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Preconception genome medicine: current state and future perspectives to improve infertility diagnosis and reproductive and health outcomes based on individual genomic data

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BACKGROUND: Our genetic code is now readable, writable and hackable. The recent escalation of genome-wide sequencing (GS) applications in population diagnostics will not only enable the assessment of risks of transmitting well-defined monogenic disorders at preconceptional stages (i.e. carrier screening), but also facilitate identification of multifactorial genetic predispositions to sub-lethal pathologies, including those affecting reproductive fitness. Through GS, the acquisition and curation of reproductive-related findings will warrant the expansion of genetic assessment to new areas of genomic prediction of reproductive phenotypes, pharmacogenomics and molecular embryology, further boosting our knowledge and therapeutic tools for treating infertility and improving women's health.

OBJECTIVE AND RATIONALE: In this article, we review current knowledge and potential development of preconception genome analysis aimed at detecting reproductive and individual health risks (recessive genetic disease and medically actionable secondary findings) as well as anticipating specific reproductive outcomes, particularly in the context of IVF. The extension of reproductive genetic risk assessment to the general population and IVF couples will lead to the identification of couples who carry recessive mutations, as well as sub-lethal conditions prior to conception. This approach will provide increased reproductive autonomy to couples, particularly in those cases where preimplantation genetic testing is an available option to avoid the transmission of undesirable conditions. In addition, GS on prospective infertility patients will enable genome-wide association studies specific for infertility phenotypes such as predisposition to premature ovarian failure, increased risk of aneuploidies, complete oocyte immaturity or blastocyst development failure, thus empowering the development of true reproductive precision medicine.

SEARCH METHODS: Searches of the literature on PubMed Central included combinations of the following MeSH terms: human, genetics, genomics, variants, male, female, fertility, next generation sequencing, genome exome sequencing, expanded carrier screening, secondary findings, pharmacogenomics, controlled ovarian stimulation, preconception, genetics, genome-wide association studies, GWAS.

OUTCOMES: Through PubMed Central queries, we identified a total of 1409 articles. The full list of articles was assessed for date of publication, limiting the search to studies published within the last 15 years (2004 onwards due to escalating research output of next-generation sequencing studies from that date). The remaining articles' titles were assessed for pertinence to the topic, leaving a total of 644 articles. The use of preconception GS has the potential to identify inheritable genetic conditions concealed in the genome of around 4% of couples looking to conceive. Genomic information during reproductive age will also be useful to anticipate late-onset medically actionable conditions with strong genetic background in around 2–4% of all individuals. Genetic variants correlated with differential response to pharmaceutical treatment in IVF, and clear genotype–phenotype associations are found for aberrant sperm types, oocyte maturation, fertilization or pre- and post-implantation embryonic development. All currently known capabilities of GS at the preconception stage are reviewed along with persisting and forthcoming barriers for the implementation of precise reproductive medicine.

WIDER IMPLICATIONS: The expansion of sequencing analysis to additional monogenic and polygenic traits may enable the development of cost-effective preconception tests capable of identifying underlying genetic causes of infertility, which have been defined as 'unexplained' until now, thus leading to the development of a true personalized genomic medicine framework in reproductive health.

Key words: genomic sequencing / whole-exome sequencing / whole-genome sequencing / reproductive genetics / IVF/ICSI outcomes / oocyte and embryo genetic defects / preconception carrier screening / infertility / genetic diagnosis / polygenic medicine

Introduction

Since the completion of the first human genome project almost 20 years ago (Lander *et al.*, 2001; Venter *et al.*, 2001), the analysis of millions of individual genomic sequences, primarily within research studies or, more recently, clinical care, have been facilitated by technological advancements and dramatic cost reduction in DNA sequencing. As a consequence of these studies, we have come to know that each person harbours 4–5 million variants in their genome, which contribute to each person being genetically unique (Auton *et al.*, 2015). Most of these variants have no effect on health, whereas some of them modulate disease risk or responsiveness to a specific drug. A general consensus surrounds the possibility of enabling a deeper appreciation of individual disease risk and pathogenic causes through personal genome sequencing, ultimately providing more accurate treatment plans and patient care, a strategy now commonly defined as 'precision medicine' (Aronson and Rehm, 2015). More specifically, the term of 'genomic medicine' (GM) is often employed when the use of genomic information is the primary consideration for determining the risk and predisposition to a specific disease, its diagnosis and prognosis, and the most effective therapeutic approach to be employed, without much consideration of environmental and lifestyle variability (Doble *et al.*, 2017).

One's global genetic inheritance is currently investigated mainly using two technical approaches: through high-density DNA microarrays, which allow the detection of millions of common genetic variation for a fraction of what they cost only a few years ago (tens of dollars per analysis) (Gunderson *et al.*, 2005), or through massive parallel DNA sequencing technologies (e.g. next-generation sequencing, NGS), which can provide billions of short sequencing DNA reads within a very short turnover (a few hours or one day) (Shendure *et al.*, 2017). Although more expensive (hundreds to a thousand dollars, depending on the extent of the sequencing target), NGS has the advantage of detecting and reporting not only common variants (which could be investigated through microarrays), but also rare and novel pathogenic mutations in a single analytical run. Despite it being only a few years since these technologies have fully matured, the number of genomes analysed through microarrays or genome sequencing has reached impressive heights, with estimations climbing from 1 million in 2014 to 3 million in 2016 and over 12 million in 2018 (Shendure *et al.*, 2017). Big data generation from genome-wide sequencing (GS) is revolutionizing medical research, providing concrete evidence on the genetic foundation of heterogeneity of disease and its molecular causes, thus delivering the means for refined diagnoses and increased capacities to medically manage a growing number of costly and debilitating conditions.

Current applications of genomic medicine

As a first application, GM has been particularly effective for high penetrance conditions characterized by a strong genetic background, such as neurodevelopmental and diseases with Mendelian inheritance. Over 7000 Mendelian or monogenic disorders are estimated to cumulatively

affect 1–3% of live births. From a healthcare standpoint, these conditions have a far larger footprint, as they correspond to a much larger proportion of the general morbidity and mortality rate (e.g. up to 71% of paediatric hospital admissions) (Baird et al., 1988; McCandless et al., 2004; Chong et al., 2015). In 2013, a landmark study showed that a significant portion of cases (over 30%) that could not be solved through a conventional diagnostic work-up could be reconciled by whole-exome sequencing (WES) (Yang et al., 2013). This proportion is most likely to increase as monogenic diseases and their pathological genomic variations are revealed through large-population genomic studies. Indeed, more recent data produced by whole-genome sequencing (WGS) has shown that the diagnostic yield can be improved to almost 50% of cases (Salfati et al., 2019).

Today, genomic testing allows the accurate assessment of a couple's reproductive risk using preconception carrier screening (PCS) and preimplantation genetic testing (PGT) or conventional prenatal testing during pregnancy (i.e. amniocentesis and chorionic villi sampling). Indeed, PCS for recessive genetic diseases has been a remarkable step forward in reproductive risk assessment from the time when couples, especially those with a known serious genetic disorder running in the family, could not access a molecular diagnosis to support reproductive management. As a result of preconception genetic testing and subsequent informed family planning, a reduction between 47% and 90% was reported in the incidence of serious monogenic conditions such as cystic fibrosis (CF) and β-thalassemia (Cunningham and Marshall, 1998; Turner et al., 2007), which also allowed the restoration of patients' reproductive confidence and autonomy. Today, millions of couples have already undergone PCS. A complementary application gaining a lot of attention in recent years is newborn screening. The ultimate purpose of newborn screening is not limited to produce a timely diagnosis and start specific treatments at an early stage but also to provide the family with a definitive explanation about the cause of the disease, thus avoiding costly and time-consuming work-ups and consultations scattered in the complexity of the healthcare system during the first years of the baby's life (Fig. 1).

Moreover, individuals' genetic information can be employed to estimate their risk of developing late-onset diseases and their likelihood to benefit from targeted preventive care and intensive monitoring and screening. A noteworthy set of genes are those selected by the American College of Medical Genetics (ACMG) considered to be adequately 'medically actionable' to be reported as secondary findings (SF) in clinical genomic sequencing tests undertaken for other primary indications (e.g. BRCA1/2) (Kalia et al., 2017).

Genomics-based precision medicine research includes both 'pharmacogenomic' studies (concerning the identification of genetic variants that influence drug pharmacokinetics) and genetic studies that aim to define disease 'endotypes' that might indicate diverse underlying aetiopathologies and associated optimal treatment strategies but lie under the same diagnostic umbrella. For example, *ivacaftor* is a drug that alters the activity of the transmembrane conductance regulator channel; this drug is licensed for use in a small proportion of patients affected by CF (4–5%) who shows a specific mutation in the protein-encoding gene. In oncology, genomic profiling of tumours is becoming a standard approach to predict patient's response to therapies. Interestingly, molecular biomarkers for targeted and personalized treatment were incorporated in about 39% of global oncology trials in 2018 (The IQVIA Institute, 2019). Overall, the combination of genomic research and cancer medicine is enabling the translation of DNA-based findings into increased understanding of pathological mechanisms and development of new drugs (Fig. 1).

On the contrary, the application of genomic screening for common multifactorial diseases is more limited, resulting in only a few robust polygenic risk scores (PRSs) available and used in clinical practice. However, the perspective of genomic prediction for polygenic conditions is remarkable. Indeed, a noteworthy proportion of the general population is affected by non-communicable diseases (NCDs), which are estimated to cause premature death in approximately 26% of the world population (World Health Organization, 2018). The WHO estimates that 80% of NCDs can be attributed to cardiovascular and respiratory disease, cancer and diabetes (i.e. polygenic disorders). The aggregated risk for a multifactorial disease can be conferred by thousands of common genetic variants. In response to this challenge, algorithmic platforms able to accurately determine PRSs from thousands of informative single-nucleotide polymorphisms (SNPs) and capture the likelihood of developing the disease are being developed. A landmark recent study by Khera et al. (2018) demonstrated how PRS can be successfully employed to characterize individual predisposition to five common diseases, showing that ∼20% of the population had a 3-fold increase in polygenic risk for one or more of them. This level of risk was comparable to that conferred by rare, high-penetrance variants associated with monogenic disorders. Furthermore, genome-wide association studies (GWAS) have identified drug-repurposing opportunities, allowing drugs already approved for specific indications to be employed in the treatment of alternative pathogenic targets (Pushpakom et al., 2019). However, while PRSs have numerous potential applications in disease risk prediction, a careful design and implementation is required, especially with respect to equal applicability across populations and ethnicities. Most of current PRSs have been developed based on data from populations of European descent and although some scores can be generalized across ancestries, it is apparent that their predictive power decreases with ethnical divergence and consequent variation in minor allele frequencies, and with changes in environmental contexts (Duncan et al., 2019). In addition, GWAS are able to identify genomic regions involved in the regulation of certain traits and diseases. However, the specific genes and pathways often remain undetermined. To date, definitive evidence for causal variants and target genes for most of polygenic traits are still lacking and the diagnostic application of PRSs is certainly in its infancy. If PRSs were to be integrated into treatment and diagnostic decision-making processes or public health interventions, the development of PRSs uniformly representing different ancestries and populations would be essential to ensure equality in access to health care programs and maximize benefits for patients.

