The Genetics of Male Infertility

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PREFACE

Male infertility is a common and severe health problem. Infertility not only affects one's ability to have children, but also has emotional, psychological, family, and societal effects. Despite the prevalence and significance of this health problem, resources and attention have not been sufficiently focused on this important issue.

Approximately 7% of men suffer from infertility, and the incidence may be increasing. Of those affected, roughly 40% have idiopathic infertility. It is likely that the majority of those patients have genetic abnormalities that are the cause of their infertility. However, it is important to remember that there are genetic ramifications for essentially all infertile male patients. For example, it is likely that there are genetic predispositions to pathologies such as varicoceles, and environmental factors almost certainly modulate the underlying condition. The understanding of the genes involved in spermatogenesis, sperm maturation, and normal sperm function is key, but we must also focus on better methods of accelerating advances into meaningful clinical diagnostic tests and therapies.

During the past 20 years, significant improvements in technology have advanced the treatment of male infertility. The primary advance has been intracytoplasmic sperm injection (ICSI) in conjunction with in vitro fertilization. Although this technological leap has allowed thousands of men to father a child who otherwise would have been unable to do so, the scientific study of the causes of male infertility has not kept pace. In fact, the clinical application of ICSI proceeded without sufficient scientific study of its safety to the offspring, or the future genetic ramifications.

We currently stand at a point in history in which new tools are available to evaluate genetic diseases. The completion of the Human Genome Project has ushered in an era of unprecedented momentum and ability to tackle the complex issues in the genetics of male infertility. New tools include in vitro methodologies, *in silico* technologies, and new model organisms. Together these advances portend great possibilities.

In January 2006, an international symposium was held at the University of Utah Campus in Salt Lake City to address the genetic causes of male infertility and the translation of the knowledge to the clinical realm. Twenty-one researchers and clinicians, and an international audience of

experts in the field, reviewed the study of the genetics of male infertility, the tools available in the laboratory and clinic, the current state of knowledge, and the future of research and translation into clinical diagnostics and treatments. This book is the result of the symposium. The book is intended as a review of our current understanding of genetic causes of male infertility, a guide to evidence-based clinical applications, and a preview of future possibilities.

Douglas T. Carrell, PhD

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I Methods and Tools for the Study of the Genetics of Male Infertility

1 The Genetics of Male Infertility in the Era of Genomics

Tools for Progress

Douglas T. Carrell, PhD

Summary

The histories of progress in the fields of genetics and andrology are rich and include many breakthroughs. The era of genomics, initiated with the completion of the Human Genome Project, is upon us and offers many new tools for better understanding the genetics of male infertility. Genomic breakthroughs give us a better understanding of structural components of DNA, new types of genetic polymorphisms, regulation of gene expression, and the identity of genes involved in male infertility. The advances we have seen in genomics are key to facilitating some of the studies needed to gain a better understanding of the genetics of infertility, but researchers in this field can better maximize resources and tools through focused collaboration on studies of major issues.

Key Words: Male infertility; genomics; medical resequencing; consortium; gene; spermatogenesis; Human Genome Project.

1. GENETICS AND ANDROLOGY: COLLABORATION BETWEEN TWO FIELDS

With the recent passing of the 50th anniversary of the publication of Watson and Crick's elucidation of the structure of DNA, much attention has been focused on the rich history of the field of genetics. From the identification of DNA as the molecule responsible for heredity in 1944 to the completion of the Hapmap Project last year, the history of genetics is marked by regular advances in techniques and understanding that have fueled the hope of future therapies to alleviate suffering and provide a higher quality of life. Although those hopes have not been realized as quickly as desired and often predicted, recent breakthroughs, largely accelerated by the Human Genome Project (HGP), have raised

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expectations higher than ever before. It is clear that we are currently in an era of genomics, an era in which advances in genetic tools are shaping the methods and capabilities available to treat disease.

