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REVIEW

The search for SNPs, CNVs, and epigenetic variants associated with the complex disease of male infertility

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Understanding the genetic basis of idiopathic male infertility has long been the focus of many researchers. Numerous recent studies have attempted to identify relevant single nucleotide polymorphisms (SNPs) through medical re-sequencing studies in which candidate genes are sequenced in large numbers of cases and controls in the search for risk or causative polymorphisms. Two major characteristics have limited the utility of the re-sequencing studies. First, reported SNPs have only accounted for a small percentage of idiopathic male infertility. Second, SNPs reported to have an association with male infertility based on gene re-sequencing studies often fail validation in follow-up studies. Recent advances in the tools available for genetic studies have enabled interrogation of the entire genome in search of common, and more recently, rare variants. In this review, we discuss the progress of studies on genetic and epigenetic variants of male infertility as well as future directions that we predict will be the most productive in identifying the genetic basis for male factor infertility based on our current state of knowledge in this field as well as lessons learned about the genetic basis for complex diseases from other disease models.

Keywords epigenetics, genome-wide association study, male infertility, microsatellite instability, mutation, polymorphism

Abbreviations SNP: single nucleotide polymorphism; CTCF: CCCTC-binding factor; CNV: copy number variant; GWAS: genome-wide association study; CFTR: cystic fibrosis transmembrane conductance regulator; FASLG: Fas ligand TNF superfamily, member 6; JMJD1A: lysine-specific demethylase 3A; TEX15: testis expressed 15; BRDT: bromodomain, testis-specific; PRM: protamine; MSI: microsatellite instability; SCO: Sertoli cell only.

Introduction

The search for genetic variants responsible for complex diseases has been an active area of study for decades. Early studies involved linkage mapping [Botstein et al. 1980] followed by targeted sequencing of candidate genes or genomic regions [Panoutsopoulou and Zeggini 2009]. The completion of the Human Genome Project in 2003 [Collins et al. 2003] and the development of a number of high throughput tools for genetic analysis have greatly accelerated this research by enabling targeted medical re-sequencing studies and genome-wide association studies (GWAS). However, despite the numerous genetic studies and significant collaborative efforts involved in some large scale GWAS efforts, the genetic causes of most complex diseases have proven elusive [Manolio et al. 2009].

Evidence across a broad spectrum of complex diseases that includes diabetes, cardiovascular disease, neurological disorders, and a number of cancers, suggests that a single common genetic variant is rarely responsible for the disease phenotype of most affected individuals [Frazer et al. 2009; Manolio et al. 2009]. While common variants such as SNPs and copy number variants (CNVs) have been found to display strong associations with many diseases, these variants generally only account for a very small percentage of disease heritability [Manolio et al. 2009; Wain et al. 2009]. A growing body of evidence suggests that the 'common disease, common variant' model does not apply to the majority of complex diseases, and that the identification of rare variants will likely be needed to understand the genetics of most common diseases. Due to rapid progress in the production of ultra-high density DNA arrays and next generation sequencing technologies, the identification of rare variants involved in the etiology of common diseases is becoming a practical option [Mardis 2009].

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In spite of the prevalence and societal costs of infertility, genetic studies of male infertility have lagged behind the study of many other common diseases, largely as a result of the lack of funding. In spite of this lag, considerable effort has been made over the past few years to identify genetic variants associated with male infertility. The majority of studies have employed the gene-targeted sequencing approach, however, recent work by our laboratory has included a pilot genome-wide association study, and a follow-up study of higher throughput target analysis to identify novel SNPs associated with azoospermia and severe oligozoospermia [Aston and Carrell 2009; Aston et al. 2010]. Results from these studies indicate that male factor infertility, like most complex diseases, does not fit the 'common disease, common variant' model, and that like most common diseases studied so far, the identification of variants responsible for most cases of male infertility will be dependent on the evaluation of rare polymorphisms and CNVs.

