Novel Variants in *UBE2B* Gene and Idiopathic Male Infertility

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ABSTRACT: The *UBE2B* gene encodes ubiquitin-conjugating enzyme, which is involved in DNA repair. *Ube2b* knockout mice were found to be infertile because of structural abnormality of sperm. However, there is no genetic study on the role of the *UBE2B* gene in human fertility; therefore, the present investigation was designed to study genetic variations in the *UBE2B* gene and its role in human male infertility. Sequence analyses of the *UBE2B* gene in 530 infertile (350 azoospermic, 105 oligoasthenoteratozoospermic, and 75 oligoasthenozoospermic) and 300 fertile control men revealed the presence of 5 substitution single-nucleotide polymorphisms (SNPs) in 221 individuals (199 infertile [37.5%] and 22 fertile [7.3%] men). Of these, 2 (g.5197:T>G; g.9157:A>G) of the 5 substitutions were novel and observed only in infertile men. Distribution of haplotypes TA, TG, GA, and GG are not uniform between the patient and the control group of this study. Interestingly, our study suggests that the haplotype TG conferred significantly increased risk for male infertility (odds ratio = 5.07, 95% CI = 1.29–23.29, p = .007). *In silico* analysis of SNPs that were specific to infertile men predicted that these SNPs lead to defective splicing by destroying or creating the potential binding site of splicing factors or causing alteration in predicted regulatory sequences. In the light of the above, our study suggests that the *UBE2B* gene is associated with male infertility in Indian men, hence, providing evidence for additional genetic factors for male infertility.

Key words: Haplotype, SNPs, ubiquitin, ubiquitin-conjugating enzyme.

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In humans, about 15% of couples are infertile for various reasons, of which male factors account for 50% of the cases. In approximately 30%–50% of all cases of azoospermia or severe oligozoospermia, etiology is idiopathic (Krausz and Forti, 2000). Y chromosome microdeletions in *AZF* (azoospermic factor) regions were found to be the major genetic cause (10%) of male infertility (Shah et al, 2003; Thangaraj et al, 2003a). Deletion of *DAZ* gene copy(ies) and mutation in an autosomal gene *DAZL* have also been reported in association with male infertility (Reynolds and Cooke, 2005; Yang et al, 2005). In addition, mutations in the mitochondrial DNA have also been found to be associated with impaired sperm motility (Thangaraj et al, 2003b; Rani et al, 2006). However, the underlying genetic cause for the large proportion of infertile men remains largely unknown.

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Ubiquitin (UB) is a small and highly conserved protein, present apparently in all eukaryotic cells. It is covalently attached to abnormal and short-lived proteins that are destined for ATP-dependent proteolysis in eukaryotic cells. UB is first activated by the ubiquitin-activating enzyme E1. Ubiquitin-conjugating enzyme E2 (ubiquitin carrier protein) transfers the activated ubiquitin to the substrate bound to E3, a member of the ubiquitin ligase family (Ciechanover, 1996). This ubiquitin pathway, by which proteins are tagged for degradation and ubiquitin-mediated proteolysis, plays a major role in a broad array of basic cellular processes (Tongaonkar et al, 2000). Among these are regulation of the cell cycle, differentiation and development, biogenesis of organelles, DNA repair, modulation of cell surface receptors, ion channels, signaling pathways, stress responses, and removal of damaged or misfolded proteins (Hochstrasser, 2001). Several investigations have clearly indicated the correlation between the UB pathway of protein degradation and spermatogenesis (Roest et al, 1996; Bebington et al, 2001).

Ube2b knockout mice were found to be infertile because of abnormal sperm morphology and immotility (Roest et al, 1996). Derailment of spermatogenesis became overt during the postmeiotic condensation of

Table 1. *Geographical origin and ethnic/linguistic affiliation of azoospermic, oligoasthenoteratozoospermic, oligoasthenozoospermic, and normozoospermic individuals*

Geographical Origin of Samples	Ethnic/Linguistic Affiliation	Infertile Men				Normozoospermic Controls
		Azoo	OAT	OA	Total	
West Bengal	Indo-European/Tibeto-Burman	112	15	13	140	110
Bihar	Indo-European	40	2	4	46	35
Madhya Pradesh	Indo-European	35	7	6	48	25
Assam	Tibeto-Burman	38	0	6	44	20
Bangladesh	Indo-European	29	15	18	62	21
Uttar Pradesh	Indo-European	30	13	6	49	18
Orissa	Indo-European	25	8	2	35	23
Andhra Pradesh	Dravidian	21	41	15	77	23
Chhattisgarh	Austro-Asiatic	20	4	5	29	25
Total		350	105	75	530	300