Although the term *andrology* was sporadically used as far back as 150 yr ago, the use of the term to denote the study of male reproduction and infertility was coined and commonly accepted in 1951, 2 yr before the elucidation of the structure of DNA *(1)*. Since that time, the evaluation and treatment of male infertility have evolved from simple techniques to evaluate sperm characteristics to a better understanding of underlying endocrinology to today's common use of intracytoplasmic sperm injection, chromatin evaluation, and sperm function assays, and the initiation of candidate gene evaluation. The interaction of genetics and andrology has been continual and productive throughout the past, bringing breakthroughs such as the identification of sexual differentiation abnormalities, Y-chromosome microdeletions, and DNA nicks and breaks. However, with the completion of the HGP and our entrance into the era of genomics, it is clear that many of the major concerns facing those studying male infertility will likely be solved using the techniques and tools the field of genomics is producing.

The era of genomics does not have a start date, however, it is clear that the genomics movement gained great momentum in 1990 with the planning of the HGP, and was officially ushered in by the initial publication of the sequence of the human genome in 2001 *(2–4)*. The HGP has spawned other major initiatives, such as the Hapmap Project, which is described in Section 3, and the Encyclopedia of DNA Elements project, a study that aims to identify all control mechanisms involved in a representative sample (~1%) of the genome *(5)*. Major consortia have been formed to study these and other big-issue questions, such as the role of the environment in gene function and the genetics of cancer. It is apparent that the progress made in genomics is largely a result of unprecedented collaboration of various specialties (sequencing, bioinformatics, statisticians, classical genetics, etc.) and this model of collaboration could benefit most areas of biomedical research.

Major questions in the study of male infertility include: What are the genes involved in normal spermatogenesis, sperm maturation, and sperm function? Can we identify what polymorphisms or mutations result in infertility, and if so, how can we screen and treat patients better? What are the regulators of normal gene expression during spermatogenesis? What role does abnormal meiotic recombination and segregation play in male infertility? What effect does abnormal DNA nicks and breaks have on embryogenesis? What is the role of abnormal protamines in infertility and does it relate to imprinting or epigenetic defects? What is the role of the environment, diet, and other factors in

the variation of the degree of pathology seen in different individuals (i.e., varicoceles, smoking effects, etc.)? These and many other important questions will largely be addressed through genetic studies. Proteomics, physiology, endocrinology, and other fields of study will assist in the quest, but it is likely that many of the large leaps made in the study of male infertility will be largely because of genetic advances, lessons learned, and the technologies developed from the HGP. Therefore, it is important to remember not only the advances spurred through the genomics revolution, but also the significant and unique collaborative efforts used in the process.

2. THE CONTRIBUTION OF THE HGP

The HGP was initiated with great hope that the sequencing of the human genome would yield tremendous advances in the understanding of gene function and the etiology of human diseases *(6)*. However, it is likely that, at this time, many of the major breakthroughs of the HGP are in the basic understanding of the human genome. Foremost is the identification of 20,000–25,000 genes, a number much lower than previously predicted *(2,7)*. Previous studies have estimated that at least 2000 genes may be involved in normal spermatogenesis, a strikingly high percentage of the total complement of human genes *(8)*.

Although the number of genes in the human genome is smaller than expected, the diversity of gene products is larger. It is estimated that as many as 35–60% of genes undergo alternative splicing, which increases the diversity of the proteome and the complexity of regulatory and functional mechanisms. Additionally, the data indicate a surprisingly narrow range in the number of genes found in a comparison of humans and other animals.

Another basic finding from the HGP that highlights the increased diversity of products of the genome is the common transcription of nonprotein-coding RNA. Some of these RNAs may simply be the result of alternative 5′ start sites during transcription, or may they may be involved in regulatory mechanisms, but it is obvious now that nonprotein-coding RNAs are essential to normal cellular function. More than 800 human micro-RNA "genes" have been identified and appear to be essential to normal development and metabolism *(9)*. The micro-RNAs are apparently an essential regulator of gene expression and very relevant to sperm function *(10)*. The mechanisms and functions of micro-RNAs are a current area of major research, and addressed in Chapters 3 and 4.

