

Review

# The Role of Number of Copies, Structure, Behavior and Copy Number Variations (CNV) of the Y Chromosome in Male Infertility

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**Abstract:** The World Health Organization (WHO) defines infertility as the inability of a sexually active, non-contracepting couple to achieve spontaneous pregnancy within one year. Statistics show that the two sexes are equally at risk. Several causes may be responsible for male infertility; however, in 30–40% of cases a diagnosis of idiopathic male infertility is made in men with normal urogenital anatomy, no history of familial fertility-related diseases and a normal panel of values as for endocrine, genetic and biochemical markers. Idiopathic male infertility may be the result of gene/environment interactions, genetic and epigenetic abnormalities. Numerical and structural anomalies of the Y chromosome represent a minor yet significant proportion and are the topic discussed in this review. We searched the PubMed database and major search engines for reports about Y-linked male infertility. We present cases of Y-linked male infertility in terms of (i) anomalies of the Y chromosome structure/number; (ii) Y chromosome misbehavior in a normal genetic background; (iii) Y chromosome copy number variations (CNVs). We discuss possible explanations of male infertility caused by mutations, lower or higher number of copies of otherwise wild type, Y-linked sequences. Despite Y chromosome structural anomalies are not a major cause of male infertility, in case of negative results and of normal DNA sequencing of the ascertained genes causing infertility and mapping on this chromosome, we recommend an analysis of the karyotype integrity in all cases of idiopathic fertility impairment, with an emphasis on the structure and number of this chromosome.

**Keywords:** Copy number variations (CNV); karyotype; mosaicism; epigenetics; aneuploidy

## 1. Introduction

The World Health Organization (WHO) defines infertility as the inability of a sexually active, non-contracepting couple to achieve spontaneous pregnancy within one year [1]. Many studies focused on the possible causes of infertility, and it has been proven that, globally, both sexes are equally involved [2]. Traditionally, male infertility has been divided into three major etiological categories: Pretesticular, testicular, and post-testicular [3]; however, another new clinically based classification has been recently proposed, considering: (a) The hypothalamic–pituitary axis function, (b) quantitative spermatogenesis, (c) qualitative spermatogenesis, and (d) ductal obstruction or dysfunction [4], all of these including both genetic and non-genetic causes. Focusing also on the clinical characteristics of the patient and not only on the anatomical/temporal characteristics of infertility might be a better approach in the diagnosis and treatment of this condition, since male infertility can be caused by several, different factors that may affect semen quality in very different ways, so that this quality can be seen as the final output of different pathophysiological mechanisms. These two classification methods are summarized in Table 1 to highlight similarities and differences better. Male fertility can be influenced by congenital or acquired urogenital anomalies, malignancies, urological infections, varicocele, genetic abnormalities, endocrine disorders and immunological factors [5]. However, it is not uncommon to diagnose idiopathic male infertility in men with normal anatomy, no history of familial fertility-related diseases and a normal panel of values as for endocrine, genetic and biochemical markers: In fact, in 30–40% of cases no male infertility-associated factor is found [5]. Azoospermia is the absence of spermatozoa from the seminal fluid and can be caused by non-obstructive (80–85%) or obstructive events (15–20%) although these values may significantly vary according to different studies [6]. Obstructive azoospermia can be further divided into (a) intratesticular (post-inflammatory or post-traumatic), (b) epididymal (secondary to epididymitis or chronic infections), (c) vas deferens (post-vasectomy), (d) ejaculatory duct (cystic or post-inflammatory), and (e) distal seminal ducts (local neuropathy) obstruction [7]. Finally, environmental factors, such as tobacco smoking and assumption of steroids, seemingly also play a role in altering male fertility [8]. Prognostic factors for male infertility involve the analysis of semen quality, which includes both sperm quantity and quality. Table 2 reports the minimum values of semen features, according to the WHO data, for normozoospermic men [9]. Recent studies demonstrate a general decline of semen quality in Western countries [10] without a clearly identified cause. Age is another cause of decreased semen quality, which affects the genetic quality of sperm (damaged DNA), its volume, spermatozoa motility and morphology [11]. Thus, in the standard laboratory tests (Table 2), semen quality is defined as follows: Oligozoospermia if there are <15 million spermatozoa/ml; asthenozoospermia if there are <32% of progressively motile spermatozoa; teratozoospermia if there are <4% of normal forms. It is also important to evaluate if infertility is primary or secondary, i.e., if the couple has become pregnant at least once in the past; this helps to discriminate congenital from acquired causes.

**Table 1.** Comparison between the traditional and newly proposed, clinically based classification of male infertility etiology.

<b>Traditional Classification</b>	<b>Examples</b>
Pre-testicular causes	Hypogonadotropic hypogonadism Pituitary diseases Coital disorders
Testicular causes	Varicocele Cryptorchidism Noonan Syndrome Vanishing Testis Syndrome Myotonic dystrophy 46,XX testicular disorders of sex development 47,XYY Syndrome Klinefelter's Syndrome Y chromosome microdeletions Sertoli Cell-Only Syndrome (germ cell aplasia) Gonadotoxins Systemic diseases Testis injury Idiopathic infertility
Post-testicular causes	Male reproductive tract obstruction Disorders of sperm function or sperm motility Disorders of coitus
<b>Clinically Based Classification</b>	<b>Examples (Top: Genetic Causes; Bottom: Non-Genetic or Mixed Causes)</b>
Hypothalamic–pituitary axis	Kallmann's Syndrome Ablative treatments (e.g., surgery or radiation); pituitary adenomas; tumors of the CNS; infection; infiltrative disease; empty sella syndrome; autoimmune hypophysitis; abuse of anabolic steroids; testosterone-replacement therapy; use or abuse of opiates and their analogues
Quantitative Spermatogenesis (i.e., affecting the production and numbers of sperm)	Gross chromosomal/karyotype anomalies; submicroscopic deletions (such as AZF deletions); Klinefelter's Syndrome (47,XXY); 46,XX male syndrome; isodicentric Y chromosome; structural aberrations of the autosomes; X-linked genetic anomalies (such as <i>AR</i> or <i>TEX11</i> gene mutations) Varicocele; previous cytotoxic chemotherapy or radiotherapy; mumps; viral orchitis; testicular torsion; gonadal malignancy; severe scrotal trauma; some common medications; severe systemic illness; cryptorchidism
Qualitative Spermatogenesis (i.e., affecting the characteristics of sperm, such as motility, fertilization, and genetic competency)	Globozoospermia (e.g., <i>SPATA16</i> , <i>PICK1</i> , <i>DPY19L2</i> gene mutations); macrozoospermia (e.g., <i>AURKC</i> gene mutations); ageing Oxidative stress; inflammation; infection; autoimmune reaction against the spermatozoa; phospholipase C $\zeta$ deficiencies
Ductal obstruction or dysfunction	Congenital bilateral absence of the <i>vas deferens</i> (CBAVD) (e.g., <i>CFTR</i> gene mutations) Vasectomy; epididymal occlusion; previous inguinal hernia repair with inadvertent interruption or scarring of the <i>vasa</i> ; spinal cord injury affecting ejaculation; retrograde ejaculation; erectile dysfunction; Young's Syndrome

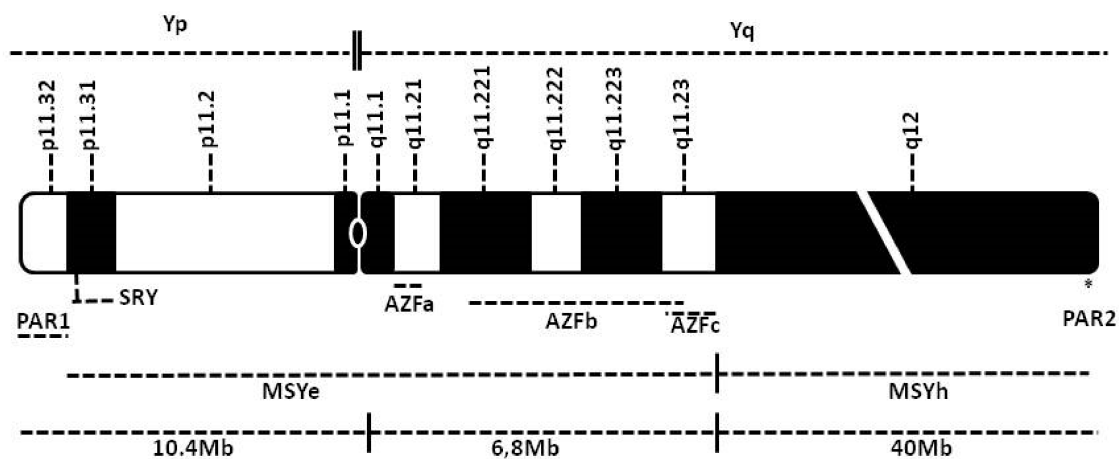
**Table 2.** Semen characteristics in normozoospermic men. Data is according to the World Health Organization reference values [9]. Data indicate lower reference limits, the fifth centiles (with 95% confidence intervals). Time to pregnancy (TTP) is defined as the number of months elapsed between stopping contraception to achieving a pregnancy [12].

