Searching for candidate genes for male infertility

B. N. Truong[1], E.K. Moses[2], J.E. Armes[3], D.J. Venter[3,4], H.W.G. Baker[1]

[1]Melbourne IVF Centre, [2]Pregnancy Research Centre, Department of Obstetrics and Gynaecology, Royal Women's Hospital, University of Melbourne, Victoria 3053, Australia

[3]Departments of Pathology and Obstetrics/Gynaecology, University of Melbourne, Victoria 3010, Australia

[4]Murdoch Children's Research Institute, Royal Children's Hospital, Victoria 3052, Australia

Asian J Androl 2003 Jun; 5: 137-147

Keywords: **male infertility; genetics; spermatogenesis; human genome; database; tissues; array**

Abstract

Aim: We describe an approach to search for candidate genes for male infertility using the two human genome databases: the public University of California at Santa Cruz (UCSC) and private Celera databases which list known and predicted gene sequences and provide related information such as gene function, tissue expression, known mutations and single nucleotide polymorphisms (SNPs). **Methods and Results:** To demonstrate this in silico research, the following male infertility candidate genes were selected: (1) human BOULE, mutations of which may lead to germ cell arrest at the primary spermatocyte stage, (2) mutations of casein kinase 2 alpha genes which may cause globozoospermia, (3) DMR-N9 which is possibly involved in the spermatogenic defect of myotonic dystrophy and (4) several testes expressed genes at or near the breakpoints of a balanced translocation associated with hypospermatogenesis. We indicate how information derived from the human genome databases can be used to confirm these candidate genes may be pathogenic by studying RNA expression in tissue arrays using *in situ* hybridization and gene sequencing. **Conclusion:** The paper explains the new approach to discovering genetic causes of male infertility using information about the human genome.

1 Introduction

1.1 The human genome

The sequencing of the Human Genome has opened new opportunities for identifying disease causing mutations. In this paper we demonstrate the new computer based (in silico) searching methods the human genome databases for male infertility candidate genes using the publicly funded Human Genome Project (which is managed by the University of California at Santa Cruz (UCSC) (http://genome.ucsc.edu/goldenPath/decTracks.html) and the privately funded, Celera Genome library (http://www.celera.com).

The UCSC data present over 95 % complete of the human genome. About 90 % of the sequence is highly accurate in a "finished" state and only about 10 % is "draft" quality. UCSC itself does no sequencing but collects and puts sequence information from other sources together and presents them online. The UCSC associated centres use the overlapping clones method to map and sequence the genome. This method sequences small fragments from specific chromosomes. These sequences are then aligned in the genome using BAC markers [1, 2].

The Celera genome library is claimed to be the most complete and accurate. DNA in this library is extracted from volunteers of different ages and both sexes. The genome was broken down into several sequenced at the same time and put back into their proper order. This method is called the shotgun sequencing technique [1, 3].

Both the UCSC and Celera genome databases provide information about gene structures including exons and introns, start and stop codons, sequences of transcripts and proteins. They also give gene and protein functions, tissue expression, related diseases and links to papers in journals and comparative data from other organisms. Other powerful services in these libraries include sequence comparison tools named BLAT in UCSC and BLAST or sequence analysis in the Celera library. These programs are used to compare sequences with the genome database in the libraries. Although these libraries should contain the same information, annotation of the genes on the chromosomes are different so that most of the information from one library cannot be used simply to search in the other. Also, some genes are annotated in one library but not in the other.

The UCSC and Celera genome websites also link with other databases such as GenBank, Online Mendelian Inheritance in Man (OMIM) and PubMed. The functions of these libraries are described briefly in Table 1.

Table 1. Description of databases and search tools.

Web-site	Description
Database (http://www.celera.com)	This website provides information about gene structure including start and stop codons, exon and intron maps and transcript size. Other information includes gene functions, tissue expression, protein composition, mutations and comparative data from other model organisms. Searching in this website can be performed using gene symbols,position on chromosome, sequence tagged site (STS) and Bacterial Artificial Chromosome (BAC) makers.
UCSC (http://genome.ucsc.edu/goldenPath/decTracks.html)	Information and searching in this library are very similar to those in Celera, although the information comes from different sources and some terminology is different.
GeneBank (http://www.ncbi.nlm.nih.gov/Genbank/index.html)	GenBank is a genetic sequence database, which collects all publicly available DNA sequences and serves them together online. A new release is made every two months. Currently, GenBank contains more than 17 billion bases from over 100,000 organisms. Searching for sequences on the GenBank website is possible usinggene names, gene symbols and GenBank account (if known).
OMIM (http://www.ncbi.nlm.nih.gov:80/entrez/query.fcgi?db=OMIM)	This database is a catalogue of human genes and genetic disorders, with descriptions of the diseases and references. It also contains copious links to libraries of papers, sequences and additional resources at NCBI and elsewhere. Searching OMIM is by disease or gene name or code (MIM number), chromosome position or cytoband [3].

