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REVIEW ARTICLE

The Genetic and Genomic Landscape of Human Reproductive Disorders: An Overview with Our Experience

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ABSTRACT

Genetics and genomics play a role in the causation of various human diseases. A large number of human reproductive disorders also arise as a result of genetic and genomic abnormalities. Reproductive disorders associated with predominantly genetics and genomic abnormalities are infertility, early pregnancy loss, congenital malformations, difference or disorder of sex development and reproductive cancers. The genetic etiology of human reproductive disorders is increasing with improved molecular biology techniques such as DNA microarray and next-generation sequencing.

Infertility is one of the significant areas of reproductive disorders where genetics/genomics plays a substantial role and may result from chromosomal, copy number variation, Yq & pseudo autosomal region microdeletion/microduplication, gene mutation (monogenic, oligogenic, polygenic), multifactorial, epigenetic, mitochondrial, etc. abnormalities. All idiopathic infertile couples should be screened for genetic disorders before assisted reproduction to prevent transmission, if any, in offspring. Pregnancy wastage in early pregnancy is very high (about 70%) and is mainly related to chromosome number, copy number variation, and some monogenic or epigenetic abnormalities. Therefore, all early pregnancy loss cases should also be tested for genetic causes. Congenital malformations are structural defects in embryo, fetus, or newborns and affect about 3% (major malformations) of all births. The malformations could be due to the abnormalities of chromosomes, copy number variation, monogenic, oligogenic, multifactorial, or environmental. Array CGH &/or NGS should be used as the first step to screen congenital malformations. Differences/disorder of sex development is a developmental defect in which the determination and/or differentiation of chromosomal, gonadal, or phenotypic/anatomic sex is abnormal. It is a common disorder and is primarily related to genetic abnormalities. Therefore, a precise diagnosis, mainly through an array CGH and/or NGS, is crucial for the proper management to prevent future psychosexual problems and another birth with the disorder. Cancer is a genomic disorder characterized by genomic instability (due to a defect in DNA repair mechanism), uncontrolled replication (due to lack of response to inhibitory factors/loss of contact inhibition), neo-angiogenesis, invasion and metastasis. All cancer cases should be investigated for genomic markers (both hereditary and somatic) for precise diagnosis, prognosis, and genetic counseling. In this review, we will try to evaluate the role of genetics and genomics in the above-mentioned reproductive disorders, along with genetic & genomic techniques used and reproductive counseling in addition to our experiences.

Keywords: Reproductive Disorders, Reproductive Technologies, Genetics & Genomics, Array Comparative Genomic Hybridization, Whole Exome Sequencing, Reproductive Genetic Counselling

Introduction

Reproductive disorders are diseases of the reproductive system. Genetics and genomics are a branch of medicine dealing with the structure, function, evolution, mapping, and alterations of genes and genomes. Genetics is the study of genes, variations, and heredity. A gene is a portion of DNA that contains instructions for synthesizing proteins that make the individual function. Gregor Mendel was the first to study genetics scientifically in the 19th century. A genome is a complete set of cell DNA. The genome was derived from the German word "Genom" and credited to Hans Winkler in 1920.[1] Tom Roderick invented the term genomics in 1986.[2]

Genetics is the study of a specific gene and its role in inheritance in physiology and pathology. Genes regulate the synthesis of messenger RNA and, thus, proteins. Genomics is the deciphering and analysis of genomes, both structural and functional. The proteins comprise body structures and mediate signals between cells, thus, biological functions. To apply for genomics and genetics role in human reproduction and its disorders, a new branch of medicine has appeared as Human Reproductive Genetics & Genomics. It deals with genetics/genomics of the human reproductive process and its disorders and its associations with epigenetics and mitochondrial DNA. Genetics and epigenetics are becoming more critical with reproduction following the advent of non-invasive prenatal as well as preimplantation tests besides *in vitro* fertilization, intracytoplasmic sperm injection (ICSI), and in vitro maturation/culture of gametes as these procedures lead to more genetic/ epigenetic abnormality in offspring since it bypasses natural protective mechanisms.

