Mice with Y chromosome deletion and reduced Rbm genes on a heterozygous Dazl1 null background mimic a human azoospermic factor phenotype

T.Vogel, R.M.Speed, P.Teague and H.J.Cooke

MRC Human Genetics Unit, Western General Hospital, Crewe Road, Edinburgh EH4 2XU, UK

To whom correspondence should be addressed

A subset of azoospermia or oligozoospermia patients have microdeletions in defined regions of their Y chromosome, namely the AZFa, b, and c regions. Candidate genes in humans that may cause the azoospermia factor (AZF) phenotype have been assigned to these regions and can include the DAZ and RBM genes. Part of the variability in the AZFc phenotype might be due to interaction between the effects of deleting the DAZ and RBM genes. We mimicked human deletions of RBM and DAZ in the mouse by crossing male mice with a deleted Y chromosome with a reduced number of Rbm genes (Yd1) to heterozygote Dazl1 null female mice to study the interaction of the Dazl1 and Rbm or other genes located in the Yd1 deletion interval. Dazl–/–Yd1 animals showed a significant reduction in the sperm count (P < 0.001), an increase of abnormal sperm heads and prominent mid-piece defects of the tails compared to either mutation alone (P < 0.001). Hence, Dazl1 and the genes removed on the Yd1 chromosome are active in different pathways contributing to different stages of spermatogenesis. Reduction of Dazl1 and Rbm genes as well as/or deletion of the Y chromosome in mice gives rise to a phenotype similar to the heterogeneous AZFc phenotype observed in humans.

Key words: AZF/Dazl1/Rbm/spermatogenesis/Y chromosome

Introduction

The human Y chromosome can be divided into recombining and non-recombining parts: the pseudo-autosomal regions contain genes with homologues on the X chromosome and the remainder which is excluded from recombination and carries genes which are mostly male specific. While the former genes are mainly ubiquitously expressed, many genes localized in the non-recombining region are restricted to a testis specific expression and these are considered to serve a role exclusively in testis determination and male germ cell development (Lahn and Page, 1997). These genes are scattered over the euchromatic regions on both of the Y chromosome’s arms but particularly deletions in Yq11 are relatively frequent in the human population and are often associated with infertility through non-obstructive azoospermia or severe oligozoospermia (reviewed by Vogt, 1998; Krausz and McElreavey, 1999). It was therefore initially postulated that an azoospermia factor (AZF) was localized in this region (Tiepolo and Zuffardi, 1976) and through recent refinement of molecular techniques and markers in Yq11 the AZF has been subdivided into at least three non-overlapping regions, designated as AZFa, b, c (Vogt et al., 1996). About 13% of men with non-obstructive azoospermia have microdeletions in the AZFc region, deletions in AZFa and b are less frequent (summarized in Vogt, 1998; Krausz and McElreavey, 1999). Testis biopsies of a small cohort of patients with azoo- or oligozoospermia have been investigated and different histological phenotypes have been associated with deletions of AZF intervals (Vogt et al., 1996). These authors suggest that deletions in the AZFa region are associated with Sertoli cell only syndrome, AZFb deletions with a premeiotic maturation arrest and AZFc phenotypes with a heterogeneous phenotype including Sertoli cell only syndrome as well as pre- and postmeiotic maturation defects. Results from different studies are not consistent with a direct link between phenotype and deletion interval (Pryor et al., 1997), and histology cannot predict the particular Y deletion present.

Several genes have been mapped into each of the AZF intervals (Lahn and Page, 1997) but for most of them their individual contribution to the AZF phenotype remains to be determined. However, biochemical characterization of some gene products and functional studies of different animal homologues led to the assignment of at least one strong candidate gene for each microdeletion interval, namely DFFRY (Brown et al., 1998) for AZFa, RBM (Ma et al., 1993; Elliott et al., 1997) for AZFa, and DAZ (Reijo et al., 1995) for AZFc, all of which may cause azoospermia if deleted.