1.2 Causes of male infertility

It is estimated that about 13 % of couples in Western societies seek treatment for infertility and 40 % have male causes [4]. Some genetic causes of male infertility are known [5]. However the causes of most types of male infertility are unclear (Table 2). Some known aetiologies cause only infertility, while others affect general health. For example men with Yq microdeletions, XXY karyotype and androgen insensitivity usually have testicular atrophy and may have signs of androgen deficiency [6]. Myotonic dystrophy and cystic fibrosis usually present with other clinical characteristics such as weakness, myotonia and failure to thrive in infancy or severe respiratory infections. In contrast, the infertility from hypospermatogenesis and congenital absence of the vasa, are incidental (OMIM).

Table 2. Classification of causes of male infertility.

Treatment categories	Causes of male infertility
Untreatable sterility (13 %)	Primary seminiferous tubule failure (12.9 %) including:
	(1) Sex chromosome aneuploidies: XYY, XXY
	(2) Single gene defects: Yq microdeletions and androgen receptor defects
	(3) Past undescended testes
	(4) Past X irradiation/cytotoxic therapy
	(5) Past mumps orchitis
	(6) Idiopathic
	Structural defects of sperm: teratozoospermia and zero motility (0.1%)
Potentially treatable (12 %)	Genital tract obstructions
	Sperm autoimmunity
	Gonadotrophin deficiency or suppression
	Coital disorders

	Reversible drug , toxin or illness effects
Untreatable subfertility (75 %)	Oligozoospermia (35 %)
	Asthenozoospermia and teratozoospermia (35 %)
	Normal semen but defective sperm-oocyte interaction (5 %)

The new reproductive technologies can help some of these men to father their own children. However, there is a concern about passing on mutations and an infertility condition to future generations [7]. Further-more, discovery of new genetic causes of male infertility may have therapeutic implications. Therefore, it is necessary to study the aetiology and modes of transmission of infertility [8].

Before the human genome databases became available, candidate genes for male infertility were detected by classical clinical genetic methods or animal model studies. Classical methods involved studies of families and individual who presented with specific male infertility phenotypes or karyotype analysis [6, 9-23]. The latter lead to the discovery of many chromosome abnormalities including sex chromosome aneuploidies such as XXY and XYY syndromes [6, 9-11] and autosomal chromosome translocations [12-14]. Classical methods also identified numbers of single gene defects such as mutations of genes for hormones or receptors, the genes involved in clinical syndromes associated with infertility such as myotonic dystrophy and cystic fibrosis and microdeletions on the human Yq chromosome causing severe defects of spermatogenesis [15-20].

More recently, gene defects have been suggested from animal models such as transgenic gene knockout mice and Drosophila mutations. These include casein kinase 2 alpha knockout with defective development of the acrosome [22, 23], and spermatogenic arrest at the primary spermatocyte stage in the fruit fly due to mutations in the boule gene [24] (described below). However, genes affecting testicular development or spermatogenesis in animals do not necessarily have a human homologue. For example, mice with inactivation of cyclic AMP response element modulator t (CREMt) have germ cell arrest at the level of round spermatids. Although many studies proved that CREM is involved in the maturation of human spermatids, few if any men with germ cell arrest have been found to have defects in this gene [25, 26]. Similarly, mice with mutation of the insulin-like hormone 3 (Insl3) gene have abnormal gubernaculum growth and bilateral cryptorchidism [27-29]. However, sequencing of this gene in boys with cryptorchidism has not identified a high rate of mutation of this gene [30]. Therefore, animal studies do not always help in the detection of relevant human genetic defects [31].

1.3 Male infertility candidate genes

1.3.1 Dystrophia Myotonica Protein Kinase (DMPK) gene: Males with Myotonic Dystrophy (MD) have reduced fertility due to progressive primary seminiferous tubule failure [32, 33]. MD is usually caused by the expansion of trinucleotide (CTG)n repeats in DMPK gene on chromosome 19q13.3. Interestingly this gene does not appear to have a role in spermatogenesis. Theoretically the CTG expansion may cause distortion of chromosome structure and dysregulation of genes adjacent to DMPK, particularly the dystrophia myotonica containing WD repeat motif (DMWD) or dystrophia myotonica related number 9 (DMR-N9) gene [34, 35]. The latter gene is 1.2 kbp upstream of DMPK and strongly expressed in testis and may have a regulatory function during spermatogenesis [34-36]. Jansen *et al*, (1995) [37] found that DMR-N9 mRNA is expressed in all neural tissues early in mouse embryogenesis and in mature testis tubules where it is restricted to spermatocytes of stages VIII to XII of the spermatogenic cycle. He concluded that the DMR-N9 gene is a candidate for being involved in the mental and testicular manifestations of MD. Therefore, DMR-N9 can be seen as a candidate gene for hypospermatogenesis in men without MD [36-39].