Parameter	Value (Interval)
Time to pregnancy (TTP)	12 months (upper limit)
Semen volume	1.5 mL (1.4–1.7)
Total spermatozoa number	39 million per ejaculate (33–46)
Spermatozoa concentration	15 million per mL (12–16)
Vitality	58% live (55–63)
Progressive motility	32% (31–34)
Total motility (progressive + non-progressive)	40% (38–42)
Morphologically normal forms	4.0% (3.0–4.0)

In this review, we discuss the male sterility caused by alterations in the shape, overall content or behavior of the Y chromosome, and describe how the size variation of specific regions of this chromosome or Y chromosome aneuploidies, irrespectively of point mutations in coding gene sequences, affects the male fertility.

## 2. Overview of the Y Chromosome Evolution and Structure

The two most important features of the genetic behavior of human Y-chromosomes are the lack of homologous chromosome exchanges for many of its regions (except for PAR1 and PAR2, as described below), and a male-limited transmission of the male-specific Y (MSY) segment [13] (Figure 1). Sex chromosomes are derived from autosomes [14–16], and encode the master-sex determining proteins, i.e., Testis Determining Factor (TDF) on the Y (see the section ‘The Sex-Determining Region of the Y (SRY)’ and Androgen Receptor (AR) on the X [17]. Massive gene decay and transposition have characterized the Y chromosome evolution [18,19], which only contains 3% of the genes of its ancestor [20]; for comparison, the Y chromosome harbors 54 protein-coding genes, while the X chromosome hosts ~700 [21]. Consequently, compared to the other human chromosomes, the Y chromosome has a limited number of genes, since they tended to degenerate during the evolution [22,23], showing a high degree of variability, among which copy number variants contribute the greatest predicted functional impact [24,25]. Overall, the Y chromosome is considered the relic of ancient autosomes that became the ancestors of both allosomes through sequential chromosomal fusions and rearrangements [20]. This karyotype evolution paralleled that of the genetic sequences mapping on both sex chromosomes, which indeed are enriched in dosage-sensitive genes, mirroring the gene dosage compensation mechanism typical of mammalian sex chromosome biology [20,26]. In particular, it has been shown that the genes in common between the sex chromosomes are enriched for important regulatory functions and predictors of haploinsufficiency [20,27,28]. The Y chromosome (comprising over 57 million base pairs); contains genes and highly repetitive DNA sequences, which harbor pseudogenes without any known function [29].



**Figure 1.** Overview of the Y chromosome organization. Rows from the top: (1) Short (Yp) and long (Yq) arms of the Y chromosome; (2) numbered cytogenetic bands; (3) chromosome banding according to the US National Library of Medicine, retrieved on October 25, 2018, at <https://ghr.nlm.nih.gov/chromosome/Y#idiogram> (note: The region q12 is shortened (white cut), due to its large extension compared to the rest of the chromosome); (4) localization of the *SRY* gene, of the PAR regions (PAR2 is indicated by an asterisk, due to its small size) and of the AZF regions; (5) extension of the euchromatic (MSYe) and heterochromatic (MSYh) portions of the MSY region; (6) approximate length in megabases (millions of base pairs, Mb) of the short arm, euchromatic portion of the long arm and heterochromatic portion of the long arm.

### 3. Mosaics and Y Chromosome Structural Anomalies

#### 3.1. Mosaics

Because of a mis-segregation of the sex chromosomes during the very early stages of development, some individuals may become mosaics, i.e., in the simplest case they harbor a mixture of cells whose karyotype is either 46,XY or 45,X [30]. The status of these patients' gonads is largely unpredictable, and deeply influences their phenotype and sexual habitus. The most extreme cases are bilateral nonfunctional rudimentary streak gonads (female habitus; typical symptom: Missing puberty) and bilateral small scrotal testes (male habitus; typical symptom: Infertility consequent to azoospermia or severe oligospermia). All possible intermediate situations have been described. As a consequence, the patient's habitus may be male, female or ambiguous, depending on the extension, localization and time of formation of mosaic patches, or based on other, rarer mechanisms, such as chimerism [31]. The gonadal function is, in turn, influenced by the number of cells that either have or do not a Y chromosome; indeed, there seems to be a minimum threshold of Y-bearing cells in the gonadal ridge that allows the development of a male habitus [32]. Notably, the percentage of SRY-positive cells in the gonads may be significantly different from that of the peripheral blood cells (the standard tissue used for karyotyping) because the embryological origin of the latter cells is in the mesoderm, while the former cells arise extragonadally in connection with the endoderm [33]. A similar situation is described for buccal cells of ectodermic origin [32]. Relying on the formation of mosaics in patients with rearranged, dicentric Y chromosomes, some authors suggest that a greater instability of the aberrant Y chromosome might promote its loss during the cell divisions of the germline, thus, promoting the development of a female habitus [34].

Among all mosaic patients, those with a male phenotype are characterized by bilaterally descended testes and represent 11–12% of the total [35–37]. The diagnosis of male mosaic patients usually occurs during fertility evaluations, upon discovery of azoospermia and elevated serum gonadotropins levels, consistent with testicular function failure [30]. Because of the increased risk of gonadoblastoma formation, present medical guidelines suggest removing the non-functional gonads, especially if this condition is diagnosed during childhood.

We conclude this section by recalling a recent work showing the connection between Y chromosome mis-segregation and its subsequent rearrangement(s) [38]. This study demonstrates that, in some cases (and if not simply lost), the mis-segregating Y chromosome, in the time frame of three consecutive cell cycles, is first included in a micronucleus, then fragmented, and finally, its fragments are re-joined through the error-prone Non-Homologous End Joining (NHEJ) mechanism (similarly to its autosomic counterparts [39]). This outcome sets the molecular and cytological bases for—at least some-of the chromosome aberrations described below.

### 3.2. Chromosome Rings and Isodicentric Chromosomes

Approximately half of the mosaic patients have an abnormal Y chromosome, shaped like a ring. Ring chromosomes of the Y (rY) have no free extremities (telomeres) and are shaped as circular DNAs. This rearrangement may be due to several causes, including telomeric fusions or terminal deletions of both arms that are subsequently (erroneously) attached together by DNA repair mechanisms. In the latter case, usually, rY-bearing cells do not have the Y-derived, terminal, acentric fragments, which are lost during cell cycles; the length of these lost fragments is extremely variable. rY are intrinsically unstable in the genome and prone to mis-segregation during cell division, mainly because of the sister chromatids entanglement during the replication phase, causing anaphase lags and chromosome breakage, leading to further rearrangements. In these cases, the mosaic usually is a consequence of a previously formed rY. These patients' cells either have a numerically normal (46,X,r(Y)) or aneuploid (45,X; i.e., the Y chromosome is missing) karyotype. In a minority of these patients' cells, there is the complementary situation, i.e., they have two rY chromosomes (47,X,2r(Y)), but this happens sporadically, because these cells also have increased genome instability. In other cells, the Y chromosome is further rearranged to create a single ring from two or more Y chromosomes, harboring a variable number of centromeric sequences [40]; this situation is sporadic and very unstable as well. In addition, interstitial deletions inside the rY are relatively frequent, even in distinct cells from the same patient [30]. All these conditions usually emerge *de novo* after fertilization or during the father's meiosis, because of the sterility of patients with rY. The only exception, to the best of our knowledge, was described by Arnedo and collaborators in 2005 [41]. As a general rule, mosaic patients with a male habitus manifest gonadal failure and short stature, as well as increased prevalence of cardiorenal malformations and germ cell tumors [35,37]; otherwise, especially in the absence of additional, very long interstitial deletions, mosaic subjects are normal males, and their phenotypes are mainly related to the extension of Y-linked terminal lost sequences and to the total amount of aberrant cells.