Despite a growing number of screening programmes for deletions in infertile men involving the AZF regions, the link between RBM and DAZ deletions and impaired spermatogenesis is still formally unproven. All microdeletions described so far encompass a substantial amount of DNA and there is evidence that genes other than RBM or DAZ might also be affected by the deletions (Lahn and Page, 1997; Wong et al., 1999). Moreover, deletions within the AZFc region that apparently do not include the DAZ gene family have been reported in azoospermic individuals (Najmabadi et al., 1996; Stuppia et al., 1997; Foresta et al., 1997).

Proof of a causative link between RBM and DAZ genes and impaired spermatogenesis would be the detection of point mutations, small intragenic deletions or rearrangements in these genes, which should give rise to the same phenotype as observed in men with microdeletions of Yq11. This goal has not been achieved to date but is of importance as concerns arise about the functionality of the DAZ genes in general (Vereb et al., 1997; Agulnik et al., 1998).
Table I. Categories and numbers counted of normal and abnormal sperm heads. \( n \) = total number of sperm heads counted. The \( Daz^{TM1Hgu} \) allele is referred to as \(-\), the wild type \( Daz^{1} \) allele as \(+\); \( wt \) = wild type.

<table>
<thead>
<tr>
<th>Nr. genotype</th>
<th>normal</th>
<th>slightly abnormal</th>
<th>grossly abnormal</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>( % )</td>
<td>( % )</td>
<td>( % )</td>
</tr>
<tr>
<td>1 (+/-) ( y^{wt})</td>
<td>195</td>
<td>97.5 ( \pm 6.2 )</td>
<td>3</td>
<td>1.5 ( \pm 2.5 )</td>
</tr>
<tr>
<td>2 (+/-) ( y^{wt})</td>
<td>180</td>
<td>88.83 ( \pm 3.6 )</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>3 (+/-) ( y^{wt})</td>
<td>173</td>
<td>69.17 ( \pm 3.6 )</td>
<td>4</td>
<td>16</td>
</tr>
<tr>
<td>4 (+/-) ( y^{wt})</td>
<td>180</td>
<td>28.25 ( \pm 3.1 )</td>
<td>7</td>
<td>17</td>
</tr>
<tr>
<td>5 (+/-) ( y^{dl})</td>
<td>124</td>
<td>23.05 ( \pm 3.1 )</td>
<td>15</td>
<td>11</td>
</tr>
</tbody>
</table>

Figure 1

Figure 2
The individual features of these genes have hampered the investigation of the controversial role of RBM and DAZ as AZF: RBM is a multigene family of at least 20–40 micro-heterogeneous members on the Y chromosome. While a minimal critical region for RBM expression in AZFb has been determined (Elliott et al., 1997), it is difficult to assess whether deletions or sequence variations affect ‘the’ essential gene./

The situation for DAZ is even more complicated as the micro-heterogeneous multigene organization on the Y comes together with a highly related homologous gene on chromosome 3, DAZL1 (Saxena et al., 1996), whose function in relation to DAZ is poorly understood.

Two approaches have been used to make genotype-phenotype correlations of the candidate genes for AZF: one is to investigate the biochemical properties of RBM and DAZ, which both seem to be involved in RNA metabolism (Houston et al., 1998; Elliott et al., 1998), the other is to address this question in model organisms that are more accessible to study defined alterations of the genes. DAZ homologues of Drosophila (boule) (Eberhart et al., 1996), Xenopus (Xdazl) (Houston et al., 1998), and C. elegans (da2l1) (Karashima et al., 1997) have all been shown to function in male and/or female germline-specific processes. The mouse is, however, the only animal model in which both genes have been investigated. The conserved Y chromosomal location of Rbm (Elliott et al., 1996) has hampered a simple knock-out experiment, but asymmetrical pairing and exchange of Yp-Sxr has produced a sex reversed mouse line with deletions of the 25–50 Rbm copies (Capel et al., 1993; Laval et al., 1995). In one of these lines, namely Yd1, the number of residual Rbm genes was estimated as 2–4. The introduction of an Sry transgene into this line produced males with only low level transcription of Rbm paired with an increased proportion of abnormal sperm heads. The sperm count and the overall fertility of these animals was unaffected (Mahadevaiah et al., 1998). These authors attribute the observed sperm head abnormalities to the lack of Rbm function. However, since the deletion interval of the Yd1 chromosome is estimated to encompass 3–4 Mb, disruption of genes other than Rbm may also be involved in the Yd1 phenotype.