In addition to the genetic causes of male factor infertility, epigenetic variation may be causative of disease [Kanwal and Gupta 2010]. Recent work by several groups has focused on the epigenetic architecture of sperm chromatin and the evaluation of epigenetic contributions to normal fertility, as well as the effect of epigenetic variations observed in some infertile men [Arpanahi et al. 2009; Filipponi and Feil 2009; Hammoud et al. 2009b; 2009c]. It is likely that this exciting new area of study will also uncover some variants associated with, and possibly causative of, male infertility.

This review discusses recent progress in the study of genetic and epigenetic variation associated with male infertility. The data indicate that the discovery of variants responsible for male infertility will be a complex undertaking requiring the evaluation of genetic, epigenetic, and environmental factors that are still not well understood. However, given the explosion in technology and bioinformatics capabilities and rapidly decreasing costs, the future study of genetic causes of male infertility appears promising.

Genetic components of male infertility

As with the majority of other complex diseases, early research in male infertility focused on the evaluation of its genetic components. The best-documented genetic causes of male infertility include microdeletions in the Y chromosome [Reijo et al. 1995], Klinefelter's syndrome [Lanfranco et al. 2004], chromosomal abnormalities [Martin 2008], and mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene [Cuppens and Cassiman 2004].

The Human Genome Project yielded a complete reference sequence along with annotation for the majority of genes in the human genome [Collins et al. 2003]. This coupled with dramatic advancements in the ability to produce genetically modified animal models via homologous recombination [Tamowski et al. 2010; Thomas et al. 1986] resulted in an explosion of new information regarding gene sequence and function. Other tools such as expression arrays gave further insight into the genes important in a host of physiological processes, including spermatogenesis [Schultz et al. 2003]. With this information, many researchers have undertaken gene re-sequencing studies in an effort to identify SNPs or gene mutations associated with various etiologies of male infertility, with non-obstructive azoospermia and oligozoospermia receiving the most attention [Matzuk and Lamb 2008].

A large number of genes important in spermatogenesis have been re-sequenced in cohorts of infertile men and fertile or normospermic controls (Table 1). In general, candidate gene re-sequencing studies have not proven productive in identifying risk loci for the majority of diseases studied. Many of these studies have reported significant associations between specific SNPs or gene mutations and male infertility, however, in most cases these genetic variants have either not been followed up with validation studies in larger groups of men, or follow-up studies have failed to confirm a given association [Nuti and Krausz 2008; Tuttelmann et al. 2007].

Our laboratory recently undertook a pilot genome-wide SNP association study in which we genotyped normospermic controls and azoospermic and severely oligozoospermic patients for over 300,000 SNPs [Aston and Carrell 2009]. While this pilot study had a sample size greater than nearly all previously reported re-sequencing studies, in reality GWAS studies generally require a much larger sample size. Despite the relatively small sample size in the pilot study, several relatively strong associations were detected (Table 2). Importantly, a larger follow-up study was designed to genotype the most significant SNPs from the pilot study ($n = 84$) along with SNPs with published associations with azoospermia or oligozoospermia ($n = 21$) as well as other candidate SNPs located in genes required for spermatogenesis ($n = 67$) [Aston et al. 2010]. This follow-up study was undertaken in collaboration with two European laboratories using more thorough and complete phenotyping than is typical of published re-sequencing studies. This included physical examination, medical history, scrotal ultrasound, endocrine workup, karyotype, and Y chromosome microdeletion screening. Following elimination of markers with a low call rate based on a strict internal quality control process, a total of 70 SNPs with strong or marginal associations detected in the pilot microarray study were analyzed. Of those, four SNPs displayed improved associations after genotyping the additional samples (Table 2). The yield of four markers from a GWAS that improve in statistical significance in a follow-up study is promising and these variants are currently being studied further.