Abbreviations: Azoo, azoospermic; OAT, oligoasthenoteratozoospermic; OA, oligoasthenozoospermic.

chromatin in spermatids. Roest et al (1996) stated that the findings in the knockout mice provided a parallel between yeast sporulation and mammalian spermatogenesis and strongly implicated UBE2B-dependent ubiquitination in chromatin remodeling in the human.

In another study, Baarends et al (2003) determined that the primary spermatocytes of *Ube2b* knockout mice underwent increased apoptosis during meiotic prophase. In the absence of UBE2B, the structure and telomere localization of synaptonemal complexes were altered within the nuclei of pachytene and diplotene spermatocytes. The number of foci containing the mismatch DNA repair protein MLH1 was increased and reflected a consistent 20%–25% increase in crossover frequency in mutant spermatocytes. The authors concluded that the UB-conjugating activity of UBE2B is required within the synaptonemal complex and for meiotic recombination in spermatocytes. Thus, it is now evident that *Ube2b* knockout mice are infertile because of abnormal sperm morphology and motility. Therefore, we undertook this study to assess the role of the *UBE2B* gene in infertile Indian men because there has been no report on this gene before this study.

Materials and Methods

Samples

We have analyzed 530 infertile men from the Indian subcontinent (Table 1), attending the infertility clinic at the Institute of Reproductive Medicine (IRM), Kolkata, India, and Infertility Institute and Research Centre (IIRC), Hyderabad, India. A team consisting of urologists and andrologists performed a detailed clinical investigation of all the patients, and recorded the complete case history of each individual. All the infertile men were also subjected to karyotyping and endocrinological assays (follicle-stimulating hormone, luteinizing hormone, testosterone, prolactin, and thyroid-stimulating hormone). Testicular biopsy and further histological analysis was done for azoospermic men wherever possible.

The cases were classified as having no sperm in the ejaculate (ie, azoospermia, n = 350; and low sperm count [oligozoospermia, sperm count < 20 million/mL], n = 180). Of the latter category, 75 patients presented with <50% motile sperm (oligoasthenozoospermic) and 105 individuals with low motility and abnormal sperm shape and size (<30% sperm with normal morphology), known as oligoasthenoteratozoospermia. Microdeletion analysis of the Y chromosome was carried out for all the cases (Thangaraj et al, 2003a, unpublished data). Patients who did not exhibit obstruction, endocrinological defect, pelvic injury, major illness, karyotype abnormality, or Y chromosome microdeletion were included in the study. The age of infertile men ranged between 26 and 42 years. Three hundred randomly selected and ethnically matched fertile men (sperm count > 20 × 10^6/mL) were included in this study as controls (Table 1). All the 300 control men had fathered at least one child each, and their fertility was proven by short tandem repeat (STR)–based (Profiler plus; Applied Biosystems, Foster City, California) DNA fingerprinting. Blood samples (5 mL) from each infertile and fertile control man were collected with their informed written consent. Genomic DNA was isolated using the protocol described in our earlier study (Thangaraj et al, 2002). This study was approved by The Institutional Ethical Committee.

Analysis of the UBE2B Gene

The DNA sequence of the *UBE2B* gene (accession number ENSG00000119048) was downloaded from the Ensembl database (www.ensembl.org), and 6 pairs of primers covering the entire coding region and the exon-intron boundaries and 2 internal primers (forward) for sequencing were designed with GeneTool software (Table 2). The primers were synthesized with the use of an ABI392 oligosynthesizer (Applied Biosystems, Foster City, California). PCRs were performed according to the following amplification conditions: 94°C for 7 minutes; 35 cycles of denaturation at 94°C for 45 seconds, annealing at 60.5°C or 65.5°C (Table 2) for 30 seconds, extension at 72°C for 1 minute, and a final extension at 72°C

Table 2. *Primers (sequences) used for amplification and sequencing of the UBE2B gene*