In addition to a better understanding of the diversity of the genome and its messenger RNA products, genomic advances have improved our understanding of several structural components of the genome. One such discovery is the presence of ultraconserved elements (UCEs;

ref. *11*). UCEs are sequences of at least 200 bp with complete homology between the human, mouse, and rat sequences. Thus far, 481 human UCEs have been identified. Their function has not been entirely worked out; however, it appears that they contain enhancer elements *(6,12)*. Given their evolutionary conservation, it seems likely that the UCEs play a vital role in gene expression regulation.

Another finding is that the genome contains "gene deserts," which are regions of 3 megabases or more that are devoid of genes *(3)*. The regions do not appear to be a result of the normal statistical distribution of the genes, which raises interesting questions as to their function. At this point, the only possible function of these regions is the possible identification of enhancers for lateral genes *(13)*. Nobrega et al. *(14)* have experimentally removed two such deserts in mice, with no apparent effect. Additionally, there is at times a clustering of functionally related genes of nonrelated origin *(15)*. It would appear that an evolutionary advantage might sometimes be found in the clustering of functionally related genes into "neighborhoods," with obvious implications for coordinated expression regulation.

Studies have found that the genome is polymorphic in a structural sense on a much larger scale than previously thought *(16)*. Using comparative microarray technology, large differences in copy number variation were shown, and it was suggested that large-scale DNA variations of up to several hundred kilobases were responsible. Several studies have since shown that these deletions and other changes are relatively common and more than 1000 such polymorphisms have been identified *(16–19)*. The studies that identified these polymorphisms used different assays and had small overlaps, indicating that the ideal assays to identify the polymorphisms are not yet known, and that there may be many more polymorphisms to be found *(20)*. This exciting find is likely to have profound implications in many areas, including a better understanding of polymorphic phenotypes, including infertility.

3. THE IDENTIFICATION AND EVALUATION OF CANDIDATE MALE INFERTILITY GENES

The great promise of the HGP is in the identification and evaluation of candidate genes in patients. Previous estimates have predicted that about 10% of the genes in the human genome may be related to spermatogenesis and fertility *(8)*. Those estimates are based largely on animal studies, with human data recently beginning to significantly add to the pool. Table 1 is a current list of genes known to affect male fertility.

Table 1
Genes That Cause Male Infertility When Targeted

Gene symbol	*Gene name*	*Reproductive phenotype*
ADAM1a	A disintegrin and metallopeptidase domain 1a	Asthenospermia, penetration defect
ADAM2	A disintegrin and metallopeptidase domain 2	Sperm–egg fusion defect
ADAM3	A disintegrin and metallopeptidase domain 3; cyritestin	Sperm–zona fusion defect
AKAP4	A kinase (PRKA) anchor protein 4	Abnormal tail morphology, asthenospermia
Acr	Acrosin	Sperm are not capable of binding and penetrating the zona pellucida
Acvr2	Activin receptor-type IIA	Small testes, delayed fertility
ACOX	Acyl-Coenzyme A oxidase 1, palmitoyl	Leydig cell hypoplasia, small testes, abnormal spermatogenesis
ADFP	Adipose differentiation -related protein	Male infertility
Arl4	ADP-ribosylation factor-like 4	Significantly reduced testicular weights and sperm counts
AFF1	AF4/FMR2 family, member 1	Male subfertility (decreased litter size)
AFF4	AF4/FMR2 family, member 4	Enlarged seminal gland, small testis, azoospermia, arrest of spermatogenesis, abnormal epididymis morphology
Man2a2	α-mannosidase IIx	Defect in adherence of spermatogenic cells to Sertoli cells; germ cells prematurely released from the testis
Amhr2	AMH receptor	Abnormal semal differentiation
Npepps	Aminopeptidase puromycin-sensitive	Asthenospermia, abnormal tubules

(Continued)

