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Research Article

Prevalence and Patterns of Y Chromosome Microdeletions in Patients with Differences of Sex Development and 45,X Mosaicism

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ABSTRACT

Background: Sex development in mammals is regulated by a complex interplay of genetic and epigenetic mechanisms. Deviations in this process can lead to Differences of Sex Development (DSD), including sex chromosome DSD conditions such as 45,X/46,XY mosaicism. Y chromosome microdeletions, particularly in the Azoospermia Factor (AZF) region, have been associated with this condition due to their potential to cause Y chromosome instability, leading to the formation of 45,X cell lineages. This study investigated the frequency and patterns of Yq microdeletions in subjects with sex chromosome DSD and 45,X mosaicism.

Methods: We analyzed 28 patients with sex chromosome DSD, all of whom had a 45,X cell lineage identified in their karyotype. Yq microdeletions were assessed using conventional and multiplex PCR, targeting 32 Sequence-Tagged Sites (STS).

Results: Yq microdeletions were identified in 13 patients (46.4%). Most deletions were observed in the AZFc subregion, with ten of eleven STSs tested in this region being absent.

Conclusion: This study corroborates previous findings linking Yq microdeletions with sex chromosome disorders and chromosomal instability. The high frequency of deletions in the AZFc subregion underscores its role in the development of 45,X cell lineages and highlights its importance for patient counseling and fertility management.

Keywords: Azoospermia factor region; Y chromosome microdeletions; 45,X/46,XY mosaicism; Differences of sex development; Sex chromosome DSD

INTRODUCTION

Mammalian sex development is controlled by a sophisticated interplay of regulatory genes, signaling proteins, hormones, and their cellular receptors, with the orchestration of genetic and epigenetic mechanisms playing a crucial role [1-3].

Typically, chromosomal sex dictates the progression of undifferentiated and bipotential gonads into XX female (ovary) or XY male (testis) gonads during sex determination. Subsequently, sex differentiation occurs, leading to the formation of internal and external genitalia. These stages collectively delineate the gonadal, hormonal and phenotypic sexes. Nevertheless, deviations in sex determination or differentiation may result in congenital conditions known as Differences/Disorders of Sex Development (DSD) [1-4].

Sex chromosome DSD is one of the three categories within the DSD classification that also include the 46,XX DSD and 46,XY DSD. The chromosomal DSD group warrants special attention because it includes patients commonly assessed in clinical practice and who may exhibit a wide range of phenotypes. In affected individuals, cytogenetic analyses play a major role in detecting abnormalities from the typical sex chromosome complement, which include sex chromosome aneuploidy or mosaic sex karyotypes [5-7].

Variations in sex chromosome composition can lead to atypical genitalia, disorders in pubertal development, gonadal failure, infertility, and other physical anomalies and dysmorphic features [8]. Turner syndrome (45,X and variants), Klinefelter syndrome (47,XXY and variants), sex chromosome mosaicism (45,X/46,XY and variants), as well as chimerism (46,XX/46,XY) are the diagnoses encompassed in this DSD subgroup [7,9].

The genetic mechanisms involved in chromosomal DSD are not yet completely understood, but Y instability has been implicated in some of these conditions [10]. Yq microdeletions, frequently identified in

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men with azoospermia or severe oligospermia, are also observed in subjects with Klinefelter Syndrome (KS) or 45,X/46,XY mosaicism [1,11,12]. These deletions often occur in the Azoospermia Factor (AZF) region, which includes the sub-regions AZFa, AZFb, and AZFc, and may predispose to Y chromosome instability, potentially leading to its complete loss and the formation of 45,X cell lineages [1,13-16].

The present study aimed to clarify the frequency and patterns of Yq chromosome microdeletions in 28 Brazilian patients with sex chromosome DSD and 45,X cell line mosaicism, and correlate them with their phenotype.

MATERIAL AND METHODS

Subjects

Twenty-eight patients with sex chromosome DSD and 45,X cell line mosaicism were included in the study. All of them were followed up at a single Brazilian Endocrinology Service at HC-FMUSP. Retrospective data including gender assignment, external genitalia characteristics, pubertal development, gonadal histology, and the presence of gonadal tumors were collected from medical records and presented in the Table 1. The patients were distributed in the female and male groups according to their assigned gender.

Cytogenetic analysis

Peripheral blood karyotypying was performed according to standard protocols, at least 20 G-banded chromosome metaphases were analyzed.

