Human Sex Determination

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ABSTRACT

Human sex determination is a fascinating topic, particularly at the level of molecular genetics, as it represents an excellent paradigm for mammalian organ development. Recent progress has seen the addition of several new pieces to this developmental jigsaw puzzle.


THE Y-LINKED SEX-DETERMINING GENE: SRY

In 1990, the SRY (sex-determining region of Y) gene was isolated from the human Y chromosome (Sinclair et al., ’90) and was subsequently shown to be TDF. Not only were mutations detected in the SRY gene of XY females, indicating that SRY is required for normal testis development (Berta et al., ’90; Jager et al., ’90), but mice transgenic for Sry developed into sex-reversed males, despite an XX karyotype (Koopman et al., ’91). Thus, among Y-derived sequences, SRY is both required and sufficient for male sex determination.

As the testis-determining gene, SRY must act in conjunction with other genes to direct testis determination. The precise temporal and spatial expression profile of SRY implies the existence of other genes “upstream” in the testis-determining pathway, which regulate its expression. The presence of a 79-amino acid HMG box DNA binding motif in the SRY protein suggests that it regulates genes “downstream” in the pathway. In addition, only 10% of XY females with gonadal dysgenesis have a mutation in the SRY gene (Hawkins et al., ’92), and a small proportion of XX males do not carry SRY. Together, these data emphasise the importance of other genes in the cascade leading to testis development.

In humans, SRY is a single exon gene with multiple transcription initiation sites. The SRY transcript has been detected in human adult testis and in lower amounts in other adult male and fetal tissues (Clepet et al., ’93). It is not clear whether SRY has a role in the development of other (non-gonadal) tissues. However, XY females with mutations in SRY do not display any phenotypes that could be associated with a more general role for SRY.

Comparison of human SRY and mouse Sry protein shows conservation of the HMG box but no homology outside this region. Striking differences between mouse and human SRY protein exist, particularly at the C terminal. In the mouse, the C-terminal of Sry is 252 amino acids long (in humans, it is only 69 amino acids) and contains a glutamine/histidine-rich domain that mediates transactivation in vitro. No such region has been identified in human SRY protein, suggesting that it may act as a repressor rather than an activator of transcription. Amongst primates, SRY sequences have evolved rapidly outside the HMG box, indicating that only this conserved motif has an important function (Ramkisson and Goodfellow, ’96).

With the discovery of SRY/TDF, it was hoped that the pathway leading to mammalian testis development could be unravelled. The properties of SRY have made the task of finding these other genes difficult, however. It is now clear that SRY is part of a large family of transcription factors that are all related by the presence of an HMG box. Genes that encode proteins with greater than 60% sequence similarity to the SRY HMG box are known as SOX (SRY-like HMG box) genes.

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circumstances.
also act as a repressor of transcription in other
transfections have demonstrated tran-
scriptional activation of a testis-specific gene by
SRY protein (Cohen et al., '94). However, this
does not exclude the possibility that SRY may
also act as a repressor of transcription in other
X-LINKED SEX-REVERSING GENE: DAX-1
The isolation of the SRY gene exploited the ge-
etic analysis of sex-reversed XX male patients,
who had minute portions of the Y chromosome
translocated to the X chromosome (Palmer et al.,
'89). Other sex-reversal syndromes are known
that involve X-linked and autosomal chromosome
rearrangements resulting in a failure to develop
testis. Duplications of the Xp21 region have been
shown to cause XY female development (Bern-
etstein et al., '80). This suggested the presence of a
gene on the X chromosome called DSS (dosage-
sensitive sex reversal), which has been limited to a
160-kb region at Xp21 (Bardoni et al., '94). Two
active copies of the DSS gene are believed to over-
ride the testis-determining signal, resulting in the
development of ovaries and an XY female (King
et al., '95). The Xp21 region is also involved with
AHC (adrenal hypoplasia congenita), which re-
results in the failure of the adrenals to form pro-
perly. Further investigation of the DSS region led
to the isolation of the gene DAX-1 (DSS-AHC-
critical region of the X chromosome, gene 1),
which encodes a novel member of the nuclear hor-
mone-receptor superfamily (Zanaria et al., '94).
This raises the possibility that DAX-1 may be
both the DSS gene and the AHC gene. However,
there may be other genes within the defined mini-
mal region on Xp21. Mutations in DAX-1 have
been shown to cause adrenal hyperplasia but do
not appear to affect testis development, as no
mutations have been found in a screen of XY fe-
males (Muscatelli et al., '94).

