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Azoospermic men with deletion of the *DAZ* gene cluster are capable of completing spermatogenesis: fertilization, normal embryonic development and pregnancy occur when retrieved testicular spermatozoa are used for intracytoplasmic sperm injection

J.P.Mulhall[1], R.Reijo[2], R.Alagappan[2], L.Brown[2], D.Page[2], R.Carson[3] and R.D.Oates[1,4]

[1]Department of Urology, Boston University School of Medicine, Boston, [2]Howard Hughes Medical Institute, Whitehead Institute and Department of Biology, Massachusetts Institute of Technology, Cambridge and [3]Reproductive Science Center, Waltham, MA, USA

[4]To whom correspondence should be addressed at: DOB Suite 606, 720 Harrison Avenue, Boston, MA 02118, USA

Some men with non-obstructive azoospermia harbour fully formed spermatozoa within their testicular tissue that can be used to achieve pregnancy via intracytoplasmic sperm injection (ICSI). Recently, Reijo *et al.* (1995) provided compelling evidence that the *DAZ* gene cluster is a strong candidate for one of the elusive azoospermia factors (*AZF*) located on the long arm of the Y chromosome. The *DAZ* gene cluster is deleted in 13% of azoospermic men and a small percentage of severely oligozoospermic men. Vertical transmission from father to son of *AZF* region deletions has also been described. Presumably these fathers were oligozoospermic. This led us to ask whether the azoospermic male with deletions of the *AZF/DAZ* region can also complete minimal spermatogenesis and whether any spermatozoa found could participate in fertilization, embryo development and pregnancy. Three out of six (50%) of the azoospermic men with *AZF/DAZ* deletions had spermatozoa identified within their harvested testicular tissue. When these spermatozoa were used for ICSI, fertilization occurred in 36% of injected oocytes. This compared favourably with testicular spermatozoa retrieved from non-obstructive azoospermic men without *AZF/DAZ* gene deletions. In one case, a twin conception resulted, which represents the first term pregnancy reported using spermatozoa from an *AZF/DAZ* deleted azoospermic male. Therefore it is necessary to take the possibility of transmission of infertility or sterility to our patients' offspring seriously when utilizing today's reproductive technologies, as spermatogenesis in men with *AZF/DAZ* deletions is by no means an exceptional occurrence.

Key words: AZF/ICSI/azoospermia/spermatogenesis/Y chromosome

Introduction

Intracytoplasmic sperm injection (ICSI) has revolutionized the treatment of males with spermatogenic defects, allowing men who previously would have been unable to father children to achieve biological paternity (Van Steirteghem *et al.*, 1993). At its inception, ICSI was used in cases where normal spermatogenesis was associated with excurrent ductal system obstructive lesions or in cases with low numbers of spermatozoa present within the ejaculate. More recently the use of ICSI has expanded dramatically, so that it is also being employed to aid men who harbour only small numbers of nearly immotile spermatozoa within their testicular parenchyma (Abuzied *et al.*, 1995; Bourne *et al.*, 1995; Devroey *et al.*, 1995a,b).

As our therapeutic strategies continue to evolve, we are increasingly able to overcome the most severe male reproductive deficiencies that prevent couples from conceiving naturally. This new-found ability has outstripped our understanding of the basic pathophysiology underlying the conditions that we are circumventing. Consequently, there are many questions to be answered. Is there a genetic basis for a particular patient's non-obstructive azoospermia? If so, can it be transmitted to either female or male offspring? What are the risks and rates of such transmission? Are there other somatic deficiencies, gross or subtle, that might also be passed along? Are the minimal number of spermatozoa found in the patient capable of contributing to fertilization, embryo development, and pregnancy? At present, there are few answers to these important queries. This study was designed to look at one genetic cause of spermatogenic deficiency, deletion of the azoospermia factor (*AZF*) region on the long arm of the Y chromosome (Yq), and answer, if possible, some of the questions posed above. In addition, by examining clinical information pertaining to affected patients, we can gain insight into the function of the *DAZ* gene, a strong candidate for *AZF* (Figure 1).