Perhaps the most striking and important data from the genotyping study involved the analysis of SNPs previously reported in the literature to be associated with male infertility. In the bead array follow-up study these reported SNPs were evaluated in a study with a larger sample size and more extensive phenotyping than provided in the majority of initial re-sequencing studies, which is imperative in obtaining accurate and clinically significant data. However, only one of 21 SNPs previously reported to be associated with male infertility (Fas ligand TNF superfamily, member 6: FASLG) was

Table 1. Genes for Which Re-sequencing Studies Have Identified Polymorphisms Associated With Male Infertility.

Category	Gene	Reference	Category	Gene	Reference
Sp funct	AHR	[Merisalu et al. 2007]	Endocr	LHCGR	[Simoni et al. 2008]
Sp funct	AHRR	[Watanabe et al. 2004]	Meiosis	LIMK2	[Kuzmin et al. 2009]
Sp funct	APOB	[Peterlin et al. 2006]	Endocr	LIPE	[Kuzmin et al. 2009]
Endocr	AR	[Hiort et al. 2000]	Sp funct	MDR1	[Drozdzik et al. 2009]
Sp funct	ARNT	[Merisalu et al. 2007]	Meiosis	MEI1	[Sato et al. 2006]
Sp'gen	BCL2	[Steger et al. 2008]	Meiosis	MLH3	[Ferras et al. 2007]
Dev/Diff	BMP4	[Hu et al. 2004]	Sp'gen	MS	[Lee et al. 2006]
Dev/Diff	BMP8	[Hu et al. 2004]	Meiosis	MSH5	[Xu et al. 2010]
Meiosis	BRCA2	[Zhoucun et al. 2006]	Sp'gen	MTHFR	[Singh et al. 2005]
Sp funct	CASP8	[Ji et al. 2009]	Sp'gen	MTRR	[Lee et al. 2006]
Dev/Diff	CFTR	[Anguiano et al. 1992]	Sp'gen	NANOS3	[Kusz et al. 2009]
Sp'gen	CREM	[Vouk et al. 2005]	Sp'gen	OAZ3	[Christensen et al. 2006b]
Sp funct	CRISP2	[Jamsai et al. 2008]	Sp'gen	ODC	[Christensen et al. 2006b]
Sp'gen	CSNK2A2	[Christensen et al. 2006a]	Sp'gen	POLG	[Jensen et al. 2004]
Sp'gen	CYP1A1	[Fritsche et al. 1998]	Sp'gen	PRDM9	[Irie et al. 2009]
Sp'gen	DAZL	[Tung et al. 2006]	Sp funct	PRM1	[Tanaka et al. 2003]
Sp'gen	EIF5A2	[Christensen et al. 2005]	Sp funct	PRM2	[Tanaka et al. 2003]
Sp funct	eNOS	[Yun et al. 2008]	Meiosis	REC8	[Griffin et al. 2008]
Meiosis	ERCC1	[Ji et al. 2008]	Endocr	SBF1	[Kuzmin et al. 2009]
Endocr	ESR1	[Galan et al. 2005]	Sp'gen	SDHA	[Bonache et al. 2007]
Endocr	ESR2	[Aschim et al. 2005]	Sp'gen	SPATA16	[Dam et al. 2007]
Sp'gen	FAS	[Ji et al. 2009]	Sp'gen	SPATA17	[Miyamoto et al. 2009]
Sp'gen	FASLG	[Wang et al. 2009]	Meiosis	SPO11	[Christensen et al. 2005]
Sp'gen	FHL5	[Christensen et al. 2006c]	Endocr	SRD5A2	[Peters et al. 2009]
Meiosis	FKBP6	[Zhang et al. 2007]	Dev/Diff	SRY	[Premi et al. 2006]
Endocr	FSHB	[Grigorova et al. 2010]	Meiosis	SYCP3	[Miyamoto et al. 2003]
Endocr	FSHR	[Shimoda et al. 2009]	Sp'gen	TAF7L	[Akinloye et al. 2007]
Endocr	GNRH	[Layman et al. 2002]	Sp'gen	TBPL1	[Kuzmin et al. 2009]
Sp'gen	GOPC	[Christensen et al. 2006a]	Sp'gen	TNF	[Tronchon et al. 2008]
Sp'gen	GRTH	[A et al. 2006]	Sp funct	TNP1	[Miyagawa et al. 2005]
Sp funct	GSTM1	[Aydemir et al. 2007]	Sp funct	TNP2	[Miyagawa et al. 2005]
Sp funct	GSTT1	[Wu et al. 2009]	Sp'gen	TSSK4	[Su et al. 2008]
Sp'gen	H2BFWT	[Lee et al. 2009]	Sp'gen	TSSK6	[Su et al. 2010]
Dev/Diff	INSL3	[Yun et al. 2007]	Meiosis	UBE2B	[Suryavathi et al. 2008]
Sp'gen	KIT	[Galan et al. 2006]	Sp'gen	USP26	[Stouffs et al. 2005]
Sp'gen	KITLG	[Galan et al. 2006]	Sp funct	XPC	[Liang et al. 2010]
Dev/Diff	LGR8	[Foresta and Ferlin 2004]	Sp'gen	XRCC1	[Gu et al. 2007]
Endocr	LH	[Ramanujam et al. 2000]	Sp'gen	YBX2	[Hammoud et al. 2009]
Endocr	LHR	[Martens et al. 1998]			

