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46,XY disorder of sex development and developmental delay associated with a novel 9q33.3 microdeletion encompassing *NR5A1*

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Abstract

Steroidogenic factor 1 (SF1) is a nuclear receptor encoded by the *NR5A1* gene. SF1 affects both sexual and adrenal development through the regulation of target gene expression. Genotypic male and female SF1 knockout mice have adrenal and gonadal agenesis with persistent Müllerian structures and early lethality. There have been several reports of *NR5A1* mutations in individuals with 46,XY complete gonadal dysgenesis (CGD) or other disorders of sex development (DSD) with or without an adrenal phenotype. To date microdeletions involving *NR5A1* have been reported in only two patients with DSDs. We report a novel microdeletion encompassing *NR5A1* in a patient with 46,XY DSD and developmental delay. The phenotypically female patient initially presented with mild developmental delay and dysmorphisms. Chromosome analysis revealed a 46,XY karyotype. A 1.54 Mb microdeletion of chromosome 9q33.3 including *NR5A1* was detected by array CGH and confirmed by FISH. Normal maternal FISH results indicated that this was most likely a *de novo* event. Since most *NR5A1* mutations have been ascertained through gonadal or adrenal abnormalities, the additional findings of developmental delay and minor facial dysmorphisms are possibly related to haploinsufficiency of other genes within the 1.54 Mb deleted region. This report further confirms the role of *NR5A1* deletions in 46,XY DSD and reinforces the utility of aCGH in the work up of DSDs of unclear etiology.

Keywords

NR5A1; SF1; 46,XY disorder of sex development; 46,XY DSD; 9q33 microdeletion

1. Introduction

Steroidogenic factor-1 (SF1) is a nuclear receptor that affects both sexual development and adrenal development through the regulation of target gene expression [1]. Genotypic male

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and female SF1 knockout mice have gonadal and adrenal agenesis, persistent Müllerian structures, and a reduced lifespan, probably due to adrenocortical insufficiency [2, 3]. SF1 is encoded by the *NR5A1* gene, and the first three published human mutations in this gene were all missense mutations [4–6]. The first patient had a heterozygous mutation causing 46,XY complete gonadal dysgenesis (CGD) and adrenal failure [4]. The second was a heterozygous mutation in a 46,XX prepubertal girl presenting with adrenal failure only [6]. Both papers excluded a dominant negative mechanism of action for the reported mutation. Despite the second report, a screen of patients with idiopathic adrenal failure demonstrated that *NR5A1* mutations are unlikely to be common causes of adrenal failure without a disorder of sex development (DSD) [7]. The third patient presented similarly to the first but was the product of a consanguineous marriage and was homozygous for a presumably milder *NR5A1* missense mutation [5].

Since these initial discoveries, there have been other reports of various types of *NR5A1* mutations (including missense, nonsense, and frameshift) in individuals with different forms of 46,XY DSD without an adrenal phenotype [8–11]. For example, Lin et al. found missense mutations in four 46,XY individuals. Two had female genitalia, one with inguinal testes and remnant Müllerian structures and the other with labial testes and no Müllerian structures. A third patient had clitoral enlargement, labioscrotal folds, labioscrotal testes, and remnant Müllerian structures. The last patient had a small phallus, hypospadias, high scrotal testes, and absent Müllerian structures [8]. Recently, there was a report of a small (3.1 to 4.8 kb) deletion encompassing two exons of *NR5A1* in a patient with 46,XY DSD lacking an adrenal phenotype [12]. In addition, missense changes, in-frame deletions, frameshift, and nonsense mutations in *NR5A1* were found in 46,XX females with isolated ovarian insufficiency [11, 13]. The DSD phenotypes can be quite variable even within the same family demonstrating that a single *NR5A1* allele can have varied expressivity [11, 14]. Furthermore, heterozygous missense mutations were discovered in males with moderate to severe spermatogenic failure [15]. The missense mutations associated with male infertility were in or near the HINGE domain of the SF1 protein. Only two microdeletions detected by array comparative genomic hybridization (aCGH) involving *NR5A1* have been reported. The first is a 3 Mb deletion encompassing several genes including *NR5A1* and *LIMX1B* (the gene responsible for nail-patella syndrome) in a 46,XY female described to have genitopatellar syndrome and developmental delay. She was reported to have clitoromegaly, inguinal ovotestes with attached Müllerian and Wolffian duct remnants, and dysmorphic facies [16]. The second is an approximately 970 kb microdeletion including the *NR5A1* gene in a patient with isolated 46,XY DSD including clitoromegaly, no fusion of the labia majora, a shallow vaginal entrance, and gonads palpable in the labium [17].