Besides the further rearrangements of the ring chromosomes, Y chromosomes showing two or more centromeric sequences (isodicentric Y chromosomes, idicY) are also found in the absence of rY. Depending on the Y portion that is present, idicY can be associated with either a male or female phenotype [42,43]. These aberrant chromosomes arise, in most cases, as an output of homologous crossing-over between opposing arms of palindromes on sister chromatids [43,44]. These ectopic recombination events occur at nearly all Y-linked palindromes, excluding the smaller one (P7), although this might be just a consequence of the relatively small number of patients analyzed. In a minority yet a significant proportion of patients, idicY formation follows the recombination events occurring within the pericentromeric heterochromatin [43,44]. idicY are found at relatively high frequency in men with non-obstructive azoospermia, and this strong phenotype is likely due to the loss of important genes, mapping on the Y chromosome, consequent to the rearrangement. Interestingly, mosaicism (45,X-46,X,idicY) in these individuals is high [45]; in addition, there is the chance that one of the two centromeres in the Y chromosome is functionally inactive, in a direct relation with their distance (i.e., the longer the distance, the higher the probability of inactivation) [44]. Because of the intrinsic genomic instability of dicentric chromosomes, it is likely that both phenomena (loss of the aberrant Y chromosome and inactivation of the second centromere) occur because idicY-bearing cells are less viable and more unstable than the aneuploid ones.

### 3.3. Chromosome Translocations

Male patients may also have a 46,XX karyotype; they are identified as 46,XX testicular Disorder of Sex Development (DSD) [46], a.k.a. de la Chapelle syndrome [47]. The incidence of this condition is estimated to be 1/20,000 to 25,000 male births [48,49], and, to date, only a few hundred patients have been described [50]. Most of them show rearranged chromosomes, with a variable portion of the Y chromosome short arm—bearing the *SRY* gene—translocated to another chromosome. The most frequent occurrence is an X-Y translocation, consequent to an aberrant crossing-over during the father's meiosis [51]. However, also autosome-Y (A-Y) translocations have been reported. If these translocations are not genetically balanced (the far more frequent event), several phenotypes are present, due to the missing loci from both the Y and the autosome involved. Instead, in a few cases, it has been shown that a balanced A-Y translocation without significant DNA loss might be transmitted across several generations without impairing the male fertility [52]. About 80% of the translocated X-Y individuals have normal pubic hair growth after puberty and normal penis sizes, but usually also have small testicles, and their infertility is caused by azoospermia, consequent to AZF loci loss. Gynecomastia is present in about 30% of cases [53]. The male habitus of these individuals is typically attributed to the presence of the *SRY* gene, which acts as a dominant determinant of sex development; indeed, the *SRY* gene function is positive in approximately 80% of these individuals [53]. However, the mere presence of *SRY* is not sufficient for masculinization, as X inactivation in XX males may play an important role in *SRY*-positive XX males. This occurs because the *SRY*-bearing X chromosome is activated during the development in 90% of cases, while in the remaining cases *SRY* is inactivated together with the X chromosome it is attached to, thus, causing masculinization failure [54]. *SRY*-negative, infertile men have been reported as well [55–57]. In these patients, the virilization is not directly due to the Y chromosome behavior, but to a mutation that either causes the over-expression of X-linked or autosomal genes promoting masculinization (such as *SOX3*, *SOX9*, *SOX10*) or the down-regulation of autosomal genes blocking it (such as *WNT4* and *RSP01*) [58,59]. On the X chromosome, a similar habitus is caused by loss-of-function mutations in the Androgen Receptor (AR) coding gene [17,50]. Since masculinization, in these cases, is not strictly driven by the Y chromosome, we will not discuss them.

## 4. Y Chromosome Aneuploidies

### 4.1. Disomy of the Y

Y chromosome hyperploidy is important for male infertility, since they can unveil the possible role of additional—but otherwise, wild type—copies of genes mapping on Y. Differently from what described in the previous section, in this case, all patients' cells have the same karyotype since the mis-segregation occurs before fecundation, i.e., in the father's germ line. During meiosis, homologous chromosomes segregate in secondary spermatocytes and then sister chromatids further segregate in spermatids. Meiosis impairment may cause chromosome mis-segregation and, if sex chromosomes are involved, the resulting gametes will produce, upon fecundation, aneuploid individuals. Most commonly, the offspring will be affected either by Turner (45,X karyotype, female habitus) or by Klinefelter syndrome (47,XXY karyotype, male habitus). Both syndromes are considered a consequence of the meiotic behavior of the X chromosome [60] either in the father or in the mother; thus, they are outside the scope of this review.

Sporadically, during the father's meiosis II, the two sister chromatids of the Y chromosome do not segregate, causing the formation of an aberrant sperm having 24 chromosomes and containing two Ys. If this sperm fertilizes a normal egg, the individual will have a 47,XYY karyotype (a condition known as XYY syndrome or Jacobs syndrome); thus, the XYY syndrome is truly related to Y chromosome aneuploidy [61]. This happens in about 1 in 1,000 newborn boys [62], and is the most common sex chromosome aneuploidy in males after the Klinefelter syndrome. The exact causes of this mis-segregation are unknown, but the meiotic recombination between X and Y may play a role [63], as well as other, unidentified causes that affect multiple chromosome segregations at the same

time [64]. Indeed, the meiotic recombination-dependent mis-segregation of X and Y chromosomes is a known cause of male infertility in otherwise karyotypically normal men [65]. More generally, sperm aneuploidy of the sex chromosomes affects fertile men as well, though without impairing their ability to procreate [66]. The phenotype of 47,XYY subjects is generally almost normal, except for a slightly taller stature, variable cognitive disorders and, more rarely, motor skill impairment [60,67,68]. The fertility of these individuals is controversial. Some reports showed that often there is no significant difference between XYY males and control groups with regard to the number of children, and the children of XYY males were as healthy as those of the control group [69,70]. This explains why usually this condition is discovered only in adults. Other researchers, instead, report fertility impairment, generally due to an increased incidence of chromosomally abnormal spermatozoa; these men have variable sperm counts, ranging from almost-normospermia to oligoasthenoteratozoospermia, to azoospermia [61,71] (and references therein). In some cases, secondary infertility has been described [61]. Cases of infertility of these individuals are explained, by some authors, as a consequence of the specific loss of one copy of the Y chromosome before meiosis or of specific degeneration of aneuploid gametes through apoptosis or necrosis [72–75]. Other authors, instead, showed that a couple of Y chromosomes could synapse in meiosis, thus, escaping the meiotic sex chromosome inactivation which, in turn, would keep active genes that should not be such, hence, inducing fertility impairment [76]. Finally, the karyotype of some patients is a mosaic of 46,XY and 47,XYY cells. They may have very mild or no fertility issues, mainly depending on the count of 47,XYY cells in their gonads; if the 47,XYY count is very low, these patients might not be classified as mosaics at all [61].

#### 4.2. Sex Chromosomes Multisomy

More complex situations have been described, with males having tetrasomy or pentasomy of sex chromosomes and carrying multiple copies of the Y chromosome [77]. The 48,XXYY syndrome is generally considered the most common variant of the Klinefelter syndrome (47,XXY); however, it has been suggested that this condition is different [78,79]. The incidence of this condition is estimated between 1:50,000 [80], and 1:18,000 [81] male births. The associated phenotypes mostly resemble those of patients with 47,XXY karyotype [77]. Similarly to 47,XXY patients, some 48,XXYY subjects may be unaware of their condition, which, sometimes, is diagnosed at a later age [77]. These men typically show hypergonadotropic hypogonadism (as in Klinefelter individuals) with increased follicle-stimulating and luteinizing hormones, decreased testosterone and small testes, [77,82]. The infertility of these men is explained by the state of their gonads. Their testes show hyalinization of the seminiferous tubules, hyperplasia and fibrosis of interstitial (Leydig) cells, Sertoli-cell-only syndrome with lack of spermatogenesis [77,83,84]. The primary event for the generation of such individuals is a double sex chromosome mis-segregation in their fathers' meiosis I and II or a contemporary mis-segregation of sex chromosomes in both parents [84]; a double sex chromosome mis-segregation in very early stages of embryonic development is another possible explanation.

