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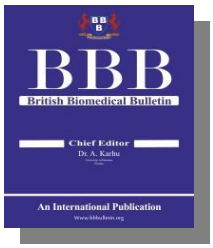
BRITISH BIOMEDICAL BULLETIN



Original

Analysis of Y-Chromosome in Several Ambiguous Genetalia Patients in Indian Population

Mandar Bhattacharya*[1], Shrestha Chakroborti[2], Sharanagata Maiti[1], Pranami Bhaumik[1], Poulami Majumder[1], Priyanka Ghosh[1] and Subrata Kumar Dey[1]

[1]*Department of Human Genetics, Maulana Abul Kalam Azad University of Technology*
[2]*DSR Genome Technologies Pvt. Ltd*

A R T I C L E I N F O

Received 17 Aug. 2015
Received in revised form 30 Aug. 2015
Accepted 05 Sep. 2015

Keywords:
Ambiguous genital,
Disorder of sex development,
Azoospermia factor,
Sequence tagged sites markers.

Corresponding author: Mandar Bhattacharya
South 24 Parganas, West Bengal, India.

E-mail address: mandarbhattacharya@gmail.com

A B S T R A C T

Objective: Disorder of sexual development (DSD) relates to a large number of sexual ambiguities in children. Various genetic factors play a major role in genital formation and any misregulation in genital formation will lead to defective genitalia. Here our goal was to check the status of azoospermia factor region (AZF) amongst sexually ambiguous babies.

Methods: Nine (9) DSD patients were included in this study and clinical information was collected accordingly. Initial 17-OH progesterone analysis and karyotyping were done after collecting peripheral blood. Genomic DNA was isolated and PCR reactions were performed using sY81, sY86, sY127, sY128, sY 134, sY 254, sY255 and SRY.

Result: Our study revealed the presence of azoospermic region (AZF)- Y chromosomal fragments in all of the cases via PCR techniques. XY karyotype cases showed variable degree of AZF deletions; on the other hand XX karyotypes possess presence of AZF fragments.

Conclusion: Irrespective of karyotype, AZF region fragments might contribute to the development of sexual disorder.

Acronyms:

AZF=Azoospermia factor
DSD=Disorder of Sex development
CAH= congenital Androgen Hyperplasia
EEC= Exstrophy-Epispadias complex
PGD= partial Gonadal Dysgenesis
MGD=Mixed Gonadal Dysgenesis

Introduction

Human sex development is a complex process which involves several finely regulated steps such as sex determination (XX/XY), sexual organ differentiation and organ development. Presence of sex specific hormones plays an important role as well. Any form of mis-regulation can lead to sexual development disorders or specifically known as development of sexual disorders or DSD.

Wide arrays of DSDs have already been reported for example female and male pseudohermaphrodism, true hermaphrodism and gonadal dysgenesis, hypospadias, androgen synthesis deficiency etc. Female pseudohermaphrodites exhibit 46, XX chromosomal array as well as virilization of external genitals. A common cause of female pseudohermaphrodism is congenital androgen hyperplasias (CAH)[1]. Male hermaphrodites bear 46, XY genotype with a variable degree of female genital appearances. Male sex hormone (androgen) synthesis is mostly related to this type of DSD. Both male and female tissue is observed in true hermaphrodites. However, majority of those candidates have 46, XX genotypes. Gonadal dysgenesis is the condition where both of the gonads (pure gonadal dysgenesis) or any one of the gonads (mixed gonadal dysgenesis) is/are dysfunctional. Hypospadias and epispadias are related to abnormal opening of urethra.

Yp deletion especially SRY portion leads to malformation of testis and Yq deletion leads to infertility in men[2]. As women don't have Y chromosome, effect of SRY and AZF is restricted to male only. As DSD harbors wide array of ambiguity, several instances of modified Y chromosome have been reported[3]. So our goal was to check Y chromosome status of DSD patients with different clinical features.

Materials and methods

Subjects

Our study includes total 9 patients with different form of DSDs. Patients were sent by Dr. BC Roy Memorial Hospital for Children, Kolkata for chromosomal analysis. 17-OH progesterone level was confirmed by standard ELISA kit.

Subject 1AB was a 1 year old child diagnosed with palpable gonads in the labioscrotal fold region and a micropenis. The overall status of case1AB was good and no other abnormality was observed. 17-OH progesterone level was found out to be normal.