1.3.2 Casein kinase 2: Xu *et al*, 1999 [23] showed that protein casein kinase 2 alpha (Csnk2a) is preferentially expressed in late stages of mouse spermatogenesis. Male mice whose Csnk2a gene has been disrupted by transgenesis are infertile with globozoospermia (acrosomeless sperm). Human globozoospermia is a rare condition (~0.1 % of all male infertility patients) of unknown mode of inheritance which is characterised by the complete absence of the acrosome, and sperm with small spherical heads and results from a failure of differentiation of a sperm specific proacrosomal granule of the Golgi complex [40]. While occasional globozoo-spermic sperm are seen in the semen of some fertile men, total absence of the acrosome is a very rare condition. The murine studies pose the question whether mutations in either of the two human CSNK2A and B genes cause human globozoospermia.

1.3.3 Boule gene: Microdeletions of the long arm of the Y chromosome (Yq) involving RNA binding protein genes such as Deleted in AZoospermia (DAZ) cause spermatogenic defects [24, 41, 42]. These genes are related to a Drosophila spermatogenic gene called boule. Eberhart *et al* (1996) [24] discovered the Drosophila boule gene on chromosome 3R. This gene also encodes an RNA binding protein that has 19 % similarity in protein sequence and 59 % similarity in structure of the RNA binding site with human DAZ. Mutation of the Drosophila boule gene causes male sterility from spermatogenic arrest. Xu *et al* (2001) [22] discovered a Drosophila boule homolog on human chromosome 2q33 which was named as the human BOULE gene. Human BOULE and Drosophila boule genes have 42 % similarity in protein sequence and 80 % similarity in structure of RNA binding site. Both genes also have testis specific expression at a late stage of spermatogenesis. Because the genetic causes of human spermatogenic arrest are largely unknown and the phenotype is very close to Drosophila boule, human BOULE is an important candidate gene for germ cell arrest at the primary spermatocyte stage.

1.3.4 Genes at the break points of balanced autosomal translocations: Balanced autosomal translocations are often associated with defects of spermatogenesis, especially spermatocyte meiosis anomalies resulting in azoospermia or oligospermia, usually without other severe defects because the number of genes in the genome is normal [43]. It is hypothesised that the translocation affects the expression and function of genes located at or near the breakpoints that may have a role in spermatogenesis [44]. Another possibility is that the translocation may interfere with pairing of chromatids during meiosis and lead to defects in this process [45].

2 Methods

As indicated above, mutations or defects in expression of DMR-N9, Casein kinase 2 α, BOULE and genes at the break point of the translocations are strong candidates to cause human infertility. In this study, we outline how to search for information about these candidate genes using the two human genome libraries. This includes (1) finding information about specific genes and adjacent genes using the gene names and/or symbols, (2) identifying candidate genes in specific regions of chromosomes and (3) BLAST searching using known sequences from other databases.

2.1 Gene names and symbols

Three candidate genes will be focused on: CSNK2A, BOLL and DMR-N9.

Searching on the Casein Kinase 2 alpha gene name and CSNK2a gene symbol in the UCSC library gave two results, which are CSNK2A2 on chromosome 16 and CSNK2A1 on chromosome 20. Searching on BOLL gene symbol in the Celera library under the Genes function gives only one result which is BOLL on chromosome 2 (Figure 1). As described below, the position of CSNK2A genes, chr16: nucleotide numbers 48586460-48626376 and chr20: nucleotide numbers 451790-512411 and BOLL gene, chr2:195523036-195580975, can be used to detect related information. Other casein kinase 2 alpha genes can be searched for by chromosome region or BLAT programs.

Figure 1. BOLL gene was detected on chromosome 2 in Celera library using gene name in Celera library. (Assembly date: 22 May 2002)

Because the UCSC library used the information from one of their collaborating centres (Ensemble) for the DMR-N9 gene, the gene name in this database is ENST00000270223 instead of DMR-N9 or DMWD. Therefore, the gene position cannot be detected using DMR-N9 or DMWD gene symbols in the UCSC library. The gene can be located by using symbol DMPK of the adjacent gene. Searching on the DMPK gene symbol in the UCSC library gives one DMPK gene on chr19. By widening the DMPK location from chr19: 46663863-46677274 to chr19: 46663863-46687274, the upstream gene DMWD is disclosed (Figure 2). Moreover, because this gene does not have a Celera's gene name and symbol, location of this gene in Celera library also is detected via DMPK gene. Position of DMPK gene, which is chr19: 43783033-43796780, can be detected using Celera's Genome Map programme. Searching under Genome Map's position programme in Celera with chromosome 19 and nucleotide numbers from 43.78 Mb to 43.8 Mb, the DMWD gene is disclosed (Figure 3).

