1 On the Genetic Origin of Sex Differences

Our children inherit a set of 23 chromosome pairs carrying the genetic information from mother and from father. Out of these 46 human chromosomes, the majority are matched for maternal and paternal genes, the so-called homologous pairs of genes. The exception is seen for the sex chromosome pair. In sons, the male Y-chromosome combines with the female X-chromosome, while daughters have two copies of the X-chromosome, one from the mother and one from the father. The genes on one of the two X-chromosomes that daughters inherit are silenced to ensure that X-gene dosage for sons and daughters always match.

The sex chromosomes took their name from early cytogenetic studies when it was observed that males have one chromosome, named the Y-chromosome, which is very much smaller than its partner, the X-chromosome. Male mammals possess an XY sex chromosome complement (heterogametic sex) and females have two XX chromosomes (homogametic sex). These cytogenetic studies revealed that the possession of a Y-chromosome determined the development of the male's testes, and in the absence of a Y-chromosome in the female, an ovary would develop. From this early stage of development forwards, sexual differentiation was considered to be dependent on the hormones produced by the testes or the ovaries. Of course, these steroid hormones have never been considered to be directly coded for by specific genes, but they are an epigenetic product of gene activity, and in turn they are able to activate genes by way of their protein receptors. These steroid receptors are coded for by the genome, and these receptor genes reside on many chromosomes within many different cell types. It is important to appreciate that these early cytogenetic studies represented a

description of what takes place, and not an explanation of the mechanisms involved. There were many gaps in our knowledge, not least of which concerned those genes residing on the sex chromosomes which coded for producing the female and male gonads. The gonads in turn ensured production of the sex hormones. Investigating the evolutionary history of sex determination across different species was not a great deal of help for our understanding of the origins of mammalian genetic sex determination. In amphibia, reptiles and birds, the female is the heterogametic sex. Moreover, in some reptiles, there is a lack of any chromosomal differences between the sexes, and ambient temperature during the period of egg incubation has been found to be the epigenetic sex-determining factor (Matsumoto *et al.,* 2013). There are also certain exceptions to normal gonadal development in mammals, with sex reversal in wild rodent species that lack any Y-chromosome (Jiminez *et al.,* 2013). Evidence for what the missing genes from the Y-chromosome might be have come from studies of a rodent found in Japan (the Amami Spiny rat). This Spiny rat has no Y-chromosome (XO/XO), nor the sex-determining *SRY* gene (Kuroiwa *et al.,* 2011). In the Spiny rat, mechanisms for sex determination are underpinned by multiple copies of the autosomal *Cbx2* gene in the male, but not in females. In the male Spiny rat, these multiple copies of the *Cbx2* gene are responsible for producing the testes.

A single gene on the mammalian Y-chromosome, the so-called *SRY* male sex-determining gene, was identified in 1990. This *SRY* gene is now known to be primarily responsible for testes development, and thereby production of androgenic hormones which promote the creation of a male phenotype and one which differs from that of the female (Cortez *et al.,* 2014). This presence of a single Y-chromosome has not always provided the underpinning for sex differences even among different mammalian species. In the evolutionary history of our recent mammalian ancestors, represented in modern times by the Australian Duck-Billed Platypus, sex determination is a function of many genes across multiple chromosomes

(Ferguson-Smith & Rens, 2010). So what have been the advantages to providing the modern male mammal with a unique Y-chromosome and a unique *SRY* sex-determining gene? Other than male sex determination, few advantages have accrued from possession of a single Y-chromosome. Indeed, the Y-chromosome has suffered extensive gene loss, with only 3% of its ancestral genes functionally surviving. Moreover, it has recently been found that with the use of assisted reproduction in mice, live offspring can be produced lacking the entire Y-chromosome long arm (Yamauchi *et al.*, 2014). Moreover, these progeny developed as males by using only two genes from the Y-chromosome, namely *SRY* and the spermatogenic proliferation factor *Eif2s3y*.

Two main processes are believed to have resulted in the evolutionary loss of genes from the Y-chromosome. Genes in that region of the Y-chromosome, which is non-recombining with the X-chromosome, are inactivated and lost as a consequence of point mutations, DNA deletions and the accumulation of nucleotide insertions. Moreover, the higher rate of mutations on the Y-chromosome is due to the many rounds of replication undertaken during sperm production, and the absence of DNA repair enzymes. Some experts have even postulated that the human Y-sex chromosome may eventually disappear (Sun & Heitman, 2012). There is, however, no need for male concern in the near future. This 3 per cent of remaining Y-chromosome genes are functionally coherent, enriched for maintaining gene dosage stability, and have remained relatively stable for the past 25 million years.

