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PHYSIOLOGY

DAZLA: An Important Candidate Gene in Male Subfertility?

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Purpose: *To study the role of the autosomal candidate gene* DAZLA *(*D*eleted in* AZ*oospermia* L*ike* A*utosome) in male subfertility.*
Methods: *We reviewed clinical data of subfertile men with oligozoospermia or azoospermia, mostly candidates for intracytoplasmic sperm injection (ICSI). Mutation detection was performed using polymerase chain reaction followed by single strand conformation polymorphism analysis. All shifted bands were analyzed by sequencing.*
Results: *We searched for mutations in 44 subfertile men. Nine subfertile men were included, because family history showed that their brothers also faced fertility problems. In these men a possible autosomal gene defect may contribute to their fertility problem. No mutations were found, except for two polymorphisms in intron 4 and 5.*
Conclusion: *At this moment it does not seem relevant to search for possible mutations in the* DAZLA *gene in clinical practice.*

KEY WORDS: *DAZLA*; genetics; ICSI; male subfertility.

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INTRODUCTION

Genetic factors may play an important role in the etiology of severe oligozoospermia or azoospermia causing male subfertility. Chromosomal aberrations, microdeletions of the Y chromosome, and cystic fibrosis transmembrane receptor gene (CFTR) mutations have been described in men with severe subfertility (1). Of particular interest is a study from Lilford *et al*. (2), showing a possible autosomal recessive mode of inheritance in male subfertility. The *DAZLA* (*D*eleted in *AZ*oospermia *L*ike *A*utosomal) gene on human chromosome 3 shares a high degree of homology with the DAZ gene (*D*eleted in *AZ*oospermia), which is sometimes deleted in men with azoospermia or severe oligozoospermia. It is suggested that the DAZ genes arose from the transposition of *DAZLA* to the Y chromosome followed by repeated amplification and pruning (3). Its testis specific expression and its homology to DAZ support the role of *DAZLA* in spermatogenesis. Disruption of the *DAZLA* gene in mice leads to loss of germ cells in both ovary and testis and absence of gamete production, demonstrating that *DAZLA* is essential for the differentiation of germ cells in mice (4).

It might be argued that mutations in the human *DAZLA* gene are responsible for some of the (familial) cases of male subfertility. Since the genomic structure of *DAZLA* is known, mutation detection is possible, but to our knowledge no such studies have been reported till now. In this study, we searched for mutations in the *DAZLA* gene in severe subfertile men, using single strand confirmation polymorphism (SSCP) and sequencing techniques.

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Table I. PCR Primers for the *DAZLA* Gene

Exon	Primers (5′–3′)	Fragment length (bp)
Exon 2	F: TGTAAAACGACGGCCAGTCTGAGCCTGAACTAACTTAGAATG	377
	R: CAGGAAACAGCTATGACCGTTTGTAACAGGGCCCAAATC	
Exon 3	F: TGTAAAACGACGGCCAGTTAAAATTAAAATTTTTGAATGCTG	332
	R: CAGGAAACAGCTATGACCAGAGCTGGCAATAAACTTTTATCC	
Exon 4	F: TGTAAAACGACGGCCAGTATTTTAGTCATGATCACTTCCG	371
	R: CAGGAAACAGCTATGACCTGTAGTTCATGAACCTAGGTGC	
Exon 5	F: TGTAAAACGACGGCCAGTTCCAAGTCTTGGAAGTAAAGAC	268
	R: CAGGAAACAGCTATGACCGATAAGCACCTTTTTGAAAAGC	
Exon 6	F: TGTAAAACGACGGCCAGTCAATCAGGAAACAAAATTTATG	353
	R: CAGGAAACAGCTATGACCCCACAGAAGGTACGATGACTAC	
Exon 7	F: TGTAAAACGACGGCCAGTTTTTCATATTTTTGTTATATTGGG	285
	R: CAGGAAACAGCTATGACCATGACAAACCATTCAGACAATTTG	
Exon 8	F: TGTAAAACGACGGCCAGTTATTATAACAACAAAGGAGCCAGC	301
	R: CAGGAAACAGCTATGACCTAGGCATATATGACATGGAAAACG	
Exon 9	F: TGTAAAACGACGGCCAGTTTAGCTTTTTGAAGAATAAGTGGC	399
	R: CAGGAAACAGCTATGACCTTTCTTTTACTATTTGGTCAAGCC	
Exon 10	F: TGTAAAACGACGGCCAGTGAAAGAGTGGTCTTTACATTAGTG	363
	R: CAGGAAACAGCTATGACCAACTACATTATGTCAAGGTTCAGC	
Exon 11	F: TGTAAAACGACGGCCAGTAGAAATTTTCAGTAAAGTAAAAGG	246
	R: CAGGAAACAGCTATGACCAGCTTAATATTCAAAACCAGCAAC	

MATERIALS AND METHODS

Patient Selection

The study population was selected from a group of 300 intra cytoplasmic sperm injection (ICSI) men attending our fertility clinic since 1996, with abnormal sperm parameters varying from severe oligoasthenoteratozoospermia (OAT) to azoospermia.