Abbreviations: Dev/Diff: development and differentiation; Endocr: endocrine; Sp funct: sperm function; Sp'gen: spermatogenesis. Many of the genes listed have been the subject of multiple re-sequencing studies. In those cases, the associated reference is for the earliest study identified.

significantly associated with infertility. Marginally significant associations were found for a handful of nonsynonymous SNPs located in genes involved in spermatogenesis including SNPs in lysine-specific demethylase 3A (JMJD1A), testis expressed 15 (TEX15), and bromodomain, testis-specific (BRDT; Table 2) [Aston et al. 2010].

The general lack of strong associations and low validation rates obtained in the GWAS and follow-up genotyping study argues strongly against the 'common disease, common variant' model for spermatogenic defects. It is important to emphasize that nearly all of the SNPs studied from previous costly and time consuming medical re-sequencing studies from numerous laboratories were not associated with male infertility in our highly phentotyped study population of infertile European men. Therefore, two important conclusions may be proposed. First, medical re-sequencing studies with low sample sizes and poor phenotyping, i.e., quality control (including studies that do not include ethnically homogenous populations or do not adequately screen for all known causes of male infertility and include strictly idiopatic cases of a specific phenotype) are likely to yield little valuable information, and may in fact dilute the literature with erroneous data. Researchers and editors should be strict in the design and publishing of such studies. Second, the data clearly indicate the importance of the search for rare variants through genome-wide sequencing studies, and the importance of further study of other types of variation, including copy number variation and epigenetic variation. While genome-wide sequencing has previously been prohibitively costly, next generation sequencing technologies and study design variations have brought the possibility of genome-wide sequencing to the realm of possibility [Munroe and Harris 2010].

Epigenetic components of male infertility

Epigenetics is the study of changes other than the primary sequence that affect gene expression, including the

Table 2. SNPs With Associations With Azoospermia or Severe Oligozoospermia Based on the Pilot Microarray Study or the Follow-up Study.

