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Review

Human sex-determination and disorders of sex-development (DSD)

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ABSTRACT

Several new genes and pathways have been identified in recent years associated with human errors of sex-determination or DSD. *SOX* family gene mutations, as well as mutations involving *GATA4*, *FOG2* and genes involved in MAP kinase signaling have been associated with virilization in 46,XX individuals or with 46,XY gonadal dysgenesis. Furthermore, mutations involving another key gene in sex-determination, *NR5A1*, are now known to be an important cause spermatogenic failure in the male and ovarian insufficiency in the female. These new findings offer insights into human sex-determination and highlight important differences between the human and mouse model.

This review will critically examine the evidence linking gene mutations, especially *MAP3K1*, to non-syndromic forms of human 46,XY gonadal dysgenesis or XX testicular/ovotesticular.

Contents

1. Introduction

DSD, defined as 'congenital conditions in which the development of chromosomal, gonadal, or anatomical sex is atypical' encompasses a wide spectrum of phenotypes [1]. This definition includes errors of primary sex-determination; 46,XY complete or partial gonadal dysgenesis (CGD, PGD; complete or partial absence of testis-determination) or 46,XX testicular DSD which refers to a male with testis and a normal male habitus and 46,XX ovotesticular DSD refers to individuals that have both ovarian and testicular tissue in the gonads. Our understanding of the genes involved in sex-determination and the mechanisms involved has improved dramatically over the past 10 years, however in cases of DSD a molecular diagnosis is still only made in only around 20% of DSD (excluding those cases where the biochemical profile indicates a specific steroidogenic block) [1]. Current data indicate that causal gene mutations can be found in around 50% of the patients who have errors of primary sex-determination. This review will focus on the gene mutations that result in human pathologies of primary sex-determination.

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2. 46,XY gonadal dysgenesis

2.1. SRY and SOX9

Approximately 15% of all cases of 46,XY CGD carry mutations in the Y-linked testis-determining gene *SRY* with the majority of these mutations localized within the HMG-domain [2]. A few rare cases of gonadal dysgenesis with small interstitial deletions 5′ and 3′ to the *SRY* open-reading frame have also been described [3,4]. In most cases the *SRY* mutations are *de novo* but some are inherited from an apparently normal and fertile father. Functional studies suggest that these inherited *SRY* mutations are hypomorphs that show partial biological activity compared to the baseline properties of wild-type protein [5]. Thus, in these familial forms the incomplete penetrance could be caused by stochastic effects around a threshold level of biological activity required for testis formation. In one exceptional case a *de novo* p.Gln2Ter mutation was reported in a woman with premature menopause [6]. The patient reported menarche and normal breast development at age of 13–14 years and had regular monthly menses until age 17, when she began oral contraceptives until she was 25 years. At that time she developed irregular menses that continued for 2 subsequent years as she attempted to get pregnant. This suggests that when the human gonad cannot form a testis it tries to develop as an ovary. Usually, in XY gonadal dysgenesis, the ovarian tissue degenerates during early development or post-natally to form a streak of fibrous tissue, but in some individuals the ovarian tissue persists until puberty or beyond.

Campomelic dysplasia (CD), characterized by skeletal defects and typical facial appearance, is associated testicular dysgenesis in about 75% of affected XY individuals [7]. *Sox9* plays both an essential role in the specification and differentiation of mesenchymal cells toward the chondrogenic lineage through transcriptional modulation of *Col2a1*, the major matrix protein of the mature cartilage as well as establishing Sertoli cell identity in the developing testis immediately following the expression of SRY. Many mutations have been reported in *SOX9* associated with CD and more recent studies have focused on potential regulatory elements that may also cause DSD. The developmental timing and tissue-specific transcriptional regulation of *SOX9* is highly complex and involves multiple elements located in flanking regions of at least ∼1 Mb upstream and 1.6 Mb downstream. In the upstream region, translocations and inversion breakpoints associated with CD fall within two clusters located ∼400 kb apart [8]. Patients with these rearrangements generally have a milder phenotype than the intragenic mutations [8,9]. Large (>1 Mb) duplications 5′ to *SOX9* that may lead to *SOX9* misexpression are associated with brachydactyly-anonychia (symmetric brachydactyly of the hands/feet, hyponychia or anonychia) [10]. Pierre Robin sequence, a craniofacial disorder characterized by micrognathia, cleft palate and macroglossia with normal testis development in 46,XY cases is associated with a 75 kb deletion located 1.38 Mb upstream and a deletion located 1.56 Mb downstream of *SOX9* [11].