Our study focused on a population of men with non-obstructive azoospermia, all of whom were tested for *AZF/DAZ* region deletions. Those with deletions of the *AZF/DAZ* region, who also underwent testicular tissue harvesting (testicular sperm extraction, TESE), were culled out as group I, while those that did not possess a detectable deletion and proceeded to TESE formed group II. By looking at group I specifically, we examined the following questions: (i) how many azoospermic men with *AZF/DAZ* deletions have whole spermatozoa within their testicular parenchyma, (ii) what is the morphological appearance of those spermatozoa, (iii) are they capable of inducing fertilization and pronuclear formation after introduction into the oocyte cytoplasm during ICSI, (iv) can they contribute the centrosome and other putative factors required for early embryo development, and (v) can those spermatozoa ultimately be part of a normal pregnancy? Unfortunately, the number of men with *AZF/DAZ* deletions is small at present, but by comparing groups I and II we can compare the efficiency of spermatozoa from *AZF/DAZ* deleted men

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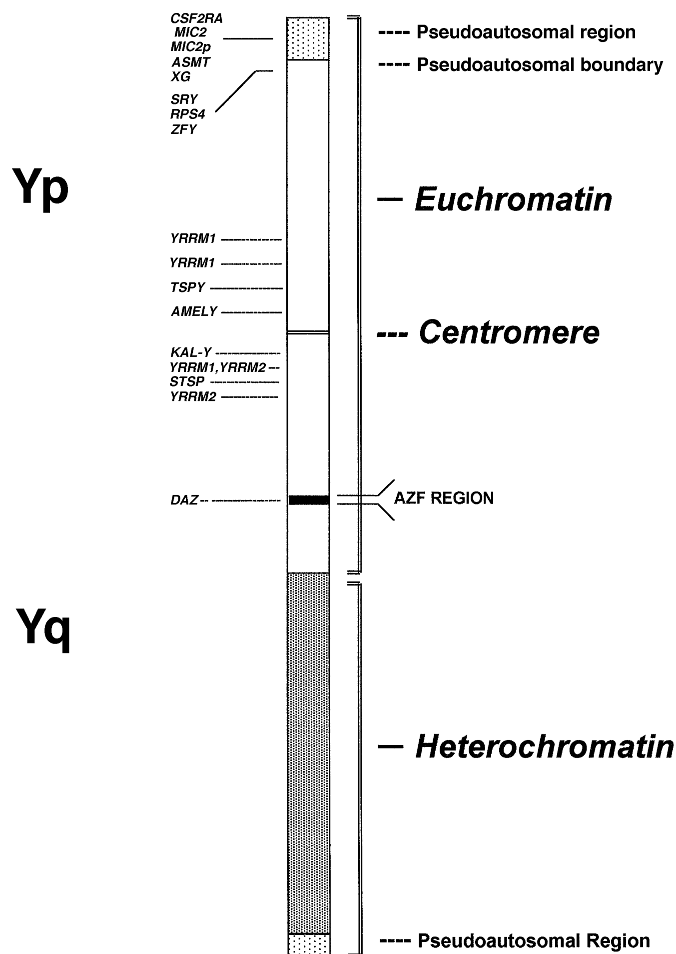

Figure 1. The Y chromosome with its various known regions and genes labelled. The *AZF/DAZ* region is indicated.

during ICSI, to that of spermatozoa from men not deleted for *AZF/DAZ*. With this information, we can tentatively speculate upon the possible role that the *DAZ* gene cluster might play in the regulation of the complex human spermatogenic process.

Materials and methods

Patient population

A total of 83 patients with a history and physical examination that revealed small, soft testes, normal epididymides and vasa deferentia as well as elevated serum FSH indicating non-obstructive azoospermia was counselled concerning possible genetic aetiologies for their azoospermia and underwent *AZF/DAZ* region microdeletion analysis (*vide infra*). Eight patients had deletions of their *AZF/DAZ* region (9.6%), of whom six underwent TESE, constituting group I. In the remaining 75 patients no *AZF/DAZ* deletions were detected. Of this latter cohort, 28 patients had TESE performed, thus forming group II. Testicular tissue analysis and processing (*vide infra*) were performed in a blinded fashion with regard to deletion status. The mean age of the overall group was 34 years, and no significant difference existed in age between groups I (mean age 33) and II (mean age 34). The mean partner age was 36 years and no significant difference existed in this parameter between groups I and II, 37 and 36 years respectively.