Primer	Primer Sequence	Annealing Temperature, °C	Product Size, bp
UBE-1F	ACTCAGGGGTGGATTGTGA	65.5	636
UBE-1R	CACAAAGGGGAAGGTGTCTG		
UBE-2F	TGTCTGTAGGTGTTTACGTGGC	65.5	347
UBE-2R	GTGCTGGGCCAAAACTCTAA		
UBE-3F	GAAAATCCAGGGCTAAGGTG	65.5	356
UBE-3R	AAGCTGCTGTGGGAAGAAGT		
UBE-4F	CCGAACCAACGTTGACATACT	65.5	340
UBE-4R	CACATGGACATGGAAGCAAC		
UBE-5F	GGGAACATTCAAACCATAGCA	65.5	438
UBE-5R	TGCAGACTGGATCCCAAGTA		
UBE-6F	AATATATTCCATATCTGACCCCTG	60.5	2031
UBE-6R	AGAGGGCAGACCCTCATAGC		
UBE-6F1	GATTTTTAAATTGGAGAAAAGCA	60.5	Internal sequencing primers
UBE-6F2	AGGCACATATTGGAGGGAAA		

for 7 minutes (Table 1). PCR products were checked on 2% agarose gel, stained with ethidium bromide, and visualized under an ultraviolet transilluminator. The amplicons were further subjected to direct sequencing (Thangaraj et al, 2002) with a BigDye Terminator cycle sequencing kit (version 3.1, Applied Biosystems) and 3730 DNA analyzer (Applied Biosystems).

Statistical Analysis

PEPI (Programs for Epidemiologic Analysis) CASECONT (Abramson and Gahlinger, 2001) was used to perform statistical analysis to determine whether any single-nucleotide polymorphism (SNP) identified in this study is associated with male infertility. The odds ratio (OR) and 95% confidence interval (CI) were calculated to measure the strength of association between genotype frequencies and infertility. P values $<.05$ were considered statistically significant.

Haplotype Analysis

Haplotypes were inferred from unphased genotypes with the program PHASE version 2.1 (Stephens et al, 2001; Stephens and Scheet, 2005), which was run with default settings (100 iterations, with 100 burn-in, and a thinning interval of 1). A case control association analysis was performed by PHASE v2.1 to test the hypothesis that the haplotypes were randomly distributed between the infertile and fertile men. χ^2 tests were used to test individual haplotypes for their over- or underrepresentation in infertile vs fertile men.

In Silico Analysis

To evaluate whether SNPs present exclusively in infertile men and are the potential cause for the defect in recognition/binding sites for the proteins responsible for proper splicing, we have analyzed those sites with ESEfinder, release 3.0 (http://rulai.cshl.edu/cgi-bin/tools/ESE3/esefinder.cgi), which evaluates the presence of exonic splicing enhancer (ESE) at the mutation sites. The ASD Workbench wrapper (http://www.ebi.ac.uk/asd-srv/wb.cgi) tools PPT analysis, BP analysis, Splicing Rainbow, and Regulatory Sequences were used to identify the presence of polypyrimidine tracks, branch point sites, binding sites for splicing factors, and exonic splicing enhancers/silencers (ESE/ESS) or intronic splicing enhancers/silencers (ISE/ISS), respectively, at the mutation sites. ESEfinder 3.0 searches the ESE motifs corresponding to 4 serine/arginine-rich (SR) proteins, wherein a Splicing Rainbow tool searches for the SR proteins as well as hnRNP motifs.

Results

Genetic Analysis of the UBE2B Gene

Sequence analyses of the *UBE2B* gene revealed 5 SNPs (Table 3). Of these, g.5197:T>G in intron 3 and g.9157:A>G in exon 4 were found to be novel. The other changes observed were g.-293:T>G in the 5′ untranslated region (UTR); g.19765:T>G and g.20016:A>G in 3′ UTR were reported in the HapMap database (International HapMap Project, publication date unknown). A total of 14 infertile men were found to have the novel g.5197:T>G transition (panel B in the Figure; Table 3), of which 10 (2.86%) were azoospermic; the remaining 4 (3.8%) were oligoasthenoteratozoospermic. Another novel change g.9157:A>G (panel C in the Figure; Table 3) was found in 5 azoospermic men, but not in other categories of infertile men. This transition does not cause any amino acid change; however, its role in infertility cannot be ruled out because it was not found in any of the noromzoospermic individuals. Another substitution, g.19765:T>G (rs11538104), was observed in the 3′ UTR, of 6 azoospermic individuals (1.71%). Interestingly, none of the normozoospermic individuals were polymorphic for any of these 3 sites (Table 3).

