Genomics and Genetics of Human and Primate Y Chromosomes

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Abstract
In mammals, the Y chromosome plays the pivotal role in male sex determination and is essential for normal sperm production. Yet only three Y chromosomes have been completely sequenced to date—those of human, chimpanzee, and rhesus macaque. While Y chromosomes are notoriously difficult to sequence owing to their highly repetitive genomic landscapes, these dedicated sequencing efforts have generated tremendous yields in medical, biological, and evolutionary insight. Knowledge of the complex structural organization of the human Y chromosome and a complete catalog of its gene content have provided a deeper understanding of the mechanisms that generate disease-causing mutations and large-scale rearrangements. Variation among human Y-chromosome sequences has been an invaluable tool for understanding relationships among human populations. Comprehensive comparisons of the human Y-chromosome sequence with those of other primates have illuminated aspects of Y-chromosome evolutionary dynamics over much longer timescales (> 25 million years compared with 100,000 years). The future sequencing of additional Y chromosomes will provide a basis for a more comprehensive understanding of the evolution of Y chromosomes and their roles in reproductive biology.
INTRODUCTION

Spermatogenic failure: extremely low or absent sperm production, often defined as either nonobstructive azoospermia or sperm density of $<5 \times 10^6$ ml$^{-1}$

Y chromosomes have evolved independently in multiple groups of animals and plants and consequently are bewilderingly diverse. However, all Y chromosomes share two defining features: restriction to the male germ line and limited recombination with a homologous partner during meiosis. Only the Y chromosomes of human and two of our closest relatives—chimpanzee and rhesus macaque—have been completely sequenced, and we focus on these Y chromosomes here.

It has been known for half a century that in mammals a Y chromosome is required for male sex determination (32, 57). But apart from sex determination, Y chromosomes were considered genetic wastelands, not only because of their obvious degeneration in the form of deletions and loss of gene function compared with X chromosomes (21, 85, 106) but also because of numerous failed attempts to identify human Y-linked genes and phenotypes through classical genetic methods such as pedigree and linkage analysis (73, 124).

Genomic studies of the human Y chromosome since the 1980s, which culminated in the publication of the complete human Y-chromosome sequence in 2003 (122), overturned this “wasteland” view of the human Y and revealed its biological and medical importance. With the human Y sequence in hand, researchers have elucidated the mechanisms behind the large-scale Y-chromosomal rearrangements that cause spermatogenic failure and other disorders. In addition, variation in the human Y sequence has been an important tool for tracing the relationships between human populations and for illuminating their migration patterns. The chimpanzee (52, 53) and rhesus (51) Y-chromosome sequences have enabled comprehensive comparative analyses that have shed light on Y-chromosome evolution. These comparisons have given us insight into the evolutionary history of primate Y chromosomes over the last 25 million years and insight into the future prospects of human Y chromosomes. However, because the highly repetitive nature of Y chromosomes makes them tremendously difficult to sequence, they have lagged behind in the genomics revolution. Nevertheless, we expect that sequencing additional Y chromosomes from diverse animal species, especially model organisms used in studies of spermatogenesis, will provide an essential foundation for further elucidation of the biological functions of Y chromosomes.

OVERVIEW OF THE HUMAN Y CHROMOSOME

The complete sequencing of the male-specific region of the Y chromosome (MSY) in humans (Figure 1) has overturned the long-held view of Y chromosomes as barren genetic wastelands. Indeed, the human MSY is surprisingly rich in genes, many of which have been implicated in important biological functions such as sperm production. Details about the biological and medical relevance of the human MSY and its evolution are discussed in later sections, following a brief introduction to the unique characteristics of the human Y chromosome.

The human Y chromosome is heterogeneous, consisting of five highly distinct types of sequence (122) (Figure 1)—pseudoautosomal, heterochromatic, X-transposed, X-degenerate, and ampliconic. The pseudoautosomal regions are located at the extreme termini of the Y and X chromosomes and constitute a small fraction of the total Y-chromosome sequence (81). During meiosis, recombination between the Y and X chromosomes is normally restricted to these regions. Notably, the human Y chromosome is the only Y chromosome characterized thus far with a second pseudoautosomal region. The remainder of the Y chromosome, the MSY, has no partner for homologous recombination and is transmitted clonally, from father to son, solely through the male germ line. This review focuses on the human MSY and the orthologous regions of the chimpanzee and rhesus Y chromosomes. A significant fraction of the human MSY comprises several discrete blocks of heterochromatic sequence, including a single $\sim40$-Mb mass of heterochromatin on the long arm.
Figure 1
Sequence classes, genes, and palindromes on the human Y chromosome. (a) Schematic representation of the entire human Y chromosome, with the male-specific region (MSY) indicated. (b) A more detailed representation that focuses on the euchromatic MSY and excludes the major heterochromatic block on Yq. Palindrome arms are shown as pairs of dark blue triangles. Arms belonging to the same palindrome are indicated by adjacent, opposite-facing triangles. Positions of protein-coding genes are indicated directly below the chromosome by vertical lines. The TSPY gene family, indicated by a rectangle below the chromosome, is arranged in a tandem array with 23–64 copies of a 20.4-kb repeat unit. Each repeat unit contains a single copy of the TSPY gene. (c) Locations and sizes of recurrent deletions within the human MSY that cause (or, in the case of the gr/gr deletion, predispose to) spermatogenic failure. Abbreviations: PAR1, short-arm pseudoautosomal region; PAR2, long-arm pseudoautosomal region.

The euchromatic portions of the human MSY comprise the X-transposed, X-degenerate, and ampliconic regions (Figures 1 and 2). The X-transposed regions, which originated from an X-to-Y transposition event that occurred 3–4 Mya (94), span 3.4 Mb and contain only two genes. The X-degenerate and ampliconic regions constitute the most prominent euchromatic MSY sequence classes and have distinct evolutionary trajectories and gene repertoires (69, 122). The X-degenerate sequences are a deteriorated version of the X chromosome. They are sparsely populated with single-copy genes, 16 in total, most of which are expressed throughout the body and likely serve basic cellular housekeeping functions.

