Do you know anyone who has been diagnosed as having PWS or may have had five of the following characteristics?
Floppiness at birth.
Initial failure to thrive or difficulty in sucking.
The development of severe overeating and rapid weight gain in early childhood.
Obesity or the need for weight control.
Problems with sexual development (for example, undescended testes, delayed periods).
Some learning disability (mental handicap).
Small hands and feet.
Short stature or the need for growth hormone.
An abnormality of chromosome 15.

Mosaicism for 45,X cell line may accentuate the severity of spermatogenic defects in men with AZFc deletion

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EDITOR—Over the past 10 years, several authors have reported microdeletions in the long arm of the Y chromosome (Yq) in men with idiopathic, non-obstructive azoospermia or severe oligospermia. These microdeletions were clustered on the Yq fragment previously described as the azoospermia factor region (AZF).[1] More recently, a number of genes expressed specifically in the testes and mapping to AZFa, AZFb, or AZFc subregions have been cloned.[2–4] One of the approaches to understanding the role of these genes in human spermatogenesis is to look for a correlation between the lack of given AZF genes and the particular spermatogenic defect in the phenotypes of the patients. However, attempts to find such a correlation have failed so far. Instead, a broad spectrum of phenotypes ranging clinically from azoospermia to severe oligospermia and histologically from Sertoli cell only syndrome (SCOS) to hypospermatogenesis has been described in association with AZFc deletions.[5 6]

A recent study found chromosomal aberrations in 15% of azoospermic patients.[7] However, in papers focusing on the analysis of AZF microdeletions in patients with idiopathic infertility,[2 3 5 8–30] systematic, bilateral, histological, molecular, and cytogenetic analyses in the same large group of patients was rarely carried out, thus limiting information on the coexistence of AZF deletions and chromosomal aberrations.

In this study, we propose and test the hypothesis that chromosomal defects may often accompany AZF deletions and cause the lack of a genotype-phenotype correlation in human male idiopathic infertility. We also attempt to evaluate the nature of the spermatogenetic failure associated with isolated AZFc deletions. For this purpose, we performed a dual genetic analysis of karyotypes and molecular status of the AZF region along with bilateral testicular histological evaluation in 94 patients with non-obstructive, idiopathic infertility and azoospermia, severe oligospermia, or oligospermia.

Material and methods

Sixty five men with azoospermia (lack of sperm cells in semen), 23 men with severe oligospermia (fewer than 5×10^6 sperm cells/ml semen), and six with oligospermia (5-10 $\times$ 10^6 sperm cells/ml semen), all of them of Polish origin, were included in the study.

Histological analyses of biopsies from both testes of 77 patients were performed in formalin fixed paraffin embedded tissue blocks. Sections were cut at 4 μm thickness and stained with haematoxylin-eosin.

Chromosome studies were carried out on peripheral blood lymphocytes of 93 out of 94 patients using GTG, FPG, CBG, and QFQ banding. Karyotypes were analysed in at least 100 metaphases.

DNA was isolated from 10 ml of peripheral blood leucocytes of the patients and, when available, also from the fathers or other male relatives on the paternal side. For molecular analysis, genomic DNA was amplified by PCR using primers specific for 23 Y chromosome specific sequence tagged site (STS) markers (19 mapping to AZFa, AZFb, and AZFc and four mapping to short arm of the Y chromosome) according to conditions described in the Genebank entry.

