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ORIGINAL ARTICLE

A to G transitions at 260, 386 and 437 in *DAZL* gene are not associated with spermatogenic failure in Indian population

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Summary

The autosomal *DAZL* (*D*eleted-in-*A*zoospermic-*L*ike) gene, mapped to the short arm of the human chromosome 3, is the precursor for the Y-chromosomal *DAZ* cluster, which encodes for putative RNA-binding proteins. Mutations in the *DAZL* have been reported to be associated with spermatogenic failure in Taiwanese population but not in Caucasians. As there was no study on Indian populations, we have analysed the entire coding sequences of exons 2 and 3 of *DAZL* in a total of 1010 men from Indian subcontinent, including 660 infertile men with 598 non-obstructive azoospermia, 62 severe oligozoospermia and 350 normozoospermic fertile control men, to investigate whether mutation(s) in the *DAZL* is associated with male infertility. Interestingly, none of our samples (1010) showed A386G (T54A) mutation, which was found to be associated with spermatogenic failure in Taiwanese population. In contrast, A260G (T12A) mutation was observed in both infertile and normozoospermic fertile control men, without any significant association with infertile groups ($\chi^2 = 0.342$; $p = 0.556$). Similarly, we have found a novel A437G (I71V) mutation, which is also present in both infertile and normozoospermic fertile control men without any significant difference ($\chi^2 = 0.476$; $p = 0.490$). Our study clearly demonstrates the complete absence of the A386G (T54A) mutation in Indian subcontinent and the other two mutations – A260G (T12A) and A437G (I71V) – observed are polymorpic. Therefore, we conclude that these mutations in the *DAZL* gene are not associated with male infertility in Indian subcontinent.

Introduction

It has been estimated that about 5% of men, although healthy, are infertile due to various reasons (World Health Organization Report, 1999), including the genetic factors. Several genes responsible for spermatogenesis have been mapped onto azoospermia factor regions (*AZF*a, b and c) on the long arm of the Y chromosome, the deletion of which have been implicated in causing infertility in 8–15% of the males (Ma *et al.*, 1993; Reijo *et al.*, 1995; Thangaraj *et al.*, 2003a). However, in majority of the azoospermia cases, the aetiology is still not known. The *DAZ* gene cluster present on the Y chromosome has an autosomal homologue, known as *DAZL* (*DAZ* like), located on the short arm of the chromosome 3 (Saxena *et al.*, 1996; Yen *et al.*, 1996). Both *DAZ* and *DAZL* genes encode RNA-binding proteins and have 83% identity in the coding region of the cDNA (Burd & Dreyfuss, 1994; Tsui *et al.*, 2000). The *DAZ* gene (Y chromosome-specific) was found to be present only in human, great apes and old world monkey, whereas the other mammals, including mice, have only the *Dazl* (autosomal copy) gene (Saxena *et al.*, 1996). It has been demonstrated by gene knockout study that disruption of the *Dazl* gene causes loss of germ cells and complete absence of gametogenesis, suggesting that the *Dazl* is essential for the differentiation of germ cells (Ruggiu *et al.*, 1997). Slee *et al.* (1999) have demonstrated that the activity of the

Dazl gene is regained when they introduced a human *DAZ* gene into *Daz*-null allele mice testifying the high degree of functional conservation between the *DAZ*, *DAZL* and *Dazl* genes. Considering this, a few studies have been carried out to investigate the functional significance of the *DAZL* gene in male infertility. Teng *et al.* (2002) identified, for the first time, a T54A (A386G) mutation in the *DAZL* gene, which is associated with susceptibility to severe spermatogenic failure in Taiwan population. However, the subsequent studies have failed to find the T54A mutation in infertile men (Bartoloni *et al.*, 2004; Becherini *et al.*, 2004; Tschanter *et al.*, 2004); in fact, these studies have shown the presence of A260G mutation in both infertile and normozoospermic fertile control men. As, there was no study on infertile men from India to demonstrate the role of *DAZL* gene in male infertility, we have analysed 660 infertile men and 350 normozoospermic fertile controls from Indian subcontinent to assess the phenotypic effect of *DAZL* mutations.

