Diagnosis of Idiopathic Primary Infertility in a Consanguineous Infertile Family using Clinical and Molecular Cytogenetic Techniques

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ABSTRACT.

Background: Male infertility is a multifactorial medical problem affecting around 7% of the male population in whom 1% are affected by Non Obstructive Azoospermia (NOA). Despite of advanced diagnostic techniques, majority of the infertility cases remain idiopathic .Therefore more evidence based scientific research is required to investigate the underlying cause of this medical problem. The objective of the current study was to investigate the etiology of idiopathic primary infertility in an extended consanguineous infertile family using clinical and molecular cytogenetic diagnostic techniques.

Material and methods: A comprehensive questionnaire was filled from the available members and blood was sampled from 3 infertile patients and 2 controls brothers along with parents of a consanguineous infertile family. Semen analyses, Reproductive hormones, Multiplex PCR, cytogenetic and molecular analyses were performed. Moreover, testicular biopsy from one patient was collected and subsequently hematoxylin and eosin staining along with meiotic surface spreading was performed on testicular section.

Finally, we did Whole Exome Sequencing (WES) to identify any genetic variants in the idiopathic infertile subjects and fertile controls.
Results: Among the 4 infertile male individuals, testicular histological analysis of one patient (IV:8) revealed spermatocyte arrest. Subsequently, abnormal pachytene was observed and the overall population of normal pachytene was significantly reduced in patient as compared to control. WES approach was used to find out the possible genetic cause of idiopathic primary infertility and we have identified a novel homozygous missense recessive mutation in ZNF621 (exon3:c.82C>G, p.L28V) that was co-segregating with infertility phenotype. Identified mutation was predicted deleterious by various softwares which might be associated with spermatogenic failure and primary infertility.
Discussion: In summary, all the studied subjects had normal 46XY karyotype and no AZF microdeletion was found .We detected a novel genetic variant in an unknown gene ZNF621 which might be associated with disrupted spermatogenesis and infertility. Further research on large scale with recent evidences are required to diagnose the underlying cause of idiopathic primary male infertility and link the unknown role of ZNF621 in spermatogenesis and normal progression of meiosis.
Conclusion: Despite extensive laboratory investigations, millions of infertile men are affected by non-obstructive azoospermia, in whom many of them are diagnosed as idiopathic. A sound proportion of these affected individuals may be investigated by WES at the point of care. While screening this family, we have found a novel missense recessive mutation in an unknown ZNF621 gene by screening an extended consanguineous infertile Pakistani family. Further large scale research is recommended to explore the unknown role of ZNF621 and its association with idiopathic primary infertility.

Keywords: Idiopathic Infertility; WES; Spermatogenesis; ZNF621; Karyotyping; Multiplex PCR

INTRODUCTION

Infertility is a heterogeneous medical problem, affecting around 10 to 15% couples of the reproductive age globally. World health organization defines infertility as the inability of a couple to conceive within 1 year of unprotected intercourse (Organization, 2001). More than 186 million people are suffering from infertility all around the world including in Pakistan (Inhorn and Patrizio, 2015). Subsequently, male factors account for about 50% cases of total infertility in human (Mirfakhraie et al., 2011), (Yousefi-Razin, Nasiri and Omrani, 2016). Male infertility is caused by multiple factors such as cryptorchidism, varicocele, injuries, obstructed semen transport system, infections and malignancies of testes. However, in about 40% infertile male the underlying etiology is still remained elusive or idiopathic (de Kretser, 1997).

Spermatogenesis is a highly intricate and coordinated process. Several cellular events occur in the course of spermatogenesis which include: (1) proliferation of spermatogonial stem cells, (2) pre-meiotic differentiation of undifferentiated spermatogonia to differentiated type A and type B spermatogonia and finally differentiation of type B spermatogonia to pre-leptotene spermatocytes, (3) meiosis, and (4) spermiogenesis and eventually spermiation (Seshagiri, 2001), (Hess and de Franca, 2009), (Cheng and Mruk, 2010), (O'Donnell, Nicholls, O'Bryan, McLachlan, and Stanton, 2011). Proper progression of spermatogenesis is regulated by numerous genes and