Importantly, before being employed on any patient, all emerging genetic tests should be evaluated by the same frameworks and analytical processes adopted to review currently available genetic tests (e.g. the ACCE standards addressing analytical and clinical validity, clinical utility and concomitant ethical, legal and social implications) (National Academies of Sciences, Engineering, and Medicine, 2017). Main technical assessment criteria include (i) analytical validity (the accuracy in variant detection by a given test), (ii) clinical validity (the accuracy between the presence of variant and the onset of a disease, or its risk of onset), and (iii) the clinical utility (the value of genetic information for making clinical decisions, such as implementing behavioural changes

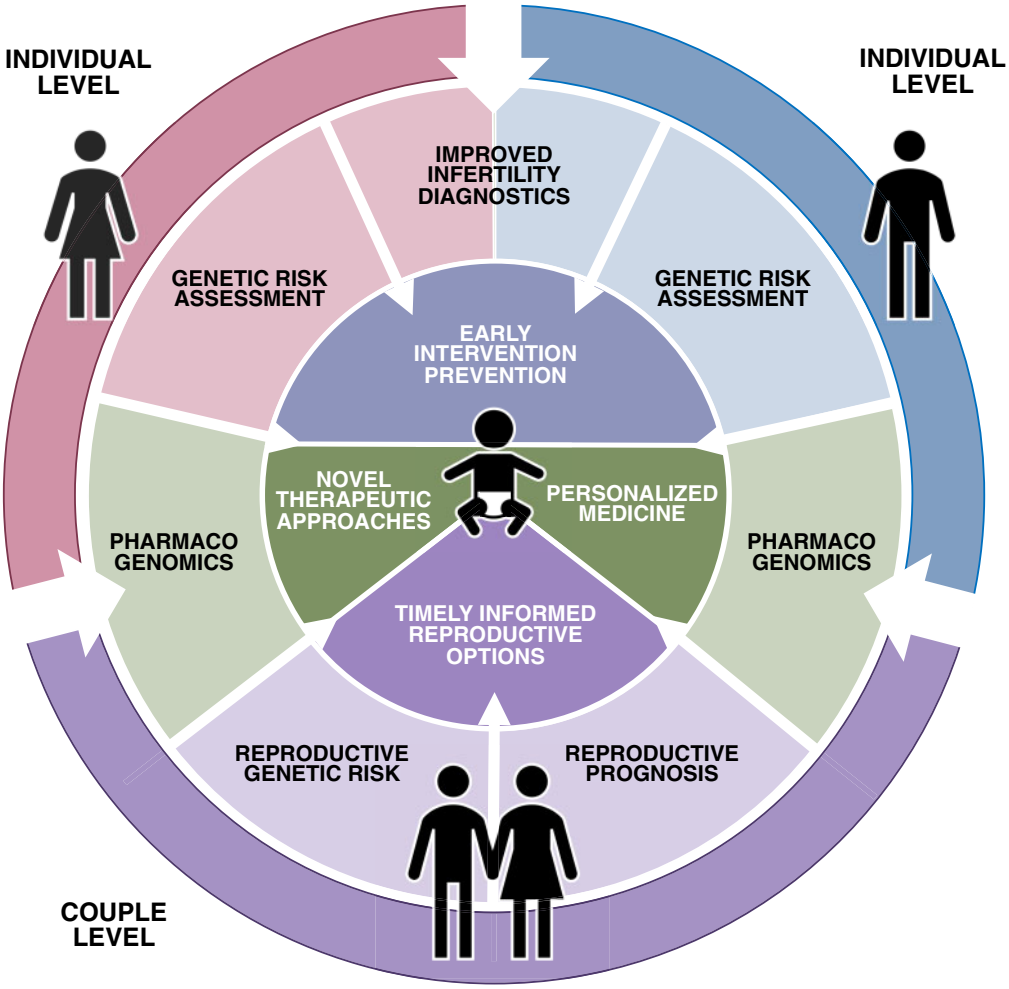

Figure 1. Applications of preconception genomic medicine. Preconception genomic assessment can provide clinical utility both at the individual and at the couple levels. At the individual level, genomic information can be used to identify causes of infertility as well as health risk for conditions unrelated to fertility. Specific variants may be used for tailoring pharmacological treatment for either infertility or general health. At the couple level, combined genomic information can be used to identify potential risk of transmitting recessive genetic conditions to the offspring, thus allowing for timely informed decisions on reproductive strategies.

or opting for a risk-mitigating intervention). In the context of genetic testing for disease risk prediction, clinical utility particularly depends on the existence of interventions that can effectively reduce risk among people identified at high-risk. Today, many genetic tests and scores will enable 'prognosis without promise', that is, there is no known way of reducing the associated risk. Finally, related ethical, legal and social issues should be examined for all tests revealing genetic information, especially in regards to the perpetuity of genetic identity and the persisting issue of variant interpretation.

Towards genome-based reproductive medicine

Infertility is one of the most common pathological conditions among individuals aged between 20 and 45 years, affecting 10–15% of couples (Mascarenhas *et al.*, 2012). More than 48 million couples worldwide suffer from infertility, demonstrating its global health impact (Mascarenhas *et al.*, 2012). GS at the preconception stage holds great promise to improve both infertility diagnosis and management and the health of the future children in the coming years. In some cases, the genetic bases of infertility in males and females have been precisely identified, ranging from the presence of chromosomal rearrangements in the parental karyotype, to single gene defects affecting a variety of biological pathways involved in reproduction, where infertility can either be an isolated phenotype or part of a syndromic spectrum.

Of note, unidentified genetic variations may be responsible for a significant portion of total idiopathic infertility cases, accounting for at least one in five couples (Shah *et al.*, 2003). Despite the current lack of evidence for genetic causes of human infertility, genetic variants in more than 600 genes have been associated with decreased fertility in animal models (Matzuk and Lamb, 2002; Oud *et al.*, 2019). This lack

of knowledge is primarily explained by the low reproductive rate of infertile individuals which subsequently reduces the number of large families available for genetic investigation in humans. In addition, the genetic heterogeneity of infertility, combined with the limited systematic uptake of genomic approaches for reproductive assessment, has slowed progress on this front.

In the past, the inability to precisely characterize some specific infertility phenotypes represented a missing window of investigation. Since its first successful application over 40 years ago, the number of IVF and ICSI cycles has increased every year. To date, more than 5 million babies have been born as a result of ART treatment (Adamson *et al.*, 2013). ART and *ex vivo* conception have made it possible to evaluate phenotypic differences in oocyte maturation, fertilization and early embryonic development and to more finely investigate the genetic basis of infertility. Furthermore, IVF also provides a direct method to reveal specific phenotypes associated with recurrent implantation failure or early pregnancy loss following embryo transfer, an outcome that is not directly accessible in spontaneous conceptions. Indeed, recent applications of GS to families with history of infertility, have led to the discovery of new genes and novel variants involved in specific human infertility phenotypes affecting isolated functions of gametes or very singular mechanisms necessary for early preimplantation embryo development (Alazami *et al.*, 2015; Xu *et al.*, 2016a; Sang *et al.*, 2018). These family-based approaches and recent GWAS (Chernus *et al.*, 2019) have revealed a small number of human genes that are crucial for early stages of reproduction. Genome sequencing and other nucleic acid-based technological advancements (e.g. genome editing, specific pathways modulators, RNAi, etc.), will provide the biological evidence required not only for advancing our understanding of reproduction and infertility but also for the development of novel preventive and therapeutic approaches to overcome and treat biologically imposed reproductive barriers. In the same way, the definition and subsequent detection of single gene defects that produce phenotypes incompatible with reproductive success, whether natural or through ART, will demonstrate their clinical utility by informing patients about their extremely low chances of conception in a timely manner, thus allowing them to make alternative choices and to save time and resources required for multiple rounds of blind IVF and relate procedures (Alazami *et al.*, 2015; Krausz *et al.*, 2018). Furthermore, underpinning the genetic causes of infertility will inform on the risk of transmitting the same reproductive phenotype to the next generation, thus improving long-term awareness on infertility.

Of note, infertility is also not necessarily an isolated condition exclusively involving the reproductive axis but is often linked with other conditions. It has been established that infertile individuals are less healthy than fertile ones, with increased risk for cardiovascular disease, cancer and diabetes (reviewed in Cedars *et al.* (2017)). For instance, variations in age at menarche and natural menopause have associations with occurrence of infertility, cardiovascular and metabolic disorders, obesity and breast, ovarian and endometrial cancer. Although the mechanisms responsible have not yet been possible to define, GWAS have revealed a series of genes and pathways with known roles in DNA damage/repair and impacting simultaneously ovarian follicle health, ageing and cancer risk. Similarly, epidemiological studies have shown associations between male factor infertility and increased cancer risk with potential underlying genetic lesions linking the two conditions (Hanson *et al.*, 2018). The hypothesis that fertility and overall health share common or intersecting physiological pathways, and that molecular dysregulation of a common pathway can disrupt both systems, provides, if confirmed, a valuable opportunity to combine fertility/preconception genetic evaluation with general health assessment. With growing attention to infertility by society and institutions, an unprecedented opportunity to reach people during their reproductive years is arising. This time-specific approach would make contact with individuals when they are highly motivated to protect their current and future health, yet young enough to make impacting changes to their lifestyle and mitigate the risk associated with late-onset diseases. Accordingly, a wide adoption of genetic investigations during the reproductive years can be seen as a window into future health and as an opportunity for proactive and generalized preventive measures.

Although an interesting approach from a diagnostic standpoint, WES/WGS of embryo biopsies is not yet an applicable strategy for clinical application due to concerning limitations in sequencing accuracy when only a few cells are analysed. On the other hand, GS can consistently be performed on larger DNA samples isolated from buccal cells swab and blood collections, offering reliable means for parental testing and reproductive risk assessment.

In this context, the term 'preconception genomic medicine' can be generally used to subtend the whole spectrum of information attainable by GS application in the preconception stage, being intended as the primary consideration for determining infertility diagnosis, disease risk and predisposition for both the couple and the future newborn as well as the selection and prioritization of the best reproductive therapeutic options. This approach considers how genomics is being applied in the reproductive field, providing illustrative examples of how couples seeking reproduction might boost their chances to conceive by exploiting personal and couple level genomic information, ranging from advanced diagnostics of infertility to reproductive risk assessment for Mendelian diseases to genomic risk assessments and IVF prediction for certain extreme phenotypes. These examples are then merged with common operational challenges precluding deep integration of genomics into clinical practice, as well as with strategies currently being developed to overcome organizational hindrances, necessary for enabling genomics to be a part of everyday patient care.

For pharmacogenomics applications in IVF and the genetic basis of miscarriages, we refer to recent manuscript where these topics have been well covered (Colley *et al.*, 2019; Conforti *et al.*, 2019).

Expanded carrier screening and reproductive risk assessment

It is estimated that one of the 1300 recessive genetic condition known today affects a minimum of three children per every 1000. In fact, diseases with recessive inheritance might be relatively rare when considered individually but when accounted as a group, they become epidemiologically significant. Carrier screening (CS) identifies the presence of healthy carriers by molecular detection of DNA pathogenic variants revealing the genetic risk of severe childhood recessive conditions for couples trying to conceive, thus allowing for reproductive autonomy and informed decision-making (Achterbergh *et al.*, 2007; Human Genetics Commission, 2011; Delatycki *et al.*, 2020).

CS can be introduced at different stages of life. Among these, the more generally considered stages include (i) prenatal, (ii) preconceptional and (iii) pre-marital/pre-relationship. Each stage has specific advantages and disadvantages. For instance, the prenatal stage appears to be easier to implement as pregnant women usually enter the healthcare system and receive medical attention, thus CS can be comprehensively offered and provided to individuals who have immediate necessity. However, the range of choices available to patients (reproductive autonomy) at this stage are very limited as the pregnancy is already ongoing and only chorionic villi sampling (CVS)/amniocentesis can be attempted following pregnancy termination in case of positive results. Nonetheless, in conditions for which effective therapeutic interventions exist, awareness of foetal carrier status may also allow early initiation of medical treatment at birth or during pregnancy (Nazareth *et al.*, 2015). By anticipating the testing process to preconception stage, the individual's reproductive autonomy is maximized, and more meaningful choices become available, including PGT during IVF treatment, the use of non-carrier donor gametes or refraining from conceiving and adoption. In PCS for couples, partners can be screened simultaneously or sequentially (based on the genes that are found mutated in the first partner). The simultaneous approach provides the largest amount of information in the most time-effective manner. In fact, sequential screening entails several limitations including increased reporting times and associated costs (which negatively impact anxiety levels) and a diagnostic value that is disproportioned towards the first person being tested (usually the female partner) and does not allow cascade evaluation in the relatives of the second partner (Capalbo *et al.*, 2019).

