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Evidence based distribution of protamine 2 genes polymorphism variants in infertile and fertile males in Southwestern Nigeria.

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Abstract

Protamine 2 is a major core protein of spermatozoa and is important for maintaining male fertility. The aim of the study was to determine the distribution of protamine 2 gene polymorphisms variants in infertile males in Southwestern Part of Nigeria. This is a cross sectional study, consisted of volunteered infertile and fertile male attendees of known Fertility Clinics in Lagos Metropolis, Lagos, Nigeria. The infertile male subjects recruited, were fifty-seven (57) in number and the fertile male subjects (control) were thirty-five in number (35) and were within the age range of 30-59 years old. Semen specimen was collected from each subject in line with WHO guideline for semen analysis into sterile semen containers through masturbation. Sperm DNA from subjects' sperm cells was extracted by chemical method (protein kinase buffer), quantified using nanodrop 1000 Spectrophotometer and amplified using the PRM II F: 5-AGGGCCCTGCTAGTTGTGA-3' PRM II R: 3'- CAGATCTTGTGGGCTTCTCG -3' primers on an ABI 9700 Applied Biosystem thermal cycler at final volume of 25 µL for 35 cycles. Sequencing was done using the BigDye Terminator kit on a 3510 ABI sequencer. The results of genetic testing on the flank; 5'-UTR of the PRM 2 gene showed the following variants with polymorphisms and frequencies as follow: rs2069880951 (A>T /T>A) 6 (6.6%), rs1382451565 (C>T) 5 (5.4%), and rs935520555 (G>C) 5 (5.4%), rs1479789045 (G/C>A/ A>G) 4 (4.3%), rs570570800 (C>T/ G>T) 3 (3.3%), rs1281533806 (C>A) 3 (3.3%), rs1434703461 (C>T/ G>T) 3 (3.3%), rs2069881018 (G>C) 3 (3.3%), rs549835830 (G>C) 2 (2.2%), rs2069880888(C>T) 2 (2.2%), rs997110743 (G>C/C>G), rs1236501997 1 (1.1%), rs119399669 (C>T) 1 (1.1%), and rs1052575569 (G>A/C>A) 1 (1.1%). In this study, there were novel variants of protamine 2 gene polymorphism, identified with reference to the reference sequence of NCBI data base in the infertile and fertile male subjects.

Keywords: Distribution; Infertile; Fertile Male subjects; Protamine 2 gene; SNPs Variants

1. Introduction

Male infertility refers to a sexually mature male's inability to impregnate a fertile female after 12 months of regular unprotected sexual intercourse[1]. Male infertility is commonly due to deficiencies in the semen. In Nigeria, the prevalence of primary infertility is 5% and secondary infertility is 8% [2]. The causes of infertility vary and have been linked to environmental factors, occupational related, genetics and infectious diseases [3]. In about 50% of cases, the cause of male infertility cannot be determined [4].

It was shown that any abnormality in protamines genes, reduction of protamines transcript and protamines deficiency may play a key role in infertility in males [5]. For example, the outcomes may cause an undesirable influence on sperm counts [6].

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Protamines are the major sperm proteins. Protamines and DNA were isolated and discovered from the sperm more than a century ago by Friedrich Miescher [7]. They are the most abundant sperm nuclear proteins in many species and act by packaging the paternal genome [8, 9]. They are proteins with a high content of positively charged amino acids, particularly arginine (48% in human protamines).

Protamine 2 is critical for maintaining male fertility [10]. Studies for the relationship between male infertility and PRM2 polymorphisms are inconclusive [11].

2. Material and methods

2.1. Study area

Study was carried out in Lagos metropolis in Lagos State, Nigeria.

2.2. Ethical approval

Ethical approval was obtained from Health Research Ethics Committee, College of Medicine, University of Lagos, Nigeria. Also, written permission was obtained from various Clinics authorities and the participants were requested to fill informed consent before being recruited for the study.

2.3. Scope of experimental design

This is a cross sectional study involving 92 volunteered males' subjects, recruited after screening.

2.4. Volunteer group

Ninety-two (92) subjects were recruited in the study.

2.5. Control group

This group consisted of thirty-five (35) male fertile subjects within the age range of 30-59 years.

2.6. Experimental group

This group consisted of fifty-seven (57) infertile males within the age range of 30-59 years.

2.7. Inclusion Criteria

According to World Health Organization guidelines, the inclusion was based on the sperm total motility of less than 32% for the infertile male subjects and the control male subjects with total motility greater than 32% [12].