Design category	Marker	Test	GWAS p	Follow-up p	Combined p
Pilot	rs3105782	AvO+N rec	1.4×10^{-7}	4.5×10^{-1}	8.2×10^{-2}
Pilot	rs6068020	AvN dom	4.5×10^{-7}	1.0	2.5×10^{-2}
Pilot	rs10841496	AvN dom	5.3×10^{-7}	2.8×10^{-1}	7.4×10^{-2}
Pilot	rs9814870	OvN add	6.8×10^{-7}	CR	NA
Pilot	rs6476866	OvA+N rec	7.6×10^{-7}	4.1×10^{-1}	1.6×10^{-3}
Pilot	rs2063802	AvN all	9.5×10^{-7}	6.7×10^{-1}	3.2×10^{-2}
Pilot	rs1399645	AvN all	9.5×10^{-7}	7.5×10^{-1}	2.5×10^{-2}
Pilot	rs4954657	AvN all	9.5×10^{-7}	7.5×10^{-1}	2.5×10^{-2}
Pilot	rs11707608	AvN all	1.2×10^{-6}	3.5×10^{-1}	1.6×10^{-3}
Pilot	rs12920268	OvA+N all	1.5×10^{-6}	CR	NA
Pilot	rs2032278	AvO+N rec	1.8×10^{-6}	1.6×10^{-1}	1.5×10^{-1}
Pilot	rs2976084	OvA+N all	2.4×10^{-6}	6.6×10^{-1}	2.4×10^{-3}
Pilot	rs215702	AvO+N rec	2.6×10^{-6}	5.8×10^{-1}	5.5×10^{-2}
Pilot	rs9825719	OvA+N all	3.6×10^{-6}	CR	NA
Pilot	rs10848911	AvN dom	3.7×10^{-6}	CR	NA
Pilot	rs4541736	OvA+N all	4.0×10^{-6}	4.8×10^{-1}	1.6×10^{-1}
Pilot	rs4695097	AvO+N rec	4.7×10^{-6}	Fail	NA
Pilot	rs2290870	AvO+N rec	5.9×10^{-6}	4.8×10^{-1}	2.2×10^{-2}
Pilot	rs4343755	AvO+N rec	5.9×10^{-6}	CR	NA
Pilot	rs1545125	AvO+N rec	6.4×10^{-6}	9.6×10^{-2}	2.9×10^{-1}
Pilot	rs4484160	OvA+N all	1.5×10^{-5}	3.2×10^{-1}	3.5×10^{-1}
Follow-up	rs5911500	OvN add	4.8×10^{-5}	4.2×10^{-3}	8.3×10^{-7}
Follow-up	rs10246939	AvO+N dom	2.1×10^{-4}	5.1×10^{-3}	1.6×10^{-5}
Follow-up	rs11204546	A+OvN add	2.4×10^{-4}	4.6×10^{-2}	1.9×10^{-4}
Follow-up	rs2059807	OvN all	1.1×10^{-3}	4.7×10^{-2}	3.2×10^{-4}
Published	rs763110	OvA+N dom	NA	1.6×10^{-4}	NA
Spermatogenesis	rs34605051	AvO+N rec	NA	7.7×10^{-4}	NA
Spermatogenesis	rs3088232	OvA+N rec	NA	6.4×10^{-3}	NA
Spermatogenesis	rs323344	A+OvN all	NA	8.2×10^{-3}	NA
Spermatogenesis	rs323345	A+OvN all	NA	1.2×10^{-2}	NA
Spermatogenesis	rs5764698	AvO+N dom	NA	1.9×10^{-2}	NA
Published	rs1801131	OvA+N rec	NA	2.4×10^{-2}	NA
Spermatogenesis	rs631357	OvA+N rec	NA	2.4×10^{-2}	NA
Spermatogenesis	rs35397110	AvO+N dom	NA	2.5×10^{-2}	NA
Spermatogenesis	rs2030259	AvO+N all	NA	2.6×10^{-2}	NA

SNPs are listed in order of significance based on *p*-value in the genome-wide study and then by follow-up *p*-value. Design category: Pilot: the most significant SNP associations based on the pilot genome-wide study; Follow-up: SNPs with significant associations based on the follow-up study; Spermatogenesis: non-synonymous SNPs located in genes involved in spermatogenesis; Published: SNPs selected based on previously published associations with spermatogenic defects. Test: describes the comparison made for association testing as well as the genetic model applied for association testing. A, azoospermic; O, severe oligozoospermic; N, normospermic; AvN, azoospermic versus normospermic; OvN, severe oligozoospermic versus normospermic, A+OvN, azoospermic + severe oligozoospermic versus normospermic, AvO+N, azoospermic versus oligozoospermic + normospermic; OvA+N, oligozoospermic versus azoospermic + normospermic; add: additive; all: allelic; dom: dominant; rec: recessive; NA: SNPs not selected for follow-up based on initial microarray results. CR: SNP not tested for association due to low genotyping call rate; Fail: unable to design BeadXpress assay for this SNP.