The human DAZ genes are a recent acquisition to the Y chromosome and are only found in humans and old world monkeys. The murine homologue Dazl1 (Reijo et al., 1996; Cooke et al., 1996) however is located on chromosome 17, and readily accessible for knock-out experiments. Heterozygote DazlTm1Hgu/+ animals have a reduced sperm count and show minor sperm tail defects, but are fertile. The reproductive organs of homozygous males and females are devoid of meiotic germ cells and DazlTm1Hgu/DazlTm1Hgu animals are therefore infertile (Ruggiu et al., 1997).

Since both DAZ and RBM are multigene families in man and since deletions of the Y chromosome can include both DAZ and RBM genes simultaneously and to varying extents (Pryor et al., 1997; Elliott et al., 1997), we reasoned that a part of the variability in phenotype might be due to interaction between the effects of deleting the two genes and/or other genes located in the vicinity. To mimic this in the mouse we crossed the Yd1 chromosome on a DazlTm1Hgu/+ background to investigate the resulting phenotype. Since a Y chromosomal localization of RBM is conserved in mammals, it is likely that genes interspersing the RBM family in humans might also be found in similar location in mouse. Hence, although the Yd1 deletion is not fully characterized with respect to affected genes other than Rbm, the same genes might be removed by human AZF deletions. If Dazl1 and Rbm (or other gene products) are acting in different molecular pathways these mutations might be expected to have additive or synergistic effects which might be expected to lead to infertility, whereas if acting in the same pathway the effect of combining the mutations might be minimal.

Figure 2. Histology. (a–d) Sections at stage VII of spermatogenesis. (a) +/+ Yw, normal with all germ cell types present. (b) DazlTm1Hgu/+ , all germ cell types present, but regions showing reduced round spermatids and absent elongating spermatids. Tubular lumen larger than normal. (c) +/+ Yd1, all germ cell types present, but slight reduction in round and elongating spermatids. (d) DazlTm1Hgu/+ Yd1 (number 8), all germ cell types present, but a reduction in round and elongating spermatids. Tubular lumen larger than normal. (e–j) DazlTm1Hgu/+ Yd1 (numbers 10 and 11). (e) Sertoli cell only tubule, with interstitial cell hyperplasia. (f) Pachytene arrest. (g) Apoptotic nuclei (arrows). (h–j) Syndactyly with micro- and macro-somatic aggregations (arrows). (k–l) Pachytene spermatids in tubules showing pachytene arrest. (l) Round spermatid symplast in tubule showing some round spermatid development. a–g: bar = 10 µm, h–j: bar = 40 µm.

Materials and methods

Mice

The control and DazlTm1Hgu/+ mice were maintained on a random bred MF1 (OLAC) background. The Yd1 males were produced as previously described (Capel et al., 1993; Laval et al., 1995) by BMC, at the MRC Radiobiology Unit. The DazlTm1Hgu/+ Yd1 males were produced by crossing male Yd1 and female DazlTm1Hgu/+ . The control animal was 9 weeks, all other males investigated were 13–14 weeks of age.

Sperm analysis

Sperm counts per epididymis were carried out as previously described (Searle and Beechey, 1974). Spermatozoa for morphology analysis were released from the epididymis into phosphate-buffered saline, the suspension smeared onto clean slides and allowed to dry. Slides were then fixed in methanol for 10 min at room temperature (RT) and air-dried. Preparations were silver-stained (Howell and Black, 1995).
observed in DazlTm1Hgu / H11001 (1997), the sperm count was considerably reduced, being only 7\%/ H11001 I and II were calculated from the mean square errors in the analyses of contrasts between the four classes. Homogeneity of variance tests necessary for the (GLM) analysis of variance assumed a 2\%/ H11003 eosin by standard methods. Sections 6\%/ H11001 thick were prepared from testes fixed in Bouin’s solution and then wax embedded. They were stained with haematoxylin and eosin by standard methods. Statistical analysis The results were analysed using Minitab12. General linear model (GLM) analysis of variance assumed a 2\times 2 cross-classification with the mean squares adjusted to allow for unequal replications of each genotype. Standard errors appropriate to each class shown in Tables I and II were calculated from the mean square errors in the analyses of variance and are employed in t-tests (with 7 degrees of freedom) of contrasts between the four classes. Homogeneity of variance tests (Bartlett’s and Levine’s) indicated that data transformation was only necessary for the ‘grossly abnormal’ percentages of sperm heads. These were analysed using a natural log transformation, hence standard errors are not shown in Table I. Results Sperm counts Sperm counts and testis weights for the different genotypes investigated are highly correlated (correlation coefficient of 0.969; Table II).