proteins of the hypothalamic-pituitary-testicular axis and alteration in genetic factors play a significant role in the impairment of spermatogenesis like oligozoospermia and azoospermia (Toshimori et al., 2004), (Massart, Lissens, Tournaye, and Stouffs, 2012), (Ferlin et al., 2007). However, still the genetic players of spermatogenesis are needed to be discovered. Due to variable phenotypes of infertile males, reliable and more effective molecular cytogenetic diagnostic techniques are required to identify the magnitude of possible genetic mutation in the entire human genome, leading to infertility. Whole Genome Sequencing (WGS) and Whole Exome Sequencing (WES) are efficient approaches used in DNA sequencing studies to identifying novel genetic mutations in various Mendelian disorders (Tenenbaum-Rakover et al., 2015), (Yang et al., 2014).

In the present study, we recruited a consanguineous Pakistani infertile family consisting of 4 infertile brothers suffering from idiopathic primary infertility for the last several years. Testicular biopsy of one infertile Non Obstructive Azoospermic (NOA) patient revealed spermatocytes development arrest. Synaptonemal complex Surface Spreading (SC) indicated higher proportion of leptotene, zygotene and abnormal pachytene. After WES variant filtration, we found a novel homozygous missense recessive mutation in third exon of ZNF621, c.82C>G, p.L32V, predicted to be deleterious by several in-silico tools. Western blotting of testicular tissue showed punctate bands. Subsequently, Sanger sequencing confirmed the detected mutation indicating autosomal recessive mode of inheritance co-segregating in the family. To the best of our knowledge, for the first time we have found a novel variant in ZNF621 gene, which might be associated with infertility in this family.

MATERIALS AND METHODS

Study subjects

A consanguineous Pakistani infertile family comprising of five individuals (four males and one female) were suffering from idiopathic primary infertility was recruited in this study. An informed written consent was obtained from all the participants. Two consecutive phenotype assessment tests like semen analyses, reproductive hormones analyses, karyotyping and multiplex PCR was performed for all patients (IV: 1, IV: 8, IV: 10 and IV: 12).

Study ethical approval

This present study was approved by the Institutional Ethical Review Committee and ASRB (Advanced studies research board) and conducted in the department of Biotechnology of Abdul Wali khan University Mardan, Khyber Pakhtunkhwa, Pakistan and University of Science and Technology of China (USTC) following ethical committee guidelines of Helsinki declaration (Code of ethics of the World Medical Association for performing Human experimentation. Declaration of Helsinki, 1964, 1972) (Rickham, 1964). DNA was extracted from EDTA whole blood using QIAGEN Protease DNA extraction kit from Germany following standard protocol of the manufacturer. The concentration of isolated DNA was confirmed by 1000 nanodrop spectrophotometer from Thermo Fisher Company and the quality of DNA was visualized on 1.5% agarose gel.

Histological and immunohistochemical analysis

Testicular biopsy from one nonobstructive azoospermic (NOA) patient (IV: 8) was performed. Tissues were immediately fixed in Bouin's solution for H&E staining or in 4% paraformaldehyde for immunohistochemistry. The tissues were embedded in paraffin block. Hematoxylin and eosin (H and E) staining was performed as described previously (He, Kokkinaki, Jiang, Dobrinski, and Dym, 2010). Fluorescence microscope (Nikon ECLIPSE 80i, Tokyo, Japan) and Image Pro-Plus version 6.0 software (Media Cybernetics Inc., Bethesda, MD, USA) were used for taking images and analysis of results, respectively (Figure 1).