The reproductive risk for recessive diseases is particularly elevated in consanguineous couples and geographically isolated populations. Indeed, individuals of Ashkenazi Jewish descent have commonly undergone carrier status screening as teenagers or prior to commencing a personal relationship. The Ashkenazi Jewish population is renowned to be at a high risk of specific genetic conditions due to high carrier rates (CRs) for several recessive condition typically observed less frequently in the general population, such as Type 1 Gaucher disease (CR 1 in 15, OMIM #230800) and Tay-Sachs disease (CR 1 in 27; OMIM #272800). Targeted strategies have been effectively employed in several geographies affected by specific genetic diseases (e.g. CF in USA, Australia and Italy; beta-thalassemia in Cyprus, Sardinia, Israel and Turkey), drastically reducing the incidence of the condition in newborns (Angastiniotis and Hadjiminas, 1981; Kaback, 2000). Despite ethnical specificity of certain disorders, conditions such as CF (OMIM #219700) are common across all populations and regions, warranting global guidance for genetic screening (ACOG, ACMG). The extended capabilities and cost-effectiveness of current DNA sequencing technologies have made possible the transition from targeted CS panels to expanded carrier screening (ECS) panels, which allow pan-ethnic and simultaneous testing of tens to hundreds recessive conditions in a single analysis. Through the ECS approach, the presence of the most frequent, early-onset and severe diseases can be tested with faster turnaround and reduced costs while avoiding potential racial stigmatization, thus providing a more effective and universal testing strategy with higher diagnostic yield for identifying at risk couples (ARCs). Furthermore, pan-ethnic strategies can definitively overcome the known limitations and inaccuracies related to self-reporting of ethnicity. Indeed, several studies have highlighted the unreliability of ethnicity classification based on patient self-reports when compared with the actual genetic ancestry (Shraga *et al.*, 2017; Kaseniit *et al.*, 2020).

Notably, the systematic application of a multigene panel including CF, fragile X syndrome and spinal muscular atrophy in routine preconception and early pregnancy programs in Australia showed significant benefits in decision-making compared with reliance on family history alone. In fact, testing healthy individuals with no known family history for these three conditions resulted in a combined affected pregnancy rate comparable to the population risk for Down syndrome (Archibald *et al.*, 2018). The increased diagnostic yield of ECS over a conventional CS approach has been also widely demonstrated across different ethnicities (Haque *et al.*, 2016). In general, well-developed and optimized ECS panels have identified 3–4% of tested couples being at risk for highly penetrant severe conditions with childhood onset (Haque *et al.*, 2016; Capalbo *et al.*, 2019) and to date, millions of couples worldwide have undergone preconception risk assessment using ECS, particularly in the context of IVF (Bell *et al.*, 2011; Hogan *et al.*, 2018; Beauchamp *et al.*, 2019).

ECS has been also largely employed to minimize the transmission of inheritable conditions to the ensuing baby in third party reproduction. Indeed, it is reasonably more crucial to employ ECS in cases where gamete donation is sought because gametes from a donor are generally being employed to create multiple families (especially in case of sperm donation). To minimize the risk that an affected child is born from third-party reproduction, two main approaches could be adopted. One involves the exclusion of donors who carry any recessive pathological condition. However, it has been shown that, if employed, this strategy would result in a significant shortage of gametes being available for third-party reproduction (Bell *et al.*, 2011; Silver *et al.*, 2016; Mertes *et al.*, 2018), thus inevitably increasing costs and limiting the access to gamete donation programs. Alternatively, a more effective approach commonly employed involves matching donors and recipients based on their genetic information. In this context, due to the individual risk for recessive X-linked inherited conditions, particular care should be placed in screening pathogenic variants on the X chromosome of oocyte donors. This strategy maintains the practice of gamete donation viable whilst minimizing the risk of passing a genetic disease to the conceptus.

Broader aspects of ECS implementation

Despite the general consensus behind the theoretical utility and benefits provided by PCS, large scale clinical implementation could pose major challenges for healthcare professionals and general stakeholders (Cornel *et al.*, 2011; Mayor, 2011). In fact, responsible implementation of ECS requires expert evaluation of specific but interconnected and equally important aspects involving technical, ethical, legal and social frameworks (Rowe and Wright, 2020).

A main ongoing debate about ECS involves (i) which conditions should be included in test panels and (ii) which testing strategy should be employed for screening (Henneman *et al.*, 2016). While several scientific societies have developed professional recommendations for ECS panel composition aimed at maximizing the test's diagnostic efficacy, most laboratories employ a test based on an a priori gene list leading to controversial results (Stevens *et al.*, 2017). Among the recommendations, the severity of the disease and the presence of a well-validated gene-disease clinical association are necessary requirements

for improving clinical utility of the ECS panels. Arguably, the inclusion of genes associated with very low or undetermined carrier frequency, low or unknown testing sensitivity, and mild or incompletely penetrant phenotypes have led to the development commercially available ECS panels with questionable clinical utility. Notably, a recent comparative analysis across 16 ECS gene panels from different providers identified extreme heterogeneity in ECS composition, with only three conditions being present in all the platforms (Chokoshvili et al., 2018). For this reason, the creation of comprehensive benchmarks for ECS panels should be expertly produced to facilitate and promote appreciation of testing capabilities by providers and patients.

Another important argument of debate for the broader implementation of ECS programs is the ideal CR threshold to consider when developing ECS gene-panels. Although professional societies have identified 1/100 as a reasonable value, recent studies have shown that larger but well-calibrated ECS panels (CR < 1/100) hold the potential to increase the diagnostic yield of ARCs compared with current recommendations (Bell et al., 2011; Haque et al., 2016; Capalbo et al., 2019). These data also suggested that the 1/100 cut-off did not perform equally across ethnicities, resulting in substantial differences in residual risk and treatment disproportions (Haque et al., 2016). Considering that with current NGS protocols employed for ECS, the inclusion of rarer conditions does not increase test cost, a rational benchmark for minimum CR for inclusion in testing panels is still an ongoing debate.

On the contrary, now that ECS is being increasingly performed using GS as primary methodology (Punj et al., 2018), there is general consensus that, in order to maximize the test's clinical utility, only variants with pathogenic/likely pathogenic (P/LP) classification should be reported. However, it is crucial that efforts are made in updating classification of variants with unknown significance (VUS) by widening population frequencies databases, especially for affected individuals (Fridman et al., 2020), so that their clinical significance can be fully considered and their diagnostic yield increased. For example, a recent study performed on an Ashkenazi Jewish cohort showed that the inclusion of P/LP*cVUS combinations in the ECS variant analysis led to significantly higher rate of ARCs (Fridman et al., 2020). Across genes included in the panel, the proportion of ARCs due to a P/LP*P/LP and P/LP*cVUS configuration was 2.7–3.8% and 6.8–7.5%, respectively, highlighting the importance of VUS reclassification in improving PCS diagnostic yield and reducing residual risk.

Finally, from a technical standpoint, it is worth noting that some well-established genetic variations associated with increased reproductive disease risk (such as repeats expansion of FMR1 gene and ex7 deletion in SMN1) need to be investigated with separate molecular methodologies as standard GS approaches fail to detect them.

From a societal point of view, the best strategies for entry point of testing process, patients' engagement frameworks and equal access opportunity are currently being debated in coordination with broader policies for GM application (detailed below in this article). Joint statements for healthcare providers and clinical laboratory personnel to promote education and guidance on the use of genomic screening approach have been released by several scientific societies (Edwards et al., 2015). However, considering the wide heterogeneity of socioeconomical and medical conditions, these protocols are usually required to be specifically incorporated in any different country with the collaborative action of all stakeholders involved, including government and scientific societies and laboratories in order to guarantee equity of access and standardized and validated ECS frameworks.

Optimized cost-effectiveness of treatments is a main priority in modern medicine and a current strong global commitment for equity and sustainability (GBD 2015 SDG Collaborators et al., 2016) and systematic implementation ECS in the society holds great promises in this sense. A 14-condition NGS-based ECS panel was found to be cost-effective by one study (Azimi et al., 2016). Recently, two additional studies have simulated the cost effectiveness of ECS in the context of Australian single payer healthcare system and in the context of patients with predominantly private coverage in the USA. In all case-studies, preconception ECS was foreseen to minimize the pressure of Mendelian diseases on healthcare expenditures in a more cost-effective manner compared with minimal or no screening (Beauchamp et al., 2019; Zhang et al., 2019a). In summary, ECS represents one of the most effective and advanced applications of preconception GM worldwide today and is expected to grow in application in coming years.

Individual level information from preconception GS: medically actionable findings

In addition to learning about prospective foetal disease risk ('primary' findings), preconception GS also has the potential to reveal genetic information not directly associated with patient's infertility condition or reproductive treatment. Since GS is a phenotype-agnostic test expected to reveal around 100 genuine loss-of-function variants (MacArthur et al., 2012), additional information with potential interest for individuals at reproductive age (i.e. secondary findings) may become available (Krier and Green, 2013). These results can be made available to consenting patients, in particular in cases where therapeutic and preventive options are available (actionability). As discussed above, infertility is not necessarily a condition confined to the reproductive axis but can have systemic ramifications. Thus, when GS is employed as analytical tool for preconception genomic application, expanding the genetic evaluation to 'exome slices' related to additional chronic conditions with onset during the adult life (e.g. cardiomyopathy and cancer), can be seen as an opportunity for interdisciplinary and preventive patient care.

It is obvious that full disclosure of all potentially pathogenetic variants is conceptually and logistically impractical, nonetheless, complete nondisclosure of secondary findings with defined health implications may also be seen as unethical (MacArthur et al., 2012; Lohn et al., 2014). Instead of incidental findings, the term 'secondary findings' has been adopted to specifically designate those conditions that are actively sought and systematically defined based on a list of genes compiled by professional societies. The ACMG has promulgated (and refined over time) a set of 59 genes designated as 'medically actionable' and which should be included in GS reports, when ECS testing is employed for other purposes (Kalia et al., 2017). These clinically relevant secondary findings are detected in approximately 1–3% of individuals undergoing GS, regardless of family history (Amendola et al., 2015; Gambin et al., 2015). These pathogenic variants might not show symptoms for most of an individual's lifespan and could therefore be compatible with early intervention and prevention strategies to reduce mortality and long-term morbidity.

Typical examples of 'medically actionable' genes recommended as reportable secondary findings by the ACMG include *BRCA1* and *BRCA2*. Pathogenic variants of these genes have been found strongly associated with early-onset breast and ovarian cancers. Knowledge of their presence could be used to effectively reduce their associated risk of developing malignant growths by employing timely interventions (e.g. mastectomy, oophorectomy). In other scenarios, frequent screening could be used to mitigate the risk of colon cancer associated with the presence of pathogenic variants of *BMPR1A* and *SMAD4*. For cardiomyopathy-causing genes, patients with pathogenetic variants in genes such as MYH7 may have annual electrocardiogram and echocardiography. Despite the usefulness of these guidelines, some critical challenges remain to be resolved. For example, actionability became over time one of the main criteria used to determine whether or not to report a secondary finding. Despite the efforts in developing semi-quantitative metrics for the objective assessment of medical actionability, this approach has been criticized due to the lack of prospective cost-effectiveness data on asymptomatic carriers (Berg *et al.*, 2016; Ormond *et al.*, 2019). Indeed, effectiveness and cost-benefit are not guaranteed by the mere availability of a medical intervention, demonstrated by the lack of evidence in prospective clinical trials for several interventions for conditions listed on the ACMG catalogue (Richer and Laberge, 2019).