This overview will summarize genetic/genomic technological advances and their use in evaluating reproductive disorders. We will then review the literature and discuss our work briefly on the relationship between genetics/genomics and reproductive disorders causing infertility, early pregnancy losses (EPL), congenital malformation, differences/disorders of sex development (DSD), and reproductive cancers. Reproductive genetic counseling is also briefed in the review.

Genetics and Genomics

Genetics and Genomics play essential roles in the causation of human disease. It plays a role in infertility, EPL, malformation, DSD and cancer. It affects fertility by influencing hormonal homeostasis, gametogenesis, and gamete quality & quantity. The genetic and genomic basis of infertility can result from chromosomal abnormalities, Yq copy number variations (CNVs), i.e., microdeletion/ microduplication or pseudoautosomal region (PAR) CNVs, other autosomal & sex chromosome CNVs, monogenic, oligogenic, polygenic, multifactorial, mitochondrial and epigenetic abnormalities. With the advent of improved molecular biology techniques, the genetic and genomic basis of the disease is coming up with an increasing number of disorders. This is also observed with reproductive disorders and will soon change previous estimates of genetic contribution because many genes are expressed in the reproductive system. It is essential to know the underlying genetic etiology as this information can be utilized for identifying abnormality before disease onset, preventing disease, planning treatment at/before the onset of disease, and predicting the offspring's health. The genome can be studied in various ways, either through cell culture (conventional cytogenetics) or molecular (molecular cytogenetics) methods. These are karyotyping, comparative genomic hybridization, DNA microarray (array comparative genomic hybridization/aCGH, SNP microarray), whole genome sequencing (WGS)/ whole exome sequencing (WES)/ targeted gene panel sequencing primarily by next-generation sequencing (NGS), etc.

Advances in genetics and genomics are mainly due to innovations in molecular biology, instrumentation technology, computation, and bioinformatics.[3,4] Genetics and Genomics is now new scientific discipline. The present form of genetics and genomics has matured into a multidisciplinary science based heavily on molecular biology and bioinformatics. The resolution has improved to the extent of a single base pair and sensitivity by many folds. Most importantly, present genomic technological advances have been freed from the dependence on cell culture and, most importantly, freed from subjectiveness. But most importantly, now complete automation without human interference is possible. The current wave is now moving to DNA chips and/or sequencing for the identification of sub-microscopic chromosome alterations as well as variant analysis. These molecular techniques are now providing far more information at higher resolution. These newer techniques can be applied throughout the cell cycle in non-dividing, gamete, dead, and fixed cells.[5-7] High sensitivity, specificity, speed, and the ability to provide information even on a single cell have made these newer molecular approaches a powerful tool in modern reproductive genetics and genomics.[8] Genome approaches used widely are conventional cytogenetics, SKY FISH/M FISH, comparative genomic hybridization (CGH), PRINS (primed in-situ synthesis/labeling), array CGH (aCGH)/SNP microarray, next generation

