The molecular pathways underlying early gonadal development.

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Abstract

The body of knowledge surrounding reproductive development spans the fields of genetics, anatomy, physiology and biomedicine, to build a comprehensive understanding of the later stages of reproductive development in humans and animal models. Despite this, there remains much to learn about the bi-potential progenitor structure that the ovary and testis arise from, known as the genital ridge (GR). This tissue forms relatively late in embryonic development and has the potential to form either the ovary or testis, which in turn produce hormones required for development of the rest of the reproductive tract. It is imperative that we understand the genetic networks underpinning GR development if we are to begin to understand abnormalities in the adult. This is particularly relevant in the contexts of disorders of sex development (DSDs) and infertility, two conditions that many individuals struggle with worldwide, with often no answers as to their aetiology. Here, we review what is known about the genetics of GR development. Investigating the genetic networks required for GR formation will not only contribute to our understanding of the genetic regulation of reproductive development, it may in turn open new avenues of investigation into reproductive abnormalities and later fertility issues in the adult.
The distinction between sexes is one of the most obvious examples of dimorphism in the animal kingdom, and highlights one of the most crucial fate decisions made *in utero*; to develop as a male or a female. This fate decision to follow either developmental trajectory is an essential process that determines the reproductive success of all sexually reproducing animals (Fig. 1). There are a number of different mechanisms underlying sex development that can be broadly categorized as either genetically sex-determined or environmentally-determined (Capel 2017). Typically, mammals fall within the genetically sex-determined category. Traditionally, sexual development has been viewed as two distinct processes, gonadal sex determination (decision of the gonad to become a testis or ovary) and sexual differentiation (establishment of testicular/ovarian structures, accessory sex structures and secondary sexual characteristics), whereby genital ridge (GR) formation was included only as a step in gonadal sex determination or excluded from the broader picture all together. The focus of this review is formation of the GR in mice and humans, and consequences of failure of its development, rather than sex determination, which has been reviewed extensively elsewhere (Capel 2017; Greenfield 2015).

**Genital ridge formation**

The mammalian GR is derived from intermediate mesoderm as paired structures that lie on either side of the dorsal mesentery in the coelomic cavity (Fig. 2). Formation of the mammalian GR begins with increased proliferation of coelomic epithelial cells on the ventromedial surface of the mesonephroi. Each mesonephros also contains the mesonephric duct (also known as the Wolffian duct), a primordial urogenital tissue that will give rise to the male epididymis, vas deferens and seminal vesicles following male sex determination (Hannema and Hughes 2007; Shaw and Renfree 2014). In addition, the paramesonephric duct (also known as the Müllerian duct) is also present in the mesonephros, running in parallel to the mesonephric duct (Fig. 2A). This is the female equivalent to the mesonephric duct, which will form the fallopian tubes, uterus, and part of the vagina following female sex determination (Acien 1992). Together, the mesonephros and genital ridge are known as the urogenital ridge (UGR).

Proliferation of the coelomic epithelial cells on the ventromedial surface of the mesonephros creates a dense, pseudostratified epithelial cell layer (Gropp and Ohno 1966; Pelliniemi 1975; Wartenberg, et al. 1991). Alongside the proliferation of the coelomic epithelium, the underlying basement membrane becomes fragmented, allowing many epithelial cells to ingress dorsally, towards the
mesonephros (Fig. 2B) (Karl and Capel 1998; Kusaka, et al. 2010). As these cells delaminate from
the coelomic epithelium, they undergo an epithelial-to-mesenchymal transition (EMT) (Kusaka et
al. 2010) and as mesenchymal cells, begin to populate the space between the coelomic epithelium
and the mesonephros (Karl and Capel 1998; Schmahl, et al. 2000). These mesenchymal cells of the
GR are precursor cells that can differentiate into somatic support cells and interstitial/stromal cell
lineages of the early sexually differentiated gonad (Ito, et al. 2006; Karl and Capel 1998; Mork, et
al. 2012).

In the testis, additional cells are also recruited into the GR region from the mesonephros to augment
the population of mesenchymal cells. Cell-tracing and organ culture studies in mice revealed that
these largely contribute to the endothelial cell population for the establishment of the male vascular
just prior to gondal sex determination are primordial germ cells (PGC), the precursors to the germ
cells that become sperm and oocytes later in life. PGCs arise near the yolk sac and travel, via the
hindgut, to the GR as a result of chemotaxis (Doitsidou, et al. 2002).

Genital ridge formation in mice
Most of the work investigating mammalian gonad development has been performed on mice. It is
assumed that events in the human embryo, as well as other Eutherian mammals, follows the same
basic pattern, albeit with some differences in the timing and anatomy.

The process of GR formation, starting with the proliferation of the coelomic epithelium, begins at
about E9.5 in mice (Hu, et al. 2013) which equates to about 5 weeks of gestation in humans (Jost
1972). The GR itself is not morphologically evident until about E10.0 - 10.5 where a clear
distinction between the GR and the mesonephros can be seen under light microscopy. From E10.5
to 11.5, further proliferation of the GR coelomic epithelium and mesonephros expands the overall
size on the ventral side of the mesonephros (Fig. 2A). The initiation, proliferation and expansion of
the GR is indistinguishable between XX and XY embryos, and the size of the GR is determined by
the length of the embryonic trunk (Wainwright, et al. 2014). At around E11.5-12.0, the molecular
events that determine gonad fate occur, prompting the gonad to follow either a testis or ovary
specific developmental trajectory from this time-point. The first sign of sexual dimorphism of the
early-differentiated gonad becomes evident in XY gonads at about E12.5, when testis cords can be
The GR is not only a progenitor tissue for the gonad, but also the adrenal cortex, a critical endocrine tissue that synthesizes and secretes a variety of steroid hormones to maintain body homeostasis and regulate the stress response (Walczak and Hammer 2015; Yates, et al. 2013). Consequently, several genes required for gonad development are also important for adrenal gland development (Bandiera, et al. 2013; Gut, et al. 2005; Katoh-Fukui, et al. 2005; Luo, et al. 1994; Tevosian, et al. 2015). The medulla and the cortex of the adult adrenal gland have separate origins; the medulla is derived from neural crest cells, whereas the cortex is derived from cells located at the anterior-most region of the GR, indicating it has an intermediate mesoderm origin (Hatano, et al. 1996). Beginning at E10.5, a small cluster of cells at the anterior region of mesenchyme separates from the GR primordium and moves dorso-medially to form the adrenal anlage (Hatano et al. 1996; Val and Swain 2010). From E11.5 to 12.5, neural crest cells invade the adrenal anlage, and aggregate in the centre to form the medulla. Mesenchyme cells, hypothesized to be of a coelomic epithelial origin, form a fibrous capsule around the composite adrenal primordium by E14.5 (Xing, et al. 2015).