A further challenge is variant interpretation. A key distinction between secondary findings and conventional monogenic disorders is that with secondary findings, the individual does not report any signs or symptoms of the disease. Of note, not all genes that could be used for secondary findings diagnosis have been as extensively detected and characterized as *BRCA1/BRCA2*. Nonsense variants can be easily interpreted as pathogenic as the encoded protein is missing the remaining amino acids downstream to the mutation. However, missense and other types of mutations may be difficult to interpret and are generally catalogued as VUS. The clinical relevance of a VUS is often confusing for physicians and patients, who also have to deal with significant stress levels associated to the diagnostic uncertainty (Starita *et al.*, 2017). This obstacle is lessened, but by no means solved, by systematic public data sharing and collation which serves to increase the number of cases for rare variants. In order to investigate the impact of VUS-determining missense mutations on protein function, *in vitro* testing experiments could be employed to determine the biological activity (or inactivity) (Starita *et al.*, 2017).

Phenotypic penetrance also presents a critical challenge to secondary findings. Penetrance of a pathogenic variant is defined as the proportion of individuals carrying the mutation who manifest the associated disease in some degree of severity. Penetrance of mutations was generally estimated in family cohorts of affected individuals (Cooper *et al.*, 2013) although overestimation of penetrance was common due to complimentary genetic features that increased phenotype manifestation. Significant national and commercial efforts have been made to establish large biobanks to help researchers improve population health studies (such as the UK Biobank and All of Us) and potentially correct penetrance estimates based on homogeneous population sampling.

In the context of preconception GM, few data are available to investigate the implications of secondary finding reporting in couples seeking reproduction. Of note, in the only study addressing the secondary finding carrier burden in an infertile population, Capalbo *et al.* (2019) did not find an increased risk for 59 ACMG genes in IVF patients compared to gamete donors acting as controls. Thus, infertility does not appear to be associated with an increased detection of pathogenic variants in 59 secondary findings of the ACMG gene list. Future studies will need to extend the causative analysis between infertility and chronic diseases beyond the established ACMG gene list to potentially identify shared genes and pathways between reproductive function and other late-onset chronic diseases.

Remarkably, while the majority of tested individuals are keen to receive information on medically actionable secondary findings and the report of positive results has been shown to have minor to no negative effect on participants (Hart *et al.*, 2019), the implications associated to disclosing secondary findings in reproductive couples and gamete donors has not yet been sufficiently investigated.

In this setting, positive results from secondary findings analysis will likely result in a higher uptake of preimplantation/prenatal genetic testing. Indeed, recent mainstream adoption of cancer predisposition genetic assessment in Europe has triggered a growth in demand for PGT for monogenic disease (PGT-M) for cancer-predisposing conditions. In the 2016 European Society of Human Reproduction and Embryology (ESHRE) data report, breast cancer ranked second among all conditions tested with PGT, surpassing CF and other most common recessive conditions (De Rycke *et al.*, 2017). In 2014, developments in WES/WGS applications for ECS led the ESHRE task force in genetics to broaden its view on PGT-M and include in the scope of PGT also the health of third generations (De Wert *et al.*, 2014). In this context, disclosure of secondary findings could be considered as a commitment to enhance patient reproductive autonomy, and allowing couples carrying oncogenic conditions to decide on whether to undergo PGT-M to minimize the risk of transmission to their offspring. Nonetheless, the possibility of an over-uptake of preimplantation/prenatal diagnosis due to the disclosure of secondary findings as well as local equal access to PGT-M services will need to be evaluated in more depth (Vaz-de-Macedo and Harper, 2017).

For gamete donors, the applicability of secondary findings reporting is further complicated. Although donors may find information on secondary findings an additional compensation derived from undergoing medical treatments for the benefit of third parties, the psychological impact that may derive from becoming aware of certain genetic inheritance may reduce gamete availability and/or increase donor programs costs. Clinical utility and the implications of returning secondary findings in the reproductive medicine context will require closer investigation.

Preconception genomics for infertility diagnostics and personalized reproductive treatment

Genetic factors in male infertility

Male infertility is a multifactorial condition affecting around 7% of the general male population. According to a recent classification of impaired male reproductive function, a major role for genetic factors is indisputable since they are present in all aetiological categories and

overall account for about 15% of male infertility (Tournaye et al., 2016). Men with non-obstructive azoospermic are at the highest risk (20%) of being carriers of numerical and structural chromosomal anomalies such as Klinefelter syndrome and Y chromosome-linked Azoospermia Factor deletions (AZF; Krausz and Riera-Escamilla, 2018). In addition to karyotype and AZF deletion screening, the routine genetic diagnostic armamentarium includes also the mutational screening of the *CFTR* gene in selected patients affected by congenital agenesis of vas deferens (CAVD) (Bieth et al., 2020). In about 40% of cases of oligo/azoospermia, the aetiology remains unknown and is referred to as idiopathic infertility. In these cases, genetic factors are likely to play an important role since about 2000 genes are predicted to be involved in spermatogenesis pathways, but only a minority of them have been identified so far. In fact, all previous efforts to discover recurrent monogenic causes with Sanger sequencing were largely unsuccessful. The recent widespread use of NGS allowed the validation of previous candidate genes and the identification of novel genetic causes in all four aetiological categories.

In fact, WES was crucial for the identification of a growing number of novel candidate genes for congenital hypogonadotropic hypogonadism (CHH), monomorphic terato/asthenozoospermia and CAVD. Excellent recent reviews provide comprehensive updates on the genetic aspects of these diseases (Bieth et al., 2020; Butz et al., 2020; Cangiano et al., 2020; Toure et al., 2020). As far as CHH is concerned, it accounts for about 3–5% of infertility cases. To date, mutations in more than 50 genes involved in the development or migration of the GnRH neurons or the neuroendocrine regulation of GnRH secretion or action have been described (Cangiano et al., 2020). Screening of candidate CHH gene panels including, on average 35–41 genes, has an approximate diagnostic yield of 40% (Krausz and Riera-Escamilla, 2019; Butz et al., 2020).

Monomorphic isolated qualitative spermatogenic disturbances are even more rare and are typically found in consanguineous families. This category includes globozoospermia (round-headed, acrosomeless spermatozoa), various morphological abnormalities of sperm flagella (MMAF) and macrozoospermia (or sperm macrocephaly, characterized by large-headed and multi-flagellated spermatozoa). The genetic diagnosis of pure globozoospermic forms is obtained in 70% of patients, and in 80% of cases is due to deletions and mutations in *DPY19L2* (Ray et al., 2017). Other genes, such as *CCDC62* and *ZPBP*, have been recently described in globozoospermic men but they need further validation in independent cohorts (Oud et al., 2020). In macrozoospermia, a frameshift deletion in the *AURKC* gene (c.144delC) is observed in over 85% of cases (Ray et al., 2017). Rarely, a stop gain mutation in exon 6 (p.Y248X) is described in patients of European origin (Krausz et al., 2018). The consequences of altered *AURKC* are sperm tetraploidy which precludes normal embryogenesis. MMAF is defined as a combination of morphological abnormalities of the sperm flagella leading to asthenoteratozoospermia (Ben Khelifa et al., 2014). To date, 18 novel genetic causes of MMAF have been discovered, whose mutations account for up 30–60% of the cases (for review see, Toure et al., 2020). Finally, in *CFTR* negative CAVD patients, a novel X-linked gene, *ADGRG2*, has been recently discovered. The screening for the two genes allows the genetic diagnosis over 90% of bilateral CAVDs (for review see, Bieth et al., 2020).

As stated above, NGS-based studies were relatively successful in patients from consanguineous families and in rare and highly specific spermatogenic or developmental defects. However, the major challenge in our field is the identification of genetic factors in the most common infertility category, i.e. in patients affected by isolated primary testicular dysfunction.

The monogenic space: towards the discovery of monogenic causes of idiopathic oligo/azoospermia.

The genetic dissection of the primary quantitative defects of spermatogenesis recently started with the first NGS studies focused on familial cases of azoospermia from inbred families. Quantitative spermatogenic disturbances are associated with various semen phenotypes ranging from oligozoospermia (reduced sperm number in the ejaculate) to non-obstructive azoospermia (NOA). Almost 75% of all infertile men belong to this aetiological category. To date, a total of 41 genes have been proposed as monogenic causes of isolated quantitative spermatogenic defects. Among them, 35 are candidate genes for NOA and 6 are candidate genes for oligozoospermia (see Table I and Supplementary Table SI). NOA is a largely heterogeneous pathological condition in terms of testis histology, which implies a polygenic aetiology. In fact, NOA can be the consequence of the complete absence of germ cells (Sertoli cell-only syndrome, SCOS), arrest of the spermatogenesis at different levels (i.e. spermatogonia, spermatocyte or spermatid) or hypospermatogenesis.

Although maturation arrest is relatively rare, over 75% of novel monogenic causes have been reported in NOA patients with maturation arrest and more than half of these genes encode for essential meiotic proteins (see Table I, Supplementary Table SI and Fig. 2).

Currently, three genes (*TEX11*, *TEX14* and *TEX15*) belonging to the testis expressed sequence gene family (TEX), are the top recurrent meiotic arrest genes. The X-linked TEX11 regulates DNA recombination and formation of chromosome synapses and crossovers (Adelman and Petrini, 2008). Mutations in this gene have been reported in 12 NOA men in 6 independent cohorts making this gene the most frequent monogenic cause of NOA (Table I). The knock-out mouse models of the three TEX genes provide further evidence for a robust cause–effect relationship between pathogenic mutations in these genes and NOA.

The large majority of NOA genes are recessive or X-linked (30/35) and near half of them were reported exclusively in consanguineous families (12/30). In general, for the recessive autosomal mutations, there is a strong evidence for their causative role since the mouse phenotype mirrors the one observed in humans. Only a few genes have been described (*DMRT1*, *SYCP3*, *SYCP2*, *HSF2*, *PLK4*) as autosomal dominant (AD). The attribution of a cause-effect relationship is rather complex since the heterozygous mouse model is fertile for four of them (*DMRT1*, *SYCP3*, *SYCP2*, *HSF2*) and functional studies demonstrating the dominant effects of the mutations are lacking for *DMRT1* and *SYCP2*. The only gene for which both the functional analyses and the mouse phenotype are concordant for an AD effect is *PLK4*, reported in a patient with SCOS.

Overall, the clinical validity for the large majority of the 41 genes do not reach 'strong/definitive' evidence according to a gene-disease scoring system proposed by Smith et al. (2017). The introduction of a given gene into diagnostic testing is based on a number of criteria such as (i) the pathogenicity of the identified variant; (ii) multiple unrelated

Table I List of recurrently mutated genes in non-syndromic NOA.