sequencing (NGS), etc. [3-5,9,10] CGH is a molecular technique that allows comprehensive analysis of all chromosomal imbalances (relative copy number as gains or losses) in the entire genome at cytogenetic resolution by single test without metaphase preparation & cell culture of the test samples.[5,11] The strategy of CGH is based on the co-hybridization of differentially labeled whole genomic tests and control DNA in equal ratios on normal metaphase spread. If the test DNA contains additional copies of DNA material, hybridization will reveal the higher signal intensity of test DNA at the corresponding target region. Similarly, deletion/monosomy will give rise to lower signal intensities. CGH, although simple and cheap to perform, is labor intensive and challenging as karyotyping of DAPI banded chromosomes is highly subjective, hence, getting replaced by array CGH. Array CGH is carried out on the principle of CGH with some modifications, i.e., co-hybridization on DNA spots/ arrays rather than metaphase chromosomes. Following the hybridization of differentially labeled tests and reference genomic DNA to the target sequences on the microarray, the slide is scanned to measure fluorescence intensities at each target on the array. The normalized fluorescent ratio for the test and reference DNA is then plotted against the position of the sequence along the chromosomes. Gains or losses across the genome are shown by values higher or lower than the standard 1:1 ratio. The resolution depends on the size, number of targets, and position of targets on the genome. This procedure is rapid (24-36 hours), provides a complete genome view at a very high resolution, and does not depend on live cells or cell cultures. It detects most microscopic and sub-microscopic chromosomal changes from any DNA source in a single experiment without prior knowledge of abnormalities.[12] The aCGH is recommended by several authorities as the first line of test in evaluating multiple malformations, developmental/mental retardation and prenatal diagnosis. Another vital application of aCGH is in the field of cancer. SNP microarray is a further refinement of aCGH to detect mosaicism, parental origin, zygosity, translocation, and better resolution (<100 kb). NGS technology utilizes amplified or single-molecule templates for sequencing massive parallel fashion. This increases throughput by several orders of magnitude. There are three primary levels of sequencing analysis: targeted genes panel, whole exome, and whole genome. It detects almost all principal types of genome alterations, including nucleotide substitutions, small insertions, deletions, copy number alterations, aneuploidy, mosaicism, parental inheritance, chromosomal rearrangements, including balanced translocation and microbial/foreign DNA integration. This method is becoming a severe challenge for all other genomic & conventional methods as this does not require cell culture, non-subjective nature of interpretation, and provides more information quickly besides automation. Microarray/NGS-based genetics and genomics have become the primary choice to directly analyze all chromosomes/ genes in one action.

Copy Number Variations

Copy number variations (CNVs) are structural variations of DNA segments, spreading widely in the genome. Chromosomal rearrangements can result in deletions or duplications. Many rearrangements occur in specific genome regions, suggesting specific mechanisms, although no genome region is exempted. The CNVs are produced from abnormal nonallelic (another chromosome) homologous recombination (NAHR) in areas with high homology within the genome.[13] NAHR induces an unequal crossing over, leading to a duplication of a DNA segment in one and a deletion of a DNA segment in the other chromosome, thus producing CNVs. Meiotic non-disjunction events in carriers of balanced translocations may also lead to a disturbance of gene dosage (CNVs) in offspring. It refers to the genetic events involving the number of copies of a particular gene or DNA segment of an individual. CNVs are sub-microscopic DNA segments (1 kb or more) of duplications and deletions of the genome and linked with disease or just variations of the genome.[14-16] Other studies defined a CNV as a DNA segment from 50 bp to several Mb.[17] CNVs can cause overt disease (pathogenic CNVs), predispose to disease, or have no effect (benign CNVs). CNVs sit at the interface between microscopically visible rearrangements and point mutations and are increasingly being assessed using microarray methods. Various studies have shown that CNVs may affect as low as 5% to as high as 30% of the human genome.[18,19]

There are various techniques for detecting CNVs. These are array CGH (aCGH), SNP microarray, or whole genome sequencing (WGS) besides MLPA (multiplex ligation-dependent probe amplification) & FISH (fluorescence in situ hybridization) techniques. Small CNVs are frequently benign, but large (>250 kb) CNVs are frequently associated with pathological conditions. CNVs are implicated in many human disorders, such as cancer, and to a lesser extent, with multiple malformations, developmental disorders, neuropsychiatric disorders, reproductive disorders, etc. It affects gene function mainly through dosage effects. CNVs alter gene function through fusion, disruption, and long-range regulation effects.[20] CNVs can be used

as diagnostic/prognostic biomarkers for various diseases such as cancer, cardiovascular disease, neuropsychiatric (especially 22q11.2 CNV in autism, attention deficit hyperactivity disorder, and schizophrenia), or reproductive disorders.[21,22] The functional consequences of some of the CNVs (e.g., 15q11-13) depend on the parental origin of the deletion. If the deletion comes from the mother, the child will suffer from Angelman syndrome. In contrast, if the deletion is inherited from the father, the child will suffer from Prader-Willi syndrome, as genes are differentially expressed depending on parental origin.[23] CNV promotes tumorigenesis and chemotherapy resistance also. CNVs are crucial in the causation of inter-individual differences, i.e., genetic diversity and adaptive evolution.[24]

