SHORT COMMUNICATION

Single nucleotide polymorphism of Arylsulfatase D gene (ARSD) and their association with male infertility

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In human, six Arylsulfatase D (ARSD) genes (ARSD A-F) exist, out of which four are clustered on short arm of X-chromosome that are linked with male fertility factors involved in regulating spermatogenesis. Our aim was to find out the role of ARSD gene based on next generation sequencing (NGS), whose role in male infertility still remains obscure. Clinically the case of male infertility was diagnosed on the basis of semen analysis and hormone profile, confirming non-obstructive azoospermia. Karyotype was prepared and microdeletions of Y-chromosome were detected using STS markers. No mutations were identified either in karyotype or using STS markers. Interestingly, the finding of the NGS based analysis revealed four different single nucleotide gene polymorphism (SNP) of ARSD gene rs111939179 C→T, rs73632978 G→A, rs73632977 A→T, rs73632976 C→T in heterozygous conditions. The results enhance our current understanding of the genetic basis of male infertility and provide helpful clues for designing future studies.

Key Words: Arylsulfatase D; Karyotype; STS markers; Microdeletion of Y-chromosome; Male infertility; Next Generation Sequencing.

DESCRIPTION

Reproductive health has become a challenge to the scientist and clinicians in developing countries. Male factor alone contribute>50% of all infertility cases [1]. To understand the genetic basis of male infertility researchers put significant efforts to identify "novel mutation" over the last decade with emerging technologies. Genetic causes of male infertility are limited to Y-chromosome microdeletions and Klinefelter syndrome, together accounting for 10-20% of the cases of severe spermatogenic failure. Undoubtedly, amongst genetic factors, deletion of AZFc is known to play crucial role to regulate infertility in males [2-4]. Both ends of the Y-chromosome contain pseudoautosomal regions (PARs) that join up with the X-chromosome during crossing over and involved in regulation of spermatogenesis. Further, molecular deletion studies of Y-chromosomes (Yq11.21, Yq11.22, Yq11.23) based on sequence tagged sites (STS) have identified in the loci responsible for the normal production of sperm. Y-chromosomal imbalance contributes to about 14% of azoospermic and 5% of oligozoospermic conditions in male infertility with abnormal seminograms [5].

There is scanty in the literature on ARSD gene mutation and their association to the male infertility. ARSD is located within a cluster of X-chromosome and related to the pseudoautosomal region of Y-chromosome [6]. Based on GWAS (Genome-Wise Association Study), ARSD gene is in close neighborhood of X-chromosome [7] and associated with sperms motility (unpublished data). Single nucleotide polymorphism (SNP) within ARSD shows that it is involved in sperm motility in animal [8], but the data on humans is still lacking. In the present report, we are reporting for the first time SNP of ARSD gene identified by Next generation sequencing in the clinically diagnosed case of male infertility. Our findings are "novel" and herein we report for the first time the association of four different SNP's of ARSD gene in male infertility which needs further verification as a "genetic marker" in male infertility.

A clinically diagnosed case of non-obstructive azoospermia (NOA) of 38 years was referred to Cytogenetic and Molecular Genetics Laboratory, Department of Pathology/ Lab Medicine from OPD of Obstetrics and Gynecology of AIIMS Patna for cytogenetics and molecular genetic studies. This study was approved by Institute Ethical Committee (IEC) of AIIMS Patna. Endocrine malfunctions are more prevalent in infertile men than in the general population. The blood plasma sample from the infertile patient was used for the study of hormones profile (testosterone/ luteinizing/follicle stimulating hormone) using standard routine laboratory methods (ELISA). Chromosomal analysis was performed from peripheral blood lymphocytes. After 72hours culture (RPMI 1640, Phytohaemagglutinin-M, Fetal bovine serum and antibiotic), the cells were harvested, hypotonised and fixed using 3:1 methanol: acetic acid. At least 20 well spread metaphases were analyzed and chromosomal abnormalities were characterized by GTG banding [9]. Karyotypes were prepared according to the recommendations of ISCN 2013 using applied spectral imaging software (Genesis USA). Genomic DNA was isolated using standard kit (Promega, USA) according to the manufacturer's instructions. Whole-exome sequencing was performed with Illumina Hiseq 2000 platform with 101 bp paired-end reads. Alignment to the reference genomes (hg19 for human) was performed using Burrows-Wheeler Aligner (BWA). After Next-Generation Sequencing data pre-processing (local realignment, duplicate marking and base quality recalibration) using GATK. We identified single nucleotide variants (SNVs) and small insertions/deletions (indels) in our samples using MuTect and Somatic Indels Detector (present in GATK), respectively. The resulting variants the following additional filters: 1) the minimum read depth=10, 2) minimum number of alternative reads=7 and 3) at least 25% of all reads covering the position variant allele with exclusion of non-coding, synonymous and highly repetitive regions was performed.

