



UNIVERSITÀ
DEGLI STUDI
FIRENZE

FLORE
Repository istituzionale dell'Università degli Studi di
Firenze

Y chromosome polymorphisms in medicine.

Questa è la Versione finale referata (Post print/Accepted manuscript) della seguente pubblicazione:

Original Citation:

Y chromosome polymorphisms in medicine / C. KRAUSZ; QUINTANA-MURCI L; G. FORTI. - In: ANNALS OF MEDICINE. - ISSN 0785-3890. - STAMPA. - 36(2004), pp. 573-583.

Availability:

This version is available at: 2158/212115 since:

Publisher:

primo editore: Publisher Finnish Medical Society Duodecim, Helsinki editore attuale: Taylor & Francis

Terms of use:

Open Access

La pubblicazione è resa disponibile sotto le norme e i termini della licenza di deposito, secondo quanto stabilito dalla Policy per l'accesso aperto dell'Università degli Studi di Firenze (<https://www.sba.unifi.it/upload/policy-oa-2016-1.pdf>)

Publisher copyright claim:

(Article begins on next page)

Y chromosome polymorphisms in medicine

Csilla Krausz¹, Lluís Quintana-Murci^{2,3} and Gianni Forti¹

Ninety-five percent of the length of the human Y chromosome is inherited as a single block in linkage from father to male offspring as a haploid entity. Thus, the Y chromosome represents an invaluable record of all mutations that have occurred along male lineages throughout evolution. For this reason, Y chromosomal DNA variation has been mainly used for investigations on human evolution and for forensic purposes or paternity analysis. Recently, Y chromosomal polymorphisms have been applied in molecular medicine from the perspective of male-specific (spermatogenic failure, testis and prostate cancer) and prevalently male-associated (hypertension, autism) diseases. The absence of recombination on the MSY (male-specific Y) region means that polymorphisms, located in this region, are in tight association with potential functional variations associated with Y-linked phenotypes. Thus, an indirect way to explore if Y chromosome genes are involved in the etiology of a specific disease is the definition of Y chromosome haplogroups in patients versus disease-free and/or the general population. Data on patients with reduced sperm count and prostate cancer indicate that the 'at risk Y haplogroup' may be different in different populations. The situation is rather contradictory for other male-specific or male-associated diseases and further multicenter – possibly multiethnic – studies are needed.

Keywords: medicine; polymorphisms; reproductive medicine; spermatogenesis; Y chromosome; Y haplogroups

Ann Med 2004; 36: 573–583

Introduction

While the X chromosome is shared by both sexes, the presence of the Y chromosome in somatic cells

represents a unique peculiarity of males. Since its first description in 1921 (1), the scientific community has been faced with divergent opinions about its role and importance in humans. Due to the abundance of tandemly repeated satellite DNA and the apparent paucity of gene content, the Y chromosome was considered for a long time to be a 'genetic wasteland', necessary only for sex determination. This has led to propose its future extinction in an evolutionary context (2, 3). This view has been challenged by the recent identification of both an unexpected number and variety of Y chromosome genes, many of which with an ubiquitous expression, and by the presence of a conversion-based system of gene copy 'correction', acting to preserve Y genes from the gradual accumulation of deleterious mutations and thus ensuring their continuity in time (4, 5). The human Y chromosome is classically divided into two functionally distinct regions: 1) the pseudoautosomal regions (PAR1 and PAR2), which are homologous with X chromosome sequences and are responsible for correct pairing between the two sex chromosomes during male meiosis; 2) the male specific region Y (MSY), previously called the 'non-recombining region Y' (NRY), in which, in certain parts, instead of classical recombination 'intrachromosomal gene conversion' (non-reciprocal transfer) takes place (5). This region comprises 95% of the length of the chromosome (Fig 1).

With the exception of the two PAR regions, the Y chromosome is inherited in block from father to male offspring as a haploid entity and thus it represents an invaluable record of all mutations that occurred along male lineages throughout evolution. For this reason, the Y chromosome is considered as a reliable 'story-teller' of population origin and dispersals (6). The MSY region is the male counterpart of mitochondrial DNA (mtDNA) which is maternally inherited and does not undergo recombination.

The extent of the MSY region is roughly 63 Mb, but only 23 Mb are transcriptionally active (euchromatic portion). A total of 156 transcription units have been identified with 78 protein coding units and 78 putative non-coding units (4). The protein coding

From the ¹Andrology Unit, Department of Clinical Physiopathology, University of Florence, Florence, Italy, ²CNRS URA1961, ³Unit of Molecular Prevention and Therapy of Human Diseases, Institut Pasteur, Paris, France.

Correspondence: Csilla Krausz, MD PhD, Andrology Unit, Department of Clinical Physiopathology, University of Florence, Viale Pieraccini 6, 50139 Florence, Italy. Fax: +390554271371. E-mail: c.krausz@dfc.unifi.it

units are the product of 27 genes, 12 of which are expressed ubiquitously and 11 are exclusively, or predominantly, expressed in testes. The majority of these genes are involved in male specific functions, such as male sex determination (SRY) and spermatogenesis (i.e., genes of the azoospermia factor (AZF) regions of the long arm of the Y chromosome). Consequently mutations/deletions of these genes lead to sex reversal or spermatogenic failure (7–9). In addition, loss or rearrangements of the Y are also associated with a number of other phenotypes, such as Turner stigmata (10), skeletal anomalies (11) and the development of a rare type of gonadal cancer (gonadoblastoma) (12). The MSY also contains genes with housekeeping cellular activities (4, 13) and are probably involved in functions other than male reproduction. The same genes may be responsible for the sexual dimorphism observed in certain pathologies.

Y chromosomal DNA variation has been mainly used for investigations on human evolution and for forensic purposes or paternity analysis, these issues being extensively reviewed elsewhere (13–15) In this mini-review, we will focus our attention on the recent applications of Y chromosomal polymorphisms in molecular medicine from the perspective of male-specific (spermatogenic failure, testis and prostate cancer) and prevalently male-associated diseases (hypertension, autism).

Y chromosome haplogroups and association studies

Polymorphic markers on the MSY region define Y chromosome lineages (or haplogroups) which are monophyletic groups of Y chromosomes sharing allelic states at slowly mutating binary markers. Until now, more than 200 Y-specific single nucleotide polymorphisms (SNPs) have been identified providing, together with a number of indels (insertions/deletions), a detailed and robust phylogenetic tree of Y chromosomal lineages (13, 16). Multiallelic markers, such as Y chromosome microsatellites, which have a higher mutation rate (around 2×10^{-3} per locus per generation), define haplotypes within each of the haplogroups and give insights into the internal diversity of a given haplogroup. In addition, microsatellite diversity is very useful to evince more subtle relationships among populations that may not be detected by simple haplogroup analysis.