Duplication of the DSS locus interferes with
testis determination. DSS is not essential for tes-
tis formation, however, because 46 XY individu-
als deleted for this region developed normal male
genitalia (Ramkisson and Goodfellow, '96). Con-
sequently, DSS is more likely to play a role in
ovarian development. In the mouse, Dax-1 is ex-
pressed in the somatic cells of the genital ridge
at 11.5 d.p.c. in both males and females (Swain
et al., '96). This is the same time at which Sry
is expressed in the male genital ridge. At 12.5
d.p.c., Dax-1 is turned off in the male gonad, but
it is maintained in the female gonad. This expres-
sion profile supports a role for DAX-1 in
ovary development and is also consistent with
DAX-1 being equivalent to DSS (Swain et al.,
'96). The overlapping expression profile of Dax-
1 and Sry in the male gonad raises the possibil-
ity that Sry may act by inhibiting Dax-1 activity.
Determining the role of the DAX-1 gene in dos-
age-sensitive sex reversal will require the pro-
duction of transgenic XY mice carrying multiple
copies of Dax-1.

AUTOSOMAL SEX-REVERSING GENES
Three autosomal loci on chromosomes 9, 10,
and 17 have been implicated in sex reversal. De-
etions of loci on chromosome 9p and 10q can
result in XY females with dysgenic ovaries
(Bennett et al., '93; Wilkie et al., '93). The other
autosomal sex-reversing locus, SRA1, resides on
chromosome 17q and is associated with campo-
melic dysplasia (CD) (Tommerup et al., '93).
Campomelic dysplasia is a rare (0.05–2 per
10,000 live births) but often fatal skeletal mal-
formation syndrome that characteristically re-
results in a bowing or angulation of the long bones,
small scapulae, a deformed pelvis, and a miss-
ing pair of ribs. In addition, common craniofa-
cial features include micrognathia, cleft palate,
a flat nasal bridge, and hypertelorism. Non-skel-
etal defects include the absence of olfactory bulbs
and a variety of cardiac and renal anomalies.
Most CD patients die shortly after birth from
respiratory distress caused by a small thoracic
cage and the narrow airways that arise from de-
fective tracheobronchial cartilage (Mansour et
al., '95). Three-quarters of XY CD patients de-
develop as phenotypic females or intersexes (Hou-
ton et al., '83). Some of these XY female CD
patients also show gonadal dysgenesis, which in-
dicates that the gene for CD is also involved with
testis development.
Campomelic dysplasia: SOX9

Two groups set out to clone the translocation breakpoints at 17q in sex-reversed CD patients and identified the SOX9 gene in this critical region (Foster et al., '94; Wagner et al., '94). Just prior to this work, mouse Sox9 had been isolated and mapped to mouse chromosome 11 (Wright et al., '95). The region of mouse chromosome 11 containing Sox9 is homologous to human chromosome 17q, which is the site for the CD and autosomal sex-reversing loci. Furthermore, Sox9 was shown to be expressed during embryogenesis before and during cartilage deposition; consistent with a role in skeletal development (Wright et al., '95). SOX9 was analysed for mutations in 15 CD patients by single-strand conformation polymorphism (SSCP) and by direct sequencing (Foster et al., '94; Wagner et al., '94). Other studies have now brought the number of CD patients analysed for mutations in SOX9 to 28 (Cameron and Sinclair, '97). A variety of mutations were identified, including: mutations in consensus splice sites, missense and frameshift mutations producing premature stop codons, and amino acid substitutions. All these mutations appear to result in a non-functional SOX9 product. Furthermore, these mutations affect a single allele, suggesting a dominant mode of inheritance for this syndrome. Consequently, campomelic dysplasia and autosomal sex reversal may result from haploinsufficiency of SOX9.

Human SOX9 is expressed in a wide variety of adult tissues including heart, brain, kidney, prostate and testis (Foster et al., '94; Wagner et al., '94). In the human fetus, SOX9 is expressed in brain, testis, and chondrocytes of the hypertrophic zones of developing long bones and ribs (Wagner et al., '94). The SOX9 HMG box shows a 71% similarity at the amino acid level with the SRY HMG box (Foster et al., '94). SOX9, however, differs in having two introns and is the only SOX gene to date that is not comprised of a single exon. In addition to the HMG box DNA binding domain, SOX9 contains a transcription-activating domain (Sudbeck et al., '96). Given these features, SOX9 probably functions as a transcription factor in regulating developmental pathways. Interestingly, most of the patients described with SOX9 mutations would probably not produce the transactivating domain of the protein.