Molecular study

Genomic DNA samples were obtained from peripheral blood leukocytes using a modified salting-out technique from all patients [17]. Furthermore, DNA samples from the fathers of four patients (n-18, 19, 22, 26) were included in the study, bringing the total sample size to 32 subjects. The total of genomic DNA used in all reactions ranged from 150 to 200 ng.

We screened for Yq microdeletions using conventional and multiplex Polymerase Chain Reaction (PCR) techniques. Thirty-two Sequence-Tagged Sites (STSs) were tested, with 26 analyzed in pairs and the remaining six (DYZ3, sY611, sY113, sY105, sY106, and Y6HPc54pr) tested individually. The number of STSs analyzed in the AZFa, the region between AZFa and AZFb, AZFb, and AZFc subregions were five, five, ten, and eleven, respectively (Table S1).

To ensure accuracy, we used 650 bp fragments from the *CYP21A2* gene as an internal control for all reactions. We included samples from a fertile man as a positive control and from a fertile woman as a negative control in each reaction. Conventional and multiplex PCR were standardized to a total volume of 25 and 27 µL respectively. Reagents included 10x PCR Buffer (TransGen Biotech Co.,), 10 mM dNTPs (deoxyribonucleotide triphosphates; Invitrogen), 0.5 U Taq DNA Polymerase (TransGen Biotech Co.,) and BSA (bovine serum albumin), which was used only in the multiplex PCRs. The Applied Biosystems Veriti 96-Well Thermal cycler (Thermo Fisher) was used for all reactions. For multiplex PCR, the samples were subjected to an initial denaturation step at 94°C for five minutes followed by 50 cycles of 94°C for 30 seconds, an annealing step for 45 seconds at the selected temperature for each pair of STSs, and an extension step at 62°C for two minutes. The final extension was at 68°C for five minutes.

For conventional PCR, we did an initial denaturation step at 94°C for five minutes followed by 35 cycles of 30 seconds at 94°C, an annealing step for one minute at the selected temperature for each STS, and an extension step at 72°C for one minute. The final extension was at 72°C for ten minutes. The multiplex and traditional PCR products were analyzed using 2% and 1% agarose gel electrophoresis respectively, both in 1x TAE buffer. Samples with detected AZF microdeletions were retested to confirm the results.

RESULTS

All patients in this study displayed a 45,X lineage along with a second lineage containing a Y chromosome, which was either intact or exhibited rearrangements and/or breaks, as identified by cytogenetic analyses of peripheral leukocyte karyotypes. The most frequently observed karyotype was 45,X/46,XY in 24 patients (85.7%), while the remaining patients showed 45,X/46,X,i(Yq), 45,X/46,XY/46,X,idic(Y), 45,X/46,XY/47,XXY, and 45,X/46,XY/47,XYY karyotypes (Table 1).

Fifteen patients were assigned as female at birth and 13 as male. All patients assigned female at birth had a 45,X/46,XY karyotype, with four displaying clitoromegaly and two having atypical genitalia. One patient who was initially assigned female had his assigned sex changed to male, patient 16 at age three (Table 1). None of the patients underwent a change in social sex during puberty or adulthood, and none exhibited gender dysphoria.

The main clinical complaint among girls was short stature (11/14) and among boys was atypical genitalia (12/14). Bilateral gonadectomy was performed in all female and in six male patients. Histological studies were conducted on the gonadal tissue of 23 out of 28 patients. Among them, 41 gonads were found and were classified as undifferentiated dysgenetic gonads (streak; n-25, 21 in female, 4 in male patients), dysgenetic testes (n-7, 6 in male and one in a female patient) and testis (n-nine, 7 in male and in 2 female patients) (Table 1). In five patients, one of the gonads was not identified. Five patients had undergone gonadectomy in another medical center and the gonadal tissue was not available.

Testicular germ cell tumors were identified in three female patients, all of them had unilateral gonadoblastoma (subjects 3, 8 and 11) and one had bilateral dysgerminoma (n-3). At the time of gonadal tumor diagnosis, the chronological age of these patients ranged from seven to 20 years.