Other, more complex conditions exist, but are extremely rare. However, since the phenotype of these patients depends on the polysomy of both sex chromosomes, the specific involvement of the Y chromosome is unclear. In this context, patients with a polysomy of the Y chromosome alone are more informative. In 48,XYYY patients, sometimes the diagnosis is made as adults; the oldest diagnosed case that we are aware of was a 52 years old subject affected by infertility and other, minor problems [85], indicating that this condition may be considered *mild*, at least in some circumstances. Indeed, the phenotype associated with an XYYY karyotype is inconsistent; genitalia are apparently normal, but show hypogonadism with azoospermia in most adult patients [77].

We were able to find only seven cases of 49,XYYYYY patients without mosaicism, two of whom were adults. In an older report [86] the affected man (30 years old) showed azoospermia, elevated basal gonadotropins and an exaggerated testosterone rise following HCG stimulation. A newer report describes a 26 years old man originally diagnosed at the age of 4 months [87]; he was at first classified as a non-mosaic, but subsequent analyses showed that the 49,XYYYYY karyotype was present in ca.



86–87% of his cells, the other cells being 45,X [88]. Genitalia were normally developed [87], and the gonadal function was normal as well [88], but, unfortunately, no additional data are available about his physiologic and spermatogenesis status. Nonetheless, data from mosaic patients suggest that the spermatogenesis of 49,XYYYY individuals is strongly impaired [89].

The paucity of data on patients having Y chromosome polysomy does not allow fully understanding the role of this chromosome on male infertility or in other phenotypes associated with supernumerary Ys; however, these patients are generally in better physical conditions than those with supernumerary Xs.

## 5. The PAR Regions

The human Y chromosome hosts two pseudoautosomal regions (PARs), named PAR1 and PAR2, which consist of 5% of the entire chromosome [90], and an MSY region (also known as non-recombining region on Y, NRY) that does not recombine with the X chromosome [91] (Figure 1). PAR1 and PAR2 recombine during meiosis with their homologous regions on the X chromosome, although with different dynamics, compared with the rest of the genome [92]. Indeed, genes located within the PARs are inherited, and genetically behave similarly to autosomal genes. Taken together, the two PARs host at least 29 genes [14], 24 in PAR1 and 5 in PAR2. PAR1 is 2.6 Mb long and maps on the short arm tips of both X and Y chromosomes in humans and other great apes; PAR1 escapes X inactivation, and mutations in its genes are known to cause short stature [93], growth retardation [94], and mental disorders [95,96]. PAR2 is located at the tips of the long arms and is much shorter, spanning only 320 kb [97]. The role of PAR1 in the recombination is crucial for male fertility. In mice, it has been demonstrated that recombination impairment leads to X-Y mis-segregation in meiosis [92]. Similarly, in humans, a fertility impairment has been described, which is caused by PAR1 deletions [98,99], and, likely, is due to a comparable X-Y meiotic mis-segregation [100–102]. Instead, PAR2 is not necessary to fertility, as shown in individuals bearing deletions of the Y chromosome [97]. This feature of PAR2 can be explained by assuming that (a) there are no genes inside it that are directly involved in male fertility, and (b) the recombination role of PAR1 is sufficient to assure the correct segregation of the sex chromosomes. In a minority (2%) of the human population, also a PAR3 has been described [103], but its functional role has not been elucidated yet. However, because of its low prevalence, PAR3 is unlikely to harbor sequences important for chromosome segregation or genes having any role in male fertility.

Some genes mapping in the PARs are expressed in testes, but they are also always expressed in other tissues, and their exact role in the spermatogenesis—if any—is currently unknown [104]. These genes map both in PAR1 (16 genes) and PAR2 (2 genes), but the limited data available about phenotype alterations based on their copy number variations (CNV) and their involvement in male fertility is still unclear. Here we only recall the debate regarding the *SHOX* gene, mapping in PAR1. In 2011, Jorgez and collaborators [105] reported the occurrence of *SHOX* gene deletion in 5.4% of men with AZF deletions and a normal karyotype, hypothesizing a correlation between PAR rearrangements and AZF microdeletion formation. However, subsequent studies did not confirm this association between Y-chromosomal microdeletions and *SHOX* haploinsufficiency [106,107].

## 6. The Sex-Determining Region of the Y (SRY)

The Y chromosome promotes sexual differentiation in humans. In fact, its presence (or absence) is strictly linked to the development of primary and secondary sexual features, due to the activity of the *Sex-determining Region Y* (*SRY*) gene, which comprises a 35-kb region of Y-specific DNA adjacent to the pseudoautosomal boundary [108] (Figure 1). This intronless sex-determining gene encodes a transcription factor, known as Testis-Determining Factor (TDF), which is a member of the high mobility group (HMG)-box family of DNA-binding proteins and is responsible for the initiation of male sex determination during embryonic life. This protein switches the undifferentiated genital ridge towards testis development: The absence of the *SRY* gene or the functional impairment of the TDF protein leads to the development of a female habitus, irrespective of the karyotype. This