In contrast to the X-transposed and X-degenerate sequences, the ampliconic sequences are highly repetitive, gene-dense, and functionally specialized (Figure 2). They are composed entirely of long stretches of duplicated sequence, most often arranged in palindrome structures that house multicopy gene families with testis-specific expression patterns. There are roughly 60 ampliconic genes in total, representing nine distinct gene families. Some of the ampliconic gene families comprise highly diverged and amplified versions of X-linked progenitors (10, 26, 27, 68, 82, 122); others derive from transpositions or retropositions of autosomal genes (71, 115). In the human MSY, the ampliconic regions are highly prone to large-scale deletions, inversions, and duplications, some of which have severe phenotypic consequences, including clinically low sperm count (7, 37, 66, 102, 104). The chimpanzee and rhesus MSYs also contain X-degenerate and ampliconic sequences (51–53), as do several nonprimate mammalian MSYs (14, 24, 45, 46, 86, 96). Indeed, there is...
Table 2

<table>
<thead>
<tr>
<th>Sequence class</th>
<th>Defining characteristics</th>
<th>Aggregate length (Mb)</th>
<th>Number of coding genes</th>
<th>Number of noncoding transcription units</th>
<th>Number of transcription units per Mb</th>
<th>Nucleotides in interspersed repeats (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>X-transposed</td>
<td>99% DNA identity to X</td>
<td>3.4</td>
<td>2</td>
<td>0</td>
<td>0.6</td>
<td>60</td>
</tr>
<tr>
<td>X-degenerate</td>
<td>Single-copy gene or pseudogene homologs of X-chromosome genes</td>
<td>8.6</td>
<td>16, most expressed widely</td>
<td>4</td>
<td>2.2</td>
<td>57</td>
</tr>
<tr>
<td>Ampliconic</td>
<td>Lengthy similarity to other MSY sequences</td>
<td>10.2</td>
<td>60 (in 9 families), expressed primarily in tests</td>
<td>74 (9 single-copy, 65 in 15 families), expressed primarily in tests</td>
<td>13.3</td>
<td>36</td>
</tr>
</tbody>
</table>

Figure 2

Characteristics and origins of three major euchromatic sequence classes in the human male-specific region of the Y chromosome (MSY): X-transposed (pink), X-degenerate (yellow), and ampliconic (blue). Panel a shows general features and characteristics of each class; panel b represents the evolutionary histories of genes within each sequence class. X-degenerate genes derive from shared X-Y ancestry; most have widespread cellular housekeeping functions. Ampliconic genes have become amplified and specialized for sperm production on the Y and derive from either X-Y ancestral genes or autosomal progenitors. X-transposed genes were transposed from the X to the Y 3–4 Mya. Adapted with permission from Reference 122.

Gene conversion: recombination between similar DNA sequences in which the donor DNA sequence overwrites the acceptor sequence

Inter- and intrachromatid recombination: recombination that occurs between sister chromatids (intrachromatid) or between sequences in the same chromatid (intrachromatid)

evidence for a phylogenetically widespread role for ampliconic sequences in spermatogenesis: In mouse, deletions that remove portions of the highly repetitive long arm of the Y chromosome cause defects in sperm production, although the genes involved are unrelated to human MSY ampliconic genes (15, 22, 135).

Ampliconic repeats are often arranged as palindromic or inverted repeat structures. Repeat copies are often highly similar to each other (usually >99.9% identity). Many of the human MSY palindromes have counterparts on the MSYs of other apes, so the high inter-arm identity cannot be due to the recentness of the palindromes’ formation (111). Rather, frequent arm-to-arm nonreciprocal recombination, or gene conversion, has maintained near identity, which might have played an important role in preventing the decay of the genes that reside within this sequence (111, 122). The ampliconic repeats provide attractive targets for inter- and intrachromatid reciprocal recombination (crossing over) as well, generating inversions, deletions, and duplications, many of which have severe phenotypic outcomes (66, 72, 102, 103, 127). The repetitive nature of the ampliconic sequences also has important consequences for Y-chromosome evolution.

THE HUMAN MSY AND DISEASE

After discovery of the human Y chromosome in 1921 (95), the following decades saw multiple attempts to assign phenotypes and biological functions to the Y chromosome. None succeeded (73, 124). More recently, however, several genes and mutations in the MSY have been definitively tied to human disease. These
successes relied mainly on molecular-genetic and genomic approaches, and in the study of MSY mutations that affect spermatogenesis, they depended critically on the availability of the complete and accurate sequence of the human MSY’s ampliconic regions.

**The SRY Gene and Sex Reversal**

Not until 1959 did researchers realize that the human Y chromosome must bear a dominant, testis-determining gene—a gene that, when present, causes the embryonic gonad to develop into a testis (32, 57). Thirty years later, this gene and its mouse ortholog were identified (43, 121) and dubbed SRY (for sex determining region Y). The approximate location of SRY (Figure 1b) was initially inferred based on analysis of naturally occurring genomic translocations and deletions. These included translocations of the tip of the Y chromosome (which contains SRY) to the X chromosome, resulting in XX males (3, 25, 44). Complementary evidence for the location of SRY came from the feminizing effects of deletions of the distal short arm of the Y chromosome (28, 80, 107). Eventually these investigations led to the cloning of SRY in human and mouse (as reviewed in 39).

Since then there have been numerous reports of women with a normal male karyotype (47,XY) whose embryonic gonads failed to develop into testes (9, 128). This phenotype is referred to as XY gonadal dysgenesis, because although the gonads do not develop into testes, neither do they develop into functioning ovaries. XY gonadal dysgenesis can result from point mutations in the SRY gene (17) or from the total loss of SRY, often due to crossing over between the MSY and the X chromosome (75). In addition, the presence of portions of the Y chromosome in women with gonadal dysgenesis predisposes them to a germ-cell tumor (see sidebar, Human MSY Function Beyond Sex Determination and Spermatogenesis).

**Severe Deficits in Spermatogenesis**

Several regions of the human Y chromosome are essential for normal levels of spermatogenesis (Table 1). The lack of spermatogenesis in XX males does not in and of itself demonstrate the presence of essential spermatogenesis genes on the MSY, because the presence of a second X chromosome in men (47,XXY karyotype, Klinefelter syndrome) is sufficient to almost completely suppress sperm production (35). Instead, the presence of essential spermatogenesis genes on the human Y chromosome was first suggested by the observation that some men with absent or extremely low levels of sperm production (spermatogenic failure) bear cytogenetically visible, and thus very large, deletions affecting the long arm of the Y chromosome (132). This result was subsequently confirmed and refined by molecular studies that showed the absence of particular Y sequence-tagged site (STS) markers in men with spermatogenic failure (99, 100, 144). Proof that these deletions were causal came from the fact that they were almost always de novo (not inherited) and were never observed in men with normal spermatogenesis. We now know that recurrent deletions of several regions of the MSY always result in spermatogenic failure (Table 1). These deletions have only spermatogenic phenotypes,
Table 1  Recurrent human variants in the male-specific region of the Y chromosome (MSY) that affect spermatogenesis