2.8. Exclusion Criteria

Male subjects with any of the following criteria were excluded from the study, varicocele, cryptorchidism, iatrogenic infertility, testis trauma, previous genital infections, and exposure to chemotherapeutics or radiation, Klinifelter's Syndrome, cystic fibrosis, addiction to smoking, alcohol drinking and environmental exposure like driving job, miners, bakers, and workers of chemical plants).

2.9. Specimen collection and processing

Semen specimen was collected from eligible subjects (test subjects and control subjects) into universal sterile plastic containers by masturbation after abstinence period of 72 hours. The seminal plasma specimens were obtained by centrifugation at 4500 rpm for 10 minutes and transferred into a new clean leak free container and stored at -70 ºC prior to analysis.

2.10. Confirmation of subjects' fertility statuses

Computer-assisted sperm analyzer machine was used to confirmed infertile and fertile male subjects.

2.11. Extraction of sperm DNA

Sperms DNA were extracted chemically using protein kinase and purified using Zymo-spin 11C-XL column. The sperm DNA was finally eluted into the new micro centrifuge tube and was stored at -20 °C for downstream reactions.

2.12. Sperm DNA quantification

The extracted genomic DNA was quantified using Nano drop 1000 spectrophotometer, Protamine 2 genes from the semen samples were amplified using PRM2 F;5 AGGGCCCTGCTAGTTGTGA-3' and PRM2 R-3' CAGATCTTGTGGGCTTCTCG -5' primers on a ABI 9700 Applied Biosystem thermal cycler at a final volume of 25 microliters for 35 cycles. The product was resolved on a 1% agarose gel at 130V for 30 minutes and visualized on blue light imagine system for a 900 base pair product size.

2.13. Amplification of protamine 2 genes

Protamine 2 genes from the semen samples were amplified using the PRM II F: 5-AGGGCCCTGCTAGTTGTGA-3' PRM II R: 3'- CAGATCTTGTGGGCTTCTCG -5' primers on an ABI 9700 Applied Biosystems thermal cycler at a final volume of 25 microliters for 35 cycles. The PCR mix included: The X2 Dream tag Master mix supplied by Inqaba, South Africa (tag polymerase, DNTPs, MgCl), the primers at a concentration of 0.4M and 50ng of the extracted DNA as template. The PCR conditions were as follows: Initial denaturation, 95 C for 5 minutes; denaturation, 94 C for 30 seconds; annealing, 61 C for 30 seconds; extension, 72 °C for 30 seconds for 35 cycles and final extension, 72 °C for 5 minutes. The product was resolved on a 1% agarose gel at 130 V for 30 minutes and visualized on blue light imaging system for a 900 bp product size.

2.14. Protamine 2 Gene Sequencing

Sequencing was done using the BigDye Terminator kit on a 3510 ABI sequencer by Inqaba Biotechnological, Pretoria South Africa. The sequencing was done at a final volume of 10 µL. the components included 0.25µL BigDye® terminator v1.1/v3.1, 2.25 µL of 5× BigDye sequencing buffer, 10 µM Primer PCR primer, and 2-10ng PCR template per 100 bp. The sequencing conditions were as follows 32 cycles of 96 °C for 10 seconds, 55 °C for 4 minutes.

3. Results

Table 1 Frequency distribution of protamine 2 gene variants in sperm cells of infertile and fertile subjects

Variant	Locus	SNP	Number	Frequency	% Frequency
rs2069880951	16, 18, 20	A>T /T>A	6	0.066	6.6
rs570570800	28, 40	C>T/ G>T	3	0.033	3.3
rs935520555	36, 38	G>C	6	0.066	6.6
rs1382451565	28, 40	C>T	5	0.054	5.4
rs1281533806		C>A	3	0.033	3.3
rs1479789045	4	G/C>A/ A>G	4	0.043	4.3
rs549835830	6	G>C	2	0.022	2.2
rs997110743	21	G>C/C>G	1	0.011	1.1
rs1434703461	28	C>T/ G>T	3	0.033	3.3
rs2069881018	14, 16,	G>C	3	0.033	3.3
rs1236501997	46		1	0.011	1.1
rs119399669	60, 65	C>T	1	0.011	1.1

Keys: G = Guanosine, C = Cyosine T=thymine, A = Adenine, > = replaces

Genetic testing on the flank region; 5'-UTR of the PRM 2 gene and comparison with NCBCI database revealed variants with SNPs and frequencies as summarized in Table 1.