Here we present a novel 1.54 Mb microdeletion including *NR5A1* in a 6 ½ year-old patient with 46,XY DSD, mild developmental delay, minor dysmorphisms, and normal adrenal function. This aberration was identified by aCGH and confirmed by fluorescence *in situ* hybridization (FISH). Maternal FISH analysis indicated that the microdeletion most likely occurred *de novo*. This report expands upon the range of mutations associated with *NR5A1*, and illustrates the value of aCGH studies in patients presenting with unusual or syndromic disorders of sexual development.

2. Clinical report

2.1 Patient

A 6 ½ year-old 46,XY female with mild developmental delay and minor dysmorphisms presented for reevaluation due to a new twin pregnancy in her mother. The patient's antenatal course was uncomplicated and she met her early developmental milestones on time. At 2 ½ years she lost communication skills and previously acquired language.

Subsequent developmental evaluation resulted in the diagnosis of Pervasive Developmental Disorder, Not Otherwise Specified (PDD-NOS). Karyotype at age 3, done for developmental delays, was reported to be 46,XY. On further evaluation, pelvic sonogram did not identify a uterus or gonads. A second study a year later also did not identify uterus, ovaries or gonadal tissue in the pelvis or inguinal regions. Gonadal testosterone showed poor response to hCG stimulation but normal T to DHT ratio. AntiMüllerian hormone, FSH, LH, and inhibin B were in the prepubertal range. Adrenal hormone production after ACTH stimulation was normal. Sequencing of the *AR, SRD5A2, SRY, NR5A1* and *WT1* genes did not detect any pathogenic mutations. Detailed discussion with the mother included recommendations for explorative surgery and removal of gonadal tissue if found. The mother decided to defer the surgery until the child was older. Both parents were healthy, non-consanguineous, and there were no fertility problems or developmental delays in the family. At age 6 ½ years, the patient was evaluated again. She was receiving physical, speech and occupational therapies and had made developmental progress. Patient's facial features included prominent forehead, long palpebral fissures, slightly cupped ears, malar flattening, full mouth, and 3 to 4 small café-au-lait macules over the trunk. She was phenotypically female with normal height (−0.064 SDS for females) and normal prepubertal female external genitalia at Tanner stage I. The mother's present twin pregnancy was spontaneously conceived, with normal 46,XY karyotype on both twins. At this visit, aCGH testing was ordered with informed consent as part of the clinical evaluation of the patient.

2.2 Array CGH

Patient aCGH was performed on a previously described custom microarray according to the manufacturer's instructions (Agilent Human CGH custom 4 × 44k; Agilent Technologies, Santa Clara, CA, USA) [18]. This array has enriched subtelomere coverage with an average resolution of 5 kb in the subtelomeres and a 125 kb resolution throughout the remaining genome. The data were analyzed with DNA Analytics 5.0.14 software (Agilent Technologies). Aberrations were identified using the DNA Analytics software via the Aberration Detection Method-1 algorithm with a sensitivity threshold of 6.0 and a data filter that rejected aberrations that did not include at least 4 probes with a $\log_2$ ratio ± 0.25. All quality control metrics passed.

2.3 FISH

FISH using the 9q subtelomere probe (9q TelVysion, Spectrum Orange, Abbott Molecular, Des Plaines, IL, USA) and the BAC RP11-412C6 probe (Spectrum Green, Empire Genomics, Buffalo, NY, USA) was performed according to manufacturer's instructions. Twenty-five interphase nuclei and ten metaphases were scored for the presence or absence of two signals per probe and a normal control was examined in addition to the patient, prenatal, and maternal samples. All FISH analyses were performed using an ImagePoint cooled CCD video camera (Photometrics, Tucson, AZ, USA) with a Nikon, Labophot-2A fluorescence microscope (Nikon, Melville, NY, USA). Digital image analyses were performed using a CytoVision Probe system and FISH software (Applied Imaging), and all preparations were counterstained with DAPI (Vector Labs, Burlingame, CA, USA).

