Title:

A systematic review and standardized clinical validity assessment of male infertility genes

Running title:

Systematic review monogenic causes of male infertility

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Abstract:

Study question: Which genes are confidently linked to human male infertility?

Summary answer: Our systematic literature search and clinical validity assessment reveals that a total of 67 genes are currently confidently linked to 81 human male infertility phenotypes.

What is known already: The discovery of novel male infertility genes is rapidly accelerating with the availability of Next-Generation Sequencing methods, but the quality of evidence for gene-disease relationships varies greatly. In order to improve genetic research, diagnostics and counseling, there is a need for an evidence-based overview of the currently known genes.

Study design, size, duration: We performed a systematic literature search and evidence assessment for all publications in Pubmed until June 2018 covering genetic causes of male infertility and/or defective male genitourinary development.

Participants/materials, setting, methods: Two independent reviewers conducted the literature search and included papers on the monogenic causes of human male infertility and excluded papers on genetic association or risk factors, karyotype anomalies and/or copy number variations affecting multiple genes. Next, the quality and the extent of all evidence supporting selected genes was weighed by a standardized scoring method and used to determine the clinical validity of each gene-disease relationship as expressed by the following six categories: no evidence, limited, moderate, strong, definitive or unable to classify.

Main results and the role of chance: From a total of 23,031 records, we included 1,286 publications about monogenic causes of male infertility leading to a list of 471 gene-disease relationships. The clinical validity of these gene-disease relationships varied widely and ranged from definitive (n=36) to

strong (n=12), moderate (n=33), limited (n=86) or no evidence (n=154). A total of 150 gene-disease relationships could not be classified.

Limitations, reasons for caution: Our literature search was limited to Pubmed.

Wider implications of the findings: The comprehensive overview will aid researchers and clinicians in the field to establish gene lists for diagnostic screening using validated gene-disease criteria and identify gaps in our knowledge of male infertility. For future studies, the authors discuss the relevant and important international guidelines regarding research related to gene discovery and provide specific recommendations to the field of male infertility.

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Keywords:

Male infertility, spermatogenic failure, genetics, clinical validity, gene-disease relation, gene panel, next-generation sequencing, systematic review

Introduction

Introduction

Infertility, a common disorder with a world-wide prevalence affecting 15% of all couples in the reproductive age, is defined as the inability to conceive within one year of unprotected sexual intercourse (Zegers-Hochschild et al. 2009). It is suggested that approximately 7% of the male population is affected by a factor of infertility and that these collectively explain half of all infertile couples (Krausz and Riera-Escamilla 2018; Irvine 1998; Winters and Walsh 2014).

The etiology of infertility is highly heterogeneous, which is not surprising when considering that both male and female reproductive systems need to function in a combined and precisely coordinated fashion in order to conceive a child. Appropriate genetic regulation is one of the most important and indispensable prerequisites to control the coordination and timing of sexual development and fertility. Because of the complexity of the gamete development, the interference of a genetic origin is suspected. Studies aiming to elucidate the genetic basis of fertility defects in both human and mice have defined numerous crucial pathways for male infertility, including sexual differentiation, development of the genitourinary system and gametogenesis (Krausz and Riera-Escamilla 2018; Jamsai and O'Bryan 2011). Currently more than 900 male infertility genes have been described in the Jackson Laboratory's Mouse Genome Informatics (MGI) database (http://www.informatics.jax.org/), and 2,300 testis-enriched genes are currently known in human (Schultz, Hamra, and Garbers 2003).

Genetic testing in infertility

It is currently thought that at least 15% of all human male infertility patients can be explained by genetic defects (Krausz and Riera-Escamilla 2018). Since the discovery of an extra X chromosome in Klinefelter patients (47,XXY) as the first genetic cause of infertility in the late 1950's (Ferguson-Smith et al. 1957; Jacobs and Strong 1959), more than 3,500 papers have been published on the genetics of male infertility, implicating various common genetic origins as well as hundreds of other genes in

82 male infertility. Despite these large numbers, genetic diagnostic testing is usually confined to
83 karyotyping, AZoospermia Factor (AZF) deletion screening and Cystic Fibrosis Transmembrane
84 conductance Regulator (*CFTR)* mutation analysis and leaves a vast majority of patients unexplained.
85 Currently a genetic diagnosis is reached in about 4% of all infertile males - a number that has not
86 increased since the late 1990's (Tuttelmann, Ruckert, and Ropke 2018; Johnson 1998). This is in sharp
87 contrast to the increase in diagnostic yield seen for other conditions with a strong genetic
88 component and increase in large-scale technologies for genetic testing since then (Rehm 2017;
89 Tuttelmann, Ruckert, and Ropke 2018). Importantly, the lack of a genetic diagnosis limits clinicians in
90 providing personalized information about the potential success of Assisted Reproductive
91 Technologies (ART), resulting in many couples undergoing these invasive procedures such as
92 TEsticular Sperm Extraction (TESE), without any chance of success. ART may lead to a situation where
93 infertility becomes an inherited condition. Therefore, a lack of genetic diagnosis limits counseling for
94 couples involved with regard to the reproductive health of offspring that can be conceived by ART
95 (Belva et al. 2016).

96 The diagnostic yield for genetic testing in male infertility remains low for several reasons. Firstly, the
97 condition is highly heterogeneous and thousands of genes are thought to play a role in
98 spermatogenesis (Schultz, Hamra, and Garbers 2003). High-impact mutations in any of these genes
99 will always remain at very low frequency in the population because of their impact on fitness. This
100 means that in order to find recurrently mutated genes and confidently linked novel genes to
101 infertility, one has to screen large cohorts of patients for pathogenic variants in large numbers of
102 genes. This has been laborious and expensive for a long time due to limitations of traditional genetic
103 assays such as Sanger sequencing. Since the first introduction of Next-Generation Sequencing (NGS)
104 in 2005, the technology has evolved to allow rapid and affordable sequencing of large amounts of
105 DNA (Metzker 2010). This has expedited sequencing of large gene panels, all coding genes (the
106 exome) and even whole genomes (Payne et al. 2018). In contrast to other fields in medical genetics
107 and oncology where NGS has revolutionized disease gene identification as well as genomic

diagnostics, the use of NGS in male infertility has only recently commenced and its use in routine diagnostics is still very limited.

The second reason underlying a disappointingly low diagnostic yield for male infertility is that the interpretation of genetic data is hampered by gaps in our understanding of the biology of male spermatogenesis and (in)fertility. This urgent need for better understanding of cellular, molecular biochemical and genetic mechanism(s) is highlighted by a recent study of the World Health Organization, who listed this need as one of the key areas of research focus (Barratt et al. 2017).

Clinical validity assessment of gene-disease relationships

With the introduction and advances in genomics, the number of genes associated to male infertility has expanded in recent years. However, the amount of genes confidently linked to disease is still very limited in comparison to developments in other genetic diseases such as intellectual disability (Tuttelmann, Ruckert, and Ropke 2018; Vissers, Gilissen, and Veltman 2016). This is caused in part by a lack of solid evidence linking variation in individual genes to human male infertility. The notion of sub-optimal quality of evidence in male infertility research is not limited to genetic studies but is considered a general concern in the field of reproductive biology (Barratt 2016; Evers 2013; Glujovsky et al. 2016).

In order to robustly link gene dysfunction to disease, one needs to consider multiple levels of evidence. This is especially important since insufficient, inconclusive and low-quality evidence may result in incorrect and misleading conclusions about gene-disease relationships. Moreover, if this wrongful gene-disease relation is not identified and corrected, it may lead to inappropriate diagnoses and even mismanagement and counseling of infertile couples involved. Furthermore, these incorrectly characterized genes may complicate follow-up research by contaminating candidate disease gene lists and pathway analyses.

To evaluate genetic variant pathogenicity, standard guidelines such as those provided by the American College of Medical Genetics and Genomics (ACMG) are invaluable (Richards et al. 2015). Their well-established framework combines variant allele frequency in control populations, computational prediction programs such as SIFT (Kumar, Henikoff, and Ng 2009) and PolyPhen (Adzhubei et al. 2010), as well as functional evidence and variant segregation evidence to classify sequencing variants into (likely) benign, pathogenic or variants of uncertain significance (VUS). These guidelines, however, have been developed for diagnostic purposes and are based on the assumption that the causal link between the gene and the condition has already been established. So whereas individual genetic variants may be considered pathogenic, unfortunately, the majority of candidate genes in male infertility still have questionable evidence and cannot be confidently linked to human disease.