Gene repression as well as gene expression in mammalian gonadal development has become a complex and important process in the understanding of sex differences. Recent studies have found that so-called polycomb repressive complexes play a crucial role in regulating repression of certain genes involved in sexual development (Katoh-Fukui *et al.*, 2012). Interestingly, male development results from the repression of female ovarian-determining genes followed by the expression of the male *SRY* gene. Female ovarian development is

very much an active process, while male testes development depends upon repressing female ovarian development. It is the expression of *Cbx2* gene in the male which represses maternal transcription from taking place and thereby development of the ovary. Mapping analysis of this gene's transcription has identified some 1600 targets for *Cbx2*, many of which code for proteins known to be involved in disorders of sexual development (Eid *et al.*, 2015). Hence, genetic determination of sex differences is now known to be extremely complex, and not just dependent on the *SRY* gene in males (Figure 1.1). *SRY* is essential for male testes development, while the *Cbx2* complex allows the testes-determining *Sox9* gene to be expressed by the inhibition of *FoxL2*, another gene actively engaged in female ovarian development. *Cbx2* is expressed in a parent of origin manner, with multiple copies being produced in the male, but not in the female (Tardat *et al.*, 2015).

The mammalian X-chromosome, in contrast to the Y-chromosome, has been proposed to enable important selection pressures to operate for mammalian evolution due to the accumulation

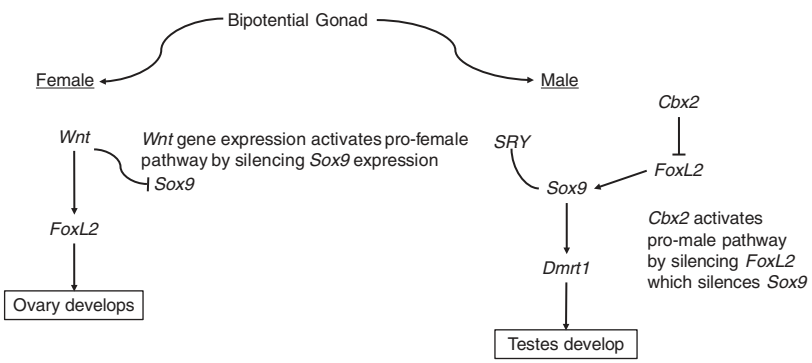

FIGURE 1.1 Overview of ovaries and testes development. The bipotential gonad develops into the testes by the *Cbx2* gene blocking the *FoxL2* gene, thereby allowing the *SRY* gene to activate *Sox9* expression and testes development.

of placental and brain-expressed genes. The X-chromosome has also acquired and amplified gene families that are expressed in the male testes. Moreover, the X-chromosome of males is always inherited from their mother, and has thereby gained a specialisation for male reproduction via the matriline and by the acquisition of new genes. These new genes acquired on the X-chromosome have tended to be of importance for regulation of gene networks that govern the expression of gene targets across multiple chromosomes throughout the genome. Thus, the sum total of the male's genetic capacity to determine masculinity is no longer solely present on the single Y-chromosome, although the Y-chromosome still plays an integral role in this process through the male *SRY* gene and the spermatogonadial proliferation factor.

The extensive gene loss from the male Y-chromosome has taken place in multiple steps. This loss was never sufficient to prevent the Y-chromosome from combining with the X-chromosome, but it certainly reduced the ability for natural selection to operate on this Y-chromosome. The Y-chromosome is always inherited through father to son, and is thus unique in that this chromosome never passes through the female germline. The consequences of such inheritance provide a focus for selection pressures to be exclusively specialised for, and primarily beneficial to, male functions. However, the majority of mutational changes which do occur to genes on the Y-chromosome are not advantageous. There is a sound biological explanation for this finding; because of the multiple cell divisions the sperm germ cells undertake, then the greater is the risk for mutational errors arising (Bachtrog, 2013). Males are particularly vulnerable to genetic errors because billions of sperm are produced by the multiple cell divisions that occur in the testes during the reproductive lifetime of a male. When compared with the relatively few cell divisions that are required to produce the full complement of female eggs (oocytes) prior to female birth, then there is considerably greater risk for accumulating male germline mutations. Not only are there more of these mutations occurring in the male germline, but there

is no opportunity for DNA repair in the male germline. Unlike the X-chromosome, the male Y-chromosome does not possess a homologous partner. Thus, there is no duplicate for the Y-chromosome against which mismatch DNA repair can be undertaken. This only remains possible for those very few duplicate genes that are present on both the X- and Y-chromosomes, but these genes are also under regulatory control by the maternal X-chromosome and are advantageous to both sexes.