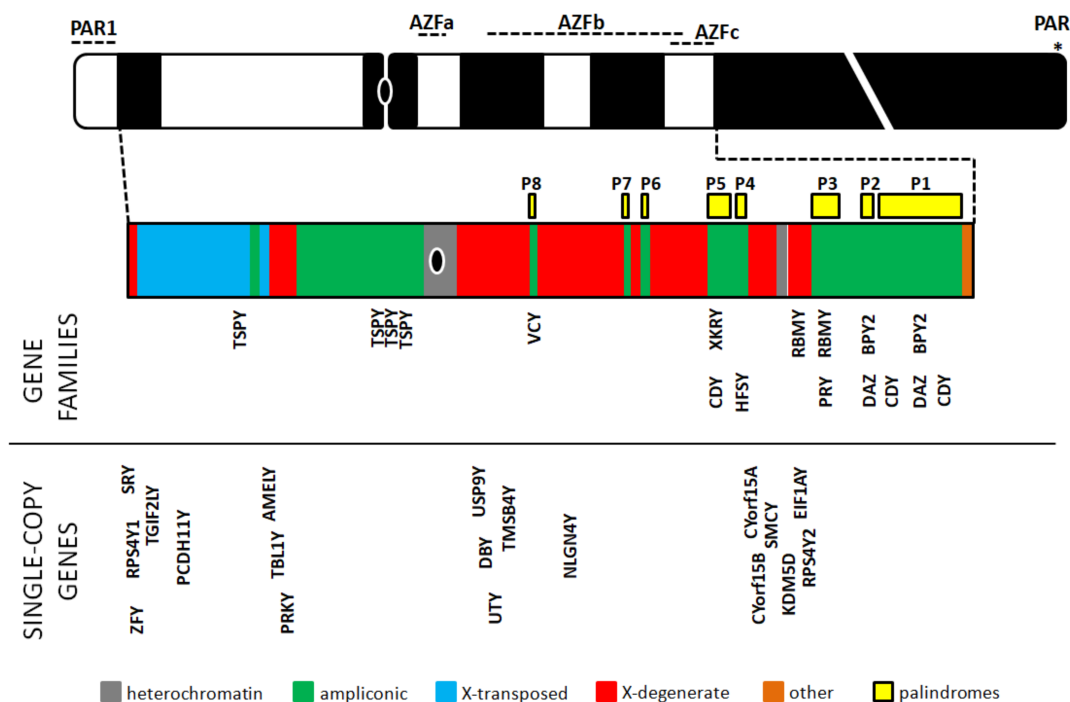
developmental switch is promoted by alternative genetic cascades, including female sex-determining genes *R-Spondin 1 (RSPO1)*, *Wnt Family Member 4 (Wnt4)/ $\beta$ -catenin* and *Forkhead Box L2 (Foxl2)* [109]. The major role of *SRY* is to achieve, through enhancer sequence binding, a sufficient expression of the gene *Sox9*, in order to induce the Sertoli cell differentiation, which in turn leads to testis formation and prevents the development of female reproductive structures (uterus and fallopian tubes) [110]. Deletions or translocations of *SRY* cause disorders of sex development (DSD) with dysgenic gonads in mutated individuals: Affected patients may have external genitalia that are not clearly male or female (ambiguous genitalia) or other abnormalities of the genitals and reproductive organs, either in karyotypically male (XY) individuals (in case of *SRY* absence/total impairment) or in karyotypically female (XX) patients (in case of *SRY* translocation on the X chromosome or on the autosomes). Failures in the gonad development may result not only in infertility, but also an increased risk of germ cell tumours (GCT), such as gonadoblastoma and various types of testicular GCT [111,112]. Indeed, recent studies demonstrate that either the loss of the Y chromosome or the ectopic expression of Y-linked genes is closely associated with various male-biased diseases, including selected somatic cancers [113]. Amongst the most important disorders caused by mutations of the *SRY* gene we mention here the Swyer Syndrome (XY karyotype, female habitus with gonadal dysgenesis) and the XX male syndrome, in which part of the Y chromosome is translocated on the X chromosome as the result of unequal recombination in the distal parts of the short arms of the sex chromosomes during the paternal meiosis, determining *SRY*-positivity [114]. Nonetheless, in some cases with complex chromosome rearrangements or mosaicism, the mere presence of *SRY* seems insufficient to determine the full development of male genitalia [115,116]. It has been shown that, at least in some cases, two copies of wild type *SRY* do not affect male fertility and that tandem duplication of *SRY* and Y chromosome aneuploidy are independent events [117]. Unfortunately, the same report does not clarify the possible role of multiple *SRY* copies, since in patients with 4 to 16 copies of the gene, its DNA sequence was not wild type and/or X0/XY mosaicism was present, sometimes resulting in a female habitus (see also the section ‘Mosaics and Y structural anomalies’) [117]. In conclusion, due to its dominant action, CNV of *SRY* affect the phenotype only when *SRY* is missing from the Y chromosome or when it is translocated onto another chromosome; instead, to the best of our knowledge, no phenotypic effect is induced by additional, wild type copies of *SRY* alone (tandem duplications). DSD in the presence of multiple copies of *SRY* were either associated with point mutations, and other gene sequence alterations [117] or a clear cause-effect relationship between the former and the latter was not demonstrated [118]. As such, *SRY* is not a good candidate gene for searching causes of male infertility; its mutations (gene deletions or point mutations, mostly inside the HMG region) usually induce a female—or, at most, an ambiguous—phenotype. Instead, in the case of a male phenotype, the most frequent cause is the presence of mosaicism beyond a certain threshold, for which, as above, we refer the reader to the section ‘Mosaics and Y Chromosome Structural Anomalies’.

## 7. The MSY Euchromatic (MSYe) Region

### 7.1. Overview of the MSYe Region

The MSY region (euchromatin plus heterochromatin) (Figure 1) represents 95% of the Y chromosome (the remaining 5% are the PAR regions) and is responsible for important biological processes, such as sex determination and male fertility. Therefore, several scientists investigated MSY role in traits and diseases, such as sex determination and reversal, spermatogenesis and male infertility, urogenital malignancies, such as prostate cancer, sex-specific effects on the brain and behavior, male viability and graft-versus-host disease [20,113,119]; in some cases, these genes also show a dosage-dependent function with their X-linked counterparts [20]. Most recently, MSY has also been studied with high-throughput techniques [120,121]. Despite the lack of homology between the X chromosome and the MSY region, recent evidence demonstrates that a specialized form of recombination, namely gene conversion [122], may take place in human MSY [123–126], and that these

events occur (i) within the Y chromosome (intrachromosomal recombination by gene conversion or other mechanisms), (ii) between the Y and X chromosomes, and also, although to a lesser extent, (iii) between the Y chromosome and some autosomal sequences [127]. Inside the MSY region, there is a mosaic of heterochromatic sequences, mostly composed of satellite DNA, alternating with three main euchromatic sequences: The X-transposed, X-degenerate and ampliconic regions [128] (Figure 2). The X-transposed sequences are 99% identical to DNA sequences in Xq21; their presence in the human MSY is the result of a massive X-to-Y transposition that occurred about 3–4 million years ago, after the divergence of the human and chimpanzee lineages [129]. Having a combined length of 3.4 Mb, the X-transposed sequences exhibit the lowest density of genes among the three aforementioned sequence classes of the MSY euchromatin, only encoding for two genes [130]; the rest of these regions is made of interspersed, repeated elements [128]. The X-degenerate sequences display single-copy genes or pseudogenes, homologues of 27 different X-linked genes, having between 60% and 96% nucleotide sequence identity with their X-linked counterparts. These regions probably consist of the remnants of the ancient autosomes from which the modern X and Y chromosomes evolved, and the single-copy genes they harbor show remarkable evolutionary conservation [131]. They have an 8.6 Mb total length and contain 16 coding genes, 12 of which are ubiquitously expressed; on the other hand, out of the 11 MSY genes, which are predominantly expressed in testis, only one, the *Sex-determining Region Y (SRY)*, is located in the X-degenerate region [132]. Finally, the highly dynamic [43,44,133–135] ampliconic region has a length of 10.2 Mb, i.e., about 30% of the MSY euchromatin. It is largely composed of sequences that are very similar (more than 99.9%) to other MSY sequences; this consistency is maintained by frequent gene conversion and, despite their ample variability between species, their total amount is stably maintained throughout human lineages [121,136]. In this region there are eight massive palindromes, at least six of which contain testis-specific genes; therefore, the ampliconic region has the highest density of genes, both coding and non-coding, among the three sequence regions in the MSY euchromatin, resulting in 9 protein-coding gene families, which are responsible for 60 transcripts [137]. The content of the MSY region is highly dynamic; the MSY-associated CNV (amplifications and deletions) repeatedly happened during human evolution, at a very fast rate and in recent times. However, the overall amount of these sequences falls within a limited range even in geographically distant populations [25,138].



**Figure 2.** Organization of the euchromatic portion of the MSY region (MSYe). The picture also reports the localization of the palindromic regions P1–P8 and, for comparison with Figure 1, also PARs and AZF regions. The black oval indicates the centromere. The region q12 is shortened (white cut), due to its large extension compared to the rest of the chromosome. The colored MSYe region is slightly enlarged compared to the Y chromosome (grey lines) to enhance the readability. Gene families and single-copy genes are reported according to [128], with minor modifications.

### 7.2. Spermatogenesis-Related Genes in the Yp

Some genes mapping in the short arm of the Y chromosome (Yp) are expressed in testes, but often this is not a specific signature; in addition, the role of some of these genes in the spermatogenesis is currently unclear [21,104]. Here we recall two of these genes, for the sake of completeness (Figure 2). *Zinc finger protein, Y-linked* (ZFY) is expressed in germline, and Leydig cells [139], and mice knockout for *Zfy* genes are infertile [140]; in addition, its function in somatic cells seems minor, if any [141]. Nonetheless, the lack of known mutations of this gene alone in infertile men does not allow confirming what it does in human spermatogenesis. *Testis-specific protein Y-linked* (TSPY) is considered a proto-oncogene that antagonizes its X-linked homologue (*TSPX*) [142]. To date, the function of TSPY in the spermatogenesis is controversial [21]: Some authors hypothesize that it might act as a pro-proliferative factor in a dosage-dependent manner [143,144]; however, recently this view has been questioned [145] suggesting a possible population stratification bias [21]. Consequently, no strong evidence exists to date of a relation between Yp CNV and male infertility.

