Diverse spermatogenic defects in humans caused by Y chromosome deletions encompassing a novel RNA-binding protein gene*

Renee Reijo[1], Tien-Yi Lee[l], Pia Salo[5], Raaji Alagappan[1], Laura G.Brown[1], Michael Rosenberg[1,3], Steve Rozen[2], Tom Jaffe[1], Donald Straus[3], Outi Hovatta[6], Albert de la Chapelle[5], Sherman Silber[4] and David C.Page[1]

[1]Howard Hughes Medical Institute and [2]Center for Genome Research, Whitehead Institute and Department of Biology, Massachusetts Institute of Technology, 9 Cambridge Center, Cambridge, MA 02142, [3]Department of Biology, Brandeis University, Waltham, MA 02254, [4]In Vitro Fertilization Program, St Luke's Hospital, St Louis, MO 63017, USA, [5]Department of Medical Genetics, University of Helsinki and [6]Family Federation of Finland, Kalevankatzu 16, Helsinki, Finland

We have detected deletions of portions of the Y chromosome long arm in 12 of 89 men with azoospermia (no spermatozoa in their semen). No Y deletions were detected in their male relatives or in 90 other fertile males. The 12 deletions overlap, defining a region likely to contain one or more genes required for spermatogenesis (the azoospermia factor, *AZF*). Deletion of the *AZF* region is associated with highly variable testicular defects, ranging from the complete absence of germ cells to spermatogenic arrest with the occasional production of condensed spermatids. We found no evidence of *YRRM* genes, recently proposed as *AZF* candidates in the *AZF* region. The region contains a single-copy gene, *DAZ* (deleted in azoospermia), which is transcribed in the adult testis and appears to encode an RNA-binding protein. The possibility that *DAZ* is *AZF* should now be explored.
*Key words: AZF/DAZ/*RNA-binding protein gene/spermatogenic defects/Y chromosome deletions

Introduction

Human spermatozoa are produced via a complex developmental process. Progression from spermatogonial stem cells to mature spermatozoa requires 65 days and involves an elaborate succession of distinct cell types (Clermont, 1966; Dym, 1994). The process is punctuated by at least three mitotic and two meiotic divisions. Meanwhile, the genome is repackaged — with protamines rather than

*Previously published in *Nature Genetics* (1995) 10, 383–393 Reijo *et al.* Reprinted by kind permission.

YRRM appear to be expressed specifically in the testis. In summation, there are many molecular parallels between *DAZ* and *YRRM*.

It is tempting to speculate that testis-specific RNA-binding proteins encoded by *DAZ* and *YRRM* might function in male germ cell development. (*YRRM* may play a role in spermatogenesis even though it is not *AZF*, a locus to which attention is drawn because of its frequent deletion in human populations.) A precedent may be provided by the *Drosophila Rb97D* gene which, like human *DAZ* and *YRRM*, encodes a protein with a single RNP/RRM domain. Loss of *Rb97D* function results in the degeneration of early spermatogenic cells and azoospermia (Karsch-Mizrachi and Haynes, 1993). Indeed, there is evidence that RNA-binding proteins function in mammalian spermatogenesis. In mice, protamine expression is translationally regulated by a protein that binds the protamine mRNA's 3′-untranslated region (Kwon *et al.*, 1993), and other genes expressed during spermatogenesis may also be post-transcriptionally regulated (Hecht, 1993). It is interesting that the testes are grossly abnormal in males with fragile X syndrome, the only heritable human disease traced to a defective RNA-binding protein (Butler *et al.*, 1993; Siomi *et al.*, 1993). Perhaps RNA-binding proteins and post-transcriptional mechanisms figure prominently in the regulation of male germ cell development in mammals.

Acknowledgements

We thank B.Raphael, A.Hashem, R.Dredge, M.Velez-Stringer and C.Rosenberg for experimental and analytic contributions; A.Chandley and T.Hargreave for DNA from patients KLARD, NIKEI and KUPAU; A.McMurray and J.Segre for advice on exon trapping; the Lawrence Livermore National Laboratory for the flow-sorted cosmid library; and P.Bain, G.Fink, K.Jegalian, N.Kenmochi, B.Lahn, R.Polakiewicz and J.Seligman for comments on the manuscript. This work was supported by National Institutes of Health, US Department of Agriculture, Academy of Finland, Sigrid Juselius Foundation and the Finnish Cultural Foundation. R.R. was the recipient of a Damon-Runyon/Walter Winchell fellowship.