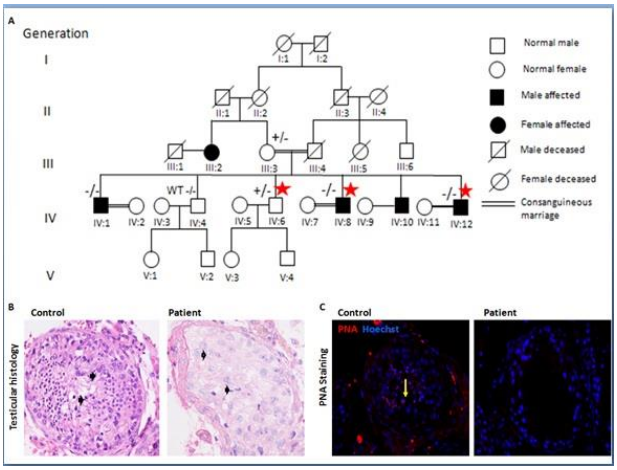

Figure 1. (A) Panel A shows the pedigree of the family. Squares represent males, circles represent females, diamonds indicate offspring and the numerals inside which indicate the number of offspring, the slash symbols denote deceased family members. Solid squares indicate male patients suffering from azoospermia, and the solid circle indicates an infertile female. Parallel slash lines indicate consanguineous marriage (B) Panel B shows representative images of H and E-stained testicular sections from a control man showing normal spermatogenesis, and patient IV:8 whose spermatogenesis arrested at spermatocyte stage (C) PNA staining images of control and patient. PNA staining (Red), showing sperm acrosome reaction, was observed in control (White arrow) while no PNA staining was observed in patient testicular section.

Synaptonemal Complex Surface Spreading (SC) of Meiotic cells

Synaptonemal complex surface spreading analyses of meiotic cells was performed by surface spreading and subsequent immunofluorescence staining as described previously (X. Jiang et al., 2015), (L. Jiang et al., 2017). Human anti-CREST (Immunovision, Springdale, AR, USA), Rabbit anti-SYCP3 (Abcam, Cambridge, UK), mouse anti-MLH1 (BD Pharmingen Biosciences, San Diego, CA), (C14 or S6os1, made in our laboratory in guinea pig against human C14) were used as primary antibodies. Alexa 555 donkey anti-rabbit (Molecular Probes, Carlsbad, CA, USA), Alexa 488 goat anti-mouse (Molecular Probes), Alexa 488 donkey antimouse (Molecular Probes) and 1-amino-4-methylcoumarin- 3-acetic acid (AMCA) donkey anti-human (Jackson Immunoresearch, West Grove, PA, USA) were used as secondary antibodies for immunostaining (Li et al., 2019). Images were captured by fluorescence microscope (Nikon ECLIPSE 80i, Tokyo, Japan).

Immunofluorescence of paraffin-embedded testicular sections

Tissue sections of the patients were visualized by immunofluorescent staining with slight modification as described previously by (L. Jiang et al., 2017), and then analyzed accordingly. Briefly, paraffin embedded tissues were first deparaffinized, followed by rehydration and direct staining step, which involved application of specific antibodies for sperm cell acrosome (Moraveji et al., 2019). After permeabilization, slides were incubated at 4˚C overnight with primary antibody Lectin PNA from Arachis hypogaea (peanut), Alex Fluor® 568 Conjugate (Invitrogen.L32458). Next day after washing with TBST, secondary antibody and Hoechst (H 33342) (Invitrogen 100M) was applied to the slides and kept for 1 hour at 37°c in a humidified box. Finally, the slides were mounted with VECTASHIELD (H-1000, Vector Laboratories). A fluorescence Olympus microscope (Nikon ECLIPSE 80i, Tokyo, Japan) connected to a CCD camera was used for capturing images and analysis was done using the Image-Pro Plus software (Media Cybernetic) (Esteves, Sharma, Thomas Jr, and Agarwal, 2007) (Figure 1C).

Whole exome sequencing and analyses of data

Genomic DNA of three individuals from the family was used for WES. Genes were captured and libraries prepared by using SureSelect Human All Exon V6 kit (Agilent), due to its sensitivity and efficiency of covering most of the coding refSeq genes (Chen, Im, and Snyder, 2015). HiSeq. 2000 sequencing platform (Illumina) was used for DNA sequencing and cluster generation. This was done by fragmentation of 50ng of genomic DNA by using QXT enzymatic procedure following adaptor tagging (Agilent). Purification of DNA fragments were done