In the 17q translocation CD patients, the breakpoints are more than 50 kb 5′ to the SOX9 gene. In these patients, no mutations were detected in the open reading frame of SOX9 (Foster et al., '94; Wagner et al., '94; Kwok et al., '95). A number of these translocation patients survived childhood, however, and may have a less severe form of the disease (Foster et al., '94; Wagner et al., '94). Discrepancies in the size of the SOX9 transcript leave open the possibility of an unidentified 5′ exon, which may carry a mutation (Foster et al., '94). Presumably, the chromosome 17q translocation causes CD and autosomal sex reversal by disrupting expression of SOX9, but confirmation of this awaits expression analysis from a rearranged chromosome.

There does not appear to be a correlation between the severity of the skeletal abnormalities and the incidence of sex reversal (Foster et al., '94; Wagner et al., '94; Kwok et al., '95, Cameron and Sinclair, '97). SOX9 mutational studies do not show any correlation between the type of mutation or its location and the presence or absence of sex reversal. Several CD patients have been described with the same mutation in SOX9 but developed either as an XY male or a sex-reversed XY female. Presumably, the variable penetrance of the syndrome results from differences in genetic background. An analysis of 30 XY females without SRY mutations or skeletal abnormalities did not detect any mutations in the SOX9 gene (Kwok et al., '95). This indicates that SOX9 mutations do not cause gonadal abnormalities without also causing defects in the skeleton.

SRY and SOX9: in the testis-determining pathway

Mutation studies on SOX9 confirm its role in CD but also place SOX9 unequivocally in the testis-determining pathway. SRY has a specific role in mammalian testis development but does not appear to be present in other vertebrates. Analysis of mouse and chicken embryos shows up-regulation of SOX9 expression in the male genital ridge just prior to gonad development. This expression pattern in both mouse and chicken implies SOX9 has been conserved in the vertebrate testis-determining pathway (Kent et al., '96). Furthermore, Sox9 expression in the mouse is specific to the Sertoli cell lineage and appears to be up-regulated shortly after Sry expression is initiated (Morais da Silva et al., '96). This temporal and spatial expression pattern suggests that SRY could regulate SOX9. As a consequence, it may be expected that SOX9 would contain within its promoter a consensus binding site for SRY, but this has not been identified to date. If SRY and
SOX9 interact, they may perform some vital function required by Sertoli cells.

**Genes required for early gonadal development (SF-1) and (WT-1)**

Steroidogenic factor 1 (SF-1) is expressed in all primary steroidogenic tissue, including the testis and ovaries. This orphan nuclear receptor is a key regulator of steroid hydroxylases (Ikeda et al., '93). In mouse, SF-1 is expressed in the urogenital ridges of both sexes at 9 d.p.c. and ceases by 12.5 d.p.c. in females. In males, however, SF-1 expression persists in Sertoli cells (Ikeda et al., '94). In mouse, SF-1 and Amh (anti-Müllerian hormone) have overlapping expression profiles in the Leydig cells of the developing testis. It has been suggested that SF-1 may regulate Amh, as in vitro SF-1 has been shown to bind a nuclear receptor consensus site in the Amh promoter (Shen et al., '94). Analysis of sf-1 knockout mice revealed that both XX and XY individuals lack adrenals and gonadal ridge development is arrested and ultimately degenerates. These knockout mice develop as phenotypic females but die soon after birth as a result of adrenocortical insufficiency (Luo et al., '94). This suggests that SF-1 may play several roles at different levels of gonad development. Initially, SF-1 appears necessary for the maintenance of the bipotential urogenital ridge and subsequently plays a role in regulating Amh in the testis and also plays an unknown function in the developing ovary.

The Wilms' tumour 1 gene (WT-1) is an oncogene associated with cancer of the kidney in children. It is also expressed in the urogenital ridge of male and female mice at 9 d.p.c. (Pelletier et al., '91a). In addition, Denys-Drash patients with heterozygous mutations in WT-1 have Wilms' tumour associated with renal failure and gonadal and genital abnormalities (Pelletier et al., '91b). This further implicates a role for WT-1 in gonadal development. Analysis of Wt-1 knockout mice showed they died in utero from failure of kidney development and also showed degeneration of the gonad (Kreidberg et al., '93). As both SF-1 and WT-1 are expressed at the same stage during embryogenesis and display similar knockout phenotypes, it seems likely they both play a role in a common pathway upstream of Sry, probably in the maintenance of the bipotential urogenital ridge (Ramkissoon and Goodfellow, '96).

**CONCLUSIONS**

In recent years, many more pieces of the human sex-determination jigsaw puzzle have come together. In 1990, only the SRY gene had a definite role in the gonad-determining pathway, but now we can add SF-1, WT-1, and DSS. A combination of strategies, including human sex-reversed patients, animal models, molecular/cellular biology, and serendipity are slowly beginning to reveal the complexity that lies behind the development of a testis or ovary.

**LITERATURE CITED**


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