Results

HISTOLOGICAL PHENOTYPES

The histological evaluation of testicular biopsies was performed in all 77 patients, 62 with azoospermia, 14 with severe oligospermia, and

Table 1 Frequency of different histological phenotypes in patients with spermatogenic failure. Testis biopsies were evaluated in 77 patients. Ten patients found with Klinefelter syndrome karyotype 47,XXY are included within the group diagnosed as Sertoli cell only syndrome

Phenotype	*No of patients*	*%*
Sertoli cell only syndrome	23	30
Maturation arrest	18	23
Mixed phenotypes	11	14
Hypospermatogenesis	6	8
Testicular atrophy	5	7
Normal histology	14	18
Total	77	100

Table 2 Results of the genetic analyses of the patients: karyotyping of blood lymphocytes and PCR detection of AZF deletions using pairs of primers complementary to 23 Y specific STS markers

Y STS marker	*Genebank accession number*	*Gene or locus*	*Deletion interval*	*AZF subregion*	*IHG8 45,X[72]/ 46,Xdel(Y) (q12)[28]*	*IHG22 46,X,del(Y) (q12)[76]/ 45,X[20]*	*IHG18 ND*	*IHG67 46,XY*	*IHG82 46,XY*	*IHG120 46,XY[92]/ 45,X[4]*	*Normal male 46,XY*	*Normal female 46,XX*
sY238	G38352	*ZFY*	1A2		+	+	+	+	+	+	+	−
sY594	G34978	*TTY1*	3C,4A		+	+	+	+	+	+	+	−
sY601	G34984	*PRY*	4A,6C,6E		+	+	+	+	+	+	+	−
sY600	G34980	*TTY2*	4A cen		+	+	+	+	+	+	+	−
sY620	G38348	*DFFRY*	5C	AZFa	+	+	+	+	+	+	+	−
sY610	G38446	DBY	5C	AZFa	+	+	+	+	+	+	+	−
sY592	G34997	UTY	5C	AZFa	+	+	+	+	+	+	+	−
sY593	G34981	TB4Y	5D		+	+	+	+	+	+	+	−
sY595	G38357	BPY1	5G		+	+	+	+	+	+	+	−
sY638	G38355	*CDY2**	5L		+	+	+	+	+	+	+	−
sY591	G34987	XKRY	5L		+	+	+	+	+	+	+	−
sY603	G34991	*EIF1AY*	5Q	AZFb	−	−	+	+	+	+	+	−
sY142	G38345	DYS230	6C	AZFb	−	−	+	+	+	+	+	−
sY143	G38347	DYS231	6C	AZFb	−	−	+	+	+	+	+	−
sY205	G38344	DAZ	6D,6E	AZFc	−	−	−	−	−	−	+	−
sY254	G38349	*DAZ*	6D,6E	AZFc	−	−	−	−	−	−	+	−
sY147	G40976	DYS232	6E	AZFc	−	−	−	−	−	−	+	−
sY202	G40973	DYS202	6E	AZFc	−	−	−	−	−	−	+	−
sY638	G38355	*CDY1**	6F	AZFc	−	−	−	−	−	−	+	−
sY241	G12006	DYS241	6F	AZFc	−	−	−	−	−	−	+	−
sY158	G12006	DYS241	6F	AZFc	−	−	−	−	−	−	+	−
sY240	G12005	DYS240	6F	AZFc	−	−	−	−	−	−	+	−
sY159	G38354	DYZ1	7		−	−	+	+	+	+	+	−
sY160	38343	DYZ2	7		−	−	+	+	+	+	+	−

+ presence, − absence of STS.
*The presence of *CDY2* and the absence of *CDY1* was detected by SSCP analysis of PCR product corresponding to the sY638 (data not shown). The sequence difference enables SSCP distinguishing between *CDY2* and *CDY1* copies.

one with oligospermia. Histological abnormalities were detected in 63 patients and in 14 patients the histology was normal. The histological phenotypes are summarised in table 1. Among 14 patients with normal histology, 10 were azoospermic, three severely oligospermic, and one was oligospermic.

AZF DELETIONS

Based on the PCR amplification of 23 STS markers specific to the Y chromosome (mostly AZF region), deletions in six patients (IHG8, IHG18, IHG22, IHG67, IHG82, and IHG120) were detected (table 2). In two cases, IHG8 and IHG22, the deletions were large, terminal, and similar in size encompassing AZFb, AZFc, and the heterochromatin region. Thus, they were detectable cytogenetically. Four other deletions, IHG18, IHG67, IHG82, and IHG120, were small, interstitial, and similar in size and spanned the AZFc subregion (table 2).