AZF/DAZ DNA analysis (Figure 2)

After describing in detail what was known regarding the *AZF/DAZ* region and its possible implications, informed consent (approved by the Boston Medical Center Institutional Review Board) was obtained and venipuncture performed. Two tubes of blood were shipped to the Whitehead Institute at ambient temperature for *AZF/DAZ* region analysis. Within 24 h, genomic DNA was prepared from peripheral leukocytes as previously described (Reijo *et al.*, 1995). Details of the polymerase chain reaction (PCR) have also been outlined (Reijo *et al.*, 1995). Essentially, PCR was performed in 96-well plates with 100 ng DNA in a 20 μl reaction containing 1.5 mM $MgCl_2$, 5 mM NH_4Cl, 10 mM Tris, pH 8.2, 50 mM KCl, 100 μM dinucleotide triphosphate (dNTP) (Pharmacia, Milwaukee, WY, USA) and 1 U *Taq* polymerase (Perkin-Elmore, Roache Molecular Systems, Branchburg, NJ, USA). PCR was initiated with a denaturation step at 94°C for 5 min followed by 35 cycles of 1 min at 94°C, 1.5 min at 58°C, and 1 min at 72°C. A final extension for 5 min at 72°C completed the PCR. Reactions were stored at 4°C until they could be run on 4% agarose gels for analysis.

The sequence-tagged-site (STS) primer pairs we employed have been published previously or will be published elsewhere (Reijo *et al.*, 1995; 1996a, b; R.Reijo, R.Alagappan and D.Page, unpublished results). The STSs used cover the entire length of the euchromatic Y chromosome and include pairs for the genes *SRY, RPS4Y, ZFY, YRRM, TSPY, AMELY, SMCY,* and *DAZ*, as well as STSs corresponding to the centromere and heterochromatic regions. The primer pairs routinely used for the *DAZ* gene cluster are shown in Table I.

As in prior studies, precautions were taken to ensure that negative PCR results were not spurious but represented the genuine absence of a Y-chromosome marker (Reijo *et al.*, 1995; 1996b). Firstly, only Y-specific PCR assays that repeatedly gave positive results when tested on 90 fertile men were used in this study, and secondly, we did not record an STS as absent from a patient unless at least three successive attempts at PCR amplification yielded negative results.

Technique of testicular tissue extraction

By coordinating the time and date of surgery with the embryology laboratory of the Reproductive Science Center of Boston, both therapeutic and diagnostic testicular biopsy were carried out. Bupivicaine® (0.25%; Astra USA Inc., Westborough, MA, USA) was used as a local spermatic cord block and was supplemented with a small amount of i.v. sedation. A 1.5 cm transverse incision was made in the left hemiscrotum and carried down to the tunica albuginea of the testis where a slightly smaller opening into the parenchyma was created. The testis was compressed and the exposed seminiferous epithelium was sharply excised. The harvested tissue sample was subdivided into 10 pieces and placed into a 12 ml conical tube containing 1 ml of test yolk buffer (TYB, Irvine Scientific, Santa Ana, CA, USA). A second tissue sample was blotted onto a glass microscope slide prior to fixation in Bouin's solution for formal histological analysis. This 'wet prep' was inspected under ×400 magnification. If any fully formed spermatozoa were seen, the incision was closed. If no spermatozoa could be detected, tissue was removed from the contralateral testis to maximize the chances that spermatozoa would be found that day. When tissue harvesting was complete, the packaged specimens were transported for processing, analysis, and cryopreservation. No complications occurred, with patients requiring minimal analgesics post-operatively.

Preparation and cryopreservation of testicular homogenates

The retrieved tissue was allowed to settle and the supernatant was aspirated and diluted to a final concentration of 10% (v/v) glycerol. It was microscopically surveyed for spermatozoa and, if any were present, was cooled to 4°C. The parenchyma itself was mixed with a 10% v/v solution of glycerol and TYB in preparation for homogenization. This step was carried out using a loose fitting glass