<table>
<thead>
<tr>
<th>Variation type</th>
<th>Phenotype(s)</th>
<th>Prevalence in affected men</th>
<th>Genes affected</th>
<th>Mutational mechanism</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>b2/b4 deletions (AZFc deletion)</td>
<td>Seminiferous tubules: Phenotype ranges from complete loss of germ cells, including the stem cells that give rise to sperm (Sertoli cell only), to partial arrest of germ-cell maturation at meiosis (91). Phenotype within seminiferous tubules can vary within the same testis. Sperm count: Uniform ascertainment is difficult. However, most men with b2/b4 deletions have azoospermia or sperm counts of $&lt; 2 \times 10^6$ ml$^{-1}$. A few have sperm counts of $&gt; 2 \times 10^6$ ml$^{-1}$ and $&lt; 5 \times 10^5$ ml$^{-1}$, with one report of $10 \times 10^6$ ml$^{-1}$ (30, 91, 120). The same man can be azoospermic or severely oligospermic in separate sperm counts (91). Fertility: Fathering children without the use of assisted reproduction is rare enough to warrant case reports (16, 19, 36, 114).</td>
<td>26/430 in azoospermia; 16/283 in severe oligospermia (91)</td>
<td>All copies of the DAZ, BPY2, CDY1, CSPG4LY, and GOLGA2LY gene families, as well as all copies of several spliced transcripts that apparently do not encode proteins (66, 102)</td>
<td>HR between 229-kb direct repeats termed the b2 and b4 repeats (66) (Figure 3)</td>
<td></td>
</tr>
<tr>
<td>P5/distal-P1 (AZFb+c deletion)</td>
<td>Azoospermia and no sperm in seminiferous tubules (38, 50, 65); one report of oligospermia (78).</td>
<td>7/602 in azoospermia (103)</td>
<td>42 genes and transcripts</td>
<td>HR or nonhomologous end joining within possible fragile sites</td>
<td>12, 78, 103, 144</td>
</tr>
<tr>
<td>P5/proximal-P1 (AZFb deletion)</td>
<td>Azoospermia and no sperm in seminiferous tubules (38, 50, 65).</td>
<td>3/602 in azoospermia (103)</td>
<td>32 genes and transcripts</td>
<td>HR or nonhomologous end joining within possible fragile sites</td>
<td>12, 78, 103, 144</td>
</tr>
<tr>
<td>AZFa</td>
<td>Azoospermia.</td>
<td>2/1,609 to 1/602 in azoospermia (103, 120)</td>
<td>USP9Y, DDX3Y</td>
<td>HR between 19-kb flanking direct repeats</td>
<td>11, 61, 127, 144</td>
</tr>
<tr>
<td>IsoYp</td>
<td>Azoospermia. There were no IsoYps in 207 men with oligospermia ($&lt; 5 \times 10^6$ ml$^{-1}$). This variation can also lead to mosaicism for 45X,0 cells and XY gonadal dysgenesis.</td>
<td>8/293 in azoospermia (72)</td>
<td>Various, depending on location of the fusion between long arms</td>
<td>Many instances due to HR between sister chromatids within palindromic sequences</td>
<td>72</td>
</tr>
<tr>
<td>gr/gr</td>
<td>In populations of European descent, gr/gr deletions increase the risk of clinically low sperm count with odds ratios versus normospermia estimated from meta-analyses of 1.7 (125), 2.3 (137), and 2.9 (140); men with gr/gr deletions have lower sperm counts than men without (Figure 6).</td>
<td>101/2,170 in azoospermia (137); 199/2,194 in oligospermia (140)</td>
<td>Some but not all copies of DAZ, BPY2, CDY1, and several noncoding transcript families</td>
<td>Ectopic HR between “red” or “green” amplicons in the AZFc region (Figure 4) as well as other HR events in inverted variants of the AZFc region (104)</td>
<td>102, 125, 137, 140</td>
</tr>
</tbody>
</table>

Azoospermia refers to nonobstructive azoospermia. Abbreviation: HR, homologous recombination.
Mechanisms by which b2/b4 and gr/gr deletions arise. (a) The organization of amplicons (large, nearly identical segmental duplications) that make up the AZFc region. Amplicons of the same color have DNA sequences that are 99.82%–99.97% identical to one another (66). b2/b4 deletions are caused by ectopic recombination and crossing over between the b2 and b4 repeats in the AZFc region. (b) Schematic representation of the b2/b4 crossover, which could occur either within a single chromatid or between sister chromatids. (c) The product of the b2/b4 crossover, an extremely abbreviated variant of the AZFc region from which 3.5 Mb have been removed. (d) Ectopic crossing over between green or red amplicons, causing gr/gr deletions. Abbreviations: cen, direction of centromere; q ter, direction of long-arm terminus of the chromosome.

indicating that much of the human MSY is highly specialized for this function.

The most common type of recurrent deletion that causes spermatogenic failure is the b2/b4 deletion (Figures 1 and 3, Table 1). This deletion occurs within the AZFc region, which comprises 4.4 Mb at the distal end of the long-arm MSY euchromatin (Figures 1 and 3). This region is remarkable for its elaborate structure of direct and palindromic repeats, and it was the first such region to be completely sequenced (66). The direct repeats in the AZFc region are responsible for the b2/b4 deletion (Figure 3) and are responsible for its relatively high prevalence, estimated as 1 out of every 4,000 men. Because, with very rare exceptions (16, 19, 36, 114), b2/b4 deletions are transmitted only via assisted reproduction (58, 60, 91), they are almost always de novo.

b2/b4 deletions remove all copies of several gene families, and we know little about the contributions of these gene families to the spermatogenic failure caused by b2/b4 deletions. This is because mutations that abrogate the function of all copies of any single gene family have not been found. Given the organization of the region (Figure 3), such deletions probably do not exist, as each copy of the gene would have to be mutated individually. However, among the gene families removed by b2/b4 deletions,
the DAZ genes are the most intensively studied, and experimental studies in mice provide circumstantial evidence that loss of the DAZ gene family is at least partly responsible for the spermatogenic failure caused by b2/b4 deletions. Although DAZ has no homologs on the mouse Y chromosome, DAZa has autosomal homologs in both human (DAZL) (115, 118, 151) and mouse (Dazl) (23, 101). Mice in which Dazl has been knocked out have severe deficits in germ cells in both testes and ovaries (112).