Development of the primordial germ cells in the GR
As previously mentioned, PGCs originate from a separate location near the yolk sac, away from the mesonephros and GR (Saga 2008). Mammalian PGCs are specified via an inductive system of signalling molecules, particularly BMP4. In mice, PGCs are induced around E6.5 (equating to approximately 2 weeks gestation in humans) from the proximal epiblast by BMP4 signalling (Lawson, et al. 1999). These cells subsequently move to and cluster at the base of the allantois/yolk sac wall, near the forming hindgut, which can be seen in mouse embryos at about E7.0 (week 3-4 human gestation) as a small population of ~45 cells (Lawson et al. 1999). As development proceeds, the hindgut folds and the PGCs migrate into the embryo proper (Hara, et al. 2009; Harikae, et al. 2013). By E9.5 the PGCs begin to migrate away from the hindgut towards the UGR and colonize the gonad between E10.0 to 11.0 (~5 weeks gestation in humans) (Fig. 2A) (Molyneaux, et al. 2001; Witschi 1948). During this mass migration of PGCs, the hindgut descends into the coelomic cavity, and the last PGCs to migrate must travel through the dorsal mesentery before entering the gonads (Molyneaux et al. 2001).

The PGCs undergo several rounds of cell division to achieve a population of about ~3000 cells by ~E11.5 (Tam and Snow 1981). Around this time PGCs begin to undergo a process known as licensing, undergoing a global change in gene expression, turning on genes required for
gametogenesis, concurrently switching off their pluripotency genes over the course of the following
days (Gill, et al. 2011; Rolland, et al. 2011; Seisenberger, et al. 2012; Stebler, et al. 2004). Following this transition, the PGCs are referred to as gametogenesis-competent cells (GCCs) and are poised to initiate either male or female differentiation, including meiosis, upon receiving cues from the somatic cells of either the forming testis or ovary and the nearby mesonephric tissue (Adams and McLaren 2002; Gill et al. 2011; McLaren and Southee 1997).

**Genes essential for initial gonad formation**

Mutational analysis using the mouse model, with some additional evidence from human clinical cases, have brought to light a number of genes that are required to initiate the formation and proliferation of the GR, as well as testis/ovary differentiation (Table 1). These include Wilms’ tumour suppressor 1 (WT1) (Hammes, et al. 2001; Kreidberg, et al. 1993), LIM homeobox gene 9 (LHX9) (Birk, et al. 2000), Nuclear Receptor Subfamily 5 Group A member 1 (NR5A1; also called Steroidogenic factor 1 (SF1)) (Luo et al. 1994), Empty-spiracles homeobox gene 2 (EMX2) (Miyamoto, et al. 1997), and GATA binding protein 4 (GATA4) (Hu et al. 2013). In mouse embryos, a homozygous null mutation in any one of these genes causes gonadal agenesis. While the function of these 5 factors have mainly been characterized in mice, mutations in three of these genes (NR5A1, WT1 and GATA4) have also been found in patients with DSD, indicating a conserved role in reproductive development (Bashamboo, et al. 2010b; Kohler, et al. 2011; Lourenco, et al. 2011; Swartz, et al. 2017). However, these genes are expressed and function in many developing organ systems, meaning the loss of function in both mice and humans produce a range of phenotypes beyond the reproductive tract (Hammes et al. 2001; Ingraham, et al. 1994; Klamt, et al. 1998; Tevosian et al. 2015). Genes known to be required for GR development often show altered expression in gene knockout lines, indicating a co-regulatory relationship exists between these critical factors (discussed further below).

It is evident that many of these critical early factors discussed below have multiple roles in reproductive development, from formation of the bi-potential gonad of both sexes, to sex-specific roles following gonadal sex determination. Currently we are lacking a detailed gene network integrating both the roles of these transcription factors, along with the signalling pathways that regulate them, to gain a broader picture of how these components together regulate cellular processes like differentiation and proliferation to control formation of the bi-potential gonad. Future
genome-wide approaches to study gene interactions will help to better define the regulatory
interactions between these proteins, and others, required for GR formation.

**Wilms Tumour suppressor, Wt1**

*Wt1* mRNA is expressed during gonad development in the coelomic epithelium and mesonephros
prior to gonadal sex determination, and in the progenitor support cells of both the developing testis
and ovary (Armstrong, et al. 1993). *Wt1*−/− mice have a recognizable gonad primordium at E11.0,
but this then degenerates due to apoptosis of somatic cells (Hammes et al. 2001; Kreidberg et al.
1993). Knockout mouse embryos, on a C57BL/6 genetic background, do not survive to parturition
due to embryonic lethality from heart defects, while knockout mice generated on other genetic
backgrounds, such as Balb/c, survive until birth (Herzer, et al. 1999; Kreidberg et al. 1993).

The *Wt1* gene encodes for a zinc finger transcription factor that functions as either an activator or
repressor of transcription, however, the structure and function of the WT1 protein varies depending
on the cell type and promoter used to transcribe the gene, RNA editing, alternative usage of
translational start sites and alternative splicing (Bruening and Pelletier 1996; Dallosso, et al. 2007;
Scharnhorst, et al. 1999). Of particular interest to gonad development are two alternate splice forms
of the WT1 protein named WT1−KTS and WT1+KTS, due to either the inclusion or exclusion of
three amino acids located between the third and fourth zinc finger domains (Hammes et al. 2001).
Mice lacking expression of the *Wt1*(−KTS) isoform have gonads markedly reduced in size and
poorly differentiated (Hammes et al. 2001), suggesting that WT1(−KTS) is the isoform required for
the proliferation and differentiation of GR cells. Knockout of the *Wt1*(+KTS) splice transcript leads
to complete male to female sex reversal and reduced *Sry* gene expression, yet ovarian development
proceeds normally (Hammes et al. 2001) (Fig. 3). For both knockout lines, the overall expression of
*Wt1* mRNA was similar to WT animals, due to increased expression of the alternative splice form
compensating for the loss of the targeted splice form (Hammes et al. 2001). Thus, this resulting
shift in isoform ratio (the ratio of -KTS/+KTS), leading to over-expression of the alternative
isoform, may also contribute to the observed knockout phenotype. However, this has not been
investigated further in mice with transgenic mouse lines. In humans, an altered splice form ratio
(increased WT1-KTS, reduced WT1+KTS isoform) does severely affect gonadal development
resulting in Frasier syndrome with streak gonads (Barbaux, et al. 1997; Klamt et al. 1998).
More recently, Chen et al., (2017) showed that the conditional inactivation of Wt1 just prior to sex-determination at E10.5 allows gonadogenesis to proceed with reduced differentiation of Sertoli and granulosa cells from somatic cell precursors. Thus, when Wt1 expression is lost from gonads at E13.5, most somatic cells develop into steroidogenic cell-types (Chen, et al. 2017) (Fig. 3). In contrast, in traditional knockout animals where Wt1 is inactive throughout development, development of the GR is blocked (Kreidberg et al. 1993) (Fig. 3). Thus, the role of WT1 alters as gonad development progresses, from being required for initial cell proliferation and growth of the GR, predominately through the WT1(-KTS) splice form, to controlling cell fate of somatic cells, and this is likely to occur through direct regulation of Nr5a1 gene expression (Fig. 4, described further in the following section).