Semen analysis was performed according to World Health Organization (WHO) guidelines (5). When more than one semen analysis was available, only the data of the first sample were used. In our center, oligoasthenozoospermic men are candidates for ICSI if their ejaculate contains less than 1.0×10^6 propulsive spermatozoa. Andrologic history and examination, family history, and hormone measurements were performed. In addition, all men were offered chromosome analysis and screening for microdeletions of the AZFa, b, and c regions of the Y chromosome (6). In cases of congenital bilateral absence of the vas deferens (CBAVD), the cystic fibrosis transmembrane receptor gene was screened for mutations. Men with a documented cause for their subfertility such as previous sterilization, testicular malignancy, CBAVD, chromosomal abnormality, or Y chromosome microdeletion were excluded from further analysis.

In this study we selected all men whose family history showed that a brother was also facing fertility problems. The remaining men included in this study were randomly selected. The local institutional review board approved this study.

Mutation Analysis

Genomic DNA was isolated from peripheral blood as described previously (7). The *DAZLA* gene has 11 exons. For exons 2–11 polymerase chain reaction (PCR) fragments were generated. Primers were designed using the published sequence of the *DAZLA* gene, GB: U77467–U77476 (Table I). Exon 1, containing only the first three coding basepairs, was not analyzed. The size of the PCR fragments varied between 241 and 399 bp. Amplified fragments were analyzed for single strand confirmation polymorphism (SSCP), using GeneGel Excel 12.5/24 Kit (Pharmacia Biotech AB, Roosendaal). Fragments were stained with the DNA Silver Staining Kit (Pharmacia Biotech AB, Roosendaal).

PCR fragments generating a shifted band were analyzed by direct sequencing using the Big dye Terminator cycle sequencing (PE Biosystems, Foster City). The sequence reactions were run and analyzed using an automated sequencer, 3700 (PE Biosystems, Foster City).

RESULTS

We selected 44 men; 9 out of these 44 men were included because the family history showed that

Table II. Testis Volume, Semen Analysis, and Hormone Measurements of the 44 Males Screened for Mutations in *DAZLA*

Patients ($n = 44$)	Testis volume (mL)	Sperm concn ($\times 10^6$ mL)	Motility (% propulsive)	FSH[a]	LH[b]	Testosterone
Local reference range	>15	>20	>50	2.0–7.5	1.8–9.5	11–45
OAT[c] ($n = 37$)	15.3 ± 0.7	3.9 ± 3.5	27.6 ± 17.9	7.3 ± 0.7	3.8 ± 0.3	15.8 ± 1.0
Asthenozoospermia ($n = 3$)	18.3 ± 2.9	51.7 ± 22.5	0	5.0 ± 2.7	2.6 ± 0.7	17.0 ± 4.6
Azoospermia ($n = 4$)	12.3 ± 1.0	0	0	14.3 ± 5.4	5.7 ± 0.8	18.0 ± 0.9

Note. Values are presented as Mean ± SD.
[a]FSH, follicle stimulating hormone.
[b]LH, luteinizing hormone.
[c]OAT, oligoasthenoteratozoospermia.

their brothers also faced fertility problems, the other 35 men were randomly selected. The main clinical characteristics, testis volume, semen analysis, and hormone measurements of these males are shown in Table II. Thirty-seven men had oligoasthenoteratozoospermia, three men had asthenozoospermia, and four men had azoospermia. The most frequently encountered abnormality was cryptorchidism ($n = 6$), followed by history of male adnexitis ($n = 4$), inguinal hernia ($n = 3$), and varicocele ($n = 2$). Clinical characteristics of the men with a brother facing fertility problems did not differ from the other men.

A total of 25 aberrant shifts were identified by SSCP in the 44 men screened for mutations in the *DAZLA* gene. Sequence analysis revealed no alteration in the coding area and the splice sites when compared to the genomic structure (8).

Shifts in exon 4 and 5 were shown to be neutral polymorphisms. At 30 bp upstream of exon 4, a variant was found (A/C), and at 28 bp upstream of exon 5, another variant was found (T/C; Fig. 1). In a control group of 20 males the same distribution of these variants was detected (not shown).