In addition to the b2/b4 deletion, there are several other less common but still recurrent types of deletions that always result in spermatogenic failure (Figure 1c). These include the P5/P1 deletions, which include part of the AZFa region but extend much more proximally (103), and AZFa deletions (144) (Table 1). As has been the case for b2/b4 deletions, it has been essentially impossible to identify the specific genes responsible for the phenotype of P5/P1 deletions. For the AZFa deletion, however, several studies show that the USP9Y gene is important (64, 126). However, AZFa deletions always result in nonobstructive azoospermia (11, 50, 61, 127), whereas mutations affecting USP9Y alone have milder phenotypes (64, 79). Therefore, it appears that additional genetic factors within the deleted region are also essential for normal levels of spermatogenesis. DDX3Y, the only other gene removed by AZFa deletions and a gene with protein expression only in the testis (29), is a prime candidate.

Isodicentric Y Chromosomes and Diverse Clinical Consequences

The ampliconic structure of much of the human MSY fosters not only recurrent deletions such as the b2/b4 deletion, but also the generation of isodicentric Y chromosomes (72). As shown in Figure 4, fusions between two sister chromatids can occur at various points in the Y-chromosome long or short arm. Inverted repeats, including palindromes, are hot spots for the ectopic homologous crossover events that often cause these fusions. The consequences of the formation of isodicentric Y chromosomes are diverse. If the isodicentric Y chromosome was formed by fusion and truncation of short arms (termed an idicYp chromosome, because the long arms are intact), then the result is XY gonadal dysgenesis and sex reversal due to loss of SRY, as discussed above. More commonly, isodicentric Y chromosomes are formed with fused and truncated long arms and with two intact short arms; these are termed idicYp chromosomes.

There can be two alternative clinical consequences of idicYp chromosomes. First, mitotic instability of the idicYps can lead to mosaicism for 45,XO and 46,XidicYp cells, which in turn can lead to XY gonadal dysgenesis and sex reversal. In addition, if all or most cells are 45,XO, the phenotype will be Turner syndrome, which involves several abnormalities, including short height, lymphedema, cardiovascular malformations, and dysgenic ovaries lacking oocytes (49). Indeed, there is circumstantial evidence that Y abnormalities, such as idicYp chromosomes, are the ultimate causes of many cases of Turner syndrome, as chromosomes with two centromeres are prone to loss during mitosis. Indeed, 75% of the X chromosomes in Turner syndrome patients are inherited from the mother (56, 138), consistent with the father having transmitted a dicentric Y chromosome that was subsequently lost during early embryogenesis. The second, alternative clinical consequence of an idicYp is spermatogenic failure. This likely stems in part from the fact that many idicYps lack distal parts of the Y-chromosome long arm containing genes essential for spermatogenesis. In addition, the presence of two short-arm pseudoautosomal regions and the absence of the long-arm pseudoautosomal region may disrupt meiotic pairing with the X chromosome and disrupt meiosis (84). Finally, mosaicism for cells completely lacking the Y chromosome might also contribute to spermatogenic failure.

Risk Factors for Deficient Spermatogenesis

In addition to the MSY deletions discussed above, which cause severe spermatogenic deficiency with nearly complete penetrance, there
Ectopic recombination and crossing over between palindromes in the human male-specific region of the Y chromosome (MSY) can form isoYp chromosomes. The crossing over occurs between sister chromatids and results in an aberrant MSY with two centromeres, two short arms, and two truncated and fused long arms. Even though two copies of the centromeric sequence are present, often only one centromere hosts a functionally active kinetochore. The examples show crossing over and fusion at (a) palindrome P1 and (b) palindrome P5, although almost all other MSY palindromes and inverted repeats have also been sites of isoYp formation (72). Fusions have also occurred in the distal Yq heterochromatin and within the centromere itself, the latter of which results in a chromosome with a single centromere and two identical short arms. Abbreviations: p, short arm of the Y chromosome; q, long arm of the Y chromosome.

Is an additional deletion that is a risk factor for low sperm count. This deletion, the gr/gr deletion, is contained wholly within the region removed by the b2/b4 deletion (102) (Figures 1c and 3d, Table 1). Unlike b2/b4 deletions, gr/gr deletions are often inherited and do not remove all copies of any gene family. Nevertheless, at least in populations of European descent, men with a gr/gr deletion have in general lower sperm counts (125, 137, 140). In Japan, however, gr/gr deletions are prevalent owing to a founder effect, many Y chromosomes descended from a single Y-bearing a gr/gr deletion. Whether this gr/gr deletion is a risk factor for spermatogenic failure in Japan has not been adequately investigated (18, 67).

Because of the MSY’s clonal inheritance, two male patients of the same pair could have a shared Y chromosome, and one patient could have a Y chromosome that is identical to his father’s Y chromosome. Therefore, more than one out of ten infertile men in whom a male partner of an infertile couple has a Y-chromosome deletion will have at least one partner who has a Y chromosome that is identical to his father’s Y chromosome. This is because the MSY is inherited as a whole. The proportions of patients with total sperm counts ≤ x (the values on the x-axis) and sperm counts significantly lower among men with the gr/gr deletion than patients with gr/gr deletions. This cumulative distribution plot shows the proportions of patients with total sperm counts ≤ x (the values on the x-axis) and sperm counts significantly lower among men with the gr/gr deletion than among men without deletions (p = 0.006; Wilcoxon rank-sum test). The patients were a series of male partners of infertile couples, regardless of sperm count. The y-axis is the proportion of patients, and the x-axis is the total sperm count (millions).
unchanged except for new mutations, MSY population genetics are very different from those of the rest of the nuclear genome. This has afforded special opportunities to understand the dynamics of large-scale MSY variation. Furthermore, the study of MSY population genetics has been heavily influenced by the use of MSY variants to provide information about genetic relationships among human populations.

**Single-Nucleotide Polymorphisms Define a Robust Genealogical Tree of Human MSYs**

A remarkable achievement of the past 15 years has been the delineation of a robust genealogical tree of human Y chromosomes based on numerous stable single-nucleotide changes scattered across the single-copy (i.e., X-degenerate) regions of the human MSY (47, 59, 62, 139) (Figure 6). Because single-nucleotide
mutations occur rarely ($3.0 \times 10^{-8}$ mutations per nucleotide per generation) (150), there are few reversions (back mutations) or recurrences (independent identical mutations at the same site). Thus, by using the chimpanzee MSY sequence to determine the ancestral state at human MSY single-nucleotide polymorphisms (SNPs), it is possible to construct a highly reliable genealogical tree of human MSYs that defines a number of distinct MSY haplogroups.