Although the DazlTm1Hgu/+ males are fertile (Ruggiu et al., 1997), the sperm count was considerably reduced, being only 45.9% (n = 3) of normal. Sperm counts for the Yd1 males were slightly impaired (Mahadevaiah et al., 1998) having a mean count that was 81.5% of normal (n = 3). The combination of heterozygous Dazl1 and Yd1 produced the most marked effect, the mean sperm count only being 16.6% of normal (n = 4). There was variation in this group, one animal resembled the Dazl1 heterozygotes, two were extremely low at 5.2% of normal (n = 2), and one was intermediate between them.

The numbers of spermatozoa from the DazlTm1Hgu/+ Yd1 mice were significantly less than both Dazl1/+ Yd1 and wild type Y (P < 0.0001) and also than the DazlTm1Hgu/+ with wild type Y (P < 0.01). Statistical analysis of variance showed that the interaction between the effects of deletion and heterozygosity were negligible (P = 0.485). The depression in the sperm count for heterozygous Y-deleted males could be adequately explained by a main effect of heterozygosity added to a smaller main effect of Y deletion. In biological terms, this means that the effects of the two mutations are not synergistic.

Sperm morphology

Heads

Two features are important for assessing head morphology. Firstly the insertion of the mid-piece into the head under normal circumstances should have a clear stepped appearance (Table I). Secondly the overall shape of the sperm head must be considered. Table I shows two examples of each of the varying types of sperm head identified. It is apparent that within such subjective categories minor variations are common. The grossly abnormal sperm heads in categories 2–6, where both assessment factors are changed, present few problems in classification. The changes in categories 1–1d are more subtle, and can be described as:

1: Normal; 1a: Mid-piece insertion normal, head showing a flattened frontal curvature; 1b: Mid-piece insertion normal, head with a rear projection above the mid-piece; 1c: Mid-piece insertion normal, curved hook of spermatozoa malformed; 1d: Mid-piece insertion abnormal, head shortened with very angular outline, hook not fully formed.

The numbers observed in each category for each genotype are shown in Table I. Categories 1c and 1d were observed predominantly in Yd1 and DazlTm1Hgu/+. Yd1 males.