Recently, the Clinical Genome Resource (ClinGen) has developed an extensive framework to assess the clinical validity of a gene-disease relationship (Strande et al. 2017). However, the overall number of validated disease genes is currently very limited (n=333) and does not contain any genes involved in male infertility. Another, more simplified and pragmatic version of this framework was recently published to more easily assess the clinical validity of gene-disease relationships (Smith et al. 2017). In this study, we applied this latter gene-disease scoring system to curate all available information on the genetics of human male infertility from 1958 up to June 2018. This analysis allowed us to objectively classify the evidence for the involvement of genes in male infertility as non-existing, limited, moderate, strong or definitive. The results from this work may be useful in both research and diagnostics, for example for developing diagnostic gene panels and hopefully help to strengthen genetic research in male infertility.

Materials and methods

Search strategy and study selection

Two independent reviewers conducted a literature search in Pubmed according to the PRISMA guidelines (Moher et al. 2009) for English articles in peer-reviewed journals. The search was performed on several occasions with the last search taking place on the 21st of June 2018 without further restrictions on publication date. The search query and screening strategy aimed to collect all records of genetics research in defective male reproductive development and function (Supplemental Table S1). We excluded all papers that did not describe human patients. Since the scope of our review is limited to monogenic causes of male infertility and/or defective male genitourinary development, we excluded papers describing chromosomal aneuploidies, complex chromosomal rearrangement or copy number variations not attributable to a single gene, polygenic and multifactorial causes, as well as variants that are associated with infertility, but do not directly influence gene function such as SNP or genome-wide association studies. We also excluded genetic disorders causing severe syndromic forms of infertility, affecting multiple organ systems (in addition to the reproductive system). This for instance excludes syndromes which compromise viability, or cause physical or intellectual disabilities to such a degree that patients are unlikely to seek for help to reproduce (Supplemental Table S1).

We included patients with delayed puberty, completely sex-reversed individuals with male phenotype (46,XX maleness) and male patients with partial virilization and a Prader score of 4, or more. We used reviews on the genetics of human male infertility to supplement our strategy with papers that were not identified in our systematic search, but did report potential gene-disease relationships. Publication inclusions of doubt were resolved by discussion and consensus between all authors.

177 Data extraction and assessment of clinical validity

178 From eligible papers presenting original data, we extracted the gene names, patient phenotypes,
179 inheritance pattern, method of discovery and whether or not single nucleotide or copy number
180 variants were identified in the genes mentioned in infertile men. After extraction of the gene names
181 from all records, we employed a recently published gene-disease scoring system to establish the
182 strength of evidence for the relationship between a gene and male infertility (Smith et al. 2017).

183 A detailed description of the evidence assessment and a assessment template are described in
184 Supplemental Table S2 and S3. In short, for each gene, we collected evidence for the most likely
185 mode of inheritance (recessive, dominant, X-linked, Y-linked) of the infertility (sub)phenotype
186 primarily based on evidence provided in the original papers and from model organisms. If the human
187 mode of inheritance was unclear, we used computational methods based on statistical learning to
188 predict the most likely mode of inheritance (Quinodoz et al. 2017; Lek et al. 2016). All variants
189 described were re-classified using the standard ACMG guidelines for the interpretation of sequence
190 variants (Richards et al. 2015). Only patients who had a variant(s) that 1) match the expected or
191 proved inheritance pattern of the disease and 2) were classified as “Pathogenic”, “Likely pathogenic”
192 or “Uncertain significance” were eligible for scoring.

193 Except for variants in *CFTR* which may be more common due to founder effects in North European
194 populations (Bombieri, Seia, and Castellani 2015), we used a maximum allele frequency of 1% in the
195 general population as a threshold value. Variants causing fully penetrant monogenic severe male
196 infertility suffer from strong selection in the general population and are unlikely to reach higher allele
197 frequencies than 1% (Eilbeck, Quinlan, and Yandell 2017). Variants that were more common were
198 classified as (likely) benign. Next to various freely available population databases such as GnomAD
199 (Lek et al. 2016), we also used an anonymized local database with exome variants found in 3,347
200 fathers of children who have been referred for trio Whole Exome Sequencing (WES) in the Radboud

University Medical Centre. The healthy fathers reflect the general Dutch population and to our knowledge conceived naturally.

In order to award points for statistical evidence in autosomal recessive (AR) forms of infertility described in families, we used the Logarithm of the ODds (LOD) scores from the original paper. If no LOD score was given, we used a simplified formula as provided by the Clinical Genome Resource Gene Curation Working Group (Strande et al. 2017). For dominant/X-linked diseases we used: $Z(LOD\ score) = \log 10 \frac{1}{0.5^{\text{segregations}}}$ and for recessive diseases we used: $Z(LOD\ score) = \log 10 \frac{1}{0.25^{\#\text{ affected individuals}-1}\ 0.75^{\#\text{ unaffected individuals}}}$. For gene function, disruption, protein interaction and model organism evidence, we critically reviewed available information from the original articles, cited papers, other (more recent) papers from Pubmed, the Protein Atlas database(Uhlen et al. 2015), the STRING database (Szklarczyk et al. 2017) and the Mouse Genome Informatics database (Smith et al. 2018). The first paper describing a variant in a potential disease gene was used as the index patient. In line with this, points for independent publications were only given from the second publication on describing variants in the same gene in unrelated patients. We then calculated the sum of the assigned points for each gene and determined the clinical validity category according to the original method (Smith et al. 2017). All genes received a denomination based on the points gathered; 1-2 points: “No evidence”; 3-8 points: “Limited”; 9-12: “Moderate”; 13-15: “Strong”; 16-17 points: “Definitive”. Similar to the publication selection process, disagreements and debatable cases were solved by consensus between all authors.

In order to prevent bias in gene-disease evaluation, a second and a third reviewer independently reviewed and verified a random selection 12 and 16 gene-disease relationships, respectively. A maximum difference of 1 point per gene-disease relationship was allowed if the classification was not altered. All other cases were discussed and re-evaluated after consensus was obtained.

Overview of biological knowledge

From all genes with at least limited evidence, we also extracted I) the reported or expected results of semen analysis (if available), II) whether the patients described are sporadic or familial cases, and III) whether the type of infertility was isolated, a reproductive organ syndrome, endocrine disorder or part of another syndrome. All genes with at least limited evidence were plotted according to their biological function.

Results:

Search strategy and study selection

With our search strategy, we aimed to identify all publications covering the genetics of male infertility, including those underlying syndromes affecting the endocrine system, disorders of sex development and genitourinary anomalies. Our search yielded a grand total of 23,031 publications that date from 1958 - 2018. Based on title and abstract, 18,095 studies were excluded because the publication was not in written English, or the study topic did not match our inclusion criteria (Supplemental Table S1). Although severe syndromes including male infertility phenotypes were excluded because affected patients are unlikely to seek for help to reproduce because of severe physical or intellectual disabilities, we included milder syndromes and syndromes affecting the reproductive organs only. A total of 4,936 publications were left. Since the scope of our systematic review is monogenic male infertility, we then excluded papers based on full-text screening which described genetic association or risk factors (n=668), AZF deletions (n=469), CNVs affecting multiple genes (n=28) or chromosomal anomalies (n=1,180). In addition, we excluded 803 publications that, based on full-text analysis in retrospect, were not covering the topic of the genetics of male infertility and we excluded 41 papers of which the full text was unavailable. We then screened the reference lists from included reviews (n=576) and were able to add another 115 publications that were not identified by our search strategy. In total, our search yielded 1,286 publications that met our inclusion criteria (Figure 1).

The systematic literature search revealed a total of 150-200 publications per year in the past 10 years and showed that the majority of publications from the last few years report on monogenic causes of male infertility (46% in 2017), followed by genetic association or risk factor analysis (28% in 2017) (Figure 2A and B). Furthermore, the absolute number of karyotype studies has been relatively stable over the past 20 years at approximately 30 publications per year.

Data extraction and evaluation of evidence

From the 1,286 included publications, we extracted 438 unique HUGO approved gene names and 471 gene-disease relationships (Figure 1). The number of gene-disease relationships is higher than the number of genes because several genes were described in multiple male infertility phenotypes. A further look into the discovery method of these gene-disease relationships showed that DNA sequencing has been the most commonly used technique for novel gene discovery and replication studies (84% of all publications). At the moment a shift from Sanger sequencing to Next-Generation Sequencing methods is taking place (Figure 2C).

We then assessed the clinical validity of each gene-disease relationship by using the simplified scoring system designed to establish the strength of a relationship between a single gene and a Mendelian disease (Smith et al. 2017) (Supplemental Table S2 and S3). In short, the scoring system takes into account the total of unrelated patients, the number of papers that reproduced the initial finding, the number of unique pathogenic variants and the evidence of gene disruption by the variant and the phenotype of model organisms.