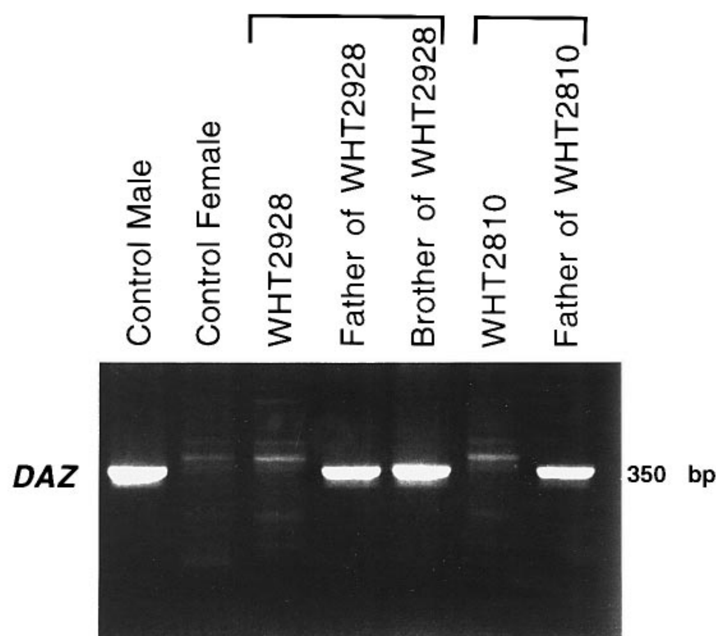

Figure 2. Results of *DAZ* gene cluster analysis in two of the patients of group I who underwent TESE/ICSI. *DAZ* is present in the normal control and in the father of both patients and the brother of WHT 2928 but is absent from WHT 2928 and WHT 2810. WHT 2928 and his wife delivered healthy twin females.

Table I. Primer pairs routinely used for the *DAZ* gene cluster

STS	Left primer	Right primer	Product size (bp)
sY254 (Reijo *et al.*, 1995)	gggtgttaccagaaggcaaa	gaccgtatctaccaaagcagca	350
sY258 (unpublished)	gttacaggattcggcgtgat	ctcgtcatgtgcagccac	125
sY465 (unpublished)	ccacccattggctcagacc	gtgaagaccccacaggcctt	280[a]

[a]Three smaller male/female common bands are observed along with the scored male specific band.

pestle as well as repeated aspiration through a 16 gauge hypodermic needle. The resultant slurry was expanded to a final volume of 5.0 ml of TYB:glycerol and searched for the presence of whole spermatozoa. Depending upon the existence and concentration of spermatozoa, either 500 µl or 1 ml aliquots were prepared and transferred to polypropylene cryovials in a sterile fashion, prior to cooling to 4°C. Supernatant and homogenized tissue vials were further cooled at –1°C/min to –7.0°C at which point ice crystal formation was mechanically stimulated. Continued cooling occurred employing a programmable unit to –150°C before transfer and storage in liquid nitrogen pending isolation of oocytes at the time of a future ICSI cycle.

Ovarian stimulation and isolation of oocytes

Leuprolide acetate (1 mg/day; Lupron®; TAP Pharmaceutical, Deerfield, Il, USA) was used to accomplish hormonal suppression and down-regulation. Either human menopausal urinary gonadotrophin (HMG; Pergonal®; Serono, Randolph, MA, USA) or pituitary follicle-stimulating hormone (HFSH; Metrodin®; Serono) was used in standard fashion to induce multiple follicular development. Transvaginal ultrasonography and serum oestradiol measurements were used to monitor follicular growth. Human chorionic gonadotrophin (HCG; Profasi®; Serono; 10 000 IU) was injected at the appropriate time to stimulate final oocyte maturation (serum oesradiol $>$1 ng/ml and/or $>$3 follicles $>$17 mm diameter). Oocytes were recovered via transvaginal aspiration 36 h after HCG administration. Cumulus cells were removed by incubation in a mixture containing hyaluronidase (80 U/ml), Dulbecco's phosphate-buffered saline (DPBS; Gibco, Baltimore, MD, USA), and bovine serum albumin (BSA; 0.1%; Sigma, St Louis, MO, USA). The denuded oocytes were maintained in 30 µl drops of IVF-50 medium (Scandinavia IVF Science AG, Goteborg, Sweden) under mineral oil until microinjection with a single testicular spermatozoon.

Technique of ICSI – sperm thaw, isolation, manipulation and injection

A vial of frozen testicular homogenate was thawed and 5 µl drops submersed in mineral oil and maintained at 37°C. Spermatozoa that demonstrated non-directional, non-progressive tail movement (indicative of viability) were preferentially selected and initially placed in adjacent beads of DPBS:BSA followed by placement in polyvinylpyrrolidone (PVP 10% w/v; Scandinavia IVF Science AG). Oocyte injection was performed under phase-contrast microscope control. Once the sperm injection was complete, each oocyte was returned to individual 30 µl drops of IVF-50 medium and kept at 37°C in an atmosphere of 5% CO_2. At 16–20 h after ICSI, the oocytes were inspected for the development of a second pronucleus, thus indicating successful fertilization. When oocytes containing two pronuclei (2PN) were observed, they were stored for another 48 h in IVF-50 medium.