Figure 2. DMPK and DMWD genes on chromosome 19.in UCSC library By widening the DMPK region in searching from chr19: 46663863-46677274 to chr19: 46663863-46687274 the upstream gene DMWD was found. (Assembly date: November 2002)

Figure 3. DMPK and DMWD genes on chromosome 19 in Celera library. Searching under Genome Maps position programme in Celera with chromosome 19 and nucleotide numbers from 43.78 Mb to 43.8 Mb, the DMWD gene is disclosed (Assembly date: 21 May 2002)

2.2 Detecting candidate genes in a chromosome region

This is especially useful for detecting interesting genes located at or near the balanced autosomal translocation breakpoints. For example, the breakpoint 6p21.33 in the 46,XY t(4,6)(q33,p21.33) karyotype of a men who presented with severe oligospermia was used to search in both data bases. In 6p21 there are about 250 and 465 genes in the UCSC and the Celera libraries, respectively. Target genes such as Zinc Finger protein (ZNF) 76, ZNF 165, Glutathione peroxidase 5, testis abundant finger protein and casein kinase 2 beta were detected in the libraries (Figures 4 and 5). These genes are strongly expressed in epididymis and testis and may have a function in spermatogenesis or sperm maturation.

Casein kinase 2 alpha genes can also be detected in searching under the 11p15, 16p 12-13 and 20p13 chromosome regions in both libraries.

Figure 4. Two candidate genes on chromosome region 6p21: casein kinase 2 beta and zinc finger protein 76, which are predominantly expressed in testis. This region in the UCSC library contains approximate 250 genes. By clicking on the gene symbols, detailed information including sequences and SNPs can be obtained. (Assembly date: November 2002)

Figure 5. Some candidate genes in the 6p21.3 region from the Celera library: zinc finger protein 165, testis abundant finger protein and casein kinase 2 beta. This region contains about 465 genes in the Celera library. The information includes gene names, symbols, locations, families and species. By clicking on gene, transcript or protein names (left column), the sequences of DNA, RNA or protein will be discovered. By clicking on the protein family assignment column (right column), other genes with the same family function will appear. (Assembly date: 22 May 2002)

2.3 BLAST searching

BLAST searching can be used to find interesting gene sequences in the human genome libraries starting with known DNA, mRNA or protein sequences from other databases such as GenBank. An example for this method is BLAST searching in UCSC library for human BOULE gene using the nucleotide sequence in GenBank, which was submitted by Xu *et al*. The result was one matching sequence on chromosomes 2 (chr2: 196791585-196848619), named BOLL (Figure 6).

Figure 6. Result of BLAST search for human BOULE gene (BOLL) in UCSC genome library. Information about SNPs such as base pair type (A, C, T or G), their location in intron or exon regions and the effect of the alternation in protein sequence can be found by clicking on the SNPs symbols. (Assembly date: November 2002)

Another example is BLAST searching for casein kinase 2 alpha genes using the two known sequences (NM001895 and NM001896) in the Celera genome library which gives one CSNK2A1 gene and one CSNK2A2 homologue on chromosomes 20 (chr20: 464659-527357), and 16 (chr16: 45801917-45841972).

2.4 Related information about genes

The previously discussed searching methods will give the basic information about target genes including gene names, gene symbols, and the exact positions of the genes. By following the directions, more details such as genomic DNA, mRNA and protein size, location, sequences, structures and functions; together with related diseases and journal articles can be found. Other information, for example mutation points and single nucleotide polymorphism (SNPs, which are sequence nucleotide variations previously documented in individuals) also are available [46]. SNPs may or may not be associated with causing disease. Information on SNPs in the UCSC library can be obtained by selecting each SNP (Figure 4). In Celera, SNPs can be found using the Human SNPs program starting with the gene position for example chr19:43783397-43804776. Information about the SNPs includes: nucleotide site and base change (Adenine, Cytosine, Guanine or Thymine), position (intron or exon), and indication of effect: silent, nonsense, or missence with altered amino acid in protein sequences, and related diseases in OMIM and Medline.

