Preface

In this book, the authors present new research in the study of sex chromosomes. Topics discussed in this compilation include the evolution of mammalian X chromosomes and X chromosome inactivation; the role of sex chromosomes in mammalian female fertility; the fate of the Y chromosome; the role of Y chromosome genes on tumor development risk in disgenetic gonads; deletion of amelogenin Y-locus; non-invasive prenatal diagnosis for fetal sex determination; and application of X chromosomal STR polymorphisms to individual identification.

Chapter I - For many years it was thought that the X chromosome in all three major lineages of mammals (monotremes, marsupials and eutherians) had a shared evolutionary history. However, monotreme and marsupial genome projects and the employment of molecular cytogenetic techniques have enabled the evolutionary history of mammalian X chromosomes to be more accurately traced. This research revealed that monotremes have a complex sex chromosome system consisting of multiple X chromosomes that share no homology to the X chromosome of therian (marsupial and eutherian) mammals. Therian sex chromosomes arose from a pair of autosomes with a subsequent addition to the X chromosome having occurred in the eutherian lineage.

The evolution of mammalian sex chromosomes has led to large, gene-rich X chromosomes and smaller, gene-poor Y chromosomes, which is thought to create an imbalance in transcriptional output between the X chromosome and autosomes in males that requires dosage compensation. This hypothesis has only been thoroughly interrogated since the advent of microarray and RNA-sequencing technologies but the findings of these studies remain controversial. However, it is more generally accepted that the dosage imbalance for X-borne

genes between the sexes requires some form of compensation; otherwise females would be expressing twice as much of an X-linked gene as their male counterparts. Compensation for this imbalance is deemed essential, as an extra copy of autosomal genes is usually deleterious. In eutherian mammals, this is achieved by transcriptionally silencing one X chromosome in female somatic cells. Fifty years of research has uncovered many of the features of this remarkable epigenetic phenomenon. However, research into X chromosome inactivation in marsupials and monotremes has lagged far behind that of their eutherian counterparts, largely due to the limited knowledge of genic content of their X chromosomes. Fortunately, genome sequencing of the platypus and several marsupial genomes has alleviated this problem and enabled such research to forge ahead at a great pace. This has led to a number of seminal findings and new hypotheses regarding the evolution of X chromosome inactivation.

Chapter II - Oogenesis takes place in the presence of two X-chromosomes in normal mammalian development. While the second X-chromosome is inactivated in all somatic cells, it is reactivated in the female germ cells prior to the onset of meiosis and remains active in the oocyte. The importance of this exception to the X-dosage-compensation rule is understandable but not formally proven. In this review, we discuss the fertility of females carrying atypical sex chromosomes, namely XO and XY. We will briefly touch on human cases, but mainly focus on mouse models. Our laboratory has been studying the mechanism of infertility in the B6.Y^{TIR} sex-reversed female mouse, which carries a single X-chromosome and an intact Y-chromosome. We review the findings from our study of this particular mouse model, with emphasis on the behaviour of sex chromosomes during the meiotic progression in the XY oocyte and the consequent ooplasmic defects leading to a failure in the second meiotic division.

Chapter III - The mammalian X and Y chromosomes were originally a homologous pair of chromosomes. The accumulation of deleterious mutations and the subsequent inactivation and loss of Y-linked genes could have led to the genetic degeneration of the Y chromosome over evolutionary time, approximately 300 million years (Myr). In modern humans, only approximately 45 Y-linked genes remain. Most of these have acquired functions that are essential for maleness, for example, sex determination or spermatogenesis. It has been proposed that the loss of human Y-linked genes is inexorable and might lead to the extinction of the entire species because of the active role that this chromosome plays in male function. The contrary opinion suggests that the remaining Y-linked genes are strictly conserved

through purifying selection. Recent comparative genomic analysis among the Y-chromosome sequences of primates, human, chimpanzee, and rhesus macaque, showed a high level of conservation of human Y-linked genes, at least over the past 25 Myr of the human lineage. However, rodents include very interesting species in which complete loss of the Y chromosome has occurred. Two species of Ryukyu spiny rat and one species of mole vole have an XO/XO sex chromosome constitution due to absence of the Y chromosome. Furthermore, in these species, a key mammalian sex-determining gene, *SRY*, was also lost upon disappearance of the Y chromosome. These XO species show us that loss of the Y chromosome would not lead to the extinction of humans, and there are several processes of Y evolution in our future.

Chapter IV - The presence of Y-chromosome material in patients with dysgenetic gonads increases the risk of gonadal tumors, especially gonadoblastoma (GB). In 1987, Page hypothesized that there is a locus, termed GBY (gonadoblastoma on the Y chromosome), which predisposes the dysgenetic gonads to developing such *in situ* tumors. Studies have been suggested that the testis-specific protein Y encoded (TSPY) repeated gene is the putative region for this oncogenic locus, and could potentially be involved in other human cancers. Hildenbrand et al. studied a patient with TS and a 45,X/46,X,mar karyotype who developed unilateral gonadoblastoma. Cytogenetic and molecular studies confirmed that the marker was derived from a Y chromosome. The authors investigated the gonadal material by immunohistochemistry for the expression of the *TSPY* gene, and the results revealed a high level of TSPY protein expression. However, one of the Y sequences most frequently used in Turner Syndrome (TS) patients screening is the *SRY* gene, because of its localization and its important role in the signaling cascade of sex determining events.

It is already well established that the presence of Y-chromosome material in patients with dysgenetic gonads increases the risk of gonadal tumors, such as gonadoblastoma and dysgerminoma, or of nontumoral androgen-producing lesions. This confers clinical importance to the detection of the Y-chromosome mosaicism in TS.