It is clear that the X-chromosome has retained or acquired many of the former male sex-related genes, including the receptor for testosterone, while other former Y-chromosome genes have been taken over by the remaining 22 autosomal chromosomes. The male hormone testosterone, the most potent of the male androgenic hormones, determines male secondary sexual characteristics by acting on the so-called androgen receptor. The gene for this receptor is expressed from the female X-chromosome in males and is, therefore, always inherited via mother (Migeon *et al.,* 1981). During mammalian evolution, the demasculinisation of the Y-chromosome became further associated with feminisation of the X-chromosome, through X-linked genes focussing expression to the ovaries (Bion & Toniolo, 2000). Thus, unlike the degenerate Y-chromosome, evolutionary positive selection has accompanied the early evolutionary progression for genes on the X-chromosome, which males always inherit from the female.

Although the Y-chromosome is essential to developing a male phenotype through the development of the testes and their production of testosterone, this hormone itself functions through the androgen receptor. As mentioned, the gene coding for this receptor has become incorporated into the female X-chromosome (Migeon *et al.*, 1981). Thus, a clinical syndrome of sexual development is seen to occur in XY males that become feminised due to a default in the androgen receptor gene on their X-chromosome. This dysfunction is known as 'testicular feminisation syndrome' (shortened to Tfm) (Wang *et al.*, 2014). Androgens are still produced by the testes in Tfm

males, but none of the somatic body tissues, or brain neurons, have the receptor that enables them to respond to this testosterone. Hence, the male brain and body of Tfm males fail to respond to male testosterone, and develops to respond for a feminine phenotype. These Tfm subjects display a feminine gender identity and, if they are reared as girls, they date and marry men. We can conclude from these findings that there are no genes on the male Y-chromosome that are sufficient in their own right to enable the development of male secondary sexual characteristics, or to induce psychological masculinity. Indeed, the mapping of brain responses to sexually arousing imagery that is seen in Tfm males provides the same responses as those for the brain of women.

It is difficult to understand why or to rationalise the claim of early reproductive biologists for 'females' to represent a default state. Their choice of terminology was inappropriate, and with a greater knowledge of the genetics underpinning sexual differentiation, this view has actually been proved to be incorrect. Indeed, it would be more accurate to define masculinity as dependent on the female germline. Certainly, the *SRY* male sex-determining gene on the Y-chromosome is essential for male reproduction, but without the androgen receptor on the maternal-inherited X-chromosome, masculinisation fails and so does male reproduction. Thus, the degenerate Y-chromosome gene content has become specialised to maintain the ancestral dosage of the few remaining homologous XY gene pairs. Moreover, the need for this X-chromosome gene pairing has been critical for the survival of these remaining Y-chromosome genes, and is essential for the segregation of X- and Y-chromosomes during male spermatogenesis. It is also the case that a higher proportion of those genes on the female X-chromosome which do have a Y-linked homologue escape X-inactivation, thereby favouring expression of the maternal copy (Sin & Namekawa, 2013). Thus, males benefit hugely and, indeed, they only maintain their masculinity through inheritance of the mother's X-chromosome. The X-chromosome is also characterised as having a disproportionately high number of

genes, which are expressed in the placenta and brain (Graves, 2010). The placenta and brain are two very important organs that have been integral to the success of human evolution and will be dealt with in subsequent chapters. However, it is important to note that the matriline has played a leading role for the co-adapted evolutionary development of these structures (see Chapter 6).