with magnetic beads and with whole exome oligonucleotides, the target regions was captured. Subsequently, the enriched libraries were amplified and quantified by using Qubit fluorometer and library size distribution measured by Bioanalyzer (Agilent). A V4 high-output flow cell (Illumina) was used for paired end sequencing of the prepared libraries. The maximum read sequencing read length was 125 bp, while capacity of read depth was more than 150 x in each targeted time. A read quality analysis of raw data was done by FastQC software (Babraham Bioinformatics). Alignment of FASTQ files to human reference genome (hg19, February 2009, build 37.5) was achieved with Novoalign V2.07.13 from the Novocraft Short Read Alignment package (http://www.novocraft.com/index.html). The output format was put to SAM and default setting was done for all choices. The SAM tools (http:/samtools.sourcforge.net/) was used to convert each SAM file to every sample to a BAM file format, then reformed and merged together. Duplication in PCR results were removed by using Picard software (http:/picard.sourceforge.net/). Subsequently, Genome analysis tool kit from the Broad Institute (http:/www.broadinstitute.org.gatk/) was used to further processing of these files. Realignment of all BAM files was done using indel realigner. Single nucleotide variants (SNVs) and small INDELs (insertion and deletions) within the captured coding sequencing intervals were called using genome analysis tool kit (GATK, s Unified Genotyper).

Genes annotation and filtration

The variants filtration and annotation was done with ANNOVAR (Wang, Li, and Hakonarson, 2010), which is based on Ensemble (http://www .ensemble. org). Those variants were selected which were within exon or exon-intron boundaries. Those variants were excluded having MAF>0.05 and found in databases: NCBI (1000 genomes browser) (1000 Genomes Project Consortium, 2015), ESP6500 (http://evs.gs.washington.edu/ EVS/), HGMD (Qiagen) and ClinVar (NCBI). Subsequently, all those variants were eliminated that were found homozygous in our in house established data comprises of 578 fertile male individuals (41 Pakistanis, 254 Chinese, and 283 Europeans). AceView (NCBI), GTEx Portal (Broad Institute) and UniGene (NCBI) databases were used for tissue gene expression evaluation. Variant conservation was tested with PhyloP (Cornell University) and Clustal Omega (EMBL-EBI). Protein change predictions were done by PolyPhen-2 HDIV, PolyPhen-2 Hvar (Harvard University), SIFT (J. Craig Venter Institute) and Mutation Taster (Charité). Based on our data base, Spermato genes is Online 1.0, (Zhang et al., 2012), and published literature, genes harboring variants were functionally annotated .Those genes with variants that are expressed at messenger RNA (mRNA) level (Fagerberg et al., 2014) with possible testicular protein detection were reserved. Variants found to be nondamaging by 50% of the above software were omitted (Tables 1 and 2).

Table 1. Physical and cytological analysis of patients and controls.

Clinical Parameters	Ref.	IV:6	IV:4	IV:1	IV:10	IV:8	IV:12	III:2
Age (years)[a]	-	36	47	41	32	55	58	71
Height/Weight (cm/kg)	-	175/75	170/64	172/60	175/63	167/50	147/45	ND
Karyotype	-	46,XY	46,XY	46,XY	46,XY	46,XY	46,XY	ND
Any other disease	-	No	No	No	No	No	No	No
Semen analysis [b]								
Semen volume (ml)	>1.5 ml	3.4±0.35	4.4±0.45	2.8 ± 0.30	2.2±0.55	2.6±0.55	2.1±0.55	-
Sperm count	>15	61	84	03	0	0	0.2	-

(millions/ml)								
Hormone analysis[c]								
Testosterone (nmol/L)	8.33-30.19	11.8	22.4	13.5	19.3	22.8	10.7	ND
FSH (mIU/ml)	M:1.4-15.4 F:19.4-144	7.93	9.68	11.37	9.46	14.82	6.59	ND
LH (mIU/ml)	M:1.2-7.8 F:19.4-144	6.45	7.76	10.45	10.34	9.86	11.20	ND
Prolactin (ng/ml)	M:3-14.7 F:19.4-144	8.74	10.63	6.91	8.83	11.37	12.89	ND
Estradiol (pg/ml)	M:0.27-0.9 F:19.4-144	ND	0.41	0.73	0.31	0.27	0.63	ND
Progesterone (ng/ml)	M:0.4-4.2 F:ND-1.13	ND	1.98	0.30	0.47	1.29	1.13	ND
AMH(ng/ml)	F:1.5-4.0	ND	ND	ND	1.76	2.10	ND	ND
Physical Examination[d]								
Testis Size (ml)	>12.5	ND	ND	R:9.38 L:11.85	R:6.81 L:7.13	-	-	-
Ovaries	-	-	-		-	ND	ND	ND
Uterus(cm*cm)		-	-	-	-	ND	ND	ND
Age of Puberty(Y)	15-18	17	16	17	16	17	16	15