Materials and methods

Study populations and clinical information

A total of 1010 Indian men were included in this study, of which 660 were infertile (598 non-obstructive azoospermia and 62 oligozoospermia patients), who were attending the infertility clinic at the Institute of Reproductive Medicine (IRM), Kolkata, India. A team consisting of urologists and andrologists performed a detailed clinical investigation and recorded the complete case history. Samples were subjected to karyotyping and endocrinological assays (follicle-stimulating hormone, luteinizing hormone, testosterone, prolactin and thyroid-stimulating hormone). Patients underwent a vasogram and testicular pathology was carried out wherever possible. Microdeletion analysis of the Y chromosome was carried out for all the cases (Thangaraj *et al.*, 2003a; Thangaraj *et al.*, unpublished data). Patients who did not exhibit obstruction, endocrinological defect, pelvic injury, major illness, karyotype abnormality or Y chromosome microdeletion were included in the present study. Three hundred and fifty men from Indian subcontinent with normal semen parameters (>20 × 10^6/mL semen fluid, normal motility and morphology), according to the World Health Organization (World Health Organization Report, 1999) guidelines, and normal levels of inhibin B testosterone, LH and FSH were included in this study as a control. All the 350 control men had fathered at least one child each and whose fertility was proven by STR-based (Profiler plus; Applied Biosystem, Foster City, CA, USA) DNA fingerprinting. Blood samples (5.0 mL) from both infertile and normozoospermic fertile control men were collected with their informed written consent.

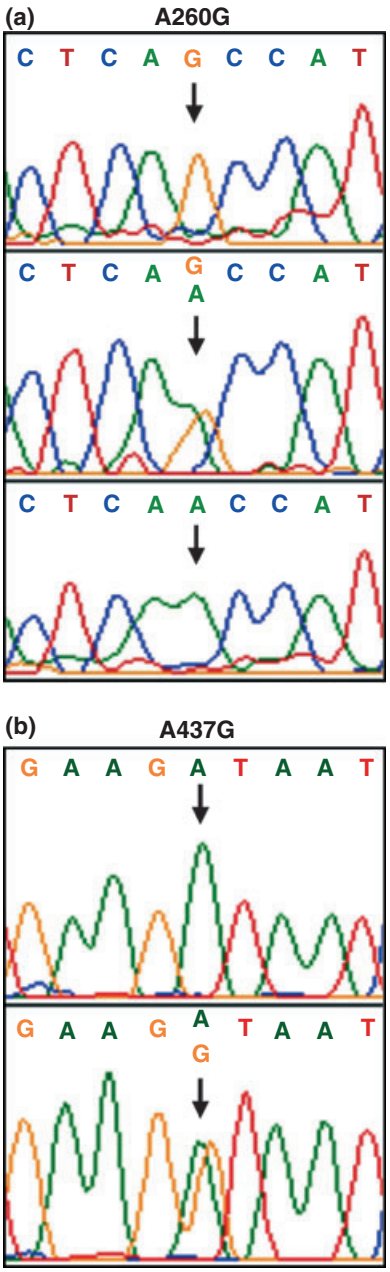

Figure 1 (a) Electropherogram showing the single nucleotide polymorphism at the nucleotide position 260 in the exon 2 of *DAZL*. Lower panel shows the homozygous wild type allele (A/A), middle panel shows the heterozygous allele (A/G) and the upper panel shows the homozygous mutant (G/G) allele. (b) Electropherogram showing the single nucleotide polymorphism at the nucleotide position 437 in the exon 3 of *DAZL*. Upper panel shows the homozygous wild type allele (A/A) and the lower panel shows the heterozygous (A/G) allele.

PCR and DNA sequencing

A pair of primers for exon 2 of the *DAZL* gene was synthesized using the information from Teng *et al.* (2002)