4. Discussion

All the SNPs of PRM2 genes found in our study subjects, were single nucleotide variant type and are non-coding transcript variant, genic upstream transcript variant, 5 prime UTR variant, intron variant in other words, this variant do not code for protein translation and therefore, not cable of producing its own proteins thus may not have contributed any significant clinical diseases or pathological consequences at the time of study in (2023). The SNPs identified are novel and have never been reported. The variant SNPs are discussed separately as follow:

The variant rs2069881018 SNP occurred because of replacing C with G (G>C) on the loci 16 and 14, on chromosome 16 at the coordinates 11276466 (GRCh38) or 11370323 (GRCh37) with canonical SPDI: NC_000016.10:11276465:C:G of the gene PRM2, LOC105371082. The functional consequence of the gene variant is a non-coding transcript variant, genic upstream transcript variant, 5 prime UTR variant, intron variant. The MAF is validated using MAF G=0./0 (ALFA, indicating 0 out of 10680 individuals) and G=0.000004/1 (TOPMED, indicating 1 in 264690).

The gene variant rs2069880951 SNP, had substitution of A with nucleotide T (A>T) occurring at loci 16, 18, and 20, on the chromosome: 16:11276464 Genome Reference Consortium Human Build 38 Organism for homo Sapiens (GRCh38) or the Genome Reference Consortium Human Build 37 Organism for homo Sapiens 16:11370321 (GRCh37) with Canonical SPDI: NC_000016.10:11276463:T:A of PRM2 gene. The functional consequence of the gene variant is a non coding transcript variant, genic upstream transcript variant, 5 prime UTR variant, intron variant. In other words, this variant do not code for protein translation and therefore, not cable of producing its own proteins thus may not have contributed any significant clinical diseases or pathological consequences. The result further indicate that the minor allele frequency (MAF) of the variant is A=0./0 and A=0.000004/1 (TOPMED) as defined by ALFA and TOPMED respectively.

Furthermore, the rs1382451565 (C>T) variant occurred because of substitution of C with nucleotide T, occurring at loci 28, 38 and 40, located on the chromosome: 16:11276438 Genome Reference Consortium Human Build 38 Organism for homo Sapiens (GRCh38) or the Genome Reference Consortium Human Build 38 Organism for homo Sapiens 16:11370295 (GRCh37) with Canonical SPDI: NC_000016.10:11276437:G:A. The variant is of the gene: PRM 2 (Protamine II), LOC105371082. The functional consequence of the gene variant is a non coding transcript variant, genic upstream transcript variant, 5 prime UTR variant, intron variant. The MAF of A=0./0 indicate occurrence of 0 out of 14050 global sample size while the MAF of 0.000023/6, indicates the occurrence 1 of this variant in 264690 in a global study-wide sample size or sampling while 0.000043/6 (GnomAD) indicates the occurrence 1 of this variant in 140278 subjects.

The variant rs935520555 SNP involves the substitution of G with nucleotide C (C>G). This variant is located on loci 36 and 38 at the chromosome: 16:11276438 Genome Reference Consortium Human Build 38 Organism for homo Sapiens (GRCh38) or the Genome Reference Consortium Human Build 38 Organism for homo Sapiens 16:11370295 (GRCh37) with Canonical SPDI: NC_000016.10:11276437:G:A. The variant is of the gene: PRM 2 (Protamine II), LOC105371082. The functional consequence of the gene variant is a non-coding transcript variant, genic upstream transcript variant, 5 prime UTR variant, intron variant. The MAF of A=0./0 indicate occurrence of 0 out of 14050 global sample size while the MAF of 0.000023/6, indicates the occurrence 6 of this variant in 264690 in a global study-wide sample size or sampling while 0.000043/6 (GnomAD) indicates the occurrence 6 of this variant in 140262 subjects.

The variant rs570570800 SNP (40G>C) or (40T>G) involved the substitution of C or G with nucleotide T on locus 40. This variant is located on the chromosome: 16:11276437Genome Reference Consortium Human Build 38 Organism for homo Sapiens (GRCh38) or the Genome Reference Consortium Human Build 37 Organism for homo Sapiens 16:11370294 (GRCh37) with Canonical SPDI: NC_000016.10:11276436:G:A, NC_000016.10:11276436:G:T. The variant is of the gene: PRM 2 (Protamine II), LOC105371082. The functional consequence of the gene variant is a non-coding transcript variant, genic upstream transcript variant, 5 prime UTR variant, intron variant.

The MAF of A=0.00187/9, indicates the occurrence 12 of this variant in 6404 in a global study-wide sample size or sampling while A=0.00935/2 indicates the occurrence 2 of this variant in 214 Vietnamese subjects.