Intrauterine transfer of embryos

On the third post-injection day (at approximately 70 h), zonal digestion was performed using acidified Tyrode's solution in selected embryos.

Table II. Results of ISCI using testicular sperm

Patient no.	No. of cycles	No. of oocytes	No. of resultant embryos	% Fertilization	Pregnancy
WHT 2928[a]	1	12	4	33	Twins
WHT 2840[a]	1	2	0	0	No
WHT 2810[a]	4	28	11	39	No
Group II	25	228	103	45	3 Ongoing

[a]Patients with *DAZ* gene cluster deletions

These embryos were then placed in the uterus, via the transcervical route, using a semi-rigid catheter.

Results

Group I consisted of those six patients with *AZF/DAZ* deletions who desired and underwent TESE. Of this group, one had a unilateral tissue extraction while the remaining five required bilateral tissue harvesting. Spermatozoa were found in three of these six patients (50%). The spermatozoa visualized were morphologically normal with heads, mid-pieces and tails demonstrating no consistent defects. All three patients who had testicular spermatozoa present in their TESE specimens (WHT 2928, WHT 2810 and WHT 2840) used their frozen-thawed samples coupled with ICSI in an attempt to achieve pregnancy (Table II). There appeared to be no overt morphological difference between the embryos generated and those obtained from ICSI cycles using ejaculated spermatozoa. In total, 42 oocytes and 15 embryos were cumulatively obtained from these six cycles, representing a 36% fertilization rate. Patient WHT 2928 and his spouse conceived and delivered healthy twin females. *AZF/DAZ* deletions were not found in the fathers of WHT 2928 and WHT 2810 indicating that they arose *de novo* in both patients (Figure 2).

Group II consisted of the 28 patients without *DAZ* region deletions who underwent TESE. Of this group, 15 patients had unilateral extraction, while the other 13 required a bilateral testicular tissue harvesting. Thirteen couples from this group have proceeded to ICSI. Three couples have achieved an ongoing pregnancy. In the 13 couples (25 cycles), a total of 228 oocytes were injected, with 103 developing into embryos for a fertilization rate of 45% (Table 1). Seventy-two of these embryos were suitable for transfer. No obvious difference was evident in morphology between these embryos and those derived from ICSI using ejaculated spermatozoa.

Discussion

Non-obstructive azoospermia: new treatment options

Non-obstructive azoospermia occurs in a small percentage of infertile men. Invariably, the underlying aetiology is primary spermatogenic dysfunction. Until recently, there was no therapy that could assist the non-obstructive azoospermic male in his quest for biological fatherhood. Innovative investigation has demonstrated that fully formed spermatozoa found scattered throughout the testicular parenchyma can serve as the source of spermatozoa for ICSI, with pregnancy occurring in around 20–30% of couples (Schoysman *et al.*, 1993; Silber *et al.*, 1995; Witt, 1995; Witt, *et al.*, 1995; Silber, 1996). With this new avenue of treatment available to men with non-obstructive azoospermia, we must acknowledge that transmissible genetic aetiologies may underly an individual's reproductive failure. In light of this genetic risk, whenever possible, we should assess the potential impact an individual's genes may have on their ability to conceive through ICSI and whether there may be any subsequent effect on future generations. At this time, we are limited in this assessment to karyotype and *AZF* region Y-DNA analysis (Reijo *et al.*, 1995; Vogt *et al.*, 1996). In the study reported herein, we related deletions of the *AZF/DAZ* region in azoospermic men to spermatozoa presence within testis tissue and to the outcome of ICSI.

AZF/DAZ deletion in the azoospermic male: spermatogenic potential?