Genes expressed in the testis do not necessarily have a role in spermatogenesis. Therefore, polymorphisms within testis-related genes may not cause spermatogenic defects. With rare diseases, candidate genes can be sequenced to detect mutations guided by available data, especially those on SNPs in human genome libraries. Knowledge of genetic mutations, altered amino acid sequences and the structure and function of the protein, may help predict the pathogenicity of alterations in candidate gene. With common diseases, pathogenicity can be supported by demonstrating altered RNA expression at a certain stage of spermatogenesis and in the testis of men with specific defects of sperm production. Altered expression of the genes may also be detected using tissue arrays.

2.5 Tissue array

Tissue arrays can be constructed from minute tissue cylinders (diameter approximately 0.6 mm) derived as punches taken from archived tissue blocks of patient testicular biopsies. Samples are taken from multiple primary paraffin blocks and placed into an empty recipient paraffin block. By this method, cores from controls as well as testicular biopsies demonstrating a number of different pathologies are placed together within a single new block (Figure 7). The major advantages of this method are: (1) improved accuracy due to identical exposure of the test and control tissues to the same experimental conditions e.g. concentrations of reagents, section thickness and labelling exposure time. (2) Rapid processing of hundreds of samples simultaneously in a few slides, rather than requiring one slide per patient biopsy, a practice which is economically beneficial. Also, many tissue sections, each containing many biopsies can be cut from each block. In situ hybridisation can then be used on these slides as a method of localising and detecting specific mRNA sequences in tissue sections using specific probes. Colourimetric in situ hybridization is easily performed on these formalin-fixed paraffin embedded sections. Four types of probes can be used: oligonucleo-tide, single stranded DNA, double stranded DNA and RNA. Oligonucleotide probes have many advantages compared with the others. They are more stable, faster to use, less expense, easier to work with and result in better tissue penetration. An approach for the candidate genes, BOULE, casein kinase 2 alpha, DMR-N9 and genes at or near the balanced autosomal translation breakpoints, can be used to illustrate the strategy in Flowchart 1.

Flowchart 1: Strategy for investigating candidate genes for human male infertility.

Hypothetically, mutations in DMR-N9, BOULE and genes at the breakpoints of translocations causing spermatogenic defects may be associated with perturbed mRNA expression in testicular tissues. Mutations in casein kinase 2 alpha genes may cause globozoospermia. However, testicular biopsies are not performed for the diagnosis of the latter condition. Because three human homologues of Cnsk2a have been discovered, it is not clear which Cnsk2a genes are expressed in human testis and thus may have a role in spermatogenesis. Therefore Polymerase Chain Reaction (PCR) or ISH on testicular tissue with probes for each of them could be performed to determine which particular Cnsk2a gene is expressed in germ cells. The detection of mutations in this Csnk2a gene in men with globozoospermia would then be done on DNA extracted from patients peripheral blood in collaboration with other groups as this condition is rare.

Specific probes for BOULE, DMR-N9 and genes on chromosome region 6p21 could be used on tissue arrays constructed using testicular biopsy material from patients with normal and abnormal spermatogenesis. The aim would be to find absent or reduced expression of human BOULE mRNA in spermatocytes in patients with abnormal spermatogenesis, and particularly, germ cell arrest at the primary spermatocyte stage. DMR-N9 mRNA may be abnormal in germ cells of some patients with hypospermatogenesis, while abnormal mRNA expression of genes on 6p21 may be seen in germ cells with other spermatogenic defects. It is predicted that the expression of these genes would be normal in germ cells with normal spermatogenesis. Specific oligonucleotide probes for human BOULE, DMR-N9 and genes at the 6p21 breakpoint region can be synthesised based on the gene sequences in the human genome libraries. The sequences would be checked for uniqueness by comparison with other homologue genes. For example, sequences for human BOULE, DAZ, DAZL and two other genes encoding RNA binding proteins were retrieved from the databases and aligned to detect a specific region of the human BOULE gene. The sequence, aacatatcac caggtttatg ctccaagtgc catcactatg cctgcgcctg tgatgcagcc tgagccaatt aaaacagtgt ggagcattca ttattaa, has 100 % identity with the human BOULE gene and is not present in DAZ or DAZL. The probes will be labelled with digoxigenin-dUTP and will be detected with DIG-antibodies. Men with reduced or absent expression in the testis would be contacted and asked to provide blood for sequencing. Gene sequencing will be performed to detect mutations.

Figure 7 illustrates a part of human testicular tissue array assembled from open and fine needle tissue aspiration biopsies with ISH using a probe for DAZL. Expression in germ cells can be seen. At higher magnification the cell types can be distinguished and variations in labelling searched for and related to the histological type of spermatogenic defect.

Figure 7. Part of human testicular tissue array assembled from open and fine needle tissue aspiration biopsies with ISH using a probe for DAZL mRNA.