The transversion g.-293:T>G (rs17167484) was observed in 37 infertile (6.2%) and 6 fertile men (2%) (panel A in the Figure; Table 3). The difference in the frequency distribution of this SNP between infertile and fertile men was statistically significant ($\chi^2 = 9.676$, $P = .002$; OR =

Table 3. *Mutations observed in the UBE2B gene of infertile and fertile men and their frequencies (%)*[a]

No.	Mutation Site	Location	Azoo (n = 350)	OA (n = 75)	OAT (n = 105)	Controls (n = 300)	χ^2 (*P* value)	Odds Ratio (95% CI)
1	g.-293:T>G	5′ UTR	26 (7.4)	2 (2.7)	9 (8.57)	6 (2.0)	9.676 ($P = .002$)[b]	3.68 (1.46–9.81)
	T/T		324	73	96	294		
	T/G		7	0	9	3		
	G/G		19	2	0	3		
2	g.5197:T>G	Intron 3	10 (2.86)	0	4 (3.8)	...	...	...
	T/T		340	75	101	300		
	T/G		5	0	3	0		
	G/G		5	0	1	0		
3	g.9157:A>G	Exon 4	5 (1.43)	...	...	...	...	...
	A/A		345	75	105	300		
	A/G		2	0	0	0		
	G/G		3	0	0	0		
4	g.19765:T>G	3′ UTR	6 (1.71)	...	...	...	...	...
	T/T		344	75	105	300		
	T/G		6	0	0	0		
	G/G		0	0	0	0		
5	g.20016:A>G	3′ UTR	96 (27.4)	7 (9.3)	34 (32.4)	16 (5.3)	53.625 ($P < .001$)	6.19 (3.52–11.04)
	A/A		254	68	71	284		
	A/G		43	3	30	11		
	G/G		53	4	4	5		

Abbreviations: Azoo, azoospermic; CI, confidence interval; OAT, oligoasthenoteratozoospermic; OA, oligoasthenozoospermic.

[a] Genotype frequencies were compared between infertile men and control subjects.

[b] Significant at $P < .05$.

3.68, CI = 1.46–9.81). Another substitution, g.20016:A>G (rs3777373) was observed in 137 infertile (23%) and 16 (5.3%) fertile men (panel D in the Figure; Table 3), with a statistically significant difference between the 2 groups ($\chi^2 = 53.625$, $P < .001$; OR = 6.19, CI = 3.52–11.04).

Haplotype Analysis

Analyses of the case and control genotypes with the use of the g.-293:T>G and g.20016:A>G sites (PHASE v2.1) revealed 4 haplotypes. Distribution of haplotypes was not uniform between infertile and fertile men. Furthermore, in fertile men frequency of the TA haplotype was high (95.83%) when compared with infertile men (82.24%), suggesting that this haplotype is advantageous for fertility. Our results suggest that haplotype TG conferred significantly increased risk for male infertility (OR = 5.07, 95% CI = 1.29–23.29, $P = .007$) if the main haplotype TA was used as the baseline genotype (Table 4).

In Silico Analysis

To evaluate the functional significance of the SNPs, which were exclusively present in infertile men, these intronic and exonic sites, along with their flanking sequence, were analyzed by various bioinformatics tools. Analysis of g.5197:T>G with the Splicing Rainbow tool predicted a binding site for SRp55 in both normal and mutated sequences, but the potential binding site for SC35, which was present in the normal sequence, was absent in the mutated sequence (Table 5). This analysis also predicted the binding sites for SRp40 and hnRNP U in the mutated sequence, but not in the normal sequence. A similar analysis performed for the exon 4 SNP g.9157:A>G showed a potential binding site for the SPp40, SC35, and ASF/SF2 in both normal and mutated sequences, but the binding site for Tra2Beta was predicted in normal, but not the mutant, sequence. We also analyzed intronic SNPs with the PPT and BP analysis tools, but none of the SNP were present in the branching point site or caused any change in the predicted polypyrimidine track.