2.4 Genomic rearrangement

Array CGH revealed a novel 1.54 Mb interstitial 9q33.3 microdeletion (Figure 1). The deleted region encompassed 10 genes including *NR5A1*. The minimally deleted region was from 125,944,826 to 127,483,435 (1.54 Mb) and the maximally deleted region was from 125,887,272 to 127,542,311 (1.66 Mb) (hg19). This deletion partially overlaps with two previously reported deletions that also include *NR5A1* [16, 17] (Figure 1). While the Database of Genomic Variants (DGV) database contains CNVs in this region detected in

healthy controls, none are as large as the pathogenic 9q33.3 deletions [19]. One study recorded in the DGV reports *NR5A1* deletions in four healthy children encompassing six interrogated single nucleotide polymorphisms (SNPs) on a SNP-based microarray (44.7 kb) [20]. However, the gender and age of these individuals are not available and without detailed clinical information, the significance is unclear.

FISH with the BAC probe RP11-412C6 confirmed the presence of the deletion in the patient and its absence in the mother as well as the cultured chorionic villi from the mother's ongoing twin pregnancy (both fetuses 46,XY) (Figure 2 and data not shown). The father was unavailable for testing. The RP11-412C6 probe hybridized to a 151 kb region containing *GPR144, NR5A1,* and *NR6A1*. The 9q subtelomere probe was used as an internal control. Only a single RP11-412C6 signal was observed in the nuclei and on metaphases of the affected individual.

3. Discussion

We report the detection of a novel 9q33.3 microdeletion in a patient with 46,XY DSD, minor dysmorphisms, mild developmental delay, and normal adrenal function. The microdeletion includes *NR5A1* (the gene encoding SF1). Mutations in this gene cause 46,XY CGD with or without adrenal failure, other 46,XY DSDs, 46,XX ovarian insufficiency, 46,XY infertility, and isolated adrenal failure in one case.

FISH analysis indicated that this aberration was not inherited from the mother. The father was unavailable for testing. It is unlikely that the father carries this microdeletion because missense mutations in specific domains of the SF1 protein have been associated with male infertility and to our knowledge, 46,XY males with dominant-acting *NR5A1* mutations are rarely fertile. Those reported belong to families segregating missense mutations causing more mild DSD phenotypes than observed in our patient [14, 21]. In one of these reports, a *NR5A1* mutation was transmitted by an unaffected father who seemed to be mosaic for the mutation [21]. It is noteworthy that the mother was carrying twins with an XY karyotype, and knowledge that the precise abnormality for the XY DSD was a *de novo* deletion provided much needed reassurance for the family.

The gonadal phenotypic spectrum of mutations associated with *NR5A1* mutations ranges from isolated male infertility or female ovarian insufficiency to 46,XY complete gonadal dysgenesis. Including this present report, the three microdeletions encompassing *NR5A1* all have relatively severe gonadal phenotypes, but none have adrenal insufficiency [16, 17]. In contrast, reported mutations within the same gene resulted in a range of findings on the gonadal phenotypic spectrum and a few have had adrenal insufficiency [2, 4, 5, 8, 9, 13, 15]. Of note, the sequencing of the *NR5A1* gene done during the patient's initial work-up, was normal. This illustrates the fact that other technologies, such as aCGH or Multiplex Ligation-dependent Probe Amplification, are needed to detect deletions or duplications of genes.

This 1.54 Mb microdeletion and the 3 Mb microdeletion reported by Schlaubitz et al. had additional phenotypic abnormalities that are likely to be due to the deletion of genes neighboring *NR5A1*, such as *LMX1B* [16] which appeared to be responsible for the patellar abnormalities in Schlaubitz et al's patient. Our patient has developmental delay and minor facial dysmorphism in addition to DSD. Since *NR5A1* mutations have not been reported to be associated with developmental delay or facial dysmorphism, one or more of the other ten genes in the 1.54 Mb deleted region is likely to be responsible. While none of these other genes have been well-characterized in humans, studies in model organisms have implicated two of them in brain development: *STRBP* and *LHX2* [22, 23].

In a study of 116 patients with DSDs of unknown etiology, Tannour-Louet et al. identified clinically relevant submicroscopic deletions and duplications in 21.5% of patients presenting with gonadal dysgenesis, ambiguous genitalia, or genitourinary defects. Recurrently occurring imbalances were found in 5p15.3, 9p24.3, 22q12.1 and Xq28 leading to identification of novel candidate genes for DSDs [24]. These and other genes, such as *NR5A1*, are likely to influence the complex sex determination and differentiation pathways by dosage sensitive effects or other mechanisms. Array CGH will therefore significantly increase the yield of diagnosis in this group of disorders. Knowledge of the precise defect will help in providing prognosis and genetic counseling.