3 Discussion

This paper describes uses of in silico searching of the human genome to detect novel candidate genes for male infertility, for which further investigation by ISH in tissue arrays can be used to detect abnormal expression of the genes during human spermatogenesis. The human genome libraries contain a large amount of information with many different applications. For example, information about tissue expression in human genome libraries helps researchers to predict potential pathology caused by the mutations and decide which tissues to study. DNA and mRNA sequences allow specific probes to be made. Also, SNPs can be used to predict individual risks for certain genetic mutations.

In considering the impact of the Human Genome databases (HGD) on medical genetics, some limitations should be acknowledged. The incompleteness of the HGD may mean important candidate genes are missing [47]. Moreover, because information in different databases varies slightly, candidate genes may not be detected by simply searching with chromosome regions or gene names and gene symbols within different databases. Limited knowledge of tissue expression of genes may lead to some candidate genes being overlooked. Also, if spermatogenic defects associated with autosomal translocations result from the translocations affecting chromosome alignment in meiosis and not altered function of genes adjacent the break point, these genes are not candidates for mutations to cause the defect of spermato-genesis.

So far, tissue arrays are mainly used in cancer research, primarily (1) to confirm involvement of mutated genes in human cancer tissues which have been already discovered by other techniques such as microarray technology, (2) to identify involvement of novel genes in different tumour types or stages of tumour development and (3) to identify the target and mechanism of drug action [48]. Compared with older methods, which used larger amounts of tissue, tissue arrays use only one or two master paraffin blocks instead of hundreds of individual blocks. The arrays may be particularly useful for collaborative studies, with researches using different probes and antibodies and molecular profiling from multi-centre clinical trial materials. Minimal tissue usage also helps in the study of rare tumours. The main concern about this method is that cores may be too small to be representative of the whole tissue pathology, particularly if there is considerable heterogeneity within the tumour. For example cores from the centre and periphery of tumours may contain different information. However, this heterogeneity is less problematic in testicular biopsies. Nonetheless, previous studies prove that with three or more cores taken from different regions of each block, the results of tissue array are 96 % to 98 % similar to previous whole tissue section methods [49]. Uninformative results of ISH arise when the genes chosen are not involved in the process or when the gene is qualitatively abnormal but the level of expression is normal. A false positive result may arise if the probed mRNA is not expressed because of a defect upstream in a regulatory pathway rather than because of a defect in the gene of interest [50-52].

References

[1] Aach J, Bulyk ML, Church GM, Comander J, Derti A, Shendure J. Computational comparison of two draft sequences of the human genome. Nature 2001; 409: 856-9.
[2] Le Hellard S, Semple CA, Morris SW, Porteous DJ, Evans KL. Physical mapping: integrating computational and molecular genetic data. Ann Hum Genet 2001; 65: 221-8.
[3] Bleck O, McGrath JA, South AP. Searching for candidate genes in the new millennium. Clin Exp Dermatol 2001; 26: 279-83.
[4] Van Assche E, Bonduelle M, Tournaye H, Joris H, Verheyen G, Devroey P, *et al*. Cytogenetics of infertile men. Hum Reprod 1996; 11 (Suppl 4):1-24.
[5] Baker HW. Management of Male Infertility. Baillieres Best Pract Res Clin Endocrinol Metab 2000; 14: 409-22.
[6] McLachlan RI, Mallidis C, Ma K, Bhasin S, de Kretser DM. Genetic disorders and spermatogenesis. Reprod Fertil Dev 1998; 10: 97-104.
[7] Kim ED, Bischoff FZ, Lipshultz LI, Lamb DJ. Genetic concerns for the subfertile male in the era of ICSI. Prenat Diagn 1998; 18: 1349-65.
[8] Silber SJ, Nagy Z, Liu J, Tournaye H, Lissens W, Ferec C, *et al.* The use of epididymal and testicular spermatozoa for intracytoplasmic sperm injection: the genetic implications for male infertility. Hum Reprod 1995; 10: 2031-43.
[9] Eskenazi B, Wyrobek AJ, Kidd SA, Lowe X, Moore D 2nd, Weisiger K, *et al.* Sperm aneuploidy in fathers of children with paternally and maternally inherited Klinefelter syndrome. Hum Reprod 2002; 17: 576-83.
[10] Smyth C, Bremner W. Klinefelter syndrome. Arch Intern Med 1998; 158: 1309-14.
[11] Wang C, Baker HW, Burger HG, De Kretser DM, Hudson B. Hormonal studies in Klinefelters syndrome. Clin Endocrinol (Oxf) 1975; 4: 399-411.
[12] Veld PA, Weber RF, Los FJ, den Hollander N, Dhont M, Pieters MH, *et al.* Two cases of Robertsonian translocations in oligozoospermic males and their consequences for pregnancies induced by intracytoplasmic sperm injection. Hum Reprod 1997; 12:1642-4.
[13] Frydman N, Romana S, Le Lorc'h M, Vekemans M, Frydman R, Tachdjian G. Assisting reproduction of infertile men carrying a Robertsonian translocation. Hum Reprod 2001; 16: 2274-7.
[14] Conn CM, Harper JC, Winston RM, Delhanty JD. Infertile couples with Robertsonian translocations: preimplantation genetic analysis of embryos reveals chaotic cleavage divisions. Hum Genet 1998; 102: 117-23.
[15] Jou S, Lin H, Pan H, Chiu Y, Li S, Lee C, *et al.* Delineation of CTG repeats and clinical features in a Taiwanese myotonic dystrophy family. Proc Natl Sci Counc Repub China B 2001; 25: 40-4.
[16] Meng MV, Black LD, Cha I, Ljung BM, Pera RA, Turek PJ. Impaired spermatogenesis in men with congenital absence of the vas deferens. Hum Reprod 2001; 16: 529-33.
[17] Sokol RZ. Infertility in men with cystic fibrosis. Curr Opin Pulm Med 2001; 7: 421-6.
[18] Augarten A, Kerem BS, Kerem E, Gazit E, Yahav Y. Correlation between genotype and phenotype in patients with cystic fibrosis. N Engl J Med 1994; 330: 866.
[19] Augarten A, Yahav Y, Kerem BS, Halle D, Laufer J, Szeinberg A, *et al*. Congenital bilateral absence of vas deferens in the absence of cystic fibrosis. Lancet 1994; 344: 1473-4.
[20] Dumur V, Gervais R, Rigot J, Delomel-Vinner E, Decaestecker B, Lafitte J, *et al*. Congenital bilateral absence of the vas deferens (CBAVD) and cystic fibrosis transmembrane regulator (CFTR): correlation between genotype and phenotype. Hum Genet 1996; 97: 7-10.