The *AZF/DAZ* region of the Y chromosome described by Reijo *et al.* (1995) contains a multiple copy gene (*DAZ*) that possesses significant homology to the *Drosophila* (*boule*) male-fertility gene (Eberhart *et al.*, 1996). It is clinically known that men with *DAZ* region deletions are mostly azoospermic and that spermatogenesis, as determined by formal histology in these men, is variable, ranging from a pattern of Sertoli cell-only to the presence of condensed spermatids in some tubules (Reijo *et al.*, 1995). Previous reports of severely oligozoospermic men with de-novo deletions (two cases; Reijo *et al.*, 1996a) and of vertical transmission of an *AZF/DAZ* deletion (two cases; Kobayashi *et al.*, 1994; Vogt *et al.*, 1996) suggested that completion of spermatogenesis (at reduced output) might be at least an occasional outcome of an *AZF/DAZ* deletion. It was not clear that this was a frequent outcome of such deletions, as the great majority of such deletions reported have been in azoospermic men. Our finding of testicular spermatozoa in three out of six *AZF/DAZ*-deleted men with non-obstructive azoospermia suggests that completion of spermatogenesis (albeit at reduced output) may be the rule rather than the exception in *AZF/DAZ*-deleted men. These data also strengthen the hypothesis that, in many cases, non-obstructive azoospermia and severe oligozoospermia are part of a continuous clinical spectrum with a common underlying genetic basis.

This study further points out the lack of a strict phenotype–genotype relationship in azoospermic men with *AZF/DAZ* deletions in regard to whether spermatozoa will or will not be found within their harvested testicular parenchyma. We found that out of six such men, three had sufficient spermatozoa in the testis for use in ICSI, whereas three had testicular parenchyma that was completely devoid of fully-formed spermato-

zoa. Although the sample size is quite small, these numbers indicate that we cannot predict the presence or absence of testicular spermatozoa in the azoospermic man based on the presence or absence of an *AZF/DAZ* deletion as currently defined. Since spermatozoa were found in some men, total loss of the *DAZ* gene cluster does not necessarily forecast complete lack of spermatogenesis within the testis of the azoospermic male. Therefore, this study expands the phenotypic expression of an *AZF/DAZ* deletion and specifically clarifies the azoospermic population. By combining the data from prior reports (Reijo *et al.*, 1996b; Vogt *et al.*, 1996) with those from the present study, we realize that deletions of the *DAZ* gene cluster may lead to non-obstructive azoospermia with complete absence of spermatogenesis, non-obstructive azoospermia with a minimal amount of spermatogenesis, or, on occasion, even severe oligozoospermia.

AZF/DAZ deletion in the azoospermic male: sperm function?

Although we cannot predict the presence or absence of spermatozoa based on *AZF/DAZ* deletion status, can we predict the ability for those spermatozoa to fertilize an egg, promote embryonic growth and result in pregnancy? Addressing this question, the sample size is even smaller, but the results are interesting to consider. It appears that the rates of fertilization and embryo development are similar when comparing testicular spermatozoa extracted from azoospermic men with *AZF/DAZ* deletions to those obtained from similar men without deletions. The few spermatozoa produced by azoospermic men with an *AZF/DAZ* deletion are, therefore, functionally competent and respond appropriately when required to participate in an array of post-fertilization events. This would be predicted, but heretofore not proven, by the demonstration of transmission of an *AZF/DAZ* region deletion from father to son (Vogt *et al.*, 1996).

AZF/DAZ deletion in the azoospermic male: DAZ gene cluster function?

The exact role of the Y chromosome *AZF/DAZ* gene cluster in male reproductive biology is still unknown and the identity of the azoospermia factor has been disputed. To date, however, the *DAZ* gene cluster is a very strong candidate for *AZF* (Reijo *et al.*, 1995; Saxena *et al.*, 1996). This gene cluster is found within the *AZF* region and is expressed in early germ cell populations (gonocytes and spermatogonia), as demonstrated in studies of laboratory mice as well as humans (Menke *et al.*, 1996; Reijo, 1996a). This localization suggests that the *DAZ* gene cluster plays a role in the proliferation, maintenance or differentiation of those early germ cell populations. Clearly, this work also demonstrates that an intact *DAZ* gene cluster is not absolutely required for completion of the later stages of spermatogenesis which involve development of sperm structure and acquisition of functional competence involving fertilization and embryonic development, even in the azoospermic male. How is it that the deletion of a single region of the Y chromosome is associated with such varied phenotypes? Two possibilities exist to explain this situation: perhaps the deletions observed are, in fact, not identical and while the *DAZ* gene cluster is deleted in all cases observed to date, other adjacent areas may lack important genes in those cases of the most profound spermatogenic failure. While this first scenario cannot be excluded, it appears to be at odds with the original Y chromosome mapping studies (Reijo *et al.*, 1995). A second explanation is that the phenotypes observed in men with *DAZ* gene cluster deletions are modified by each man's particular environment and genetic background. This latter postulate seems more plausible given the variability of sperm production from tubule to tubule within the testis of any given individual. Therefore the loss of *DAZ* gene cluster function may not smother spermatogenesis entirely as other genes and/or mechanisms may allow for some small measure of escape. Various autosomal genes or other Y spermatogenesis genes may exert alone, or in combination, this minimally protective effect. A search for other genes involved in the regulation of spermatogenesis such as in the *AZFa* and *AZFb* regions is certainly warranted in light of this phenotypic spectrum (Vogt *et al.*, 1996).