Steroidogenic factor 1 (Sf1), Nuclear receptor subfamily 5 group A member 1 (Nr5a1)

The Nr5a1 (Sf1) gene encodes a transcription factor that is expressed in the adrenal glands, coelomic epithelium, hypothalamus and anterior pituitary gland during development (Luo et al. 1994; Morohashi and Omura 1996). During UGR development, Nr5a1 gene expression is limited to the gonad primordium, where it is required for the proliferation and survival of progenitor somatic cells (Luo et al. 1994). After gonadal sex determination, Nr5a1 gene expression is restricted to testis-specific cells, namely Leydig and Sertoli cells (Luo et al. 1994). Nr5a1-/- mice show complete failure of adrenal and gonadal development, abnormalities of the pituitary and hypothalamus, and obesity (Luo et al. 1994; Shinoda, et al. 1995). The gonads of Nr5a1 knockout mice embryos do not develop beyond the early GR stage, and as a result, XY mice show complete male-to-female sex reversal (Luo et al. 1994). Gonadal regression in Nr5a1-/- embryos is due to increased apoptosis of the somatic cells (Luo et al. 1994), also observed with Wt1 loss of function mice (Hammes et al. 2001). Recent single cell RNA-sequencing (scRNA-seq) of XY somatic cells, expressing Nr5a1 (Nr5a1-GFP labelled cells) prior to (E10.5) and following sex-determination (E11.5 – E16.5), confirmed the presence of a multipotent Nr5a1+ cell population in the GR that progressively forms both the supporting and steroidogenic cell lineages from E11.5 (Stevant, et al. 2018).

Regulation of Nr5a1 gene expression plays a critical role in gonad development, with all key transcription factors described to date in GR development function in regulating correct Nr5a1 expression (Fig. 4) (Chen et al. 2017; Hu et al. 2013; Katoh-Fukui et al. 2005; Wilhelm and Englert 2002b). LHX9 and WT1(-KTS) proteins both bind to the promoter region of Nr5a1 in vitro, and together they increase reporter gene expression in a Sertoli-like (TM4) cell line (Wilhelm and
Englert 2002a), a finding replicated in Leydig-like (TM3) and C2C12 (myoblast derived) cell lines (Takasawa, et al. 2014; Val, et al. 2007). However, in primary Leydig cells WT1 overexpression repressed Nr5a1 gene expression. Further experiments confirmed that WT1 directly binds in vivo to the Nr5a1 promoter in Sertoli cells obtained from 2 weeks old mice (Chen et al. 2017). It was suggested that these conflicting results were most likely due to the use of cell lines versus primary cells in these studies (Chen et al. 2017). The TM4 and TM3 cell lines were derived from juvenile BALB/c mouse testis (Mather 1980) and both cell lines express a similar combination of cell-type gene markers (Beverdam, et al. 2003). It is also worth noting that Chen et al., (2017) used primary Leydig cells obtained from adult and juvenile mice on a mixed background (C57Bl/6:129/SvEv), given that strain background can strongly influence the resulting phenotypes (Brennan and Capel 2004; Herzer et al. 1999; Meeks, et al. 2003; Munger, et al. 2009). WT1 also may have differing roles in the regulation of Nr5a1 gene expression, as the gonad develops into a testis, perhaps being required for an initial activation of Nr5a1, with protein partner LHX9 in the early gonad and, following gonadal sex-determination, WT1 reduces Nr5a1 expression in those NR5A1+ cells that are fated to become Sertoli cells. In this case the presence or absence of certain protein-partners, for instance LHX9, would impact on how WT1 regulates Nr5a1 gene expression. Previous studies have shown that WT1 is converted from an activator to a repressor protein by the protein partners such as BASP1 (Carpenter, et al. 2004; McKay, et al. 1999). Given that NR5A1+ cells contribute to both cell populations, it maybe that Nr5a1 expression levels determine which fate, with Sertoli cells expressing significantly lower levels of Nr5a1 compared to Leydig cells (Fig. 4B; (Chen et al. 2017)).

LIM homeobox 9 (Lhx9)
The Lhx9 gene encodes a transcription factor expressed in a variety of regions within the developing mouse embryo, including the brain, heart, kidney, limb buds, and the coelomic epithelium (Birk et al. 2000; Failli, et al. 2000; Molle, et al. 2004; Oshima, et al. 2007; Retaux, et al. 1999; Smagulova, et al. 2008; Tzchori, et al. 2009; Yang and Wilson 2015). Lhx9 gene expression in the UGR is first seen in the coelomic epithelium at E9.5 and later in the gonad primordium, until sexual differentiation where its expression becomes restricted to the interstitial/mesothelial regions of testes and the cortical regions of ovaries (Birk et al. 2000). Lhx9−/− mutant mice exhibit a similar gonadal phenotype to that of Wil1(-KTS)−/− mice, whereby normal GR development and PGC migration is observed but discrete gonads fail to form and genetically male mice show complete male-to-female sex reversal of their secondary sex characteristics (Birk et al. 2000). The observed
underdevelopment of the GR results from disrupted proliferation of the gonad primordium (Birk et al. 2000), as opposed to increased apoptosis observed in the \( Wt1^{-/-} \) and \( Nr5a1^{-/-} \) mice (Kreidberg et al. 1993; Luo et al. 1994). In addition, male and female knockout offspring are infertile, with atrophic uteri, vaginas and oviducts, no male accessory sex organs, increased follicle stimulating hormone (FSH) levels, and undetectable levels of testosterone and estrogen (Birk et al. 2000). Interestingly, \( Lhx9^{-/-} \) mice show no other phenotypic abnormalities outside of gonad agenesis and male-to-female sex reversal (Birk et al. 2000; Tzchori et al. 2009), despite expression in other tissues, such as the limbs, nervous system and pancreas, during development. This maybe a result of the functional redundancy of \( Lhx9 \) with its closely related paralogue, LIM-homeobox gene, \( Lhx2 \) (Birk et al. 2000; Jurata and Gill 1998; Tzchori et al. 2009).

