

GENETICS OF MALE INFERTILITY

Sherman J. Silber, M.D.
Infertility Center of St. Louis
St. Luke's Hospital
224 South Woods Mill Road, Suite 730
St. Louis, MO 63017
Telephone: 314-576-1400
FAX: 314-576-1442
Email: silber@infertile.com

LEARNING OBJECTIVES

1. The ART physician should understand the structure of the Y chromosome, why it harbors genes required for spermatogenesis, and why it so frequently has deletions which result in azoospermia and severe oligospermia.
2. The ART physician should understand the pitfalls in interpreting standard Y deletion testing, and be able to identify those STS markers which are robust and those which could represent polymorphisms or repeat sequences (false negative and false positive results).
3. The ART physician should have an understanding of the entire range of testis specific genes whose deletions or mutations are being missed (because of multiple repeats) by standard screening.
4. The ART physician should understand how to counsel infertile males undergoing ART regarding transmission of their infertility to ICSI offspring.

PRE-COURSE QUESTIONS

1. Male infertility can be transmitted either to ICSI offspring or to their children:
 - a. If a Y deletion is detected on routine screening.
 - b. If an X chromosomal testis-specific gene has a mutation.
 - c. Possibly in the majority of cases.
 - d. If the patient has only moderate oligospermia.
 - e. All of the above.

2. Y chromosome deletions associated with azoospermia:
 - a. Are generally transmitted to the infertile patient from his father who also has a & deletion in his peripheral lymphocytes.
 - b. First occur in the infertile patient's testes as a spontaneous mutation.
 - c. First occur in the testes of the infertile patient's father even though the father's sperm are found to have no Y deletions.
 - d. Result from testicular mosaicism in the infertile patient's father.

Answers:

1. E
2. C

SUMMARY

The purpose of this lecture is to put in perspective the accumulating molecular data on Y chromosomal, X chromosomal, and autosomal spermatogenesis genes, and the genetic origin of oligospermia. The current gene search with simple STS mapping on the Y chromosome is just a starting point for locating many other spermatogenesis genes that are widespread throughout the genome. Now that the Y has finally been sequenced (2002), many more genes are being discovered that impact spermatogenesis.

The presence on the Y chromosome of testis specific genes, which arrive from autosomal homologues, or from persistence of ancestral X genes which eventually acquire male specific function, is a recurrent theme in the evolution of spermatogenesis of all animals with sex determining chromosomes. A summary of the evolutionary history of our X and Y-chromosomes explains why the Y chromosome was a good place to start in the molecular search for spermatogenesis genes. However, it is clear that numerous genes on the X chromosome as well, and on autosomes, also impinge on spermatogenesis and may thus be transmitted to ICSI offspring.

The presence of Y deletions in azoospermic and severely oligospermic ($<2 \times 10^6$) men does not prevent fertilization or pregnancy either with ICSI, or occasionally with no treatment at all. The Y deletion (and presumably infertility) is thereby transmitted to the male offspring. However, there are many spermatogenesis genes involved in male infertility, and we have barely scratched the surface with what have been (up until very recently) very gross mapping techniques.

Whether or not these currently detectable gross "microdeletions" are found in an infertile male patient does not obviate the likelihood of there being a genetic cause for his azoospermia or severe oligospermia. If a defective gene (or genes) is located on his Y chromosome, then all of the male offspring will inherit his problem. However, if genes on the X chromosome are responsible for the infertility, then daughters will be carriers and grandsons may inherit the defect. If autosomal dominant genes are the cause of the infertility, then only half of the male offspring will be infertile, and half of the daughters will be carriers. There is no way of knowing what effect, if any at all, the carrier state for male infertility will have on the daughter.

All cases of male infertility need to be considered genetic until proven otherwise, and patients so counseled. A negative Y deletion assay as currently widely practiced, and a normal 46 XY karyotype does not in any way rule out that the infertility is genetically transmissible (because these techniques have such low resolution). With sequence-based techniques we are now identifying many genes that in a polygenic fashion determine the sperm count. The current enthusiasm for STS mapping of Y deletions is just a very crude beginning, and is only identifying huge deletions. Smaller deletions and point mutations are certain to be much more common causes of male infertility.

EARLY GENETIC STUDIES OF AZOOSPERMIC AND SEVERELY OLIGOSPERMIC MEN

For several decades, it had been speculated that there was a genetic etiology to many cases of male infertility (1-2). This suspicion originally arose from cytogenetic evidence reported over 25 years ago in a very small percentage (0.2%) of azoospermic men who were otherwise phenotypically normal, but who had grossly obvious terminal Y chromosome deletions (Fig. 1a,

1b) (3). Simple karyotyping of infertile men also raised the possibility of infertility being associated with autosomal translocations (4-10). A massive summary of karyotyping results in newborn populations, reviewed by Van Assche, revealed an incidence of balanced autosomal translocations in a normal newborn population of 0.25% but an incidence of 1.3%, in infertile men (Table 1A) (4). In fact, karyotyping of oligospermic males (i.e. less than 20 million per cc) reveal a 3% incidence of some type of autosomal chromosome anomaly, either balanced Robertsonian translocations, balanced reciprocal translocations, balanced inversions, or extra markers. These translocations could conceivably be transmitted to offspring if ICSI allowed them to conceive. However, because of the limitations of the resolution of cytogenetics, and the very small percentage of these readily discernable karyotypic abnormalities found in infertile men, until recently it had been a convoluted struggle to study the genetic causes of male infertility, and the possible transmission of these genetic errors to the offspring of couples with male infertility (10).

The possibility that many more cases of male infertility might be genetic was bolstered by the failure of most clinical therapies to correct deficient spermatogenesis (1-2,11-21). The heritability of sperm count demonstrated in the wild (22-24), classic studies of naturally occurring pure sterile Y deletions in *Drosophila*, and very early molecular investigations of the Y chromosome in humans led to what has now become an intense search for genes which control spermatogenesis and which may be defective in many or most infertile males (25-29). However, only recently has the frequent genetic etiology of male infertility related to defects in spermatogenesis (not to mention obstruction) become widely acknowledged via molecular methodology (1,30-39). If male infertility is of genetic origin, its possible transmission to offspring of successfully treated infertile men is a serious social concern (1,40-61).

Y CHROMOSOME MAPPING OF INFERTILE MEN AND ICSI

With simple karyotyping, it has been known that a very small number of azoospermic men (0.2%) have large defects visible in the long arm of the Y chromosome that are not present in their fertile fathers. This implied the existence of an azoospermic factor somewhere on Yq. (3). However, smaller defects (i.e. "microdeletions") could not be discerned with those limited early cytogenetic methods (Fig. 1a,1b,1c). Therefore, defects in Yq were considered to be rare even in azoospermic men.

In 1992, comprehensive Y chromosomal maps were constructed using yeast artificial chromosomes (YACS) and sequenced tagged sites (STS), and this created the possibility for more detailed study of the Y chromosome in infertile men (62-63). Using polymerase chain reaction (PCR), a more refined search for Y chromosome deletions could be pursued by testing for as many as 52 DNA landmarks (STSs, or sequence tagged sites) across the entirety of the Y chromosome. All Y-DNA markers employed were placed on a physical map of the chromosome, the markers representing all gene families that were then known in the non-recombining region of the Y chromosome (35,62-64). Using these molecular mapping techniques, which have much greater resolution than cytogenetics, a large series of severely infertile men with clearly identified phenotypes revealed deletions in 13% of azoospermic males (36) (Fig. 2). As many as 7% of severely oligospermic men also had these same "microdeletions" (65-66). The most commonly deleted region was located in the distal portion of interval 6, subsequently referred to as AZFc (Fig. 3a &3b) (65,34,36). The higher resolution of Y mapping over karyotyping thus showed

that more than just 0.2% of azoospermic men had defects of the Y chromosome, and more than just a few percent of severely infertile men had a genetic cause for their condition.

However, because of the highly polymorphic nature of the non-recombining region of the Y (NRY), there are many “Y deletions” that are of no consequence. Only if these deletions in the infertile male are not present in his fertile male relatives, nor in hundreds of normal controls, could they be implicated as a cause of the infertility. The fertile fathers of the Y-deleted, infertile men were shown to have intact Y chromosomes, demonstrating that the deletions had arisen *de novo* and providing strong evidence that these *de novo* deletions were indeed the cause of the spermatogenic failure observed in these men. Many laboratories throughout the world have reported on these sub-microscopic deletions of the Y chromosome in azoospermic and severely oligospermic men (27,30-35,60,67-93). Nonetheless, even these popular, new molecular methodologies were crude (not sequence-based) maps, and were suspected of missing huge areas of DNA sequence.