[21] Foresta C, Moro E, Ferlin A. Y chromosome microdeletions and alterations of spermatogenesis. Endocr Rev 2001; 22: 226-39.
[22] Xu EY, Moore FL, Pera RA. A gene family required for human germ cell development evolved from an ancient meiotic gene conserved in metazoans. Proc Natl Acad Sci U S A 2001; 98:7414-9.
[23] Xu X, Toselli PA, Russell LD, Seldin DC. Globozoospermia in mice lacking the casein kinase II alpha?catalytic subunit. Nat Genet 1999; 23: 118-21.
[24] Eberhart CG, Maines JZ, Wasserman SA. Meiotic cell cycle requirement for a fly homologue of human Deleted in Azoospermia. Nature 1996; 381: 783-5.
[25] Behr R, Weinbauer GF. cAMP response element modulator (CREM): an essential factor for spermatogenesis in primates? Int J Androl 2001; 24: 126-35.
[26] Behr R, Weinbauer GF. CREM activator and repressor isoforms in human testis: sequence variations and inaccurate splicing during impaired spermatogenesis. Mol Hum Reprod 2000; 6:967-72.
[27] Adham IM, Nayernia K, Burkhardt-Gottges E, Topaloglu O, Dixkens C, Holstein AF, *et al*. Teratozoospermia in mice lacking the transition protein 2 (Tnp2). Mol Hum Reprod 2001; 7:513-20.
[28] Adham IM, Steding G, Thamm T, Bullesbach EE, Schwabe C, Paprotta I, *et al*. The overexpression of the insl3 in female mice causes descent of the ovaries. Mol Endocrinol 2002; 16: 244-52.
[29] Nef S, Parada LF. Cryptorchidism in mice mutant for Insl3. Nat Genet 1999; 22: 295-9.
[30] Lim HN, Raipert-de Meyts E, Skakkebaek NE, Hawkins JR, Hughes IA. Genetic analysis of the INSL3 gene in patients with maldescent of the testis. Eur J Endocrinol 2001; 144: 129-37.
[31] Hackstein J, Hochstenbach R, Pearson P. Torwards an understanding of the genetics of human male infertility: lessons from flies. Trends Genet 2000; 16: 565-72.
[32] Hauser W, Aulitzky W, Baltaci S, Frick J. Increasing infertility in myotonia dystrophica Curschmann-Steinert. A case report. Eur Urol 1991; 20: 341-2.
[33] Hortas ML, Castilla JA, Gil MT, Molina J, Garrido ML, Morell M, *et al*. Decreased sperm function of patients with myotonic muscular dystrophy. Hum Reprod 2000; 15: 445-8.
[34] Lia AS, Seznec H, Hofmann-Radvanyi H, Radvanyi F, Duros C, Saquet C, *et al*. Somatic instability of the CTG repeat in mice transgenic for the myotonic dystrophy region is age dependent but not correlated to the relative intertissue transcription levels and proliferative capacities. Hum Mol Genet 1998; 7:1285-91.
[35] Shaw DJ, McCurrach M, Rundle SA, Harley HG, Crow SR, Sohn R, *et al*. Genomic organization and transcriptional units at the myotonic dystrophy locus. Genomics 1993; 18: 673-9.
[36] Groenen PJ, Wieringa B. Expanding complesity in myotonic dystrophy. Bioassays 1998; 20: 901-12.
[37] Jansen G, Bachner D, Coerwinkel M, Wormskamp N, Hameister H, Wieringa B. Structural organization and developmental expression pattern of the mouse WD-repeat gene DMR-N9 immediately upstream of the myotonic dystrophy locus. Hum Mol Genet 1995; 4: 843-52.