After excluding genes that did not contain any potentially pathogenic variant or were unable to be classified, a total of 173 gene-disease relationships were curated and classified into the following categories definitive (n=36), strong (n=12), moderate (n=33), limited (n=86) and no evidence (n=6). We identified a total of 67 genes that can at least be moderately linked to a total of 81 male infertility or abnormal genitourinary development phenotypes showing autosomal recessive (n=42), autosomal dominant (n=26), X-linked (n=11) and Y-linked (n=2) inheritance patterns. Patients were found sporadic (n=15), in families (n=10) or in both (n=56) and led to either isolated (n=18), reproductive organ or endrocrine syndrome (n=49) or a syndromic form of infertility (n=14). A summary of the results is depicted in Table 1 and Supplemental Table S4; full scoring is available in Supplemental Table S5. In 154 cases, no (likely) pathogenic variants were identified and were therefore also classified as “No evidence” for involvement in human infertility without further curation of other evidence (Supplemental Table S6). In 150 cases, we could not evaluate the gene-

disease relationship because either the inheritance pattern remains unclear or suggests polygenic inheritance, the technical quality of the identification method was too poor or the exact variant information could not be retrieved (Supplemental Table S7).

The results show that the total number of confidently linked genes is growing steadily at about 3 genes per year (Figure 2D). The increase of NGS methods being used has caused an exponential growth in novel candidate genes. However, the vast majority of these are currently classified as "Limited evidence".

Overview of human genes involved in human male infertility

Taking into account that normal functioning of the male reproductive system is biologically mostly dictated by the hypothalamic–pituitary–gonadal axis functioning, the origins of male infertility can be divided in three major groups: pre-testicular, testicular and post-testicular. We grouped all genes with at least limited evidence for an involvement in human male infertility into these three groups based on their reported biological function (Figure 3) to assess whether the curated genes play a role in these biological processes.

Our results show that pre-testicular forms of infertility are mostly syndromic and caused by endocrine abnormalities, characterized by low levels of sex steroids and abnormal gonadotropin levels. Post-testicular causes include ejaculatory disorders or obstructions, which impair the transport of spermatozoa from the testis. These obstructions can be caused by a congenital unilateral or bilateral absence of the vas deferens. The most common genetic cause of obstructive azoospermia (OA) are biallelic variants in the *CFTR* gene (Anguiano et al. 1992; Culard et al. 1994; Dumur et al. 1990; Oates and Amos 1994; Patrizio et al. 1993) and variants in the recently identified X-linked gene *ADGRG2* (Patat et al. 2016).

Despite the fact that monomorphic forms of teratozoospermia are extremely rare, the majority of genes known to cause isolated testicular forms of infertility are involved in such disorder (n=8, 53%

of all 15). The number of genes confidently linked to oligozoospermia or azoospermia when mutated remains limited (n=7, 47% of all 15).

Discussion

Male infertility is a complex multifactorial condition which pathogenesis can be explained by environmental causes, urological conditions such as retrograde ejaculation, defective endocrine control of spermatogenesis such as hypogonadotropic hypogonadism or by the occurrence of genetic alteration in genes important for proper reproductive functioning. This standardized clinical validity assessment focused on the genetic causes of infertility and provides a systematic and comprehensive overview of all genes implicated as a monogenic cause of male infertility. Our study aimed to provide an overview of all currently available evidence and gene-disease relationships, as well as formulate a set of recommendations for future studies involving the genetics of male infertility.

Clinical validity of gene-disease relationships in male infertility

In our literature search, we identified 471 gene-disease relationships that were subjected to critical evaluation. Hereto we used a framework that was designed for interpretation of new research findings in a clinical context in an unbiased way (Smith et al. 2017). The method that we used is a simplified version of the extensive framework used by ClinGen to curate gene-disease relationships and results in similar evidence categories. The method was previously described and proved to be reliable, reproducible and similar to the conclusions of the ClinGen method which makes the method suitable for robust and rapid evaluation of genes in both research and diagnostic sequencing settings (Smith et al. 2017).

The clinical validity of the 471 gene-disease relationships varied widely and ranged from definitive (n=36) to strong (n=12), moderate (n=33), limited (n=86) or no evidence (n=154). A total of 150 gene-disease relationships could not be curated because the tool that we used was not suitable for the type of inheritance pattern observed: we only assessed genes with highly penetrant Mendelian inheritance patterns and excluded mitochondrial and polygenic inheritance patterns (n=45). Furthermore, in several cases the quality of the variant detection was problematic (n=5), essential information like variant information was missing (n=8), or there was insufficient evidence for

333 establishment of the inheritance pattern (n=92) in the original article(s). These results demonstrate
334 that the quality of evidence for gene-disease relationships and reporting of the results varies greatly -
335 a matter that is often not examined or acknowledged in original publications and/or literature
336 reviews.

337 The curation of all gene-disease relationships was performed with the currently available evidence
338 identified in this literature search. The results are not static and as knowledge increases over time
339 the outcome may be subjected to changes over time. Hence, we expect that a large number of the
340 genes that are currently classified as “Limited”, “No evidence” or “Unable to classify” may still play
341 an important role in male infertility and should therefore not be omitted from future genetic studies.

342 The number of candidate gene-disease relationships is growing exponentially as a result of the
343 availability of NGS methods and the first half of 2018 has already yielded more novel gene-disease
344 relationships than the full year of 2017 (Figure 2D). However, the number of confidently linked gene-
345 disease relationships is not growing at the same pace. The major reason for this is that most genes
346 have only been found mutated in single patients and functional evidence is lacking. We expect the
347 number of genes confidently linked to azoospermia to grow in the coming years by large-scale data
348 sharing, especially since this is a common form of infertility and genetic components are very likely to
349 play an important role in its etiology (Krausz and Riera-Escamilla 2018).

350 Importance of re-evaluation of evidence

351 The recent availability of large genetic population reference databases facilitates re-evaluation of
352 reported disease-associated variants and allows to determine whether the population frequency of
353 the variant is in line with a reported link to a disorder associated with reduced fitness such as male
354 infertility. Previous reports have shown that healthy participants on average have ~54 exonic variants
355 that were previously reported to be pathogenic, but based on their allele frequency were likely to be
356 misclassified (Lek et al. 2016).

The systematic re-classification of reported genetic sequencing variants in male infertility using this information resulted in some interesting observations. For example, *PICK1* is regularly mentioned as a gene that causes globozoospermia in human patients (De Braekeleer et al. 2015; Ray et al. 2017; Krausz and Riera-Escamilla 2018). However, only one patient with one homozygous variant has ever been described in the initial report of a Chinese globozoospermia patient, and no new patients were published since (Liu, Shi, and Lu 2010). The article was published in 2010, six years before the release of gnomAD which is currently the largest database with allelic information from 123,136 exomes and 15,496 genomes. The variant described in the original publication is present in 1.74% of the East Asian population (http://gnomad.broadinstitute.org/variant/22-38471068-G-A). Calculating the Hardy-Weinberg equilibrium using the incidence of globozoospermia (0.1 % of all male infertility patients) (Holstein et al. 1973) suggests that the maximum allele frequency of this variant can be 0.026%, which is much lower than the observed frequency. Importantly, this maximum allele frequency is still an overestimate as it assumes that all globozoospermia patients are explained by this *PICK1* variant. Although the gnomAD database includes females, may contain male infertility patients and the variant may have reduced penetrance, it is highly unlikely that this particular variant is causing globozoospermia in this patient based on the allele frequency.

Despite the gene-disease relation being based on the wrong data, PICK1 deficiency has been shown to result in disruption of acrosome formation in mice and *PICK1* is expressed in human testis (Xiao et al. 2009). Hence, based on these observations the gene remains an important candidate gene for human male infertility. Similar discrepancies in originally published allele frequencies and currently available allele frequencies were found in several other genes including *NLRP14* (http://gnomad.broadinstitute.org/variant/11-7060977-A-T) (Westerveld et al. 2006), *SEPT12* (http://gnomad.broadinstitute.org/variant/16-4833970-C-T) (Lin et al. 2012) and *RHOXF1* (http://gnomad.broadinstitute.org/variant/X-119243190-C-T) (Borgmann et al. 2016).

Evidence from animal models was often strong and genetic studies clearly benefit from a wealth of studies describing hundreds of well-characterized male infertility mouse models (de Boer, de Vries, and Ramos 2015; Kherraf et al. 2018). However, caution is urged in drawing conclusions about gene function and inheritance mode based on mouse models only. The mouse and human reproductive system are not identical and genes may have (slightly) different functions or transmit disease through different modes of inheritance (Lieschke and Currie 2007). For this reason, we included statistical evidence from large human datasets to supplement the evidence from animal models (Lek et al. 2016; Quinodoz et al. 2017). In case the evidence for inheritance pattern was clearly contradictory between mice and human, we did not evaluate the gene-disease relationship (n=92).