Table 2. Softwares used to predict mutation effect.

Chr	DNA change	Protein change	Mutation	Clin Var	Polyphen2(HDIV)	SIFT	Polyphen2(Hvar)
3p22.1	c.82 C>G	L28V	Missense	Pathogenic	Damaging	Damaging	Damaging

Clin Var, Polyphen 2 HDIV, SIFT and Polyphen 2 Hvar predicted Identified muatation as pathogenic and damaging.

Sanger sequencing

Sanger sequencing was used to detect the remaining variants (Figure 2) in all of the available members of the family.

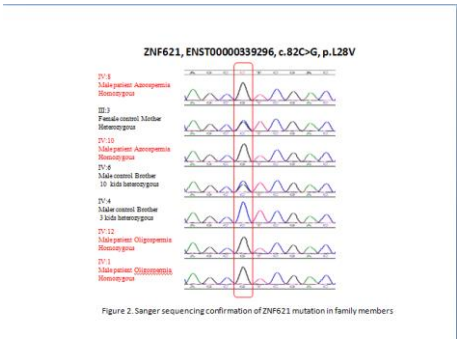

Figure 2. (A) Panel A shows the progression of meiotic prophase I detected by immunostaining for SYCP3 and SIX6OS1 on human spermatocytes. Images show leptotene, zygotene, and pachytene spermatocytes from a control man and leptotene, zygotene and abnormal pachytene spermatocytes from patient IV:3 stained for SYCP3 (red) and SIX6OS1 (green). (B) The graph showing the various population of meiotic cells in control and Patient (IV:8).

Western blotting

Western blotting of prepared testicular tissues cells was carried out according to previously described protocol (X. Jiang et al., 2015) and primary antibodies against ZNF621 (ZNF621 Antibody (NBP2-56492): Novus Biologicals) and glyceraldehyde-3-phosphate dehydrogenase. Results obtained indicated detection of proteins bands in patient's testicular tissues compared with normal control and revealed expression of testicular proteins expression. Images were taken on Image Quant LAS 4000 and quantification of bands was done using the Gel-Pro Analyzer 4.0 computer program (Media Cybernetics, Silver Spring, MD).

RESULTS

A Family history and clinical phenotypes

In our recruited family, five individuals (four males and one female) were suffering from idiopathic primary infertility (Figure 1A). All the four infertile male subjects had normal karyotype with 46, XY chromosome (Table 1), and none of them had any history of associated diseases. All of them were not addicted to smoking and alcohol drinking. Two consecutive semen analyses reports of the infertile subjects indicated non obstructive azoospermia in two patients (IV: 8 & IV: 10) and severe oligozoospermia in other two patients (IV: 1 & IV: 12). Of note, all patients had normal semen volume (>2 ml). Reproductive hormones, LH, FSH, prolactin, testosterone, estradiol and AMH, analyses revealed normal levels in all patients (Table 1). Furthermore, no Y chromosome microdeletion was detected in the studied family members. Detailed summary of primers used for Yq microdeletion is given in (Table 1). Patient's scrotal ultrasound revealed normal testicular volume and texture.

Hematoxylin and Eosin staining of testicular tissue and analysis

Testicular biopsy from one azoospermic patient (IV: 8) was processed for fixation in Bouin's solution overnight at room temperature, and then followed by embedding in paraffin wax, microtomy and H&E (hematoxylin and eosin) staining of 5 µm thin sections and finally microscopic examination. Histological examination revealed profoundly disrupted spermatogenesis process due to sperm maturation arrest at meiotic prophase 1 in patient than control showing all stages of sperm cell maturation including spermatogonia, spermatocytes and spermatids, as well as mature sperm (Figure 1B).