The last common ancestor of all extant human MSYs was found in a man living ∼100,000 years ago (119, 130, 131, 149), although there is substantial uncertainty in this date. (Estimates have varied from ∼50,000 to 110,000 years ago owing to uncertainties inherent in the statistical approaches used and in the date of the divergence between human and chimpanzee MSYS.) Although this man has been nicknamed “Y-chromosomal Adam,” many other men lived at the same time and contributed non-MSY genetic material to today’s human population. To see that this is possible, consider a man who fathers only daughters. Although his MSY is lost forever, his daughters nevertheless inherit half of his autosomal genome and his X chromosome. The special characteristic of Y-chromosomal Adam is that he was the most recent man to be connected to all living men by an unbroken line of sons, through which descendants of his MSY were transmitted to the present.

**MSY Variation Traces Patrilineal Relationships Between Men and Between Human Populations**

Because of the MSY’s clonal inheritance, MSY variation (organized into the MSY genealogy) can trace patrilineal relationships between men and between populations. As an example of tracing relationships between men, analysis of Y chromosomes combined with historical information showed that Thomas Jefferson, the third president of the United States, fathered a child by his slave, Sally Hemings (33). The MSY evidence consisted of the fact that the purported patrilineal descendant of Jefferson and Hemings had an MSY with a rare set of variants that were identical to those of patrilineal descendants of Jefferson’s paternal uncle. The conclusion that Jefferson fathered his slave’s child relied not only on molecular evidence, but also on historical evidence that ruled out Jefferson’s close patrilineal relatives, whose MSYS would have been indistinguishable from Jefferson’s.

At a larger scale, and relying on additional assumptions, the Y chromosome can be used to trace relationships among human populations. In fact, the two most recent compendia of the MSY genealogy (59, 62) have been cited >500 times, with the vast majority of these publications using MSY variation to trace relationships among human populations. To take just one example from hundreds, MSY data helped tease apart the African and Southeast Asian contributions to the population of Madagascar (54, 133). In addition, analysis of MSY variants in ancient DNA now lets us study relationships between ancient and modern populations—for example, helping to show that people living in south-central Siberia almost 4,000 years ago bore many resemblances to modern Europeans (63). These resemblances include a high proportion of Y chromosomes in haplogroup R1a1, which is now prevalent in Europe, in addition to mitochondrial and autosomal similarities. Although human MSY variation is a valuable tool in these studies, natural selection has operated on the MSY during human evolution (102, 110) and might have influenced the survival of particular Y lineages.

**High Rates of Large-Scale Mutation**

In addition to its use in tracing patrilineal relationships among populations, the SNP-based genealogical tree of human MSYS has provided a framework in which to understand large-scale mutation. For example, in the case of gr/gr deletions, the fact that deletions occurred in many branches of the Y genealogy indicates that deletion events have occurred multiple times (102). This in turn helped show that the gr/gr deletion itself, rather than some linked variant on the...
Table 2  High rates of large-scale mutation in human male-specific regions of the Y chromosome (MSYs)

<table>
<thead>
<tr>
<th>Type of large-scale variation</th>
<th>Lower bound on mutation rate per father-to-son transmission of MSY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deletions, duplications, and inversions within AZFc; we detected 11 different organizations</td>
<td>(3.8 \times 10^{-4})</td>
</tr>
<tr>
<td>Change in the length of the tandem array containing the TSPY genes (i.e., addition or deletion of repeat units); the length of this array ranges from 23 to 64 repeat units</td>
<td>(4.4 \times 10^{-4})</td>
</tr>
<tr>
<td>Change in the length of the distal-Yq heterochromatin (i.e., addition or deletion of heterochromatic sequence)</td>
<td>(2.3 \times 10^{-4})</td>
</tr>
<tr>
<td>Change in orientation of the short-arm region bounded by the IR3 inverted repeats (i.e., a mutation that inverts this region)</td>
<td>(2.3 \times 10^{-4})</td>
</tr>
<tr>
<td>Lower bound on aggregate mutation rate</td>
<td>(12.8 \times 10^{-4} (1/781))</td>
</tr>
</tbody>
</table>

We inferred mutation rates by looking at chromosomes from 47 branches of the MSY genealogy and calculating how often, at a minimum, large-scale mutations must have occurred to account for the observed patterns of large-scale variation across these 47 chromosomes. We then used the total number of single-nucleotide polymorphisms (SNPs) in the genealogy and the average number of SNPs from root to twig in the genealogical tree to estimate the total number of generations represented by the tree. Summarized from Reference 104.

same MSY, is a risk factor for low sperm count. More generally, it has been possible to infer that the human MSY is subject to high rates of several kinds of large-scale rearrangement (Table 2), rates that are 10,000 times higher than the rate at which single-nucleotide substitutions occur (104).

Y-CHROMOSOME EVOLUTION

Although the availability of the human MSY sequence overturned long-held beliefs about the functional irrelevance of the Y chromosome, comparative analyses with the X chromosome and with the MSYs of other species have provided even deeper insight into the fascinating evolutionary dynamics of these singular chromosomes.

Comparison with the X: Evolution over Hundreds of Millions of Years

The view of Y chromosomes as barren genetic wastelands was cemented in the 1960s with Ohno’s (92) proposal that present-day sex chromosomes descended from a once identical pair of autosomes that ceased recombining with each other and thus became differentiated (Figure 7). Theory holds that recombination suppression was selectively advantageous in the nascent Y chromosome because it restricted the expression of Y-linked sexually antagonistic genes to males (105, 106). There was a downside to this strategy, however: The lack of regular meiotic recombination left Y chromosomes vulnerable to gene loss and evolutionary decay (21, 106). Indeed, the present-day human MSY is substantially degenerated compared with the X in both euchromatic size (\(\sim 23\) Mb versus \(\sim 150\) Mb) and protein-coding gene content (\(\sim 78\) genes versus \(\sim 800\) genes) (109, 122). Degeneration following recombination suppression has been inferred in Y chromosomes of plant and insect species as well (13, 20, 34). Meanwhile, it has long been assumed that mammalian X chromosomes, which continue to undergo recombination in females, have been largely preserved over hundreds of millions of years of evolution (93). One major alteration was a massive autosomal translocation that added nearly 50 Mb to the ancestors of eutherian X and Y chromosomes after the divergence of marsupials (83, 109, 147, 148) (Figure 7).

A systematic analysis of the divergence between the human MSY’s surviving ancestral genes and their X-chromosome counterparts revealed that X-Y differentiation was a stepwise process (70) (Figure 7). Recombination between the X and Y chromosomes was
suppressed in stages over the course of hundreds of millions of years, and regions of the Y that were isolated from recombination with the X were immediately subject to degenerative processes. At least five discrete regions, or strata, have been identified within the human X and Y chromosomes (70, 109), with the oldest stratum containing the most divergent genes and the youngest stratum containing the least divergent genes. It was postulated that strata
were formed by large inversions on the Y chromosome, which halted productive meiotic recombination with the X chromosome within the affected regions (70). Inversion breakpoints at the boundaries of the newest strata are still recognizable, confirming this hypothesis (74). Y-chromosome stratification followed by degeneration appears to be a widespread phenomenon of sex chromosome evolution, as staggered X-Y gene pair divergences have been noted in mammals other than primates (97, 113) and even in plants (90).