It has previously been shown (Bruce et al., 1974) that sperm abnormalities can vary between 1 and 15% depending on strain. The figure of 2.5% for our control was at the lower limit. Analysis of variance showed very strong evidence (P = 0.00002) that there were fewer normal heads in the Yd1 groups than in the wild type Y-carrying groups (Table I). This was also the case for heterozygous Dazl1 males compared to males with two wild type Dazl1 alleles (P = 0.001). However, there is also considerable non-additivity since the two wild type Y-carrying groups do not differ significantly where there is strong evidence (P = 0.00004) that DazlTm1Hgu/+ Yd1 has far fewer normal heads than +/+ Yd1 animals. The DazlTm1Hgu/+ males exhibit a mean total of 11.2% abnormalities, Yd1 males 30.8% and DazlTm1Hgu/+ Yd1 males 73.8%. The increased level in Yd1 over DazlTm1Hgu/+ is due to the proportion of slight head abnormalities, the class 1a and 1b being far more frequent in the former. The elevated levels in the DazlTm1Hgu/+ Yd1 males reflect increased numbers of all classes of grossly abnormal spermatozoa, particularly the class 4 (tulip-headed spermatozoa).
The DazlTm1Hgu/− males, and while still at low levels were most frequent in the DazlTm1Hgu/+ tissue. All stages of spermatogenesis were present although multiple tails and bent or coiled tails may be due to defects in the structure of organelles such as the internal fibres or the outer sheath, and such spermatozoa have generally been considered incapable of fertilization. Multiple tails or mid-pieces (Figure 1a–c) were not seen in the control, they occurred at low levels in both the DazlTm1Hgu/+ (0.7%) and Yd1 (0.2%) males, and while still at low levels were most frequent in the DazlTm1Hgu/+ Yd1 males (2.1%) (Table III). Bent and coiled tails (Figure 1d–f) were more frequent in the DazlTm1Hgu/+ (41.1%, n = 3). They were also present in the Yd1 males but about four-fold lower in frequency (10.1%, n = 3). Unlike sperm head abnormalities the frequency of tail defects in the DazlTm1Hgu/+ Yd1 was not significantly increased in comparison with DazlTm1Hgu/+ males (31.3%, n = 4) (Table III). However in these males the defects were qualitatively more severe with extreme coiling and an apparent self-adhesion of the mid-piece and flagella (Figure 1g–l). Minor structural defects were also seen in areas of both the mid-piece and flagella (Figure 1j–l), these often splitting into two sub-components.

**Sections**

The control male exhibited normal testis histology (Figure 2a). Tubules were uniform in cross sectional diameter and interstitial tissue was normal in appearance. All the stages of spermatogenesis were present. The Yd1 males appeared virtually normal; the Yd1 chromosome, accompanied by the induction of sex reversal through an Sry transgene, leads to an increased production of spermatozoa with abnormal heads. Although our numbers for Yd1 related head abnormalities differed slightly

### Table III. Numbers counted of normal and abnormal sperm tails

<table>
<thead>
<tr>
<th>No.</th>
<th>Genotype</th>
<th>Tails</th>
<th>normal</th>
<th>abnormal</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>a</td>
<td>b</td>
<td>c</td>
</tr>
<tr>
<td>1</td>
<td>+/-Ywt</td>
<td>190</td>
<td>95 ± 4.9</td>
<td>10 5 ± 4.9</td>
<td>200</td>
</tr>
<tr>
<td>2</td>
<td>+/-Ywt</td>
<td>103</td>
<td>95</td>
<td>2</td>
<td>200</td>
</tr>
<tr>
<td>3</td>
<td>+/-Ywt</td>
<td>111</td>
<td>57.83 ± 2.8</td>
<td>88 42.17 ± 2.8</td>
<td>200</td>
</tr>
<tr>
<td>4</td>
<td>+/-Ywt</td>
<td>133</td>
<td>66</td>
<td>1</td>
<td>200</td>
</tr>
<tr>
<td>5</td>
<td>+/-Yd1</td>
<td>175</td>
<td>24 10.33 ± 2.8</td>
<td>22 10.33 ± 2.8</td>
<td>200</td>
</tr>
<tr>
<td>6</td>
<td>+/-Yd1</td>
<td>178</td>
<td>89.67 ± 2.8</td>
<td>22 10.33 ± 2.8</td>
<td>200</td>
</tr>
<tr>
<td>7</td>
<td>+/-Yd1</td>
<td>185</td>
<td>15</td>
<td></td>
<td>200</td>
</tr>
<tr>
<td>8</td>
<td>+/-Yd1</td>
<td>125</td>
<td>66 3 34.75 ± 2.5</td>
<td>58 37.5 ± 2.5</td>
<td>200</td>
</tr>
<tr>
<td>9</td>
<td>+/-Yd1</td>
<td>138</td>
<td>65.25 ± 2.5</td>
<td>58 3 34.75 ± 2.5</td>
<td>200</td>
</tr>
<tr>
<td>10</td>
<td>+/-Yd1</td>
<td>128</td>
<td>72 2</td>
<td></td>
<td>200</td>
</tr>
<tr>
<td>11</td>
<td>+/-Yd1</td>
<td>133</td>
<td>65 2</td>
<td></td>
<td>200</td>
</tr>
</tbody>
</table>

Abnormal tails are categorized as a: bent and coiled tails; b: multiple tails; and c: multiple mid-pieces. n = total number of sperm tails counted. The DazlTm1Hgu allele is referred to as −, the wild type Dazl allele as +; wt = wild type.