Table 1 ***(Continued)***

Gene symbol	*Gene name*	*Reproductive phenotype*
Ar; tfm	Androgen receptor; testicular feminization	Feminized external genitalia; hypogonadal; cryptorchidism with a block in spermatogenesis
ACE	Angiotensin I-converting enzyme; peptidyl-dipeptidase A 1	Presumed penetration defect; normal testicular histology, concentration, sperm morphology
Ace	Angiotesin-converting enzyme	Compromised ability of sperm to fertilize ova
AE2	Anion exchanger 2	Disrupted spermiogenesis, complete absence of spermatozoa in tubules
Amh	Anti-Mullerian hormone	Uteri development in males causes obstruction and secondary infertility
Apob	Apolipoprotein B	Decreased sperm count, motility, survival time, and ability to fertilize ova
Apaf1	Apoptotic protease-activating factor 1	Spermatogonial degeneration
Atm	Ataxia Telangiectasia	Germ cells degenerate; disruptions evident in meiosis I
Atxn7	Ataxin 7	Reduced fertility at 16 wk of age
AGTPbp1	ATP/GTP-binding protein 1	Oligospermia, teratospermia, asthenospermia
Atp2b4	ATPase, Ca^{++} transporting, plasma membrane 4	Infertile
Atp8b3	ATPase, class I, type 8B, member 3	Impaired sperm–egg interaction, reduced zona pellucida-induced acrosome reaction
Bbs2	Bardet-Biedl syndrome 2 homolog (human)	Sperm lack flagella
Bbs4	Bardet-Biedl syndrome 4 homolog (human)	Flagella are absent throughout the seminiferous tubules, even on cells with condensed sperm heads
BSG	Basigin	Azoospermia, arrest at meiosis I

(Continued)

Table 1 ***(Continued)***

Gene symbol	*Gene name*	*Reproductive phenotype*
Bsg	Basign	Block in spermatogenesis at metaphase I
Bax	Bc12-associated X protein	Premeiotic arrest of spermatogenesis
*Bc*16	B-cell leukemia/ lymphoma 16	Apoptosis in metaphase I spermatocytes
Bclw; Bc1212; Bc12-like 2	BCL2-like 2 protein apoptosis regulator *BCL-W*	Late meiotic arrest with loss of germ cells
	β 1-4-galactosyl-transferase	Male infertility; defects in sperm–egg interaction
Btrc	β-transducin repeat-containing protein	Meiotic arrest with multiple errors
bs	Blind-sterile	Small testis, oligospermia
Bmp4	Bone morphogentic protein 4	Absent primordial germ cell (PGC) population; defect in PGC development
Bmp8a	Bone morphogentic protein 8a	Degeneration of germ cells and epididymis
Bmp8b	Bone morphogentic protein 8b	Reduced or absent PGCs (developmental defect); postnatal germ cell defects and spermatocyte apoptosis
Bdnf	Brain-derived neurotrophic factor	Reduced male fertility
Brca1	Breast cancer 1	Spermatogenic arrest
BUB1B	Budding uninhibited by benzimidazoles 1 homolog β	Oligzoospermia
Camk4	Calcium/calmodulin-dependent protein kinase IV	Impaired chromatin packaging during spermiogenesis
Clgn	Calmegin	Defect in sperm-zona pellucida binding
	Cα(2)/Prkaca	cAMP-dependent protein kinase catalytic subunit 2 Males infertile, motility and fertilization affected
Crem	cAMP-responsive element modulator	Defective spermiogenesis with aberrant postmeiotic gene expression
Csnk2a2	Casein kinase Iia 1	Globozoospermia (no acrosomal cap)

(Continued)