DISCUSSION

In 40–60% of cases, the etiology of male subfertility remains unknown and has to be classified as idiopathic (9). It has been suggested that in cases of idiopathic male subfertility there may be a genetic origin (1). Some of these genetic factors are microdeletions of the Y chromosome and the CFTR gene.

The frequency of Y chromosomal DAZ inclusive deletions occur in a frequency of 13% in azoospermic, 7% in severely oligospermic, and 1–29% in subfertile men (6,10,11). This frequency is dependent on the definition of male subfertility and on the choice of Sequence Tagged Sites used for screening (12). There are more candidate genes on the Y chromosome, which could play a role in male factor subfertility (13–15).

Recently, an azoospermic man with a de novo point mutation in the Y chromosomal gene, USP9Y, has been described (16). This study shows the importance of the deletion of this gene as seen in subfertile men with an AZFa deletion. However, given the low frequency reported in this study (1/576 men), routine screening of this gene in ICSI candidates probably has no clinical relevance. Another common monogenic disorder present in subfertile men with CBAVD is cystic fibrosis (CF), caused by mutations in the CFTR gene. The frequency of CBAVD in subfertile men is low and the frequencies of the CFTR

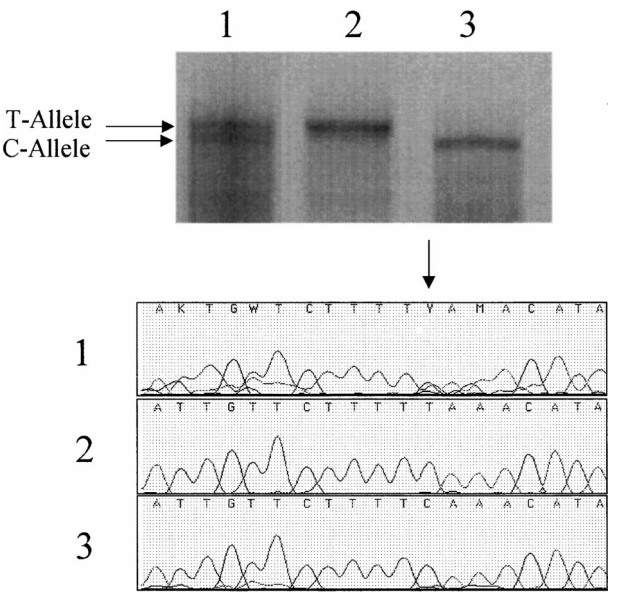

Fig. 1. SSCP and sequence analysis showing the polymorphism found in intron 5 twenty-eight base pairs upstream of exon 5 (IVS5-28T/C). Lane 1–3: patient DNA.

mutations among subfertile men do not differ from normal frequency (17).

Furthermore, an inactivating point mutation of the FSH receptor gene has been reported in some men with elevated serum FSH concentrations and abnormal sperm parameters (18). In a study of 28 men with a high level of FSH, no mutations were found in the FSH receptor gene (19). Information on the genetic basis of male subfertility is important. The cause of male subfertility is often unknown and in this group of idiopathic male subfertility, genetic factors could be involved. *DAZLA* may be one of the genes important in the pathogenesis of male subfertility. To come to an etiological diagnosis rather than a purely descriptive diagnosis is not only more satisfactory to the doctor, it will also be beneficial to patients. Coping with subfertility can be facilitated by a specific diagnosis and at risk relatives may be recognized earlier (20,21). Only by the identification of the etiology of male subfertility progress can be made toward a better therapy and prevention. In this way genetic risk for the offspring could be correctly evaluated and dealt with, especially nowadays since intracytoplasmic sperm injection circumvents a part of the natural selection mechanism.

In this study we searched for mutations in the *DAZLA* gene in male subfertility. Disruption of the *DAZLA* gene in mice leads to loss of germ cells and absence of gamete production (4) resulting in azoospermia. Mutations in the human *DAZLA* gene may lead to oligoasthenoteratozoospermia in men comparable to the effect of the deletions of the Y chromosomal DAZ gene, and may be responsible for some of the familial cases of male subfertility (2). No mutations were found, but two neutral polymorphisms were identified in the introns 4 and 5. It is not likely that *DAZLA* mutations have been missed in a majority of cases, although SSCP detects only between 80 and 90% of all possible mutations and we did not analyze exon 1, containing only the first codon. Besides that with a study group consisting of 44 subfertile men, statistical analyses shows that there is 95% certainty that the possible mutation frequency in the *DAZLA* gene is lower than 7%. Screening a larger group of patients, as has been performed for the USP9Y gene (16), might still result in finding a pathogenic mutation.