Gene	Chromosome (inheritance)	n° unrelated cases/n° of publications	Semen phenotype	Testis histology	Altered reproductive phenotype in mouse male/female	References
TEX11	Xp11 (XL)	12/6	AZO	MeiA, MA	M	Oud *et al.* (2017), Sha *et al.* (2018), Tuttelmann *et al.* (2018), Yang *et al.* (2015), Yatsenko *et al.* (2015), Krausz *et al.* (2020)
TEX14	17q22 (AR)	7/4	AZO	SCOS, MeiA; MA	M	Fakhro *et al.* (2018), Fenz Araujo et al. (2020), Gershoni *et al.* (2017), Krausz *et al.* (2020)
SYCE1*	10q26.3 (AR)	6/4	AZO	MeiA	M/F	Huang *et al.* (2015), Maor-Sagie *et al.* (2015), Pashaei *et al.* (2020), Krausz *et al.* (2020)
DMRT1	9p24.3 (AD)	7/3	AZO	SCOS; MA; n.a	M	Lopes *et al.* (2013), Tewes *et al.* (2014), Krausz *et al.* (2020)
MEIOB*	16p13.3 (AR)	5/3	AZO	MeiA	M/F	Gershoni *et al.* (2017, 2019), Krausz *et al.* (2020)
SYCP3	12q23.2 (AD)	4/3	AZO, SO	MeiA	M/F	Miyamoto *et al.* (2003), Oud *et al.* (2017), Stouffs *et al.* (2011)
USP26	Xq26.2 (XL)	4/3	AZO, SO	MeiA, n.a	M*	Lee *et al.* (2008), Li *et al.* (2020), Ma *et al.* (2016)
MEI1*	22q13.2 (AR)	3/3	AZO	MeiA	M/F	Ben Khelifa *et al.* (2018), Nguyen *et al.* (2018), Krausz *et al.* (2020)
STAG3*	7q22.1 (AR)	3/3	AZO	MeiA	M/F	Van der Bijl *et al.* (2019), Riera-Escamilla *et al.* (2019), Krausz *et al.* (2020)
TEX15	8p12 (AR)	3/3	AZO, Crypto	MA; MeiA	M	Colombo *et al.* (2017), Okutman *et al.* (2015), Wang *et al.* (2018)
M1AP	2p13.1 (AR)	11/2	AZOO, SO	MeiA, SPA; n.a	M	Tu *et al.* (2020), Wyrwoll *et al.* (2020)
FANCM*	14q21.2 (AR)	4/2	AZO, O	SCOS; n.a	M/F	Kasak *et al.* (2018), Yin *et al.* (2019)
SHOC1	9q31.3 (AR)	3/1	AZO	MeiA	M	Krausz *et al.* (2020)
SYCP2	20q13.33 (AD)	3/1	AZO, Crypto	MeiA	M/F	Schilit *et al.* (2020)
ADAD2	16q24.1 (AR)	2/1	AZO	SGA	M/F	Krausz *et al.* (2020)
*FANCA**	16q24.3 (AR)	2/1	AZO	SCOS	M/F	Krausz *et al.* (2018)
*MSH4**	1p31.1 (AR)	2/1	AZO	MeiA	M/F	Krausz *et al.* (2020)
NANOS2	19q13.32 (AR)	2/1	AZO	SCOS; MA	M/F	Fakhro *et al.* (2018)
TERB1	16q22.1 (AR)	2/1	AZO	MeiA	M/F	Krausz *et al.* (2020)
SPINK2	4q12 (AR/AD)	2/1	AZO, O, OAT	SPA	M	Kherraf *et al.* (2017)

AD, autosomal dominant; AR, autosomal recessive; AZOO, azoospermia; Crypto, cryptozoospermia; F, female; M, male; MA, maturation arrest; MeiA, meiotic arrest; n.a, not available; O, oligozoospermia; OAT, oligoasthenoteratozoospermia; SCOS, Sertoli cell-only syndrome; SGA, spermatogonial arrest; SO, severe oligozoospermia; SPA, spermatid arrest; XL, X-linked.
In bold: genes reported in more than one independent cohorts.
*Genes leading both human male and female infertility.

patients from different cohorts; (iii) availability of *in vitro* functional analysis and mouse model recapitulating the human phenotype. Since the publication of a systematic review by Oud *et al.* (2019) based on data available up to December 2018, seven novel NOA genes (*ADAD2*, *M1AP*, *MSH4*, *RAD21L1*, *RNF212*, *SHOC1*, *STAG3*, *SYCP2*, *TERB1*) and three novel severe oligozoospermia/oligo-astheno-teratozoospermia (OAT) genes (*RPL10L*, *FAM47C* and *IFT140*) have been published (see Table I and Supplementary Table SI). In addition, novel variants in TEX11, TEX14, *SYCE1*, *MEIOB*, *MEI1* and *DMRT1* were reported in independent cohorts increasing their clinical evidence (Table I). Notwithstanding, only 11/35 NOA genes reached 'moderate' or higher grade of evidence that would qualify them to be included in a diagnostic gene panel (Oud *et al.*, 2019). We expect major breakthroughs in the coming years, which will likely upgrade many of the listed genes to a sufficiently high clinical evidence for them to be included in a diagnostic gene panel.

Careful genotype to phenotype correlation in NOA cases will add a prognostic value concerning the outcome of testicular sperm retrieval. Future pre-testicular sperm extraction diagnostic/prognostic testing will be a perfect example of precision medicine in andrology.

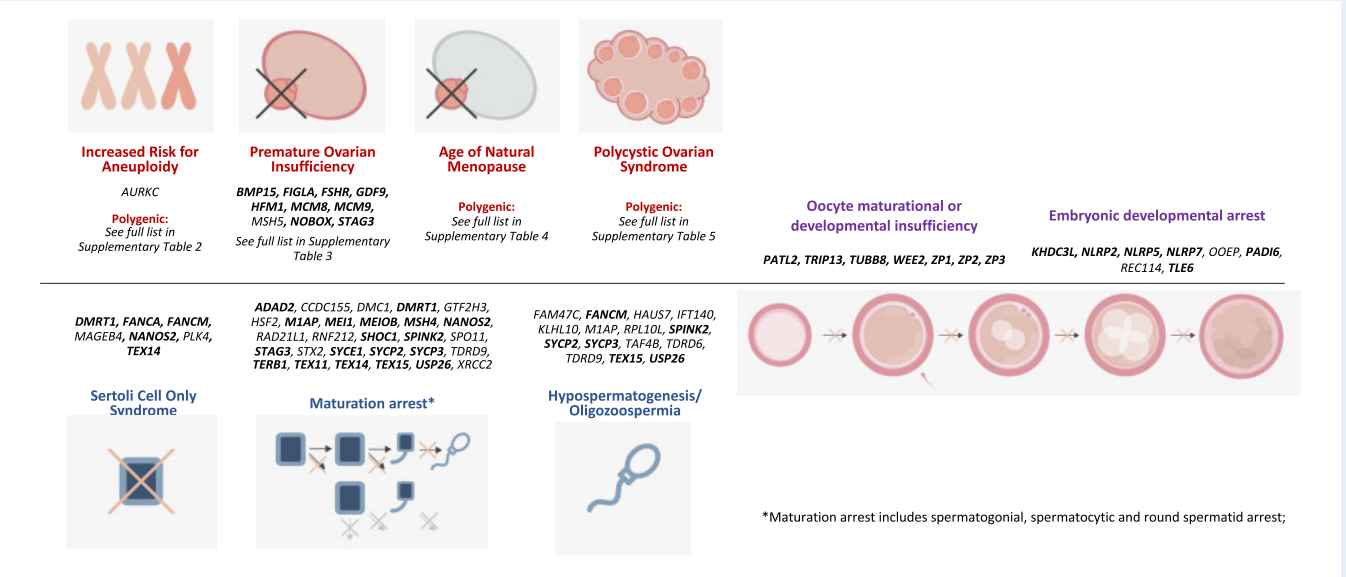

Figure 2. Genes involved in non-syndromic isolated female and male infertility and embryonic lethality. In bold, genes reported in more than one unrelated patient.

The polygenic space: oligogenic and polygenic models in male infertility.

As stated above, CHH is a multifactorial disease with over 50 candidate genes (Cangiano *et al.*, 2020) with digenic/oligogenic origin in about 20% of cases (for review see, Cangiano *et al.* 2020). The digenic/oligogenic inheritance is characterized by the combined effect of more than one heterozygous mutation in genes belonging to the same biological pathway or function. This model has not been explored in isolated spermatogenic disturbances so far. It is plausible that two or more heterozygous, rare pathogenic variants in closely related genes may act synergistically, leading to a given NOA phenotype. However, the interpretation of this model is expected to be rather complex and will require a system biology approach.

In contrast to some other multifactorial/polygenic diseases, GWAS were largely unsuccessful in finding recurrent, clinically relevant SNPs in male infertility (Krausz *et al.*, 2015). The polygenic inheritance model calls for genetic variants in multiple genes, each with a small and additive effect leading to the phenotype. Only three GWAS studies tested this model with limited evidence. Kosova *et al.* (2012) observed that some sperm parameters, birth rate and family size showed associations with multiple SNPs. These traits decreased with increasing number of risk alleles/genotypes. Two other GWAS studies proposed an excess of low frequency or rare mutations in oligo/azoospermia versus normozoospermia (Aston and Carrell, 2009; Li *et al.*, 2015). Disappointingly, the risk alleles did not overlap in the three studies hence further validation on large cohorts are needed.

Wider implications of monogenic causes of NOA.

A number of epidemiological studies are clearly indicating that azoospermic men are at higher risk of developing cancer (Hanson *et al.*, 2018). An emerging issue deriving from whole-exome analysis in NOA and severe OAT patients is related to the discovery of genes which are not only involved in spermatogenesis but also in oncogenesis. Three genes belonging to the Fanconi anaemia (FA) pathway (*FANCA*, *FANCM* and *XRCC2*) were recently reported in patients with spermatogenic failure (Kasak *et al.*, 2018; Yang *et al.*, 2018; Krausz *et al.*, 2019; Yin *et al.*, 2019). The FA pathway is involved in DNA damage repair, DNA replication and mitotic checkpoints. Hence, defects in these genes predispose to cancer. Krausz *et al.* (2019) reported an unexpectedly high frequency of 'occult' Fanconi anaemia in a specific subgroup of NOA subjects affected by SCOS (pure and partial) and borderline/mild haematological alterations. The double phenotype suggests that the proliferation and differentiation of both the haematopoietic stem cells and the primordial germ cells/spermatogonia is highly dependent on the FA pathway (Tsui and Crismani, 2019). These findings stimulate further investigations on the discovery of common genetic factors involved in severe spermatogenic impairment, bone marrow failure and cancer.

Another wider implication of the recently discovered gene defects is their potential involvement in premature ovarian insufficiency (POI). More than half of the known monogenic causes (19/35) are either already reported as causes of POI or the knock-out mouse models indicate both male and female infertility. Interestingly enough, the vast majority of these genes are involved in the meiotic process. A novel conclusion obtained is that genetic counselling should involve not only the male but also the female relatives of NOA patients.

Genetic determinants of female infertility

Unlike males, cessation of fertility affects all women as meiotic errors in oocytes reach such a high level that the majority of conceptions are aneuploid and fail to implant or develop to term (Gruhn *et al.*, 2019). Moreover, reproductive longevity is determined by the onset of menopause, which in turn has implications for comorbidities, pending upon the age of onset (Perry *et al.*, 2015; Day *et al.*, 2015b). Clinically, female infertility is caused by anovulation, ovulation of defective oocytes,

embryonic and foetal factors (that may in some cases be paternally derived) and endometrial factors. Pregnancy loss is a major cause of infertility that affects 25% of clinically recognized pregnancies, and the causes are heterogeneous including foetal aneuploidies as well as maternal endocrine and immunological factors with uncertain genetic bases and substantial comorbidities (Westergaard *et al.*, 2020). Despite this complexity, our understanding of female reproductive phenotypes has greatly improved from the advances in genomics. Case–control studies have been expanded to encompass the whole exome, and even whole genome, as opposed to being limited by targeted sequencing or gene mapping in families. This has allowed an unbiased approach to variants mapping within families as well as in cohort studies. Extensive GS has been particular insightful when our knowledge of basic science makes follow-up feasible or where a substantive base of knowledge of gene function exists. Recent examples include investigations of diverse disorders such as oocyte maturation arrest (Feng *et al.*, 2016a,b; Chen *et al.*, 2017b), aneuploidy (Nguyen *et al.*, 2017) and POI (Caburet *et al.*, 2014), reviewed in França and Mendonca (2020). Monogenic variants with high penetrance, large effects and heritability are the extreme of the genetic space. Since many female reproductive traits are common, such monogenic variants explain only a small proportion of the population. It is therefore not surprising that the field has benefitted from GWAS and the investments in biobanks. In part, this is because many female reproductive traits are straightforward to assess and can be self-reported with sufficient precision when averaged over large populations. Below, we discuss conceptual advances in the genetics of human female reproduction and we refer to Supplementary Tables SII–SV and relevant recent reviews that cover more comprehensive gene lists.