### 7.3. Yq Chromosome Microdeletions and the Azoospermia Factor (AZF) Regions

Most genes mapping in the Y chromosome belong to its euchromatic portion, and several of those directly involved in the spermatogenesis are in the proximal part of its long arm (Yq11). Three distinct regions have been described in this arm, namely AZFa, AZFb and AZFc [146] (Figures 1 and 2); a putative fourth region (AZFd) that was hypothesized by Kent-First and co-workers [147] does not exist [148]. Although AZFs harbor also functions that are not specifically required for fertility [119,149,150], their loss is usually associated with azoospermia or severe oligoasthenozoospermia and, consequently, to infertility. Indeed, AZF microdeletions are the most frequent structural chromosomal abnormalities and the major cause of CNV-related male infertility: They occur in approximately 10–15% of azoospermic

and 5–10% of severe oligospermic patients [21,151,152]. However, the phenotype associated with different AZF microdeletions may significantly vary among patients and increasing evidence points towards population-based biases [21]. Microdeletions may span one or more AZF regions, or be partial deletions of two contiguous AZF regions; in addition, because some genes inside these regions are present in multiple copies, the extent of gene families' deletions may influence the final phenotype [21,146,153,154], and assessing a direct relationship between their CNV and male fertility is sometimes problematic. The possibility that microdeletions of the AZF regions may cause other pathologies than infertility is still a matter of research; studies performed on both mosaic (45,XX/46,XY) and non-mosaic men suggest that this could be true at least for some specific microdeletions, but the scarcity of available data strongly limits what we know about this matter [21] (and references therein). All known AZF microdeletions are due to errors in the intrachromosomal recombination process [21,155–157].

The molecular analysis of AZF loci allowed the identification of several genes having a direct role in the spermatogenesis, thus, explaining infertility of men bearing microdeletions of these sequences (Figure 2).

In AZFa at least three loci, possibly important for male fertility, were identified, *Ubiquitin specific peptidase 9, Y-linked (USP9Y)*, *Dead box on Y (DBY)* and *Ubiquitously transcribed tetratricopeptide repeat gene, Y-linked (UTY)*. *USP9Y*, also known as *DFFRY*, is a protein-coding gene located on Yq11.221; it is a member of the peptidase C19 family and consists of 46 exons spanning 159 kb of genomic DNA [158]. This gene encodes a protein that is similar to ubiquitin-specific proteases, which cleaves the ubiquitin moiety from ubiquitin-fused precursors and ubiquitylated proteins. The *USP9Y* protein may play an important role at the level of protein turnover by preventing degradation of proteins by the proteasome through the removal of ubiquitin from protein–ubiquitin conjugates [159]. Considerable evidence has been collected during the past years, linking several kinds of mutations of the *USP9Y* gene to male infertility, especially to diseases like *SPGFY2* and *Y Chromosome Infertility* [160], possibly by stabilizing a specific target protein that is important for male germ cell development [161]. However, the discovery of fertile men in a family with this locus deletion [162] has shown that *USP9Y* mutations alone are insufficient to cause infertility. Instead, *DBY* is very important for the spermatogenesis [163], since it is specifically expressed in the spermatogonia [164], and is frequently mutated/deleted in infertile men. The *UTY* deletion has been associated with male infertility [163], and some of its missense mutations have deleterious effects on the spermatogenesis [165]. However, because no cases of *UTY* deletion alone have been identified, the role played by this gene in normal spermatogenesis remains to be fully understood.

AZFb contains both single copy genes and gene families. Among single copy genes, the most interesting for male fertility are *lysine (K)-specific demethylase 5D (KDM5D)* and *Ribosomal protein S4, Y isoform 2 (RPS4Y2)*. *KDM5D* encodes a histone H3 lysine 4 (H3K4) demethylase that is probably involved in male germ cell chromatin remodeling [166,167]. *RPS4Y2* is a protein-coding gene located on Yq11.223. This gene encodes a ribosomal protein specifically expressed in testis, being in contrast with the Y-linked *RPS4Y1* (located in the short arm of the Y) and the X-linked *RPS4X*, which are ubiquitously expressed paralogues [168]. One of the Y-linked copies of the Ribosomal Protein S4 is preferentially expressed during the spermatogenesis and might be important for germ cell development: In fact, the exclusive expression pattern of *RPS4Y2* may indicate the functional role of this protein in posttranscriptional regulation of spermatogenesis [169,170]. As for gene families, potential candidates for fertility control are *XK, Kell blood group complex subunit-related, Y-linked (XKRY)*, *heat shock transcription factor, Y-linked (HSFY)*, *RNA binding motif protein, Y-linked, family 1, member A1 (RBM1A1)*, and *PTPN13-like, Y-linked (PRY)*. *XKRY* is located at position Yq11.222. Its encoded protein is called Testis-specific XK-related protein, Y-linked, which is similar to *XK* (X-linked Kell blood group precursor), a putative membrane transport protein. Dysfunctions or deletions of the *XKRY* gene are associated with spermatogenic failure and male infertility. *XKRY* shows a testis-specific expression [171,172], but its contribution to male spermatogenesis is considered marginal as its deletion is heritable [173]. *RBM1A1*, located on

Yq11.223, encodes for a male germ cell-specific RNA-binding protein associated with spermatogenesis. *RBMY1A1* has a testis-specific expression [174,175]; its role in the AZFb deletion-related phenotype has been described [176,177]. This protein is thought to function as a splicing regulator during spermatogenesis and microdeletions of the *RBMY* gene family are strongly associated with male infertility [177]. *RBMY* has also a homologue on the X-chromosome, named *RBMX*: *RBMX* and *RBMY* are members of an ancient pair of genes located on the sex chromosomes that encode RNA-binding proteins involved in the splicing. These genes have differentiated and evolved separately: *RBMY* has acquired a testis-specific function, while *RBMX* is ubiquitously expressed [178]. To investigate the number of functional genes, *RBM* expression has been studied through RT-PCR of *RBM* transcripts and characterizing *RBM* cDNA clones from six *RBM* subfamilies; of them, only the *RBMI* subfamily is actively transcribed. A total of six *RBMI* genes were identified, which produce four polypeptides [179]. *RBMY1A1* CNV is associated with sperm motility, i.e., its low copy number (<6) increases the risk of asthenozoospermia [180]. *HSFY* belongs to the heat shock factor family that has been shown to be implicated in spermatogenesis both in animals and humans. This gene maps at position Yq11.222. It has been discovered that two identical and functional full-length copies of *HSFY* map inside the palindrome P4, whereas, four similar sequences mapping in two clusters in palindrome P1 of AZFc and P3 seem to represent pseudogenes [181]. Deletions of this gene may be involved in azoospermia or oligospermia [182], suggesting its implication in unexplained cases of idiopathic male infertility [183]. More precisely, it has been shown that alterations of spermatogenic cell differentiation may be associated with altered expression of *HSFY* in the testis, leading to deteriorated spermatogenesis processes [184]. Overall *HSFY* is another strong candidate for infertility issues, since (i) it is expressed in testes, especially in round spermatids [182,185], (ii) its protein levels are low in spermatogenic cell samples from patients showing maturation arrest [184,186], and (iii) one azoospermic patient has been described, who had a small AZFb deletion, including only the two copies of *HSFY* [183]. Nevertheless, this interpretation is not final and has been challenged by some groups [173]. *PRY* is a protein-coding gene family, which has been proposed as a candidate spermatogenesis effector because of its essential role in the regulation of apoptosis [187]. It is located at position Yq11.223 and is specifically expressed in testis. Five exons were identified, by comparison of the cDNA sequence with the genomic sequence: Therefore, the presence of two full-length copies in AZFb (*PRY1* and *PRY2*) and two shorter versions of the *PRY* gene containing exons 3, 4 and 5 in AZFc (*PRY3* and *PRY4*) were discovered analyzing GenBank-derived clones on the Y chromosome [188]. *PRY* encodes a protein, which has a low degree of similarity with protein tyrosine phosphatase, non-receptor type 13 (PTPN13). It is expressed in testicular tissue and in the ejaculated sperm; furthermore, immunocytochemistry on testicular tissue showed the expression of the *PRY* gene in a small number of spermatozoa and spermatids. In the male ejaculate of 18 infertile couples, the *PRY* protein was found in 1.5–51.2% of spermatozoa and in most of the sperm precursor cells, suggesting its involvement in apoptosis of spermatids and spermatozoa [189]. Large deletions of AZFb, including *PRY1* and *PRY2*, seem to cause complete meiotic arrest, which leads to male infertility [190], whereas, patients with deletions of all the AZFb genes, except *PRY* and *RBMY*, display hypo-spermatogenesis [191]; these data validate *RBMY* and *PRY* central importance in male fertility processes [192]. For some authors, *PRY* might be an important infertility marker, more than a cause of infertility *per se*. In fact, its expression is irregular and restricted to few cells in the normal germ line, but the *PRY* protein is typically over-expressed in sperms having DNA fragmentation [188], which shows its apoptotic involvement [189].

