NR5A1/SF-1 and development and function of the ovary
NR5A1/SF-1, développement et fonction ovarienne

A. Bashamboo *, K. McElreavey

Human Developmental Genetics, Institut Pasteur, Paris, France

Available online 14 April 2010
Presented by J. Young

Résumé
L’insuffisance ovarienne prématu rée (IOP) est définie par l’arrêt des menstruations associé à une ascension des gonadotrophines résultant d’une diminution de la fonction ovarienne avant l’âge de 40 ans. L’incidence de l’IOP est de 1 % chez les femmes avant 40 ans et de 0,1 % avant 30 ans. Il apparaît qu’une forte composante génétique est associée aux IOP. Cependant, les variations et mutations des gènes influençant l’IOP demeurent encore mal caractérisées. NR5A1, membre de la super famille des récepteurs nucléaires est un régulateur transcriptionnel majeur des gènes impliqués dans l’axe hypothalamo-hypophyso-gonado-surrénalien. Les souriceaux nouveau-nés déficients en NR5A1 sont à la fois dépourvus de gonades et de glandes surrénales et ont une expression altérée des gonadotrophines. NR5A1 est aussi exprimé dans de multiples types cellulaires de l’ovaire fœtal, postnatal, prépubertaire et mature. Jusqu’en 2008, 18 mutations du gène NR5A1 ont été rapportées chez l’homme. Trois de celles-ci étaient identifiées chez des individus présentant une insuffisance surrénale, deux étant associées à un caryotype 46,XY et la troisième à un caryotype féminin 46,XX avec conservation de la fonction ovarienne. Les autres mutations étaient associées à des anomalies variées du développement testiculaire sans évidence d’insuffisance surrénalienne. Nous avons identifié 19 autres mutations de NR5A1 incluant des mutations dans quatre cas familiaux, comportant des individus de caryotype 46,XY aussi bien que des IOP. Une analyse supplémentaire de 25 cas sporadiques d’IOP a révélé deux autres mutations. L’analyse fonctionnelle a montré que chaque protéine mutante présentait une altération de la transactivation sur les promoteurs gonadiques. Ces études ouvrent de nouvelles perspectives sur le rôle de NR5A1 dans le développement et la fonction ovarienne et laissent penser que ses mutations pourraient correspondre à une cause non négligeable d’IOP.

Keywords: Insuffisance ovarienne prématu rée ; Caryotype ; NR5A1

Abstract
Primary ovarian insufficiency (POI) is defined as cessation of menstruation with associated elevation of gonadotropin levels as a result of decreased ovarian function before the age of 40. The incidence of POI is 1% in women prior to age 40, and 0.1% prior to age 30. There is evidence of a strong genetic component associated with POI. However, the gene mutations/variations influencing POI still remain uncharacterized. NR5A1, a member of the nuclear receptor superfamily, is a key transcriptional regulator of genes involved in the hypothalamic-pituitary-gonadal steroidogenic axis. Newborn mice deficient in NR5A1 lack both gonads and adrenal glands and have impaired expression of pituitary gonadotrophins. NR5A1 is also expressed in multiple cell types in the fetal, postnatal, prepubertal and mature ovary. Until 2008, 18 NR5A1 mutations were described in the human. Three of these were identified in individuals with adrenal insufficiency, two associated with 46,XY disorders of sex development (DSD) and the third a 46,XX female with conserved ovarian function. Other mutations were associated with various anomalies of testis development with no evidence of adrenal failure. We have identified further 19 mutations in NR5A1 including mutations in four familial cases having individuals with 46,XY DSD as well as POI. A further analysis of 25 sporadic cases of POI revealed two additional mutations. Functional analysis revealed that each mutant protein had altered transactivational properties on gonadal promoters. These data reveal novel insights into the role of NR5A1 in ovarian developmental and function and indicate that mutations of the NR5A1 gene may be a significant cause of human ovarian insufficiency.