methylation of DNA and modifications to DNA-binding proteins. Recent evidence has indicated that the epigenetic 'marks' on the DNA and chromatin of sperm may be important to the function of sperm after fertilization [Brykczynska et al. 2010; Carrell and Hammoud 2010; Hammoud et al. 2009b; Miller et al. 2010; Nanassy and Carrell 2008]. Accumulating evidence by groups evaluating the epigenetic status of mature sperm suggests a programmatic localization of retained histones, histone modifications, and DNA methylation that appears to be important in the regulation of genes involved in early embryogenesis. Whether epigenetic variation in a given individual may be causative of infertility or simply a secondary result of other abnormalities remains to be answered. However, it appears that the budding field of epigenetics may be yet another area in which individual variants may affect male infertility and should be considered together with genetic variations.

Certainly the best-documented link between epigenetics and male infertility involves protamine packaging of the sperm genome. Sperm chromatin compaction is increased twenty-fold compared with somatic cells following the replacement of 90–95% of histones in the genome by the highly negatively charged and arginine-rich nucleoproteins, protamine 1 (PRM1) and PRM2 [Balhorn 2007]. Integration of PRM1 and PRM2 into the sperm genome during the elongation phase of spermatogenesis normally occurs in a strictly controlled 1:1 fashion. Significant deviations in the PRM1 to PRM2 ratio have been associated with reduced sperm counts, motility, morphology, and fertilization capacity as well as increased DNA fragmentation [Carrell et al. 2007; Oliva 2006]. Importantly, it appears that abnormal protamination may also be associated with decreased implantation potential in IVF patients with a low PRM1/PRM2 ratio [Aoki et al. 2006].

A recent study conducted by our laboratory evaluated the localization of the small proportion of histones that remained bound to the sperm genome following histone replacement by protamines. The aim was to determine whether histone retention was simply a result of incomplete replacement by PRMs or whether the localization of retained histones was programmatic and of functional importance [Hammoud et al. 2009b]. We found strong enrichment of retained histones in the promoter regions of spermatogenesis genes, genes involved in early development, some micro RNAs, and imprinted genes. To contrast, as expected since protamine replacement occurs in 90–95% of the sperm genome, protamine-bound regions were not enriched in any specific gene families. These data indicate a possible programmatic retention of histones, but any such functions would likely be dependent on further epigenetic marks such as histone modifications and/or DNA methylation.

Analysis of the epigenetic modifications of retained histones revealed an additional layer of epigenetic programming in male germ cells. Many retained histones in sperm are bivalently marked similar to the pattern present in stem cells [Bernstein et al. 2006], meaning they contain both activating and silencing epigenetic marks, poising them for rapid gene activation or silencing, and the gene promoters are demethylated [Hammoud et al. 2009b]. Strikingly, 'poised' genes are largely genes involved in embryogenesis and development, and generally the DNA is demethylated. Preliminary studies from our laboratory indicate that some male factor IVF patients that produced extremely fragmented embryos and/or embryos that arrested by the 8-cell stage may have altered patterns of histone retention and marking of the histone variants for some developmental gene promoters.

In agreement with this work, similar results have recently been confirmed in both the human and the mouse [Brykczynska et al. 2010]. Additionally, another group employed a different approach based on the sequencing of endonuclease-sensitive regions of sperm DNA in mice and men to determine that histones were retained at regulatory regions, gene promoters, and CCCTC-binding factor (CTCF) sites in both species [Arpanahi et al. 2009; Hammoud et al. 2009b].