Table 1 ***(Continued)***

Gene symbol	*Gene name*	*Reproductive phenotype*
Catsper1	Cation channel of sperm 1	Asthenospermia, normal count and testis weight
Catsper2	Cation channel of sperm 2	Capacitation defect
Cnot7	CCR4-NOT transcription complex, subunit 7	Abnormal testis morphology, testis hypoplasia
Cd59b	CD59b antigen	Teratozoospermia, oligozoospermia, asthenozoospermia
Cks2	CDC28 protein kinase regulatory subunit 2	Male and female germ cells arrest at anaphase I
Cenpb	Centromere protein B	Hypogonadal and have low sperm counts
Cldn11; Osp-11	Claudin 11	No tight junctions between Sertoli cells
Csf1	Colony-stimulating factor (macrophage)	Reduced testosterone
Gja1; C43	Connexin 43	Small ovaries and testes; decreased numbers of germ cells from E11.5
Ros1	c-ros protoncogene	Sperm motility defects
Crsp	Cryptorchidism with white spotting, deletion region	Azoospermia
Cutl1; CDP/Cux	Cut-like 1	Severely reduced fertility
Ccna1	Cyclin A1	Block in spermatogenesis before the first meiotic division
Ccnd2	Cyclin D2	Fertile with decreased testis size
p27Kip1; Cdkn1b	Cyclin-dependent inhibitor 1b	Fertile with testicular hyperplasia
p57kip2; Cdkn1c	Cyclin-dependent inhibitor 1c	Surviving mice show sexual immaturity
p18Ink4c; Cdkn2c	Cyclin-dependent inhibitor 2c	Leydig cell hyperplasia and reduced testosterone production
p19ink4d; Cdkn2d	Cyclin-dependent inhibitor 2d	Testicular atrophy and germ cell apoptosis
Ccne1	Cyclin E1	Testicular hypoplasia
Ccne2	Cyclin E2	Testicular hypoplasia

(Continued)

Table 1 ***(Continued)***

Gene symbol	*Gene name*	*Reproductive phenotype*
Cdkn2d	Cyclin-dependent kinase inhibitor 2D (p19, inhibits CDK4)	Increased germ cell apoptosis, small testis
Adam3	Cyritesin	Altered sperm protein expression and adhesion defects during fertilization
CYP17	Cytochrome P450 17α-hydroxylase/ 17,20-lyase	Abnormal morphology, reduced motility, sexual behavior
Cyp11a	Cytochrome P450, 11a, cholesterol side-chain cleavage	Males feminized with female external genitalia, underdeveloped sex organs; gonads degenerate
Cyp19	Cytochrome P450, 19, aromatase	Early spermatogonial arrest, Leydig cell hyperplasia, and defects in sexual behavior
Cpeb	Cytoplasmic polyadenylation element-binding protein	Disrupted germ cell differentiation and meiosis I synaptonemal complex formation
Tial1	Cytotoxic granule-associated RNA-binding protein-like 1	PGCs lost by E13.5
Dax1 (Nr0b1)	Orphan nuclear receptor	Progressive degeneration of the germinal epithelium
Ddx4	DEAD (Asp-Glu-Ala-Asp) box polypeptide 3, Y-linked (DBY) Symbol-DDX3Y, AZFa region; VASA homolog	Defective proliferation/ differentiation of PGCs
Dazl	Deleted in azoospermia-like	Reduced germ cells; differentiation failure and degeneration of germ cells
Dhh	Desert hedgehog	Complete absence of mature sperm; defects in Sertoli-to-Leydig cell signaling
Dmc1h	Disrupted meiotic cDNA 1 homolog	Defects in chromosome synapis in meiosis
	DNA polymerase λ	Asthenozoospermia

(Continued)

Table 1 ***(Continued)***

Gene symbol	*Gene name*	*Reproductive phenotype*
Dnaja1	DnaJ (Hsp40) homolog, subfamily A, member 1	Small testis, tubal degeneration
Dms	Dominant male sterility	Testicular degeneration, azoospermia
Dspd	Dominant spermiogenesis defect	Teratozoospermia, oligozoospermia
Dmrt1	Doublesex and Mab-3-related transcription factor 1	Defects in postnatal testes differentiation; disorganized seminiferous tubules and absence of germ cells
Spo11	DPO11 homolog	Defects in meiosis
Cnahc1	dynein heavy chair 7	Defects in sperm flaggelar motility
Ube2b	E2B ubiquitin-conjugating enzyme; HR6B	Alterations in sperm chromatin structure, an incomplete meiotic arrest, abnormal sperm morphology
Egr1; NGFI-A	Early growth response 1	Lack of LH
Egr4	Early growth response 4	Germ cells undergo apoptosis during pachytene stage
Esgd12d	Early spermiogenesis defective 12d	Some epididymal sperm present, asthenozoospermia, teratozoospermia
Elk1	ELK1, member of ETS oncogene family	Asthenozoospermia
Emk	Elkl motif kinase	Infertile
Emx2	Empty spiracles homolog 2	Defective development of gonads and urogenita tracts
Esr1	Estrogen receptor (ER)α	Develop disruptions of the seminiferous epithelium because of abnormal epididymal function, no ejaculations
Esr2	ERβ	Fertile, but develop prostate hyperplasia
Etv4	Ets variant gene 4 (E1A enhancer-binding protein, E1AF)	Severe oligozoospermia
Etv5	Ets variant gene 5	Early testicular degeneration
Fanc	Fanconi anemia complementation group A	Hypogonadism, reduced fertility