Finally, inside the AZFc region, the candidate genes for infertility are *Chromodomain Y*, *Y-linked* (*CDY*), *Basic charge*, *Y-linked*, *1* (*BPY1*), *Basic protein Y2*, *Y-linked* (*BPY2*) and *Deleted in Azoospermia* (*DAZ*). *CDY* is a human Y-chromosomal gene family expressed exclusively in the testis and implicated in male infertility; it is located at position Yq11.23. Its protein is localized in the nucleus of late spermatids, where histone hyperacetylation takes place; in fact, *CDY* protein has histone acetyltransferase activity, with a preference for histone H4 [193]. Since histone hyperacetylation is thought to facilitate the transition in which protamines replace histones as the major DNA-packaging protein, this feature

offers a plausible mechanism to account for spermatogenic failure in patients bearing deletions of the *CDY* genes [194]. A strong association exists between loss of the *CDY1a* sequence family variant and male infertility ( $p = 0.002$ ); overall, this genetic alteration, due to gene deletion or conversion, may represent a major risk factor for male infertility [195]; a similar scenario has been described for *CDY1b* [196]. *VCY*, also known as *Basic Protein Y 1 (BPY1)*, is a multi-copy gene family located on Yq11.221. This gene family has multiple members on both X and Y: Members of the *VCX* and *VCY* family share a high degree of sequence identity, except for a 30-nucleotide unit that is tandemly repeated in X-linked members, but is present only once in Y-linked members [171]. Therefore, all these members are expressed exclusively in male germ cells, especially in the nuclei of germ cells of the seminiferous epithelium [197]. The protein encoded by this gene is called Testis-specific basic protein Y 1 and is found only in the testicular tissue; it may be involved in the spermatogenesis or play a role in sex ratio distortion; however, its direct role in male fertility, if any, is currently unknown. Interestingly, it has been shown by a yeast two-hybrid assay that *VCY* interacts with acidic ribosomal protein P0, suggesting its possible involvement in the regulation of ribosome assembly during spermatogenesis [197]. *BPY2* is a protein-coding gene mapping in Yq11.223; it belongs to the *VCX/VCY* gene family as well, although it is not related to *BPY1* [198], and it is also known as *VCY2*. *BPY2* contains eight exons, with the initiating ATG codon in exon 4; its encoded protein (Testis-specific basic protein Y 2) interacts with ubiquitin protein ligase E3A and may be involved in male germ cell development and male infertility—in fact, its deletions are frequently found in infertile men with severe oligozoospermia or azoospermia [199]. *BPY2* possible role in male infertility upon duplication has been suggested as well [200]. *BPY2* encodes a highly charged testis-specific protein that localizes to the nucleus of all-male germ cells [197,201]. Immunohistochemical analyses showed that *BPY2* localized to the nuclei of spermatogonia, spermatocytes, and round spermatids, but not in elongated spermatids. At the ultrastructural level, *VCY2* was expressed in the nucleus of human ejaculated spermatozoa [201]. Although *VCY* role in the spermatogenesis is unclear, some authors suggest its potential involvement in the cytoskeletal regulation of the spermatogenesis, based on its interaction with a protein (namely, variable charge, Y chromosome 2 interacting protein-1 (*VCY2IP-1*), also known as *MAP1S*) having a high degree of homology with the Microtubule-Associated Proteins (*MAPs*) *MAP1A* and *MAP1B* [202]. *DAZ*, of autosomic origin [203], is a multicopy gene family mapping at Yq11.223 and is organized in a repeated cluster containing four gene copies (*DAZ1-4*). Each gene copy contains at least seven tandem copies of a 2.4-kb repeat unit that encodes 24 amino acids, with *DAZ1* paired with *DAZ2*, and *DAZ3* paired with *DAZ4* [204]. The *DAZ* proteins are all encoded by a strongly repeated region of the Y chromosome and appear to be very similar to each other; thus, it is very likely that the described interactions with any *DAZ* protein may involve all four proteins. *DAZ* genes belong to a greater family of genes, which includes also *BOULE* (or *BOLL*, boule-like) and *DAZL* (deleted in azoospermia-like) genes that map on autosomes and may serve as *backup genes*, which would help to preserve a residual spermatogenesis in males with *AZFc* deletions that remove the Y-linked *DAZ* genes [205]. Complete deletions of *DAZ* genes have been associated with severe disruption of spermatogenesis [206], and might be one of the causes of a condition called Spermatogenic failure Y-linked 2 (*SPGFY2*) (OMIM ID: # 415000). The four *DAZ* genes are expressed in the spermatogonia and encode for 3'-UTR RNA-binding proteins, which regulate the RNA translation during the germline cell progression to meiosis and the formation of haploid germ cells [207,208]. The proofs of the *DAZ* pivotal function in spermatogenesis are several, since its protein localizes in the late spermatids and spermatozoa tails [209,210], and the frequency of its deletion in infertile men is high [211]. However, the role of the *DAZ* gene deletion alone in the spermatogenesis impairment, without additional genetic alterations, has been questioned [118,212]. Besides the well-known spermatogenesis impairment caused by *DAZ* genes loss, some research indicates that also a primary duplication of *DAZ* genes may cause male fertility disorders [200,213–216]. This suggests that this gene family CNV (and, possibly, the total amount of copies of genes found in the *AZFc* region, such as *BPY2* and *CDY1*) might be under strict genetic control. These observations about *DAZ* and *BPY2* genes might—at least partially—explain

the impaired fertility of men with duplication/hyperploidy of the Y chromosome without recognized, specific gene mutations, i.e., men with supernumerary, wild type, Y-linked DNA sequences (see the sections 'Mosaics and Y Chromosome Structural Anomalies' and 'Y Chromosome Aneuploidies').

## 8. The MSY Heterochromatic Region (MSYh)

The distal portion of Yq, mapping at Yq12 and totaling roughly 40Mb [128], excluding the PAR2 region (Figure 1), is essentially composed of constitutive heterochromatin and mainly contains two highly repetitive families of satellite sequences, called DYZ1 and DYZ2, made up of about 5000 and 2000 copies of each satellite, respectively [23,217]. These DYZ regions account for approximately 50% of the total Y chromosome DNA [218]. Patients with either longer or shorter MSY have been reported [219–221] (and references therein) and, to a certain extent, these differences fall within the normal Y-length polymorphisms [222,223]. However, in many cases either this is not the only karyotype anomaly or patients are mosaics for such alterations; thus, discriminating the role of Y-linked aberrations in the patients' phenotype—if any—is difficult. In addition, according to some authors, even in cytogenetically Yq negative individuals, it is possible to detect residual DYZ1 sequences (as short as 3.4Kb) using suitable probes, and this condition is still compatible with fertility [224]. Similarly, DYZ2 residual sequences were detected in samples from patients with small Y chromosomes [225], but not from others having the same anomaly [217]. There are chances that residual copies of both DYZ1 and DYZ2 were also present in samples classified as negatives, but they remained undetected because of the relatively low resolution of the used techniques (Southern blot, cytogenetics, restriction enzyme digestion). Altogether, these data suggest a minor role, or no role at all, for DYZs in male development, also considering that the MSY heterochromatic region may be present in healthy females as a consequence of Y-autosome translocations [226]. However, others suggest a role at least for DYZ1. According to these authors, DYZ CNV may be connected with recurrent spontaneous abortion and early embryo growth arrest both when DYZ repeats significantly increase or decrease [227]; moreover, a reduced number of copies of DYZ1 was found in infertile men, although a clear cause-effect relationship is missing [118]. Reports describing variations of the MSYh region are rare [221,228]; MSYh evolution is relatively recent and probably started after speciation of humans from their common ancestor [229]. Indeed, variations in the amount of DYZ sequences have been reported, and their estimated frequency seems higher than for similar events in the rest of the genome [230]. However, in all described cases, the variation does not go beyond a few repeated units, and the size variation of the Y chromosome is, on average, quite limited. Therefore, the MSYh region might be under evolutionary pressure, either because it fulfils a yet unknown function and/or because there are mechanisms that preserve its length within a certain range. It is useful to recall here the study of Jehan and collaborators [231], who found a developmental stage- and tissue-specific transcription of DYZ1 at week 36 of pregnancy and in adults, inside the testis. This DYZ1 transcript has a stretch of 67 nucleotides, which almost perfectly match (66/67) the 5'UTR of *CDC2L2*  $\beta$  *sv13* isoform mapping in 1p36.3. In addition, the DYZ1 transcript found in the testis is chimeric and originated by *trans*-splicing [232], joining the DYZ1 homologous stretch with the *CDC2L2*  $\beta$  *sv13* coding sequence; this transcript is testis-specific, while the transcript from the chromosome 1 is found in the brain, but not in the testes. The functional role of the DYZ1 RNA is unclear.