Monogenic and polygenic variants in human aneuploidy: pleiotropic effects of regulators of meiotic recombination and chromosome segregation.

Aneuploidy, the gain or loss of a whole chromosome, is common and affects between 20% and 85% of human oocytes The rate of aneuploidy follows a U curve that shapes the natural fertility curve over the reproductive life span of women between menarche and menopause (Gruhn *et al.*, 2019). Maternal age, meiotic recombination as well as socioeconomic and occupational status are implicated in the predisposition to aneuploidy in human oocyte (Hou *et al.*, 2013; Hunter *et al.*, 2013; Ottolini *et al.*, 2015; Keen *et al.*, 2020). In women of advanced reproductive age (>35 years), meiotic recombination accounts for 20% of the variance in aneuploidy incidence, making it a significant factor (Ottolini *et al.*, 2015). Meiotic recombination together with sister chromatid cohesion tether homologous chromosomes together in meiosis I until their separation at ovulation and extrusion of one set of chromosomes in the first polar body (reviewed in Capalbo *et al.* (2017)). A recent GWAS study of Down syndrome assessed genes implicated in recombination and cohesin genes that had been identified in human population studies. The analysis identified signals consistent with polygenic regulation of chromosome segregation by meiotic recombination and cohesin genes (Chernus *et al.*, 2019) (Supplementary Table SII and Fig. 2). A different approach has also suggested that monogenic variants may contribute to age-independent aspects of aneuploidy. A study using targeted sequencing of Aurora kinase B and C in women with high or low levels of embryonic aneuploidies for their age identified one variant of each gene (Nguyen *et al.*, 2017). The AUR B p. L39P variant was discovered in a woman of advanced maternal age, who had lower aneuploidy rates in her embryos compared to the age-matched population-average. Functional follow-up revealed that the variant increased the protein's ability to regulate chromosome alignment when injected into wildtype mouse oocytes. The second variant, AURKC p. I79V, did not show an effect in mouse oocytes, suggesting that (i) this variant is neutral, (ii) the mouse oocytes may not be sensitive to this change or (iii) the variant affects segregation in embryonic mitosis, since embryo (not oocyte) aneuploidy was assessed. AURKC may be particularly important for male spermatogenesis and perhaps plays a less prominent role in human oocytes, since several mutations in North African families are associated with macrospermatozoa and tetraploidy, whereas women harbouring these mutations are fertile (Dieterich *et al.*, 2007). Overall, GS for aneuploidy in oocytes might provide breakthroughs but will require functional validation and will likely only explain a small proportion of the population variability.

The emergence of a continuous genetic space: monogenic and polygenic factors in premature ovarian insufficiency, diminished ovarian reserve and poor responders.

POI is a complex disorder characterized by amenorrhoea, low oestrogenic levels, elevated levels of gonadotropins and onset of menopause below the age of 40 years. POI can be syndromic or non-syndromic and affects 1–2% of women. Clinical investigations include karyotypic assessment as well as *pre-FMR1* expansions that explain up to 15% of the cases. However, the onset of reproductive senescence is continuous, characterized by progressive depletion of oocytes, and clinical definitions of diminished ovarian reserve (DOR) and poor responders in IVF treatment also map onto the continuous age at natural menopause (ANM) curve.

In the monogenic space, over 90 genes have been identified as implicated in POI (Supplementary Table SIII and Fig. 2). However, they explain a very small proportion of cases and are typically discovered in consanguineous families. For example, *SGOL2* is associated with POI by a single proband from a consanguineous family, where pathogenic variants that affect hearing co-segregate (Perrault syndrome; Faridi *et al.*, 2017). Furthermore, individuals with biallelic pathogenic variants in DNA repair genes such as *MCM8*, *MCM9*, *XRCC4* and *MSH5* show non-syndromic POI (Table II and Fig. 2). The *MCM8* or *MCM9* genes were first reported in homozygous women with hypergonadotropic primary amenorrhoea, hypothyroidism absent or very small ovaries and growth retardation. In cases of POI with early-onset, rare monogenic variants are likely to be causative. Carriers of pathogenic mutations in *BRCA1* and *BRCA2*, which predispose to breast and ovarian cancers, are also at increased risk of POI (Titus *et al.*, 2013). The >90 genes that are implicated in POI were recently reviewed (Yatsenko and Rajkovic, 2019), and they are inferred to have functions in meiosis, DNA repair, granulosa cells and steroidogenesis. Without appropriate cell lines, functional follow-up remains challenging and is typically based on mouse models in which the entire gene is deleted (e.g. Caburet *et al.*, 2014). As GS becomes more affordable and common, other variants may appear. However, their low frequencies suggest unique founder mutations, which may require individual functional analyses.

In the polygenic space, GWAS has revealed that the genetic space that explains the ANM is continuous and determined by two major genetic networks: hypothalamic signalling and the DNA damage

Table II Genes associated with non-syndromic female infertility and embryonic lethality.

Gene (OMIM)	Chromosome (inheritance)	n° of unrelated cases identified	Ethnicity/region	Phenotypic spectrum	References
BMP15 **(300247)**	Xp11.22 (XL, AD/AR)	25	Unspecified, Indian, Chinese	POI	Di Pasquale *et al.* (2006), Dixit *et al.* (2006), Wang *et al.* (2010), Zhang *et al.* (2018), Mayer *et al.* (2017)
FIGLA **(608697)**	2p13.3 (AR)	6	Chinese	POI	Zhao *et al.* (2008), Chen *et al.* (2018), Yuan *et al.* (2019a)
FSHR **(136435)**	2p16.3 (AR)	8	Brazilian, Chinese	POI	França *et al.* (2017), Liu *et al.* (2017), He *et al.* (2019), Liu *et al.* (2019)
GDF9 **(601918)**	5q31.1 (AR)	6	Brazilian	PA	Dixit *et al.* (2005), França *et al.* (2017)
HFM1 **(615684)**	1p22.2 (AD)	3	Unspecified, Chinese	POI	Wang *et al.* (2014), Zhe *et al.* (2019)
MCM8 ***(608187)***	20p12.3 (AR)	9	Arabic, unspecified, Tunisian	POI	AlAsiri *et al.* (2015), Desai *et al.* (2017), Bouali *et al.* (2017)
MCM9 **(610098)**	6q22.31 (AR)	12	Unspecified, French	POI	Wood-Trageser *et al.* (2014), Desai *et al.* (2017), Yang *et al.* (2019)
MSH5 **(603382)**	6p21.33 (AR)	2/1		POI	Guo *et al.* (2017)
NOBOX **(610934)**	(AR/AD)	4	Unspecified, Chinese, Brazilian	PA, POI	Qin *et al.* (2007), Li *et al.* (2017), França *et al.* (2017)
STAG3 **(608489)**	7q22.1 (AR)	10	Arabic, unspecified, Chinese, Brazilian	POI	Caburet *et al.* (2014), Colombo *et al.* (2017), He *et al.* (2018), França *et al.* (2019)
AURKC **(603495)**	19q13.43	1		Aneuploidy	Nguyen *et al.* (2017)
KHDC3L **(611687)**	6q13 (AR)	8 1 (Wang)	Asian for PEDA	Hydatiform mole/PEDA	Nguyen and Slim (2014) (Review), Wang *et al.* (2018)
NLRP2 **(609364)**	19q13.42 (AR)	5	Asian	PEDA/single imprinting syndromes or MLID	Mu *et al.* (2019)
NLRP5 **(609658)**	19q13.43 (AR)	3	Asian	PEDA/single imprinting syndromes or MLID	Mu *et al.* (2019), Xu *et al.* (2020a,b)
NLRP7 **(609661)**	19q13.42 (AR)	86		Hydatiform mole	Nguyen and Slim (2014) (Review), Soellner *et al.* (2017)
PADI6 **(610363)**	1p36.13 (AR)	5	Asian	PEDA/single imprinting syndromes or MLID/recurrent hydatidiform mole	Xu *et al.* (2016b), Wang *et al.* (2018)
PATL2 **(614661)**	15q21.1 (AR)	19	Asian, North African	Meiotic arrest/fertilization failure/PEDA	Liu *et al.* (2020), Maddirevula *et al.* (2017), Christou-Kent *et al.* (2018), Huang *et al.* (2018), Wu *et al.* (2019)
REC114 **(618421)**	15q24.1 (AR)	2	Asian	Abnormal fertilization and PEDA	Wang *et al.* (2020)
TLE6 **(612399)**	19p13.3 (AR)	4	Asian	PEDA, recurrent biochemical pregnancy loss	Alazami *et al.* (2015), Maddirevula *et al.* (2020), Wang *et al.* (2018)
TUBB8 **(616768)**	10p15.3 (AD/AR)	61	Asian, Mexican	Meiotic arrest/fertilization failure/PEDA/implantation failure	Feng *et al.* (2016a,b), Lanuza-López *et al.* (2020), Chen *et al.* (2019), Huang *et al.* (2017), Chen *et al.* (2017a), Wang *et al.* (2018)
TRIP13 **(604507)**	5p15.33 (AR)	4	Chinese	Meiotic arrest	Zhang *et al.* (2020b)
WEE2 **(614084)**	7q34 (AR)	16	Asian	Meiotic arrest	Sang *et al.* (2018), Zhao *et al.* (2019), Zhang *et al.* (2019)

Continued

Table II Continued

Gene (OMIM)	Chromosome (inheritance)	n° of unrelated cases identified	Ethnicity/region	Phenotypic spectrum	References
ZP1 **(195000)**	11q12.2 (AR)	19	Asian, Arabic	Meiotic arrest/empty follicle syndrome/oocyte morphologic abnormalities	Huang *et al.* (2014), Dai *et al.* (2019), Maddirevula *et al.* (2020), Sun *et al.* (2019), Yuan *et al.* (2019b), Okutman *et al.* (2020), Xu *et al.* (2020a), Zhou *et al.* (2019), Yang *et al.* (2019)
ZP2 **(182888)**	16p12.3-p12.2 (AD)	5	Asian	Meiotic arrest	Dai *et al.* (2019), Zhou *et al.* (2019), Liu *et al.* (2017)
ZP3 **(182889)**	7q11.23 (AD)	6	Asian	Meiotic arrest	Chen *et al.* (2017c), Liu *et al.* (2017), Zhou *et al.* (2019)

MLID, multi-locus imprinting disturbance; PA, primary amenorrhoea; PEDA, preimplantation embryonic development arrest; POI, premature ovarian insufficiency. In bold, OMIM gene access numbers.

response (Day *et al.*, 2015b). This view is well-supported by the identification of a range of highly penetrant POI genes that affect DNA repair and hypothalamic–pituitary–ovarian (HPO) signalling. Moreover, several recent studies have identified genetic variants with predicted pathogenicity in two or more genes in women with POI (Patiño *et al.*, 2017; Jolly *et al.*, 2019). The number of genes and proportion of heritability explained are currently modest (Supplementary Table SIV, Fig. 2 and Day *et al.*, 2015b). Nevertheless, GWAS has identified two major genetic networks that seem to regulate the age at which natural menopause occurs (ANM), including the DNA damage response and repair (DDR) as well as HPO signalling. Because GWAS assesses phenotypes on a continuum, understanding the relative polygenic risks associated with the DDR and HPO signalling will be important, because it may require designing combination treatments for women with DOR or poor responders, pending upon their genetic susceptibility. Models for PRSs are still in their infancy. There are currently no PRSs for POI, DOR or poor responders, and for any interventions, it would be essential to understand the molecular mode-of-action of the genetic variants and networks. In particular, GWAS not only predicts early-onset menopause but also later onset, and the latter prediction, that natural reproductive longevity can be achieved by genetic manipulation of the DDR and HPO, still needs to be demonstrated in appropriate models. There is reason to think that PRSs may become a part of the clinical evaluation and that interventions could be developed, since a range of mouse models preserve ovarian function in response to chemotherapy and other exogenous DNA damage inducing agents (e.g. Myers *et al.*, 2014; Yang *et al.*, 2017a; Nguyen *et al.*, 2018; reviewed in Sonigo *et al.* (2019)). However, current PRSs can only predict a difference of few months in the reproductive lifespan of women, resulting in poor clinical utility (Perry *et al.*, 2015). Further understanding of the genetic causes can be important in designing and implementing intervention strategies. Nearly 600 trials or pilot studies have been conducted on poor response to gonadotrophin stimulation in IVF treatment. Stratification on whether the women might carry higher genetic risk scores for HPO signalling or DNA repair might make a critical difference in treatment. If the underlying cause is poor response because the majority of follicles are defective in DNA repair and therefore senesce or undergo apoptosis during follicular growth, then interventions that rely on inhibiting apoptosis or designing interventions that reduce the load of DNA damage in the follicle might prove more fruitful for this group of women.