We may, to some extent, gain an overall picture of the functional role played by the sex chromosomes in humans from clinical syndromes. Such experiments of nature include Turner's syndrome in females who are lacking one X-chromosome (XO). Understanding this requires a little more genetic information (Knickmeyer & Davenport, 2011). Females have two X-chromosomes of which one is suppressed by the long non-coding RNA (*Xist*) gene, resulting in the process called X-inactivation (Lee & Barolomei, 2013). Silencing the genes of one X-chromosome in females ensures that the balance for gene dosage is secured across both sexes, as males have only one X-chromosome. The loss of one X-chromosome in females (as in Turner's syndrome) produces a phenotype that differs according to which parent, mother or father, provided the inheritance for their only remaining X-chromosome. Only 3 per cent of pregnancies are viable with XO embryos. Most of these pregnancies terminate very early in the first trimester (Urbach & Benvenisty, 2009) due to failures in placental development. This is due to loss of the maternal X-chromosome. Generally speaking, Turner's syndrome (XO) survivors are usually missing the paternal X-chromosome, which impairs brain functioning, especially in the visual–spatial domain, and in mathematics. Those exceptionally few XO females that do survive placental dysfunctioning, due to the loss of the maternal-inherited X, have lower scores on most social and cognitive measures, and magnetic resonance imaging (MRI) scans show their neocortex to be thicker in the temporal region. Female patients missing the paternal X have enlargement of grey matter in the frontal cortex, but show little evidence of brain dysfunction. Again, we may conclude that it is primarily the contribution of the female X-chromosome which

provides the expression of genes to ensure adequate placental development and advanced brain functioning.

Males with an additional X-chromosome (XXY), named Klinefelter's syndrome after its discoverer, possess testes but no sperm are produced. Their brains are also changed, and they develop autistic symptoms with decreased brain activation in areas of the frontal cortex (Viana *et al.*, 2014). These frontal regions of the brain are important to social cognition, and this is revealed in XXY males who are unable to make correct recognition of affect (happiness, sadness, anger) from the examination of facial expressions. It is, therefore, not simply those genes functionally engaged in female and male reproduction that have accumulated on the female X-chromosome, but also those genes in the brain that are especially involved with social and intellectual abilities.

New gene-sequencing technologies have recently identified certain gene mutations associated with X-linked intellectual disability. These pathological variant genes have been identified in those females with skewed X-inactivation, resulting in their X-chromosome genes being expressed only when they are inherited from the father's X-chromosome. There is also a male over female predominance for Autism Spectrum disorders (ASDs) according to which of the X-chromosome's genes are expressed (Hoffbuhr *et al.*, 2002). ASDs comprise a complex group of behaviourally related disorders that are primarily found in males and are genetic in origin. One of the genes that has been identified on the X-chromosome that is important in ASD is the gene responsible for DNA methylation (*MeCp2*). This gene is required for silencing other genes during brain development, thereby regulating the spatial and temporal expression of developmentally important genes. Specific mutations to the *MeCP2* gene results in reduced brain size (primarily cortex and cerebellum) producing impaired social interactions (Hoffbuhr *et al.*, 2002). These so-called Rett syndrome patients share many of the neurological symptoms of autistics, while specific *MeCp2* mutations have been further identified in association with neuropsychiatric

disorders, such as schizophrenia. Skewing of X-inactivation in favour of the healthy X-chromosome avoids the *MeCp2* mutation and hence expression of the syndrome in females, but this is not possible for males, who have only the one X-chromosome.

Returning to the sex steroid hormones and their influence on behaviour, pioneering work on small-brained rodents (rats and mice) in the 1960s identified a critical period for sexual differentiation of the male brain around the time of birth (reviewed in de Vries & Sodersten, 2009). A single injection of testosterone to female rat pups at this critical time is sufficient to depress their feminine sexual behaviour later in life. Moreover, if at this later stage their ovaries are removed, and this neonatal androgenised female is now given the male hormone testosterone, then there is an adult enhancement of male patterns of behaviour. These experiments led to the conclusion that late in the embryonic life of males, the central nervous system is sexually undifferentiated, and future masculine behaviour is organised by hormones secreted by the immature testes (Herbert, 2015).These studies provided a biological basis for explaining sexual dimorphic behaviour, and it was tempting to relate such findings to humans. However, the rat brain has little resemblance to the human or any primate brain, and when similar experimental studies were undertaken with monkeys that possess a much larger executive brain than rodents, the outcome was very different. When female monkeys were prenatally treated with the hormone testosterone, they continued to show female patterns of behaviour when they reached maturity and were paired with males, albeit at considerably reduced levels (Herbert 2015). Importantly, these females did not exhibit masculinised behaviour, and they were delayed in starting their ovulatory menstrual cycles. In this context, the monkey's brain is very different when compared to the rodent, and a significant effect of social rearing is found which appears to be more consequential. Early social experiences can themselves have a major influence on the development of sexual behaviour in monkeys. This ranges from complete sexual inadequacy resulting from early separation of the male