Spermatocyte Synaptonemal Complex Spreading (SC) of meiotic cells and immunostaining

Subsequently, spermatocyte surface spreading was performed to investigate the progression of meiosis prophase I. We observed higher number of abnormal pachytene cells in patient (IV:8) as compared to control (Figure 2A). Consistently, the number of pachytene cells was significantly lower in patient than control (Figure 2 B). Further indicating that progression of meiosis was disrupted at pachytene stage.

Whole exome sequencing and analyses of data

WES (whole exome sequencing) was performed in patients (IV: 6, IV: 8 and IV: 12), to identify the genetic etiology of idiopathic primary infertility in this family. The analysis of WES data is given in Supplementary Figure S1. Briefly, after WES, all those variants were excluded that show higher genomic frequency MAF >0.05 in human genetic variation databases (1000 Genomes, ESP6500, or ExAC) or were homozygous in our 578 in-house fertile male controls, leaving in 905 variants in 594 genes. After eliminating the variants in genes considered to be nonfunctional in spermatogenesis based on Spermatogenesis Online 1.0 (Zhang Y, et al., 2013) and literature survey, variants in genes that are detected at the mRNA level (≥ 1 FPKM in testes and >10 FPKM in at least one tissue) (Fagerberg et al., 2014) or at the protein level in testes (Uhlén et al., 2015) were reserved. Variants within exons or exon–intron boundaries were kept and other excluded, reducing the number to 17 variants in 14 genes, followed by selecting variants within genes expressed in testis. Variants within genes predicted to be non-deleterious by >50% software covering them were omitted, reducing the number to 7 variants in 7 genes. Sanger sequencing for these 7 variants was performed in all the available family members. After Sanger sequencing, we identified 1 variant with novel homozygous missense recessive mutation, c.82C>G, p.L32V, in the third exon of an unknown gene ZNF621 which is predicted to be deleterious by several in-silico tools, recessively co-inheriting with idiopathic infertility in this family (Table 2) Thus, we considered that this Pathogenic Variant (PV) in ZNF621 was the most plausible candidate associated to idiopathic infertility in this family. Based on these results, it is suggested that the identified PV in infertile patients may be associated with unexplained infertility in this family.

Confirmation of mutation by Sanger sequencing

A novel homozygous single base substitution of c.82C>G causes replacement of leucine with Valine (p.L32V) in the third exon of ZNF621, predicted to be highly damaging was shared by patients in WES data. This variant was not observed in any variation database. To confirm inheritance pattern of identified mutation in all available family members, Sanger sequencing was performed on amplified PCR products. Subsequently, Sanger sequencing result analysis confirmed the segregation of mutation with infertility phenotype as four patients were homozygous recessive while mother was heterozygous and their normal siblings were either heterozygous or wild type (Figure 3).

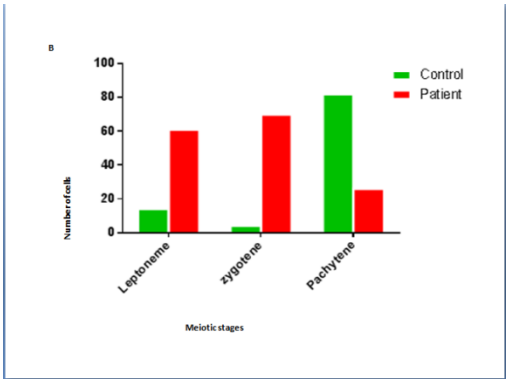

Figure 3. Sanger sequencing chromatogram showing results of two controls (IV:6 and IV:4) and their mother (III:3) while four patients (IV:8, IV:10, IV:12 and IV:1). Controls and mother were heterozygous while all patients were homozygous for ZNF621 mutation.