Comparison with Other Primate Ys: Evolution over Tens of Millions of Years

Signs of degeneration are observed in even the most recently emerged Y chromosomes, such as those in some plant and fly species (5, 31, 77). The evolutionary mechanisms causing gene loss depend on the number of genes on an MSY, and all well-supported evolutionary models indicate that MSYs with large numbers of genes lose them much more rapidly than MSYs with few genes (4, 21). Therefore, ancient Y chromosomes, such as those in mammals, should lose genes only rarely. Nevertheless, flawed linear extrapolations of gene loss have often been used to predict the continued rapid loss of Y-linked genes in humans and, as a consequence, the evolutionarily near-term demise of human Y chromosomes (1, 40, 41, 129).

The complete sequences of two additional primate Y chromosomes—those of the chimpanzee and rhesus macaque (51–53)—provide empirical data that clearly refute the Y-demise hypothesis. Through a comparison of ancestral gene contents across species, it is evident that the human MSY has not lost any genes since the human-chimpanzee lineages diverged roughly 6 Mya: There are no genes on the chimpanzee MSY that were lost from the human MSY (52, 53). In addition, comparison with the rhesus MSY, which gives a 25-million-year view into human MSY evolution, indicates that the gene content has been largely maintained even over this longer time frame (51). Within the oldest four strata, the ancestral gene contents of human and rhesus are identical, meaning that no genes were lost within these regions in the past 25 million years. Gene loss has occurred only in the newest stratum, which formed just prior to the split of the human and rhesus lineages and occupies only ~3% of the MSY (109).

A comparison with the same region of the X chromosome indicates that only one of the original seven genes remains intact on the human MSY and that only two genes remain intact on the rhesus MSY. These data confirm that the rate of MSY gene loss was probably rapid initially but slowed dramatically over time, and in the oldest strata has apparently come to a halt. A study of amino acid variation in X-degenerate genes among >100 men representing worldwide diversity revealed that these genes, which have been retained on the MSY for tens or hundreds of millions of years, are under strong purifying selection (110). Thus, it seems unlikely that they will disappear from the population. Interestingly, whereas the rhesus MSY also retained its genes over the past 25 million years, the chimpanzee MSY suffered significant gene loss (six genes in the 6 million years since the human and chimpanzee lineages diverged) (52, 53), signifying a recent acceleration in the pace of decay in the chimpanzee lineage.

A second key insight emerging from the full-scale comparison of MSYs is the divergent evolutionary dynamics at work within the X-degenerate and ampliconic sequence classes. Although the X-degenerate regions are largely conserved between human, chimpanzee, and rhesus, the ampliconic sequences have undergone dramatic restructuring and renovation, and have followed completely different evolutionary trajectories in each lineage (51, 52) (Figure 8). The proportion of the euchromatic MSY that is occupied by ampliconic sequence in human, chimpanzee, and rhesus varies substantially (45%, 57%, and 5%, respectively). The ampliconic region of the chimpanzee MSY is not only larger than that of human but structurally more complex. In the human MSY, the majority of repeat structures are
palindromes, which have a repeat unit copy number of 2. In chimpanzee, most of the repeats are present in 4 or more copies, and the most abundant repeat has 13 copies. By contrast, the rhesus MSY is surprisingly lacking in ampliconic sequence and may represent a “minimal Y chromosome.” It is evident that primates Y chromosomes are not homogeneous substrates for evolution. Instead, the distinct classes of MSY sequence, which play different functional roles in males, are under very different types of selective pressure. Perhaps the rapid evolution of the ampliconic sequence is driven by positive selection targeting spermatogenesis genes, ampliconic structures, or both. The rearrangement-prone nature of ampliconic structures may also contribute to the rapid evolution of these regions.

**SEQUENCING Y CHROMOSOMES**

Although the complexity of ampliconic regions makes them difficult to sequence, the accurate and complete sequence of the human MSY has yielded numerous biomedical returns. These returns stem from the identification of repeat structures on the MSY that are the cause of recurrent deletions and isodicentric chromosome formation, both of which have clinical consequences. Here we explore in more depth the challenges of sequencing Y chromosomes and the technical approaches that have successfully generated accurate sequences of the ampliconic regions of these chromosomes.

**Why Y Chromosomes Fall Short in Whole-Genome Sequencing**

The genomes of 249 animals have been or are in the process of being sequenced (88), and plans to sequence thousands more are in the pipeline (48). All but a handful of these genome sequences were assembled using the relatively cheap and fast whole-genome shotgun (WGS) approach (89). Remarkably, however, only the human, chimpanzee, and rhesus macaque Y chromosomes have been sequenced to date (51–53, 122). The reasons that Y chromosomes lag so far behind the remainder of the genome are twofold; one is technical and one is biological. The technical reason stems from the desire to adequately represent the X chromosome in WGS assemblies. Therefore, female (XX) genomes are chosen as sequencing targets so that the X chromosome achieves the same coverage as the autosomes. The biological reason for the lack of Y-chromosome sequence is due to the complex and repetitive nature of Y chromosomes, the scale of which is not seen elsewhere in the genome. For reasons described in detail below, WGS approaches are not suitable for the assembly of such intricate sequences.

All mammalian Y chromosomes studied thus far contain stretches of ampliconic sequence that are composed of long, highly similar repeats (52, 122; H. Skaletsky, unpublished information). In human, the amplicon repeat unit length is typically hundreds of kilobases and up to 1.5 Mb, and the repeat units differ by only 1 base pair (bp) per 5–10 kb on average (99.98%–99.99% identity) (122). Repetitive sequences are not restricted to Y chromosomes but are found throughout the genome, where they are known as segmental duplications. This classification conventionally encompasses any sequence with a repeat unit of >1 kb and sequence identity of >90% (6). Therefore, the ampliconic sequences on Y chromosomes can be thought of as extreme segmental duplications with especially long repeat units and the highest sequence identities.