(Continued)

Table 1 ***(Continued)***

Gene symbol	*Gene name*	*Reproductive phenotype*
Fancc	Fanconi anemia complementation group C	Hypogonadism, compromised gametogenesis
Fancg	Fanconi anemia complementation group G	Hypogonadism, compromised gametogenesis
Adam2	Fertilin β	Altered sperm protein expression and adhesion defects during fertilization
Fgf9	Fibroblast growth factor 9	XY male-to-female sex reversal; phenotype ranges from testicular hypoplasia to complete sex reversal
Fkbp6	FK506-binding protein 6	Absence of normal pachytene spematocytes
Fmr1	Fragile-X mental retardation syndrome 1 homolog	Macroorchidism
Fishb	FSH hormone β-subunit	Decreased testis size
Fshr	FSH receptor	Decreased testis size
Gpr 106	G protein-coupled receptor 106	Crsp males homozygous for trans gene integration exhibit a high intra-abdominal position of the testes, complete sterility
Gpr64	G protein-coupled receptor 64	Enlarged testis, azoospermia
Gcl	Germ cell-less homolog (Drosophila)	Asthenozoospermia, teratozoospermia (giant heads with multiple tails), oligozoospermia
Gdnf	Glial cell line-derived neurotrophic factor	Depletion of stem cell reserves; spermatogonia differentiate
GAPDS	Glyceraldehyde 3-phosphate dehydrogenase-*S*	Severely decreased sperm motility
Cga	Glycoprotein hormone α-subunit	Hypogonadal because of FSH and LH deficiency
GRTH/ Ddx25	Gonadotropin-regulated testicular RNA helicase	Arrest of spermiogenesis, elongation failure
*iPLA(2)*β	Group VIA phospholipase A2	Reduced motility, impaired fertilization, unable to fertilize

(Continued)

Table 1 ***(Continued)***

Gene symbol	*Gene name*	*Reproductive phenotype*
Gdf7	Growth differentiation factor-7	Defects in seminal vesicle development
Ghrhr	Growth hormone-releasing hormone receptor	Idiopathic
Gdi1	Guanosine diphosphate dissociation ihibitor 1; Rho GDI α	Impaired spermatogenesis, vaculolar degeneration in males
HSFY	Heat shock factor Y	Deleted in individual with idiopathic azoospermia
Hsp70-2	Heat shock protein 70-2	Meiosis defects and germ cell apoptosis
Hfe2	Hemochromatosis type 2 (juvenile; human homolog)	Sterility
Tcf1	Hepatocyte nuclear factor (HNF-1α) transcription factor 1	Vestigial vas deferens, seminal vesicles and prostate, impaired spermatogenesis, no mating behavior
Hmga1	High mobility group AT-hook 1	Abnormal Sertoli cells, abnormal epididymis morphology
Hmgb2	High mobility group box 2	Sertoli and germ cell degeneration and immotile spermatozoa
H3f3a	Histone 3.3A	Reduced copulatory activity and fewer matings result in pregnancy
H2afx	Histone H2A family, member X	Pachytene stage arrest in spermatogenesis; defects in chromosome segregation and MLH1 foci formation
Hrb	HIV-1 Rev-binding protein	Round-headed spermatozoa lack an acrosome (Globozoospermia)
Hoxa10	Homeobox A10	Variable infertility; cryptorchidism
Hoxa11	Homeobox A11	Males have malformed vas deferens and undescended testes
HOOK1	Hook homolog 1	Teratozoospermia and decapitation
HE6/ GPR64	Human epididymal protein 6	Dysregulation of efferent ductule fluid reabsorbtion, stasis of spematozoa within the ducts