Our data suggest that the *DAZLA* gene is not a major contributor to male subfertility. At this moment routine screening in ICSI men for possible mutations in the *DAZLA* gene does not seem relevant in clinical practice.

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REFERENCES

1. Tuerlings JH, Kremer JA, Meuleman EJ: The practical application of genetics in the male infertility clinic. J Androl 1997;18:576–581
2. Lilford R, Jones AM, Bishop DT, Thornton J, Mueller R: Case-control study of whether subfertility in men is familial [see comments]. BMJ 1994;309:570–573
3. Saxena R, Brown LG, Hawkins T, Alagappan R, Skaletsky H, Reeve MP: The DAZ gene cluster on the human Y chromosome arose from an autosomal gene that was transposed, repeatedly amplified and pruned. Nat Genet 1996;14:292–299
4. Ruggiu M, Speed R, Taggart M, McKay SJ, Kilanowski F, Saunders P: The mouse Dazla gene encodes a cytoplasmic protein essential for gametogenesis. Nature 1997;389:73–77
5. World Health Organization: WHO Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction. Cambridge, UK, Cambridge University Press, 1992
6. Kremer JA, Tuerlings JH, Meuleman EJ, Schoute F, Mariman E, Smeets DF: Microdeletions of the Y chromosome and intracytoplasmic sperm injection: From gene to clinic. Hum Reprod 1997;12:687–691
7. Miller SA, Dykes DD, Polesky HF: A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988;16:1215
8. Chai NN, Phillips A, Fernandez A, Yen PH: A putative human male infertility gene DAZLA: Genomic structure and methylation status. Mol Hum Reprod 1997;3:705–708
9. de Kretser DM: Male infertility [see comments]. Lancet 1997;349:787–790
10. Reijo R, Lee TY, Salo P, Alagappan R, Brown LG, Rosenberg M, Rozen S, Jaffe T, Straus D, Hovatta O, de la Chapell A, Silber S, Page DC: Diverse spermatogenic defects in humans caused by Y chromosome deletions encompassing a novel RNA-binding protein gene. Nat Genet 1995;10:383–393
11. Silber SJ, Alagappan R, Brown LG, Page DC: Y chromosome deletions in azoospermic and severely oligozoospermic men undergoing intracytoplasmic sperm injection after testicular sperm extraction. Hum Reprod 1998;13:3332–3337
12. Simoni M, Bakker E, Eurlings MC, Matthijs G, Moro E, Muller CR: Laboratory guidelines for molecular diagnosis of Y-chromosomal microdeletions. Int J Androl 1999;22:292–299
13. Schnieders F, Dork T, Arnemann J, Vogel T, Werner M, Schmidtke J: Testis-specific protein, Y-encoded (TSPY) expression in testicular tissues. Hum Mol Genet 1996;5:1801–1807
14. Kent First M, Muallem A, Shultz J, Pryor J, Roberts K, Nolton W: Defining regions of the Y-chromosome responsible for male infertility and identification of a fourth AZF region (AZFd) by Y-chromosome microdeletion detection. Mol Reprod Dev 1999;53:27–41
15. Lahn BT, Page DC: Functional coherence of the human Y chromosome. Science 1997;675–680
16. Sun C, Skaletsky H, Birren B, Devon K, Tang Z, Silber S: An azoospermic man with a de novo point mutation in the Y-chromosomal gene USP9Y. Nat Genet 1999;23:429–432

17. Tuerlings JH, Mol B, Kremer JA, Looman M, Meuleman EJ, Meerman GJ: Mutation frequency of cystic fibrosis transmembrane regulator is not increased in oligozoospermic male candidates for intracytoplasmic sperm injection. Fertil Steril 1998;69:899–903
18. Tapanainen JS, Aittomaki K, Min J, Vaskivuo T, Huhtaniemi IT: Men homozygous for an inactivating mutation of the follicle-stimulating hormone (FSH) receptor gene present variable suppression of spermatogenesis and fertility. Nat Genet 1997;15:205–206
19. Tuerlings JH, Ligtenberg MJ, Kremer JA, Siers M, Meuleman EJ, Braat DD: Screening male intracytoplasmic sperm injection candidates for mutations of the follicle stimulating hormone receptor gene. Hum Reprod 1998;13:2098–2101
20. Meschede D, De Geyter C, Nieschlag E, Horst J: Genetic risk in micromanipulative assisted reproduction. Hum Reprod 1995;10:2880–2886
21. In't Veld PA, Halley DJ, van Hemel JO, Niermeijer MF, Dohle G, Weber RF: Genetic counselling before intracytoplasmic sperm injection [letter]. Lancet 1997;350:490