References

Andersson, M. *et al.* (1988) Y:autosome translocations and mosaicism in the aetiology of 45.X maleness: assignment of fertility factor to distal Yq11. *Hum. Genet.*, **79,** 2–7.

Borgaonkar, D.S. and Hollander, D.H. (1971) Quinacrine fluorescence of the human Y chromosome. *Nature*, **230,** 52.

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

Butler, M.G. *et al.* (1991) Anthropometric comparison of mentally retarded males with and without the fragile X syndrome. *Am. J. Med. Genet.*, **38,** 260–268.

Carr, D.H., Haggar, R.A.S. and Hart, A.G. (1968) Germ cells in the ovaries of XO female infants. *Am. J. Clin. Pathol.*, **49,** 521–526.

Clermont, Y. (1996) Renewal of spermatogonia in man. *Am. J. Anat.*, **118,** 509–524.

Duyk, G.M., Kim, S., Meyers, R.M. and Cox, D.R. (1990) Exon trapping: a genetic screen to identify candidate transcribed sequences in cloned mammalian genomic DNA. *Proc. Natl. Acad. Sci. USA*, **87,** 8995–8999.

Dym, M. (1994) Spermatogonial stem cells of the testis. *Proc. Natl. Acad. Sci. USA*, **91,** 11287–11289.

Fisher, E.M.C. *et al.* (1990) Homologous ribosomal protein genes on the human X and Y chromosomes: escape from X inactivation and possible implications for Turner syndrome. *Cell*, **63,** 1205–1208.

Fitch, N., Richer, C.-L., Pinsky, L. and Kahn, A. (1985) Deletion of the long arm of the Y chromosome and review of Y chromosome abnormalities. *Am. J. Med. Genet.*, **20,** 31–42.

Foote, S., Vollrath, D., Hilton, A. and Page, D.C. (1992) The human Y chromosome: overlapping DNA clones spanning the euchromatic region. *Science*, **258,** 60–66.

Haldi, M. *et al.* (1995) Large human YACs constructed in a *rad52* strain show a reduced rate of chimerism. *Genomics*, **24,** 478–484.

Hartung, M., Devictor, M., Codaccioni, J.L. and Stahl, A. (1988) Yq deletion and failure of spermatogenesis. *Ann. Genet.*, **31,** 21–26.

Hecht, N.B. (1993) In Desjardins, C. and Eing, L.L. (eds), *Cell and Molecular Biology of the Testis.* Oxford University Press, New York, NY, USA, pp. 400–432.

Holland, J., Coffey, A.J., Giannelli, F. and Bentley, D.R. (1993) Vertical integration of cosmid and YAC resources for interval mapping on the X-chromosome. *Genomics*, **15,** 297–304.

Hull, M.G.R. *et al.* (1985) Population study of causes, treatment, and outcome of infertility. *Br. Med. J.*, **291,** 1693–1697.

Johnson, M.D., Tho, S.P.T., Behzadian, A. and McDonough, P.G. (1989) Molecular scanning of Yq11 (interval 6) in men with Sertoli cell-only syndrome. *Am. J. Obstet. Gynecol.*, **161,** 1732–1737.

Karsch-Mizrachi, I. and Haynes, S.R. (1993) The *Rb97D* gene encodes a potential RNA-binding protein required for spermatogenesis in *Drosophila*. *Nucleic Acids Res.*, **21,** 2229–2235.

Kenan, D.J., Query, C.C. and Keene, J.D. (1991) RNA recognition: towards identifying determinants of specificity. *Trends Biochem.*, **16,** 214–220.

Kobayashi, K. *et al.* (1994) PCR analysis of the Y chromosome long arm in azoospermic patients: evidence for a second locus required for spermatogenesis. *Hum. Mol. Genet.*, **3,** 1965–1967.

Kozak, M. (1986) Point mutations define a sequence flanking the AUG initiator codon that modulates translation by eukaryotic ribosomes. *Cell*, **44,** 283–292.

Kwon, Y.K., Murray, M.T. and Hecht, N.B. (1993) Proteins homologous to the *Xenopus* germ cell-specific RNA-binding proteins p54/p56 are temporally expressed in mouse male germ cells. *Dev. Biol.*, **158,** 90–100.