Microdeletions in the Yq chromosome were identified in 13 out of 28 patients (46.4%) (Figure 1). Out of these, six were assigned as female and seven as male. Among the six patients with testicular tissue identified in the histological study, only one presented an AZFc deletion (sY3168). None of these 13 patients exhibited microdeletions in the AZFa subregion and only one patient had a single deleted marker located in the region between AZFa and AZFb (sY102). Microdeletions in the AZFb subregion were detected in four patients, with at least one of the following eight STSs being deleted: sY105, Y6PHc54pr, sY117, sY1287, sY1752, sY125, sY127 and sY134.

The highest frequency of microdeletions was observed in the AZFc subregion, with all 13 patients showing deletions in at least one marker within this region. Ten out of eleven STSs in the AZFc subregion were deleted: sY143, sY1191, sY149, sY152, sY254, sY147, sY255, sY158, sY157, and sY3168. The loss of sY3168 was the most common, occurring in 12 patients (92.3%), followed by sY1191 and sY158, each seen in eight patients (61.5%) (Figure 2).

No silent microdeletions were found in any of the four fathers included in this research. Patient 22 was the only one who presented deletions among the four patients whose father's DNA was available.

Table 1: Clinical and histological data from the patients with sex chromosome differences of sex development and 45,X cell line mosaicism included in this study.

Patient	Diagnosis	Sex assignment	Karyotype	Main clinical complaint	External genitalia	Gonads (left/right)	Preserved gonads	Presence of tumor
1	45,X/46,XY DSD	F	45,X/46,XY	Short stature	F	NA	No	No
2	45,X/46,XY DSD	F	45,X/46,XY	Short stature	F	DG/DG	No	No
3	45,X/46,XY DSD	F	45,X/46,XY	Short stature	F*	DG/DG	No	Unilateral gonadoblastoma and bilateral dysgerminoma
4	45,X/46,XY DSD	F	45,X/46,XY	Short stature	F	NA	No	No
5	45,X/46,XY DSD	F	45,X/46,XY	Short stature	F*	DG/DG	No	No
6	45,X/46,XY DSD	F	45,X/46,XY	Short stature	F	DG/DG	No	No
7	45,X/46,XY DSD	F	45,X/46,XY	Short stature	F	DG/DG	No	No
8	45,X/46,XY DSD	F	45,X/46,XY	Short stature	F*	DG/DG	No	Unilateral gonadoblastoma
9	45,X/46,XY DSD	F	45,X/46,XY	Delayed puberty	F	DG/DG	No	No
10	45,X/46,XY DSD	F	45,X/46,XY	Syndromic phenotype	A	DG/DG	No	No
11	45,X/46,XY DSD	F	45,X/46,XY	Delayed puberty and clitoromegaly	F*	T/DG	No	Unilateral gonadoblastoma
12	45,X/46,XY DSD	F	45,X/46,XY	Short stature	F	DT/DG	No	No
13	45,X/46,XY DSD	F	45,X/46,XYqh	Short stature	F	DG/DG	No	No
14	45,X/46,XY DSD	F	45,X/46,XY	Short stature	F	DG/T	No	No
15	45,X/46,XY DSD	M	45,X/46,XY	Atypical genitalia	A	DT/DT	No	No
16	45,X/46,XY DSD	F to M	45,X/46,XY	Atypical genitalia	A	T/T	Left testis	No
17	45,X/46,XY DSD	M	45,X/46,XY	Atypical genitalia	A	DT/DG	No	No
18	45,X/46,XY DSD	M	45,X/46,XY	Atypical genitalia	A	DG/T	Right testis	No
19	45,X/46,XY DSD	M	45,X/46,XY	Atypical genitalia	A	NF/T	No	No
20	45,X/46,XY DSD	M	45,X/46,XY	Atypical genitalia	A	T/DG	No	No
21	45,X/46,XY DSD	M	45,X/46,XY	Hypogonadism	A	NF/DT	No	No
22	45,X/46,XY DSD	M	45,X/46,XY	Atypical genitalia	A	NF/DG	No	No
23	45,X/46,XY DSD	M	45,X/46,XY	Atypical genitalia	A	NA	No	No
24	45,X/46,XY DSD	M	45,X/46,XY	Atypical genitalia	A	NA	No	No
25	45,X/46,XY DSD	M	45,X/46,X,i(Yq)	Atypical genitalia	A	T/DG	No	No
26	45,X/46,XY DSD	M	45,X/46,XY/46,X,idic (Y)	Short stature	M	T/T	Right and left testes	No
27	Sex chromosome DSD	M	45,X/46,XY/47,XXY	Atypical genitalia	A	DT/NF	No	No
28	Sex chromosome DSD	M	45,X/46,XY/47,XYY	Atypical genitalia	A	DT/NF	No	No

Note: *DSD-Differences of Sex Development; F-Female; M-Male; A-Atypical; F*-female with clitoromegaly; T-testis; DT-Dysgenetic testis; DG-Dysgenetic gonad; NA-the information is Not Available; NF-the gonadal tissue was Not Found during the surgical procedure. The lines presented in gray represent the patients in whom microdeletions in Yq were identified.