**Empty spiracles homeobox 2, Emx2**

\( Emx2 \) encodes a transcription factor that is the mouse homolog of the \( Drosophila \) head gap gene empty spiracles (\( ems \)). The \( Emx2 \) transcript is expressed in the dorsal telencephalon, mesonephros and coelomic epithelium (Miyamoto et al. 1997; Yoshida, et al. 1997). \( Emx2^{-/-} \) knockout mice embryos exhibit normal PGC migration into the UGR region, but the initial thickening of the coelomic epithelium is not prominent and the GR soon degenerates (Miyamoto et al. 1997). These mutants completely lack gonads, genital tracts and kidneys (Miyamoto et al. 1997). The gonadal dysgenesis phenotype in \( Emx2^{-/-} \) mice is a result of impaired cell migration from the coelomic epithelium through the basement membrane, as well as increased apoptosis (Kusaka et al. 2010). Interestingly, \( Nr5a1 \) gene expression is also significantly affected in \( Emx2^{-/-} \) embryos (Kusaka et al. 2010), suggesting that \( Emx2 \) acts upstream of \( Nr5a1 \) in the developmental cascade, but how \( Emx2 \) regulates \( Nr5a1 \) transcription, whether directly or indirectly remains unclear.

**GATA-binding protein 4, Gata4**

GATA4 is a transcription factor that is essential for the development of multiple organs such as the heart, foregut, liver, ventral pancreas and the UGR (Hu et al. 2013; Kuo, et al. 1997; Molkentin, et al. 1997; Viger, et al. 1998). \( Gata4 \) gene expression in the GR was originally linked to a role in testis differentiation through activating transcription of \( Sry \) together with WT1 (Tevosian, et al. 2002). Later studies revealed that \( Gata4 \) is expressed prior to any other gonadal factor, initially in the anterior half of the coelomic epithelium at E9.5 and expanding to the posterior region by E10.2 (Hu et al. 2013). \( Gata4 \) knockout mice show no signs of gonadal initiation, with the coelomic
epithelium remaining as an undifferentiated monolayer, indicating that Gata4 is required for the initial thickening of the coelomic epithelial layer (Hu et al. 2013). In these knockout mice, Lhx9 and Nr5a1 gene expression is lost, but Wt1 and Emx2 gene expression is unaffected (Hu et al. 2013). This suggests that GATA4 acts not only upstream of the Lhx9 gene, but also Nr5a1, possibly even directly regulating both genes, as binding sites for GATA4 have been identified in the proximal promoter regions of these two genes (Hu et al. 2013; Smagulova et al. 2008; Tremblay and Viger 2001).

**Genes with minor but essential roles in GR development**

Other genes have been implicated in early genital ridge development, although mice knockout strains for these genes exhibit a less severe phenotype than that of the null phenotype of the key genes described above. While the loss of function of the following genes does not cause termination of gonad development, it does result in underdevelopment of the gonads, often in combination with sex-reversal of the secondary sex characteristics.

**Transcription factor 21 (Tcf21 or Pod1)**

TCF21, also known as epicardin, capsuling, or transcription factor 21, belongs to the basic helix-loop-helix family of transcription factors. It is expressed by mesodermal cell types including heart proepicardial cells, kidney and visceral smooth muscle as well as the endodermal gastrointestinal tract (Lu, et al. 2000; Miyagishi, et al. 2000; Quaggin, et al. 1999; Quaggin, et al. 1998). Tcf21 knockout mice die in the perinatal period due to lung, kidney and cardiac defects, but also display gastric and splenic defects (Cui, et al. 2003; Funato, et al. 2003; Plotkin and Mudunuri 2008). In addition, Tcf21 XX and XY KO mice have irregular shaped gonads, the urogenital tracts of both XX and XY mice indistinguishable and XY pups had feminized genitalia (Cui, et al. 2004). Consistent with this phenotype, Tcf21 gene expression is initially detected throughout the GR at the bi-potential stage and continues to be expressed in the gonads of both sexes, with expression slightly higher in the testes following sex-determination (Tamura, et al. 2001). While the gonad does form in the Tcf21+/− embryo, morphological defects such as a shortened length were observed by E11, and vascular abnormalities were observed by E12.5 (Cui et al. 2004), suggesting a defect early in gonadal development. Further studies focused on expression analyses, these suggested a negative regulatory relationship exists between Tcf21 and Nr5a1, as Tcf21 KO results in increased Nr5a1 expression and an expanded Leydig cell population (Miyagishi et al. 2000). Despite an increase in
Leydig cell numbers, the XY KO genitalia was feminized and the gonads fail to descend from an abdominal position, suggesting that testosterone levels were low, either due to later apoptosis of the gonadal tissue or a not all steroidogenic enzymes were expressed correctly (Cui et al. 2004). TCF21 also appears to repress \textit{Nr5a1} gene expression in the adrenal gland, with \textit{in vitro} studies revealing that TCF21 directly represses \textit{Nr5a1} gene expression, through binding at E-box sequences located in the \textit{Nr5a1} promoter (Franca, et al. 2015; Franca, et al. 2013).

Interestingly, the Sertoli cells do differentiate in the \textit{Tcf21} KO line but there was some evidence that this cell population was reduced in number (although it was not quantified) (Cui et al. 2004). TCF21 can cause a sex-reversal like phenotype \textit{in vitro} using a rat primary ovarian cell culture system. Over-expression of \textit{Tcf21} causes these cells to express Sertoli-like gene markers, in a similar pattern to that observed with \textit{Sry} over-expression (Bhandari, et al. 2011). Additionally, the \textit{Tcf21} gene has proposed to be a direct target of \textit{SRY} (Bhandari et al. 2011). Together, these results suggest that TCF1 acts downstream of \textit{Sry} to promote Sertoli cell expansion and repress steroidogenic cell lineages.