**Tails**

Sperm tails can be divided into two regions, the mid-piece and the flagellum. Primary defects in each region such as multiple tails and bent or coiled tails may be due to defects in the structure of organelles such as the internal fibres or the outer sheath, and such spermatozoa have generally been considered incapable of fertilization. Tubules containing apoptotic nuclei (1:100). The multinucleate aggregations (symplasts) were seen in a few tubules (3:100) (Figure 2h–j). These have been reported in a variety of situations (MacGregor et al., 1990; Odorisio et al., 1998) where knock-out or transgenic technologies have disrupted normal spermatogenesis. They may be related to a destabilization of the cytoskeletal apparatus maintaining the integrity of intercellular bridges between the developing germ cells (Russell et al., 1990). They usually originate from round spermatids, but other spermatogenic nuclei have been implicated (Ross et al., 1998). Males 10 and 11, with the lowest sperm counts, showed the most severely impaired spermatogenesis. Tubular size was variable and interstitial tissue increased in both cases. Smaller tubules were occasionally Sertoli cell only (Figure 2e), while others showed pachytene arrest (Figure 2f). Round and elongating spermatids were severely reduced in larger tubules. The lumen of such tubules was also bigger than those of the other genotypes being filled with Sertoli cell cytoplasm. Tubules containing apoptotic nuclei (Figure 2g) were more frequent (12:100 and 26:100), as was the presence of symplasts (Figure 2h–j) in these males (26:100 and 32:100). The remaining male, number 9, was intermediate between these extremes, showing impaired spermatogenesis.

**Discussion**

This study was aimed (i) to investigate the resulting phenotype of combined genetic modifications of Dazl1 and Rbm gene number in order to provide more evidence justifying a classification of both of them as candidates for an AZF, and (ii) to compare the phenotype with those of infertile or subfertile human individuals.

We have shown that the Dazl1 heterozygote phenotype is a reduction of sperm output and tail abnormalities mainly affecting the mid-piece. The replacement of a normal Y with the Yd1 chromosome, accompanied by the induction of sex reversal through an Sry transgene, leads to an increased production of spermatozoa with abnormal heads. Although our numbers for Yd1 related head abnormalities differed slightly

3027
from the figures reported by Mahadevaiah et al. (1998), the overall picture is the same. Mahadevaiah and colleagues’ report is based on spermatocytes entering the epididymis. In contrast, we isolated spermatozoa from the entire epididymis. These different sources of spermatozoa might account for the differing values of abnormal spermatozoa obtained rather than age dependent variations.

Apart from the assigned predominant phenotypes we observed a small overlap in the phenotypes of the mutations, i.e. the number of abnormal sperm heads was slightly increased in DazlTm1Hgu/ compared to wild type animals, and a small proportion of spermatozoa in Ydl-carrying animals exhibited tail defects. Ydl animals have also a lower sperm count than wild type animals.

These slightly overlapping phenotypes were enhanced when the Ydl chromosome was crossed onto a DazlTm1Hgu/+ background resulting in a significant drop of the sperm count, an increase of abnormal sperm heads and more prominent midpiece defects of the tails. These data suggest a model in which Rbm genes (or in the case of the Ydl chromosome all genes in the deletion), and Dazl, affect at least three different phenotypic pathways in mouse spermatogenesis. It is difficult to assess whether both genes act in the same or in different molecular pathways. However, since all observed phenotypes seem to be more severe in DazlTm1Hgu/+ Ydl animals, this suggests an additive or synergistic effect, and we assume that Rbm and Dazl are mainly acting in different molecular pathways. Since the phenotypes of both mutations on their own overlap only slightly, we conclude that the contribution of each gene to the main pathway of the other is only small.