Discussion

Although several causes of male infertility have been identified, the majority remain idiopathic. Impaired spermatogenesis can only be explained in a very small portion of infertile patients, which is not surprising in that male infertility is thought to be a complex disorder in which multiple genes are involved, as suggested by gene targeting studies in *Drosophila* and mice (Grootegoed et al, 1998; Hackstein et al, 2000; Venables and Cook, 2000). In this study, we have sequenced all the exons, including the intron-exon boundaries of the *UBE2B* gene in infertile and fertile men belonging to Indian subcontinent (Table 1), to find the SNP(s) that could be responsible for the abnormal semen profile. The samples we used were from different geographical regions of India, belonging to 4 major linguistic groups

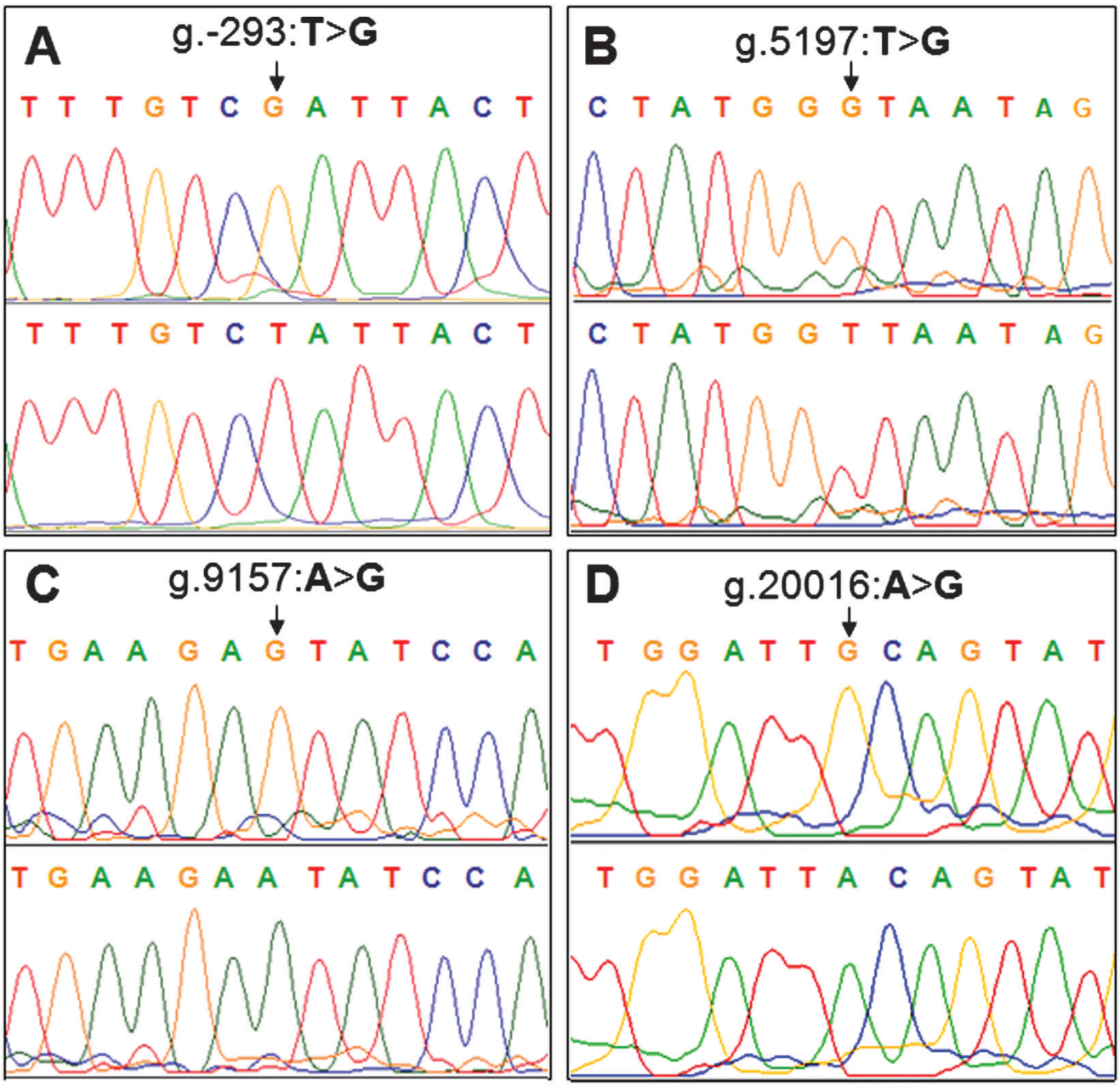

Figure. (**Upper panel**) Electropherogram showing single-nucleotide polymorphisms (SNPs) in infertile men. (**Lower panel**) The electropherograms of the fertile control men. (**A**) g.-293:T>G transversion in 5′ UTR. (**B**) g.5197:T>G transversion in intron 3. (**C**) g.9157:A>G transition in exon 4. (**D**) g.20016:A>G transition in 3′ UTR.

Table 4. *Haplotype frequencies (%) estimated for case and control groups*[a]

No.	Haplotype	Haplotype Frequency, %					χ^2 (*P* Value)[b]	Odds Ratio (95% CI)
	g.-293:T>G g.20016:A>G	Azoo	OAT	OA	All Cases	Controls		
1	T A	75.29	81.43	90	82.24	95.83	. . .	Reference
2	T G	18.29	14.29	7.33	13.3	2.67	7.272 (*P* = .007)	5.07 (1.29–23.29)
3	G A	3.43	0.48	2.67	2.19	0.67	0.503 (*P* = .478)	2.34 (0.16–66.50)
4	G G	3	3.81	0	2.27	0.83	0.503 (*P* = .478)	2.34 (0.16–66.50)

Abbreviations: Azoo, azoospermic; CI, confidence interval; OAT, oligoasthenoteratozoospermic; OA, oligoasthenozoospermic.

[a] Haplotype frequencies were compared among all case and control subjects.

[b] The *P* value was analyzed for statistical significance by χ^2 test, and $P < .05$ was considered statistically significant.

Table 5. *Splicing proteins and binding site sequences as predicted by the "Splicing Rainbow" tool*[a]

No.	SNP	Location	Splicing Rainbow		Binding Site Sequence
			Normal	Mutant	
1	g.5197:T>G	Intron 3	SRp55	SRp55	TTTCTATGG(**T/G**)TAATAGGCCAAGTTAAAAG
			SC35	...	GG**T**TAATA
			...	**SRp40**	TGG**G**TAATAGG
			...	**hnRNP U**	GG**G**TAATAGG
2	g.9157:A>G	Exon 4	SRp40	SRp40	A(**A/G**)TATCCAAATA
			SC35	SC35	AGA**A**TAT and TGAAGA**G**TA
			Tra2Beta	...	CTGAAGA**A**TAT
			ASF/SF2	ASF/SF2	CTGAAGA**A**TATCC and CTGAAGA**G**TA

Abbreviation: SNP, single-nucleotide polymorphism.

[a] Proteins that show differences between normal and mutated sequences are shown in bold letter type.