The absence of recombination on the MSY region means that polymorphisms located in this region are in tight association with potential functional variations associated with Y-linked phenotypes. Although the Y chromosome is not generally considered in genome-wide studies, the non-recombining nature of

Key messages

- With the exception of the two PAR regions, the Y chromosome is inherited as a single block in complete linkage from father to male offspring as a haploid entity and is in tight association with potential functional variations associated with Y-linked phenotypes.
- Polymorphic markers on the MSY region define Y chromosome lineages (or haplogroups) which are monophyletic groups of Y chromosomes sharing allelic states at slowly mutating binary markers.
- There is increasing evidence showing that male reproductive fitness is greatly influenced by DNA variations/deletions of the Y chromosome, therefore Y haplogroup definition may have a direct application in reproductive medicine.
- The identification of a predisposing or protecting Y haplogroup can also be considered as a starting point for a better understanding of differences in disease susceptibility between the two sexes and to the identification of genes involved in physiological processes other than male reproduction.

most of the Y offers the possibility to study the relationship between the Y-gene content and a number of diseases. Thus an indirect way to explore if Y chromosome genes are involved in the etiology of a certain disease is the definition of Y chromosome haplogroups in patients *versus* disease free and/or the general population. If a Y-linked genetic factor predisposing males to develop a disease phenotype is present in a given population, two possible explanations can be given. First, a functional variation predisposing to a given disease could have appeared before the generation of the polymorphisms that define Y chromosome haplogroups. In this case, all Y chromosomes belonging to these haplogroups will contain the susceptibility variant. The alternative possibility is that a predisposing variation arose on a single Y chromosome haplogroup background. In this case, due to the absence of recombination on the Y chromosome, the susceptibility variant would be in strong association with the polymorphisms defining the haplogroup. As a consequence, the frequency of this haplogroup would be higher in subjects with the given disease compared to the control population. The majority of Y polymorphisms can be tested through PCR (polymerase chain reaction)-based assays (16), which makes it relatively easy to explore

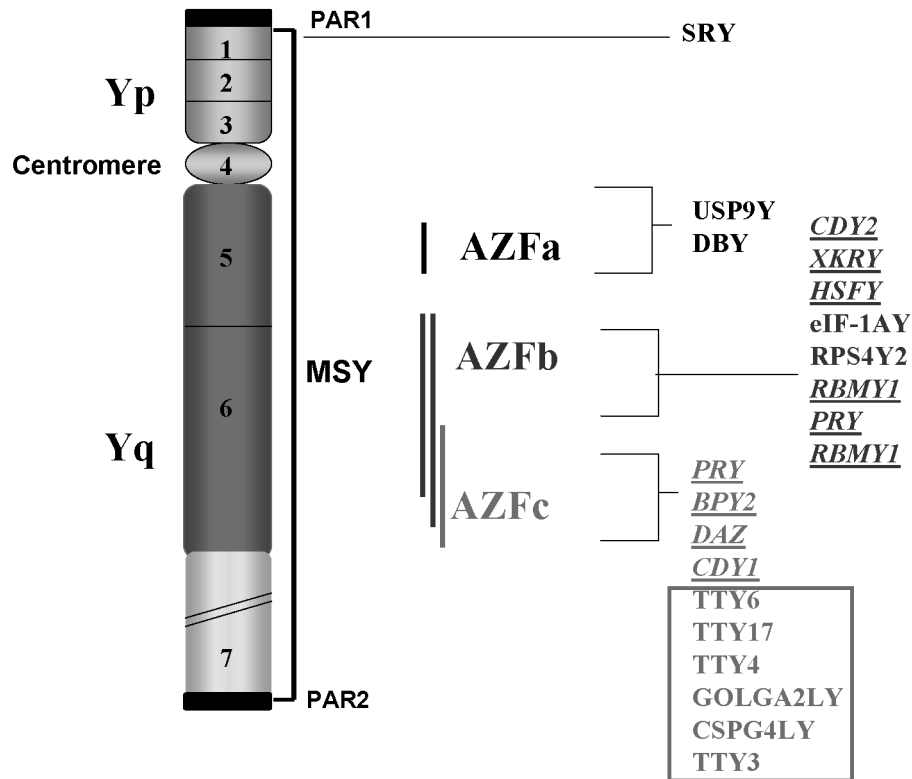


Figure 1. Genes of the three azoospermia factor (AZF) regions. Deletions of these regions result in spermatogenic failure. Multi-copy genes are in *italic*, whereas non-italic letters indicate single copy genes. Underlined genes are expressed exclusively in the testis, whereas non-underlined are expressed ubiquitously. Transcription unit families are reported in box. AZFb deletions (two subtypes) overlap with the AZFc region. Yq = long arm of the Y chromosome; Yp = short arm of the Y chromosome; PAR = pseudoautosomal region; MSY = male-specific Y with an extension of 63 Mb.

the role of Y chromosome background in predisposing to different pathological conditions.

Reproductive medicine

The role of the Y chromosome in reproductive medicine is indisputable since it contains the master gene of testis determination (SRY) and a number of genes with specific functions in spermatogenesis (Fig 1). The first relationship between infertility and Y chromosome background has been described in a rare form of azoospermia due to the so called XX male syndrome (17). This syndrome, in more than 80% of cases, is the result of an ectopic recombination between the short arms of the X and Y chromosomes, leading to the translocation of Y material, including the gene SRY, to the X chromosome. A particular European Y haplogroup has been defined where the risk of X-Y ectopic exchange, leading to the XX male syndrome, is increased (17) (Table 1). Y chromosomes belonging to this haplogroup bear a paracentric inversion involving an XY homologous recombining sequence which represents a putative mechanism for the predisposition to XX maleness.

One of the most frequent genetic causes of severe

oligozoospermia (<5 million spermatozoa/ml) and azoospermia (absence of spermatozoa in the ejaculate) are Y chromosome microdeletions. The Y microdeletions associated with infertility occur in specific regions of the long arm of the Y, called azoospermia factor (AZF) regions (8, 18). The complete absence of the most proximal region (AZFa) is associated with the most severe phenotype (complete absence of germ cells in the testis), whereas the deletion of the entire AZFb region is associated with spermatogenic arrest (in general at the stage of spermatocytes) (19). The deletion of the most distal AZFc region can cause different grades of testicular failure, with an important variability between individuals, ranging from the absence of germ cells in the testis to the presence of spermatozoa in the ejaculate (oligospermia). Considering that the frequency of men with severe impairment of spermatogenesis in the general population is about 2.5% and the average frequency of microdeletions in this group is around 5%, it can be estimated that around one man in 2000 bears Y chromosome deletions. The mechanism by which AZF microdeletions originate can be traced to intrachromosomal recombination between repetitive elements, situated at the border of different AZF regions (AZFa, AZFb, AZFc) (20, 21). Sequence

Table 1. Association studies dealing with Y chromosome polymorphisms and male specific or prevalently male associated diseases. Studies indicated with * have been performed in the general population. The association^o found in reference (49) is specific for the Japanese population. n.r. = not recorded.