Sperm head abnormalities and more strikingly sperm output vary between individual animals in DazlTm1Hgu/+ Ydl mice, whereas little variation is seen in all other genotypes studied here. It has been reported that Y chromosomes from different genetic backgrounds can affect the total percentage of sperm head abnormalities in inbred mouse strains (Krzanowska, 1976). An increase in specific types of abnormality has not been observed in the study of Krzanowska, in contrast to the situation observed in DazlTm1Hgu/+ Ydl animals. In these mice, the increase of specific abnormalities, e.g. the tulip-shaped class 4, is therefore not an effect of different genetic backgrounds, but is caused by reduced activity of the affected Y chromosomal genes and Dazl.

A variant penetration of the phenotype in DazlTm1Hgu/+ Ydl was also observed in testis sections. Some animals, i.e. number 8, were only slightly affected by the reduction of functional Dazl1 and Rbm genes and exhibited a mild phenotype that resembled a DazlTm1Hgu/+ phenotype. The tubules of these animals had germ cells of all stages and a complete depletion of germ cells was never observed. In contrast, very badly affected animals (numbers 10 and 11) showed only few tubules with complete spermatogenesis but predominantly tubules with arrested pachytene stages or Sertoli cells only. Fertilization ability of these most severely affected animals has not been investigated.

The variation in severity of the double mutant phenotype could also be caused by strain-dependent segregation of different modifier genes. Although initially generated by modifying a C129-derived allele, the Dazl null allele was maintained on a random bred MFI1 strain. Crossing of this null allele in different inbred strains did not result in a variable phenotype (data not shown). Thus the Dazl1 null phenotype seems not to be susceptible to the action of modifying genes. Variations of sperm output in different animals has not been observed for the Ydl chromosome, which was also crossed onto the MF1 background (Mahadevaiah et al., 1998). However, involvement of modifying genes cannot be ruled out to contribute to the observed individual variations in DazlTm1Hgu/+ Ydl males.

The penetration of the AZF phenotype is also apparent in human patients with deletions in the AZFc, ranging from Sertoli cell only to meiotic or spermatid maturation arrests that lead to absence of mature (azoosperma) or only few spermatozoa (oligozoosperma). In some cases increased rates of dysmorphic sperm heads (oligoasthenoteratozoospermia) have also been observed (Vogt et al., 1996).

This variability might point to an involvement of other factors apart from the single candidate gene DAZ, influencing the severity of the AZFc phenotype in humans. Those factors can be environmental, epigenetic or genetic in nature.

The data presented in this study, however, show that the variable AZFc phenotype in humans is mimicked in the mouse by copy number reduction of Dazl1 and Rbm, and/or additional Y chromosomal genes. Assuming similar functions during spermatogenesis for Rbm and Dazl1 in humans and mouse, this observation provokes the question as to whether RBM is also involved in the AZFc phenotype. According to the data presented by Elliott and colleagues (1997) RBM expression from the minimal critical region in AZFb is unaffected in the AZFc patients studied, excluding a direct involvement of RBM itself. Another possible explanation would be the involvement of other genes that are localized in the vicinity of or in the murine Rbm cluster itself. Hence, the similar phenotype observed in this study would not reflect an effect mediated by the copy number reduction of Rbm but by the removal or silencing of those different genes, which might on the other hand be localized in the AZFc region on the human Y. Deletions in AZFc also reduce the distance between the heterochromatic region of the Y chromosome and active genes. Hence, position effects evoking silencing of active genes to various extents might contribute to the variability of the AZFc phenotype in some individuals.

In summary, this study has shown firstly that Dazl1 and Rbm and/or other genes localized in the Yd1 deletion interval have multiple targets that contribute to different areas of the establishment and progression of spermatogenesis. Secondly, that a reduction of active gene product of those genes gives rise to a phenotype similar to the heterogeneous AZFc phenotype observed in humans.

Acknowledgements

The authors would like to thank P. Bourgoyne for providing Ydl mice, M. Taggart and the animal technicians of the MFAA, Edinburgh for supporting mouse work, P.T.K. Saunders and M. Ruggiu for critical comments on the manuscript. This work was supported by the MRC and The Wellcome Trust through a grant to T.V. (no. 055047).
References


Received on May 17, 1999; accepted on September 10, 1999

An AZFc-like phenotype in deleted Y, Dazfl +/- mice