Recommendations for genetic testing in male infertility

During our study, we noted that international guidelines for nomenclature and interpretation of sequencing variants were often not followed even long after the introduction and world-wide acceptation of these guidelines (den Dunnen et al. 2016; Richards et al. 2015). We identified several errors in nomenclature of sequencing variants and in some cases the variants were not named in a meaningful and unequivocal manner rendering them unusable for assessment. This is in conflict with the FAIR Data Principles for data management and stewardship (Wilkinson et al. 2016). Furthermore, many publications did not mention the expected or proven inheritance pattern or made doubtful conclusions about the mode of inheritance.

In order to ensure efficient sharing and downstream use of newly identified sequencing variants and genes, it is crucial to report variants in an unambiguous and standardized way. In adherence to the standard ACMG guidelines and the best practice guideline of Dutch Genome Diagnostic Laboratories, we have made a list of recommendations for future reporting of novel male infertility variants (Supplemental Table S8). Furthermore, our literature study shows that the quality of evidence of a gene-disease relationship varies greatly. We recommend the use of public and local genomic reference databases, statistical and functional experiments to build evidence for causality

(Supplemental Table S9). Due to the sporadic nature of some forms of male infertility, it can be very challenging to acquire multiple patients with variants in the same gene. There are multiple online platforms such as Matchmaker Exchange available for researchers and clinicians which have proven to successfully match patients and uncover rare and novel causes of disease (Philippakis et al. 2015).

The genetics of human male infertility: overview and future perspectives

Our work shows that the field of genetics of male infertility is rapidly expanding due to the introduction of NGS methods (Figure 2). However, currently of all 471 gene-disease relationships described, only 17% (n=81 gene-disease relationships involving 67 genes) have been at least moderately linked to the disease and an additional 18% (n=86 gene-disease relationships involving 84 genes) are candidate gene-disease relationships with only limited evidence for involvement of the gene in a male infertility phenotype (Table 1, Supplemental Table S4; Figure 3). Caution should be warranted when using genes with limited or no evidence for diagnostic screening.

Similar to other fields in medical genetics, the field of genetics in male infertility has largely focused on inherited variation. Our analysis indicates that 52% of all gene-disease relationships with at least moderate evidence for an involvement in male infertility show an autosomal recessive inheritance pattern (n=42 of 81 gene-disease relationships involving 40 genes). Importantly, many of these genes have been identified in consanguineous families and many of these are associated with very specific and rare sperm defects. It is therefore unlikely that these genes will play a major role in the more common quantitative sperm defects encountered in outbred populations. In contrast, our analysis revealed that only 32% of all gene-disease relationships (n=26 of 81, involving 20 genes) with at least moderate evidence for causing male infertility has an autosomal dominant inheritance pattern, most of which are syndromic presentations.

It may perhaps not be surprising that there is only a limited number of autosomal dominant genes described for male infertility, as pathogenic variation in these genes can only be passed through the maternal line. Importantly, however, studies in intellectual disability and developmental delay have

recently pointed to an important role for *de novo* germline mutations resulting in autosomal dominant disease (Vissers, Gilissen, and Veltman 2016). The *de novo* mutation hypothesis for male infertility is further underscored by the fact that *de novo* chromosomal and structural variations are well-known causes of male infertility: Klinefelter syndrome (47,XXY) and AZF deletions almost exclusively occur *de novo* (Lanfranco et al. 2004; Colaco and Modi 2018). The role of *de novo* point mutations, however, remains unexplored in male infertility so far. At the moment, only 3 autosomal dominant genes are moderately linked to isolated male infertility (*DMRT1*, *KLHL10*, *SYCP3*). Unfortunately, for none of these genes parental samples were studied to find out whether the variant was paternally or maternally inherited or occurred *de novo*.

Genetic testing in diagnostic settings

In clinics, genetic testing is offered to infertile men to establish a molecular diagnosis that can be used predict the potential success of fertility treatment options, such as In Vitro Fertilization (IVF), IntraCytoplasmic Sperm Injection (ICSI) combined with TEsticular Sperm Extraction (TESE) or PErcutaneous Sperm Aspiration (PESA) and the risk of transmitting infertility to the next generation. Several studies have shown that men with male factor infertility experience more negative emotional impact such as depressive symptoms, stigma and reduced self-esteem, than men whose partners were infertile or men of couples diagnosed with unexplained infertility (Fisher and Hammarberg 2012). The standard use of NGS in the diagnostic work-up of male infertility could lead to more men receiving a diagnosis, or explanation, and therefore possibly influence this emotional burden in a positive way.

The recommendations for genetic testing during the diagnostic work-up of male infertility have only minimally changed over the last 20 years and most of these recommendations still focus on the well-known and common causes of male infertility that were already known in the 1990's (Barratt et al. 2017; Jungwirth 2018). For cost-efficiency, there are guidelines to help stratify patient groups to receive pre-conceptive genetic tests such as karyotype analysis, AZF deletion tests or a screening for

pathogenic variants in a single gene involved in a specific phenotype such as CBAVD or Kallmann syndrome. A recent World Health Organization study on the diagnosis on male infertility suggested to at least perform karyotyping and AZF deletion tests in men with non-obstructive azoospermia or extreme oligoasthenoteratozoospermia (OAT) without a history of a known cause of spermatogenic failure such as chemotherapy, varicocele, orchitis or bilateral cryptorchidism (Barratt et al. 2017). However, after stratification, in approximately 40% of all male infertility patients no genetic cause is found with the above mentioned tests (Krausz and Riera-Escamilla 2018) and this strongly suggests that much more genetic research is required and at the same time the use of other diagnostic assays should be considered.

Testing all patients for all genetic anomalies was very costly for a long time. However, in light of the recent developments of novel sequencing technologies, it is now possible to consolidate one or multiple tests in a single NGS assay which will help to cut the costs. The first examples of NGS-based screening methods have been described for male infertility (Oud et al. 2017; Fakhro et al. 2018; Patel et al. 2018). The European Society of Human Genetics (ESHG) and the European Society for Human Reproduction and Embryology (ESHRE) have recently made a recommendation for developing and introducing new tests, specifically for extended carrier screening (Harper et al. 2018). Genetic tests should be designed to achieve high clinical validity, establish clinical utility, minimize secondary findings such as carriership of cancer-predisposing variants as the capacity of follow-up counseling is limited and furthermore they emphasized that providers should take into account that there are inter- and intra-population and individual differences for genetic risk and disease. For example, when considering the effect of immigration of non-European populations on counseling and interpretation of uncommon disease-associated variants (Harper et al. 2018).

With the current rise of NGS in the field of male infertility, the number of novel pathogenic variants and genes will grow rapidly (Figure 2). The identification of novel disease genes allows for selection of genes for male infertility gene panels. For diagnostic purposes, gene panels should contain genes

with a minimal level of evidence of involvement with disease. We recommend to include genes with an evidence classification of at least "Moderate" for the composition of diagnostic gene panels. We recommend the inclusion of genes involved in syndromic forms of male infertility. The severity of several syndromes relies on the damaging effect of the mutation(s) and the spectrum may span combinations of recognizable associated features to isolated infertility. It is therefore possible that patients referred to for infertility suffer from a mild syndrome which features were missed or not present upon anamnesis.

Conclusion

In this clinical validity assessment, we evaluated a total of 471 gene-disease relationships involving 438 genes with reported monogenic association to male infertility and identified 81 gene-disease relationships with at least moderate evidence for a role in male infertility. Both our results as well as our objective approach and recommendations may aid the robust and rapid identification and incorporation of novel genes in male infertility diagnostics.

Author roles

M.S.O., L.V., L.E.L.M.V, and J.A.V. designed this study and L.R., L.E.L.M.V. and J.A.V. supervised this study. M.S.O. and L.V. selected studies for the inclusion and evaluation of quality. M.S.O. evaluated the quality of all included publications and L.V. reviewed and verified the results. Disagreements in the inclusion and evaluation process were solved by consensus between all authors. All authors made substantial contributions to the interpretations of the results. M.S.O. and L.V. prepared the figures and M.S.O., L.V. and R.M.S. wrote the first draft of the manuscript. All authors contributed to the revision process.

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The authors have nothing to disclose.