Screening for Y-chromosome specific sequences is important in the follow-up of TS patients, considering that it is correlated to the proven risk of gonadal tumors development. Thus, the early detection of such events could be of great importance to preventing of gonadal tumor development in TS patients.

The *SRY* gene plays a pivotal role in the signaling chain that occurs during embryonic development, functioning as an activator and also being regulated

by several genes. Moreover, it is of fundamental importance in cell differentiation and, consequently, in the determination of the gonadal microenvironment, which starts interacting in the presence of androgens. This way, genes that perform in sex differentiation and development would have an altered expression in the dysgenetic gonads of TS patients, possibily implying in gonadal tumorigenesis. Significant difference in the dysgenetic and controls gonads regarding the expression of genes *OCT4*, *SRY*, and *TSPY* in both gonads of a case characterized by temporal exposure of dysgenetic gonads to the Y-chromosome sequences. This fact could lead to neoplastic development as a result of accumulated modifications in the gonadal microenvironment, especially under the hormonal activity that characterizes the pubertal period, even in the absence of histopathologic abnormalities of the gonads.

Chapter V - Accurate gender determination is widely used and it is crucial in many scientific disciplines, especially in profiling for DNA databasing, forensic casework (e.g., identifying the gender of biological material in stains of unknown origin), analysis of archeological specimens, preimplantation/prenatal diagnosis and post-natal diagnosis (e.g., X-linked diseases or children with ambiguous genitals). Today, molecular techniques, also based on length variation in the X–Y homologous amelogenin gene (AMELX and AMELY), are used for sex determination. In humans, the amelogenin gene is a single copy gene located on Xp22.1–Xp22.3 and Yp11.2 and it is sufficiently conserved, so the simultaneous detection of the X and Y alleles using polymerase chain reaction can lead to gender determination. There is a size difference of 6 bp between the X and the Y genes in the most widely used PCR primer set. The presence of two amplified products indicates a male genotype, while a single amplicon implies a female genotype. Several studies, published since 1998, have shown that normal males may be typed as females with this test because the amelogenin gender test may not always be concordant with true male gender: AMELY deletions may result in no amplification product and normal males being typed as female with the test (negative male).

To date literature data have supported that the null allele is the result of a larger deletion on the short arm of the Y chromosome and that this occurs in different percentages in different population groups. Considering the consequences of the result obtained using only the amelogenin marker and the potential related interpretation difficulties, the gender misinterpretation may be troublesome in some cases, both in clinical practice and forensic caseworks.

Different strategies have been proposed to solve this misinterpretation, such as the use of additional markers to resolve the possible occurrence of

AMEY deletion. In this paper we propose a review of the incidence in failures of gender testing among different populations and the different strategies proposed in literature in case of doubt regarding the presence of deleted AME in the DNA profile.

Chapter VI - Clinical indications for fetal sex determination include risk of X-linked disorders, a family history of conditions associated with ambiguous development of external genitalia and some fetal ultrasound findings. It is usually performed in the first trimester from fetal material obtained through CVS and is associated with an approximately 1% risk of miscarriage. Ultrasound fetal sex determination is often performed after 11 weeks of gestation.

Diagnosis of fetal sex was one of the earliest developed tests for non invasive prenatal diagnosis (NIPD) from the 7th week of gestation using cell free fetal DNA (cffDNA) circulating in maternal plasma. The majority of reported studies are based on quantitative real-time PCR (RT-qPCR) analysis of the SRY gene, achieving sensitivities of 90–100%, with an extremely low incidence of false positive results. False negative results are usually due to failure of amplification of the SRY gene in male fetuses caused by undetectable levels of cffDNA in maternal plasma. A gender independent fetal marker is therefore necessary to verify the presence of fetal DNA sequences. Female fetuses are not detected directly, but by the absence of Y chromosome specific sequences.

Fetal sex determination by cffDNA analysis is currently performed clinically in many centers in order to avoid conventional invasive testing in pregnant women at risk for X-linked and endocrinal disorder.

Chapter VII - The human DNA markers most commonly utilized in individual identification are autosomal short tandem repeat (STR), followed by Y-chromosome STRs and mitochondrial DNA. X-chromosomal short tandem repeat (X-STR) loci may efficiently complement autosomal markers in paternity testing, especially in deficient paternity cases with female offspring, and in kinship analysis involving large and incomplete pedigrees. X-STR loci are located on the non-recombining region of the X chromosome and are inherited as a block of linked haplotypes. Due to its unique inheritance pattern, the X chromosome is a potential candidate for forensic and human identity testing applications. Currently, more than 40 X-STRs have been established as forensic markers, and a large number of population data have been published. X-STR haplotyping can be of particular help in kinship testing in deficient paternity cases where a DNA sample from one of the parents is not available for testing. The ideal technique for X-STR typing is multiplex PCR, because

as the number of polymorphic loci examined increases, the probability of identical alleles being present in two different individuals decreases. Multiplex systems have been developed in order to apply X-STRs efficiently for paternity testing. In view of the wide application of these markers, several X-STRs multiplex PCR systems have been validated for individual identification, which include four to thirty markers. Multiplex with greater number of markers are being developed to obtain a high degree of discrimination. Samples from a mass disaster site or from a crime scene exposed to environment are often not only highly degraded but also in very scarce quantities, making it difficult for scientists to perform multiple PCR analyses. Analysis of degraded DNA samples using mini X-STR multiplex systems will offer high efficacy for personal identification.