In four cases (IHG22, IHG67, IHG82, and IHG120), deletions were found to be de novo, and all STS markers absent in the patients were present in their father. DNA samples of close male relatives of patients IHG8 and IHG18 were not available and therefore the de novo status could not be tested.

KARYOTYPING

Patients IHG8, IHG22, and IHG120 were mosaic: They were carrying one cell line 46,XY

Table 3 Phenotypes of the five patients with spermatogenic failure carrying deletions within AZF region

	IHG8 Azoospermia Maturation arrest complete		*IHG22 Azoospermia Maturation arrest complete*		*IHG18 Azoospermia Maturation arrest incomplete*		*IHG67 Severe oligospermia Maturation arrest incomplete*		*IHG82 Azoospermia Hypospermatogenesis*	
Histological evaluation	*R*	*L*	*R*	*L*	*R*	*L*	*R*	*L*	*R*	*L*
Evaluation of seminiferous tubules										
Thinning of the germinal epithelium (proportional hypoplasia of all germ cells)	+	+	−	−	−	−	−	−	+	+
Peritubular fibrosis	+	+	−	+	−	+	−	−	−	−
Tubular hyalinosis (hyalinisation)	−	−	+	−	−	−	−	−	−	−
Thickening of the basal membrane	−	−	−	−	+	+	−	−	−	−
Change in tubule diameter	−	−	−	−	−	−	−	−	−	−
Evaluation of the germinal epithelium										
Spermatogonia	↓	↓	↓	↓	↓	↓	N	N	↓	N
Spermatocytes 1st order	↓	↓	Single	Few	↓	↓	N	N	↓	↓
Spermatocytes 2nd order	−	−	−	−	Single	Single	Single	Single	↓	↓
Spermatides	−	−	−	−	−	−	−	−	↓	↓
Sperm cells	−	−	−	−	−	−	−	−	↓	↓
Sertoli cells	N	N	N	N	N	N	N	N	N	N
Evaluation of the interstitial tissue										
Leydig cells	N	N	N	N	N	N	N	N	N	N
Inflammatory infiltrates	−	−	−	−	−	−	−	−	−	−
Fibrosis	−	−	−	−	−	−	−	−	−	−
Evaluation of extension of the lesions										
Focal					+	+				
Diffused (generalised)	+	+	+	+			+	+	+	+

+ presence, − absence, N = normal appearance, ↓ = decrease in number or size, L = left, R = right testis. Patient IHG120 had azoospermia but no histological evaluation of the testis.

with a large terminal deletion (IHG8 and IHG22) or small interstitial deletion (IHG120) and a second cell line (45,X) lacking the entire Y chromosome (table 2). Among patients with no AZF deletions, chromosomal aberrations were detected in 30% of cases. In 18 subjects, aberrations of the sex chromosomes were found including 10 47,XXY cases, whereas autosomal aberrations were present in 10 males (Wojda *et al*, submitted).

GENOTYPE–PHENOTYPE CORRELATION

Both patients with large terminal deletions (IHG8 and IHG22) were found to have azoospermia and complete maturation arrest lacking secondary spermatocytes, whereas three patients with small interstitial AZFc deletions (IHG18, IHG67, and IHG82) had milder phenotypes: azoospermia or severe oligospermia with incomplete maturation arrest (IHG18 and IHG67) or hypospermatogenesis (IHG82) (table 3). In addition, the pattern of incomplete maturation arrest of patient IHG18 was focal and not generalised (table 3). Despite significant representation of the 45,X cell line, especially in patients IHG8 and IHG22 (table 2), no gonadal degeneration, short stature, webbing of the neck, lymphoedema, mental retardation, or any other Turner stigmata, or other somatic abnormalities, such as genital ambiguity or gynaecomastia, were present in any of our six Y deleted patients. The height of the men with Y deletions was: 170 cm (IHG8), 173 cm (IHG18), 168 cm (IHG22), 175 cm (HG67), 170 cm (IHG82), and 180 cm (IHG120).