Phenotype	Population	No. of subjects analysed		Y marker(s)	Main finding	Reference
		Patients	Controls			
XX maleness	European	24	81	3	Association	17
AZFdeletions	European	50	50	9	No association	23
AZFdeletions	European	73	299	11	No association	22
AZFdeletions	Japanese	6	84	15	No association	29
Oligo/azoospermia	Japanese	51	57		No association	
Azoospermia	Japanese	106	156	3	Association	28
Oligo/azoospermia	Italian	74	216	8	No association	30
Teratozoospermia						
Oligo/azoospermia	Danish	43	128	3	Association	26
Sperm count	Italian	41	n.r.	11	No association	31
Testis cancer	English	43	179	7	No association	47
Prostate cancer	Japanese	90	99	1	Association	48
Prostate cancer	Multi-ethnic	131	1157		Association ^o	49
Blood pressure*	Australian	409*		1	Association	57
Blood pressure*	Polish	155*		1	Association	58
	Scottish	762*		1	Association	
Hypertension	Japanese	110	104	1	No association	60
Hypertension	Spanish	208	178	2	No association	61
Hypertension + myocardial infarction	Spanish	76	132	2	Association	
Cholesterolemia*	Polish	1288*		1	Association	59
Autism	European	111	140	10	No Association	66

differences or different orientation of the repeated blocks may predispose or protect against a specific type of deletion. An example is the polymorphic deletion of L1PA4 element which confers a higher homology between the two retroviral blocks in the flanking sequences of the AZFa region and thus may, in theory, facilitate homologous recombination (Fig 2b and 3). This deletion is considered a polymorphism and reaches high frequencies in populations from the Middle East. Similarly to AZFa region, there could be differing susceptibilities to the other AZF deletions depending on the Y chromosome structure. This hypothesis has been tested in two studies by comparing Y chromosome haplogroup distributions in case and control groups. Men carrying Y chromosome microdeletions from Ireland, Scotland, Germany, Italy, Spain, Holland and Denmark were haplotyped and the haplotype distribution was then compared to infertile men without microdeletions (22) or compared to an unselected male control population (23). Y chromosome microdeletions were found to occur on different Y chromosome backgrounds and there was no significant difference between Y chromosome haplogroup distribution in the study and control groups. However, due to the relative rarity of AZFa deletions (5% of the total deletions), the number of this type of deletion included in both studies was small and, despite the apparent lack of association, the relationship between Y chromosome back-

ground and microdeletion formation cannot be ruled out.

The classical identification of Y microdeletions is based on simple PCR analyses of a few representative Y-specific markers able to detect deletions of entire regions (24, 25). Apart from classical microdeletions, other Y anomalies, not detectable through routine analysis, can in theory cause spermatogenic failure. Among these, partial gene copy deletions of multi-copy genes or rearrangements/inversions occurring in non-coding sequences but with possible functional effects on gene expression, are the most obvious candidates. These types of alterations may segregate with certain Y backgrounds, therefore the definition of Y chromosome haplogroups in patients with reduced sperm counts could be again the first step for identification of patients with 'Y-related' factors, other than AZF deletions, leading to spermatogenic failure. In this context, Y haplogroup distribution has been compared between men with reduced sperm count *versus* the general population in three different populations – Danish, Japanese and Italian. In Denmark, a study of men with $<20 \times 10^6$ sperm/ml revealed a significant overrepresentation of the lineage K(xL,N,O1,O3c,P), a haplogroup previously known as haplogroup 26 (26) (Fig 3). This haplogroup is found in Danish men with unexplained reduced sperm counts at a frequency of almost 28%, whereas in the general population it is present only at 1%–5%