Repeated sequences such as those found on Y chromosomes and elsewhere in the genome cannot be accurately assembled using WGS approaches. Sequence reads that are shorter than the length of a given repeat unit, such as those produced by both Sanger-sequencing (~500 bp per read) and next-generation (76–300 bp per read) WGS approaches, are not long enough to distinguish different copies of that repeat unit, even when using paired reads. Any assembly algorithm will lump all reads deriving from the repeat family together if the between-unit identity is modestly high. The read length is simply too short to provide genomic context for distinguishing repeats. A systematic investigation
Figure 8

Structural overview of three primate Y chromosomes. In the center are representations of the overall structure of each of the Y chromosomes, with male-specific regions (MSYs) bracketed. For the human Y, the given length excludes the major long-arm heterochromatin. (b,c) Organization of selected ampliconic regions in the human and chimpanzee MSYs. Each “flavor” of amplicon is represented by a different color. The colors used in the human and chimpanzee plots reflect interspecies homology. (a,d) Triangular dot plots of the ampliconic regions. These plots are constructed by comparing the selected sequence with itself and then constructing a two-dimensional plot to highlight regions of identity. One copy of the sequence is on each axis. A dot is plotted for every 200-bp window of sequence that shares 100% identity with another 200-bp sequence. The plot is then rotated 45°, and only one half of the plot is shown. Vertical lines represent inverted repeats, and horizontal lines represent direct repeats.

of the representation of all types of repeats, including segmental duplications, in the de novo WGS assemblies of two human individuals (76) exposed a dramatic artifactual loss of duplicated sequences (>99% missing) (2), confirming the intractability of Y ampliconic sequence to WGS approaches.

Assembling Y-Chromosome Sequences

The human, chimpanzee, and rhesus Y chromosomes were sequenced using a specialized clone-by-clone-based sequencing strategy known as single-haplotype iterative mapping.
and sequencing (SHIMS), which is described below (51, 52, 66, 122). The SHIMS approach was also used to sequence the ampliconic regions of the chicken Z chromosome, which was the first bird sex chromosome to be fully sequenced (8). Bacterial artificial chromosomes (BACs) are the clones of choice because of the large amount of foreign DNA that they can incorporate (150–200 kb). The initial step in SHIMS is to construct a provisional BAC contig map: First, BACs containing Y-chromosome inserts are identified in a genomic BAC library, usually by spotting the BACs on filters and then hybridizing with Y-specific probes. Second, sets of clones containing overlapping segments of DNA are identified using several techniques, including (a) STS content assayed by polymerase chain reaction (PCR); (b) BAC-end sequences, which are Sanger-sequencing reads derived from both ends of the insert sequence; and (c) fingerprint contig analysis, a technique that utilizes the unique restriction enzyme fragment pattern of BAC inserts to detect overlapping BACs. After the contig map is constructed, a “tiling path” of overlapping, nonredundant BACs is then selected for sequencing.

The next steps in the procedure are what differentiate SHIMS from conventional clone-based sequencing approaches (Figure 9). Overlapping regions from finished BAC sequences are scrutinized for mismatches, or sequence family variants (SFVs), which indicate that the BACs are from different copies of a repeat and are therefore not true neighbors. If mismatches are consistently identified, then the

**Figure 9**
Iterative mapping and sequencing procedure. The example shown is for an amplicon with two copies. (a) A bacterial artificial chromosome (BAC) map that spans the region of interest is constructed, and a set of nonredundant overlapping BACs, or tiling path, is selected for sequencing (BACs in the tiling path are shown in **green**). (b) BACs are sequenced, and overlapping sequences from neighboring BACs are scrutinized for any mismatches or sequence family variants (SFVs, shown in **yellow** or **orange**). (c) If SFVs are found, then the remaining BACs in the contig are screened by polymerase chain reaction (PCR) and sequencing to determine their SFV patterns. (d) BACs are sorted into separate contigs, representing different copies of the same repeat, based on overlapping SFV patterns. The newly selected BACs are sequenced, and the assembly now represents complete copies of the two segmental duplications ( **gray arrows** ) in the region.
Fluorescence in situ hybridization (FISH): method to detect and visualize specific DNA sequences in chromosomes within individual cells.

BACs are separated because they are derived from two different copies of a repeat. True neighbors for each BAC are then identified by "typing" candidate BACs (located in the original BAC contig map or through further library screening) for SFV content. If the same SFV is found in a candidate BAC, then this BAC is selected for sequencing to extend the original BAC sequence. If new SFVs are found, the repeat copy number is expanded and further neighbors need to be identified. The process of sequencing, SFV typing, and BAC screening is iterated until all copies of the repeat are identified and completely sequenced with no mismatches between overlapping BACs. The copy number of the repeat can be confirmed using fluorescence in situ hybridization (FISH) on interphase cells in some cases. The more complex the repeat architecture of a Y chromosome, the more iterative mapping and sequencing is required: 135 SFVs were typed for the human Y chromosome (122), whereas 372 SFVs were typed for the more ampliconic chimpanzee Y chromosome (32) (Figure 8).

The success of iterative mapping and sequencing relies on two important components—selection of tiling paths with sufficient overlap between BACs and the use of a single chromosome (or haplotype) as a template. For single-copy regions, BAC tiling paths can be chosen to minimize overlap, thereby reducing the amount of redundant sequence generated. However, for ampliconic regions of Y chromosomes, extensive BAC overlaps (usually 30 kb) are required to detect rare mismatches between highly similar sequences. In addition, distinguishing repeat copies on the Y chromosome is only possible if a single Y is used as a sequencing template. Otherwise, differences between repeat copies would not be distinguishable from polymorphisms between different Y chromosomes. The haploid nature of Y chromosomes in males enables this single-haplotype template sequencing approach.

Sequencing each individual BAC is the major bottleneck for each iteration of the procedure. Assembling the sequence of a single BAC from multiple Sanger-sequencing reads is a disappearing capability that depends on expensive Sanger sequencing and a prohibitive amount of expert hands-on attention and manual sequence editing. Instead, it should be possible to pool dozens of BACs together in a single next-generation sequencing run and obtain >100-fold sequence coverage for each clone. The difficulty will arise in the assembly of the short sequence reads into continuous individual BAC sequences. However, if care is taken in building pools that contain readily distinguishable BACs (i.e., from different species or divergent regions of the chromosome), then the abundant sequence coverage should make up for the short read length and allow for a largely automated assembly of the sequence. The dramatic savings in both cost and time promised by this new approach to BAC sequencing could have a tremendous impact on sequencing not only Y chromosomes but also many other complex but biologically interesting genomic regions.

CONCLUDING REMARKS

Since 1990, there has been remarkable progress in understanding human and primate Y chromosomes and their genes. The sequencing of the human MSY required extraordinary efforts but was a major milestone in this process. We now can discern several major conceptual themes that revolve around human and primate MSYs.

