www.sphinxsai.com

ChemTech

International Journal of ChemTech Research
CODEN (USA): IJCRGG ISSN : 0974-4290
Vol.6, No.8, pp 4033-4037, September 2014

ICSET-2014 [6th – 7th May 2014]
International Conference on Science, Engineering and Technology

Screening for polymorphism in exon 12, USP9Y gene of Azoospermic patients

Ambili Narikot, Dhanya Gangadharan, Ch Reenee, Rajiv S, Nishu S and Abilash V.G*

Division of Biomolecules and Genetics School of Bio Sciences and Technology, VIT University, Vellore - 632014, TamilNadu, India

***Corres.author: abilash.vg@vit.ac.in, Tel.: +919840364805**

Abstract: Azoospermic patients are reported to have either complete or partial deletions of USP9Y gene ecoding Ubiquitin specific peptidase 9, Y-linked (USP9Y) enzyme, located on the q arm of Y chromosome. The gene is known to be involved in normal spermatogenesis; however, its function is not well understood. In this study, we amplified a specific region of exon-12 of USP9Y gene from 22 azoospermic patients using polymerase chain reaction and then PCR products were screened for polymorphism using single strand conformation polymorphism (SSCP). SSCP analysis showed band variation in one azoospermic patient.
Keywords: Azoopermic, USP9Y, PCR, SSCP, Polymorphism.

Introduction

The USP9Y gene is located on AZFA region of the long (q) arm of the Y chromosome at position 11.2[1] Out of 792 kb span of AZF interval, 170 kb is spanned by USP9Y gene which consists of 46 exons [2]. USP9Y gene encodes Ubiquitin specific peptidase 9, Y-linked enzyme and is a member of the peptidase C19 family. This protein is known to play an important role in normal human spermatogenesis, although its precise function is not known[3] Complete or partial loss of USP9Y gene and single nucleotide polymorphisms have shown to result in severe Sertoli-cell-only phenotype with no visible germ cells [4,5.]

It may be either due to an early defect in germ cell progression, or germ cell degeneration which lead to blockage in spermatogenic progression[6.] Azoospermia and severe oligospermia have been associated with deletion in USP9Y patients [7.] USP9Y gene, therefore, could be linked with proper spermatogenesis progression in humans[8].In this study, we planned to screen for any mutation in exon-12 of USP9Y gene in azoospermic patients.

Materials and Methods

Patients and control

22 azoospermic men and 1 fertile male control samples were recruited from Sandya fertility centre, Vellore for present study. Blood samples were collected in EDTA vacutainers from all the patients. The age groups of azoospermic men ranged from 25 to 40 years. With the help of an experienced Gynaecologist a

detailed case history and clinical examination of every patient were carried out. The life styles and habits of the patients were recorded including smoking and alcohol drinking as Presented in table 1. Semen analysis was routinely performed on the male partner of couple coming for infertility treatment. After semen analysis only confirmed azoospermia cases were included in this study. Blood samples from each azoospermic patient were collected by the physicians with the written consent.

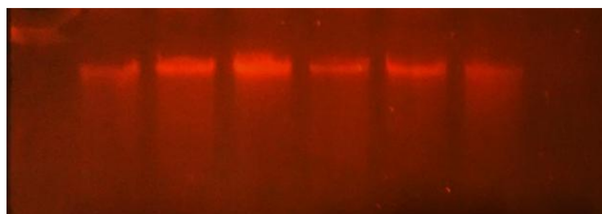
Table 1: showing the clinical features and lifestyle habits of azoospermic patients.

S.No	Azoospermic Cases	Age at Reporting (Years)	Sex	Sperm count	Smoking	Alcohol consumption
1	AY1	30	Male	Nil	Yes	No
2	AY2	27	Male	Nil	Yes	Yes
3	AY3	25	Male	Nil	Yes	No
4	AY4	34	Male	Nil	No	No
5	AY5	38	Male	Nil	Yes	Yes
6	AY6	40	Male	Nil	Yes	Yes
7	AY7	27	Male	Nil	No	No
8	AY8	28	Male	Nil	Yes	Yes
9	AY9	40	Male	Nil	Yes	No
10	AY10	31	Male	Nil	No	Yes
11	AY11	36	Male	Nil	Yes	No
12	AY12	38	Male	Nil	No	No
13	AY13	40	Male	Nil	Yes	Yes
14	AY14	29	Male	Nil	Yes	No
15	AY15	27	Male	Nil	Yes	Yes
16	AY16	25	Male	Nil	No	No
17	AY17	37	Male	Nil	No	Yes
18	AY18	35	Male	Nil	Yes	Yes
19	AY19	32	Male	Nil	Yes	Yes
20	AY20	38	Male	Nil	No	No
21	AY21	39	Male	Nil	No	Yes
22	AY22	29	Male	Nil	No	No
23	C	31	Male	Normal	No	No

DNA extraction:

4 ml of intravenous blood was sampled from all the patients using EDTA coated vaccutainer. The genomic DNA was extracted from peripheral blood by using Gill et,al method[9]. Qualitative analysis of DNA was carried out by 0.8% Agarose Gel Electrophoresis (Fig.1).