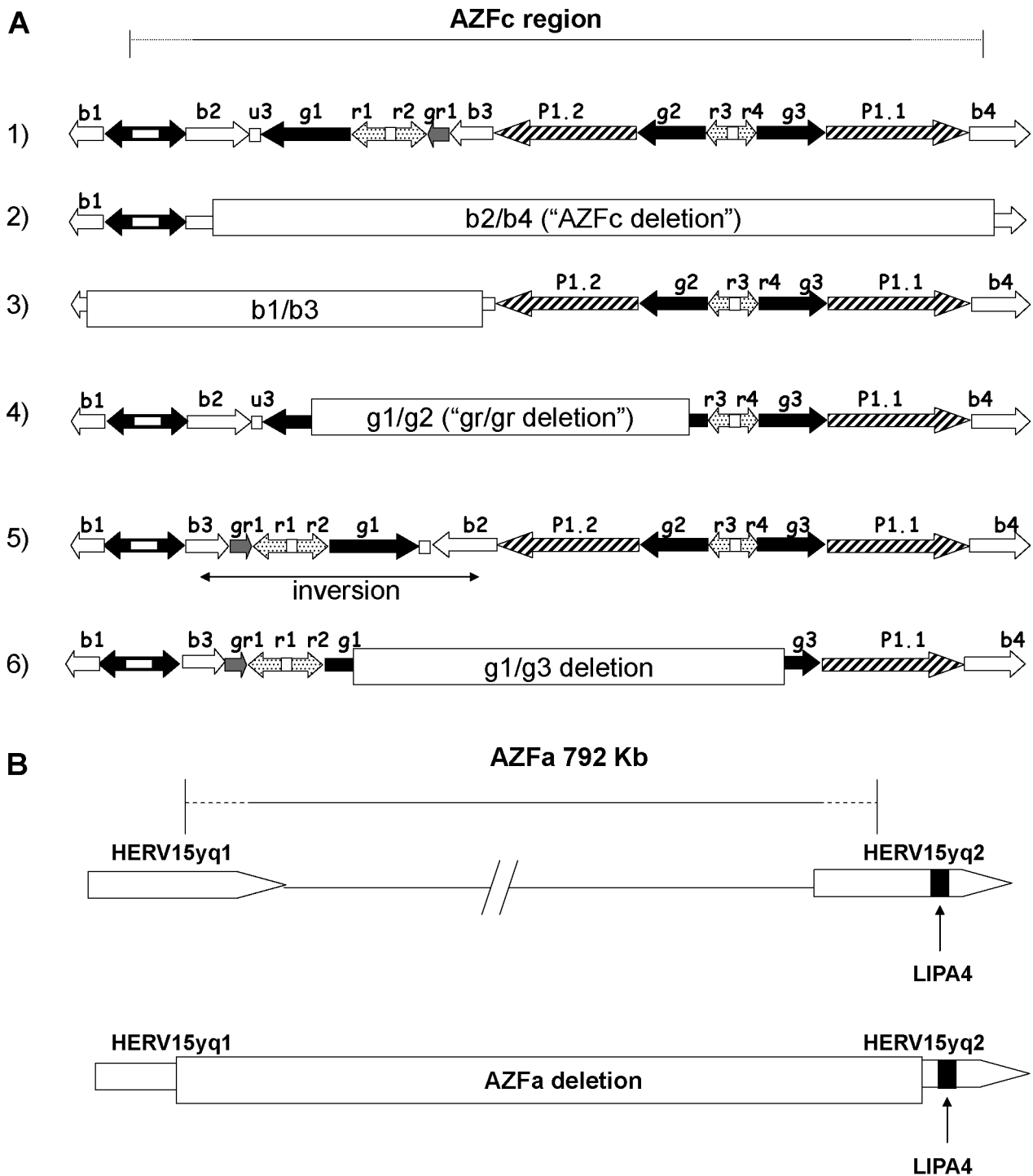


Figure 2A. Schematic representation of AZFc region. 1) the AZFc region contains a number of repeated sequences with the same orientation (narrow with the same shade or motives) which through intrachromosomal recombination may lead to deletions. 2) 'complete' AZFc deletion removing the entire AZFc region; this deletion is specific for spermatogenic failure. 3), 4) and 6) 'partial' deletions occurring between repeated homologous sequences inside the region; 4) 'gr/gr' deletion is considered a 'risk factor' for oligozoospermia; 5) an inversion between b2 and b3 allows intrachromosomal recombination between blocks g1 and g3 leading to deletion type 'g1/g3'; 6) This partial deletion has no pathogenic significance. **B.** Schematic representation of AZFa region. The AZFa region contains two blocks of homologous retroviral sequences at the border (HERV15yq1 and HERV15yq2). HERV15yq2 contains an insertion of a LINE1 3' UTR sequence block (LIPA4). The deletion of LIPA4 element confers a higher grade of homology between the two retroviral sequences and thus may predispose to AZFa deletion. Y chromosome lineages containing the inversion (A-5) and the deletion of LIPA4 are indicated in Figure 3.

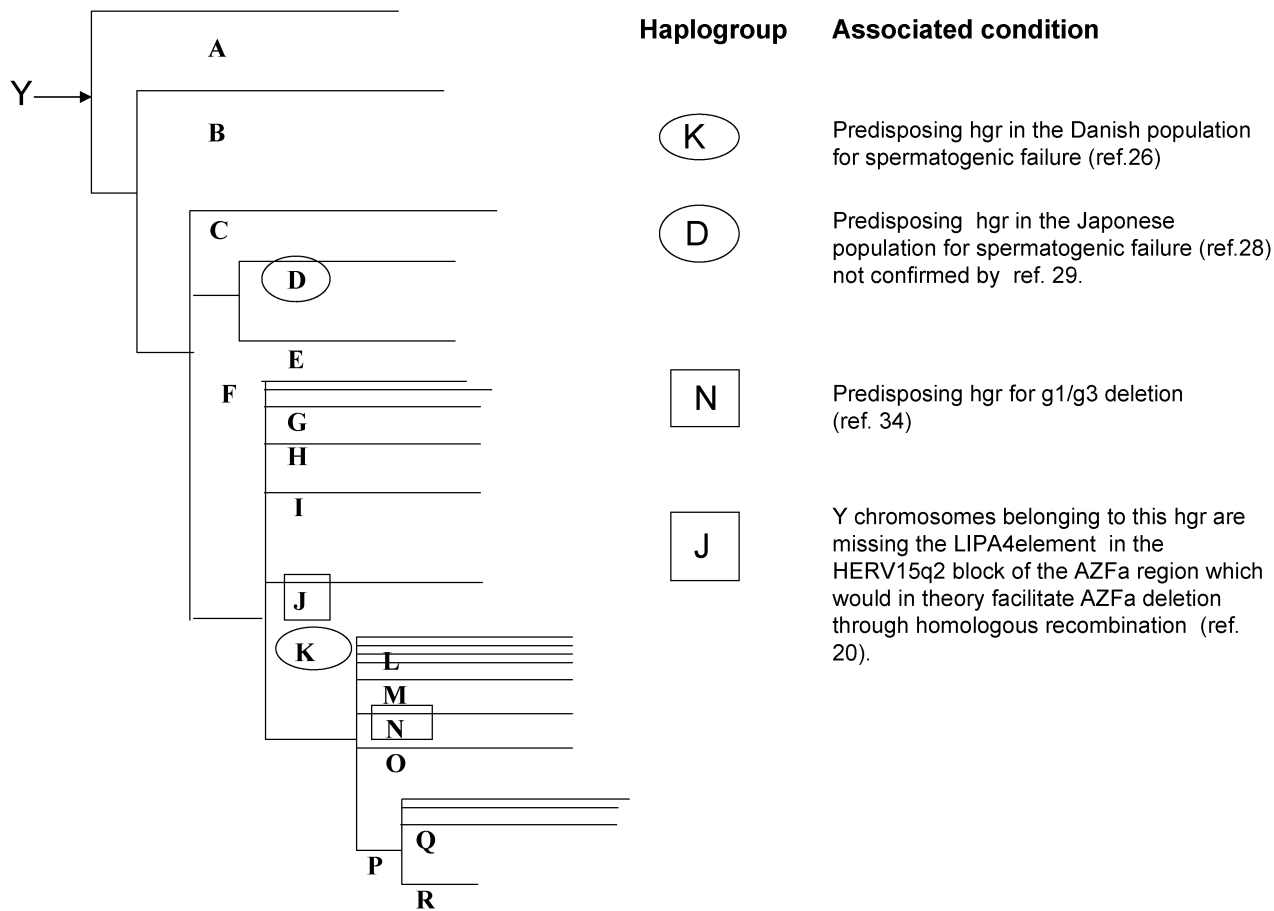


Figure 3A. Simplified tree of human Y chromosomal binary haplogroups (hgrs) showing the 18 main Y lineages. Each lineage contains several sublineages leading to a total of 153 binary haplogroups (not shown, see reference (16)). **B.** Examples of predisposing haplogroups in relation to sperm production (hgr N and hgr D) and to deletion formation (hgrs N and J).

(27). Similar types of studies have been performed using study and control Japanese populations with mixed results. Y chromosome haplogroup associated with reduced sperm counts and an increased risk ($\times 2$) of azoospermia was reported in Japanese males (28) but not confirmed in a similar study on men of Japanese descent (29). This lineage, known as haplogroup D (Fig 3), represents a Y chromosome variant not observed in European and African populations and seems to be specific of East-Asian populations showing considerable geographic substructuring. Two Italian studies (30, 31) also failed to find a relationship between infertility (30) or sperm counts (31) and Y chromosome haplotypes. There are a number of possible explanations for the apparent discrepancies among these studies. First of all, sample sizes are generally small in all studies, representing therefore, one of the major problems of reproducibility of association studies (32). Another critical point concerns patient and control selection. In this regard there are a number of possible confounding factors, such as: 1) reliability of sperm count when a single semen analysis is performed per individual; 2)

controls selected on their 'fertility status' rather than on their normal sperm count; 3) insufficient clinical characterization of patients and the consequent inclusion of men with andrological abnormalities in which a role for a Y component would not be expected; and 4) clustering of haplogroups due to population substructure.

