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Exon structure of the DAZ/SPGY gene in Yq11 and identification of its ancestor homologue SPGYLA on chromosome 3

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DAZ and SPGY1 genes isolated from the AZFc region in Yq11 are expressed exclusively in germ cells. They are deleted in 4-12% of azoo/oligozoospermic patients.The basic goal of all experiments in this study was to contribute to the resolution of the molecular complexity of the human DAZ/SPGY gene locus in distal Yq11 and to isolate its proposed autosomal ancestor. This goal was met by analysing the DAZ/SPGY exon-intron structure and identification of its ancestor gene SPGYLA on the short arm of chromosome 3. These results were achieved as follows.

PCR with specific primers from the N-terminal part of the Y linked DAZ and CT340 sequences (autosomal DAZ candidate copy) identified five groups of cDNA clones from the cDNA pool isolated by crosshybridization to SPGY1. The first group comprised truncated cDNAs lacking the N-terminal, the second group was characterized by DAZ homologous cDNA clones containing only part of the N-terminal sequence region; the third and fourth group comprised CT340 homologous cDNA clones characterized by different parts of the N-terminal sequence region; the fifth group was represented by one cDNA clone (CT351) containing a larger N-terminal region as known from DAZ gene. Complete sequence analysis of CT351 provides strong evidence that DAZ and SPGY1 cDNAs belong to the same gene structure in Yq11 which consequently was designated as the DAZ/SPGY gene. The CT340 homologous cDNA clones support our view of an autosomal as well as X chromosomal DAZ/SPGY gene copy with a distinct sequence structure.

A PCR based cloning-sequencing strategy was found to be an efficient way for the determination of the DAZ/SPGY exon/intron structure and the identification of its boundary sequences essential for exon specific mutation analysis in SSCP (Single-Strand-Conformation-Polymorphism) experiments. There are 10 DAZ/SPGY exons of different

lengths (exon 1: 270 bp; exon 2: 147 bp; exon 3: 92 bp; exon 4: 52 bp; exon 5: 64 bp; exon 6: 140 bp; exon 7: n x 72 bp; exon 8: 35 bp; exon 9: 99 bp; exon 10: 917 bp) interrupted by 9 introns (intron 1: 7-8 kb; intron 2: 300 bp; intron 3: 600 bp; intron 4: 500 bp; intron 5: 89 bp; intron 6: 1.4 kb; interspersed intron 7: n x 2.3 kb; last intron 7: 800 bp; intron 8: 4.5 kb; intron 9: 4 kb). Assuming that DAZ/SPGY is a single copy gene with 24 copies of exon 7, its gene structure covers about 80 kb of genomic DNA in Yq11.

The autosomal DAZ/SPGY copy was identified by sequence analysis of the CT340 homologous cDNA clones. CT355 is the only full length sequence (2921 bp) containing a complete open reading frame which encodes an RNA binding protein with 289 aminoacids. CT355 was mapped to the short arm of chromosome 3 by FISH experiments and was designated as SPGYLA (**SPGY**-**L**ike-**A**utosomal). The chromosomal localization was confirmed by PCR with CT355 specific primers on genomic DNA samples of a hamster-human hybrid cell panel containing different sets of human chromosomes. Like DAZ/SPGY, SPGYLA was found to be expressed specifically in human testis.

Most intriguing appears the result that the SPGYLA coding sequence is more homologous to that of the homologous mouse gene Dazla than to that of the homologous human Y chromosomal DAZ/SPGY gene. SPGYLA, like the mouse gene Dazla, contains only one 72 bp exon 7 unit. Furthermore SPGYLA contains a 130 bp sequence region (CTA box) present in Dazla but not in DAZ/SPGY. The SPGYLA sequence structure was also found in the Drosophila gene boule. These indicate that SPGYLA is highly conserved, and suggest that SPGYLA has a function in human spermatogenesis similar to that of Dazla in mouse spermatogenesis or that of boule in Drosophlia spermatogenesis.

Zoo-blot experiments using genomic DNA samples of different mammalian indicated that the DAZ/SPGY gene locus on the human Y chromosome may have first translocated from its ancestor autosomal SPGYLA genome position during primate evolution. The time range of this translocation event was estimated to be around 35-50 million years ago, after the divergence of platyrrhini (new world monkeys) and before the divergence of catarrhini (old world monkeys). Therefore one may speculate that the DAZ/SPGY gene has a primate specific spermatogenesis function different from that of SPGYLA but most likely interacting with it.

Initial genomic analysis of the CTA box sequence unit in SPGYLA revealed that it contains the 3´end of SPGYLA exon 8 (16 bp). The first 35 nucleotides of this exon are homologous to exon 8 of the DAZ/SPGY gene. Consequently, SPGYLA exon 8 is 16 nucleotides longer than exon 8 of DAZ/SPGY. The homologous 16 nucleotide stretch was identified in the 5´region of intron 8 in the DAZ/SPGY gene which means that evolution to human has created a DAZ/SPGY specific 5´end splicing signal (CTA→GTA) in the ancestral exon 8 sequence. The other 114 bp of the CTA box were identified as an unique exon of SPGYLA (exon 9). The DAZ/SPGY exon 9 therefore becomes SPGYLA exon 10 and the DAZ/SPGY exon 10 represents SPGYLA exon 11.

SSCP experiments were performed to search for point mutations in exons 1, 2 and 4 of the DAZ/SPGY in 70 patients and in exon 9 of the SPGYLA gene in 150 patients. These cases were selected for a normal karyotype and absence of microdeletions in Yq11. Sterility of these men (azoospermia or severe oligozoospermia) could not be explained by any clinical diagnostic tool (idiopathic sterility). The SSCP experiments failed to find any point mutation

in the DAZ/SPGY and the SPGYLA. Although not all exons have been analysed in this study, the rate for point mutations in DAZ/SPGY and SPGYLA is expected to be lower than that observed for the complete deletion of DAZ/SPGY in distal Yq11.