An association of genetic factors based on karyotyping and PCR based genetic analysis of STS markers associated with male infertility was performed as described earlier. Study was performed including the case of clinically diagnosed non obstructive azoospermia (NOA). Hormonal profile showed significant increase in serum FSH level (37.87IU), testosterone (3.25IU) when compare with normal values. According to ISCN (2013) the normal karyotype showing 46, XY chromosomal complements with high resolution of GTG banding, visualization of both euchromatin and heterochromatin using ASI 3.6 software (USA). Furthermore, no mutations were detected using STS markers of (AZF a-c) belonging to microdeletion of Y-chromosome. Curiosity further developed to identify genetic mutations using NGS based analysis. To identify further involvement of causative mutation in the patient, we selected rare SNVs or Indels by four steps of filtering: 1) basic filtering: variants with insufficient

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sequence coverage (sequencing depth<8 x coverage and Phred-like quality score<30 were ruled out; 2) frequency filtering: SNVs or InDels with allelic frequencies>0.05 in the 1000 Genomes Project dataset and proprietary exome sequencing dataset were excluded; 3) function region filtering: variants in the intron or untranslated region were discarded with the exception of splice site mutations or variants recorded in the Human Gene Mutation Database; and 4) clinical phenotype filtering: pedigree co-segregation of disease phenotypes were considered to confirm the causality of the variant. NGS was performed to identify different genes involved during spermatogenesis in male fertility. Interestingly, data base analysis showing a variety of "new mutations" of ARSD gene variants: rs111939179 C→T, rs73632978G→A, rs73632977 A→T, rs73632976 C→T in heterozygous conditions with different frequency of mutation (Table 1).

Table1 X-Chromosome gene mutation in male infertile case.

S.N.	Name of the Gene	dbSNP ID	SNP analysis	Zygosity	%frequency of variation	Remark
1	ARSD	rs111939179	C→T	Heterozygous	40.5	NA
2	ARSD	rs73632978	G→A	Heterozygous	73.7	Deleterious
3	ARSD	rs73632977	A→T	Heterozygous	70.0	Deleterious
4	ARSD	rs73632976	C→T	Heterozygous	64.2	Deleterious

In human NOA associated infertility is linked to the variety of chromosomal aberrations [10]. Molecular pathology of male infertility is highly complex because of mitotic and meiotic events that work together during spermatogenesis. Although, the Y-chromosome encodes gene(s) regulating events of spermatogenesis, the genes assigned on autosomes are also linked with spermiogenesis. Human genome shows that spermatogenesis is highly complex process and associated with a large number of genes crosslinked to X, Y and autosomes. NGS is therefore a comprehensive powerful tool and efficient for diagnosis of disease such as infertility. Present study expands the pathogenic spectrum of new mutation that are involved in increasing risk of infertility. An ARSD gene shows six isoforms (ARSD A-F) out of which four are clustered at distal region of the short arm of X- chromosome (Xp22.3). These genes are originated through a series of evolutionary duplication events that occurred in ancestral pseudoautosomal regions of Y-chromosome [11]. The isoforms of ARSD gene encodes 48 amino acid peptides and transcribe in similar boundaries of exon-intron during metabolism in different tissues. However, the pathophysiology of ARSD protein during translation in testis is still unknown [12]. Many male fertility genes are assigned on the X- chromosomes and the study suggest the possible linkage of these SNPs interface during cell proliferation and maintenance of spermatogenesis. Present study based on NGS identifies four ARSD SNP mutations, out of which three are deleterious in nature, however, the functional aspect of the fourth is unknown and needs further determination.

In human population, more than 32% case of infertility the causative factors remain obscure and fall in the category idiopathic infertility. Cytogenetic studies have been successful in revealing some causes. However, most NOA cases are still idiopathic. The frequency of karyotypes variation differs in population due to epigenetic factors. The formation of ring chromosome and translocation are rare events involving autosomes in fertility. During spermatogenesis interaction of X & Y genes are expressed at various stages of sex differentiation in male at the time of cellular differentiation (mitotic, meiotic and post meiotic) finally leads to develop healthy haploid mature spermatozoa.

However, recent advancement for the identification of Mendelian genes, NGS explore more than 30 Mendelian disease genes with recessive and dominant in nature. NGS data can be used to discover linkage analysis and zygosity intervals and to determine copy number variation of specific intervals [13,14]. Present study identified "novel" SNP of ARSD genes regulating spermatogenesis, single nucleotide variants (SNVs [15-17]. Although, there is a high likelihood of identifying significant variants, since approximately 85% of disease-causing mutations are thought to occur in gene coding regions [18,19]. Therefore, present study explores the knowledge of non-coding pseudoautosomal region (PARs) during pairing between X & Y chromosomes that interfere with spermatogenesis and fertility [20,21]. Interestingly, arylsulfatase D gene (ARSD) shows with three different SNP's in harmful forms to the case of NOA, but the fourths SNP's role is still not known and needs further evaluation in disease condition. However, future studies are warned by increasing large sample size to explore the functional aspect of pseudo autosomal regions and confirmation of epigenetic regulation during pre and post meiotic events during spermatogenesis.

In summary, our study provides new evidence based linkage study between PARs of X & Y chromosome act as an independent "risk" associated to SNPs of ARSD gene polymorphism. The identification of causative mutations is important for the management of male infertility.

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