Other possible clues about the MSYh role in the human genome come from a 30 year old report by Nazarenko and co-workers [233]. They studied 55 men with an almost complete deletion of the MSYh region and reported that the Y-linked heterochromatin has a phenotypic effect on a significant number (20 out of the 80 analyzed) of quantitative anthropometric features. Other reports propose a link between excess of total genomic heterochromatin and male infertility, including both autosomal (chromosomes 1, 9, 16, 18) and Y-linked heterochromatin [234] (and references therein). Altogether, these articles suggest that there are both upper and lower limits to the Y-linked heterochromatin length, with (i) excessive heterochromatin possibly causing (or participating to some extent to) male infertility and (ii) insufficient heterochromatin causing various morphological and physiological changes.



Some authors propose that epigenetic regulation may involve the Y chromosome heterochromatin, with DNA-binding chromatin regulators sequestered to the lengthy Y chromosome heterochromatic domains [235]. This would resemble what happens in other organisms, such as *Drosophila melanogaster*, where polymorphic variations of the Y chromosome heterochromatin result in genome-wide gene expression variations [236]. It is possible to hypothesize that the CNV of the Y-linked heterochromatic sequences, in combination with other heterochromatic, autosomal sequences, have a quantitative role on gene expression. It would follow that the total amount of genomic constitutive heterochromatin might influence the transcription of target genes by indirectly acting as a repository for chromatin-modifying proteins, which in turn may affect male fertility in specific genetic backgrounds.

## 9. Discussion

Male fertility is determined by several, complex, interconnected events that include the action of single genes (mapping in different genome locations, such as *SOX9* or *AR*), the activity of gene families (e.g., those in the *AZF* loci), and epigenetic factors, such as (1) DNA modifications, (2) non-coding RNAs altering gene expression or acting via *trans*-splicing, and (3) constitutive heterochromatin that may interfere with the availability of transcription factors and/or chromatin-modifying proteins. Available data support the idea that both qualitative and quantitative factors have a role in these events and considering any one of them, without accounting for the others, is insufficient, sometimes, to explain male fertility impairment. Single point mutations or gene deletions might be identified as possible causes of spermatogenesis failure in many idiopathic patients; however, in many others, while the wild type gene sequences are preserved, their total amount is altered. CNV are one of the major causes of human genome variability and, essentially, they are the deletion or the duplication of the original sequence (or parts of it), without any additional modification (mutation), consequent to unequal crossing-over between or within chromosomes. CNV are frequently associated to both Mendelian and more complex conditions and are based on several, different molecular events, such as gene disruption, gene fusion and gene expression modification consequent to chromosome sequence relocation [237]. CNV may have positive consequences for species, from an evolutionary perspective, being the basis for the acquisition of new genetic functions after gene duplication or exon shuffling [237]. However, at an individual level, growing evidence supports the existence of loci that may impair male fertility as a consequence of CNV and which map on the autosomes, as well as on the X chromosome [238–243]. Here we recapitulate results that Y-linked genes may affect male fertility through the same mechanism.

We have discussed structural and/or numerical anomalies of the Y chromosome that may deeply influence the male fertility, independently of the sequence of protein-coding genes harbored in this chromosome. Indeed, some genes mapping on the Y chromosome have a direct role in male fertility [104], so that any chromosomal anomaly (deletions, some unbalanced translocations) causing underexpression or loss of such functions is able to impair the male ability to procreate. In this perspective, the *SRY* gene and *AZF* loci can be seen as models. Much less clear is the situation when there are additional copies of one or more genes, as in aneuploidies, duplications or some unbalanced translocations, having a wild type sequence. For some Y-linked genetic functions (such as *SMCY*), it has been shown a dosage-dependent fertility impairment, although not directly related to the male physiology. However, there are also studies supporting a direct relationship between CNV of other genes—such as *RBMY1* and *DAZ*—and male sterility. Accordingly, it is possible to hypothesize that other male fertility-related Y-linked genes are under dosage control as well. Indeed, for some of them, either an up- or down-regulation has been demonstrated in infertile men [244], although it is unclear if this is a cause or rather a consequence of infertility. While, for most of these genes, a dosage-dependent fertility impairment has not yet been established, it would be in good agreement with the escape from meiotic sex chromosome inactivation and related consequences on male fertility described by Royo and collaborators [76].

Sometimes, the structure of the Y chromosome is at least as important as its gene content, and it might even be more crucial than it. PAR1 does not harbor genes ‘specifically’ affecting male fertility,

but its absence causes sex chromosomes mis-segregation and, consequently, infertility. PAR1 resembles ‘chromosome structural information’, like centromeres and telomeres, whose function relies on their structure, as well as on their sequence, as for the karyotype stability. Essentially, PAR1 does not promote male fertility through its gene content, but because of its ability to recombine with the X chromosome. Consequently, an increase in the number of PARs, as it occurs in sex chromosomes aneuploidies, could impair the ability of sex chromosomes to correctly pair and segregate in meiosis. The PAR2 deletion does not impair fertility, but patients lacking PAR2 have an intact PAR1 region. It would be interesting to check if cells with a deletion of PAR1, but a complete PAR2 are still able to correctly segregate the sex chromosomes in meiosis; of course, this could be tested *in vivo* only in mosaic men, because of the importance of PAR1 genes for patients’ health. Similarly, the apparent contradiction between the stability of the MSYh region length and its lack of protein-coding genes might underline some not yet understood role for the Y-linked constitutive heterochromatin as a whole, which is not linked to specific sequences contained in it, but is sufficient to put this region under evolutionary constraints preventing major variations of its size. The MSYh region is highly dynamic, being able to gain and lose sequences very quickly, yet the overall amount of MSYh-linked chromatin in different populations is, in most cases, relatively constant. Indeed, the few available data indicate that, in most cases, the Y chromosome multisomy causes severe sperm count impairment. This outcome fits quite well the findings of Yakin and collaborators, who described a correlation between total genomic heterochromatin and male fertility [234], suggesting that the Y chromosome might also influence the expression of (likely, several) genes involved in male fertility through epigenetic mechanisms. Further studies and more patients are necessary to validate this hypothesis.

## 10. Conclusions

The Y chromosome role in human physiology, despite the low number of its genes compared to other chromosomes of similar size, is far from simple. The genes harbored in Y are involved not only in male fertility, but also in several other functions, such as neurological activities, morphogenesis and development. In addition, it has been demonstrated that in most cases (excluding genes mapping in PARs) the genes of this chromosome do not behave in a Mendelian fashion and are influenced by the genetic background of the host organism. Epigenetic mechanisms are likely involved too, thus, further complicating the genetic background in which the Y chromosome operates. All these aspects possibly make the Y the most complicated chromosome with respect to human reproduction. In cases of male infertility where an evident, common cause of this inability is missing (e.g., point mutations of known genes or physiological/morphological conditions), a genetic analysis of the Y chromosome content is strongly advised, since the mere sequencing of fertility-related genes might not disclose karyotype anomalies, especially in the case of supernumerary, wild type copies of known genes or when there are alterations in its chromatin content/structure. Genes mapping on the Y chromosome act in a complex fashion and a strong effort is required to comprehensively understand their role and interaction with multiple genetic pathways.

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