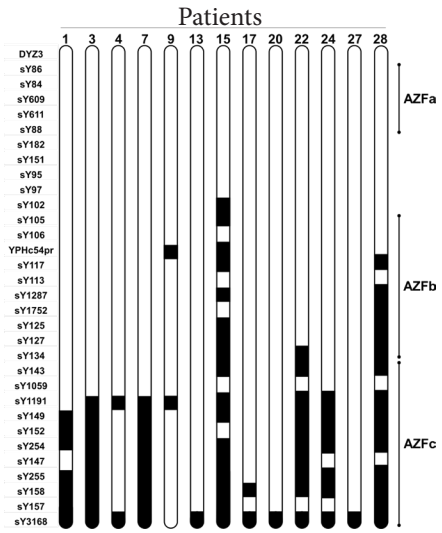

Figure 1: Schematic diagram of Yq Sequence-Tagged Sites (STS), AZF regions and the different deletion patterns of each patient in this study. On the left, the analyzed STSs are listed, categorized by their respective regions on the Y chromosome (AZFa, AZFb and AZFc). The columns represent the 13 patients with sex chromosome DSD and 45,X cell line mosaicism in whom the microdeletions were identified. Black indicates the absence of the specific fragment, while white indicates the presence of the fragment.

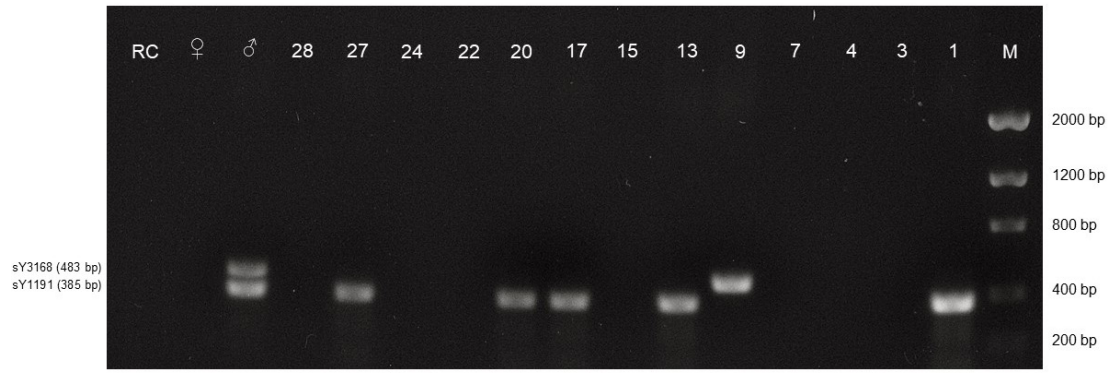

Figure 2: Agarose gel (2%) electrophoresis of multiplex PCR amplified products for markers sY3168 and sY1191 in all patients, in whom microdeletion were detected in these regions. Patients: 1, 13, 17, 20 and 27 showed only sY1191-STS (band size-385 BP); patient 9 showed only sY3168-STS (band size-483 BP) and patients 3, 4, 7, 15, 22, 24 and 28 had no amplification for either marker. M-Molecular weight marker (Low DNA Mass Ladder); Patients-1, 3, 4, 7, 9, 13, 15, 17, 20, 22, 24, 27, 28; ♂-Male control; ♀-Female control; RC-PCR reaction control.

DISCUSSION

The AZF region comprises three sub-regions AZFa, AZFb, and AZFc each essential for spermatogenesis [15]. This region appears to be more susceptible to present breakpoints due to the presence of repetitive palindromic sequences. These deletions have been previously associated with increased Y chromosome instability, which can lead to its complete loss and the formation of 45,X cell lineages [1,16,18-21].

Previous studies reported Yq microdeletions in 27% to 71.4% of patients with sex chromosome DSD and a 45,X/46,XY karyotype or its variants [1,16,18,20]. However, these abnormalities were not identified in patients with 46,XY partial gonadal dysgenesis [1].