The DAZ gene cluster was identified within the most commonly deleted region, AZFc (36,94). DAZ genes were shown in humans to be transcribed specifically in spermatogonia and early primary spermatocytes (95). Autosomal DAZ homologues were also found in *Drosophila* (the Boule gene), in mice (DAZLA), and in fact in frogs and even worms (Table 2). These autosomal DAZ gene homologues were found to be necessary for spermatogenesis in every species studied (28,96-97). In the human, Y chromosome DAZ, located in the AZFc region, was found to be in the midst of an area of multiple nucleotide sequence repeats. It was later found to be present in four near-identical copies (99.9%) in the AZFc region (98). The presence on human chromosome 3 of DAZLA, an autosomal homologue of the human Y chromosomal DAZ, is what allows a small degree of spermatogenesis to survive in the majority of AZFc-deleted men. However, men with larger deletions that extended beyond AZFc had no sperm at all (65). Recently it has been shown that smaller deletions, which take out only two copies of DAZ result in milder spermatogenic defects than the classic AZFc deletion which takes out all four copies of DAZ (99-100). This indicates that there is a polygenic dosage effect of multiple genes that might control spermatogenesis.

Early ICSI studies showed a clear trend toward larger deletions causing more severe spermatogenic defects than smaller deletions (65,101). These studies suggested that possibly several genes in different areas of the Y chromosome might play an important role in spermatogenesis. In fact, some of the earliest studies of deletion on the human Y chromosome unveiled a different gene (RBM) in the AZFb region (27,26,102,64,73). Although there are numerous copies and pseudogenes of RBM on the Y, most of which are nonfunctional, there appears to be a functional copy in the region just proximal to AZFc, with no “rescue” homologues elsewhere. These early results supported the concept, that numerous genes on the Y chromosome, in addition to those of the AZFc region, impinge on spermatogenesis (64). As might have been expected, many more genes have now been identified in AZFc and elsewhere on the Y by detailed sequencing studies (64,102-103). Thus, multiple spermatogenesis genes apparently contribute to and modify the severity of the spermatogenic defect in Y-deleted men.

These early deletions on the Y were called “micro” deletions only because they could not be discerned by karyotyping. But they were indeed huge deletions (102). It was correctly hypothesized that smaller deletions, or point mutations might very well be present both in AZFc

and elsewhere, but the repetitive nucleotide sequences which characterize much of the Y chromosome made it very difficult with standard STS markers to define smaller deletions (104).

The unusually repetitive sequence structure of the AZFc region of the Y plagued even the first attempts at constructing a physical map with YAC's, because repetitive STS's could not be accurately placed in what was then called deletion intervals 6D-6F. Even the size of AZFc (without an accurate sequence) was controversial (0.5 to 2 Mb) (62,105-106). Efforts to find point mutations along the Y chromosome, have also been thwarted by the presence of multiple copies of genes in these regions with numerous Y specific repeats that in the absence of a complete sequence made the detection of specific nucleotide errors almost impossible to detect. The Y chromosome, and specifically the most commonly deleted area, such as AZFc, defied sequencing by the usual methods. Therefore, the AZFa section of the Y was initially selected to study in detail, because of the apparent absence of multiple gene copies or Y-specific repeats in that region (104,107).

AZFa, has a completely different, more conventional and non-repetitive structure than AZFb or AZFc, making this much less commonly deleted region of the Y an ideal starting-off point. Therefore, the AZFa region of the Y chromosome was the first region of the Y to be sequenced, and two functional genes were identified, USP9Y and DBY (104,107). Sequencing of these two genes in 576 infertile men and 96 fertile men revealed several sequence variants most of which were inherited from the fertile father and of no functional consequence. However, in one case a de novo point mutation was found on USP9Y (a four base pair deletion in a splice-donor site, causing an exon to be skipped and protein truncation). This mutation was absent in fertile relatives and represented the first case of a point mutation causing a single gene defect associated with spermatogenic failure. This particular region of the Y was more amenable to such a mutation search because of the lack of sequence repeats which plague the rest of the Y chromosome. This finding offered a hint at what we might find if we were able to search for more subtle gene defects in the larger areas of the Y chromosome where most of the testis specific genes have been located, such as AZFb and AZFc (64,102).

Studying AZFa also provided a good model for the interaction and overlapping functions of multiple genes which sheds light on the "polygenic" nature of the genetic control of spermatogenesis. When the entire AZFa region is deleted, taking out both DBY and USP9Y, there is a more severe spermatogenic defect and the patient is azoospermic. However, when there is only a specific point mutation of the USP9Y gene, we observed maturation arrest with a few pachytene spermatocytes developing into mature sperm in a few seminiferous tubules. Thus, the loss of DBY (the only other gene in the AZFa region) exacerbates the spermatogenic consequences of the loss of USP9Y. This finding in the AZFa region runs parallel to previous observations that larger Y deletions (which take out more genes) are associated with a lesser likelihood of finding sufficient sperm for ICSI (65).

Shortly after we began our Y chromosomal mapping study of infertile men, intracytoplasmic sperm injection (ICSI) with testicular and epididymal sperm retrieval methods for azoospermia were developed (1,44-46,48-49,51,54,60,108). Men with the most severe spermatogenic defects causing azoospermia in the ejaculate could now have children. Thus, at the very moment in time that we had an effective treatment for severe male infertility, the reality that male infertility is often of genetic origin, also became generally recognized. Subsequently it was

demonstrated that these Y deletions would be transmitted to offspring as a result of ICSI (54-55,59,65). When sperm were recoverable in azoospermic or oligospermic men, there was no significant difference in fertilization or pregnancy rate with ICSI whether the man was Y-deleted or not (Table 1B). Large defects resulted in complete azoospermia but smaller defects were associated with the recovery of some sperm sufficient for ICSI, and even occasionally spontaneous offspring as well (109).

WHY THE “Y”?

Why should the initial molecular efforts at defining the genetic causes of male infertility have concentrated on this difficult Y chromosome with all of its confounding repeats, polymorphisms, and degenerating regions? The answer lies in the evolutionary history of the X and Y chromosome. Over the course of the last 240-320 million years of mammalian evolution, the X and the Y chromosome have evolved from what was originally a pair of ordinary autosomes (Fig. 4) (64,103,110-115). During that evolution, just as most of the ancestral X genes were decaying because of the lack of meiotic recombination of the developing X and Y-chromosomes, genes which control spermatogenesis arrived (by transposition or retroposition) from autosomes to the Y (Fig. 5). Once on the Y, these formerly autosomal genes amplified into multiple copies, and achieved “greater prominence” (94,103). Spermatogenesis genes that arrived on the Y, but came originally from autosomes, include the well studied DAZ and CDY genes (94,98,116). Other spermatogenesis genes on the Y have persisted from their original position on the X and later became modified, and developed specific spermatogenic function on the Y. These genes also were amplified into numerous copies on the Y, such as the RBM genes (117-121).

Although DAZ is a very ancient, well-conserved gene, readily found to be functional in autosomes of *c. elegans* (worms), *Drosophila* (fruit flies), *Xenopus* (frogs), and rodents, it is only found on the Y chromosome of old world monkeys, apes, and humans (Table 2). In earlier mammals and in non-mammalian species, DAZ homologues are otherwise purely autosomal. RBM genes, however, are found on the mammalian Y as far back as the Y’s origin, as evidenced by its presence on the Y of marsupials even before the divergence of eutherian from non-eutherian mammals. Thus, RBM had its origin on the ancestral autosomes that evolved into the mammalian X and Y-chromosomes. The ancestral RBM that remained on the X chromosome (RBMX) retained its “widespread” function, whereas RBM-Y, which persisted on the receding Y chromosome, evolved a male-specific function in spermatogenesis amplified from 9 exons to 12 exons, and into numerous copies, most of which are nonfunctional. But at least one of them, RBMY-1 in the AZFb region, retained its testis-specific function (119,121-123).

Indeed, even the SRY gene (the male sex-determining locus) is considered to originate from the SOX3 gene on the ancestral X prior to differentiating into the SRY male sex-determining gene. In fact, the evolution of a non-recombining male determining gene (SRY) is what actually began the whole process of the Y chromosome’s evolution. SOX-3 is a gene on the X chromosome which inhibits SOX-9 also on the X chromosome. SOX-9 (on the X chromosome) is the gene that actually activates male sex determination. SOX-3 evolved into SRY on the ancestral Y chromosome. SRY inhibits SOX-3 from suppressing SOX-9, and thus determines whether the SOX-9 cascade of events leading to the formation of a testis takes place. That was the beginning of the transformation of an ordinary pair of autosomes into the modern X and Y. (Fig. 6) (113-114,116,122,124).

Genes associated with the non-recombining SRY region that were specifically beneficial for male function or antagonistic to female function, flourished on the evolving Y chromosome because it was a “safe harbor,” without the detrimental effect of meiotic recombination which would have otherwise allowed male-specific genes to be expressed in females (64,103,116,125). In this way, “male benefit” genes have arrived and accumulated on the evolving Y chromosome over many millions of years via the three mechanisms of: “transposition” from an autosome via

translocation, “retroposition” from an autosome via reverse transcription, and “persistence,” i.e., male modification of function from what was originally a gene on the ancestral X. This process gives the Y chromosome a very unique type of “functional coherence” not seen elsewhere in the human genome (Fig. 7) (64). However, like with SRY, we should not be surprised to find that many genes which are male-specific could be on the X as well, and sprinkled throughout the genome.