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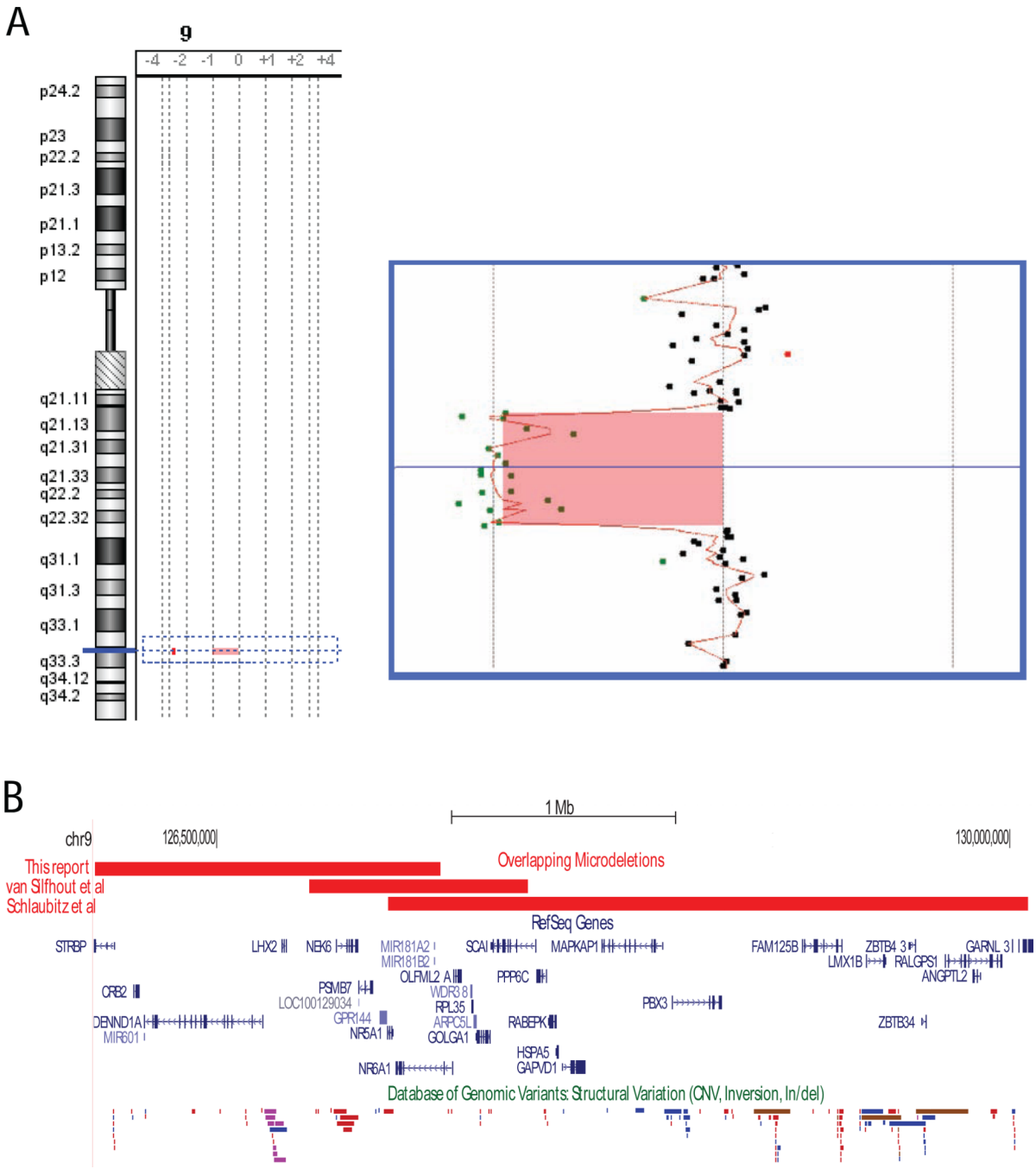

Figure 1.

Array CGH detection of a novel 9q33.3 microdeletion in the patient. (A) On the left is an ideogram of chromosome 9. The area outlined by the dotted box is enlarged on the right. The Y-axis is genomic position and the X-axis is the $\log_2$ ratio of the signal of the patient relative to the control. Black filled circles represent probes with a $\log_2$ ratio between −0.25 and +0.25. Red filled circles are probes with a $\log_2$ ratio greater than 0.25 and green filled circles are probes with a $\log_2$ ratio less than −0.25. The red line is the moving average and the shaded region is the area called as an aberration by the Agilent software. (B) The pertinent region is depicted on the hg19 assembly as adapted from the UCSC genome browser (http://genome.ucsc.edu/) [25]. The 9q33.3 deletion detected in this report is

compared to the other deletions reported in the literature. The color coding of the Database of Genomic Variants (DGV) is as follows: blue indicates gains, red indicates losses, brown indicates both gains and losses have been detected, and purple represents inversions [19].