\textit{Sine oculis related homeobox 1/4 (Six1/Six4)}

\textit{Six1} and \textit{Six4} genes belong to the mammalian homolog of the \textit{Drosophila sine oculis homeobox} family of transcription factors, containing a distinctive SIX domain (required for protein-protein interactions) and homeodomain (Kawakami, et al. 2000). \textit{Six1} and \textit{Six4} genes are located in the same genomic regions within about 100 kb of one another, and have highly overlapping tissue expression during mouse development (Boucher, et al. 1996; Esteve and Bovolenta 1999; Fougerousse, et al. 2002; Laclef, et al. 2003; Ozaki, et al. 2001). \textit{Six1/4} double KO mice show different phenotypes compared to either \textit{Six1} or \textit{Six4} single KO mice, highlighting regions of redundant function in the development of limbs, skeletal muscle, sensory neurons and kidney (Giordani, et al. 2007; Grifone, et al. 2005; Kobayashi, et al. 2007; Konishi, et al. 2006). The suggested functional redundancy between the two genes is further exhibited as both genes share a common DNA binding site, MEF3 (Kawakami et al. 2000; Kumar 2009). In particular, only \textit{Six1/4} double KO mice embryos display smaller gonads and adrenal glands (Fujimoto, et al. 2013; Kobayashi et al. 2007). Out of the essential GR genes listed previously, only \textit{Nr5a1} gene expression is significantly reduced, a finding corroborated through reporter assays showing that SIX1/4 is able to transactivate \textit{Nr5a1} transcription (Fujimoto et al. 2013). Furthermore, it was also shown that SIX1/4 is able to activate \textit{Fog2} gene expression and that interaction, together with
GATA4, regulates Sry gene expression (Fujimoto et al. 2013). Overall, this indicates that SIX1/4 is required necessary for sufficient Nr5a1 gene expression in the early gonad and, a loss of Six1/4 gene expression, reduces Nr5a1 gene expression and this may be responsible for the Six1/4 KO undersized gonad phenotype. Nonetheless, there is still sufficient Nr5a1 gene expression to form the gonad primordium in Six1/4 KO embryos.

**Chromobox homolog 2, Cbx2 (mouse polycomb group member, M33)**

CBX2 is a component of the polycomb group complex of regulatory proteins involved in the repression/silencing of genes. In mice, Cbx2/- knockout mice show XY gonadal male-to-female (testis-to-ovary) sex reversal, and XX animals have smaller or absent ovaries (Katoh-Fukui et al. 2005). Additionally, mutants show defects in adrenal and splenic development, which was shown to be a result of a reduction in the expression of Nr5a1 (Katoh-Fukui et al. 2005). This has led to the suggestion that CBX2 acts as an upstream regulator of Nr5a1 gene expression. Cbx2 has also been identified as a factor contributing to the differentiation of the testis through indirect regulation of Sry (Katoh-Fukui, et al. 2012). Genome-wide identification of CBX2 target genes in a human Sertoli-like cell line suggests that CBX2 acts to stimulate male-specific genes, while suppressing female-pathway genes (Eid, et al. 2015). In humans, a reported case study of a 46, XY child with female internal and external genitalia and histologically normal ovaries found a compound loss of function mutation in the coding region of CBX2 (Biason-Lauber, et al. 2009). This study further lends support to the role of CBX2 in the trans-activation of Nr5a1 and its role in both the GR and testis developmental pathways (Fig. 4A).

**Insulin receptor, Insr & insulin-like growth factor type 1 receptor, Igf1r**

Insulin and its related growth factors IGF1 and IGF2 regulate a variety of physiological processes including metabolism, stimulation of cell proliferation, differentiation and survival (Efstratiadis 1998). Their function is mediated by two membrane-associated tyrosine kinase receptors, the insulin receptor (INSR) and the IGF type 1 receptor (IGF1R). The genes for Insr, Igf1r and insulin receptor related receptor (Irr) have previously been shown to be necessary for the testis determination pathway, as mutant mice lacking all three genes show male-to-female sex reversal and decreased Sry and Sox9 gene expression (Nef, et al. 2003). Recently it has been shown that Insr and Igf1r, but not Irr have roles in GR development, whereby mice lacking both Insr and Igf1r have reduced proliferation of the GR prior to sex determination but also extensive down-regulation of hundreds of genes associated with adrenal, testicular and ovarian development (Pitetti, et al. 2013).
As a result, these mice embryos exhibit agenesis of the adrenal cortex, along with male-female sex reversal due to a delay in Sry gene up-regulation. Interestingly, ovarian differentiation is also delayed in these mice, leaving the GR in an undifferentiated state until about E16.5 when the ovarian program is eventually initiated (Pitetti et al. 2013). Among the genes down-regulated are Wt1, Lhx9 and Nr5a1, indicating that genes essential for GR development are under the influence of insulin/IGF signalling (Pitetti et al. 2013), but only partially dependent on this signalling pathway, as GR development is only hindered by decreased progenitor cell numbers.

Pre-B-cell leukemia homeobox 1, Pbx1

Pbx1 encodes a TALE (three amino acid loop extension) class homeodomain transcription factor that has been shown to be involved in a number of processes during mammalian embryogenesis including, skeletal development and patterning (Selleri, et al. 2001), maintenance of haematopoiesis (DiMartino, et al. 2001), pancreatic development (Kim, et al. 2002), and kidney and adrenal development (Schnabel, et al. 2003). The GR of Pbx1 null mice are also smaller due to decreased proliferation of the progenitor cells in the gonad primordium (Schnabel et al. 2003). Further experiments found that this is due to the down-regulation of Nr5a1 gene expression; PBX1 also up-regulates Nr5a1 during adrenal development (Schnabel et al. 2003). The adrenal gland primordium is initially part of the GR but then later buds off from the very rostral end around E10.5 (Pitetti et al. 2013; Schnabel et al. 2003). Therefore, perturbed Pbx1 gene expression prior to budding likely affects Nr5a1 expression in the rostral GR, if not the whole GR, causing reduced Nr5a1 expression and decreased proliferation of somatic progenitor cells.

Odd-skipped related 1, Odd1

Odd1 gene encodes a zinc finger transcription factor homologous to the Drosophila Odd-skipped class of transcription factors that are involved in embryonic patterning and tissue morphogenesis (Wang, et al. 2005). Targeted gene knockout of Odd1 revealed that this gene functions in both heart and intermediate mesoderm development (Wang et al. 2005). Odd1<sup>−/−</sup> embryos have severe heart malformations, and completely lack adrenal glands, kidneys and gonads, all of which derive from intermediate mesoderm (Wang et al. 2005). Although gonad development was not the focus of this study, they did show that in early development the GR was hypoplastic (Wang et al. 2005), likely due to increased apoptosis observed with the developing kidney. These hypoplastic GRs appeared to degenerate, as no visible gonad structures were observed by E15.5 (Wang et al. 2005). Although no further investigation of Odd1 with regards to urogenital development has been done since Wang
et al. (2005), it is unclear if the GR phenotype was an effect of Odd1 acting either directly in the
GR or indirectly, potentially acting via down regulation of Wt1 gene expression which also has an
apoptotic phenotype when deleted (Hammes et al. 2001).