(forward: 5′-CCTGTGTATCTAATTATGATG-3′; reverse: 5′-CCTTAAGTTTGTAACAGGGCC-3′) and primers for exon 3 were designed and synthesized (forward: 5′-TGA-AAGAAATTAACACAGCAACAA-3′; reverse: 5′-GGGGG-AGAAATTGTCACATCAT-3′) using an ABI392 Oligo synthesizer (Perkin Elmer, Foster City, CA, USA). PCR of each sample was performed in a 0.2 mL thin-walled tube using 50.0 ng of DNA, 5 pM of each primer, 200 μM dNTPs, 10x PCR buffer containing 1.2 mM $MgCl_2$ and 1 unit of AmpliTaq Gold (Perkin Elmer). Amplification was carried out in a MJ Research Thermal Cycler (Waltham, MA, USA) using the following cycling condition: 94 °C for 5 min, 35 cycles at 94 °C for 1 min, 60 °C for 1 min and 72 °C for 1 min and a final extension at 72 °C for 10 min. Amplicons were directly sequenced using an ABI3700 DNA analyzer (Thangaraj *et al.*, 2003b). The *DAZL* sequences of the infertile and normozoospermic fertile control men were edited and compared with the reference sequence using AutoAssembler software (Applied Biosystems, Foster City, CA, USA).

Results

Analysis of exons 2 and 3 of the *DAZL* gene in a total of 1010 men, including 660 infertile and 350 normozoospermic control men revealed the complete absence of a previously reported A386G mutation in exon 3 of DAZL gene in Indian subcontinent. A260G mutation leading to T12A in exon 2 was observed in 54 individuals (Fig. 1a; Table 2), of which, two (azoospermic) men showed homozygous mutation A/A260G/G, while the rest were heterozygous A/A260A/G (Table 2). Similar analysis on 350 normozoospermic fertile control men also revealed the presence of A260G mutation in 25 individuals, of which heterozygous mutation was found in 21 individuals and homozygous in four individuals. Also a new A437G mutation was observed in four infertile men (azoospermic) and one normozoospermic fertile control men (Fig. 1b; Table 2). This mutation replaces isoleucine with valine (I71V), which are abundant in the β-strand and both are neutral-hydrophobic-aliphatic amino acids.

Table 1 Geographical origin and ethnic/linguistic affiliation of the azoospermic, oligozoospermic and normozoospermic individuals

S. no	Geographical origin of samples	Ethnic/linguistic affiliation	Infertile men			Normozoospermic control men, total
			Azoospermia	Oligozoospermia	Total	
1	West Bengal	Indo-European/Tibeto-Burman	163	9	182	119
2	Bihar	Indo-European	97	15	112	45
3	Madhya Pradesh	Indo-European	89	4	93	31
4	Assam	Tibeto-Burman	62	6	68	39
5	Bangladesh	Indo-European	53	7	60	33
6	Uttar Pradesh	Indo-European	39	3	42	25
7	Orissa	Indo-European	45	2	47	23
8	Jharkhand	Dravidian	27	3	30	19
9	Chhattisgarh	Austro-Asiatic	23	3	26	16
Total			598	62	660	350

Table 2 Nucleotide position of the single nucleotide polymorphisms in exon 2 and 3 of *DAZL* gene and genotype of both infertile and normozoospermic fertile men

Nucleotide position	Exon	Samples	Genotype			Total	χ^2	*p*-value
			AA wild	AG hetero	GG homo			
260	2	Azoospermia	548	48 (8.0)	2 (0.33)	598	0.342	0.556
		Oligozoospermia	58	4 (6.5)	–	62		
		Infertile men	606	52 (7.9)	2 (0.30)	660		
		Normozoospermic	325	21 (6.0)	4 (1.14)	350		
437	3	Azoospermia	594	4 (0.67)	0	598	0.476	0.490
		Oligozoospermia	62	–	–	62		
		Infertile	656	4 (0.60)	0	660		
		Normozoospermic	349	1 (0.29)	0	350		

The values given in parenthesis are expressed in percentage.
AA, homozygous wild type allele; AG, heterozygous allele; GG, homozygous mutant allele.

Discussion

Although the Y chromosome microdeletion was found to be the major factor for the genetic causes of the male infertility, there were a few reports giving evidence of the involvement of the autosomal gene (*DAZL*) in spermatogenesis. Initial evidence came from Drosophila, that the loss of boule (*Dazl*) resulted in a meiotic arrest and azoospermia (Eberhart *et al.*, 1996). Another study demonstrated that the disruption in the *Dazl* gene led to the loss of germ cells and complete arrest of spermatogenesis in mouse (Ruggiu *et al.*, 1997). The above studies suggested that the *DAZL* was essential for the germ cell differentiation. This was supported by another study, where a human *DAZ* transgene was introduced into *Dazl*-null mice, which was partially rescued from null phenotype (Slee *et al.*, 1999).