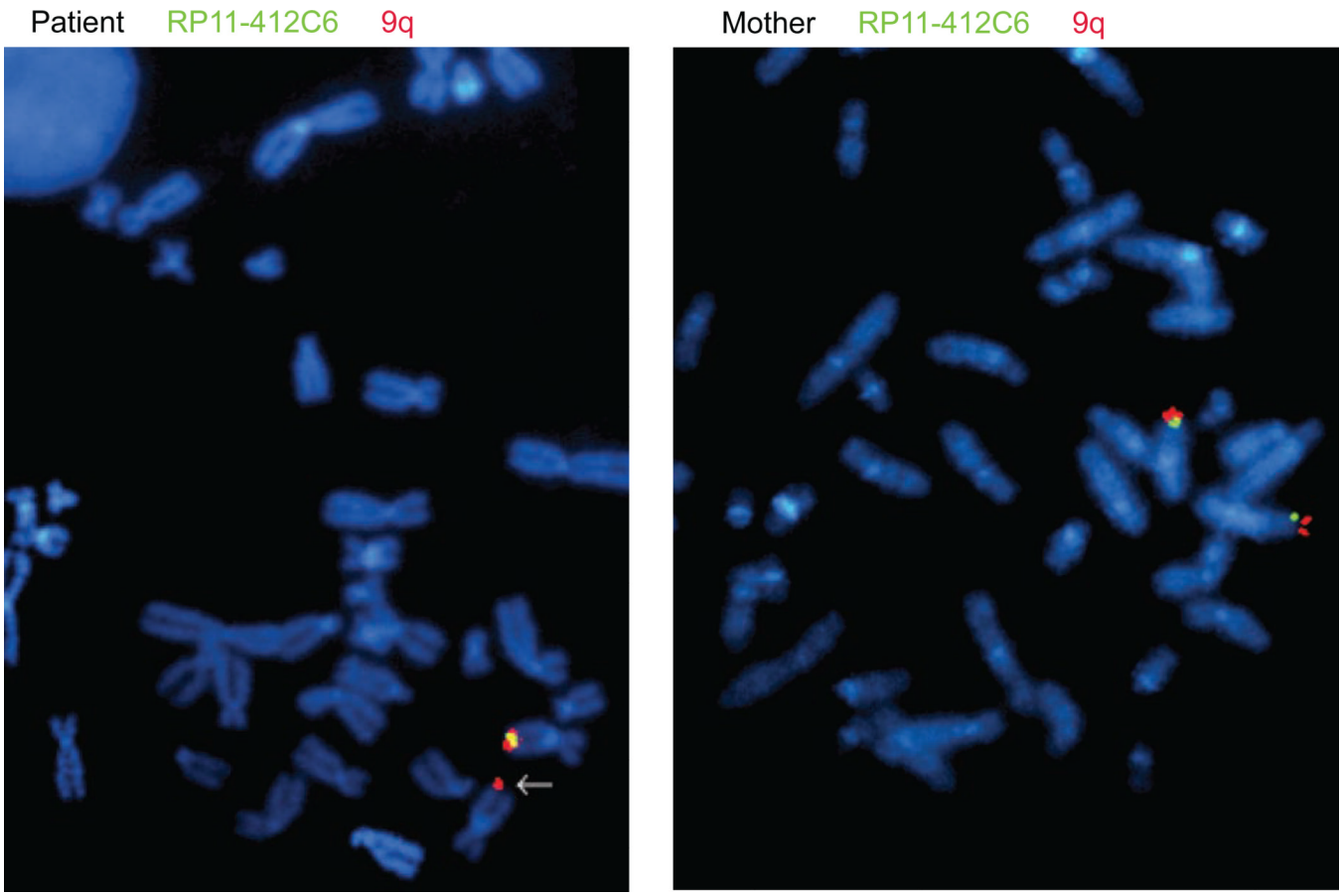

Figure 2.
FISH confirms the 9q33.3 microdeletion in the patient but not in the mother. Representative metaphase spreads of the patient (left) and mother (right) are depicted. The arrow head points to the chromosome 9 with a deleted RP11-412C6 signal.