Subject 2TB was a 1 year old preterm child (7months) with ambiguous genetalia and extremely low birth weight. Subjects father was treated with azoospermia and lower uterine segment cesarean section was done while birth. Subject's inguinal region was swollen and appears cord like 2-3 cm long urethra and bifid scrotum was observed. Subject was a positive case of hypospadias. No 17-OH imbalance was observed.

Subject 3SM was four years and ten months old at the time of study. Subject suffered precocious puberty and obesity.

Scrotal sac was empty and subject had undescended testicles. Left testis was hypoplastic and lied deep near inguinal ring but right testis cannot be delineated in right inguinal region. Several hormonal imbalance had been observed (FSH 0.08mIU/mL; LH <0.5mIU/mL; testosterone 1.78 ng/ml). 17-OH-progesteron level was very high (12.29 ng/ml) compared to reference values[4,5,6].

Subject 4KK was two and half year old male child with hypospadias opening. Subject 5PP (two year old) was also a male patient of hypospadias defect. Both of these patients were otherwise good in condition. 17-OH progesterone level was found within the normal range.

Subject 6JS was three years old at the time of study. Subject suffered cardiac disorder (pentalogy of fallot presented cyanosis), respiratory distress, and lower vision in both eyes and ambiguous genital with normal level of 17-OH progesterone.

Subject 7TH was a one year old diagnosed with Exstrophy-Epispadias complex (EEC). No palpable gonads were observed. Normal values of 17OH progesterone were observed.

Subject 8RS was a three year old child with inguinal hernia. Gonads were palpable. USG showed normal uterus structures but both the ovaries were absent and 17OH-progesterone level was normal.

9MB was a year old child with hypospadias defect however no imbalance in 17-OH progesterone was detected. No other abnormalities were visualized as well.

Collection of Blood

2 ml peripheral blood was collected in an EDTA anti-coagulant tube.

Karyotyping

Standard karyotyping methods using colcimid was followed and at least 30 plates per samples were prepared and analyzed. IKAROS software was used to make karyotypes.

Polymerase Chain reaction

After genomic DNA isolation from peripheral blood, PCR were performed using Y chromosome specific STSs: sY81, sY86, sY127, sY128, sY 134, sY 254, sY255 and SRY as per previously described[7,8]. Primer sequences are listed in **table 1**.

Deletion of an STS was confirmed after three consecutive fail in amplification process. A positive control (a male 5 year old child) and a negative control (a female 5 yr old child) were included in the study.

For all patients, information and blood was collected under strict anonymity and only after receiving consents from parents.

Result

Karyotype result showed 6 patients with XY genotype, 2 (subject 8RS and 4KK) with XX genotype and another 1(subject 1AB) with mosaic genotype **table 2**.

PCR result yielded random presence of AZF specific STS markers among those 9 cases. Out of 6 patients with XY genotype only 4 were positive for sY14 (SRY). Subject 1AB (XX/XY) were positive for all three AZF region and SRY as well. Subject 8RS (XX) were SRY negative and only AZFb positive. Subject 4KK exhibited most of the deletion and it retained only 1 marker in AZFb region. This patient was also found negative for SRY. Patient 9MB with genotype XY was negative to AZFc and SRY region. Patient 7THwas negative for AZFb and positive for SRY. Both 5PP and 6JS were positive for all three AZF regions along with SRY part **table 3**.

Discussion

Yq deletion is a major area of interest because of its association with

sexual development. AZF deletion and correlation with infertility is an established fact[9-12]. However, only handfuls of studies have indicated a potential role of chromosomal deletion among DSD patients. Male specific STSs were found positive among Brazilian partial gonadal dysgenesis (PGD) patients. However, 40% mixed gonadal dysgenesis (MGD) patients did exhibit Yq deletion in AZFb and AZFc regions[13]. High frequency (63%) of Y deletion was observed in gonadal DNA among dysgenesis patients. 45X DSD male patient, a unique case, revealed 7q distal deletion[14], which also been observed in a 3year old multiple malformation DSD patient[15]. In another detail study, 67% XY DSD and 50% XX DSD found positive for SRY and one rare Klienfilter patient found out to be AZF deleted[16]. A study among Turkish cryptorchidism children revealed no AZF region deletions[17]. Therefore the cause of undescendend testicular symptoms might not be due to azoospermia factor region. AZFb and AZFc deletion is directly involved in meiosis impairment[18]. In our study, no XY genotype showed complete AZFa or AZFb deletion on contrary to AZFc which was absent in 2 XY patients. 4KK and 5PP, both were diagnosed with same form of hypospadias but drastically different in terms of karyotype and AZF analysis. Karyotype of subject 4KK was XX, however fragments of Y-chromosome were observed albeit only 1 marker was present. Case 8RS is unique in terms of karyotype and clinical features. A striking presence of AZFb region and other female features shows that Y chromosome may play a bigger role in DSDs who are not necessarily to be male.