(Continued)

Table 1 ***(Continued)***

Gene symbol	*Gene name*	*Reproductive phenotype*
Bc1X; Bc121	Hypomorph	PGCs are lost by E15.5
Inha	Inhibin a	Granulosa/Sertoli tumors, gonadotropin hormone-dependent
Inpp5b	Inositol polyphosphate-5-phosphatase	Sperm have reduced motility and reduced ability to fertilize eggs; defects in fertilin β processing
Igf1	Insulin-like growth factor 1	Hypogonadal and infertile; disrupted spermatogenesis and vestigial ductal system, defects in mating behavior
Insl3	Insulin-like hormone 3	Bilateral cryptorchidism results in abnormal spermatogenesis
Izumo1	Izumo sperm–egg fusion 1	Normal zona penetration, abnormal oolema binding
JunD; Jund1	Jun D proto-oncogene	Anomalous hormone levels and sperm structural defects
Klhl10	Kelch-like 10 (Drosophila)	Sertoli cell only
Kitl	Kit ligand	Defect in PGC migration/ survival
Kit	Kit receptor	White spotting null mutation causes PGC defects
Ggtp	λ-Glutamyl transpeptidase	Hypogonadal and infertile; phenotype corrected by feeding mice *N*-acetylcysteine
LGR8 (GREAT)	Leucine-rich repeat-containing G protein-coupled receptor	Intra-abdominal cryptorchidism and sterility
Lep; ob/ob	Leptin	Obese and infertile with hypogonadotrophic hypogonadism
Lepr; db/db	Leptin receptor	Obese and infertile with hypogonadotrophic hypogonadism
Lgr7	Leucine-rich repeat-containing G protein-coupled receptor	Spermatic apoptosis at meiotic stage 12
Lipe; HSL	Lipase, hormone-sensitive	Multiple abnormalities in spermatogenesis
Lhcgr	LH receptor	Underdeveloped sex organs and infertility; spermatogenesis

(Continued)

Table 1 ***(Continued)***

Gene symbol	*Gene name*	*Reproductive phenotype*
		arrested at round spermatid stage
Smad5; Madh5	MAD homolog 5	Developing embryos lose PGCs
Smad; Madh1	MAS homolog 1	Developing embryos lose PGCs
Mell1	Mel-transforming oncogene-like 1	Decreased fertilization and embryogenesis
Mitf	Microphthalmia-associated transcription factor	Reduced male fertility
Morc	Microrchidia	Early arrest in meiosis and germ cell apoptosis
Mtap7; E-MAP-115	Microtubule-associated protein	Abnormal microtubules in germ cells and Sertoli cells
Mlh3	MutL homolog 3 (*E. coli*)	Increased sperm aneuploidy, increased arrest at pachytene
Mlh1	MutL homolog 1	Meiotic arrest and genomic instability
Msh4	MutS homolog 4	Prophase I meiotic defects apparent at the zygotene/ pachytene stage; germ cells lost within a few days postpartum
Msh5	MutS homolog 5	Zygotene/pachytene meiotic defects with aberrant chromosome synapsis and apoptosis
Myhl1; A-myb	Myeloblastosis oncogene-like 1	Germ cell meiotic arrest at the pachytene stage
NKCC1; Slc12a2	Na(+) –K(+) –2C1(–) cotransporter; solute carrier family 12, member 2	Low spermatid counts and compromised sperm transport
Nkd1	Naked cuticle 1 homolog (Drosophila)	Oligozoospermia
Nanos2	Nanos homolog 2 (Drosophila)	Azoospermia
Nanos3	Nanos homolog 3 (Drosophila)	Increased germ cell apoptosis, no germ cells were detected in the testes by E15.5