Western blotting

In order to check its specific expression in testes, Western blotting was carried out on patient testes lysate as described previously (X. Jiang et al., 2015). Primary antibody against ZNF621 (ZNF621 Antibody (NBP2-56492): Novus Biologicals) and B-actin (served as control) were used. Images were captured on Image Quant LAS

4000 and quantification of bands was done using the Gel-Pro Analyzer 4.0 computer program (Media Cybernetics, Silver Spring, MD). The representative image showed the presence of 50 KDa band on membrane in control and patient testes lysate (Figure 2). These results confirmed the testis specific expression of ZNF621 that validated PV in the family.

DISCUSSION

Infertility is clinically and genetically a very heterogeneous medical problem affecting both genders equally in human. Male patients suffering from primary infertility usually exhibit phenotype of azoospermia as well as severe oligozoospermia associated with impaired spermatogenesis. Nevertheless, the underlying cytogenetic and molecular genetics basis of idiopathic male infertility is still less understood. It is important to note that genetically unexplained infertility often vary between different ethnic groups, therefore, the current case-control study is designed to diagnose idiopathic primary infertility in a consanguineous infertile family through clinical and molecular cytogenetic techniques. This is the first ever study conducted in Pakistan on such an extended infertile consanguineous family with total 10 siblings (six brothers and four sisters) in whom five individuals (four brothers and one female, aunt) were suffering from idiopathic primary infertility. Physical and clinical features of all the four infertile brothers confirmed azoospermia as well as severe oligozoospermia as revealed by semen analyses. Of note, reproductive hormone profiles, testicular volume and texture were normal in infertile patients. Additionally, karyotype analyses of these individuals' revealed normal 46, XY chromosomes. Moreover, no Y chromosome microdeletion was detected in multiplex PCR results. Furthermore, all of them had no obvious associated diseases. Therefore, these findings strongly suggest that the identified homozygous missense mutation in ZNF621 in four infertile brothers may be associated with spermatogenic defect that is transmitted through autosomal recessive mood of inheritance.

ZNF621 (zinc finger protein 621) belongs to zinc finger protein family is a gene located at chromosome position 3p22.1 in humans, having 5 exons, encodes a 439 amino acid protein. ZNF621 is involved in regulating the formation of essential proteins required for the process of spermatogenesis and detected genetic mutation may be associated with defects in spermatogenesis (Yatsenko et al., 2015). Here, we observed spermatogenesis defects in ZNF621 mutation carriers ranging from severe oligospermia to azoospermia confirmed from the testicular biopsy of one azoospermic infertile patient with variation in ZNF621. To the best of our knowledge, this is first study on an unknown ZNF621 gene mutation in patients with azoospermia and severe oligospermia. Of note, due to absence of mouse homolog for this gene, and lack of sound database, we could not carry out functional expression and mechanistic studies in mouse model. Synaptonemal Complex (SC) spreading analysis revealed an abnormal distribution of spermatocytes at zygotene and pachytene stages during crossing over of homologous chromosomes in meiotic prophase I. These findings further suggest that chromosomal recombination during meiosis looks normal although there are some abnormal number of pachytene and zygotene distribution, the underlying problem of infertility in this patient could be sperm maturation arrest in post meiotic stages.

In case of spermatogenic Maturation Arrest (MA) testicular histology of the patients indicates incomplete spermatogenesis in seminiferous tubular. Non Obstructive Azoospermic (NOA) individuals with MA are more affected by genetic defects which alter normal spermatogenesis and in most cases no identifiable cause has been documented (Hung, King, and Schlegel, 2007). Identification of genetic etiology of NOA with MA is a challenging task because of multiple factors, lack of experimental animals harboring azoospermia with MA, heterogeneous nature of infertility and paucity of human testicular tissues for experimental research work (Okada et al., 2008).

CONCLUSION

In conclusion, while working on this family, we discovered missense recessive mutation in a novel ZNF621 gene. Furthermore, we screened an extended consanguineous fertile Pakistani family and confirmed that homozygous mutation in an infertile male could lead to NOA and post-meiotic sperm maturation arrest. Further evidence based research and cell line experiments on ZNF621 gene with large sample size from diverse ethnicities are required to identify and investigate the pathological role of this genetic mutation in male infertility. Such investigations may include screening of large numbers of families with idiopathic infertility and the generation of KO mouse models using knock-in alleles that mimic the missense mutation found in patients with NOA and sever oligozoospermia.

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