Genital ridge formation in humans

Much of what is known of human gonadal development is based on early embryology work and
case studies of individuals with abnormal characteristics stemming from improper sexual
development as a result of disruption of genes involved in this process. Recently, as sequencing
technologies have become more affordable and require less material, more studies are using human-
derived samples to study human sex development at the molecular level, mostly through studies of
cases of Disorders of Sexual Development (DSD) (Del Valle, et al. 2017; Houmard, et al. 2009; Li,
et al. 2017; O'Shaughnessy, et al. 2007; Ostrer, et al. 2007). These are congenital disorders that
arise as a consequence of atypical chromosomal, gonadal or anatomical sexual development
(Hughes, et al. 2006), including some of the aforementioned genes involved in GR development.
The term DSD replaced old and ambiguous terms such as intersex, hermaphroditism and pseudo-
hermaphroditism previously used to describe such disorders in humans (Dreger, et al. 2005; Lees
and Tuch 2006). DSD phenotypes include a broad range of conditions such as failure of gonad
formation, mixed gonadal tissue (male- and female-specific cell types within the tissue), ambiguous
genitalia and failure of secondary sexual characteristics to develop normally (Cools, et al. 2006).

There is limited data on the incidence of DSDs, but it is estimated that the overall worldwide
incidence of DSDs is 1 in 5,500 (Damiani 2007; Houk and Lee 2008).

In humans, the gonad develops from the coelomic epithelium (Fig. 5), and the first signs of sex-
differentiation are observed between 6-7 weeks with the formation of testicular cords (Gruenwald
1942; Wyndham 1943). Germ cells are first detected in the hindgut dorsal mesentery at 4 weeks
(Carnegie Stage 12 (CS12)), and migrate into the genital ridge by 5 weeks (CS16) (Mckay, et al.
1953). Gondal sex-determination occurs around 41-42 days in human embryos, signified by the up-
Mamsen, et al. 2017). Genes required for steroidogenesis and secreted factors such as AMH are
essential for the sex-specific development of genitalia and the reproductive tract, with their
expression commencing between 54 - 57 dpc (CS23). Genes essential for genital ridge development
in mice are also expressed at similar levels in both XX and XY gonads (LHX9, EMX2, WTI and
GATA4), further supporting a role for these factors in early human gonad development (Del Valle et
Despite their apparent early essential roles in gonad development, the loss of function of genes required for sex development typically results in a range of phenotypes from complete gonad dysgenesis to adult infertility. Mutations in some of these early gonad-development genes are also responsible for some cases of human idiopathic infertility in cases with apparently normal gonad development. Like DSDs, infertility is a condition associated with severe emotional and mental stress, particularly in societies where there is a social emphasis placed upon the ideal of having biological children (Ashraf, et al. 2014). The mechanisms of infertility in males and females are varied in both origin and functional impact, and often these conditions are thought to have a underlying genetic component (Zorrilla and Yatsenko 2013). Only 6-18% of infertility cases have identifiable genetic causes (mainly sex-chromosome abnormalities) indicating that for many cases of infertility (and subfertility), like DSD, the underlying genetic factors are yet to be elucidated.

Genes with mutations commonly identified in DSD studies include androgen receptor and synthesis genes (Androgen receptor (AR), CYP17A1, SRD5A2), NR5A1(SF1), WT1, GATA4, SRY, DAX1 and CHD7 (Kremen, et al. 2017). Those patients with DSD and a complete gonad dysgenesis phenotype carry mutations in SRY, MAP3K1, DHH and NR5A1 genes (Ono and Harley 2013). While these genes are also critical for mouse gonadal development, largely supporting the use of the mouse as a model for human gonad development, there are several exceptions. For example, sequencing screens have found gain of function mutations in the Mitogen-activated protein kinase kinase kinase 1 (MAP3K1) gene, including patients with streak gonads and female genitalia (Baxter, et al. 2015; Eggers, et al. 2016; Granados, et al. 2017; Loke, et al. 2014; Pearlman, et al. 2010), suggesting a requirement for MAP3K1 function in gonad development for both sexes. However, even although the mouse orthologue, Map3k1, is expressed in the E11.5 gonad mouse, knockout mice have only minor testicular abnormalities, indicating a minimal role in XY gonadal development (Warr, et al. 2011). This stresses the importance of additionally developing mouse models that replicate human gene mutations to study phenotype, as often human gene mutations do not result a complete loss of gene/protein function but rather gain of function.

Mutations in NR5A1 were identified in 4% of patients examined with unexplained male infertility (Bashamboo, et al. 2010a; Ropke, et al. 2013) and in female patients with in premature ovarian failure (Lourenco, et al. 2009). Novel NR5A1 mutation I (R92W) leads to 46, XX ovotestis (SRY-negative) (Baetens, et al. 2017b; Igarashi, et al. 2017). The types of NR5A1 gene mutations and phenotypes associated with these disorders are reviewed in (Ferraz-de-Souza, et al. 2011).
mutations have also been found in male and female cases of infertility, with ‘normal’ gonadal
development (Nathan, et al. 2017; Seabra, et al. 2015). Humans are not the only species to present
with variable phenotypes; in mice, the genetic background or strain strongly influences the adult
phenotype of many genes linked to gonadal development. In one such example, gene knockout of
Gadd45g (growth arrest and DNA-damage-inducible protein 45g) on a mixed genetic background
(129/C57BL/6), 20% of the XY homozygous mice developed as infertile males, whereas on a
C57BL/6 (B6) background 100% of XY mice were sex-reversed (Johnen, et al. 2013). Genetic
background also influences the phenotype of Nr5a1, Fgf9 and Wt1 null mice (Brennan and Capel
2004; Meeks et al. 2003). The B6 mouse strain is more likely to result in a male to female sex-
reversal phenotype than the 129S1/SvImJ (129S1) strain, and differences in gonadal gene
expression between these strains are observed even prior to sex-determination at E11.5 (Colvin, et
al. 2001; Munger et al. 2009). Therefore, even though studies have identified genes critical to the
early steps of gonad development, a loss of function mutation does not necessarily lead to complete
gonadal dysgenesis. In at least some cases, genetic background and possibly environmental factors
determine the phenotypic consequences of loss of function mutations in these genes.

Future directions: unravelling the molecular pathways of early gonad development

The advent and affordability of high-through put sequencing technologies and new methods of
replicating organ development in vitro, together will be valuable research tools in propelling
forward both genetic and cellular biology into early GR development.