Given the apparent importance of the epigenetic status of sperm, a number of recent studies have evaluated DNA methylation patterns of specific genes or promoters in sperm. Early studies evaluating the methylation status of two imprinted genes in sperm DNA from normozoospermic and oligozoospermic patients reported abnormal methylation of H19 in a subset of oligozoospermic men [Marques et al. 2004]. Similar methylation abnormalities have subsequently been reported for a number of other imprinted loci in the sperm of infertile men [Boissonnas et al. 2010; Hammoud et al. 2009c; Houshdaran et al. 2007; Kobayashi et al. 2009; 2007; Marques et al. 2008; Poplinski et al. 2009]. In addition, abnormal methylation patterns have been reported in a few non-imprinted genes in the sperm of infertile men [Houshdaran et al. 2007]. Our laboratory is currently evaluating the association of other epigenetic modifications with male factor infertility [Hammoud et al. 2009c].

Genomic instability and male infertility

A growing amount of evidence suggests male infertility is not an isolated deficiency in sperm production, rather it may be one symptom in a systemic problem in some cases. A decade ago it was observed that mutation rates were higher in DNA from testicular tissue of men with maturation arrest than those with obstructive azoospermia [Nudell et al. 2000]. Two recent epidemiological studies have found an association between reduced life expectancy and reduced semen parameters [Jensen et al. 2009] and increased comorbidities in infertile men compared with fertile controls [Salonia et al. 2009]. Another recent paper reported an increased risk for developing high-grade prostate cancer compared with men from the general population [Walsh et al. 2010]. While these examples are all anecdotal, they do indicate that wide scale genomic dysregulation might be a feature of some types of male infertility. Therefore, in our GWAS study we evaluated the incidence of minor alleles genome-wide as a possible measure of genomic differences between fertile, nomozoospermic men and infertile men. Strikingly, we reported a significantly increased incidence of minor alleles in azoospermic men based on this genome-wide genotyping scan [Aston and Carrell 2009].

Genomic instability can be broadly classified in two categories: chromosome instability and microsatellite instability (MSI) [Aguilera and Gomez-Gonzalez 2008]. Gross chromosomal abnormalities involving chromosome instability, particularly sex chromosome aneuploidies are frequent causes of male infertility. Klinefelter's syndrome (XXY) occurs at a frequency of approximately 1 in 500 live births but is responsible for about 14% of azoospermic cases [Walsh et al. 2009]. Similarly XYY and XX male syndromes result in male infertility. In addition, autosomal chromosome aneuploidies and chromosomal translocations have been associated with male infertility [Nagvenkar et al. 2005; Walsh et al. 2009]. Y chromosome microdeletions are another well-documented example of gross chromosomal abnormalities leading to male infertility [Vogt et al. 1992].

MSI results in expansions or contractions in repetitive DNA regions and is a hallmark feature of a number of different cancers [de la Chapelle 2003]. It occurs as a result of defects in mismatch repair genes, replication slippage, or during homologous recombination [Aguilera and Gomez-Gonzalez 2008]. Despite the established prevalence of chromosomal instability in male infertility, MSI has received much less attention in the context of male infertility. A single study evaluated the incidence of MSI in the testes of 41 testicular failure patients and 20 controls. Seven microsatellite loci were amplified from blood and testes DNA, and microsatellite lengths were compared between tissues, within individuals. The authors found a significantly higher incidence of MSI in patients with Sertoli cell only (SCO) syndrome compared to other patient groups or controls [Maduro et al. 2003].

We recently evaluated the incidence of MSI in oligozoospermic men by comparing blood- and sperm-derived DNA across five microsatellite regions. While the differences were not statistically significant, we found a trend toward increased MSI in oligozoospermic men (unpublished data). These preliminary data offer some indication that microsatellite instability might be an important etiology in some classes of male infertility. It is likely that this realm of study will expand and may offer some important hints as to one possible cause of the origin of some genetic variants associated with male infertility.

Future directions

Of critical importance in identifying genetic variants associated with a particular disease is comprehensive phenotyping of all patients and controls. Y microdeletion screening, karyotype analysis, and physical examination are necessary to eliminate any potential known genetic causes of male infertility. Particularly in case control association studies, ethnic and phenotypic homogeneity greatly strengthen the power of the study to detect true genetic variants. It is apparent that many of the re-sequencing studies reported in the literature are deficient in careful phenotyping, and it is likely that these deficiencies have lead to erroneous data. We encourage increased care in characterizing patients and controls in the study of genetic causes of infertility.