(Table 1) that practice strict endogamy and maintain social and cultural identity (Thangaraj et al, 2005) and, hence, are prone to genetic disorders.

We identified 2 novel SNPs (g.5197:T>G, g.9157:A>G) observed only in infertile men and 3 known SNPs (g.-293:T>G [rs17167484], g.19765:T>G [rs11538104], and g.20016:A>G [rs3777373]), of which the g.19765:T>G substitution was found only in azoospermic men, whereas the other 2 were found in both infertile and fertile men. However, the differences in the frequency of these mutations (g.-293:T>G and g.20016:A>G) between cases and control groups were statistically significant (Table 3). Because the Ube2b knockout mouse has been shown to have maturation arrest, we tried to correlate the histological phenotype in 18 azoospermic men (for which the biopsy results were available) with the mutations observed; however, we did not find any correlation. One possible reason could be availability of fewer samples for histological studies. The genotype frequencies of the known SNPs (g.-293:T>G, g.19765:T>G, and g.20016:A>G) in various populations, including this study, are shown in Table 6. The g.19765:T>G transversion was found only in azoospermic samples (1.71%) in this study; hence, we strongly suggest that this SNP might be associated with spermatogenic failure in Indian populations. Considering the unique ethnic origin of Indian populations, it is not surprising. India is known for its human diversity, having 4635 anthropologically well defined populations. Each population follows strict endogamy, social and cultural traditions; therefore, we presume that these SNPs might have originated in situ in Indians. Similar findings are quite common between different ethnic populations. We have earlier demonstrated that the A386G in the *DAZL* gene is not associated with male infertility on the Indian subcontinent (Thangaraj et al, 2006), whereas Teng et al (2002) earlier reported that this mutation was associated with severe spermatogenic failure in the Taiwan population. Similarly, expansion of the CAG repeat length was reported to be associated with male infertility (Patrizio et al, 2001), whereas, we did not find any significant difference in the CAG repeat length between infertile and fertile men (Thangaraj et al, 2002). This trend is not only observed in the genes related to infertility, but it also true for other genes involved in sex determination (Singh et al, 2006), alcoholism (Bhaskar et al, 2007; Rao et al, 2007), and mitochondrial disorders (Rani et al, 2006; Vanniarajan et al, 2006). Interestingly, g.9157:A>G and g.19765:T>G polymorphisms were present only in azoospermic