Although the patients studied presented similar karyotypes, the number of patients evaluated in each study varied, as did the number and regions of STS studied in the different cohorts. This heterogeneity may have contributed to the differing results obtained by the authors (Table S2). In our study, we evaluated the largest number of STS located in the AZF region in blood-derived DNA samples from 28 Brazilian patients, identifying microdeletions in 46.4% of the cases.

Out of the 32 STSs analyzed, 19 (59.3%) were deleted in at least one patient. Among these, ten deletions (52.6%) were in the AZFc subregion, eight (42.1%) in AZFb and only one (5.3%) between AZFa and AZFb. These findings align with literature reports indicating a higher incidence of microdeletions in the AZFc subregion compared to other AZF regions in the 45,X/46,XY DSD patients [1,16,18,21].

Three STSs in the AZFc subregion were crucial for detecting microdeletions in our study cohort. STS sY3168 revealed deletions in 42.8% of patients, sY1191 in 28.5%, and sY158 in 28.5%. These findings also align with previous studies, highlighting the importance of using a comprehensive range of STSs to detect microdeletions [1,16]. Notably, in cases 4, 13, 17, 20 and 27, microdeletions would not have been identified without testing these regions, underscoring their critical value in comprehensive detection [22].

The AZFc subregion is highly dynamic and particularly prone to rearrangements or deletions, which can disrupt spermatogenesis [23]. Among infertile men, the most frequently identified Y chromosome microdeletions occur in the AZFc subregion. The complete absence of this locus leads to oligozoospermia or azoospermia, with histological

findings that include Sertoli cell-only syndrome, maturation arrest, or hypospermatogenesis [24].

However, partial AZFc deletions have been observed in some fertile men, suggesting potential redundancy of AZFc genes. Nonetheless, specific polymorphic deletions are associated with reduced sperm counts and infertility in certain populations [13,25-27].

This variability suggests that the impact of AZFc deletions on fertility may be modulated by the genetic context and population-specific factors. Moreover, studies have shown that paternal AZF deletions are consistently inherited by male offspring, with the size of the deletion either remaining unchanged or increasing, often resulting in the transmission of the infertility phenotype to the next generation [26,28,29].

Unfortunately, faced limitations due to the small number of paternal DNA samples obtained (4 out of 28). Moreover, only one of the affected children in this group was found to have Yq microdeletions. Consequently, we were unable to evaluate the potential contribution of cryptic paternal Y microdeletions to the phenotype of these 45,X/46,XY DSD patients.

Liu et al., reported AZFb and AZFc microdeletions in a patient with a 45,X/46,XY karyotype, but not in his father, who had a typical 46,XY karyotype [29]. This observation is similar to our finding in the only father-son pair studied, where the father had no deletions, while the son (n-15) carried an AZFb+c microdeletion. Another aspect reported in the literature is the association between Y chromosome AZFc microdeletions and an increased risk of embryo aneuploidy, likely due to the mitotic instability caused by the microdeletion [18,21,22,30-34]. However, other studies do not make this association, showing that embryos from men with Y chromosome deletions do not have a higher risk of developing chromosomal abnormalities or other adverse health conditions [23,35-37].

It is important to note the findings of Álvarez-Nava et al., who analyzed DNA samples extracted from both gonadal and blood tissues of 45,X/46,XY DSD patients [18]. They identified several deleted fragments in the gonadal DNA that were not present in the blood cell DNA, resulting in a higher frequency of microdeletions (63.6%, seven out of 11 gonads) in the gonadal samples than blood (27.2%, three in 11 samples). This raises the question of whether using DNA samples from gonadal tissue could provide more insights into the phenotypic variability of patients.

CONCLUSION

This study identified a high frequency of microdeletions in the AZFc subregion of the Y chromosome in Brazilian patients with 45,X/46,XY karyotypes and its variants. These findings suggest a potential link between the loss of specific Yq segments, chromosomal instability, and the formation of 45,X cell lineages. The results underscore the importance of detecting Yq deletions to guide sex chromosome DSD patients counseling and manage fertility.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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STATEMENT OF ETHICS

This study was approved by the Ethical Committee of the Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (HC-FMUSP) (CAAE n 24372). Written informed consent was obtained from all patients or their parents or legal guardians who agreed to participate in our research.

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