FUNCTIONAL REPRODUCTIVE ANATOMY OF THE X AND Y CHROMOSOME

Translocations occur on a relatively frequent basis in any species. Over evolutionary time, this results in conserved, homologous genes of different species residing in completely different parts of the genome and in a relatively mixed-up array of genes in every chromosome, where structural proximity has little or no relationship to function (64). However, these random transpositions (which over the course of time result in a chaotic lack of apparent organization of the genome) have also allowed direct acquisition by the Y of genes that have a common function to enhance male fertility. Selective pressures favor the process of spermatogenesis genes concentrating on the non-recombining portion of the Y chromosome in association with the male sex-determining gene, SRY, particularly if these genes are of little benefit to females or actually diminish “female fitness” (94,110-113,125-127,129).

Quite interestingly, the X chromosome, unlike autosomes, and unlike the Y chromosome, has been remarkably conserved in all mammals, with little mixing of genes from elsewhere. This is because of the selection against disruption of development of the X-inactivation process in the evolution of the X and Y (128). In a later section, I will explain more lucidly the phenomenon of X-inactivation. Suffice it to say at this junction, unlike the autosomes, the X chromosomes of all mammals are remarkably similar, and the Y chromosome has evolved into a specific functional organization not seen elsewhere in the genome.

Genes which arrived on the Y, or which persisted on the degenerating Y from the ancestral X, and gained prominence on the Y, underwent paradoxical processes of amplification, producing multiple copies, and degeneration because of the failure of recombination. DAZ (as has been discussed) was the first such gene which was identified in the AZFc region of the Y chromosome by our initial Y mapping in azoospermic men (36). DAZ represents the first unambiguous example of autosome-to-Y transposition of a spermatogenesis gene, which is representative of a generalized process that affects many other spermatogenesis genes, and indeed possibly explains the relatively poor state of affairs of human spermatogenesis compared to that of other animals (94,125). Autosome-to-Y transposition of male fertility genes appears to be a recurrent theme in Y chromosome evolution throughout all species. The autosomal DAZ gene (in humans called DAZ-L) is located on human chromosome 3, and on mouse chromosome 17. At some point during the evolution from new world to old world monkey, about 30 million years ago, this DAZ gene arrived on the Y by transposition from what is now human chromosome 3, and there multiplied to produce four almost identical gene copies. This process was first depicted for DAZL and DAZ. However, there are now known to be other previously autosomal genes or gene families on the Y that are expressed specifically in the testis, and are also likely to play a major role in spermatogenesis (Table 3) (64).

The CDY gene arrived on the AZFc region of the Y chromosome in a different fashion than DAZ, via reverse transcription (103). The autosomal CDY gene (CDY-L) is located on mouse chromosome 13 and on human chromosome 6. CDY's intron-free homologue found its way to the human Y actually prior to the arrival of DAZ, sometime after the prosimian line of primates separated off, approximately 50 million years ago (Fig. 8). It did so by reverse transcription and, therefore, has very few introns in marked contrast to CDYL, its autosomal homologue on chromosome 6, which is intron-rich (103). The RBMY-1 gene, located on the "AZFb" region of the Y chromosome, had its origin in our ancestral X chromosome, "persisted" on the Y, and there it was modified into a testis-specific gene (116,118-121,113,96). As the evolving Y chromosome underwent decay because of lack of recombination, these "persistent" genes (which were originally X chromosomal) diverged in sequence on the Y, and those which had "male benefit" functions flourished (Table 4).

The AZFa region of the Y chromosome is a little more complicated. As the ancestral Y began to recede in comparison to its paired X, it did so in stages and "strata" over about 320 million years (Fig. 9). There are four clearly definable strata on the X chromosome that decrease in X-Y homology according to how early in their history they failed to recombine (103). As a given stratum of the X failed to recombine with its Y counterpart, homologous X-Y genes in that stratum diverged in sequence structure (Fig. 6). The most recent areas of non-recombination of X genes is located most proximally on the X and the most ancient areas of non-recombination are located most distally on the X. The AZFa region of the Y chromosome diverged from the X fairly recently in its evolutionary history and, therefore, has a much more conventional sequence structure, with much greater homology to its counterpart on the X. The two genes in AZFa (USP9Y and DBY) both play an important role in spermatogenesis, in that deletion of AZFa results in a complete absence of sperm. Yet they have very close homologues on the X, and are still ubiquitously transcribed (104,107).

Regardless of the method of arrival of spermatogenesis genes to the non-recombining portion of the Y, this region would inevitably face, and likely succumb, to powerful degenerative forces during subsequent evolution (94). Saxena postulated that, "perhaps the rate of acquisition of male fertility genes approximates the rate of subsequent degeneration, resulting in an evolutionary steady state. In contrast to the extreme evolutionary stability of the X chromosome, at least in mammals, individual male infertility genes might not be long lived, in an evolutionary sense, on the Y chromosome."

Our emphasis on the Y chromosome for locating spermatogenesis genes to help in elucidating the causes of male infertility makes sense, because the Y has collected for us genes that otherwise would be hidden throughout the genome. However, it would be naïve to assume, in view of the evolutionary history of the X and the Y, that there are not equally powerful components for regulating spermatogenesis located also on the X chromosome and on the autosomes. Some have speculated that the instability of the Y chromosome may lead to an inexorable decline in sperm production in the evolution of any species, unless there is either sperm competition within the mating pattern of the species, or a method of continual recruitment of new spermatogenesis genes to the Y chromosome with subsequent amplification prior to ultimate degeneration (125). The Y chromosome is a favorable place to begin a molecular search

for genes that affect male fertility. But the very reason for starting with the Y emphasizes the likelihood of finding more such genes hiding throughout the genome.

PARALLEL AND INDEPENDENT EVOLUTION OF X AND Y CHROMOSOMES IN HUMANS AND ANIMAL MODELS: THE ORIGIN OF X-INACTIVATION (e.g., worms, flies, even fish)

Sex chromosomes have evolved independently many times in different genera with the same common theme. The chromosome with the sex-determining gene progressively loses the ability to recombine with its mate, accumulates mutations, and embarks on an inexorable deterioration. For example, the mammalian Y chromosome and the *Drosophila* Y chromosome (not to mention the ZW system in avians) have nothing in common with each other except their name, and the fact that they do not recombine with their larger counterpart, which is called the X chromosome. The X and Y-chromosomes evolved completely separately and differently in each of these well studied groups of species, but remarkably they evolved via the same common evolutionary theme.

If the Y chromosome of *Drosophila* has a deletion, the *Drosophila* is sterile. If the Y chromosome of the mouse or human has a deletion, the mouse or human is sterile. In any species thus far studied, if the Y chromosome has a significant deletion, that species is sterile. However, the genes that would have been deleted on the *Drosophila* Y, or the mouse Y-chromosomes, are not the same genes that are deleted in the human Y. For example, the homologue of the human Y DAZ gene on *Drosophila* is autosomal, (the so-called “boule” gene), just as it is also autosomal in the mouse (DAZLA), and the deletion of this autosomal gene in *Drosophila*, or in the mouse, results in sterility just as readily as deletion of the *Drosophila* Y or the mouse Y chromosome (96,28,97). Deletion of the DAZ genes on the Y chromosome of humans often does not result in complete absence of spermatogenesis, possibly because the ancient DAZ autosomal homologue on chromosome 3, rescues spermatogenesis to some small extent. Deletion of certain AZFb genes, however, usually result in total absence of sperm, probably because there are no effective autosomal or X homologues to rescue spermatogenesis when these genes are deleted.

The same pattern is found in all species studied. The X and Y begin as a pair of ancestral autosomes in which a male-determining gene (which does not recombine with its homologue) begins the inexorable process of decay into what then becomes a Y chromosome. In some *Drosophila*, the Y chromosome has disappeared altogether, and the resultant XO male is sterile. Although the human Y chromosome (or for that matter, any of the mammalian Y chromosomes) has no nucleotide sequence similarity at all to the fruit fly’s Y chromosome, the same mechanism of accumulation of spermatogenesis genes to a decaying male sex-determining chromosome is operating (125). Thus, the Y chromosome of the *Drosophila*, (and to some extent even the mouse) is quite different than the Y chromosome of the human, but yet they appear to be the same because of the common two evolutionary themes in the development of the Y. One theme is its gradual decay from what was its autosomal homologue, but is now the X chromosome, and the second theme is its growth from acquisition and accumulation of male benefit specific genes from other parts of the genome.

This evolutionary mechanism of degeneration of the Y, and accumulation of spermatogenesis genes may explain the relatively high frequency of male infertility and poor

sperm quality in species (like ours) that have minimal sperm competition. It may also explain the phenomenon of X inactivation, the high frequency of XO human stillbirths, and the survival of some XO concepti as Turner's Syndrome children.