Polygenic space: common shared genetic networks and phenotypes, the case of PCOS.

One of the emerging themes in female reproductive traits is pleiotropy, in which a gene or genetic network influences multiple phenotypes. Co-occurring phenotypes, or comorbidities, are common and a good example of this is polycystic ovarian syndrome (PCOS). PCOS is the most common reproductive metabolic disorder affecting around 15% women worldwide. The condition is characterized by chronic anovulation and hyperandrogenism, i.e. excessive androgen synthesis by the thecal cells in the ovary, as well as many well-known comorbidities (Kosova and Urbanek, 2013; Azziz *et al.*, 2016; Gajbhiye *et al.*, 2018; Yatsenko and Rajkovic, 2019). A study of Dutch monozygotic twins suggested that PCOS is highly heritable ($h^2 \approx 70\%$) with a complex genetic inheritance pattern. The proportion of heritability accounted so far in the general population by GWAS is <10% (Vink *et al.*, 2006). A large number of candidate gene case–control studies have identified variants in genes involved in metabolism (*ADIPOQ*, *FTO*, *VDR*, *SOD2*), androgen biosynthesis and regulation (*AR*, *CYP1A1*, *CYP11A*, *SRD5A1*, *SRD5A2*, *SHBG*), insulin signalling (*IRS1*, *IRS2*, *PPARG*, *CAPN10*) and folliculogenesis (*FS*, *ESR*, *ESR2*; *LHCGR*) (Supplementary Table SV). While candidate gene approach studies are informative to explore the genetic origin of the disease, they do not provide a proper understanding of molecular mechanisms underlying complex polygenic disease and frequently have not been properly powered or replicated in independent cohorts. Since 2011, several GWAS studies have allowed a more extensive discovery of many novel genetic loci and SNPs involved in various reproductive, metabolic and endocrine processes that are associated with PCOS. The first GWAS study in Han Chinese women led to the discovery of three novel genetic loci (*LHCGR*, *THADA*, *DENND1A*) (Chen *et al.*, 2011). Another study again in Han Chinese women identified eight novel genetic loci (*FSHR*, *HMGA2*, *SUMO1P1*, *TOX3*, *YAP*, *RAB5B* and *c9orf3*) (Shi *et al.*, 2012). A GWAS in a European ancestry population implicated three new genetic loci involved in folliculogenesis (*ARL14EP-FSHB*, *GATA-NEIL2*), while a second study of women of European descent found associations with insulin signalling (*ERBB2*, *ERBB3*, *ERBB4*) (Day *et al.*, 2015a) (Supplementary Table SV). A recent meta-analysis of multiple published genome association studies identified three novel loci involved in immune response and mitosis (*MAPRE-1*, *PLGRKT*, *ZBTB16*) (Day *et al.*, 2018). A recent discovery-validation GWAS in PCOS cases from electronic health records identified a novel variant near *SOD2*

and two novel intronic variants in *ERBB2* and *WWTR1*. Further association tests showed that the SNP near *SOD2* is associated with polycystic ovaries and hyperandrogenism, while the SNPs in *ERBB2* and *WWTR1* are associated with oligomenorrhoea and infertility (Zhang *et al.*, 2020a). Although around 100 genetic loci and variants have been discovered to this date, no proven diagnostic pathogenic variants in genes for PCOS have been reported and a more thorough understanding of molecular mechanisms leading to the multifactorial aetiology of PCOS is needed, as it is still unclear how these processes would result in anovulation.

Prediction of extreme IVF outcomes from parental genome in cases of idiopathic infertility

Isolated mutations affecting genes involved in fundamental processes of oocyte fertilization and embryo development are all potential candidate for the study of infertility. Widespread use of IVF and ICSI has made it possible to identify subtle phenotypic changes in these processes (i.e. total oocytes maturation failure, total embryonic developmental arrest) and to investigate the genetic causes of infertility that otherwise would remain concealed. Recent applications of GS in families with post-IVF infertility phenotypes, have led to the discovery of new genomic variants affecting unique functions of gametes or very singular mechanisms necessary for early embryo development (Alazami *et al.*, 2015; Nishimura *et al.*, 2019). Often, these families with recurrent IVF failure cycles due to stage-specific embryogenesis failure present with an indistinctive history of infertility combined with normal values for typically investigated during conventional infertility work-up, such as markers of ovarian reserve and semen analysis. Anticipating these genetic defects into the course of preconception genomic analysis will help by avoiding multiple failed IVF cycles and associated costs and times.

It will also represent a starting point for developing precision reproductive medicine applications to overcome biologically imposed barriers. For fully penetrant conditions, identifying genetic causes of infertility in parents will also provide information on their offspring's risk of developing infertility later in life. This type of information will be useful for deploying timely preventive actions and tailored infertility treatment strategies for their offspring, potentially reducing financial resources and time required to achieve conception.

Genetic defects in oocyte maturation and function.

Several genotypes associated with oocyte maturation defects have been reported in recent years, spanning from genes involved in the meiotic process to factors essential for functional cytoplasmic maturation (Fig. 2).

The first genetic breakthrough came from a Chinese study in which WES identified women who had inherited a defective AD variant of *TUBB8* from their father, resulting in maturation failure (Feng *et al.*, 2016a,b) (Table II). The *TUBB8* gene is a primate-specific beta-tubulin subunit necessary for meiotic spindle assembly in oocytes, but not in sperm. In the original Chinese families, all women with pathogenetic variants in TUBB8 failed to extrude the first PB and displayed evidence of disorganized spindle at confocal microscopy analysis. Recently, likely pathogenetic variants in *TUBB8* have been reported also in three Mexican females under treatment for primary infertility and characterized by the production of immature oocytes during IVF (Lanuza-López *et al.*, 2020). Of note, additional investigations highlighted that different variants in *TUBB8* also cause multiplicity of phenotypes affecting developmental competence from human oocytes through to early preimplantation embryos, eventually showing developmental arrest at the cleavage stage (Feng et al., 2016a,b ; Chen *et al.*, 2017a). Furthermore, mutational screening for *TUBB8* has been subsequently performed on a large cohort of patients showing recurrent oocyte immaturity, resulting in the identification of potentially pathogenic sequence variants in TUBB8 in about 30% of cases (Huang *et al.*, 2017; Chen *et al.*, 2019). The authors have proposed that *TUBB8* mutation screening might not only be a genetic diagnostic marker for patients with complete oocyte maturation arrest, but also a biomarker for metaphase II oocytes competence. However, larger data on the general population of reproductively competent patients are necessary to exclude false positive association and selection biases, as well as assessing *TUBB8* genetics in different ethnicities.

Homozygous loss-of-function variants in *PATL2* gene have been detected in women with primary infertility characterized by complete oocyte immaturity in multiple IVF cycles (Maddirevula *et al.*, 2017; Huang *et al.*, 2018; Wu *et al.*, 2019; Liu *et al.*, 2020). *PATL2* is an RNA-binding protein highly expressed in human GVs and MI oocytes, controlling gene expression processes at a large scale.

Following WES, Christou-Kent *et al.* (2018) recently reported an subset of six cases of women with *PATL2* homozygote pathogenic variants and primary infertility caused by oocyte maturation deficiency. Furthermore, transcriptomic analysis of oocytes from wild type and Patl2-/- animals showed the absence of Patl2 caused the deregulation of the expression levels of a large number of highly relevant genes involved in oocyte maturation and early embryonic development (Christou-Kent *et al.*, 2018).

Homozygous loss-of-function variants in *WEE2* were also reported in relation to female infertility due to oocyte's inability to complete metaphase II and form pronuclei, resulting in total fertilization failure (Sang *et al.*, 2018; Zhao *et al.*, 2019; Zhang *et al.*, 2019b). Sang's study included four affected individuals carrying homozygous loss-of-function missense mutations or homozygous frameshift protein-truncating mutations. Fertilization failure phenotype followed a Mendelian recessive inheritance pattern. Interestingly, phenotype rescue was attempted by directly injecting WEE2 cRNA into oocytes of one proband during a new ICSI cycle. All of the seven oocytes in the control group did not fertilize after ICSI, while all four oocytes injected with WEE2 cRNA fertilized successfully and two of them developed into chromosomally normal blastocysts on Day 6. The effects of WEE2 on fertilization were therefore confirmed by this study, suggesting the possibility for future treatment options for individuals with defective *WEE2* gene.

Very recently, pathogenetic variants in TRIP13, a fundamental component of the spindle assembly checkpoint with AAA-ATPase activity, have been described in five patients from three independent families showing oocyte maturation arrest and in one patient from another family showing abnormal embryo development (Zhang *et al.*, 2020b). Of note, homozygous pathogenic variants in *TRIP13* are known causes of Wilms tumours in children. Although homozygote variants were detected in the infertile women tested, no sign of Wilms tumour was observed for these subjects, suggesting a variable expressivity and clinical manifestation of genetic variants in TRIP13. Remarkably, functional

analysis highlighted reduced TRIP13 protein levels and increased accumulation of TRIP14 and downstream target HORMAD2 in both HeLa cells and in infertile patient-derived lymphoblastoid cells. Chromosome mis-segregation assays showed that variants do not have effects on mitosis, supporting the lack of association between the genetic variants causing infertility with Wilms tumour. The authors have also provided evidence of phenotype rescue by injecting TRIP13 cRNA into oocytes collected from a patient with complete oocyte maturation arrest. In this trial, 13 out of the 22 oocytes collected in a new IVF cycle were injected with TRIP13 cRNA. All injected MI oocytes extruded PB1, and eleven of them showed a successful fertilization and seven further progressed to the blastocyst stage. In contrast, all the untreated control oocytes failed to develop into mature gametes.

Successful interaction between a single sperm and the zona pellucida as well as the activation of a blocking mechanism that prevents the fusion of additional sperm with the oocyte is critical for fertilization. Likely pathogenic variants in genes encoding for the human zona pellucida structure have been found in individuals with idiopathic infertility undergoing IVF. Women harbouring homozygote loss-of-function variants in *ZP1* showed a variable phenotype characterized by the complete absence of the zona pellucida around their oocytes (Huang *et al.*, 2014; Okutman *et al.*, 2020) or by empty follicle syndrome phenotype, denoting phenotypic variability associated with *ZP1* genotypes (Yang *et al.*, 2017b; Dai *et al.*, 2019; Sun *et al.*, 2019; Yuan *et al.*, 2019b; Maddirevula *et al.*, 2020). Empty follicle syndrome has also been described in infertile women carrying a heterozygous pathogenic variant in the *ZP3* gene (Chen *et al.*, 2017c), while homozygote pathogenic variants in ZP2 showed a dysfunctional zona pellucida (Dai *et al.*, 2019). Of note, for the *ZP3* gene, a female-specific heterozygote inheritance pattern has been documented. Accordingly, infertile women with *ZP3* genetic defects can originate following *de novo* mutation or by paternal transmission of the mutated allele (Dai *et al.*, 2019).

Of note, some of the discussed genetic defects mainly produce oocyte maturation impairment; however, they have been occasionally shown to be compatible with embryo formation. Nonetheless, the ensuing embryos failed to develop, highlighting variable expressivity and phenotypic expansion to early embryonic arrest.