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Figure descriptions:

Figure 1: PRISMA flow chart. Our search and screening strategy to identify publications and genes eligible for clinical validity assessment. AR: Autosomal Recessive; AD: Autosomal Dominant; XL: X-linked; YL: Y-linked

Figure 2: Genetic studies in male infertility. A) Graphical overview of genetic studies in male infertility. B) Graphical representation of type of genetic research in male infertility. C) The use of Sanger Sequencing and Next Generation Sequencing for the discovery of genes in male infertility. D) Increase of genes linked to human male infertility.

Figure 3: Biological overview of the genetics of male infertility. The color of each gene indicates the amount of evidence: Brown: Definitive; Red: Strong; Orange: Moderate.

Table 1: Results gene-disease relationships; only showing at least moderate evidence

Identification

Records identified through Pubmed search on the 21st of June 2018:
n= 23,031

Screening

Abstract and title screening:
n= 23,031

Excluded
n=18,095
1. Publication not in English: 2,505
2. Study not in humans: 4,562
3. Study topic irrelevant: 11,028

Eligibility

Full-text screening:
n=4,936

Excluded
n=3,189
4. Association study: 668
5. AZF deletion: 469
6. Multiple genes affected: 28
7. Karyotype anomaly: 1,180
8. Irrelevant: 803
9. Full text unavailable: 41

Reviews included:
n=576

Additional articles from reference lists:
n=115

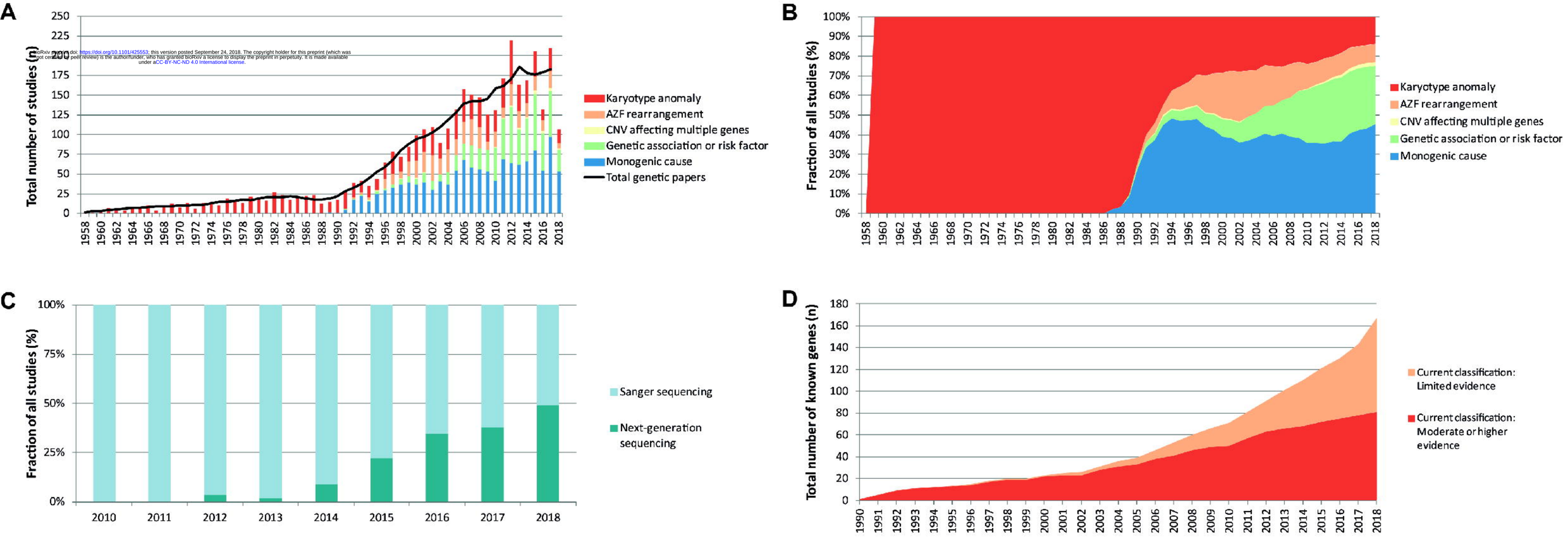
Inclusion

Papers included:
n=1,286

Gene names and disease names extracted from publication:
Number of genes=438
Number of gene-disease relationships: 471

Evidence assessment

	Definitive: n=36	Strong: n=12	Moderate: n=33	Limited n=86	No evidence: n=154	Not evaluated: n=150
AR	22	4	16	51	95	N/A
AD	8	4	14	27	45	
XL	5	3	3	7	14	
YL	1	1	0	1	1	



Pre-testicular, abnormal development of reproductive organs:
AMH AMHR2 AR NR5A1 SRD5A2 WT1 MAMLD1 SRY HSD17B3
SOX3 SOX9 RSPO1

Pre-testicular, abnormal hypothalamus development and function: **ANOS1 CHD7 FGFR1 KISS1R PROKR2 TACR3** ANOS1 FGF8 PROK2 SOX10 **SOX2** CCDC141 GNRH1 SEMA3A WDR11

Hypothalamus
GnHR

Pre-testicular, pituitary gland dysfunction: **GNRHR LHB** FSHB FSHR

Anterior Pituitary
FSH LH

Pre-testicular, adrenal gland dysfunction:
CYP11A1 CYP11B1 CYP19A1 CYP21A2 NR0B1 CYP17A1 HSD3B2

Testicular, Leydig cell dysfunction: **LHCGR**

Leydig (Interstitial) cells
Testosterone

Pre-testicular, cryptorchidism: INSL3

Post-testicular, hydrocele/spermatocele:

Testicular, Sertoli cell only syndrome: FANCA

Post-testicular, abnormal development of urethra/ vas deferens: **CFTR** ADGRG2 BNC2

Post-testicular, fertilization defect:

Embryo/ Fetus	Postnatal stage	Puberty	Continues throughout life
Migration	Growth/proliferative phase (spermatocytogenesis)	Differentiation/Growth	Maturation/ Differentiation

	Mitosis (2n)					Meiosis I (2n, 4c)						Meiosis II (1n, 2c)	Spermiogenesis (1n, 1c)	
Primordial germ cell precursors	**Primordial germ cells (PGS)**	**Gonocytes**	**Spermatogonial stem cells (SSC)**	**A-spermatogonia**	(Progenitor A dark-spermatogonia | Progenitor A pale-spermatogonia | Committed A pale-spermatogonia)	**Intermediate spermatogonia**	**B-spermatogonia**	**Primary spermatocytes**	(Preleptotene spermatocytes | Leptotene spermatocytes | Zygotene spermatocytes | Pachytene spermatocytes | Diplotene spermatocytes)	**Secondary spermatocytes**	**Spherical or round spermatids**	**Sperm cell (spermatozoon)**

Pre-meiotic arrest

AR APOA1

Meiotic arrest

TEX11 SYCP3 TEX15

Spermiogenesis defect

AURKC CFAP43 CFAP44 DNAH1 DPY19L2 SUN5 CCDC39 LRRC6 CCDC40 CEP290 CFAP69 DNAAF2 PIH1D3 SPATA16

Spermatogenesis defect (stage unknown)

NR5A1 TRIM37 KLHL10 CDC14A DMRT1 NLRP3

Colour coding of genes: Definitive Strong Moderate

Table 1: Results gene-disease relationships; only showing at least moderate evidence