The ancestral autosome which is to become the X chromosome develops a process first of hyperactivation, and then X inactivation, to make up for the decay of homologous alleles on what is now becoming the Y chromosome (129,128). As the X retained, and the Y gradually lost, most of these ancestral genes, expression of the X had to, at first, be increased to compensate for the male's loss of these genes, and X inactivation had to develop in the female for X genes whose Y homologue had eventually disappeared (Fig 10). The problem of X chromosome dosage differences in males and females is solved by inactivation in the female of one of the two X chromosomes combined with up regulation of the remaining X chromosome in females and the single X chromosome of males. This mechanism also insured the remarkable conservation and similarity of the X chromosome in all mammalian species.

An understanding of the evolution of X-homologous Y genes losing their general cellular functions, requiring up regulation, and inactivation of X genes on one of the two female X chromosomes, helps to clarify the different stages of evolutionary development of the mouse and human Y. The RBMY gene is a testis-specific male benefit spermatogenesis candidate gene. RBM's homologue on the X (RBMX) developed no male specific expression, but retained its general cellular housekeeping function. Thus, RBMX would be expected to behave like an X gene with no Y counterpart and probably undergo X inactivation (Table 5). As a related example, human ZFX and ZFY genes are very closely related homologues on the X and Y chromosome, both of which have general cellular housekeeping functions that are critical for life. Therefore, ZFX escapes X-inactivation in the female and ZFY is, therefore, probably one of the Turner genes (64,129). However, the mouse is quite different. In the mouse, ZFY appears to have evolved a male-specific function, and, therefore, ZFX in the mouse has a general housekeeping function not shared with its Y homologue. Thus, ZFX in the mouse is X-inactivated, even though in the human it is not.

Similarly, RPS4X and RPS4Y are another homologous pair of genes on the X and Y, both of which have equivalent housekeeping functions in the human. Therefore, in the human RPS4X, similarly to ZFX, is not subject to X-inactivation because there are functional transcripts in men, from the X and Y, and in women from the two X's. In mice, however, RPS4Y has not only lost its function in evolution, but has degenerated out of existence. Therefore, in the mouse RPS4X is subject to X-inactivation, just as most of the genes on the X chromosome in all animals require X-inactivation if they don't have a functioning homologue on the Y.

This summary of the evolutionary history of our X and Y chromosome explains why the Y chromosome was a good place to start in our molecular search for spermatogenesis genes. However, it is clear that numerous genes from throughout the genome, though less well studied, also impinge on spermatogenesis, and may thus be transmitted to ICSI offspring.

KARYOTYPE OF INFERTILE MALES AND OF ICSI OFFSPRING

The incidence of cytogenetically recognizable chromosomal abnormalities in the offspring of ICSI patients is acceptably very low, but much greater than what would be anticipated in a normal newborn population. Follow-up of the first 1,987 children born as a result of ICSI has

been meticulously studied and reported by the originators of ICSI in the Dutch-Speaking Free University of Brussels (Bonduelle et al., 1995, 1996, 1998a, 1998b, 1999). In 1,082 karyotypes of ICSI pregnancies, 9 (0.83%) had sex chromosomal abnormalities, including 45,X (Turners), 47, XXY (Klinefelter's), 47, XXX and mosaics of 47, XXX, as well as 47, XYY (Table 6). This is a very low frequency of sex chromosomal abnormalities, but nonetheless is four times greater than the expected frequency of sex chromosomal abnormalities in a newborn population (0.19%). Obviously the 45,X and 47,XXY children will be infertile (0.5%). Four (0.36%) of the 1,082 offspring had de novo balanced autosomal translocations or inversions. These children were apparently normal, but this incidence of de novo balanced autosomal translocations is five times greater than what would be anticipated in a normal newborn population (0.07%), and these children might also be suspected of growing up to be infertile (0.36%).

There were ten cases of translocations inherited from the infertile male (.92%), and these children are also likely to be infertile. Nine of these ten were balanced translocations in normal newborns. The one (0.09%) unbalanced translocation, was diagnosed at amniocentesis and was terminated. Since approximately 2% of oligospermic infertile males have chromosomal translocations (compared to a controlled population of 0.25%), it is not surprising that 0.9% of ICSI offspring would inherit such a translocation from their father (Van Assche et al., 1996). Thus, on purely conventional cytogenetic evidence, approximately 2% of ICSI offspring might be expected to share their father's infertility.

The remarkable five-fold increase in de novo balanced translocations among ICSI offspring (0.36% compared to 0.07%) is of great concern. Only 20% of balanced translocations are de novo, and 80% are inherited (130). De novo balanced translocations are usually of paternal origin (84.4%) and obviously most of the inherited balanced translocations in ICSI patients would come from the father (10,131). Balanced translocations which are associated with male infertility thus originally arose de novo in the testis of an otherwise fertile father, or his paternal ancestors, in 0.07% of a control population. Much more frequently, de novo balanced translocations (albeit still a low percentage of only 0.36%) arise in the testis of infertile men undergoing ICSI and are transmitted to their offspring. The deficient testis appears not only to be at risk of transmitting inherited autosomal cytogenetic defects, but also of producing a greater number of de novo cytogenetic defects.

The incidence of congenital abnormality in ICSI children (2.3%) is no greater than in every normal population studied (5-9). Even the few reported ICSI offspring of Klinefelter's patients have been chromosomally normal (132-135). There is no greater incidence of autosomal aneuploidy than what is predictable from maternal age. Sex chromosome aneuploidy (0.83%) in ICSI offspring is not an unacceptably high incidence, although it is clearly greater than normal (0.19%). Thus, the evidence based on cytogenetic and pediatric follow-up of ICSI offspring is very reassuring, despite the probable occurrence of infertility and sex chromosomal disorders in a small percentage of cases.

Y DELETION STUDIES OF ICSI OFFSPRING

Microdeletions on the long arm of the Y chromosome do not appear to adversely affect the fertilization or pregnancy results either in severely oligospermic men, or in azoospermic men from whom sperm were successfully retrieved (65). There have been concerns registered that the

ICSI results might be poorer with Y-deleted men, but in larger series, that has not been the experience (90,136) (Table 1B).

Thus far, all of our male offspring from Y-deleted men have had the same Y deletion as their infertile father (59). Fathers, brothers, and paternal uncles of the infertile men, were also examined for Y deletions and fertility. Y deletions in our infertile males were for the most part de novo. That is, the fertile fathers of infertile Y-deleted patients had no Y deletion. However, all male offspring from ICSI procedures involving these Y-deleted men had their father's Y deletion transmitted to them without amplification or change (Fig. 11).

The idea that the Y deletion would be transmitted to the son is not as obvious as it might at first seem. If a few foci of spermatogenesis in the testis of a severely oligospermic or azoospermic Y-deleted man were present because of testicular mosaicism, it would seem very possible that the few areas of normal spermatogenesis within such a deficient testis of a Y-deleted man might actually have a normal Y chromosome. In that event, one could have expected the sons of these patients undergoing ICSI not to be Y-deleted. For example, thus far all the sons of Klinefelter's patients have been normal 46, XY (132-135). Thus, it is not at all obvious, intuitively, that this Y deletion had to be transmitted to the son. However, increasing experience seems to indicate that the Y deletion of the sterile father is, in fact, transmitted to the son, and we no longer have to just speculate about it.

It remains to be determined whether non Y-deleted fertile or infertile men have mosaic deletions in their testis. If so, then de novo Y deletions would also be found more frequently in the brothers of our Y-deleted patients, or in ICSI offspring of infertile men (even those who have no Y deletion) than would otherwise be expected to occur in a normal newborn population (30). However, what we now know from the detailed sequence studies of the AZFa and AZFc regions of the Y chromosome gives us a much better picture of how Y deletions commonly occur, and how they are transmitted to offspring.

SEQUENCING OF THE Y CHROMOSOME, MECHANISM OF DE NOVO APPEARANCE OF Y DELETION, AND TRANSMISSION OF MALE INFERTILITY TO FUTURE GENERATIONS

The first region of the Y chromosome that was completely sequenced was AZFa because it was a region of the Y with very little repetitive sequences, and was relatively amenable to study (104). Now, the more daunting AZFc region (with large areas of sequence identity) has also been very recently sequenced (102). The sequence of AZFa, revealed it to span approximately 800,000 nucleotide bases (800 KB), and was bounded on each side by a proximal breakpoint area and a distal breakpoint of around 10,000 bases (10 KB) of 94% sequence identity with each other. Furthermore, the sites of these breakpoints (even with conventional mapping) in most infertile men with AZFa deletions were indistinguishable from each other. Within these 10 KB breakpoint regions, the site of AZFa deletion almost uniformly fell within smaller domains (447 BP to 1285 BP) of these 10,000KB breakpoints that exhibited absolute sequence identity (107). Indeed, the sequencing of deletion junctions of most AZFa-deleted patients revealed that homologous ("illegitimate") recombination had occurred between identical areas of proviruses that bounded each side of this 800,000 nucleotide region, allowing the entire intervening segment to drop out. The repeat areas of absolute sequence identity proximal and

distal to a common area of deletion clarifies the mechanisms for Y deletions to occur so frequently. With AZFa, the sequence repeats are caused by an ancient intrusion of a retrovirus into that region of the human Y. For AZFc, the situation is similar but occurs for a different reason on a vast scale of unprecedented and much more massive lengths of repeats.