Preimplantation embryonic developmental arrest (PEDA).

Defects in genes specifically involved in the earliest physiological processes of embryo development can cause idiopathic infertility characterized by complete embryonic lethality (Fig. 2). This outcome is typically observed in around 50% of human embryos produced via IVF (Gardner and Lane, 1997), while a minority of couples (<5%) show recurrent complete embryonic development arrest in a single or multiple IVF treatments. As it is known, early fundamental steps of embryogenesis, including remodelling of genomes, first cleavages and embryonic genome activation, are driven by maternally derived RNAs and proteins that had been accumulated during oogenesis (Yanez *et al.*, 2016). Defects in genes involved in these functions are all expected to be good causal candidates for early embryonic development arrest (Table II). Of note, pathogenic variants in several genes that make up the subcortical maternal complex (SMC) were found to be associated with a range of early embryonic lethality phenotypes, spanning from cleavage stage arrest to implantation failure and epigenetic remodelling defects. The SMC is composed by at least six proteins (OOEP, NLRP5, NLRP7, TLE6, PADI6 and KHDC3L) and is uniquely expressed in mammalian oocytes (Li *et al.*, 2008; Zhang *et al.*, 2008). It is well documented that maternal recessive pathogenic variants in *NLRP7* and *KHDC3L* are the prevalent cause of biparental, complete hydatidiform mole (BiCHM: OMIM #231090 and #614293), a rare gestational abnormality characterized by trophoblast overgrowth and absence of embryo development. Indeed, *NLRP7* or *KHDC3L* pathogenic variants are found in ~75% and 10% of BiCHM cases, respectively. Furthermore, pathogenic variants in other SMC genes, *PADI6*, *OOEP*, *NLRP5* and *NLRP2*, were also detected in BiCHM cases or in association with generalized loss of methylation (LoM) in molar tissues (Nguyen and Slim, 2014; Docherty *et al.*, 2015; Soellner *et al.*, 2017; Begemann *et al.*, 2018; Qian *et al.*, 2018).

GS studies of families with primary infertility characterized by repeated preimplantation embryo developmental failure post-IVF have identified women with homozygote or compound heterozygote pathogenic variants in near all SMC components. The first evidence of Mendelian inheritance associated with early embryo arrest was found for *TLE6*, following WGS in two Saudi consanguineous families with a female-limited infertility phenotype (Alazami *et al.*, 2015). The human variant identified was shown to abrogate TLE6 binding to the SMC components. However, in a recent WES study, different *TLE6* homozygous truncating variants were found in an individual with history of four biochemical pregnancy losses, suggesting *TLE6* homozygous genotypes can be compatible with embryo development beyond the cleavage stage (Wang *et al.*, 2018; Maddirevula *et al.*, 2020). Pathogenic variants in *KHDC3L* have been commonly associated with BiCHM but have also been found recently in infertile women with embryos arrested before reaching the blastocyst stage (Wang *et al.*, 2018). Recently, biallelic pathogenic variants in *NLRP2* and *NLRP5* have been found in five independent infertile women and three individuals from two families, respectively, all showing recurrent embryonic arrest in IVF cycles as isolated phenotype (Mu *et al.*, 2019). NLRP5 biallelic variants have been also detected in an independent family with preimplantation embryonic developmental arrest (PEDA) (Xu *et al.*, 2020b).

An extensive investigation of SMC genes was performed in families affected by PEDA revealing a homozygous premature nonsense mutation and compound-heterozygous mutations in *PADI6* (Xu *et al.*, 2016b). Subsequent mutational screening of *PADI6* in a cohort of 36 unrelated PEDA women revealed two additional compound-heterozygous individuals. Interestingly, PADI6 expression is exclusively confined to oocytes and early embryonic arrest was shown to be specifically caused by disruption of the zygotic genome activation (ZGA) process. Indeed, embryos from affected women showed reduced amounts of phosphorylated RNA polymerase II and expression levels of seven genes involved in ZGA. This evidence provides the rationale for further precision GM applications where the transient re-establishment of PADI6 function in fertilized oocytes could overcome embryo arrest caused by failed ZGA with no foreseen side effects on future embryogenesis because of the time-specific and confined expression of PADI6 in the oocyte. Finally, Wang *et al.* (2020) have very recently identified a homozygous missense and splicing pathogenic variants in *REC114*, a gene with a known critical function in the formation of double-strand breaks during meiosis. Women who were homozygous for REC114 showed multi-pronuclear zygotes and early embryonic arrest from two independent consanguineous families (Wang *et al.*, 2020).

Of note, mutations in the reported genes can only explain a fraction of patients with idiopathic infertility, and the genetic foundation of PEDA remains essentially unidentified. The majority of these monogenic variants are rare, suggestive of de-novo founder mutations in prevalently consanguineous families. Systematic genetic analysis of embryos showing lethal phenotypes is likely to reveal additional crucial genes and allow the characterization of fundamental biological processes governing early human development. In a very interesting study, Dawes and colleagues have curated a list of 3435 'candidate developmental lethal' human genes that are necessary for murine development or human cell viability (Dawes *et al.*, 2019). These genes are not associated to human disorders because they are never mutated in viable conceptions, suggesting strong candidacy for unexplained infertility and prenatal/infantile mortality. Future studies will need to assess the extent to what this panel of candidate genes contribute to primary infertility linked to embryonic lethality.

Although promising and attracting, GS studies performed so far show common limitations including cohorts' small size (often based on a single family) and the lack of information on control populations or robust functional analysis. Of note, most of these studies were performed by a single research institute and involved individuals from highly consanguineous populations, posing questions on the generalizability of their findings. In summary, although encouraging evidence has emerged for increasing the genetic diagnostic yield on isolated infertility, much larger and more comparable cohort studies are required to assess the prevalence of these monogenic conditions across multiple ethnicities, establish causality of candidate genes and precisely determine their phenotypical effects.

Current challenges and future translational research aimed at the expansion of genomics investigations with precision-medicine applications

The path to universal clinical implementation and subsequent benefit of preconception GM is met by several institutional and societal roadblocks. These challenges encompass diverse aspects that include not only technical and infrastructural shortcomings but also educational, logistic, economic and social limitations that require significant resources and extensive implementation.

One of the main challenges in widening the adoption of preconception GM resides in the lack of familiarity and understanding of its concepts and their ramifications by clinicians, medical institutions and political parties as well as the general public. For professionals, full understanding of pathogenicity variants classification, variants significance, di-genic or poly-genic inheritance, and disease penetrance is required. Furthermore, clinical utility of genomic findings needs to be entirely understood so that its aspects can be communicated to patients. In order to generate a comprehensive knowledge of these concepts and applications, mandatory training specific to GM should be introduced to new generations of clinicians and healthcare professionals at a university or post-graduate level. More urgently, the use of GM already requires a plethora of experts and specialized professionals that are currently lacking in several countries. From biostatisticians to molecular genetics specialists, from epidemiologists to genetic counsellors, these professional roles are in extremely high demand as we approach more clinical applications. In fact, these specialists will be required both to collect and curate data and subsequently assess and update correlations and degrees of evidence between the genomic and the phenotypic information, but also to educate and inform patients. Complex information regarding genetic risk may be overwhelming for some patients. Therefore, it is crucial that thorough and informative pre- and post-test counselling sessions are provided with any genetic assessment. In the context of genomic reproductive medicine, balanced professional guidance is crucial for granting patients unrestricted ability to make individual choices based on their personal values and beliefs and to maximize their reproductive autonomy. Experience-based data on actual decisions and their outcomes will be required to evaluate the most effective counselling approaches.

From a purely technical standpoint, current molecular genetics technologies offer advanced diagnostic capabilities. However, one of the biggest (and earliest) technical challenges to be negotiated is the lack of international standards for variants interpretation of GS data. The rapid evolution in understanding genomic variation, the dynamic nature of variant interpretation and the appropriate utilization of this information in clinical care will continue to challenge clinicians, laboratories, and patients (Manolio *et al.*, 2019). For this scope, the ClinVar and Human Genome Mutation (HGMD) databases (Stenson *et al.*, 2003) serve as data aggregation repositories for the international community. The NIH-funded ClinGen (Clinical Genome Resource) program convenes relevant experts to evaluate gene-disease associations and approaches to their identification (Rehm *et al.*, 2015). However, expert curation is extremely time consuming and only ~4% of variants identified in a typical GS experiment have been annotated by experts in ClinVar so far (Landrum *et al.*, 2016). Furthermore, it has been shown that even using similar variant classification schemes, variants can be interpreted differently across laboratories (Amendola *et al.*, 2016). Thus, continued efforts in variants annotation and standardization are an urgent task in genomics that will require large collaborative activities.

In order to create meaningful data repositories where both genomic and medical information can be updated and further analysed, patients undergoing genotyping will need to be followed-up. For this reason, it can be envisaged that current primary point of care (i.e. family doctor), will need to be integrated on time with a centralized electronic medical records database where the correlation between the initial genomic information derived from GS and the progression of health status can be continuously monitored.

Crucially, if legal, ethical and social rights are not developed in parallel with the technical infrastructure, this condition may affect the legitimacy of the genomic approach, as well as reduce its public acceptance. Hence, important efforts will be required in developing a framework able to maintain patient privacy while sharing data in effective ways to improve variant interpretation (Manolio *et al.*, 2013).

It is therefore vital that public frameworks will be developed carefully, considering perceptions and concerns of the community in regards of personal data privacy and confidentiality and the implementation of data access limitations to avoid potential discrimination based

on genetic inheritance. The specific characteristics of the legal and ethical structure of personal genome data management can have deep consequences in culture and society and will be crucial for protecting personal rights and privacy. For example, the life insurance industry may have an interest in accessing personal genome data, with consequences for the type of coverage and costs associated to individual predisposition to diseases. Also, non-disease traits may become of interest for several types of industries and may affect study and work opportunities as well as contribute to the creation of a hierarchical society based on genomic characteristics. Theoretical benefits of GM are fairly intuitive; however, the social and ethical ramification of personal data repositories can be alarming if not dealt with responsibly. Overall, it is crucial that the development and improvement of life-saving GM applications is counterbalanced by an equally important evolution of legal frameworks that minimize potential harms deriving from it, whether ethical, social or economic.

Conclusions

The expansion of GS analysis to additional monogenic and polygenic traits involved in reproduction and the constant reduction of sequencing costs is enabling the development of cost-effective preconception tests capable of identifying simultaneously the reproductive risk for hundreds of Mendelian conditions and improving the diagnostic yield for underlying genetic causes of infertility, which have been defined as 'unexplained' until now. This is paving the way for the development of a true precision GM frameworks in reproductive health. Also, preconception GS is improving the understanding of the genetic and molecular foundation of reproductive processes, facilitating a shift from current reactive infertility treatments to more proactive strategies for safeguarding reproductive potential. In addition, if infertility is further defined as an early marker of chronic diseases with shared underlying genetic defects, preconception GS could be employed for the assessment of individual risk for actionable disorders, thus enabling timely preventive interventions. While it is surely exciting to be at the dawn of the genomic era, a high level of responsibility must accompany the development and implementation of these ground-breaking technologies, and solid evidence of clinical utility must be demonstrated before routine application can be established.

Supplementary data

Supplementary data are available at *Human Reproduction Update* online.

Authors' roles

A.C. and C.S. developed the concept of the manuscript. A.C. and M.P. coordinated the development of the manuscript and drew the figures. C.K. and A.R.-E. wrote the male infertility section. E.R.H., V.S. and M.K.H. wrote the female infertility section. A.C. and M.P. wrote all remaining parts. C.S. reviewed the whole manuscript. All authors reviewed all sections, and provided comments and active input in manuscript drafting.

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Conflict of interest

A.C., M.P. and C.S. are employees of Igenomix, a company providing reproductive genetic services. The other authors have no conflict of interest.

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