Conclusion

One of the striking features of DSDs is ambiguous correlation between genotype and phenotype. Even an otherwise female with XX chromosome array revealed fragments for y-chromosome. Therefore random AZF region deletion might not only be responsible for infertility but for DSDs as well. However more studies need to be done to unlock the actual role of AZF region behind all sorts of DSDs

Acknowledgement

The authors gratefully acknowledge the families of patients. We are also in debt of Dr. BC Roy Memorial Hospital for Children, Kolkata and the doctors for diagnosis and characterization of disease classes.

Conflict of interest

The authors declare no conflict of interests.

Funding Source declaration

The present work is funded by Department of Biotechnology, DBT. Sanction number: 102/IFD/SAN/2841/2012-2013.

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Table 1: STS markers used to detect AZF deletion among DSD patients.

STS Marker	Primer Sequence	AZF Region
sY81	F5'-AGGCACTGGTCAGAATGAAG R5'-AATGGAAAATACAGCTCCCC	AZF-a
sY86	F5'- GTGACACACAGACTATGCTTC R5'- ACACACAGAGGGACAACCCT	AZF-a
sY127	F5'- GGCTCACAAACGAAAAGAAA R5'- CTGCAGGCAGTAATAAGGGA	AZF-b
sY128	F5'-GGATGAGACATTTTTGTGGG R5'-GCCCAATGTAAAACTGGACA	AZF-b
sY134	F5'- GTCTGCCTCACCATAAAACG R5'- ACCACTGCCAAAACTTTCAA	AZF-b
sY254	F5'-GGGTGTTACCAGAAGGCAAA R5'-GAACCGTATCTACCAAAGCAGC	AZF-c
sY255	F5'- GTTACAGGATTCGGCGTGAT R5'- CTCGTCATGTGCAGCCAC	AZF-c
SRY	F5'-GAATATTCCCGCTCTCCGGA R5'-GCTGCTGCTCCATTCTTGAG	-

Table 2: Patient information summary

Subject ID	Age	Karyotype Report	Clinical Report
1AB	1Y	XX/XY	Palpable gonads in labioscrotal fold, bilateral testis within labial fold
2TB	1Y	XY	Premature conception, under weight, 2-3 cm long penis, small scrotal
3SM	4Y10M	XY	Undescend testes, mentally retarded
4KK	2Y5M	XY	Hypospadias
5PP	2Y	XY	Hypospadias
6JS	3Y	XY	Cardiac disorder, respiratory distress, lower vision, delayed development, low set ear
7TH	1Y	XY	Exstrophy epispadias complex, No gonad seen
8RS	3Y	XX	Inguinal Hernia with palpable gonad, Uterus normal, ovary absent
9MB	1Y6M	XY	Hypospadias

Table 3: STS marker status among DSD patients

Subject ID	Karyotype Report	sY81	sY86	sY127	sY128	sY134	sY254	sY255	SRY
1AB	XX/XY	1	1	0	1	0	1	0	1
2TB	XY	1	1	0	1	1	0	1	1
3SM	XY	1	0	1	0	1	0	0	0
4KK	XX	0	0	0	0	1	0	0	0
5PP	XY	0	1	0	1	0	1	1	1
6JS	XY	1	1	1	1	1	1	0	1
7TH	XY	1	0	0	0	1	1	1	1
8RS	XX	0	0	1	1	0	0	0	0
9MB	XY	1	1	1	1	0	0	0	0

'1' represents amplification was successful i.e. presence of that particular marker, '0' represents failed amplification after three consecutive attempts, thereby indicating absence of that particular STS marker.