The observed susceptibility haplogroup identified in Denmark could be explained by negative selection acting on a rare haplotype that is associated with decreased reproductive fitness. This Y chromosome may exhibit structural rearrangements, polymorphisms in Y chromosome-specific gene coding sequences or differences in gene copy numbers, all of which may alter spermatogenic efficiency. Various environmental factors (such as diet or pollutants) may act on different classes of Y chromosome in different countries. This is also suggested by epidemiological data which indicate that reduced sperm quantity and quality is a relatively recent phenomenon that may have considerable environmental aspects (33). Studies on the functional characteristics of chromosomes belonging to this haplogroup should be done to verify

this hypothesis and to potentially elucidate its relationship with environmental factors for preventive purposes.

Finally, Y chromosome haplogroups differ from country to country and even within countries. Therefore a potential Y chromosome haplogroup associated with reduced sperm count may differ between countries. Further evidence for the importance of population-specific effect of Y polymorphisms on reproductive fitness is coming from two recent studies dealing with partial AZFc deletions (34, 35). Apart from the AZF flanking sequences, the AZFc region is particularly rich in large palindromic sequences, tandem repeats and multiple gene copies (4). Homologous recombination may, in theory, occur between each repeated sequence with the same orientation. A partial deletion, called g1/g3, which removes partially the AZFc gene content, has been reported to be in strong association with a specific Y haplogroup (hgr N) (34). This haplogroup reaches a particularly high frequency (up to 55%) in northern Eurasian populations (36). Although the deletion removes copies of multicopy genes involved in spermatogenesis, it does not seem to confer a lower reproductive potential to men belonging to this haplogroup. By contrast, another partial AZFc deletion which removes 1.6 Mb inside the AZFc region (gr/gr deletion), seems to be associated with reduced spermatogenesis (35). However, in contrast to classical AZFc deletions, which are in the majority of cases *de novo*, the gr/gr deletion is usually transmitted successfully from father to son (35). In fact, the relatively mild entity of spermatogenic failure allowed deleted chromosomes to rise to polymorphic frequency (about 2% worldwide). However, in certain populations such as the Japanese, its frequency reaches to 30% in linkage with the D2b Y lineage. These data cast doubts about the clinical importance of this type of deletion, at least as far as the Japanese population is concerned. However, it is also possible that the Y chromosome lineage D2b has acquired, besides the gr/gr deletion, a compensatory or even advantageous mutation which would change the phenotype in men bearing this haplogroup (e.g., a type of balancing selection). In certain individuals, in association with gr/gr deletion, a duplication of part of AZFc has been observed which may represent a compensatory mechanism. Similarly to gr/gr deletions, different Y chromosome backgrounds may also be responsible for the inter-individual variation in the phenotypic expression of a given AZF deletion such as the complete AZFc deletion which can be associated with both severe oligospermia or with the total absence of spermatogenic cells in the testis. Future clinical-molecular combined studies are needed to accredit this attractive hypothesis.

Y chromosome and cancer

Human Y chromosome loss and rearrangements have been associated with specific types of cancer, such as bladder cancer (37), male sex cord stromal tumors (38), lung cancer (39) and esophageal carcinoma (40), suggesting that both oncogenes and tumor suppressor genes may exist on this chromosome. Genetic mapping studies have identified a gonadoblastoma locus on this chromosome (GBY) that predisposes the dysgenetic gonads of XY sex-reversed patients to tumorigenesis. A recently identified candidate gene for GBY, the testis-specific protein Y-encoded (TSPY) has been demonstrated to be expressed preferentially in tumor cells from gonadoblastoma (41), testicular tumor (41) and from prostate cancer (42). A differential expression of some other Y genes was also reported in prostate cancers, further indicating a possible role for the Y chromosome in malignancy and cancer progression (43, 44). However, in the absence of functional data on the proteins encoded by these genes, the mechanism by which Y gene anomalies lead to cancer formation remains to be defined.

A more direct relationship appears to be between Y genes involved in spermatogenesis and testis cancer. In fact, more than 97% of testis cancers (TC) are germ cell tumors and patients with spermatogenic failure are at higher risk to develop testicular cancer than the general male population. This higher incidence cannot be explained by either local tumor or general cancer effect for two reasons: 1) the impairment of testicular function is present several years before the diagnosis; and 2) patients with other malignancies have normal, or slightly decreased, semen quality. In the light of these data, the effects of the Y chromosome background and Y deletions on testis cancer formation have been assessed recently. Data on Y microdeletion frequencies in patients affected by TC are contradictory (45, 46) whereas the role of Y chromosome background has been explored only in one population (47). The haplogroup distribution of 43 English patients presenting testicular cancer has been analyzed for 13 Y-linked polymorphisms (47) (Table 1). Their haplotype profile has been compared with a selected population of 197 controls to determine if there is an association between Y chromosome background and a predisposition to this cancer. The statistical analysis of data did not indicate significant differences between the case and control groups, thus it has been concluded that the existence of a predisposing Y haplogroup to TC is unlikely. However, it is plausible that TC may have multifactorial origin and a Y-related etiology would be present only in patients with associated spermatogenic failure. Future studies on a much larger group of patients with known sperm count

are needed to reach conclusive data about the role of Y background in this pathology.

As far as prostate cancer is concerned, a study on a Japanese population reported a linkage between prostate cancer incidence and different alleles of the human Y-linked tetranucleotide polymorphism DYS19 (48). Apparently one allele (allele C) would confer a higher susceptibility whereas another (allele D) a resistance to prostate cancer. These results should, however, be interpreted with caution since: 1) they are based on a single microsatellite marker; and 2) these markers present a high mutation rate and recurrent mutations are more likely to appear, distorting potentially the interpretation of the results. More recently, a multi-ethnic cohort study which examined the haplogroup distribution from four ethnic groups found a statistically significant predisposition to prostate cancer in one Y lineage from the Japanese population (49). These findings suggest that a Y chromosomal factor may contribute significantly to the development of prostate cancer in Japanese men.

Y chromosome and male-associated diseases

Among genetic determinants of sexual dimorphism observed for pathologies such as cardiovascular diseases, hypertension and autism, the Y chromosome seems to be an obvious candidate. This hypothesis is further supported by the expression pattern of some of the MSY genes which is compatible with functions other than male reproduction. However, neither causative loci nor specific candidate genes of the Y chromosome have been identified for these pathologies and the gender difference in their frequency could also be related to differences in sex hormones, environmental and social factors.