Lamar, E.E. and Palmer, E. (1984) Y-encoded species-specific DNA in mice: evidence that the Y chromosome exists in two polymorphic forms in inbred strains. *Cell*, **37,** 171–177.

Lefebvre, S. *et al.* (1995) Identification and characterization of a spinal muscular atrophy-determining gene. *Cell*, **80,** 155–165.

Lindsley, D. and Tokuyasu, K.T. (1980) In Ashburner, M. and Wright, T.R.F. (eds), *The Genetics and Biology of Drosophila.* Academic Press, London, UK, pp. 226–294.

Lucotte, G. and Ngo, Y.Y. (1985) p491, a highly polymorphic probe, that detects *Taq*1 RFLPs on the human Y chromosome. *Nucleic Acids Res.*, **13,** 8285.

Ma, K. *et al.* (1992) Towards the molecular localisation of the AZF locus: mapping of microdeletions in azoospermic men within 14 subintervals of interval 6 of the human Y chromosome. *Hum. Mol. Genet.*, **1,** 29–33.

Ma, K. *et al.* (1993) A Y chromosome gene family with RNA-binding protein homology: candidates for the azoospermia factor AZF controlling human spermatogenesis. *Cell*, **75,** 1287–1295.

Magram, J. and Bishop, J.M. (1991) Dominant male sterility in mice caused by insertion of a transgene. *Proc. Natl. Acad. Sci. USA*, **88,** 10327–10331.

Page, D.C. *et al.* (1987) The sex-determining region of the human Y chromosome encodes a finger protein. *Cell*, **51,** 1091–1104.

Page, D.C., Fisher, E.M.C., McGillivray, B. and Brown, L.G. (1990) Additional deletion in sex-determining region of human Y chromosome resolves paradox of X,t(Y;22) female. *Nature*, **346,** 279–281.

Rosenberg, M., Przybylska, M. and Straus, D. (1994) RFLP subtraction: a method for making libraries of polymorphic markers. *Proc. Natl. Acad. Sci. USA*, **91,** 6113–6117.

Roy, N. *et al.* (1995) The gene for neuronal apoptosis inhibitory protein is partially deleted in individuals with spinal muscular atrophy. *Cell*, **80,** 167–178.

Silber, S.J. (1989) The relationship of abnormal semen parameters to male fertility. *Hum. Reprod.*, **4,** 947–953.

Silber, S.J. (1995) Sertoli cell-only revisited. *Hum. Reprod.*, **10,** 1031–1032.

Sinclair, A.H. *et al.* (1990) A gene from the human sex-determining region encodes a protein with homology to a conserved DNA-binding motif. *Nature*, **346,** 240–244.

Siomi, H., Siomi, M.C., Nussbaum, R.L. and Dreyfuss, G. (1993) The protein product of the fragile X gene, FMR1, has characteristics of an RNA-binding protein. *Cell*, **74,** 291–298.

Skare, J. *et al.* (1990) Interstitial deletion involving most of Yq. *Am. J. Med. Genet.*, **36,** 394–397.

Straus, D. and Ausubel, F.M. (1990) Genomic subtraction for cloning DNA corresponding to deletion mutations. *Proc. Natl. Acad. Sci. USA*, **87,** 1889–1893.

Tiepolo, L. and Zuffardi, O. (1976) Localization of factors controlling spermatogenesis in the nonfluorescent portion of the human Y chromosome long arm. *Hum. Genet.*, **34,** 119–124.

Vogt, P. *et al.* (1992) Microdeletions in interval 6 of the Y chromosome of males with idiopathic sterility point to disruption of *AZF*, a human spermatogenesis gene. *Hum. Genet.*, **89,** 491–496.

Vollrath, D. *et al.* (1992) The human Y chromosome: a 43-interval map based on naturally occurring deletions. *Science*, **258,** 52–59.

Yen, P.H. *et al.* (1990) Frequent deletions of the human X chromosome distal short arm result from recombination between low copy repetitive elements. *Cell*, **61,** 603–610.

Genetics and Assisted Human Conception

André Van Steirteghem,
Paul Devroey and Inge Liebaers

Human Reproduction
Volume 11 Supplement 4 December 1996
Oxford University Press