HGNC gene name	Synonyms	Gene locus	Broad category	Disease category	Disorder	Expected result semen analysis	Cases	Type of infertility	Inheritance pattern in human	Score *	Conclusion *	First 2 references **
ADGRG2		Xp22.13	Post-testicular	Abnormal development of vas deferens	Congenital Bilateral Absence of the Vas Deferens; OMIM:300985	Azoospermia	Sporadic	Isolated infertility	XL	10	Moderate	(Patat et al. 2016; Yang et al. 2017)
AMH		19p13.3	Pre-testicular	Abnormal development of reproductive organs	Persistent Müllerian Duct Syndrome; OMIM: 261550	Oligoasthenoteratozoospermia, hematospermia	Familial/sporadic	Reproductive system syndrome	AR	17	Definitive	(Knebelmann et al. 1991; Carre-Eusebe et al. 1992)**
AMHR2		12q13.13	Pre-testicular	Abnormal development of reproductive organs	Persistent Müllerian Duct Syndrome; OMIM: 261550	Oligoasthenoteratozoospermia, hematospermia	Familial/sporadic	Reproductive system syndrome	AR	17	Definitive	(Imbeaud et al. 1995; Imbeaud et al. 1996)**
ANOS1	KAL1	Xp22.31	Pre-testicular	Abnormal hypothalamus development and function	Kallmann syndrome; OMIM:308700	Azoospermia, oligozoospermia	Familial/sporadic	Endocrine disorder/Reproductive system syndrome	XL	18	Definitive	(Franco et al. 1991; Bick et al. 1992)**
					Isolated Hypogonadotropic Hypogonadism (normosmic); OMIM:308700	Azoospermia, oligozoospermia	Familial/sporadic	Endocrine disorder/Reproductive system syndrome	XL	15	Strong	(Sato et al. 2004; Li et al. 2016)**
APOA1		11q23.3	Testicular	Pre-meiotic arrest	Testicular amyloidosis; OMIM:105200	Azoospermia, oligozoospermia	Familial/sporadic	Syndromic infertility	AD	12	Moderate	(Obici et al. 2004; Scalvini et al. 2007)**
AR		Xq12	Pre-testicular	Abnormal development of reproductive organs	Partial androgen insentitivity syndrome; OMIM:312300/300633	Azoospermia, oligozoospermia	Familial/sporadic	Reproductive system syndrome	XL	17	Definitive	(DiLauro et al. 1991; Lobaccaro et al. 1992)**
			Testicular	Pre-meiotic arrest/Meiotic arrest	Non-obstructive azoospermia; OMIM:NA	Azoospermia, oligozoospermia	Familial/sporadic	Isolated infertility	XL	17	Definitive	(Akin et al. 1991; Yong et al. 1994)**
AURKC		19q13.43	Testicular	Spermiogenesis defect	Macrozoospermia; OMIM:243060	Teratozoospermia: Macrozoospermia	Familial	Isolated infertility	AR	17	Definitive	(Dieterich et al. 2007; Dieterich et al. 2009)**
BNC2		9p22.3-p22.2	Post-testicular	Abnormal development of urethra	Hypospadias; OMIM:NA (PS300633)	Normozoospermia, oligozoospermia	Sporadic	Reproductive system syndrome	AD	10	Moderate	(Bhoj et al. 2011; Baxter et al. 2015)**
CCDC141		2q31.2	Pre-testicular	Abnormal hypothalamus development and function	Kallmann syndrome; OMIM:NA (PS147950)	Oligozoospermia, azoospermia	Familial/sporadic	Endocrine disorder/Reproductive system syndrome	AR	9	Moderate	(Kotan et al. 2014; Hutchins et al. 2016)**
CCDC39		3q26.33	Testicular	Spermiogenesis defect	Primary ciliary diskinesia; OMIM:613807	Oligoasthenozoospermia	Familial/sporadic	Syndromic infertility	AR	14	Strong	(Merveille et al. 2011;

												Blanchon et al. 2012)
CCDC40		17q25.3	Testicular	Spermiogenesis defect	Primary ciliary diskinesia; OMIM:613808	Asthenozoospermia	Familial/sporadic	Syndromic infertility	AR	11	Moderate	(Blanchon et al. 2012; Sui et al. 2016)
CDC14A		1p21.2	Testicular	Pre-meiotic arrest/Meiotic arrest/Spermiogenesis defect	Oligoasthenoteratozoospermia OMIM:NA (PS258150) with deafness; OMIM:616958	Oligoasthenoteratozoospermia	Familial	Syndromic infertility	AR	11	Moderate	(Imtiaz et al. 2018)
CEP290		12q21.32	Testicular	Spermiogenesis defect	Leber Congenital Amaurosis; OMIM:611755	Asthenozoospermia	Sporadic	Syndromic infertility	AR	9	Moderate	(Yzer et al. 2012)
CFAP43	WDR96	10q25.1	Testicular	Spermiogenesis defect	Multiple morphological abnormalities of the sperm flagella; OMIM:617592	Teratozoospermia: Multiple Morphological Abnormalities of the Sperm Flagella	Familial/sporadic	Isolated infertility	AR	16	Definitive	(Tang et al. 2017; Sha et al. 2017)**
CFAP44	WDR52	3q13.2	Testicular	Spermiogenesis defect	Multiple morphological abnormalities of the sperm flagella; OMIM:617593	Teratozoospermia: Multiple Morphological Abnormalities of the Sperm Flagella	Familial/sporadic	Isolated infertility	AR	16	Definitive	(Tang et al. 2017; Sha et al. 2017)**
CFAP69		7q21.13	Testicular	Spermiogenesis defect	Multiple morphological abnormalities of the sperm flagella; OMIM:617959	Teratozoospermia: Multiple Morphological Abnormalities of the Sperm Flagella	Familial	Isolated infertility	AR	9	Moderate	(Dong et al. 2018)
CFTR		7q31.2	Post-testicular	Abnormal development of vas deferens	Congenital Bilateral/Unilateral Absence of Vas Deferens; OMIM:277180	Azoospermia	Familial/sporadic	Isolated infertility	AR	16	Definitive	(Dumur et al. 1990; Anguiano et al. 1992)**
CHD7		8q12.2	Pre-testicular	Abnormal hypothalamus development and function	Kallmann syndrome without CHARGE phenotype; OMIM:612370	Oligozoospermia, azoospermia	Familial/sporadic	Endocrine disorder/Reproductive system syndrome	AD	16	Definitive	(Kim et al. 2008; Jongmans et al. 2009)**
			Pre-testicular	Abnormal hypothalamus development and function	Isolated Hypogonadotropic Hypogonadism (normosmic) without CHARGE phenotype; OMIM:612370	Azoospermia	Familial/sporadic	Endocrine disorder/Reproductive system syndrome	AD	16	Definitive	(Kim et al. 2008; Tommiska et al. 2014)**
CYP11A1	P450SCC	15q24.1	Pre-testicular	Adrenal gland dysfunction	Congenital adrenal insufficiency with partial 46,XY sex reversal (Prader stage 4; 5 or 6); OMIM:613743	Normozoospermia, oligozoospermia, azoospermia	Familial/sporadic	Endocrine disorder/Reproductive system syndrome	AR	16	Definitive	(Rubtsov et al. 2009; Sahakitrungruang et al. 2011)**
CYP11B1	P450C11	8q24.3	Pre-testicular	Adrenal gland dysfunction	46,XX Disorders of Sex Development (Prader scale 4; 5 or 6) due to congenital adrenal hyperplasia (11-beta-hydroxylase deficiency); OMIM: 202010	Azoospermia	Familial/sporadic	Endocrine disorder/Reproductive system syndrome	AR	17	Definitive	(Kandemir and Yordam 1997; Chabre et al. 2000)**
CYP17A1	P450C17	10q24.32	Pre-testicular	Adrenal gland dysfunction	46,XY Disorders of Sex Development (Prader stage 4, 5 or 6) due to 17-alpha-hydroxylase/17,20-lyase deficiency; OMIM:202110	Normozoospermia, oligozoospermia, azoospermia	Familial/sporadic	Endocrine disorder/Reproductive system syndrome	AR	16	Definitive	(Ahlgren et al. 1992; Imai et al. 1992)**
CYP19A1	Aromatase	15q21.2	Pre-testicular	Adrenal gland dysfunction	Aromatase excess syndrome with gynaecomastia; OMIM: 139300	Normozoospermia, oligozoospermia, azoospermia	Familial/sporadic	Endocrine disorder/Reproductive system syndrome	AD	17	Definitive	(Shozu et al. 2003; Demura et al. 2007)**
			Pre-testicular	Adrenal gland dysfunction	46,XX Disorders of Sex Development (Prader scale 4; 5 or	Azoospermia	Familial/sporadic	Endocrine disorder/Repro	AR	9	Moderate	(Marino et al. 2015;