The use of single-cell RNA-seq (scRNA-seq) has rapidly furthered our understanding of
developmental events, particularly within heterogeneous cell populations (Shapiro, et al. 2013).
Given that the GR consists of a broad variety of cellular precursors for endothelial, steroidogenic,
and supporting cell lineages, along with maturing germ cells, it is especially suitable for scRNA-seq
analysis. Li et al. undertook scRNA-seq analysis of isolated single fetal germ cells and their
surrounding ‘niche’ somatic cells from 4 – 26 week old human embryos (Li et al. 2017). Based on
gene expression profiles from this study, somatic cells in the early gonad can be divided into four
groups within each sex. XX gonadal cells are comprised of endothelial cells, and three types of
maturing granulosa cells (early- (10 weeks), mid-(10-20 weeks) and late-granulosa (20-26 weeks))
(Li et al. 2017). In XY gonads, somatic cells group into Sertoli cells, Leydig cell precursors,
differentiated Leydig cells and endothelial cells (Li et al. 2017). Systematic examination of the
expression profiles of each gonadal cell population during development will not only improve our
knowledge of the *in vivo* mechanisms of cell differentiation but also the conditions required to induce correct cell differentiation *in vitro*.

Organoid systems are becoming popular *in vitro* models of organ development (Fatehullah, et al. 2016). Isolated preparations of human somatic and germ cells can self-organize into a testicular-like organoid using an artificial scaffold to aid 3D organization (Baert, et al. 2017). These studies make use of cells that have already undergone sex-specific differentiation, as the cells are isolated from post-natal tissues (Baert et al. 2017). Recently, Sepponen *et al.*, reported using human embryonic stem cells (hESCs) culture conditions to sequentially induce the primitive streak, followed by intermediate mesoderm, and finally bi-potential-like gonadal cells expressing genes such as *LHX9*, *EMX2*, *WT1* and *GATA4* (Sepponen, et al. 2017). This study found that timing and levels of BMPs (bone morphogenetic protein), WNT/β-catenin and Activin-A signalling ligands are essential to promote differentiation of gonadal cell precursors, over other mesodermal cell types (Sepponen et al. 2017). These studies, along with new sequencing resources, lay the groundwork for future research for not only modelling the early events of human gonad development, but to also examine the functional consequences of gene mutations identified in DSD patients using cultured cells engineered with the same genetic mutation.

Several groups have taken a comprehensive targeted screening approach in order to identify genetic factors in DSD patients, this in turn may lead to the identification of novel genes required for GR development. Exome sequencing and targeted gene sequencing identified the genetic cause (classed as a functional gene mutation) in 28-38% of cases examined (Baxter et al. 2015; Dong, et al. 2016; Fan, et al. 2017; Kim, et al. 2017). Deep sequencing of 64 known and 967 candidate genes improved the genetic diagnosis rate to 43% for one patient cohort (Eggers et al. 2016). Thus, despite advances in sequencing technologies, over 50% of DSD cases remain without a genetic diagnosis.

While targeted sequencing and exome sequencing studies have focused on the protein coding regions of the genome, epigenetic processes, gene regulatory elements and non-coding RNAs (ncRNAs) are just as important for correct embryonic development. Errors in any of these gene regulatory mechanisms may underlie many cases of idiopathic DSD that lack mutations in protein coding genes. Studies in vertebrates have largely focused on identifying sex-dimorphic expression of small ncRNAs called microRNAs (miRNAs) following sex determination (Bannister, et al. 2009; Presslauer, et al. 2017; Real, et al. 2013; Wainwright, et al. 2013). There is limited information
regarding how these miRNAs function in sex determination, if their function is essential, if they act
to finely adjust gene expression levels during gonad development, and whether these, and others,
have earlier roles in the formation of the bi-potential gonad. Currently no miRNAs have been
linked to human gonad development. Long ncRNAs are another important class of ncRNAs, that
function to regulate gene expression (reviewed in (Moran, et al. 2012)). With respect to sex
development, these have been most thoroughly investigated for their role in X-chromosome dosage
compensation (Cerase, et al. 2015). It remains to be determined if autosomally encoded long
ncRNAs contribute to mammalian sex-determination and gonadal development but given their
important roles in the development of other organ systems, it is likely they have a role in some
aspects of gonadal development.

Genome regulatory elements have been difficult to identify as most lie within the non-coding
regions of the genome and can act over long distances to influence gene expression via chromatin
folding. Mutations in regulatory elements range from single nucleotide sequence variants that
prevent or reduce binding of a transcription factor to their DNA target sequence, to larger deletions,
duplications and translocations resulting in structural changes that alter chromatin confirmation and
regulatory interactions between enhancers and their target genes. Loss of function mutations in
regulatory elements located near the *SOX9* gene, a gene important for male sex-determination, are
the best-characterized regulatory changes associated with DSD (reviewed in (Baetens, et al. 2017a).
As putative regulatory elements are difficult to predict, few have been mapped for human genes
associated with early gonadal development. Recent genome-wide studies, including those mapping
the chromatin state in specific cell lineages (Mikkelsen, et al. 2007), will improve our ability to
predict if DNA changes associated with DSD lie within gene regulatory regions.

Epigenetic mechanisms such as DNA methylation and histone modifications have been implicated
2011). Loss of function of the *JMJD1A* gene, which encodes a H3K9 demethylase enzyme, results
in XY sex reversal (Kuroki et al. 2017). Some cases of XY DSD with complete sex reversal in
dogs is thought to be due to persistent DNA hypermethylation of the *Sry* gene (Jeong et al. 2016).
Therefore, it is likely that some cases of human DSD may be the result of mutations to epigenetic
regulatory factors. Regulation of gene expression through epigenetic mechanisms is also especially
sensitive to environmental influences and this impacts on many developmental programs including
sex-determination (Feil and Fraga 2012). DNA methylation levels, determined by environmental factors, is key to many naturally occurring forms of sex-reversal and environmental sex determination in animals (Capel 2017). While difficult to study with respect to human DSD, it is possible that DNA methylation may play a role balancing one sex developmental trajectory over another, and thus errors in this may lead to gonad dysgenesis or sex reversal in humans.

Summary

The complexity of reproductive development is reflected in the difficulty in assigning a genetic diagnosis in most cases of human DSD. While we know the genetic aetiology of a small number of DSDs, up to as many as 75% of individuals with a DSD will remain without a genetic diagnosis (Arboleda, et al. 2014). Even with whole genome sequencing, it is often difficult to identify functional variants and causal mutations, rendering many sequencing approaches somewhat ineffective (Fan et al. 2017). Gene expression levels, epigenetic modifiers and genetic background, along with the type of mutation and its functional consequence can all influence the resulting phenotype for both humans and mice. The future development of new technologies and improvement of existing ones will provide us with a much better understanding of the processes underlying normal gonadal development in both human and mouse models, which in turn will lead to improved diagnosis in cases of DSD and infertility.