Keywords: Primary ovarian insufficiency; DSD; NR5A1

* Corresponding author.
E-mail address: abashamb@pasteur.fr (A. Bashamboo).
Primary ovarian insufficiency (POI) is characterized by primary or secondary amenorrhea, hypergonadotrophic hypogonadism and estrogen deficiency in women younger than 40 years [1,2]. POI was previously referred to as premature menopause or premature ovarian failure and other terms used for this condition include primary ovarian failure, hypergonadotrophic hypogonadism and 46,XX gonadal dysgenesis [1,2]. Many aspects of female reproductive function are strongly influenced by genetic factors and consequently there have been repeated attempts to identify genetic mutations associated with disorders of female reproductive function. There is a wide variation in the age at which the normal menopause begins, varying from 40 years to just over 60 years. The variation of the age of onset of normal menopause has a very strong genetic component with several to many genes assumed to make an additive contribution to the variation [3,4]. A woman with one or more first-degree relatives with a history of early menopause is likely to experience early menopause herself suggesting that genes, perhaps the same genes, are also involved in POI. The incidence of familial idiopathic POI is reported to be between 4 and 31% suggesting that genetic factors play a significant role in some cases [5–8]. The wide range in occurrence may reflect genetic heterogeneity and the wide spectrum of pathologies that have included in these studies. The occurrence of POI varies considerably with ethnicity [9]. The observation that 1.4% of African-American women, 1.4% of Hispanic women, 1.0% of white women and 0.1% of Japanese women experience POI is strongly suggestive of predisposing genetic factors in some populations.

Several genetic causes of syndromic and non-syndromic forms of POI have been identified in recent years. Individuals presenting with Turner’s syndrome, or monosomy X are typically born with streak ovaries (ovarian dysgenesis) as oocytes never develop past the pachyten stage of meiosis I [10]. However, others with mosaicism, or partial X monosomy, are likely to present with POI [11,12]. Individuals with POI and partial deletions of the X chromosome have been investigated and several candidate regions/gene have been suggested. An expansion of the CGG trinucleotide repeats in the S’ region of the fragile X mental retardation 1 (FMR1) gene within the premutation range [13]. Premutation repeats range from 55 to 200 trinucleotides and increasing repeat length is correlated with decreasing age for ovarian failure [14]. Other regions on the X chromosome may also be associated with ovarian failure. These are the POF-1, POF-2 and POF-3 loci on the long arm of the X chromosome. Several candidate genes in these regions have been identified but the precise gene(s) involved has not been identified [15–17].

Mutations in genes that result in ovarian insufficiency are often associated with other somatic anomalies. These include autosomal recessive mutations in the APECED, EIF2B and GALT genes [18–20]. POI has also been observed to be associated with the blepharophimosis-ptosis-epicanthus inversus (BPES) syndrome caused by mutations in the FOXL2 gene [21,22]. Mutations in FOXL2 associated with non-syndromic forms of POI have not yet been reported. In non-syndromic forms of ovarian failure, rare recessive inactivating mutations of the follicle stimulating hormone (FSH) and luteinizing hormone (LH) receptors have been described [23–25]. Mutations in the ovary specific homeobox transcription factor NOBOX are also a rare cause of POI [26]. Factor in germline alpha (FIGLA), is a germ cell-specific basic helix-loop-helix (bHLH) transcription factor that regulates expression of zona pellucida genes as well as that of other oocyte-specific genes. In a screen of 100 individuals with POI of Chinese origin, a paternally inherited 22 bp deletion resulting a premature stop codon was identified in one case and a p.140 delN was identified in a second case [27]. These changes were not observed in a control population. Several other candidate genes have been analysed and although variants have been identified, these are either not exclusively associated with POI, for example FOXO3A and LHX8 [28,29]; or the functional consequences of these variants remain unclear as observed in the case of the genes BMP15, GDF9 and DAZL [30–34].