					6) due to aromatase deficiency; OMIM:613546			ductive system syndrome				Mazen et al. 2017)
CYP21A2	P450c21 B	6p21.33	Pre-testicular	Adrenal gland dysfunction	Classic congenital adrenal hyperplasia; OMIM:201910	Oligozoospermia, azoospermia	Familial/sporadic	Syndromic infertility/Endo crine disorder	AR	17	Definitive	(Cabrera, Vogiatzi, and New 2001; Ezquieta et al. 2007)**
			Pre-testicular	Adrenal gland dysfunction	Non-classic adrenal hyperplasia (late onset or no CAH symptoms); OMIM: 201910	Oligozoospermia, azoospermia	Familial/sporadic	Endocrine disorder	AR	17	Definitive	(Sugino et al. 2006; Nandagopal et al. 2011)**
DMRT1		9p24.3	Testicular	Sertoli cell only syndrome/Pre-meiotic arrest/Meiotic arrest	Non-obstructive azoospermia; OMIM:NA(PS258150)	Azoospermia	Sporadic	Isolated infertility	AD	9	Moderate	(Lopes et al. 2013; Tewes et al. 2014)
DNAAF2	KTU/C14 orf104/P F13	14q21.3	Testicular	Spermiogenesis defect	Primary ciliary dyskinesia; OMIM:612518	Asthenozoospermia	Familial	Syndromic infertility	AR	10	Moderate	(Omran et al. 2008)
DNAH1		3p21.1	Testicular	Spermiogenesis defect	Multiple morphological abnormalities of the sperm flagella; OMIM:617576	Teratozoospermia: Multiple morphological abnormalities of the sperm flagella	Familial/sporadic	Isolated infertility	AR	16	Definitive	(Ben Khelifa et al. 2014; Wambergu e et al. 2016)**
DPY19L2		12q14.2	Testicular	Spermiogenesis defect	Globozoospermia; OMIM:613958	Teratozoospermia: Globozoospermia	Familial/sporadic	Isolated infertility	AR	16	Definitive	(Koscinski et al. 2011; Harbuz et al. 2011)**
FANCA		16q24.3	Testicular	Sertoli cell only syndrome	Occult Fanconi Anemia; OMIM:NA (PS227650)	Azoospermia	Familial/sporadic	Isolated infertility	AR	10	Moderate	(Krausz et al. 2018)
FGF8		10q24.32	Pre-testicular	Abnormal hypothalamus development and function	Kallmann syndrome; OMIM: 612702	Oligozoospermia, azoospermia	Familial/sporadic	Endocrine disorder/Repro ductive system syndrome	AD	14	Strong	(Falardeau et al. 2008; Trarbach et al. 2010)**
			Pre-testicular	Abnormal hypothalamus development and function	Isolated Hypogonadotropic Hypogonadism (normosmic); OMIM:612702	Oligozoospermia, azoospermia	Familial/sporadic	Endocrine disorder/Repro ductive system syndrome	AD	10	Moderate	(Falardeau et al. 2008; Trarbach et al. 2010)
FGFR1	KAL2	8p11.23	Pre-testicular	Abnormal hypothalamus development and function	Kallmann syndrome; OMIM:147950	Oligozoospermia, azoospermia	Familial/sporadic	Endocrine disorder/Repro ductive system syndrome	AD	17	Definitive	(Dode et al. 2003; Sato et al. 2004)**
			Pre-testicular	Abnormal hypothalamus development and function	Isolated Hypogonadotropic Hypogonadism (normosmic); OMIM:147950	Oligozoospermia, azoospermia	Familial/sporadic	Endocrine disorder/Repro ductive system syndrome	AD	17	Definitive	(Kim et al. 2005; Pitteloud et al. 2006)**
FSHB		11p14.1	Pre-testicular	Pituitary gland dysfunction	Isolated Hypogonadotropic Hypogonadism; OMIM:229070	Oligozoospermia, azoospermia	Familial/sporadic	Endocrine disorder/Repro ductive system syndrome	AR	12	Moderate	(Phillip et al. 1998; Lindstedt et al. 1998)**
FSHR		2p16.3	Pre-testicular	Pre-meiotic arrest	Hypergonadotropic hypogonadism; OMIM:NA (PS147950)	Oligozoospermia	Familial	Endocrine disorder	AR	11	Moderate	(Tapanaine n et al. 1997; Franca et al. 2017)
GATA4		8p23.1	Pre-testicular	Abnormal development of reproductive organs	46,XY Disorders of Sex Development (Prader scale 4; 5 or 6) resulting in anomalies of	Oligozoospermia, azoospermia	Familial	Reproductive system syndrome	AD	12	Moderate	(Lourenco et al. 2011; Igarashi et

					testicular development; OMIM:615542							al. 2018)
GNRH1		8p21.2	Pre-testicular	Abnormal hypothalamus development and function	Isolated Hypogonadotropic Hypogonadism; OMIM:614841	Oligozoospermia, azoospermia	Familial/sporadic	Endocrine disorder/Reproductive system syndrome	AR	10	Moderate	(Bouligand et al. 2009; Chan et al. 2009)
GNRHR		4q13.2	Pre-testicular	Pituitary gland dysfunction	Isolated Hypogonadotropic Hypogonadism; OMIM:146110	Oligozoospermia, azoospermia	Familial	Endocrine disorder/Reproductive system syndrome	AR	17	Definitive	(de Roux et al. 1997; Layman et al. 1998)**
HSD17B3		9q22.32	Pre-testicular	Abnormal development of reproductive organs	46,XY Disorders of Sex Development (Prader scale 4; 5 or 6) resulting in anomalies of testicular development; OMIM:264300	Normozoospermia, oligozoospermia, azoospermia	Familial/sporadic	Reproductive system syndrome	AR	12	Moderate	(Neocleous et al. 2012; Costa-Barbosa et al. 2013)**
HSD3B2		1p12	Pre-testicular	Adrenal gland dysfunction	Adrenal hyperplasia due to 3β-hydroxysteroid dehydrogenase deficiency; OMIM:201810	Normozoospermia, oligozoospermia, azoospermia	Familial/sporadic	Syndromic infertility	AR	15	Strong	(Zhang et al. 2000; Welzel et al. 2008)**
INSL3		19p13.11	Pre-testicular	Abnormal development of reproductive organs	Cryptorchidism; OMIM:219050	Normozoospermia, oligozoospermia, azoospermia	Familial/Sporadic	Reproductive system syndrome	AD	12	Moderate	(Tomboc et al. 2000; Marin et al. 2001)**
KISS1R	GPR54	19p13.3	Pre-testicular	Abnormal hypothalamus development and function	Kallmann syndrome; OMIM:614837	Oligozoospermia, azoospermia	Familial/sporadic	Endocrine disorder/Reproductive system syndrome	AR	17	Definitive	(de Roux et al. 2003; Seminara et al. 2003)**
			Pre-testicular	Abnormal hypothalamus development and function	Isolated Hypogonadotropic Hypogonadism (normosmic); OMIM:614837	Oligozoospermia, azoospermia	Familial/sporadic	Endocrine disorder/Reproductive system syndrome	AR	17	Definitive	(de Roux et al. 2003; Seminara et al. 2003)**
KLHL10		17q21.2	Testicular	Meiotic arrest/Spermiogenesis defect	Oligozoospermia; OMIM:615081	Oligozoospermia	Sporadic	Isolated infertility	AD	10	Moderate	(Yatsenko et al. 2006)
LHB		19q13.3	Pre-testicular	Pituitary gland dysfunction	Hypogonadotropic hypogonadism; OMIM:228300	Oligozoospermia, azoospermia	Familial/sporadic	Endocrine disorder	AR	17	Definitive	(Weiss et al. 1992; Valdes-Socin et al. 2004)**
LHCGR	LHR	2p16.3	Testicular	Leydig cell dysfunction	Leydig cell dysfunction with hypogonadism; OMIM:238320	Normozoospermia, oligozoospermia	Familial/Sporadic	Reproductive system syndrome/Endocrine system disorder	AR	17	Definitive	(Laue et al. 1996; Misrahi et al. 1997)**
			Testicular	Leydig cell dysfunction	Male precocious puberty; OMIM:176410	Normozoospermia, oligozoospermia	Familial/sporadic	Reproductive system syndrome/Endocrine system disorder	AD	17	Definitive	(Shenker et al. 1993; Kremer et al. 1993)**
LRRC6		8q24.22	Testicular	Spermiogenesis defect	Primary ciliary dyskinesia; OMIM:614935	Asthenozoospermia	Familial/Sporadic	Syndromic infertility	AR	13	Strong	(Kott et al. 2012; Liu and Luo 2018)
MAMLD1	CXorf6	Xq28	Pre-testicular	Abnormal development of reproductive organs	46,XY Disorders of Sex Development (Prader scale 4; 5 or 6); OMIM:300758	Normozoospermia, oligozoospermia, azoospermia	Familial/sporadic	Reproductive system syndrome	XL	15	Strong	(Fukami et al. 2006; Ogata, Fukami, and Wada 2008)**