The sequence of AZFc reveals the same mechanism for deletions to occur as for AZFa, but on a grander scale. Examination of the AZFc sequence revealed symmetries of unprecedented scale and precision. Figure 12 is a compressed dot-plot of the AZFc sequence in which perfect matches of at least 500 nucleotide bases are summarized by the same color (See Figure 12). There are no single-copy sequences in the 4.5mB region analyzed. There are six distinct families of amplicons (massive repeat units) ranging from 14kb to 678kb in length, spanning the entirety of AZFc, as well as another million base pairs proximal to AZFc. There are six massive inverted repeats and three massive direct repeats. Large domains of absolute sequence identity become easy sites for dropout of large chunks of DNA, since the boundaries of absolute sequence identity can “illegitimately” recombine with each other (106,102). Amplification and inversions in the most ancient areas of divergence from the X, create a perfect environment for subsequent deletion and degeneration. The very repetitive nature of the Y chromosome, that made sequencing and finding small deletions or point mutations so difficult, is also the cause of its instability over an evolutionary time frame, as well as in our current infertile male patient population. Y deletions large enough to be detected with our currently outmoded maps occur in about 1/2000 births because of these vast areas of absolute sequence identity. However, an analysis of the complete sequence of AZFc indicates that smaller deletions, involving only limited areas of AZFc, have been completely missed until now by conventional STS mapping.

The AZFc region of the Y (which is the most common “microdeletion” site, spans 3.5 megabases of DNA, and is thus hardly a “microdeletion” (as it is often called). It is composed of three giant palindromes constructed from six families of amplicons (i.e., long areas of absolute sequence identity). This “microdeletion” site, AZFc, contains 19 transcriptional units composed of seven different gene families, only one of which is DAZ (which comes in four separate copies). This huge 3.5 mB AZFc region is bounded on each side by nucleotide sequences that are 229,000 long of near absolute sequence identity (99.9%). But within AZFc are multiple sites for smaller deletions that could not possibly be detected by current methods because the PCR would amplify from one area of AZFc even if it did not amplify from a missing area with the same sequence. Unlike AZFa, the “breakpoints” of sequence identity for AZFc did not come from an ancient retrovirus, but rather from the very nature of the evolution of the Y chromosome caused by the failure of recombination.

In retrospect, it is apparent why all previous mapping and sequencing efforts relying on techniques that include STS content analysis, restriction mapping, Southern blotting, and fluorescent in-situ hybridization, failed to decipher AZFc’s organization. These approaches simply could not distinguish amplicons that display the size and sequence similarity found in AZFc. The extraordinary structure of the AZFc complex, and presumably other testis-specific regions of the Y, could only be unraveled by accurate genomic sequencing of clones drawn from a single Y chromosome, and an analysis of sequence family variants (SFV’s) identified through such sequencing. There are many amplicon pairs (and inverted repeats) in AZFc in addition to B2/B4. There are similar repeats elsewhere on the Y outside of AZFc. This “huge” region of

what used to be called a Y “microdeletion” spans transcriptional units that appear to affect only one cell lineage, spermatogenesis. The Y chromosome is a preferential site for “gene building” of testis-specific transcriptional units. Thus, autosomal DAZ has 11 exons, whereas each one of the four copies of DAZ on the Y has 28 exons and (as well as 19 pseudoexons). It is, therefore, safe to anticipate finding a much greater number of cases of male infertility to derive from Y chromosomal aberrations. There are multiple areas on the Y that, like the AZFc region, have either direct or inverted sequence repeats. Direct repeat sequences will result in common deletions due to homologous recombination. Inverted repeats will result in “isodicentric” translocations also because of homologous recombination. Thus, we can expect to find many other, smaller deletions on the Y of infertile men that have previously escaped detection by the crude, non-sequence based STS mapping currently available.

The proximal and distal breakpoint regions of AZFc deletions correspond to just two members (B2 and B4) of one of the six-amplicon families. There are seven different families of genes with 19 separate transcriptional units within AZFc (3.5mB), and eleven families of genes with 27 separate transcriptional units within the palindromic complex that spans AZFc (4.5mB). The seven gene families within AZFc are BPY_x, DAZ, CDY-1, CSPGL4Y, GOLGA2LY, TTY-3 and TTY-4. The four additional gene families within the palindromic complex that are located proximal to AZFc (RBM1-1, PRY, TTY5 and TTY6) are located in the distal most region of what used to be called AZFb. All these genes are specifically transcribed in the testis. The “designations” of AZFb and AZFc no longer seem tenable, because the basic construction of the palindromic complex containing these repeat units and genes extends for almost one million nucleotides proximal to AZFc.

Therefore, it is likely that smaller deletions, taking out less genes, or point mutations, involving just one or two copies of identical genes that normally occur in multiple copies, could account for many more cases of male infertility, or perhaps more moderate degrees of spermatogenic failure (e.g. $> 2 \times 10^6$ to 20×10^6 sperm/cc). The large “micro-deletions” thus far reported in the literature are for the most part de novo, but certainly some men with these large deletions, despite severe oligospermia, can naturally father children (about 4%). Men with more moderate degrees of oligospermia may father children even more easily, and thus smaller Y deletions causing more moderate degrees of male infertility may be less likely to be of de novo origin. In any event, as more and more genetic causes of spermatogenic failure (severe or mild) come to light, there is an increased awareness of the likely transmission of male infertility to future generations.

THE X CHROMOSOME AND MALE INFERTILITY

It has been theorized that the Y is not the only sex chromosome that accumulates genes which benefit spermatogenesis over an evolutionary time span (137,110,138,139). As the X chromosome (240 to 320 million years ago) differentiated from the Y, the sexually antagonistic gene theory favors the emergence of genes on the X also that benefit the heterogametic sex (with mammals of course, that is the XY male) and that are detrimental to the homogametic sex (the XX female) if these genes are recessive. For example, a rare recessive evolutionary mutation on the X that favors spermatogenesis would be preferentially passed on to male offspring who by virtue of a higher sperm count would then continue to pass down this favorable X mutation in

turn to his offspring. Such a recessive mutation (favorable to spermatogenesis) in an autosome would be lost to future generations. Thus, we can also anticipate an accumulation on the X chromosome (as well as on the Y) of male benefit recessive genes.

In fact, RT-PCR subtraction studies of spermatogonia in mice have demonstrated that a large fraction of genes which are expressed exclusively in pre-meiotic male germ cells, are indeed X chromosomal in origin (140). Eleven of the 36 genes that were expressed specifically in mouse spermatogonia were found exclusively on the X chromosome. Since the X chromosome is so well conserved in all mammals (as explained earlier by the universal development of X inactivation in mammalian evolution), it seems very likely that evolution has also conferred on the human X chromosome a large portion of the burden for spermatogenesis. Thus, a search for detrimental mutations on the human X in infertile males is also likely to be very rewarding. Thus, the failure to identify a Y deletion gives no assurance whatsoever that a genetic cause for infertility won't be transmitted to the ICSI offspring either via the Y, the X, or even autosomes.

CONCLUSIONS

The presence of Y deletions does not decrease the fertilization or pregnancy rate for azoospermic and severely oligospermic ($<2 \times 10^6$) men. Thus far the sex ratio of delivered children is apparently equal and the children are karyotypically normal. However, the Y deletion (and presumably infertility) is transmitted to the male offspring (59). Although using standard STS mapping, Y deletions occur in only 10% of azoospermic and severely oligospermic men, sequenced based mapping (now just available) is certain to increase that percentage significantly.

There are most likely many spermatogenesis genes involved in male infertility, and we have barely scratched the surface with what have been, up till now, very crude mapping techniques on the Y chromosome. Whether or not these gross "microdeletions" currently reported in the literature are found in an infertile male patient, does not obviate the likelihood of there being a genetic cause for his azoospermia or severe oligospermia. If a defective gene (or genes) is located on his Y chromosome, then his male offspring will most likely inherit his problem. However, there are also many genes on the X-chromosome, and throughout the genome, that impinge upon spermatogenesis that are not thus far identified by our currently crude mapping procedures. The recognized failure of any conventional therapy to improve spermatogenesis infers a genetic origin for most spermatogenic defects (141,142,136). These numerous genes may also be responsible for many cases of male infertility. Therefore, sons, and even daughters, may inherit the defect or be carriers.