NR5A1 (also termed SF-1), a member of the nuclear receptor superfamily, is a key transcriptional regulator of genes involved in the hypothalamic-pituitary-steroidogenic axis [35,36]. The protein consists of a classical DNA-binding domain (DBD) characterized by two Cys2 zinc fingers in the N-terminal region. The first zinc finger of the DBD of NR5A1 contains a proximal (P) box conferring specificity to DNA-binding and interaction with the major groove of DNA. DNA-binding is stabilized by an A-box (FTZ box) motif that interacts with the minor groove of DNA by recognizing nucleotides flanking the core DNA-binding motif on its 5’ side [37–39]. This accessory DNA-binding region is important as NR5A1 is thought to bind to target genes monomerically rather than as a homo- or heterodimer. The protein also contains a nuclear localization signal, a ligand-binding domain (LBD) and two activation domains. The A-box is separated from the LBD by Hinge region (AA 95-225). An N-terminal AF1 region crucial for ligand-independent transactivation by other nuclear hormone receptors is needed for maximal NR5A1-mediated transcription and interaction with nuclear receptor cofactors [40]. This interaction requires phosphorylation of a serine residue, Ser203, located in the hinge region [40]. The hinge region is important for stabilizing the LBD and enhancing interaction with other proteins. Phosphorylation of Ser203 enhances the interaction of the cofactors TIF2 (GRPR/NCaA2) and SMRT (NCoR2/TRAC1) with the AF1 and AF2 regions of NR5A1, whereas sumoylation of lysines within the hinge region (Lys119 and Lys194) increase interactions with DEAD box proteins including DP103, and result in transcriptional repression [41].

NR5A1 is expressed in the nuclei of Sertoli’s and Leydig’s cells of testis and cells of the adrenal cortex [42]. In addition to the steroidogenic cells NR5A1 is also expressed in adult mice in the ventromedial hypothalamic nucleus and in the pituitary. During development, NR5A1 is first expressed in the diencephalons at E12.5, and in the forming hypothalamus between E14.5 and E17. In the developing pituitary NR5A1 is expressed at E13.5–E14.5, immediately prior to the expression of LHβ and FSHβ. In adults, expression is restricted to LH producing gonadotrope cells.

During the fetal development in human, NR5A1 is expressed from 32 dpo/CS14, initially in a pool of cells medial to the mesonephros and underlying the genital ridge. It is expressed in
the bipotential gonad and by 41 dpo/CS17, *NR5A1* expression is not sexually dimorphic in either gonads. In the developing ovary, *NR5A1* shows diffuse expression at 52 dpo/CS21 and a stronger localisation to the coelomic epithelium later in the fetal period. Expression of *NR5A1* persists in the developing human ovary [42]. This is in contrast to the mouse development where the expression of *NR5A1* decreases at the onset of sexual differentiation [43]. In cycling human ovaries *NR5A1* is expressed in the undeveloped follicles, during the preantral phase. *NR5A1* is also detected in theca interna as well as in luteinized and non-luteinized granulosa cells. It is also expressed in corpus luteum during the luteal phase as well as in both atretic follicles and degenerating corpora lutea [44].

Mice that lack *NR5A1* specifically in their pituitary, show marked hypogonadism with a 95% decrease in male and female gonad mass and an absence of sexual maturation, resulting in infertility. *NR5A1*/−/− mice that are maintained by adrenal transplantation, develop marked obesity after 8 weeks and this is correlated to a marked increase in adipose tissue. This phenotype is similar to obesity seen following lesions of the ventromedial hypothalamus obtained by surgery. These mice also have impaired expression of pituitary gonadotropins.

The most prominent phenotype seen in newborn mice deficient in *NR5A1* is the absence of both gonads and adrenal glands [35]. A mouse model with a granulosa cell-specific *NR5A1* knockout showed marked postnatal gonadal defects [45,46]. These included ovarian hypoplasia, altered uterine differentiation and defects in estrogen biosynthesis and fertility. Heterozygous mice have mild ovarian hypoplasia with normal ovarian function. Mice lacking *NR5A1* show reduced ovary size with decreased numbers of growing follicles and absence of the corpus luteum [46]. This suggests that they have impairment of the terminal stages of follicle differentiation and/or ovulation. Basal plasma FSH concentrations are elevated compared to control mice [46] and levels of AMH and aromatase expression were reduced in granulosa cells. These data suggested a key role for *NR5A1* in ovarian development and function. *NR5A1* regulates transcription of key genes involved in sexual differentiation and reproduction, including SOX9, stAR, hCYP17, P450SSC, CYP11A1, adrenocorticotrophic hormone receptor, LH b-subunit, CYP21, AntiMüllerian Hormone, aromatase and inhibin alpha either by direct interaction with the P promoter of these genes or by regulating more distant regulatory elements [47–51]. Thus, *NR5A1* has been suggested to be a ‘master-regulator’ of many aspects of adrenal, gonadal, and reproductive development and function, and relatively small changes in its activity could have clinically significant effects on one or all of these different endocrine systems.