Genomic disorders

Genomic disorders are diseases resulting from genomic rearrangements, such as deletions, duplications, insertions, inversions, and large tandem repeats. The human genome is highly dynamic and shows large-scale variations due to genomic rearrangements. The mechanisms by which rearrangements convey phenotypes (evolution, diversity, traits, susceptibility, behavior, or diseases such as microdeletion/microduplication syndrome, schizophrenia, autism, cancer, etc) are diverse and include gene dosage, gene disruption, gene fusion, position effects, mutations, etc. Rearrangements introduce variation into our genome and serve an evolutionary function. Genomic rearrangements may cause cancers, and produce complex traits such as behavior or represent benign polymorphic changes. Microarray & NGS are used to identify genomic disorders. In some cases, the individual can be found to have multiple genetic abnormalities, including modifiers. There is a concept of multiple genetic abnormalities in which several genetic disorders are identified, such as chromosomal and/or CNVs and/or gene variants, in particular with cancers, multiple malformations, or infertility.

Reproductive genetics and genomics

Genetics and genomics have a role in every discipline of medicine. The main contribution of genetics and genomics is to predict and prevent a disorder, thus decreasing its burden right from the planning of reproduction. A new branch called Reproductive Genetics and genomics has emerged to fulfill this objective. Reproductive genetics and genomics deals with the genetic contribution of the reproductive process, both natural and assisted. Now it expands to include studying epigenetic modifications of the genome and their effect on reproduction. Genetic factors are greatly responsible for infertility, EPL, malformation, DSD, and cancer. Reproductive genetics is becoming integral to today's reproductive practice due to the increased burden of reproductive disorders, particularly after assisted reproduction invention. The ideal time to apply reproductive genetics should be from the pre-conception or peri-conception period, so that prediction and/or prevention (primary and/or secondary) is possible. Advances in molecular technology (NGS and aCGH), the introduction of non-invasive prenatal testing (NIPT), prenatal diagnosis (PND) and preimplantation genetic diagnosis (PGD) have increased this drive and expectations of the public. The NIPT is an upcoming technology for screening fetal aneuploidies (in particular trisomy 21) from cell-free fetal DNA (cffDNA) present in the blood of pregnant women.[25] The NIPT can also determine paternity, fetal sex, fetal rhesus D (RhD) status, copy number variations (microdeletion/microduplication syndromes), or even single gene disorders, but professional bodies do not yet recommend it. Research is on non-invasive prenatal diagnosis (NIPD) using fetal erythroblast and trophoblast cells from maternal blood or cervical mucus.[26,27] The advent of preimplantation & prenatal diagnosis has allowed the option of having unaffected offspring in couples at risk of transmitting genetic disorders. This has immensely increased the scope of genetic testing in reproductive practice. Furthermore, rapid dissemination of information to the public and health care providers has affected daily reproductive care so much that understanding genetics is essential for all reproductive specialists, in particular, to know the risk of a genetic disorder and how to prevent it. This knowledge will protect reproductive specialists from medico-legal consequences following failure to prevent genetic disorders in a family.[28]

Reproductive Technologies

Advances in reproductive technology, like cellular reprogramming or cellular differentiation/ dedifferentiation have created another dimension for reproductive genetics. In the laboratory, stem cells can be manipulated to make specialized cells and can be used to treat disease. For example, embryonic stem cells can be differentiated into gametes (sperm/oocytes) to treat infertility. Therefore, developing a novel method for precisely controlled differentiation is crucial to facilitate its application in clinical practice. Recent progress in germline stem cell isolation and culture may provide a platform for in vitro gamete development. It may open a new era of gametogenesis in a dish and personalized infertility treatment in the coming years. For therapy with stem cells, the issue of immune compatibility arises. The breakthroughs in somatic cell nuclear transfer have raised the

possibility of generating unlimited, undifferentiated cells with potential applications (therapeutic/reproductive cloning) without immune rejection.