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Figure legends.

Figure 1. Summary of the origin of reproductive tissues within the mammalian embryo. The gonad region of the GR develops into either the testis or the ovary depending upon expression of the \textit{Sry} gene. In XY gonads, the Leydig cells produce testosterone, required for the masculinization of the genitalia and maintenance of the mesonephric duct. Sertoli cells secrete AMH, resulting in the degradation of the paramesonephric (Müllerian) duct. In the absence of androgens, the paramesonephric duct develops as the uterus, uterine tubules and upper portion of the cervix. Female genitalia also form from the genital tubercle, folds and swellings. Post-natal oestadiol synthesis by the ovary is important for the final maturation of the oocytes and secondary sex characteristics. Other tissues of the body also show sex-dimorphic gene expression such as the brain and liver, under the influence of steroid hormones and other gonadal factors such as AMH (Conforto and Waxman 2012; Maekawa, et al. 2014; Wang, et al. 2009; Wittmann and McLennan 2013; Yang, et al. 2006).

Figure 2. Schematic illustrations of the structures and components involved in early UGR development in mice. A. (i) Side view of a E11.5 embryo indicating the location of the developing GR (orange bar). (ii) A ventral view of the abdominal region focuses on the mesonephros (white) and the gonadal ridge (grey) that develops on the ventromedial surface. The aorta is shown in red. (iii) In transverse sections the mesonephros, containing mesonephric and paramesonephric ducts, are often visible. The gonad appears as a bulge facing into the coelomic cavity. At this stage the PGCs have migrated into the GR, from the hindgut via the dorsal mesentery. 

B. Starting at E9.5, the coelomic epithelium (yellow) on the ventromedial surface of the mesonephros begins to proliferate, forming a pseudostratified epithelial layer. The basement membrane becomes fragmented, allowing GR progenitor cells that have undergone EMT to migrate inward. These cells continue to proliferate, just behind the coelomic epithelium to form the bi-potential gonad. Between E10.0 -11.5, the PGCs (green) also migrate into the GR.

Abbreviations: dorsal mesentery (DM), mesonephros (M), hindgut (HG), paramesonephric duct (PMD), mesonephric tubule (MT), coelomic epithelium (CE), genital ridge (GR), germ cells (GC).
**Figure 3.** The *Wt1* gene has multiple roles in gonad development and differentiation. Two isoforms of WT1, WT1(+KTS) and WT1(-KTS) have differing roles in gonad development based on gene knockout studies. Loss of WT1(+KTS) leads to reduced *Sry* gene expression and XY sex-reversal, in contrast, deletion of WT1(-KTS) halts GR development for both sexes (Hammes et al. 2001). However, if both splice forms of WT1 are present until E10.5, the gonad primordium still develops but with altered cell fate, with most somatic cells now adopting a steroidogenic cell fate (Chen et al. 2017).

**Figure 4. Summary of the molecular relationships during early gonad formation. (A)** Summary of relationships between the core genes necessary for GR development. Relationships are based on previous studies using knockout lines where expression changes of genes were observed. Loss of expression from knockout indicated a positive regulatory relationship and increased expression suggests a negative relationship. If direct relationships are known, through studies such as ChIP-PCR and reporter assays (Chen et al. 2017; Franca et al. 2013; Katoh-Fukui et al. 2005; Wilhelm and Englert 2002b), they have been indicated by a solid line. (B) Recent studies suggest a complex regulatory relationship exist between WT1 and *Nr5a1* (Chen et al. 2017). *Nr5a1* expressing cells (*Nr5a1*) in the GR contribute to the Sertoli, interstitial and Leydig cell populations following sex-determination. In the bipotential GR, WT1 is required for *Nr5a1* gene expression. In Sertoli cells WT1 binds directly to the *Nr5a1* gene promoter and appears to reduce its expression. In Leydig cells (no *Wt1* expression), *Nr5a1* gene expression is significantly higher. Lhx9 is a candidate protein partner for WT1 (Wilhelm and Englert 2002b), it is expressed in the GR, and later in interstitial cells.

**Figure 5.** Histology sections through human embryos 36-44 days (Carnegie stages 14-18). At ~36 days the coelomic epithelium begins to thicken, and by 39 days a ridge of tissue is forming, facing into the gut cavity. Just prior to sex determination (~42 days), the mesenchyme has proliferated to form a gonad region that is now easily distinguished from the neighbouring mesonesphros. Section images were obtained from the Virtual Human Embryo resource (https://www.prenatalorigins.org/virtual-human-embryo/)
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Tables

Table 1. Summary of genes essential for GR formation
<table>
<thead>
<tr>
<th>Gene</th>
<th>Mouse UGR expression</th>
<th>Mouse gonadal phenotype</th>
<th>Human phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nr5a1</strong></td>
<td>Initially expressed in the anterior region of the coelomic epithelium. Expression domain expands in an anterior-posterior direction (Hu et al. 2013).</td>
<td>Gonad regression due to increased apoptosis. Loss of adrenal development, obesity and pituitary abnormalities XY KO mice show complete male to female sex reversal (Luo et al. 1994; Shinoda et al. 1995).</td>
<td>Range of phenotypes including 46, XY sex reversal, gonad dysgenesis, male infertility, hypospadias, adrenal insufficiency, gonad dysgenesis, premature ovarian failure (reviewed in (Ferraz-de-Souza et al. 2011)) 46, XX sex reversal, ovotestis (Bashamboo, et al. 2016; Swartz et al. 2017)</td>
</tr>
<tr>
<td><strong>Lhx9</strong></td>
<td>Coelomic epithelium and cells that move into the underlying mesenchyme (Birk et al. 2000; Hu et al. 2013)</td>
<td>Absent gonads. Reduced proliferation of the coelomic epithelium (Birk et al. 2000).</td>
<td>None reported. Limited evidence of involvement in reproductive cancers (Cervical cancer promoter methylation (Bhat, et al. 2017))</td>
</tr>
<tr>
<td><strong>MAP3K1</strong></td>
<td>Coelomic epithelium and gonad mesenchyme (Warr et al. 2011).</td>
<td>Minor testis abnormalities. (Warr et al. 2011).</td>
<td>Ambiguous genitalia streak gonads (Granados et al. 2017; Pearlman et al. 2010)</td>
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