Hypertension

It is well known that human males have a significantly higher blood pressure, morbidity and mortality in comparison with premenopausal women of comparable age (50). In two animal models of spontaneous hypertension (the spontaneously hypertensive rat – SHR – and the stroke-prone spontaneously hypertensive rat – SHRSP) males show higher levels of blood pressure than females and a role for Y chromosome of SHRSP animals for the increased blood pressure has been suggested by several genetic crosses between SHR and SHRSP animals and normotensive animals (51–53). On the other hand, data obtained from consomic rat strains with a hypertensive background and a normotensive Y have shown that these animals are easily prone to dyslipidemia (54).

Concerning the human, a possible Y factor

influencing blood pressure has been suggested by the observation that a hypertensive father but not a mother affects the blood pressure in male offspring (55). Studies from Australia and two European populations show an association between single Y chromosome polymorphisms (such as the <HindIII+/- and the YAP +/-) and blood pressure or lipid levels (56–59) whereas a lack of association has been observed for the Japanese population (60) and in men from Northern Spain (61) (Table 1). In the latest study the HindIII + Y chromosome polymorphism was reported not to be associated with differences in blood pressure but to be associated with an increased risk of an early episode of myocardial infarction among the hypertensives (61). The lack of coherence among the different studies highlights the potential risk of deducing a genetic association based on a single genetic marker. Therefore, evidence for the presence of a 'hypertension factor' on the Y remains very hypothetical and needs further studies, based on Y haplogroup definition rather than the analysis of single polymorphisms.

Autism

The male to female ratio in autism (excluding subjects with physical or brain abnormalities) is exceptionally high, reaching to 23:1. Despite this gender difference recognized since long ago, the male predisposition (or female protection) to autistic disorder remains unexplained. Numerical and structural anomalies of the sex chromosomes have frequently been reported in autistic subjects and consequently X- or Y-related factors have been hypothesized (62, 63). However, linkage analyses on the X chromosome failed to detect candidate genes and the same method cannot be applied to the non-recombining Y chromosome (MSY region) (64, 65). In order to define a Y-related component, a recent multiethnic study analyzed the Y chromosome background using a set of 10 polymorphic informative markers (66) (Table 1). No differences in haplogroup distribution have been found between controls and a large number of autistic patients. Although this finding fails to demonstrate a causative role for Y chromosome in predisposing to autism, the presence of a specific Y susceptibility gene cannot be excluded. The analysis of Y chromosome genes expressed in the central nervous system, for example SRY (sex determining region Y), ZFY (zing finger protein, Y-linked), PRKY (protein kinase Y), Y-linked protocadherin (PCDHY), would be necessary before any conclusion can be made.

Conclusions

The analysis of Y chromosome polymorphisms has

given a major contribution to the understanding of human histories, including human origins and population movements. These studies performed by population geneticists are based on the general assumption that selection is not acting on the markers under study, being therefore considered selectively neutral. Selection however seems to be acting on uniparentally inherited loci, as indicated by specific haplogroups either associated with or protecting against human diseases (14). In the light of recent data from association studies, population geneticists started to modify their vision on the neutrality of Y polymorphisms used for evolutionary purposes. On the other hand clinicians must be aware about data accumulated by population geneticists, for instance geographical and social clustering, which may affect association studies. Much of the data concerning the Y chromosome are coming from studies on male reproductive function. Haplogroups predisposing to Y anomalies leading to severe reduction of sperm production, are present at a relatively low frequency in the general population, whereas they can be over-represented in specific groups of infertile subjects (26). In this case negative selection could be acting on this type of Y chromosome lineage and it could be eventually removed from the general population.

Paradoxically, haplogroups associated with Y mutations/deletions with deleterious effect on sperm production can reach a high frequency in certain populations, despite a potential negative selection acting on them. This seems to be the case for the recently described *gr/gr* deletion of the AZFc region (35). Although, there are no conclusive data in this regard, the phenomena could eventually be explained by the contemporaneous presence of advantageous mutations (for example duplications) raised on the same Y chromosome bearing the deleterious mutation. These observations underlie the complexity of association studies between Y haplogroups and Y gene anomalies. Nevertheless, Y haplogroup studies in male reproductive pathophysiology represent an important tool for a relatively fast selection of groups of men for subsequent straightforward molecular-cytogenetic studies. It will be mainly useful for the identification of two category of subjects: 1) men in whom spermatogenic damage is likely to be related to a Y component not detectable with the routine Y microdeletion screening; and 2) subjects with a known Y gene defect (for example microdeletions) but with a less severe disease phenotype than would be expected. Given that the regions and genes

involved in spermatogenesis on the Y are now known, the search, in the first case, for deleterious, or, in the second case, for advantageous mutations/rearrangements can be then performed in the risk group.

The situation is more complex concerning diseases or pathological conditions other than spermatogenic failure, since no candidate genes on the Y chromosome have been identified for hypertension, hypercholesterolemia or autism. Moreover, the etiology of the above listed pathologies together with testis and prostate cancer is likely to be multifactorial. Another weak point concerning the association studies on hypertension, is that they are based on the analysis of one or a maximum of two polymorphisms which gives considerably less precise information about Y background than the analysis of Y chromosome haplogroups or haplotypes. Therefore, a minimum degree of lineage definition should be mandatory for studies aiming to define an 'at risk' Y chromosome haplogroup for systemic diseases. Selection bias (population substructuring) and adequate sample size for both the control and affected populations are also critical. Many of the reviewed papers lack these criteria and consequently their conclusions may be questioned. However, this problem seems to be common to other association studies dealing with different genetic loci. Ioannidis et al. (32) demonstrated by meta-analysis that a small size (with a critical sample size of less than 150) was one of the best predictors of reaching discrepancies between the first and subsequent studies. One solution to these problems could be multicenter and possibly multi-ethnic studies which may help in increasing the size of the study population.

There is increasing evidence showing that male reproductive fitness is greatly influenced by DNA variations/deletions of the Y chromosome. Therefore Y haplogroup definition may have a direct application in reproductive medicine. On the other hand, the identification of a predisposing or protecting Y haplogroup can be considered as a starting point also for a better understanding of differences in disease susceptibility between the two sexes and to the identification of genes involved in physiological processes other than male reproduction. This latter task will be facilitated by the recent identification of the sequence of the MSY, and the emerging sequence of the X chromosome which will offer a comprehensive database of genetic and sequence differences between human males and females (4).