It is clear that a negative Y microdeletion assay by currently popular methods does not rule out genetic abnormality. Therefore, in our view, genetic counseling should be provided to all infertile males, whether or not an abnormality is detected and whether or not Y deletion assays have even bothered to be performed. Although karyotyping certainly should be routinely performed for infertility patients (because of the risk of miscarriage and abnormal offspring resulting from either sex chromosome abnormalities, or unbalanced translocations), Y deletion testing may not be mandatory yet, because it is still very crude, and negative results should not be at all reassuring. Furthermore, some "deletions" may only be polymorphisms, and not of clinical significance. It is apparent that there is likely to be frequent transmission of male

infertility from the ICSI father to his male (or even female) offspring regardless of current testing. Every couple must decide for themselves whether they wish to consider this risk. In our experience, most such couples, even when well informed, choose to have ICSI despite this risk. Thus, continued long-term clinical and molecular study of ICSI offspring is mandatory.

POST-COURSE QUESTIONS

1. Y deletions (some not detectable with currently popular assays) are associated with:
 - a. Varicocele.
 - b. Moderate oligospermia.
 - c. Severe oligospermia.
 - d. Azoospermia.
 - e. B, C, and D.

2. Y deletions result from:
 - a. Varicocele.
 - b. Excessive heat.
 - c. The desire to make more money.
 - d. Illegitimate homologous recombination between large areas of absolute DNA sequence identity.
 - e. Gene mutations.

Answers:

1. E
2. D

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Table 1a: Percentage of Chromosome Abnormalities Observed in Seven Series of Infertile Men (Azoospermic and Oligospermic) Compared to Normal Newborn Population

All References	Number	Chromosomes	Sex	
			Autosomes	Total
Total (Azoospermic and Oligospermic)	7,876	295 (3.8)	104 (1.3)	399 (5.1)
Newborn Infants (Control)	94,465	131 (0.14)	232 (0.25)	366 (0.38)

Van Assche *et al.*, 1996

Table 1b: Results of ICSI in Y-Deleted Versus Y Non-Deleted Men with Severe Oligospermia (2×10^6) and Azoospermia (Non-Obstructive)

	<u>Y-Deleted (<math>2 \times 10^6</math>)</u>	<u>Not Y-Deleted (<math>2 \times 10^6</math>)</u>
# Patients (with sperm)	23	205
# Cycles (with sperm)	45	312
# Eggs	508	3291
# 2PN	(289) 57%	1849) 56%
# Pregnant	(17) 38%	(112) 36%
# Deliveries	(13) 29%	(81) 26%
# Babies	18	99
# Boys	10	43
# Girls	8	56

Table 2: Genes of the DAZ Family in Vertebrates and Drosophila

	<u>Y Chromosomal</u>	<u>Autosomal</u>
Human	DAZ cluster (at least 4 copies, > 99% identical)	DAZL (chrom. 3)
Mouse		DAZL (Dazla) (chrom. 17)
Xenopus		Xdazl
Drosophila		boule
C. elegans		daz-1

Table 3: Arrival of Spermatogenesis Genes to Y Chromosome

	<u>Y Gene</u>	<u>Ancestral Gene</u>
Transposition	DAZ (AZFc) Human-Y	DAZL (autosomal) Mouse 17 Human 3
Retroposition	CDY (AZFc) Human-Y	CDYL (autosomal) Mouse 13 Human 6
Persistence	RBM (AZFb) Human-Y and Mouse-Y	RBMX Mouse X Human X
Persistence	SRY	SOX-3

Table 4: Persistence on Y of RBM X

<u>Y Gene</u>	<u>Ancestral Gene</u>
<u>SRY</u>	<u>SOX-3</u>
Determines Male Sex	No male specific function
<u>RBM-Y</u>	<u>RBM-X</u>
Male specific function	No male specific function
Numerous copies	One copy
Many degenerate	
Same in all therian mammals	

Table 5: Persistence on Y of X Genes

	<u>Y Gene</u>	<u>Ancestral X Gene</u>
	SRY	SOX-3
	Determines male sex	No male specific function
	<u>RPS4-Y</u>	<u>RPS4-X</u>
Human	Housekeeping	Housekeeping
	Ubiquitous	Ubiquitous
	Turner Gene	No X-inactivation
Mouse	No RPS4-Y	Housekeeping
	Evolved out of existence	Is X-inactivated
	<u>ZFY</u>	<u>ZFX</u>
Human	Housekeeping	Housekeeping
	Ubiquitous	Ubiquitous
	Turner Gene	No X-inactivation
Mouse	Male specific function only	Housekeeping
	Two copies only	Ubiquitous
		Is X-inactivated

Table 6: Karyotype Anomalies in 1,082 Prenatal Diagnosis

Abnormal Karyotypes On 1,082 Prenatal Tests	Maternal		Percent	Percent in Literature
	Age (years)	Number		
<u>De novo chromosomal aberrations</u>		18	1.66	0.445
Sex-chromosomal:		9	0.83	0.19, 0.23
45, X	37			
46, XX/47, XXX	44			
47, XXX (2 children)	32, 37			
47, XXY (4 children)	26, 28, 28, 32			
47, XYY	25			
Autosomal:		9	0.83	0.21, 0.61
Trisomy 21 (5 children)	32, 33, 37, 41, 41	5	0.46	0.14
structural		4	0.36	0.07
46, XXY, t (4;5)	30x			
46, XX, t (2;15)	30			
46, XX, t (2;13)	36			
46, XX, inv (1qh)	39			
<u>Inherited aberrations</u>		10	0.92	0.47
balanced		9	0.83	0.45
unbalanced		1	0.09	0.023
<u>Total aberrations</u>		28	2.5	0.92, 0.84
<u>de novo + inherited</u>				

(Bonduelle et al., 1995; Bonduelle et al., 1996; Bonduelle et al., 1998; Bonduelle et al., 1999)

LEGENDS

- Figure 1a/1b: Karyotype of the azoospermic male with cytogenetically visible Yq deletion compared to karyotype of a azoospermic male with a normal Y chromosome.
- Figure 2: Y chromosome map based on STS interval markers and their corresponding X-homologous and testis specific gene.
- Figures 3a: Typical early deletion map of azoospermic and severely oligospermic men with chromosomal micro deletion.
- Figures 3b/3c: More refined, later deletion maps of azoospermic and severely oligospermic men.
- Figure 4: Figurative outline of evolutionary degeneration of one chromosome with the testicular determining factor (TDF) gene which doesn't recombine with its homologue, resulting eventually in a Y chromosome
- Figure 5: Over the course of evolution, the Y chromosome descended from the ancestral autosome that developed the SRY male-determining gene. The Y then attracted male-specific genes by three mechanisms. (Lahn and Page, modified, 1999b)
- Figure 6: Graphic depiction of X-homologous genes on the Y chromosome representing four different stages of a divergence from its original ancestral X showing corresponding X and Y homologous genes. Note that SOX3 and RBMX come from the earliest region of sequence divergence and correlate with the SRY gene and the RBMY gene. (Lahn and Page, 1999a)
- Figure 7: The Y chromosome has a remarkable functional coherence not seen in any other chromosomes. Genes depicted above are X-homologous because of their equal similarity to genes on the X chromosome. Genes depicted below are Y-specific genes which are testis specific, expressed only in the testis, and have no X-homologues. (Lahn and Page, modified, 1997)
- Figure 8: Whereas DAZ was transposed to the Y chromosome approximately 30 million years ago (after the divergence of old world and new world monkeys), CDY arrived on the Y chromosome much earlier (50 million years ago) by a process of reverse transcription. (Lahn and Page, 1999b)
- Figure 9: The X and the Y chromosome develop in mammals at the time of divergence the avian and mammalian line by a series of four well defined inversions. The earliest inversions (region 1 on the X) have the least similarity to their Y-homologue, and genes in the most recent area of divergence (region 4) have the greatest sequence similarity of their counterpart on the Y. (Lahn and Page, modified, 1999a)
- Figure 10: X-inactivation develops after X-linked hyper expression as a pairing mechanism between the evolving X and Y chromosomes to compensate for decay of X genes on the evolving Y. (Jegalian and Page, 1998)
- Figure 11: The AZFc Y deletion present in the azoospermic or severely oligospermic father is not present in his father, but is transmitted to his sons via ICSI. (Page, Silber, Brown, 1999)
- Figure 12a: Amplicons and palindromes in 4.5-Mb portion of the human Y chromosome that includes AZFc. Dot-plot in which region's sequence is compared with itself. This compressed, triangular plot avoids redundancies and false symmetries that would appear in a conventional square plot. Amplicons of various families (blue,

turquoise, green, red, gray and yellow arrows) are shown at the base of the plot. Each dot within the plot represents a perfect match of 500 bp. Direct repeats are shown as horizontal lines, inverted repeats as vertical lines and palindromes as vertical lines that nearly intersect the baseline. Palindromes P1, P2 and P3 are indicated, as are two smaller palindromes, P1.1 and P1.2, that lie within P1. Tinted squares reflect pairs of amplicons with nearly identical sequences; the adjacent numbers denote substitutions per 10 kb. Gray diagonal lines mark amplicon boundaries. Selected amplicons (b1, t1, t2, b2, g1, r1, r2, b3, g2, r3, r4, g3 and b4) are identified, as are sequences that occur once within the 4.5-Mb region (u1, u2 and u3). Orientation with respect to the centromere (cen) and long-arm telomere (qter) is shown. The full annotated sequence of the 4.5-Mb region is available in Web Tables A, B and C and Web Note A. *Kuroda-Kawaguchi T, Skaletsky H, Brown LG, Minx PJ, Holland SC, Waterston RH, Wilson RK, Silber S, Oates R, Rozen S and Page D. The AZFc region of the Y chromosome features massive palindromes and uniform recurrent deletions in infertile men. 2001, Nature Genetics 29, 279-286.*