(Continued)

Table 1 ***(Continued)***

Gene symbol	*Gene name*	*Reproductive phenotype*
Neurl	Neuralized-like homolog (Drosophila)	Asthenozoospermia, missing sperm heads
Nxph1	Neurexophilin 1	Infertility appears to be an artifact of homologous recombination
Nhlh2	Neuronal helix–loop–helix 2	Infertile and hypogonadal
NIR	Neuronal insulin receptor	Hypothalamic hypogonadism; impaired spermatogenesis
Nkx3-1	NK-3 transcription factor, locus 1 (Drosophila)	Accessory gland deformation
Nmp4	Nuclear matrix protein 4	Abnormal seminiferous tubule morphology, decreased spermatocytes
Nr5a1	Nuclear receptor subfamily 5, group A, member 1	Prostate hypoplasia, seminal gland hypoplasia, germ cell depletion
Ncoal; SRC1	Nuclear receptor co-activator; steroid receptor coactivator-1	Decreased responsiveness to steroid hormones in testes and prostate
Nr0b1	Nuclear receptor subfamily 0, group B, member 1	Early testicular degeneration
Nr2c2	Nuclear receptor subfamily 2, group C, member 2	Oligozoospermia, cells arrest in meiotic prophase stage/ pachytene spermatocyte stage resulting in an increase in the ratio of stage X to stage XII tubules
Nr5a1; SF-1	Nuclear receptor subfamily 5, group A, member 1; steroidogenic factor-1	Gonadal agenesis in both sexes
Ovo	Ovo protein (Drosophila melanogaster homolog)	Reduced fertility and underdeveloped genitalia
P2rx1	P2X1 receptor	Oligospermia and defective vas deferens contraction
Wip1	p53-induced phosphatase	Runting and testicular atrophy
PLCdelta4	Phospholipase C δ 4	Sperm fail to activate eggs, no calcium transients
Pi3k	Phosphatidylinositol 3′-kinase	Defects in proliferation and increased apoptosis of spermatogonia

(Continued)

Table 1 ***(Continued)***

Gene symbol	*Gene name*	*Reproductive phenotype*
Piga	Phosphatidylinositol glycan, class A	Abnormal testes, epididymis and seminal vesicles
Pss2/ Ptdss2	Phosphatidylserine synthase 2	Reduced testis weigth, some infertile males
Styx	Phosphoserine/threonine/ tyrosine interaction protein	Defects in round and elongating spermatid development
mili/piwil2	Piwi-like homolog 2	Spermatogenesis arrested in early prophase I
Pafah1b1	Platelet-activating factor acetylhydrolase, isoform 1b, β1 subunit	Azoospermia, abnormal testicular morphology
Nectin-2/ Pvrl2	Poliovirus receptor-related 2	Abnormal morphology, males are sterile
TPAP/ Papolb	Polymerase β (testis-specific)	Sperm arrest during spermiogenesis
Pea3	Polyomavirus enhancer activator 3	Normal mating behavior, but males do not set plugs or release sperm
Pms2	Postmeiotic segregation increase 2	Abnormal chromosome synapsis in meiosis
Doppel/ Prnd	Prion protein dublet	Reduced counts, motility and morphology
Adamts2	Procollagen *N*-proteinase	Defects in spermatogenesis; marked decrease in sperm within testes tubules
Prlr	Prolactin receptor	Variability in infertiity and subfertility
Prm1	Protamine 1	Protamine haploinsufficiency; abnormal spermatogenesis
Prm2	Protamine 2	Protamine haploinsufficiency; abnormal spermatogenesis
PN-1	Protease inhibitor protease nexin-1; serpine2	Abnormal seminal vesicle morphology and altered semen protein composition
Ppp1cc	Protein kinase A, catalytic subunit λ	Defects in spermiogenesis
P2rx1	Purinergic receptor P2X, ligand-gated ion channel 1	Impaired neurogenic vas deferens contraction, azoospermia
CatSper	Putative sperm cation channel	Defects in motility and fertilization

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