Until 2008, 18 pathological mutations in *NR5A1* were described in human [36]. Three of these were identified in individuals with adrenal insufficiency, two associated with 46,XY disorders of sex development (DSD) and the third a 46,XX female with conserved ovarian function [36]. Other mutations were associated with various anomalies of testis development with no evidence of adrenal failure [36]. We have identified further 19 mutations in *NR5A1* including mutations in four unusual familial cases (unpublished and [52]). These families [52] had affected individuals with 46,XY DSD as well as individuals with POI. The mode of inheritance of the phenotype in the families is consistent with either autosomal recessive or autosomal dominant transmission [52]. Genetic analysis of these families revealed a variety of mutations in the *NR5A1* gene spanning a range of phenotypes (Fig. 1).

In an analysis of a further 25 apparently sporadic cases of POI, two additional mutations were identified [52]. One girl, of Roma origin, presented at 12.5 years for short stature. She suffered from generalized seizures at day 7. She grew at −2 standard deviations for height until 1.9 years and then at −3 standard deviations. Known causes of short stature were excluded. Her karyotype was 46,XX and she was diagnosed as having ovarian failure. The analysis of the *NR5A1* gene revealed a heterozygous in-frame 9 bp deletion that results in the loss of three amino acids

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**Fig. 1.** Summary of the phenotypes associated with mutations in *NR5A1* associated with familial cases of 46,XY disorders of sex development (DSD) and 46,XX premature ovarian failure.
(p.231Leu_Leu233del) in the N-terminal region of the LBD. In silico analysis predicted a change in hydrophobicity of helix 1 of the LBD, which may result in altered protein conformation or function. A second girl, of West African (Senegalese) origin, presented at 4 months of age with hypertrophy of the clitoris. The child, born to non-consanguineous parents, had elevated FSH levels indicating ovarian insufficiency. Analysis of the NR5A1 gene revealed two closely linked heterozygous mutations (c.369G > C [p.Gly123Ala] and c.387C > T [p.Pro129Leu]) in the hinge domain of the protein.

The mutations we observed in both sporadic and familial cases were not seen in analyses of more than 2000 control samples (unpublished data and [52]). Functional analysis of these mutations, using both embryonic kidney tsa201 cells and CHO cells, revealed quantitative reduction in the transactivation of the CYP11A1 (encoding P450scC) and CYP19A1 (encoding aromatase) promoter. The mutant protein containing the p.Asp293Asn mutation detected in family 2 partially activated the CYP11A1 and CYP19A1 promoters [52] and this is consistent with the recessive mode of inheritance of the phenotype in this family with both copies of the mutant gene necessary to express the gonadal phenotype. In the sporadic case with a double mutation, the p.Pro129Leu variant abolished the transactivation properties of the protein whereas the p.Gly123Ala variant had functional activities similar to the wildtype NR5A1 protein indicating that the p.Pro129Leu variant is pathogenic.

We observed a reduction in transactivation of aromatase by the mutant proteins in our study. In the ovary, aromatase is essential for estrogen biosynthesis by granulosa cells and the dysregulation of its expression can effect the ovarian function. It is also possible that these mutations may affect the expression of several other genes that are known to be crucial for ovarian development and function, such as AMH, inhibit alpha. AMH is involved in the regulation of follicular growth and development, and inhibit alpha is important in the control of ovarian homeostatis.

