important and should be extended to larger groups of patients.

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