NLRP3		1q44	Testicular	Sertoli cell only syndrome/Pre-meiotic arrest/Meiotic arrest/Spermiogenesis defect	Muckle-Wells Syndrome; OMIM:191900	Oligozoospermia, azoospermia	Familial/sporadic	Syndromic infertility	AD	9	Moderate	(Tran et al. 2012)
NR0B1	DAX1	Xp21.2	Pre-testicular	Adrenal gland dysfunction	Congenital Adrenal Hypoplasia; OMIM:300200	Oligozoospermia, azoospermia	Familial/sporadic	Syndromic infertility/Endocrine disorder	XL	17	Definitive	(Muscatelli et al. 1994; Meloni et al. 1996)**
			Pre-testicular	Adrenal gland dysfunction	Late-onset adrenal failure or isolated hypogonadotropic hypogonadism; OMIM:NA (PS147950)	Oligozoospermia, azoospermia	Familial/sporadic	Isolated infertility	XL	17	Definitive	(Tabarin et al. 2000; Mantovani et al. 2002)**
NR5A1	SF1	9q33.3	Pre-testicular	Abnormal development of reproductive organs	46,XY Disorders of Sex Development (Prader scale 4; 5 or 6); OMIM:612965	Oligozoospermia, azoospermia	Familial/Sporadic	Reproductive system syndrome	AD	17	Definitive	(Mallet et al. 2004; Lin et al. 2007)**
			Pre-testicular	Abnormal development of reproductive organs	46,XX Disorders of Sex Development (Prader scale 4; 5 or 6); OMIM:617480	Azoospermia	Sporadic	Reproductive system syndrome	AD	10	Moderate	(Bashamboo et al. 2016; Baetens et al. 2017)**
			Pre-testicular	Leydig cell dysfunction/Sertoli cell only syndrome/Pre-meiotic arrest/ Pituitary gland dysfunction	Isolated spermatogenic failure; OMIM:184757	Oligozoospermia, azoospermia	Sporadic	Endocrine disorder	AD	14	Strong	(Ropke et al. 2013; Safari et al. 2014)**
PIH1D3		Xq22.3	Testicular	Spermiogenesis defect	Mild Primary ciliary dyskinesia; OMIM:300991	Asthenozoospermia	Familial	Syndromic infertility	XL	9	Moderate	(Paff et al. 2017)
PROK2		3p13	Pre-testicular	Abnormal hypothalamus development and function	Kallmann syndrome; OMIM:610628	Oligozoospermia, azoospermia	Familial/Sporadic	Endocrine disorder/Reproductive system syndrome	AR	15	Strong	(Pitteloud et al. 2007; Leroy et al. 2008)**
PROKR2		20p12.3	Pre-testicular	Abnormal hypothalamus development and function	Kallmann syndrome; OMIM:244200	Oligozoospermia, azoospermia	Familial/sporadic	Endocrine disorder/Reproductive system syndrome	AR	17	Definitive	(Dode et al. 2006; Sinisi et al. 2008)**
RSPO1		1p34.3	Pre-testicular	Abnormal development of reproductive organs	Palmoplantar hyperkeratosis with squamous cell carcinoma of skin and sex reversal; OMIM:610644	Azoospermia	Familial/Sporadic	Syndromic infertility/Reproductive system syndrome	AR	12	Moderate	(Micali et al. 2005; Parma et al. 2006)**
SEMA3A		7q21.11	Pre-testicular	Abnormal hypothalamus development and function	Kallmann syndrome; OMIM:614897	Oligozoospermia, azoospermia	Familial/sporadic	Endocrine disorder/Reproductive system syndrome	AD	10	Moderate	(Young et al. 2012; Hanchate et al. 2012)
SOX10		22q13.1	Pre-testicular	Abnormal hypothalamus development and function	Kallmann syndrome; OMIM:NA (PS147950)	Oligozoospermia, azoospermia	Familial/sporadic	Endocrine disorder/Reproductive system syndrome	AD	15	Strong	(Pingault et al. 2013; Vaaralahti et al. 2014)**
SOX2		3q26.33	Pre-testicular	Abnormal hypothalamus development and function	Isolated hypogonadotropic hypogonadism (normosmic); OMIM:NA (PS147950)	Oligozoospermia, azoospermia	Familial	Endocrine disorder/Reproductive system syndrome	AD	15	Strong	(Kelberman et al. 2006; Stark et al. 2011)**
SOX3		Xq27.1	Pre-testicular	Abnormal development of reproductive organs	46,XX Disorders of Sex Development (Prader scale 4; 5 or 6); OMIM:NA	Azoospermia	Sporadic	Reproductive system syndrome	XL	12	Moderate	(Sutton et al. 2011; Moalem et al. 2012)**

SOX9		17q24.3	Pre-testicular	Abnormal development of reproductive organs	46,XX Disorders of Sex Development (Prader scale 4; 5 or 6); OMIM:NA	Azoospermia	Familial/Sporadic	Reproductive system syndrome	AD	11	Moderate	(Cox et al. 2011; Vetro et al. 2011)**
			Pre-testicular	Abnormal development of reproductive organs	46,XY Disorders of Sex Development (Prader scale 4; 5 or 6); OMIM:NA (PS400044)	Oligozoospermia, azoospermia	Sporadic	Reproductive system syndrome	AD	9	Moderate	(Katoh-Fukui et al. 2015)
SPATA16		3q26.31	Testicular	Spermiogenesis defect	Globozoospermia; OMIM:102530	Teratozoospermia: Globozoospermia	Familial/sporadic	Isolated infertility	AR	9	Moderate	(Dam et al. 2007; Ellnati et al. 2016)
SRD5A2		2p23.1	Pre-testicular	Abnormal development of reproductive organs	46,XY Disorders of Sex Development (Prader scale 4; 5 or 6); OMIM:264600	Normozoospermia, oligozoospermia, azoospermia	Sporadic	Reproductive system syndrome	AR	18	Definitive	(Thigpen et al. 1992; Hiort et al. 1996)**
SRY		Yp11.2	Pre-testicular	Abnormal development of reproductive organs	46,XX Disorders of Sex Development (Prader scale 4; 5 or 6); OMIM:400045	Azoospermia	Sporadic	Reproductive system syndrome	YL	17	Definitive	(Numabe et al. 1992; Fechner et al. 1993)**
			Pre-testicular	Abnormal development of reproductive organs	46,XY Disorders of Sex Development (Prader scale 4; 5 or 6); OMIM:400044	Oligozoospermia, azoospermia	Sporadic	Reproductive system syndrome	YL	15	Strong	(Domenice et al. 1998; Assumpcao et al. 2002)**
SUN5		20q11.21	Testicular	Spermiogenesis defect	Acephalic sperm; OMIM:617187	Teratozoospermia: Acephalic spermatozoa	Familial/sporadic	Isolated infertility	AR	16	Definitive	(Zhu et al. 2016; Elkhatib et al. 2017)**
SYCP3		12q23.2	Testicular	Meiotic arrest	Non-obstructive azoospermia; OMIM:270960	Azoospermia	Sporadic	Isolated infertility	AD	11	Moderate	(Miyamoto et al. 2003; Stouffs et al. 2011)**
TACR3		4q24	Pre-testicular	Abnormal hypothalamus development and function	Kallmann syndrome; OMIM:614840	Oligozoospermia, azoospermia	Familial/sporadic	Endocrine disorder/Reproductive system syndrome	AR	16	Definitive	(Topaloglu et al. 2009; Guran et al. 2009)**
TEX11		Xp11	Testicular	Meiotic arrest	Non-obstructive azoospermia; OMIM:309120	Azoospermia	Sporadic	Isolated infertility	XL	15	Strong	(Yatsenko et al. 2015; Yang et al. 2015)**
TEX15		8p12	Testicular	Meiotic arrest	Non-obstructive azoospermia; OMIM:617960	Azoospermia	Familial	Isolated infertility	AR	12	Moderate	(Okutman et al. 2015; Wang et al. 2018)**
TRIM37		17q22	Testicular	Leydig cell dysfunction/Sertoli cell only syndrome/pre-meiotic arrest/Meiotic arrest/Spermiogenesis defect	Mulibrey nanism; OMIM:253250	Oligoasthenozoospermia, azoospermia	Familial/sporadic	Syndromic infertility	AR	9	Moderate	(Karlberg et al. 2011)
WDR11		10q26.12	Pre-testicular	Abnormal hypothalamus development and function	Kallmann syndrome; OMIM:614858	Oligozoospermia, azoospermia	Familial/Sporadic	Endocrine disorder/Reproductive system syndrome	AD	12	Moderate	(Kim et al. 2010; Izumi et al. 2014)**
WT1		11p13	Pre-testicular	Abnormal development of reproductive organs	46,XY Disorders of Sex Development (Prader scale 4; 5 or 6) without Wilm's tumor; OMIM:NA (PS400044)	Normozoospermia, oligozoospermia, azoospermia	Sporadic	Reproductive system syndrome	AD	16	Definitive	(Clarkson et al. 1993; Kohler et al. 2001)**

Abbreviations: HGNC: HUGO Gene Nomenclature Committee; OMIM: Online Mendelian Inheritance in Man; PS: Phenotype Series; AR: Autosomal Recessive; AD: Autosomal Dominant; XL: X-linked; YL: Y-linked

Full table including gene-disease relationships with “Limited evidence” and “No evidence” available in Supplemental Table S4.

*Details about the score available in Supplemental Table S5.

**Additional references are available in Supplemental Table S5