Figure 12b: Sequence-based map of STSs, deletions, transcription units and autosomal homologies. *Kuroda-Kawaguchi T, Skaletsky H, Brown LG, Minx PJ, Holland SC, Waterston RH, Wilson RK, Silber S, Oates R, Rozen S and Page D. The AZFc region of the Y chromosome features massive palindromes and uniform recurrent deletions in infertile men. 2001, Nature Genetics 29, 279-286.*

FIGURE 1a

FIGURE 1b



FIGURE 2

Continuation of the Commission's report for 2011

Number of the Commission's report

Year

Number of the Commission's report

Year

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FIGURE 3a

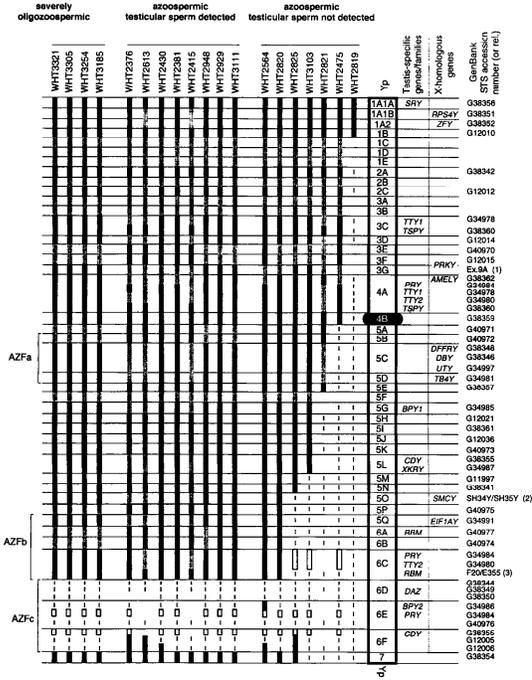
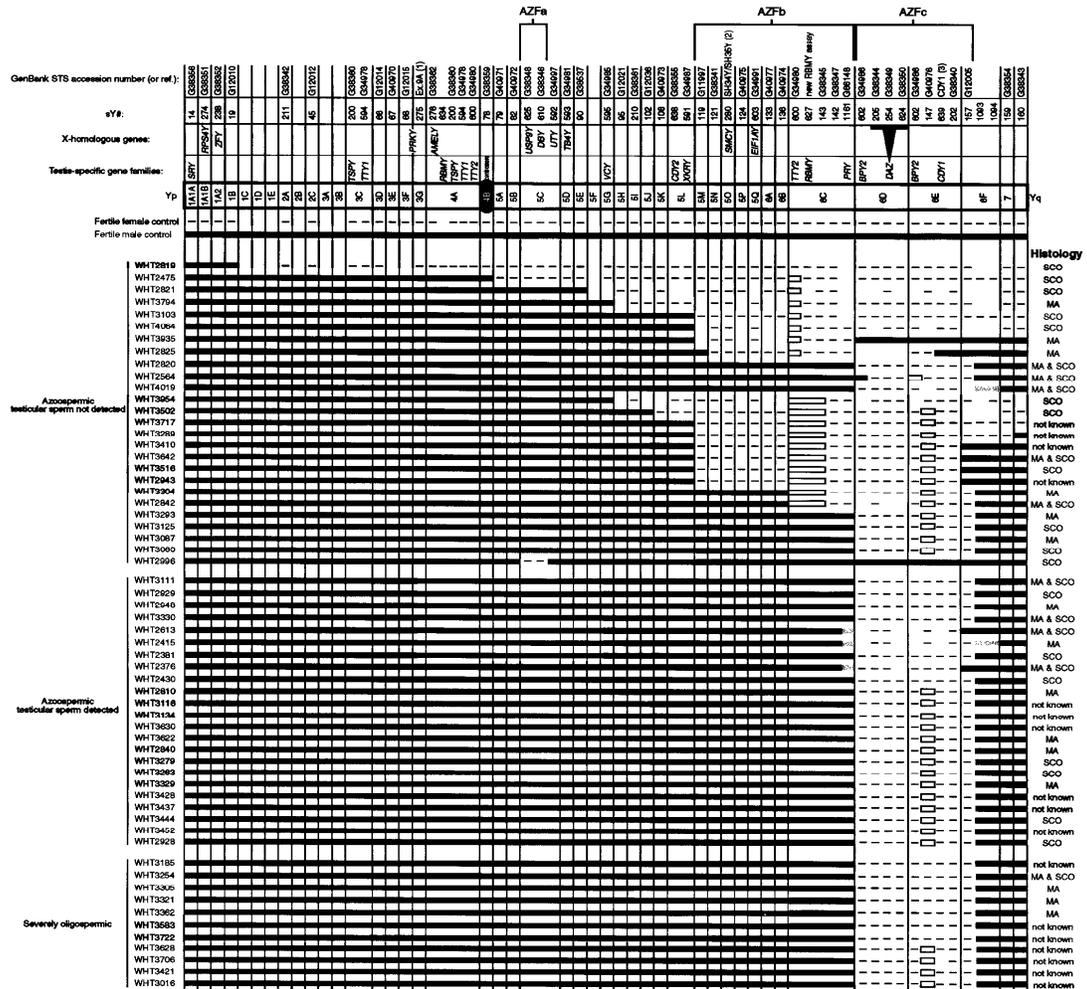


FIGURE 3c

Y Deletions and Diverse Histology in Azoospermic and Severely Oligospermic Men



The experimentally demonstrated presence of a locus in an individual is indicated by a black segment; the gray segment means "presumably" not deleted but no DNA remaining to test; experimentally demonstrated absence is indicated by a minus, and inferred absence is indicated by the absence of any symbol. White boxes represent positive PCR results, and these must be interpreted in the context of the Y-specific repeat nature of the sequences being considered. It is very likely that these positive results reflect the existence of closely related, cross-amplifying sequences in other portions of the Y chromosome.