1. Painter TS. The Y chromosome in mammals. *Science* 1921; 53:503–4.
2. Marshall JA, Human Y. chromosome, Sex determination, and spermatogenesis – a feminist view. *Biol Reprod* 2000;63:667–76.
3. Aitken JR, Marshall JA. The future of sex. *Nature* 2002;415: 963.
4. Skaletsky H, Kuroda-Kawaguchi T, Minx PJ, Cordum HS, Hillier L, Brown LG, et al. The male-specific region of the human Y chromosome is a mosaic of discrete sequence classes. *Nature* 2003;423:825–37.
5. Repping S, Skaletsky H, Lange J, Silber S, Van Der Veen F, Oates RD, et al. Recombination between palindromes P5 and P1 on the human Y chromosome causes massive deletions and spermatogenic failure. *Am J Hum Genet* 2002;71:906–22.
6. Bertranpetit J. Genome, diversity, and origins: the Y chromosome as a storyteller. *Proc Natl Acad Sci USA* 2000;97:6927–29.
7. Harley VR, Clarkson MJ, Argentaro A. The molecular action and regulation of the testis-determining factors, SRY (sex-determining region on the Y chromosome) and SOX9 [SRY-related high-mobility group (HMG) box 9]. *Endocr Rev* 2003; 24:466–87.
8. Vogt PH, Edelmann A, Kirsch S, Henegariu O, Hirschmann P, Kiesewetter F, et al. Human Y chromosome azoospermia factors (AZF) mapped to different subregions in Yq11. *Hum Mol Genet* 1996;5:933–43.
9. McElreavey K, Krausz C. Sex Chromosome Genetics '99. Male infertility and the Y chromosome. *Am J Hum Genet* 1999;64:928–33.
10. Zinn AR, Ross JL. Turner syndrome and haploinsufficiency. *Curr Opin Genet Dev* 1998;8:322–27.
11. Rao E, Weiss B, Fukami M, Rump A, Niesler B, Mertz A, et al. Pseudoautosomal deletions encompassing a novel homeobox gene cause growth failure in idiopathic short stature and Turner syndrome. *Nat Genet* 1997;16:54–63.
12. Lau YF. Gonadoblastoma, testicular and prostate cancers, and the TSPY gene. *Am J Hum Genet* 1999;64:921–27.
13. Jobling MA, Tyler-Smith C. The human Y chromosome: an evolutionary marker comes of age. *Nat Rev Genet* 2003;4:598–612.
14. Jobling MA, Tyler-Smith C. New uses for new haplotypes: the human Y chromosome, disease and selection. *Trends Genet* 2000;16:356–62.
15. Quintana-Murci L, Krausz C, McElreavey K. The human Y chromosome: function, evolution and disease. *Forensic Sci Int* 2001;118:169–81.
16. Y Chromosome Consortium. A nomenclature system for the tree of human Y-chromosomal binary haplogroups. *Genome Res* 2002;12:339–48.
17. Jobling MA, Williams GA, Schiebel GA, Pandya GA, McElreavey GA, Salas GA, et al. A selective difference between human Y-chromosomal DNA haplotypes. *Curr Biol* 1998;8:1391–94.
18. Tiepolo L, Zuffardi O. Localization of factors controlling spermatogenesis in the nonfluorescent portion of the human Y chromosome long arm. *Hum Genet* 1976;34:119–24.
19. Krausz C, Quintana-Murci L, McElreavey K. Prognostic value of Y deletion analysis: what is the clinical prognostic value of Y chromosome microdeletion analysis? *Hum Reprod* 2000;15: 1431–4.
20. Kamp C, Hirschmann P, Voss H, Huellen K, Vogt PH. Two long homologous retroviral sequence blocks in proximal Yq11 cause AZFa microdeletions as a result of intrachromosomal recombination events. *Hum Mol Genet* 2000;9:2563–72.
21. Kuroda-Kawaguchi T, Skaletsky H, Brown LG, Minx PJ, Cordum HS, Waterston RH, et al. The AZFc region of the Y chromosome features massive palindromes and uniform recurrent deletions in infertile men. *Nat Genet* 2001;29:279–86.
22. Paracchini S, Stuppia L, Gatta V, Palka G, Moro E, Foresta C, et al. Y-chromosomal DNA haplotypes in infertile European males carrying Y-microdeletions. *J Endocrinol Invest* 2000;23: 671–6.
23. Quintana-Murci L, Krausz C, Heyer E, Gromoll J, Seifer I, Barton DE, et al. The relationship between Y chromosome DNA haplotypes and Y chromosome deletions leading to male infertility. *Hum Genet* 2001;108:55–8.
24. Simoni M, Bakker E, Krausz C. EAA/EMQN best practice guidelines for molecular diagnosis of y-chromosomal microdeletions. State of the art 2004 *Int J Androl* 2004;27:240–9.
25. Krausz C, Forti G, McElreavey K. The Y chromosome and male fertility and infertility. *Int J Androl* 2003;26:70–5.
26. Krausz C, Quintana-Murci L, Rajpert-De Meyts E, Jorgensen N, Jobling MA, Rosser ZH, et al. Identification of a Y chromosome haplogroup associated with reduced sperm counts. *Hum Mol Genet* 2001;10:1873–7.
27. Rosser ZH, Zerjal T, Hurles ME, Adojaan M, Alavantic D, Amorim A, et al. Y-chromosomal diversity in Europe is clinal and influenced primarily by geography, rather than by language. *Am J Hum Genet* 2000;67:1526–43.
28. Kuroki Y, Iwamoto T, Lee J, Yoshiike M, Nozawa S, Nishida T, et al. Spermatogenic ability is different among males in different Y chromosome lineage. *J Hum Genet* 1999;44:289–92.
29. Carvalho CM, Fujisawa M, Shirakawa T, Gotoh A, Kamidono S, Freitas Paulo T, et al. Lack of association between Y chromosome haplogroups and male infertility in Japanese men. *Am J Med Genet* 2003;116A:152–8.
30. Previdere C, Stuppia L, Gatta V, Fattorini P, Palka G, Tyler-Smith C. Y-chromosomal DNA haplotype differences in control and infertile Italian subpopulations. *Eur J Hum Genet* 1999;7:733–6.
31. Paracchini S, Stuppia L, Gatta V, De Santo M, Palka G, Tyler-Smith C. Relationship between Y-chromosomal DNA haplotype and sperm count in Italy. *J Endocrinol Invest* 2002;25: 993–5.
32. Ioannidis JP, Ntzani EE, Trikalinos TA, Contopoulos-Ioannidis DG. Replication validity of genetic association studies. *Nat Genet* 2001;29:306–9.
33. Skakkebaek NE, Rajpert-De Meyts E, Main KM. Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects. *Hum Reprod* 2001;16: 972–78.
34. Fernandes S, Paracchini S, Meyer LH, Florida G, Tyler-Smith C, Vogt PH. A large AZFc deletion removes DAZ3/DAZ4 and nearby genes from men in Y haplogroup N. *Am J Hum Genet* 2004;74:180–7.
35. Repping S, Skaletsky H, Brown L, van Daalen SK, Korver CM, Pyntikova T, et al. Polymorphism for a 1.6-Mb deletion of the human Y chromosome persists through balance between recurrent mutation and haploid selection. *Nat Genet* 2003;35:247–51.
36. Jobling MA, Samara V, Pandya A, Fretwell N, Bernasconi B, Mitchell RJ, et al. Recurrent duplication and deletion polymorphisms on the long arm of the Y chromosome in normal males. *Hum Mol Genet* 1996;5:1767–75.
37. Sauter G, Moch H, Wagner U, Novotna H, Gasser TC, Mattarelli G, et al. Y chromosome loss detected by FISH in bladder cancer. *Cancer Genet Cytogenet* 1995;82:163–9.
38. de Graaff WE, van Echten J, van der Veen AY, Sleijfer DT, Timmer A, Schraffordt Koops H, et al. Loss of the Y-chromosome in the primary metastasis of a male sex cord