The double mutation (c.369G > C [p.Gly123Ala] and c.387C > T [p.Pro129Leu]) detected in the apparent sporadic case of West African origin was also observed in a case of 46,XY DSD from Brazil and in three other 46,XY individuals with gonadal anomalies from West and North Africa (unpublished data). This is suggestive of population-specific mutations indicating founder effects. This is further corroborated by the identification of a p.Pro131Leu mutation in a woman, of Tamil origin, with POI (unpublished data). The p.Pro131Leu protein lacks the ability to transactivate the MIS and Cyp11a1 promoters. The same mutation was observed in 46,XY individuals of Tamil origin with testicular anomalies, again suggesting that a single mutation from a common ancestor gives rise to a population-specific mutation (unpublished data).

The majority of 46,XX POI cases that carry pathological NR5A1 mutations have no apparent somatic anomalies. However, one of the sporadic cases described in our initial study [52] had short stature. The Tamil case carrying the p.Pro131Leu mutation also presented with somatic anomalies that include short stature and some of the stigmata associated with Turner’s syndrome. The somatic anomalies in these two cases may be caused by alterations in the biological activity of the mutant protein that affects downstream targets other than the ones that we have analysed. This merits further investigations. The variability in the expressivity of the phenotype seen in the cases, including that in familial cases may be caused by the presence of genetic modifiers. NR5A1 has been demonstrated to physically interact with numerous proteins including WT1, GATA4, SRY, SOX9, FOXL2, DAX1 to regulate gene expression through gonadal promoters [53–58]. Variant residues of any of these proteins may influence NR5A1 activity, perhaps through direct physical interaction. Similarly, nucleotide variation at target gonadal promoters may also account for range of expressivity and different phenotypes associated with NR5A1 mutations. Activity of mutant NR5A1 may also be compensated for by the presence of NR5A2. NR5A2 (also termed FTF/LRH-1) is an orphan nuclear hormone receptor that is closely related to NR5A1. The DBD of each protein share 88% amino acid identity and both have a conserved serine residue that when phosphorylated facilitates the recruitment of cofactors as well as stabilizes the LBD. NR5A2 can activate transcription of the human aromatase gene from promoter II via the NR5A1 response element [59]. NR5A2 also activates the promoters of a number of steroidogenic enzymes whose expression is also regulated by NR5A1 including CYP11A1, CYP17A1, and steroidogenic acute regulatory protein [60]. Thus it could be hypothesized that incomplete penetrance and variability in the expressivity of the phenotypes could be caused by functional redundancy between NR5A1 and NR5A2. Alternatively the phenotypic variability observed in women carrying mutations in NR5A1 could be due to other genetic modifiers in other genes that influence ovarian development and function. These may include, but are not limited to, variants in the NOBOX, FIGLA, BMP15, GDF9 and DAZL genes that have been identified [18–34,61].

The genetic analysis of patients presenting with POI reveal novel insights into the role of NR5A1 in ovarian development and function. Our studies indicate that mutations of the NR5A1 gene may be a significant cause of human ovarian insufficiency. However, there are several questions that remain outstanding:

- Is there a progressive loss of ovarian function in carriers of NR5A1 mutations?
- What is the contribution of mutations in NR5A1 to the incidence of both sporadic and familial cases of ovarian insufficiency?

The answer to these questions will require the analysis of more cases of POI and should include longitudinal studies to determine the degree of progressive loss of ovarian function in carriers of NR5A1 mutations. Our current data suggest that the incidence of NR5A1 mutations in individuals with otherwise unexplained POI is around 5% (unpublished data) and that these mutations are mainly associated with non-syndromic forms of the pathology. However, we would predict that because of mutations associated with founder effects, the incidence of mutations associated with POI may vary between populations.
Conflict of interest statement

Nothing declared.

Acknowledgements

A.B. and K.McE. are supported by the European Community’s Seventh Framework Programme (FP7/2007–2013) under grant agreement number 201444 (www.eurodsd.eu). K.McE. is supported by the ANR-GIS institut des maladies rares. This study is supported in part by Research Grant No. 1-FY07-490 from the March of Dimes Foundation (K.McE.).

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