A testis-specific enhancer *Sox9* has been mapped in mice to a 1.4 kb core region termed *Tesco* that is located 13 kb upstream from *Sox9* [12]. Both Sry and Nr5a1 (see Section 2.2) bind to the *Tesco* enhancer sequence *in vivo*, possibly through a direct physical interaction to up-regulate *Sox9* gene expression. Once Sox9 protein levels reach a critical threshold, several positive regulatory loops are initiated for its maintenance, including auto-regulation of its own expression and formation of feed-forward loops *via* Ffg9 or Pgd2 signaling [12]. Other cofactors are likely to be involved in this process but have not yet been identified. The homologous human SRY-responsive enhancer can also be activated by human SRY and SOX9 together with NR5A1 suggesting that there may be a conserved mechanism for male-specific up-regulation and maintenance of SOX9 expression in gonadal pre-Sertoli cells in human and mouse. To date, mutations involving the *TESCO* element have not been reported in association with human DSD.

Rearrangements grouped around a 600 kb locus (termed *RevSex*) upstream of the human *SOX9* gene are associated with both XY and XX DSD. Five cases of 46,XX testicular or ovotesticular DSD that carried duplications of this region and a familial case of 46,XY DSD that carried a deletion have been reported [13–15]. We identified three phenotypically normal patients presenting with azoospermia and 46,XX testicular DSD [16]. This included two brothers, who carried a 83.8 kb duplication that refined the minimal region associated with 46,XX-*SRY* negative DSD to a 40.7–41.9 kb element, which contains two predicted enhancer motifs. A proximal strong enhancer motif, which is enriched for H3K4 methylation and H3K27 acetylation, both of which are epigenetic marks that are characteristic of gene activation. The histone acetyltransferase EP300, which regulates transcription *via* chromatin remodeling binds to this element. In mice, *Ep300* is strongly expressed in the somatic cell lineages of both the XX and XY gonad during sex-determination and it can act as a co-activator of both Nr5a1 and Sox9 [17]. This enhancer motif is located between two predicted binding sites for DMRT1-binding (see Section 2.7). There is also data suggesting that deletions of an immediately adjacent and non-overlapping region are associated with 46,XY gonadal dysgenesis [18]. In our experience about 10% of cases of testicular/ovotesticular DSD and 46,XY gonadal dysgenesis have rearrangements involving the *RevSex* locus.

2.2. NR5A1

A major cause of human DSD is mutations involving the *NR5A1* gene. NR5A1 belongs to the subfamily of transcription factors known as nuclear receptor subfamily 5 (group A, member 1; NR5A1), which is highly conserved in vertebrates [19]. Like other nuclear receptors, the NR5A1 protein consists of a DNA-binding motif composed of two zinc-chelating modules that coordinate the interaction between the receptor and hormone response element [20]. NR5A1 binds DNA as a monomer, with DNA-binding stabilized *via* a 30 amino acid extension to the DNA-binding domain (Ftz-F1 or A box). The C-terminal ligand-binding domain (LBD) encompasses an AF-2 domain that cooperates with a proximal activation domain (AF-1) and is required for maximal biological activity with co-activators such as NCOA1 (SRC-1) [20]. Posttranslational modification plays an important role in modulating NR5A1 activation and repressor functions. Phosphorylation of Ser203 within the LBD enhances the interaction of the cofactors Tif2 (Grip1/Ncoa2) and Smrt (Ncor2/Trac1) with the AF-1 and AF-2 motifs. Strong transcriptional repression requires sumoylation of lysines Lys119 and Lys194, which increases interactions with DEAD box proteins including Ddx20 [21].

XY mice lacking *Nr5a1* have gonadal dysgenesis resulting in male-to-female sex reversal significantly diminished corticosterone levels, elevated adrenocorticotrophic hormone levels due to adrenal insufficiency and underdevelopment of the spleen and impaired clearance of abnormal red blood cells [22]. *Nr5a1*$^{-/-}$ mice do not express luteinizing hormone (LH) or follicle stimulating hormone (FSH) from the gonadotroph cells in the pituitaries, whilst the VMH is disorganized [23]. Mice lacking *Nr5a1* specifically in the VMH show high fat diet-induced obesity and increased anxiety-like behavioral patterns [24]. In the human, mutations involving *NR5A1* are associated with a wide range of reproductive anomalies including adrenal insufficiency and 46,XY gonadal dysgenesis, ambiguous genitalia, hypospadias, micropenis, spermatogenic failure with normal genitalia and primary ovarian insufficiency [25].

In a study of 315 men with spermatogenic failure, we identified heterozygous missense mutations in *NR5A1* in seven men with either azoospermia or severe oligozoospermia [26]. Testis