Fig.1: Qualitative analysis of extracted DNA of Azoospermia patients on 0.8% Agarose Gel Electrophoresis.



PCR Analysis:

The polymerase chain reaction (PCR) technique was used to amplify the segment of Exon-12 of USP9Y gene on the long arm of Y chromosome with a pair of primers sequence

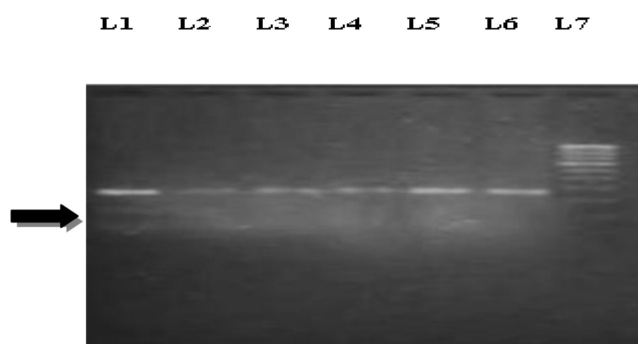
FP: (5’ GCAGGAAAACATGAAGCCAT 3’), RP:(3’CAATGACCAATTCTTTTTCATCA5’).

The lyophilized primers were ordered and received from the company (Merck, Bangalore). Polymerase chain reaction consisted of 20µl PCR reaction mixture and included 5µl 2x Red mix PCR buffer, 0.5µl of 2 picomol (pm) each forward and reverse primer, 9µl of autoclaved MilliQ water and 4µl of 10ng genomic DNA. The Red mix 2x PCR reagents was purchased from Synergy Scientific Services (Chennai). Preparation of PCR Master Mix is presented in table 2. Each sample was amplified separately in a 0.2 mL thin wall tube using an Applied Biosystems® Veriti® 96-Well Thermal Cycler, USA. A PCR condition used was as follows: initial denaturation (95°C for 5 min), subsequent denaturations (95°C for 30 sec) and extension (72°C for 30 sec) The annealing temperatures that were used for Exon-12 of USP9Y gene was 57°C for 40sec and final elongation was (72°C for 5min). PCR condition is presented in table.3. To confirm the amplification of Exon-12 of USP9Y gene of AZFa region on Y-chromosome, the PCR products were checked by electrophoresis in a 2% agarose gel containing ethidium bromide (0.5 mg/ml) and the bands were visualized under UV illumination and photographed (Fig.2).

Table 2: Preparation of PCR Master Mix amplification of Exon- 12 of USP9Y gene on Y-chromosome.

Content	**Quantity**
2X Master Mix- Red	5µl
Forward Primer	0.5µl
Reverse Primer	0.5µl
MilliQ Water	10µl
Master mix + DNA	16µl + 4µl
Total	20µl/sample

Fig 2: 2% Agarose gel showing the PCR amplification of SNP bearing region of Exon-12 of USP9Y gene in azoospermic patients.



L1: Control, L2-L6: Azoospermic patient sample, L7:100 bp ladder.

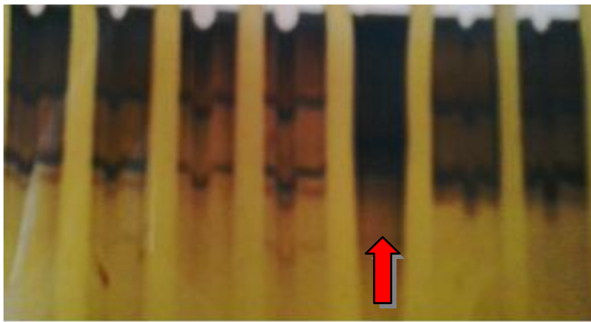
Table 3: PCR Conditions for amplification of Exon 12 of USP9Y gene on Y-Chromosome.

Steps of PCR	**Conditions**
Initiation	95^0C for 5 minutes
Denaturation	95^0C for 30 seconds
Annealing(standardized at)	57^0C for 40 seconds
Elongation (Initial Extension)	72^0C for 30 seconds
Final Extension	72^0C for 5 minutes
Hold	4^0C

Single Strand Conformation Polymorphism (SSCP)

SSCP (Single Strand Conformation Polymorphism) of the PCR product was carried out to screen the samples for the suspected mutation in Exon- 12 of USP9Y gene. 8µl of PCR products were used for SSCP which was performed by the modified and standardized protocol.[10]

Fig 3: SSCP gel indicating presence of band variation in patient sample.