- stromal tumor: pathogenetic implications. *Cancer Genet Cytogenet* 1999;112:21–5.
39. Center R, Lukeis R, Vrazas V, Garson OM. Y chromosome loss and rearrangement in non-small-cell lung cancer. *Int J Cancer* 1993;55:390–3.
 40. Hunter S, Gramlich T, Abbott K, Varma V. Y chromosome loss in esophageal carcinoma: an in situ hybridization study. *Genes Chromosomes Cancer* 1993;8:172–7.
 41. Lau Y, Chou P, Iezzoni J, Alonzo J, Komuves L. Expression of a candidate gene for the gonadoblastoma locus in gonadoblastoma and testicular seminoma. *Cytogenet Cell Genet* 2000;91:160–4.
 42. Lau YF, Lau HW, Komuves LG. Expression pattern of a gonadoblastoma candidate gene suggests a role of the Y chromosome in prostate cancer. *Cytogenet Genome Res* 2003;101:250–60.
 43. Lau YF, Zhang J. Expression analysis of thirty one Y chromosome genes in human prostate cancer. *Mol Carcinog* 2000;27:308–21.
 44. Dasari VK, Goharderakhshan RZ, Perinchery G, Li LC, Tanaka Y, Alonzo J, et al. Expression analysis of Y chromosome genes in human prostate cancer. *J Urol* 2001;165:1335–41.
 45. Bianchi NO, Richard SM, Peltomaki P, Bianchi MS. Mosaic AZF deletions and susceptibility to testicular tumors. *Mutat Res* 2002;503:51–62.
 46. Frydelund-Larsen L, Vogt PH, Leffers H, Schadwinkel A, Daugaard G, Skakkebaek NE, et al. No AZF deletion in 160 patients with testicular germ cell neoplasia. *Mol Hum Reprod* 2003;9:517–21.
 47. Quintana-Murci L, Weale ME, Thomas MG, Erdei E, Bradman N, Shanks JH, et al. Y chromosome haplotypes and testicular cancer in the English population. *J Med Genet* 2003;40:20.
 48. Ewis AA, Lee J, Naroda T, Sasahara K, Sano T, Kagawa S, et al. Linkage between prostate cancer incidence and different alleles of the human Y-linked tetranucleotide polymorphism DYS19. *J Med Invest* 2002;49:56–60.
 49. Paracchini S, Pearce CL, Kolonel LN, Altshuler D, Henderson BE, Tyler-Smith C. A Y chromosomal influence on prostate cancer risk: the multi-ethnic cohort study. *J Med Genet* 2003;40:815–9.
 50. Reckelhoff JF. Gender differences in the regulation of blood pressure. *Hypertension (DATA)* 37:1199–208.
 51. Ely DL, Turner MT. Hypertension in the spontaneously hypertensive rat is linked to the Y chromosome. *Hypertension* 1990;16:277–81.
 52. Turner MT, Johnson ML, Ely DL. Separate sex-influenced and genetic components in spontaneously hypertensive rat hypertension. *Hypertension* 1991;17:1097–103.
 53. Davidson AO, Schork N, Jacques BC, Kelman AW, Sutcliffe RG, Reid JL, et al. Blood pressure in genetically hypertensive rats. Influence of the Y chromosome. *Hypertension* 1995;26:452–9.
 54. Kren V, Qi N, Krenova D, Zidek V, Sladka M, Jachymova M, et al. Y chromosome transfer induces changes in blood pressure and blood lipids in SHR. *Hypertension* 2001;37:1147–52.
 55. Uehara Y, Shin WS, Watanabe T, Osanai T, Miyazaki M, Kanase H, et al. A hypertensive father, but not hypertensive mother, determines blood pressure in normotensive male offspring through body mass index. *J Hum Hypertens* 1998;12:441–5.
 56. Charchar FJ, Tomaszewski M, Strahorn P, Champagne B, Dominiczak AF. Y is there a risk to being male? *Trends Endocrinol Metab* 2003;14:163–8.
 57. Ellis JA, Stebbing M, Harrap SB. Association of the human Y chromosome with high blood pressure in the general population. *Hypertension* 2000;36:731–3.
 58. Charchar FJ, Tomaszewski M, Padmanabhan S, Lacka B, Upton MN, Inglis GC, et al. The Y chromosome effect on blood pressure in two European populations. *Hypertension* 2002;39:353–6.
 59. Charchar FJ, Tomaszewski M, Lacka B, Zakrzewski J, Zukowska-Szczechowska E, Grzeszczak W, et al. Association of the human Y chromosome with cholesterol levels in the general population. *Arterioscler Thromb Vasc Biol* 2004;24:308–12.
 60. Shoji M, Tsutaya S, Shimada J, Kojima K, Kasai T, Yasujima M. Lack of association between Y chromosome Alu insertion polymorphism and hypertension. *Hypertens Res* 2002;25:1–3.
 61. Garcia EC, Gonzalez P, Castro MG, Alvarez R, Reguero JR, Batalla A, et al. Association between genetic variation in the Y chromosome and hypertension in myocardial infarction patients. *Am J Med Genet* 2003;122A:234–7.
 62. Gillberg C, Winnergard I, Wahlstrom J. The sex chromosomes – one key to autism? An XYY case of infantile autism. *Appl Res Ment Retard* 1984;5:353–60.
 63. Weidmer-Mikhail E, Sheldon S, Ghaziuddin M. Chromosomes in autism and related pervasive developmental disorders: a cytogenetic study. *J Intellect Disabil Res* 1998;42:8–12.
 64. International Molecular Genetic Study of Autism Consortium (IMGSAC). A genome-wide screen for autism: strong evidence for linkage to chromosomes 2q, 7q, and 16p. *Am J Hum Genet* 2001;69:570–81.
 65. Philippe A, Martinez M, Guilloud-Bataille M, Gillberg C, Rastam M, Sponheim E, et al. Genome-wide scan for autism susceptibility genes. Paris Autism Research International Sib-pair Study. *Hum Mol Genet* 1999;8:805–12.
 66. Jamain S, Quach H, Quintana-Murci L, Betancur C, Philippe A, Gillberg C, et al. Y chromosome haplogroups in autistic subjects. *Mol Psychiatry* 2002;7:21–9.