However, in human very limited studies have been carried out on *DAZL* gene and its role in male infertility. Teng *et al.* (2002) identified two polymorphic sites in the *DAZL* gene of Taiwan population; one was A260G (T12A) of exon 2 in 3.52% and 2.59% of the infertile and fertile men respectively. Another variant was A386G leading to threonine to alanine (T54A) in the exon 3 in 7.39% of the infertile and 0.86% of the fertile men. As the A260G (T12A) mutation was present in both infertile and fertile men with almost equal frequency, Teng *et al.* (2002) suggested that this mutation is the true variant. Our study on 1010 men from Indian subcontinent (660 infertile and 350 normozoospermic fertile control men) also revealed the presence of this mutation with almost equal frequency in both infertile (8.1%) and normozoospermic fertile control men (7.4%). Hence, there was no significant association with infertility ($\chi^2 = 0.342$; $p = 0.556$). Our results also strongly suggest that the A260G (T12A) mutation is highly polymorphic and is not associated with infertility among men belonging to Indian subcontinent.

The A386G mutation observed by Teng *et al.* (2002) in exon 3 is located within the RNA recognition motif domain of the DAZL protein, which changes the amino acid from threonine to alanine (T54A). Considering the occurrence of this mutation in the highly conserved region and its high prevalence in patients with spermatogenic failure, they suggested that this mutation is associated with spermatogenic failure in Taiwanese population. However, our analysis on Indian men showed the complete absence of A386G (T54A) mutation in both infertile (660) and fertile normozoospermic control (350) men. Three independent studies conducted recently also did not find the T54A mutation in Caucasian populations (Bartoloni *et al.*, 2004; Becherini *et al.*, 2004; Tschanter *et al.*, 2004). As the A386G (T54A) mutation was observed only in Taiwanese population and absent in Caucasian populations, Bartoloni *et al.* (2004) suggested that this mutation might play a role in infertility only in Taiwanese or Asiatic populations. However, the T54A mutation was totally absent in a substantial number of samples (1010) belonging to Indian subcontinent (Table 1). Indian populations, consisting of approximately 4635 culturally and anthropologically well-differentiated groups, can broadly be divided into four different linguistic families, namely, Austro-Asiatic, Dravidian, Tibeto-Burman, and Indo-European. Each population, affiliated to one of these linguistic families, has its own social and cultural identity, and practices endogamy, which helps to maintain its genetic architecture (Thangaraj *et al.*, 2005). Despite the infertile and fertile normozoospermic men belonging to different ethnic groups of Indian subcontinent, they were consistent with the absent of the T54A mutation. Therefore, we strongly suggest that the A386G (T54A) mutation is not associated with male infertility in Indian populations and probably in most of the Asian populations. It is likely that this mutation might be associated with spermatogenic failure only in Taiwan populations and probably the Southeast Asian populations of the Mongoloid origin.

Careful analysis of the exon 3 sequence of the above samples revealed the presence of a novel A437G polymorphism in four infertile and one fertile man. This mutation replaces isoleucine to valine (I71V) in the β-strand. Both are neutral, hydrophobic aliphatic amino acids. Considering this and its occurrence in both infertile and fertile individuals ($\chi^2 = 0.476$; $p = 0.490$), we suggest that this single nucleotide polymorphism is also not significantly associated with male infertility. Further, analysis of the parents of one of the infertile men (parents of other infertile men were not available) with A437G mutation did not show this mutation, suggesting that this is a *de novo* mutation and we believe that this may be true in many cases.

In conclusion, our study on both infertile and fertile men revealed complete absence of the A386G (T54A) mutation in the Indian populations. The other two mutations – A260G (T12A) and A437G (I71V) – were found polymorphic. Therefore, we propose that these mutations in the *DAZL* gene may not be associated with male infertility in Indian subcontinent, hence it may not be necessary to analyse these in infertile Indian men other than that of the Mongoloid origin.

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References

Bartoloni, L., Cazzadore, C., Ferlin, A., Garolla, A. & Foresta, C. (2004) Lack of the T54A polymorphism of the DAZL gene in infertile Italian patients. *Molecular Human Reproduction* 10, 613–615.