Table 6. *The genotype frequency of the known SNPs in various populations*

Population	g.-293:T>G (rs17167484)		g.19765:T>G (rs11538104)		g.20016:A>G (rs3777373)	
	T/T	T/G	T/T	T/G	A/A	A/G
Sub-Saharan African	0.333	0.667	1.000	...	0.545	0.455
Hispanic	0.75	0.25	0.952	0.048	0.810	0.190
European	0.875	0.125	0.905	0.095	0.909	0.091
African American	0.25	0.75	1.000	...	0.571	0.429
Asian	1.000	...	1.000	...	0.913	0.087
Present study	0.948	0.052	0.993	0.007	0.816	0.184

Abbreviation: SNP, single-nucleotide polymorphism.

patients, which suggests that these SNPs are specifically associated with azoospermia, but not with other phenotypes of infertility (Table 3).

In this investigation, 4 haplotypes were observed based on 2 SNPs (Table 4). Distribution of haplotypes was not uniform between the infertile and fertile men. The high frequency of TA haplotype in fertile men when compared with infertile men suggests that this haplotype is advantageous for fertility, whereas the TG haplotype confers susceptibility to spermatogenic failure. The remaining 2 haplotypes (GA and GG) were observed at low frequencies in both fertile and infertile men. The absence of GG haplotype in oligoasthenozoospermic men could be attributed to their small sample size. These findings indicate that substitution of a single variant in a haplotype could significantly alter gene function. Although both of the SNPs used for the haplotype analysis were present in fertile men as well, their frequency was significantly low. We observed similar findings in other genes; for example, a total of 5 haplotypes were observed for the *NPY* gene, only 2 of which were found to occur with a high frequency in all populations (Bhaskar et al, 2007).

Gene expression profile depends on the proper transcription, which includes DNA to mRNA formation; pre-mRNA splicing and specific consensus sequences (5′ splice site, branch site, and 3′ donor site) especially are found in virtually all exon-intron boundaries (Sun and Chasin, 2000). Among the splicing factors involved in splice site choice, members of SR protein family have been studied extensively. These proteins are rich in serine and arginine residues and have an RNA recognition motif domain that recognizes several classes of specific RNA motifs, including ESEs and ISEs. The hnRNPs are another class of proteins that recognize negative regulatory elements known as ESSs and ISSs. These sequences have been demonstrated to play key roles in both alternate and constitutive splice site selection (Gabut et al, 2005). Our *in silico* analysis of intronic and exonic SNPs specifically present in infertile men predict that these SNPs affect the binding site of splicing factors (SR and hnRNP proteins), which will in turn affect the proper splicing and translation of protein. Therefore, we predict that these SNPs bring about either loss or appearance of potential binding sites for various splicing factors.

This is for the first time we report SNPs and haplotypes in the human *UBE2B* gene and its role in human male infertility. We detected SNPs in all categories of infertile men (azoospermic, oligoasthenoteratozoospermia, and oligoasthenozoospermic) in the Indian population. From this study, it is clear that disturbance in the *UBE2B* gene not only causes structural abnormalities of spermatozoa, which is seen in knockout mice (Roest et al, 1996), but also could be responsible for sperm production in humans. Therefore, our study predicted the additional genetic causes of male infertility.

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