Limited contribution of *NR5A1* (SF-1) mutations in women with primary ovarian insufficiency (POI)

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Objective: To evaluate the significance of *NR5A1* mutations in a large, well-phenotyped cohort of women with primary ovarian insufficiency (POI). Mutations in the *NR5A1* gene (SF-1) were previously described in disorders of sexual development and adrenal insufficiency. Recently, a high frequency of *NR5A1* gene mutations was reported in a small group of women with POI.
Design: Cross-sectional cohort study.
Setting: University hospital.
Patient(s): Well-phenotyped women (n = 386) with secondary amenorrhea and diagnosed with POI, including women with familial POI (n = 77).
Intervention(s): None.
Main Outcome Measure(s): The entire coding region and splice sites of the *NR5A1* gene were PCR-amplified and sequenced. The pathogenicity of identified mutations was predicted in silico by assessing Align-GVGD class and Grantham score.
Result(s): Sequencing was successful in 356 patients with POI. In total, 9 mutations were identified in 10 patients. Five of these mutations concerned novel nonconservative mutations occurring in 5 patients. Prediction of effect on protein function showed low to intermediate pathogenicity for all nonconservative mutations. The overall *NR5A1* gene mutation rate was 1.4%.
Conclusion(s): The current study demonstrates that mutations in the *NR5A1* gene are rare in women with POI. Primary ovarian insufficiency remains unexplained in the great majority of patients; therefore, continued efforts are needed to elucidate its underlying genetic factors. (Fertil Steril® 2012;97: 141–6. ©2012 by American Society for Reproductive Medicine.)
Key Words: Primary ovarian insufficiency, POI, steroidogenic factor 1, SF-1, *NR5A1*

Menopause occurs at a mean age of 51 years (1). However, 1% of women experience menopause before the age of 40 years (2). Primary ovarian insufficiency (POI), also known as premature menopause or premature ovarian failure (3), is characterized by amenorrhea for at least 4 months, occurring before the age of 40 years, along with repeated elevated FSH to a menopausal level and decreased E_2 concentrations (2). Primary ovarian insufficiency gives rise to infertility and increased risk for osteoporosis (4) and has been associated with a higher incidence of cardiovascular disease (5–7), depression (8), and possibly neurologic disease and impaired cognition (9). A positive family history for POI has been reported in 12%–50% of patients (10–12).

Multiple factors may underlie the clinical entity of primary ovarian insufficiency. Primary ovarian insufficiency should therefore be considered a multifactorial heterogeneous condition. Gonadotoxic treatments using chemotherapeutic agents and irradiation, and extensive abdominal surgery and oophorectomy may cause iatrogenic POI. Furthermore, steroidogenic cell autoimmunity has been associated with autoimmune oophoritis in POI (13, 14). A fair proportion of POI is caused by numerical or structural chromosomal abnormalities, including (full or mosaic) monosomy X (15). In addition, carrier status of the fragile X premutation (FMR1) has a prevalence of 3%–15% in women with POI (16). A recent study described mutations in the X-linked bone morphogenic protein 15 (BMP15) gene in 2% of patients with POI (17).

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However, the search for other monogenetic causes of POI proved to be challenging. Besides syndromic forms of POI, caused by genes such as *FOXL2* or *GALT* (18–20), only rare gene mutations have been identified for nonsyndromic forms of POI. These include genes involved in folliculogenesis and follicle function: *GDF9*, *NOBOX*, FSH-receptor gene (*FSHR*) and LH-receptor gene (*LHR*) (21, 22). Recently, high-throughput methods, using genomic variants such as single-nucleotide polymorphisms (SNPs) and copy number variants (CNVs), were applied to identify genetic risk loci in the complex genetic disease POI. A preliminary genomewide association study performed by our group showed a possible role for the *ADAMTS19* gene (23). We also showed that CNVs on the X chromosome do not play a central role in the pathogenesis of POI (24). Up until now, no cause can be established in the majority of women with POI (12, 25).

