The human Y chromosome: a sole survivor
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Chapter 1

Introduction

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General introduction

Once considered to be involved in multiple traits, later described as a degenerative chromosome of no scientific interest beyond sex determination, the human Y chromosome is nowadays implicated in gonadal sex reversal, Turner syndrome, graft rejection and, especially, spermatogenic failure (Skaletsky et al., 2003).

The human Y chromosome stands out from all other human chromosomes as it is only present in males and is clonally, i.e. without any change, passed down from father to son, apart from two regions that can pair and cross-over with the X chromosome, namely PAR1 and PAR2 (Figure 1). Due to this unique father-to-son transmission, the human Y chromosome was thought to be involved in nineteen traits, including hairy ears and scaly skins, at the beginning of the 20th century (Painter, 1921). By the late 1950s, these ideas were discarded and the sole purpose of the Y chromosome was believed to be sex-determination (Stern, 1957). This assumption was primarily based upon the findings that individuals with a 45, X karyotype were females and individuals with a 47, XXY were males (Jacobs and Strong, 1959; Ford et al., 1959). Apart from a Sex-determining Region on the Y chromosome (SRY), the Y chromosome was considered to be a genetic wasteland.

In 1976 a landmark study described deletions of the long arm of the Y chromosome (Yq) in men that had azoospermia, i.e. men without sperm in their ejaculate (Tiepolo and Zuffardi, 1976). The researchers postulated that an azoospermia factor (AZF) had to be present on Yq. At that time, it was not possible to identify AZF or to pinpoint it more precisely on Yq due to the limitations of the available research techniques.

**Figure 1.** Schematic diagram of the human Y chromosome. The centromere (cen) separates the short arm (Yp) from the long arm (Yq). The Male specific part of the Y (MSY) is flanked by two pseudo-autosomal regions (PAR1 and PAR2) that still undergo pairing and exchange with the X-chromosome. The long arm (Yq) of the chromosome contains a large block of heterochromatin (het) that is known to vary in size between men. In blue, the euchromatic ampliconic sequences are indicated. The position and size of recurrent Y chromosome deletions are shown as black bars. The asterisk indicates the b2/b3 deletion that can only occur as an inverted variant of the AZFc region; the location of the deletion is therefore depicted here in relation to the reference sequence.
Almost two decades later, more advanced molecular research techniques such as Polymerase Chain Reaction (PCR) were used in the hunt for the illusive AZF-gene on the Y chromosome and the first gene thought to be AZF was identified: RNA Binding Motif on the Y (RBMY) (Ma et al., 1993). Later researchers could not confirm the presence of RBMY in the AZF region, but a novel gene, called Deleted in Azoospermia (DAZ), was identified instead in this region (Reijo et al., 1995).

Since that discovery, the DAZ gene has been the subject of many studies. DAZ was originally thought to be located on Yq as a single copy gene, later to be present with copy numbers ranging between two and seven (Reijo et al., 1995; Yen et al., 1997; Glaser et al., 1997; Yen, 1998; Saxena et al., 2005), but finally determined to be normally present as a four copy gene family on the Y chromosome (Saxena et al., 2000; Kuroda-Kawaguchi et al., 2001; de Vries et al., 2002). These four genes are arranged in two clusters and within each cluster there are two copies in a head-to-head orientation. Within the DAZ family, there is intragenic variation of a 10.8 kb RNA recognition motive (RRM) which can vary in number between one and three and of a smaller 2.8 kb repeat. the DAZ repeat, that can vary in copy number and in signature (Yen et al., 1997; Skaletsky et al., 2003; Saxena et al., 2005). In 1996, it was reported that Yq contained three non-overlapping AZF regions that could be deleted in men with spermatogenic failure and which were termed AZFa, AZFb and AZFc respectively with the DAZ genes located in the AZFc region (Vogt et al., 1996).

In 2003, knowledge of the Y chromosome was propelled forward with the publication of the male-specific region of the Y chromosome (MSY) that was reported to contain 78 genes (Skaletsky et al., 2003). The MSY constitutes 95% of the Y chromosome and does not recombine with the X chromosome. The MSY is built up from heterochromatic and three types of euchromatic sequences: X-transposed, X-degenerate and ampliconic sequences. The X-transposed and X-degenerate sequences combined represent 55% of the euchromatic sequences and harbor nineteen MSY genes of which the majority is ubiquitously expressed. Their functions are so far largely unknown. The ampliconic sequences contain the remaining MSY genes, which are exclusively or predominantly expressed in the testis. These ampliconic sequences are arranged in direct and inverted repeats and in palindromes – large inverted repeats with very little intervening sequence. These structures evolved via intrachromosomal duplications and hampered for a long time Y-chromosomal mapping.