FIGURE 4

Classical Model of Sex Chromosome Evolution: Y as Decayed X

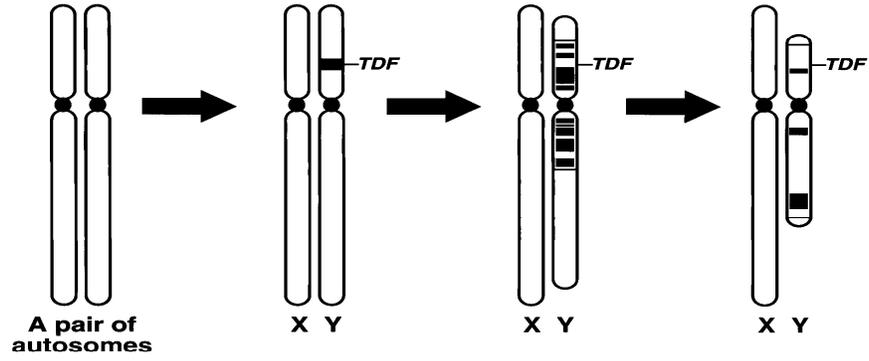


FIGURE 5

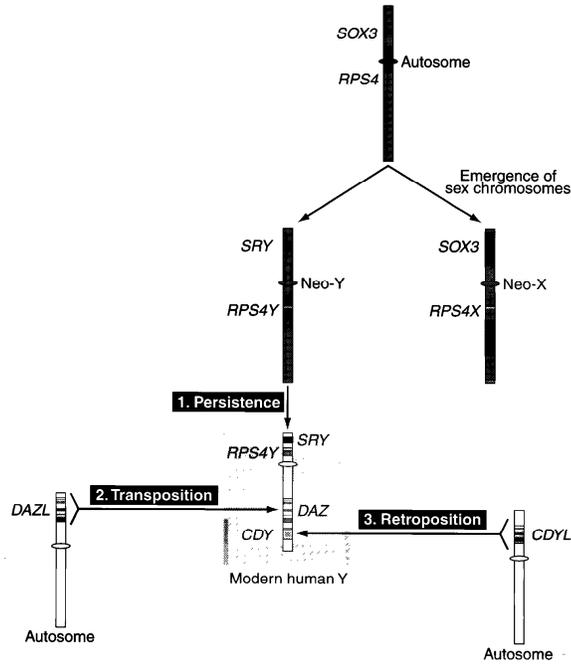


FIGURE 6

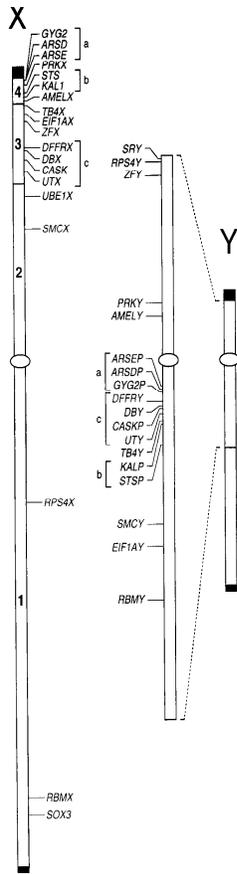


FIGURE 7

Two Gene Classes Reflect Sequence Organization of NRY

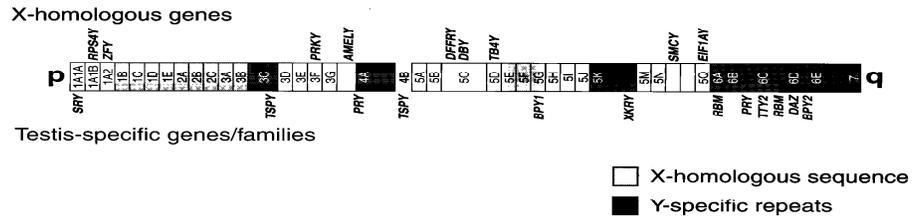


FIGURE 8

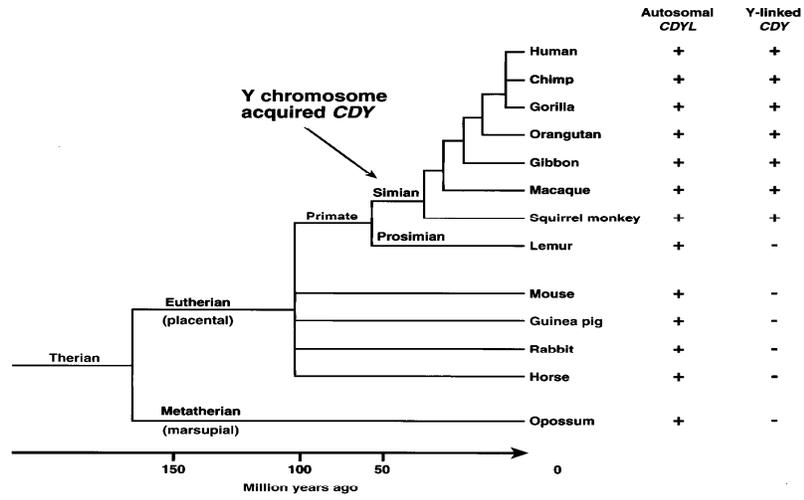


FIGURE 9

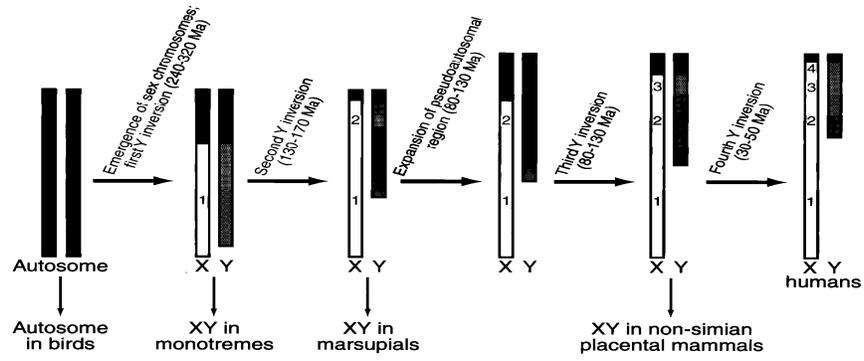


FIGURE 10

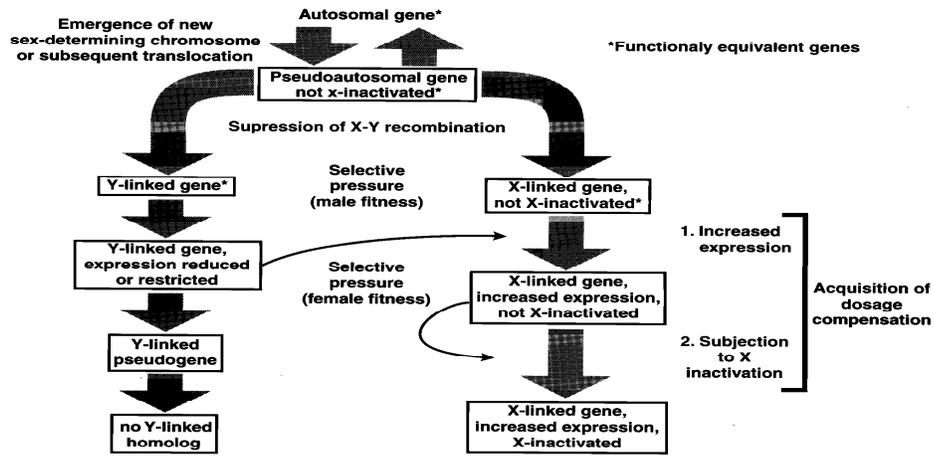


FIGURE 11

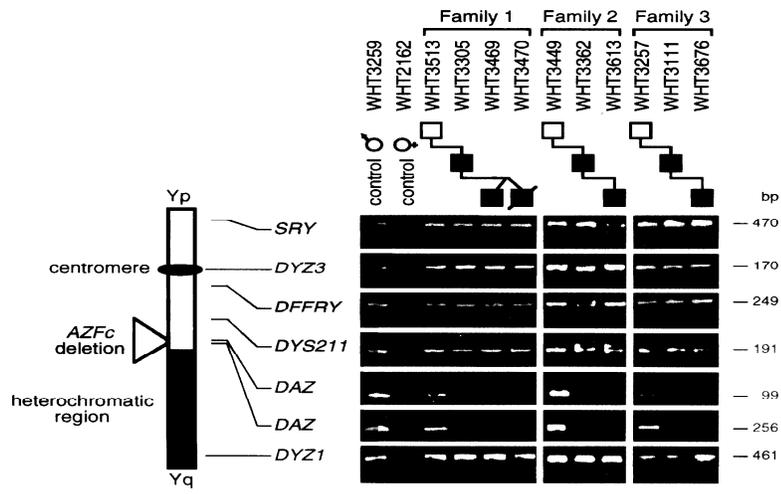


FIGURE 12a

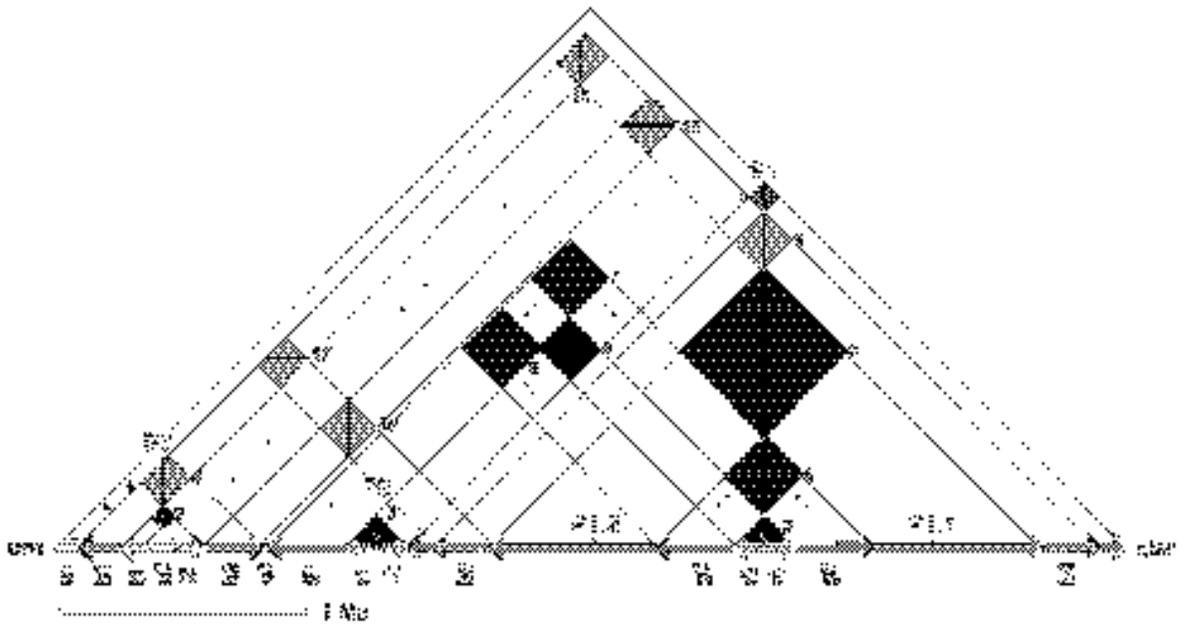


FIGURE 12b