A recent report showed that in a sample of 40 women with sporadic POI, as much as 8% carried mutations in the nuclear receptor subfamily 5, group A, member 1 (*NR5A1*) gene (26). Moreover, the same study identified mutations in the *NR5A1* gene within four families with histories of both 46,XY sexual development disorders and 46,XX POI. It should be noted that this study was different compared with the studies mentioned before, in which an association was studied between DNA markers such as SNPs and CNVs and the phenotype. In the latter study (26), the whole gene sequence was determined, thus identifying all sequence mutations and variants. The *NR5A1* gene is located on chromosome 9q33 and encodes the steroidogenic factor 1 (SF-1) protein, which regulates the transcription of genes associated with steroidogenesis, sexual development, and reproduction. Initially, *NR5A1* mutations were identified in 46,XY individuals with primary adrenal insufficiency and complete gonadal dysgenesis (27, 28). Later, *NR5A1* mutations were associated with primary adrenal failure in a 46,XX woman with intact ovaries (29). Moreover, *NR5A1* mutations are relatively frequently present in patients with 46,XY disorders of sexual development (DSD) without adrenal insufficiency, such as severe hypospadias, or male infertility (26, 30–33). At the moment, more than 30 different mutations are known.

The recently reported high mutation frequency of SF-1 mutations in a small group of women with POI in the previously published paper (26), prompted us to evaluate these results and to further establish the contribution of *NR5A1* mutations to POI. Therefore, we sequenced the complete coding regions of *NR5A1* in a large, well-phenotyped cohort of 356 women with POI, and we sought to identify and describe any new *NR5A1* mutations associated with POI.

MATERIALS AND METHODS

Patients

In 2005, a nationwide POI consortium for the screening and follow-up of patients with POI was initiated by the University Medical Center Utrecht, the Netherlands. Participating hospitals included all eight university hospitals in the Netherlands and six large regional hospitals. Women suspected to suffer from hypergonadotropic oligo- or amenorrhea were systematically evaluated in the outpatient clinic of each individual hospital, as has been described in previous studies from our group (7, 12). For the current study, all women visiting the outpatient clinic for POI were identified (n = 378). Primary ovarian insufficiency was defined as spontaneous cessation of menses for at least 4 months in women younger than 40 years of age, along with repeated FSH concentrations exceeding 40 IU/L (2).

In short, data were gathered on reproductive and obstetric history, medical history with special attention for sexual development abnormalities and adrenal dysfunction, and family history for POI, sexual development disorders, and adrenal hypoplasia. Family history covered three generations including paternal and maternal grandparents, paternal and maternal aunts and uncles, and the proband's parents and siblings. Familial POI was defined as when the index patient had at least one family member also affected by POI (12). Serum endocrine measurements included FSH and E_2. Follicle-stimulating hormone concentrations were measured using a chemoluminescence-based immunometric assay (Immulite 2000; Diagnostic Products Corp., Los Angeles, CA). Estradiol concentrations were measured using the Roche E170 Modular (Roche, Basel, Switzerland). Furthermore, androstenedione (AD), 17-hydroxyprogesterone (17-OH), DHEA, and DHEAS were measured. AD was measured after hexane-toluene extraction using an in-house RIA. Imprecision for the range 1–11 nmol/L was 7%–9%. 17-Hydroxyprogesterone was measured using after toluene extraction using an in-house RIA employing a polyclonal anti-17α hydroxy-progesterone antibody (Bioconnect/Biogenesis), and 17α hydroxy[1,2,6,7-^{3}H]-progesterone (Perkin Elmer) was used as a tracer after chromatographic purification. Imprecision was 10.5%, 8%, and 8.5% at 3.5, 10, and 38 nmol/L, respectively (n = 75). Dehydroepiandrosterone was measured after diethyl ether extraction and Celite chromatography using an in-house RIA. Imprecision for the range 3.5–30 nmol/L was 6%–12%. Dehydroepiandrosterone sulfate was measured using the Coat-A-Count DHEA-SO_4 RIA (Siemens Diagnostics, Breda, the Netherlands). Imprecision for the range 1.5–13 μmol/L was <7%. Finally, karyotyping and screening for fragile X premutation was performed. Mosaicism was defined by the presence of at least 3 or more mosaic cells per 32 analyzed cells. The study protocol was approved by the institutional review boards of the participating hospitals, and all women gave informed consent.

An additional eight patients with the diagnosis code POI were selected from the diagnostic database of the medical genetics department of the University Medical Center Utrecht, the Netherlands. All DNA samples were stored with informed consent for the purpose of future diagnostics and research.

Mutation Analysis

For each patient, a blood sample was collected in a 10-mL EDTA tube. DNA was extracted from peripheral blood leukocytes using conventional techniques and frozen at −20°C until genotyping experiments were conducted.

The entire coding region (exon 2–7) and splice sites of the *NR5A1* (SF-1) gene were PCR-amplified with primers designed with primer3 software (http://www.broadinstitute.org/genome_software/other/primer3.html) (Supplemental