Discussion

GENOTYPE–PHENOTYPE CORRELATION

Among 94 infertile but otherwise normal males, we describe six with two types of AZF deletions, large and terminal encompassing AZFb/AZFc/heterochromatin (IHG8, IHG22) and small and interstitial encompassing AZFc (IHG18, IHG67, IHG82, IHG120). In confirmation of our hypothesis that the lack of correlation between the size of AZF deletions and the phenotype of infertile men may be caused by coexistence of the AZF deletion with a chromosomal aberration, we found a mosaic 45,X cell line in three of five karyotyped AZF deleted patients (a frequency of at least 50%). Several cases of infertile males with 45,X and 46,XY cell lines with the abnormal Y chromosome were described before molecular studies of male infertility were available.[31–45] Among these cases, the aberrant Y chromosome was described as being isodicentric,[33 38–41] or a ring chromosome,[31 32 44 45] or just lacking Yq,[1 34–37 40 42 43] as in the two cases, IHG8 and IHG22, described in this study. Most of these previously described infertile males with 45,X mosaic karyotypes and a non-fluorescent Y rarely had a "pure sterility" phenotype, but usually manifested several somatic features, mostly short stature,[35 36 42 43] gynaecomastia,[34 36–38] or genital ambiguity.[42 43] In our study, however, all three patients with a 45,X mosaic cell line had a pure sterility phenotype, which was the case even in those with a high proportion of 45,X (patients IHG8 and IHG22). Another patient of this type was previously published and had 45,X in 50% of blood cells, no somatic abnormalities, and a height of 183 cm.[39] However, that report did not describe the patient's testicular histology, so phenotype-genotype correlation could not be analysed to the extent to which it was possible in our patients.

Pure 45,X chromosomal constitution is known to result in incomplete ovarian development (streak gonads in the adult). Phenotypic variability of these published 45,X mosaic infertile patients, including our patients, is probably the result of the tissue representation of 45,X mosaicism, which may to a varying degree affect the gonadal development and differentiation processes.[46] Similarly, 45,X mosaicism could enhance the infertility phenotype caused by a coexisting AZF deletion of any size. Since the representation of the 45,X line in the target tissue, that is, primordial germ cells or spermatogonia, may be different from the one observed in blood cells, the phenotype enhancement in AZFdel/45,X subjects cannot be accurately evaluated, making the correlation of the size of the AZF deletion and the infertility phenotype very difficult. Thus, patient IHG120 with 45,X mosaicism should not be compared to those who have a similar sized deletion and no 45,X cell line in blood (IHG67 and IHG82). Owing to the same limitations, 45,X mosaic patients IGH8 and IGH22 carrying large terminal deletions should not be directly compared to the rest of the patients carrying smaller interstitial deletions.

However, this study does show that AZFc microdeletions alone may result in incomplete maturation arrest in which some secondary spermatocytes and normal appearing spermatogonia are present (IHG67), or even in hypospermatogenesis (IHG82) in which all developmental stages of germ cells are found, although in fewer numbers. We were able to find this despite a broad spectrum of observed phenotypes (tables 1 and 3) and sizes of AZF deletions (table 2), which were typically associated with AZFc deletions in other studies.[5 6] Detection of such a clear genotype-phenotype correlation in our patients with isolated AZFc microdeletion was possible only when the karyotypic defect was excluded or confirmed in the patients studied and a systematic bilateral histological evaluation accompanied the AZFc deletion molecular screening. Since interphase FISH with Y probes in testicular samples from AZF deleted patients was not performed, we cannot exclude the possibility of a 45,X cell line in the target tissue. However, testicular biopsy is an invasive procedure and therefore is limited to azoospermic patients when obtaining spermatozoa for ICSI in vitro fertilisation.