Ampliconic sequences of the Y chromosome are targets for ectopic homologous recombinations and consequently can lead to Y chromosomes with inversions, deletions or duplications or to isodicentric Y chromosomes (Repping et al., 2006; Lange et al., 2009) (Figure 2). To date, of all Y chromosome recombination events, interstitial deletions have been described most often. Seven of these deletions are recurrent: AZFa, P5/proximal-P1 (AZFb), P5/distal-P1 (AZFb+c), b2/b4 (AZFc), gr/gr, b2/b3 and b1/b3 deletions (Vogt et al., 1996; Sun et al., 2000; Kuroda-Kawaguchi et al., 2001; Repping et al., 2002; Repping et al., 2003; Fernandes et al., 2004) (Figure 1). These deletions were identified and characterized using several molecular techniques, i.e. karyotyping, sequence tagged site (STS) analysis, sequence nucleotide variance (SNV) analysis, Southern blot analysis and/or fluorescence in situ hybridization (FISH). Of these seven recurrent deletions, the AZFa, P5/proximal-P1 and P5/distal-P1 deletions result in azoospermia (Oates et al., 2002). The b2/b4 deletion is found in men with azoospermia and in men with severe oligozoospermia (Oates et
The *gr/gr* deletion is a risk factor for spermatogenic failure as it is found in men with azoospermia or oligozoospermia but also, at a lower frequency, in men with normozoospermia (Visser et al., 2009). The *b2/b3* and *b1/b3* deletions do not appear to affect spermatogenesis (Fernandes et al., 2004; Repping et al., 2004).

Recently the large scale organizations of Y chromosomes from 47 different branches of the Y chromosome genealogical tree was studied (Repping et al., 2006). This study showed that rearrangements caused by homologous recombination are very common among Y chromosomes. Eighteen chromosomes with an *AZFc* structure other than the reference sequence were found. Of these eighteen chromosomes only nine had a genomic content other than the reference sequence. It was therefore hypothesised that this constraint in genomic content, shown by the relative underrepresentation of deletions and duplications compared to inversions, was due to selection.

![Diagram](image)

**Figure 2.** Schematic representations of homologous recombination events that can occur on the human Y chromosome: (A) the inversion and (B) deletion which are the result of intrachromosomal homologue recombination and the (C) duplication and deletion and (D) isodicentric and acentric Y chromosomes that are the result of interchromosomal recombination.

Despite the vast amount of research on Y-chromosome aberrations, the clinical impact has been limited to the advice of informing patients diagnosed with azoo- or severe oligozoospermia about these aberrations, the option to screen for them and the implications of finding such an aberration for the patient and for his (male) offspring. However, most men with azoo- or oligozoospermia do not carry a known Y-chromosome aberration and thus the origin of their poor semen quality still remains unknown. This warrants further research into identifying more (genetic) causes of reduced semen quality, and for obvious reasons the Y chromosome is the ideal candidate chromosome for these investigations. Of even greater importance is the lack of direct treatment of male infertility. Currently, male infertility is circumvented by applying ICSI with ejaculated or surgically retrieved spermatozoa, which is burdensome for the female partner, has limited success and is costly.
Therefore it is expected that based on data of future (genetic) research, new treatments will be developed for a direct cure of male infertility.

Before writing this thesis, known genetic causes of male infertility that originate from Y-chromosome aberrations were limited to the above described deletions. We therefore aimed at identifying additional Y-chromosome aberrations that are causative in male infertility. In doing so we focused on discovering (partial) duplications of the \textit{AZFc} region, deletions in the \textit{AZFc} region that occurred via non-homologous recombination, large scale inversions on the Y chromosome, pseudo iso-Y chromosomes, \textit{AZFc} gene copy numbers and intragenic-repeat variation of \textit{DAZ}. 
Outline of the thesis

Chapter 2 is a detailed review of the chromosomal make-up of the Y chromosome prior to the start of this thesis. In the review, reported recombination events on the Y chromosome, associated phenotypes and potential future research topics are discussed.

Chapter 3 describes the intergenic variation of the DAZ gene in men from different Y haplotypes and men with partial AZFc deletions. We used Southern blot to map intergenic variability in 47 samples originating from different branches of the Y genealogical tree and in 51 samples carrying partial AZFc deletions. In addition, we investigated whether the intergenic variation in DAZ affects semen quality in men with partial AZFc deletions.

Chapter 4 focuses on (partial) duplications of the AZFc region. We screened for these (partial) duplications in a cohort of 845 men with variable semen qualities and determined the copy numbers of DAZ, BPY2 and CDY1/CSPG4LY/GOLGA2LY via a novel qPCR method. Next we examined the effects of increased copy numbers of these genes on semen quality. In addition, we examined the origin of these duplications in relation to the Y-chromosome genealogical tree.

Chapter 5 investigates the effect of gene copy number variation in men with (partial) deletions of AZFc. We screened for these (partial) deletions via STS PCR in a cohort of 840 men with variable semen qualities and subsequently determined the copy numbers of DAZ, BPY2 and CDY1/CSPG4LY/GOLGA2LY via qPCR. Next we examined the effects of individual copy numbers of these three genes on semen quality.

Chapter 6 describes novel deletions of the proximal part of the AZFc region that do not occur via homologous recombination. We characterized these deletions using STS PCR, qPCR and Southern blot. We present a model via which these deletions can occur and examine their effect on semen quality. Furthermore, we examined their evolutionary age and occurrence over the Y genealogical tree.

Chapter 7 reports on two different events that can occur via homologous recombination between two inverted repeats of which one copy is located on Yp and one on Yq. We present models via which these events can occur and screen our databases for such cases. We test if the identified cases match our models via FISH and STS PCR.

Chapter 8 summarizes the results obtained, discusses the techniques used and suggests future research goals.
References


Chapter one