Based on models established by genome-wide association studies for other diseases, it is likely that strong associations, particularly those involving common variants such as those assayed on current SNP genotyping arrays, will require thousands or even tens of thousands of cases and controls. Furthermore, it appears that rare variants are likely key in most complex diseases. Emerging tools that allow sequencing of the entire exome or genome in a cost effective manner will dramatically change the way genome-wide studies are performed. Next generation sequencing technologies will facilitate the detection of rare variants or mutations that are never targeted by SNP genotyping arrays [Cirulli and Goldstein 2010; Glaser 2010]. The cost of whole genome sequencing currently remains prohibitively high for large cohort studies, however, the costs are rapidly dropping and have recently dropped from 'millions of dollars' to 'tens of thousands of dollars.' It is likely that within the next few years large genome-wide sequencing studies will become feasible and relatively common.

Early genome-wide sequencing studies may follow the model established in 2010 exploiting the markedly increased power to detect rare, disease-causing variants by sequence analysis of nuclear families [Roach et al. 2010]. As whole genome sequencing affordability increases further, larger case-control studies employing whole genome sequencing, rather than genome-wide SNP genotyping, will likely emerge.

While considerable progress has been made in the past few years to characterize the epigenetic landscape of the human genome in a variety of tissues including sperm, a comprehensive understanding of the functional significance of various elements of the epigenome is still a major area of research. Increased understanding of basic mechanisms and functions as well as improved tools to efficiently evaluate the numerous epigenetic marks on a genome-wide scale will accelerate progress in this area. Furthermore, the significance of noncoding DNA regions in regulatory processes is just becoming appreciated [Manolio and Collins 2009]. Many of the variants reported to be associated with other complex diseases, and from the GWAS for male infertility, are in regions very distant from genes [Aston and Carrell 2009]. Again, the understanding of the biological functions of these regions is a nascent field of study. Ultimately, the complex interaction of genetic and epigenetic variants will likely be responsible for much of the risk of male infertility [Dowell et al. 2010].

Clearly, the best starting point to efficiently identify genetic variants associated with male infertility is to begin with well phenotyped samples and well designed studies. Large numbers of samples will be necessary to identify rare variants and variants of reduced effect size. Genetic studies in other common, complex diseases such as type 2 diabetes, Alzheimer's disease, heart disease, and others have conclusively demonstrated the necessity of collaboration between groups and the development of large consortia focused on a specific disease. Obviously substantial funding will be necessary to undertake sufficiently powered studies. These goals will probably not be met by individual Andrology programs, rather by the cooperation and collaboration of many laboratories.

Conclusions

Clearly, we are in the early stages of gaining a comprehensive understanding of the genetic and epigenetic contributions to male infertility. Emerging technologies are enabling the collection of an unprecedented amount of data in a single experiment. There is great potential for these tools to yield valuable new insight, but the real value of genome-wide genetic and epigenetic analyses will only be realized by carefully designed experiments and with the proper analysis. The nature of genome-wide studies necessitates a highly phenotyped and homogenous study population. In addition, very large study groups are generally necessary to find true associations among the hundreds of thousands or even millions of pieces of data generated. Naturally, sufficiently powered genome-wide genetic and epigenetic studies will require cooperation between Andrology groups to provide a sufficient number of patients along with substantial funding to perform these studies.

With the ever-larger datasets generated from genome-wide and whole genome sequencing studies, personnel trained to properly analyze that data is of paramount importance. Future collaborations will of necessity include bioinformaticists capable of gleaning biologically meaningful information from the vast sea of data. With a clear set of goals in mind, and with cooperatively designed and executed experiments utilizing new and emerging genome-wide tools, we anticipate a flood of newly identified and validated

variants associated with male infertility within the next decade.

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