The variant rs1281533806 (46A>C) involves the substitution of C with nucleotide A on locus 46. Another variant in this category includes rs1236501971. This variant is located on the chromosome: 16:11276428 Genome Reference Consortium Human Build 38 Organism for homo Sapiens (GRCh38) or the Genome Reference Consortium Human Build 37 Organism for homo Sapiens 16:11370285 (GRCh37) with Canonical SPDI: NC_000016.10:11276427:G:T. The functional consequence of the gene variant is a non-coding transcript variant, genic upstream transcript variant, 5 prime UTR variant, intron variant. The MAF of A=0./0 indicate occurrence of 0 out of 14050 global sample size.

The rs1193996669 (60 C>T) and (65C>T) are SNV types of SNP. It is because of C nucleotide been replaced by T nucleotide. The variant on the human chromosome is located on the chromosome 16 on the coordinates of 11276414 (GRCh38) 11370271 (GRCh37). The Canonical SPDI NC_000016.10:11276413:G:A for the gene PRM2, LOC105371082. The functional consequence of the gene variant is a non-coding transcript variant, genic upstream transcript variant, 5 prime UTR variant, intron variant. The MAF of A=0.000111/1 indicate occurrence of 1 out of 8988 global sample size and GnomAD exomes of 0.000004/1, indicating 1 in 242424.

The variant rs1479789045 occurred on the loci 4. The variant is because of the substitution of A for G or A for C. On the human chromosome, it is located on the 16:11276473 of the Genome Reference Consortium Human Build 38 Organism for Homo sapiens (GRCh38) (GRCh38) or 16:11370330 Genome Reference Consortium Human Build 37 Organism for Homo sapiens (GRCh37). The functional consequence of the gene variant is a non-coding transcript variant, genic upstream transcript variant, 5 prime UTR variant, intron variant. As defined by the MAF, the frequency of occurrence of this variant as seen with ALFA is C=0./0, +C=0.000026/7 BY TOPMED, and C=0.000029/4 by GnomAD, indicating 0 in 14050 persons, 7 in 264690 and 4 in 140120 persons are with these variant globally.

The variant rs549835830 occurred on the loci 6. The variant is because of the substitution of G for C. On the human chromosome, it is located on the 16:11276474 (GRCh38) of the Genome Reference Consortium Human Build 38 Organism for Homo sapiens (GRCh38) (GRCh38) or 16:11370331 (GRCh37) Genome Reference Consortium Human Build 37 Organism for Homo sapiens. The functional consequence of the gene variant is a non-coding transcript variant, genic upstream transcript variant, 5 prime UTR variant, intron variant. As defined by the MAF, the frequency of occurrence of this variant as seen with ALFA IS G=0./0, G=0.000026/7 BY TOPMED, and G=0.000029/4 by GnomAD, indicating 0 in 14050 persons, 2 in 264690 and 2 in 140234 persons are with these variant globally. (GRCh37).

The rs997110743 variant occurred because of G been substituted for C or C been substituted for G. It is a SNV type of single Nucleotide polymorphism. It is located on the locus 21. The variant is located on the 16:11276456 (GRCh38) or the 16:11370313 (GRCh37) of the human chromosome. The Canonical SPDI is NC_000016.10:11276455:C:G for the gene PRM2, LOC105371082. The functional consequence of the gene variant is a non-coding transcript variant, genic upstream transcript variant, 5 prime UTR variant, intron variant. As defined by the MAF, the frequency of occurrence of this variant as seen with ALFA is G=0./0, G=0.000019/5 by TOPMED, and G=0.000014/2 by GnomAD, indicating 0 in 14050 persons, 5 in 264690 and 2 in 140256 persons are with these variant globally. Other frequencies include C=0.5/2 and G=0.5/2 indicating 2 out of 4 in SGDP PRJ cases.

The rs1434703461 SNP is because of C being replaced by T or G being replaced by T. The alleles of the variant gene is C>A. The variant gene is located on Chromosome 16, on the coordinates 11276451 (GRCh38) or chromosome 16 of the coordinate 11370308 (GRCh37). The Canonical SPDI: NC_000016.10:11276450:C:A of the gene PRM2, LOC105371082.). The variant is a genic upstream transcript variant, non-coding transcript variant, 5 prime UTR variant, intron variant.

5. Conclusion

In this study, there were novel variants of protamine 2 gene polymorphisms, identified with the reference sequence of NCBI database in the infertile and fertile male subjects.

Recommendations

In addition to the routine semen analysis, genetic analysis of semen proteins should be examined to ascertain causative factors for some idiopathic causes of male infertility factor.

Compliance with ethical standards

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Disclosure of conflict of interest

Authors have declared that no competing interests exist.

Statement of ethical approval

Ethical approval was issued by Health Research Ethics Committee, College of Medicine, and University of Lagos.

Statement of informed consent

Written permission was obtained from various Clinics authorities and the participants were requested to fill informed consent before being recruited for the study.

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