L1: Control, L2-L7: Azoospermic patient sample where L5 shows band variation.

Results

DNA was isolated from all the 22 azoospermic patients and control sample. Qualitative analysis of extracted DNA were checked in 0.8% agarose gel and presented in figure.1. The annealing temperature of PCR amplification was standardized at 57°C using gradient PCR technique. A segment of Exon-12 of USP9Y gene on Y chromosome was amplified for all the 22 azoospermic patients as shown in figure 2. PCR products were analysed using SSCP to screen for polymorphism in the azoospermic patients. Band variation was seen only in sample AY19 in the SSCP analysis in which polymorphism can be confirm by sequencing.

Discussion

De novo mutation in USP9Y gene causes spermatogenic failure due to formation of truncated protein[11.] This mutation was associated with non-obstructive azoospermia. The USP9Y gene was considered as one of the major Y-linked spermatogenesis genes, both for its position (AZFa region) and the previously reported genotype–phenotype correlation (infertility due to severe spermatogenic failure)[12.] But its exact role in spermatogenesis is still not known[13.]Technological progresses in molecular genetics and data from animal model and the human genome project will be an excellent background for a large scale research in the field of genetics of male infertility.

Conclusion

In our samples, only one sample showed band variation in SSCP analysis. This suggest that in this sample, there might be mutation in exon 12 while the other samples might be having mutation in different region of AZF region of Y chromosome which led to azoopermia in them. Hence, screening of exons other than exon 12 might confirm mutation in rest of the samples.

Acknowledgements

The authors would like to thank the VIT University authorities for providing all the facilities needed for this project.

References

1. Layman L.C; Human gene mutations causing infertility; J Med Genet 2002;39:153–161.
2. Hall NM, Brown GM, Furlong RA, et al. Usp9y (ubiquitin-specific protease 9 gene on the Y) associated with a functional promoter and encodes an intact open reading fram homologous to Usp9x that is under selective constraint. Mamm Genome 2003;14:437-47.
3. Lynn M. Sims and Jack Ballantyne; A Rare Y Chromosome Missense Mutation in Exon 25 of Human USP9Y Revealed by Pyrosequencing; Biochem Genet ,2008, 46,154–161.
4. Sherman J Silber and Sjoerd Repping; Transmission of male infertility to future generations: lessons from the Y chromosome, Human Reproduction; 2002,8, 217-229.

5. Chao Sun, Helen Skaletsky, Bruce Birren, Keri Devon, Zhaolan Tang[1], Sherman Silber, Robert Oates and David C. Page; An azoospermic man with a de novo point mutation in the Y-chromosomal USP9Y; Nature Genetics., 1999,23, 429–432.
6. Sophie Mazeyrat, Noëmie Saut, Vladimir Grigoriev, Shantha K. Mahadevaiah, Obah A. Ojarikre, Áine Rattigan, Colin Bishop, Eva M. Eicher, Michael J. Mitchell & Paul S. Burgoyne; A Y-encoded subunit of the translation initiation factor Eif2 is essential for mouse spermatogenesis; Nature Genetics; 2001, 29, 49–53.
7. Brown, G.M, Brown GM1, Furlong RA, Sargent CA, Erickson RP, Longepied G, Mitchell M, Jones MH, Hargreave TB, Cooke HJ, Affara NA; Characterisation of the coding sequence and fine mapping of the human DFFRY gene and comparative expression analysis and mapping to the Sxrb interval of the mouse Y chromosome of the Dffry gene;Human Molecular Genetics; 1998,7, 97–107.
8. Carlo Foresta, Enrico Moro, and Alberto Ferlin; Y Chromosome Microdeletions and Alterations of Spermatogenesis, Endocrine Reviews 22(2), 2001, 226–239.
9. Gill, P., Lygo, J. E., Fowler, S. J. and llerrett, D. J; An evaluation of DNA fingerprinting for forensic purposes, Electrophoresis; 1987, 8, 38-44.
10. Orita, M. Iwahana H, Kanazawa H, Hayashi K, Sekiya T; Detection of Polymorphisms of Human DNA by Gel Electrophoresis as SSCPs, Proceedings of the National Academy of Sciences of the United States of America; 1989, 86, 2766-70.
11. Westerveld G.H; Korver C.M; Van Pelt A.M.M; Leschot N.J; Van der Veen F; Repping S; and Lombardi M.P; Mutations in the testis-specific NALP14 gene in men suffering from spermatogenic failure; 2006,21, 3178–3184.
12. Csilla Krausz and Degl'Innocenti S; Y chromosome and male infertility; Update Frontiers in Bioscience, 2006,11, 3049 -3061.
13. Yen PH. Putative biological functions of the DAZ family, Int J Androl; 2004,27,125–129.

*****