Becherini, L., Huarducci, E., Degl'innocenti, S., Rotondi, M., Forti, G. & Krausz, C. (2004) DAZL polymorphisms and susceptibility to spermatogenic failure: an example of remarkable ethnic differences. *International Journal of Andrology* 27, 375–381.

Burd, C. G. & Dreyfuss, G. (1994) Conserved structures and diversity of functions of RNA-binding proteins. *Science* 265, 615–621.

Eberhart, C. G., Maines, J. Z. & Wasserman, S. A. (1996) Meiotic cell cycle requirement for a fly homologue of human deleted in azoospermia. *Nature* 381, 783–785.

Ma, K., Inglis, J. D., Sharkey, A., Bickmore, W. A., Hill, R. E. & Prosser, E. J. *et al.* (1993) A chromosome gene family with RNA-binding protein homology: candidates for the azoospermia factor AZF controlling human spermatogenesis. *Cell* 75, 1287–1295.

Reijo, R., Lee, T. Y., Salo, P., Alagappan, R., Brown, L. G. & Rosenberg, M. *et al.* (1995) Diverse spermatogenic defects in humans caused by Y chromosome deletions encompassing a novel RNA-binding protein gene. *Nature Genetics* 10, 383–393.

Ruggiu, M., Speed, R., Taggart, M., McKay, S. J., Kilanowski, F. & Saunders, P. *et al.* (1997) The mouse *Dazl*a gene encodes cytoplasmic protein essential for gametogenesis. *Nature* 389, 73–77.

Saxena, R., Brown, L. G., Hawkins, T., Alagappan, R. K., Skaletsky, H. & Reeve, M. P. *et al.* (1996) The *DAZ*gene cluster on the human Y chromosome arose from an autosomal gene that was transported, repeatedly amplified and pruned. *Nature Genetics* 14, 292–299.

Slee, R., Grimes, B., Speed, R. M., Taggart, M., Maguire, S. M. & Ross, A. *et al.* (1999) A human DAZ transgene confers partial rescue of the mouse Dazl null phenotype. *Proceedings of the National Academy of Sciences of the United States of America* 96, 8040–8045.

Teng, Y. N., Lin, Y. M., Lin, Y. H., Tsao, S. Y., Hsu, C. C. & Lin, S. J. *et al.* (2002) Association of a single-nucleotide polymorphism of the deleted-in-azoospermia-like gene with susceptibility to spermatogenic failure. *Journal of Clinical Endocrinology and Metabolism* 87, 5258–5264.

Thangaraj, K., Gupta, N. J., Pavani, K., Reddy, A. G., Subramainan, S. & Deepa-Selvi, R., *et al.* (2003a). Y chromosome deletions in azoospermic men in India. *Journal of Andrology* 24, 588–597.

Thangaraj, K., Singh, L., Reddy, A. G., Rao, V. R., Sehgal, S. C. & Underhill, P. A. *et al.* (2003b) Genetic affinities of the Andaman Islanders, a vanishing human population. *Current Biology* 13, 86–93.

Thangaraj, K. Sridhar, V., Kivisild T., Reddy A. G., Chaubey G., Singh V. K. *et al.* (2005). Different population histories of the Mundari and Mon-Khmer speaking Austro-Asiatic tribes inferred from the mtDNA 9-bp deletion/insertion polymorphism in Indian populations. *Human Genetics* 116, 507–517.

Tschanter, P., Kostova, E., Luetjens, C. M., Cooper, T. G., Nieschlag, E. & Gromoll, J. (2004) No association of the A260G and A386G DAZL single nucleotide polymorphisms with male infertility in a Caucasian population. *Human Reproduction* 19, 2771–2776.

Tsui, S., Dai, T., Warren, S. T., Salido, E. C. & Yen, P. H. (2000) Association of the mouse infertility factor DAZL1 with actively translating polyribosomes. *Biology of Reproduction* 62, 1655–1660.

World Health Organization (1999) WHO Laboratory Manual for the Examination of Human Semen and Sperm–Cervical Mucus Interaction. Cambridge University Press, Cambridge.

Yen, P. H., Chai, N. N. & Salido, E. C. (1996) The human autosomal gene DAZLA: testis specific and a candidate for male infertility. *Human Molecular Genetics* 5, 2013–2017.