AZF/DAZ deletion in the azoospermic male: risk to offspring?

Detailed and comprehensive counselling was offered to all couples in this study prior to TESE, including those in whom a *DAZ* gene cluster deletion was observed, so that both partners fully understood and appreciated the implications of possible *DAZ* gene cluster deletion vertical transmission. It is unknown at this time whether all male offspring of fathers with a recognizable *AZF/DAZ* deletion will also possess this deletion but the probability is certainly quite high. The underlying assumption of this postulate is that all Y-bearing spermatozoa will possess *AZF/DAZ* deleted Y-chromosomes, a fact not yet proven but anticipated based upon evidence that spermatozoa from a severely oligozoospermic male with a *DAZ* gene cluster deletion showed the same deletion (Reijo *et al.*, 1996b). The 'choice' of bearing male children predicted to be infertile/sterile is still within the jurisdiction of the couple. There is no moral or ethical standard to suggest that anyone other than the couple should be the ultimate arbiters of this decision. In counselling couples, however, we are limited in our ability to predict the ultimate sperm production capabilities of male offspring of *AZF/DAZ*-deleted men. As adults, these offspring may have no available spermatozoa for therapeutic use and possibly may be sterile rather than infertile, a consequence of the wide phenotypic variability in expression of *AZF/DAZ* deletions. Our patients' ability to conceive a son through the use of TESE coupled with ICSI does not, at this time, ensure that son's ability to conceive a child of his own. The deletions detected so far presumably arose *de novo* and were not transmitted vertically from father to patient. This suggests that therapeutic advances such as ICSI may now enable us to burden future generations with genetic constitutions that nature would routinely eliminate. An effective ethical strategy must be formed in order to deal with the difficult situations that will face both couples and reproductive clinicians. Is predictable infertility/sterility in the offspring (male or female) a rationale to prohibit procreation and the use of all the latest technological advances in reproductive biology? Or is it a legitimate reason to proceed with preimplantation genetic analysis with transfer of only those embryos that do not possess inherited infertility/

sterility? Are the children of our patients to be considered our responsibility as well? Hopefully, better definition of all genetic aetiologies underlying male and female infertility will allow us occasionally to share good news with patients, as with autosomal recessive disorders in which it will be unlikely that transmission of the full disorder will occur.

In summary, this report is the first to document that non-obstructive azoospermic men with *AZF*/*DAZ* deletions may harbour spermatozoa within their testicular parenchyma that can be retrieved for use in combination with ICSI to effect fertilization, embryo development, and pregnancy. The non-obstructive azoospermic male partner of couples undergoing TESE/ICSI should be assessed for *AZF*/*DAZ* deletions and counselled appropriately. The obvious risk for the male off-spring is infertility or sterility, realized only in his adulthood. It seems unlikely that other somatic abnormalities would occur in these children. Careful phenotypic analyses of all males born from patients with *AZF*/*DAZ* deletions will be required to answer this question definitively. Since the limited number of morphologically normal testicular spermatozoa recovered are capable of contributing to fertilization, embryo generation and ongoing pregnancy when coupled with ICSI in a fashion similar to those from men without *AZF*/*DAZ* deletions, it is unlikely that the *DAZ* gene cluster product plays a role in sperm development, with regard to morphology and ability to undergo requisite post-fertilization events. Since most testicular biopsies show severe reductions in all spermatogenic elements, it is probable that the *DAZ* gene cluster plays a pivotal role in the quantitative, rather than qualitative, regulation of the spermatogenic epithelium.

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