[38] Alwazzan M, Newman E, Hamshere MG, Brook JD. Myotonic dystrophy is associated with a reduced level of RNA from the DMWD allele adjacent to the expanded repeat. Hum Mol Genet 1999; 8: 1491-7.
[39] Eriksson M, Ansved T, Edstrom L, Anvret M, Carey N. Simultaneous analysis of expression of the three myotonic dystrophy locus genes in adult skeletal muscle samples: the CTG expansion correlates inversely with DMPK and 59 expression levels, but not DMAHP levels. Hum Mol Genet 1999; 8: 1053-60.
[40] Trokoudes KM, Danos N, Kalogirou L, Vlachou R, Lysiotis T, Georghiades N, *et al*. Pregnancy with spermatozoa from a globozoospermic man after intracytoplasmic sperm injection treatment. Hum Reprod 1995; 10: 880-2.
[41] Yen PH, Chai NN, Salido EC. The human DAZ genes, a putative male infertility factor on the Y chromosome, are highly polymorphic in the DAZ repeat regions. Mamm Genome 1997; 8: 756-9.
[42] Haag ES. Rolling back to BOULE. Proc Natl Acad Sci U S A 2001; 98: 6983-5.
[43] Bugge M, Bruun-Petersen G, Brondum-Nielsen K, Friedrich U, Hansen J, Jensen G, *et al*. Disease associated balanced chromosome rearrangements: a resource for large scale genotype-phenotype delineation in man. J Med Genet 2000; 37:858-65.
[44] Olesen C, Hansen C, Bendsen E, Byskov AG, Schwinger E, Lopez-Pajares I, *et al*. Identification of human candidate genes for male infertility by digital differential display. Mol Hum Reprod 2001; 7: 11-20.
[45] Baker HW. clinical approach to molecular genetic diagnosis in andrology. in VII International Congress of Andrology. 2001. Montreal, Canada: Medimond Inc.
[46] Hoh J, Wille A, Zee R, Cheng S, Reynolds R, Lindpaintner K, *et al*. Selecting SNPs in two-stage analysis of disease association data: a model-free approach. Ann Hum Genet 2000; 64:413-7.
[47] Bentley DR. The Human Genome Project -- an overview. Med Res Rev 2000; 20: 189-96.
[48] Liu J, Nagy Z, Joris H, Tournaye H, Devroey P, Van Steir-teghem A. Successful fertilization and establishment of pregnancies after intracytoplasmic sperm injection in patients with globozoospermia. Hum Reprod 1995; 10: 626-9.
[49] Hoos A, Cordon-Cardo C. Tissue microarray profiling of cancer specimens and cell lines: opportunities and limitations. Lab Invest 2001; 81: 1331-8.
[50] Kononen J, Bubendorf L, Kallioniemi A, Barlund M, Schraml P, Leighton S, *et al*. Tissue microarrays for high-throughput molecular profiling of tumor specimens. Nat Med 1998; 4: 844-7.
[51] Wang H, Zhang W, Fuller GN. Tissue microarrays: applications in neuropathology research, diagnosis, and education. Brain Pathol 2002; 12: 95-107.
[52] Horvath L, Henshall S. The application of tissue microarrays to cancer research. Pathology 2001; 33: 125-9.

home

Correspondence to: B. N. Truong, the Melbourne IVF Centre, Department of Obstetrics and Gynaecology, Royal Women's Hospital, University of Melbourne, Victoria 3053, Australia.
Tel: +61-3-9344 2613, Fax: +61-3-9347 1761
E-mail: b.truong@ugrad.unimelb.edu.au
Received 2002-11-04 Accepted 2003-03-10