Medically, the human MSY is critical for testis differentiation and for spermatogenesis, and testing for Y-chromosome deletions is routine in the treatment of spermatogenic failure. The human MSY is also important for transplantation medicine and is a major cause of the benign germ-cell tumor gonadoblastoma. In anthropology and human history, the MSY has been widely used to trace connections between human populations and to understand differences in migration and population genetics between males and females. Over primate evolution, X-degenerate and ampliconic sequences have had contrasting trajectories: X-degenerate...
sequences have tended to be conserved, whereas ampliconic sequences have mutated and evolved rapidly. These two sequence classes may experience very different selective pressures because of the distinct functions of the genes that reside within them—purifying selection acting to maintain the integrity of the widely expressed X-degenerate genes and positive selection influencing the evolution of the testis-expressed genes within the ampliconic sequence. Ampliconic sequences’ propensity for rearrangement may also have contributed to their rapid evolution.

A critical challenge for the future will be adapting next-generation sequencing technologies to reduce the costs of clone-by-clone-based sequencing of ampliconic regions. This will then open the door to the sequencing of additional Y chromosomes, which will illuminate both their biological roles and widespread, recurrent phenomena in Y-chromosome evolution.

SUMMARY POINTS
1. The male-specific regions of Y chromosomes—MSYs—occur only in males and are transmitted clonally from father to son.
2. The human MSY has three euchromatic sequence classes—X-transposed, X-degenerate, and ampliconic—each of which has distinct evolutionary and functional characteristics.
3. The human MSY is medically important because of its roles in sex determination, spermatogenesis, transplantation medicine, and the germ-cell tumor gonadoblastoma.
4. Ectopic homologous crossing over is a major mechanism for pathogenic and non-pathogenic large-scale variation among human MSYs and leads to the formation of deletions, duplications, inversions, and isochromosomes.
5. IdicYps can lead either to spermatogenic failure or to sex reversal and gonadal dysgenesis, possibly accompanied by Turner syndrome.
6. The genealogical tree of human MSYs has been used extensively to illuminate the connections between human populations and sex-specific differences in migration and population genetics.
7. In primate evolution, X-degenerate regions have tended to be conserved, whereas ampliconic regions have changed dramatically.
8. Although hundreds of genomes are deemed to be sequenced, only three MSYs have been fully sequenced—those of human, chimpanzee, and rhesus macaque—which testifies to the technical challenges presented by MSYs.

FUTURE ISSUES
1. It will remain difficult to study the functions of individual human MSY genes, especially for the ampliconic gene families.
2. The extraordinary reliance on the genealogical tree of human MSYs in understanding relationships among human populations is being replaced by broader approaches that make much more use of autosomal data, and MSY variation will be increasingly used to answer questions about sex-specific differences in population history and genetics.
3. Combining next-generation sequencing technologies with clone-by-clone-based approaches should enable much cheaper sequencing of additional MSYs, including their ampliconic regions.

4. The ability to more cheaply sequence MSYs of additional species will illuminate the biological functions of these chromosomes and widespread, recurrent phenomena in Y-chromosome evolution.

DISCLOSURE STATEMENT
S.R. consulted for Repromedix, which marketed a test for clinical Y deletion testing, and receives royalties for use of this test.

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LITERATURE CITED

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62. Provides the most up-to-date SNP-based human MSY genealogical tree.

66. Reports the extent, mechanism, and genes affected by b2/b4 (AZFa) deletions.

70. Provides molecular evidence that the human MSY appropriated previously pseudoautosomal sequence in stepwise fashion.

72. Shows that Y-Y recombination can form isodicentric chromosomes leading to sex reversal or spermatogenic failure.


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Contents

Human Genetic Individuality
   Maynard V. Olson ................................................................. 1

Characterization of Enhancer Function from Genome-Wide Analyses
   Glenn A. Maston, Stephen G. Landt, Michael Snyder, and Michael R. Green ....... 29

Methods for Identifying Higher-Order Chromatin Structure
   Samin A. Sajan and R. David Hawkins ...................................... 59

Genomics and Genetics of Human and Primate Y Chromosomes
   Jennifer F. Hughes and Steve Rozen ........................................ 83

Evolution of the Egg: New Findings and Challenges
   Katrina G. Claw and Willie J. Swanson .................................... 109

Evolution of the Immune System in the Lower Vertebrates
   Thomas Boehm, Norimasa Iwanami, and Isabell Hess ......................... 127

The Human Microbiome: Our Second Genome
   Elizabeth A. Grice and Julia A. Segre ...................................... 151

Functional Genomic Studies: Insights into the Pathogenesis of Liver Cancer
   Ze-Guang Han ........................................................................... 171

A Comparative Genomics Approach to Understanding Transmissible Cancer in Tasmanian Devils
   Janine E. Deakin and Katherine Belov .................................... 207

The Genetics of Sudden Cardiac Death
   Dan E. Arking and Nona Sotoodehnia ....................................... 223

The Genetics of Substance Dependence
   Jen-Chyong Wang, Manav Kapoor, and Alison M. Goate .................... 241

The Evolution of Human Genetic Studies of Cleft Lip and Cleft Palate
   Mary L. Marazita ......................................................................... 263

Genomic Analysis of Fetal Nucleic Acids in Maternal Blood
   Yuk Ming Dennis Lo and Rossa Wai Kwun Chiu ............................. 285
Enzyme Replacement Therapy for Lysosomal Diseases: Lessons from 20 Years of Experience and Remaining Challenges
R.J. Desnick and E.H. Schuchman ................................................................. 307

Population Identification Using Genetic Data
Daniel John Lawson and Daniel Falush ........................................................ 337

Evolution-Centered Teaching of Biology
Karen Burke da Silva ..................................................................................... 363

Ethical Issues with Newborn Screening in the Genomics Era
Beth A. Tarini and Aaron J. Goldenberg ...................................................... 381

Sampling Populations of Humans Across the World: ELSI Issues
Bartha Maria Knoppers, Ma’n H. Zawati, and Emily S. Kirby ....................... 395

The Tension Between Data Sharing and the Protection of Privacy in Genomics Research
Jane Kaye ......................................................................................................... 415

Genetic Discrimination: International Perspectives
M. Otlowski, S. Taylor, and Y. Bombard ....................................................... 433

Indexes
Cumulative Index of Contributing Authors, Volumes 4–13 ......................... 455
Cumulative Index of Chapter Titles, Volumes 4–13 ..................................... 459

Errata
An online log of corrections to *Annual Review of Genomics and Human Genetics* articles may be found at http://genom.annualreviews.org/errata.shtml