The association of incomplete maturation arrest phenotype with isolated AZFc deletions found in this study could also indicate that genes encoded by AZFc, for example, *DAZ* or *CDY1*, are not crucial for the establishment of the germline stem cells, the spermatogonia.

ORIGIN OF 45,X CELL LINE MOSAICISM WITH AZFC MICRODELETIONS

It was previously reported that Y chromosomes carrying several types of cytogenetically detectable aberrations, including the lack of Yq, have a tendency to be lost in the course of cell division and lead to the appearance of the 45,X mosaic cell line.[47 48] Therefore, it can be assumed that large AZF terminal deletions and the mosaic 45,X cell line in patients IHG8 and IHG22 are not independent events, and that the 45,X line resulted from a loss of the aberrant Yq chromosome.

Interestingly, in patient IHG120, the mosaic 45,X line coexists with an interstitial AZFc microdeletion. This finding seems to indicate that smaller, cytogenetically undetectable molecular defects could predispose to the loss of the entire Y chromosome too. So far, only a single case of that type has been reported,[20] although most papers describing patients screened for AZFc microdeletions did not include cytogenetic analysis, so 45,X mosaicism could not be excluded. Taken together, our data (one patient out of three) and the ones of Oliva *et al*[20] (one patient out of 10) show that at least 15% of karyotyped patients with AZFc microdeletions do carry a mosaic 45,X cell line. This seems to be less frequent than the coexistence of 45,X with the terminal deletions of Yq (both patient IHG8 and IHG22), but frequent significantly enough to be addressed in future or even retrospective studies.

In conclusion, our results underline the importance of a combined molecular and karyotypic approach as well as thorough histological analysis for proper evaluation of genotype-phenotype correlation in patients with spermatogenic failure carrying AZFc deletions.

- In infertile men with deletions within the Y chromosome azoospermia factor region (AZF), investigators have observed only a weak correlation between the size of the deletions and the severity of the associated spermatogenic defects. We hypothesise that this might result from a coexistence of the AZF deletion with a chromosomal aberration.
- A thorough bilateral analysis of testicular histology combined with genetic tests including blood karyotyping and screening for AZF deletions was performed in 94 patients with non-obstructive infertility, including 65 azoospermic, 23 severely oligospermic, and six oligospermic men.
- Abnormalities of the AZF region were identified in six patients: large terminal deletions of AZFb/AZFc/heterochromatin (two patients) and small interstitial deletions of just AZFc (four patients). Surprisingly, in five of these AZF deleted males who were karyotyped, a second cell line, 45,X, was found in three of them (a frequency of at least 50%). Interestingly, one patient had a 45,X line coexisting with just an interstitial AZFc deletion, indicating mosaic loss of an entire Y chromosome secondary to this deletion. The two patients with large deletions and a coexisting 45,X cell line had both azoospermia and complete maturation arrest with no secondary spermatocytes, whereas patients with just AZFc deletions and a pure 46,XY karyotype had less severe phenotypes, namely azoospermia or severe oligospermia and incomplete maturation arrest with the presence of secondary spermatocytes or hypospermatogenesis.
- These results show the importance of a combined histological, cytogenetic, and molecular approach, which in this study allowed the observation of an association between the incomplete maturation arrest phenotype and isolated AZFc deletions.

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Alternative centromeric inactivation in a pseudodicentric t(Y;13)(q12;p11.2) translocation chromosome associated with extreme oligozoospermia

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EDITOR—Centromeres are the specialised regions of chromosomes that ensure normal transmission of sister chromatids to each daughter cell after mitosis. Alphoid satellite DNA sequences, consisting of tandemly repeated ≅170 bp units present at all human centromeres, contain the information necessary for centromeric function,[1] despite the observation of marker chromosomes lacking detectable alphoid DNA.[2–4] Dicentric chromosomes, resulting from