Infertility

Infertility is defined in various ways by different scientific bodies. It is defined as failure to conceive after regular unprotected sexual intercourse for two years in the absence of known reproductive pathology (NICE) or failure to achieve a successful pregnancy after 12 months or more of regular unprotected intercourse (ASRM), or failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse or inability of a non-contracepting, sexually active woman to have a live birth in 5 years.[29] Infertility affects 5-15% of couples of reproductive ages.[30] The underlying etiology may be due to pathological factors in males (male infertility), females (female infertility), or both partners. About 30% of infertility are idiopathic despite extensive investigations available at present.[31] Genetics account for 15% (female) to 30% (male) of infertility cases, but this will change soon as many newer genetic/ genomic/epigenomic factors are coming up regularly. Genetic research has expanded in the last few years, and more and more genetic causes are coming up that are going to change previous estimates (about 30%) of the genetic contribution of infertility.[32] The genetic basis of infertility may result from chromosome abnormalities, Yq microdeletion/ microduplication, CNVs, monogenic, oligogenic, polygenic, multifactorial, epigenomic and mitochondrial defects.

Genetic defects associated with infertility are Kallmann syndrome, Laurence Moon Biedl syndrome, Prader Willi syndrome, Noonan syndrome, androgen receptor mutations or CAG triplet expansion, or gene defects of steroid 5 α-reductase 2, FSH-receptor, LH receptor, mitochondrial gene, etc. Some of the likely CNV hot spots are 1p31-33, 6p21, 6p22.1, Xq28, 7q31, 3p21.1, etc (testis expressed genes) and Xq27.3, Xq21.1, Xq21.33, Xq21.1, 1p22.2, 2p13.3, 3q22.3, 6p21.33, 7q22.1, 7q35, 9q33.3, 10q11.23, 10q26.3, etc (ovary expressed genes).[32] Epigenetic factors also affect reproduction and fertility from gametogenesis to birth. Epigenetic changes in gametes are critical for normal fertilization and embryonic development. Hypermethylation of promoters of genes like *MTHFR, PAX8, NTF3, SFN, HRAS, JHM2DA, IGF2, H19, RASGRF1, GTL2, PLAG1, D1RAS3, MEST, KCNQ1, LIT1, SNRPN*, etc are associated with infertility. Genomics and epigenomics are becoming more important following the development of assisted reproductive technologies (ART) such as in vitro fertilization (IVF), intra cytoplasmic sperm injection (ICSI), and in vitro maturation (IVM) of gametes as these leads to more genetic and epigenetic abnormalities in gametes & offspring. ICSI has raised major concerns about safety for the offspring since it bypasses the physiological protective mechanisms related to normal fertilization. Natural selection prevents the transmission of mutations causing infertility, while ART bypass this protective mechanism. Due to ART, the risk of genetic causes of infertility will increase in future generations.[33] Thus, the identification of genetic factors in infertile couples should become routine practice for appropriate counseling and management of the infertile couple.

Male Infertility

Males are responsible for about 50% of infertility cases.[34] About 30% of male infertility is caused by chromosomal abnormalities or specific genetic mutations of genes involved in germ cell production and function, and about 30% of male infertility is idiopathic.[35] Most idiopathic causes are probably related to genetics/genomics, as many genes are expressed in the male germ cells. Male infertility can be divided into three major groups such as pre-testicular (predominantly hypogonadotropic hypogonadism/secondary testicular failure), testicular (predominantly hypergonadotropic hypogonadism/primary testicular failure), and post-testicular (predominantly eugonadotropic hypogonadism/normal testicular function).

The pre-testicular/ hypogonadotropic hypogonadism is caused by a defect in the hypothalamo-pituitary axis secondary to infection/trauma/tumor/etc of hypothalamo-pituitary axis or genetic or idiopathic. The prevalence of idiopathic hypogonadotropic hypogonadism (IHH) ranges from 1-10 cases per 100000 births.[36] About 60% of patients with IHH present with associated anosmia (total or partial), also known as Kallmann syndrome.[37] Common genes involved with IHH are *KAL1*, *FGF8, KAL2/FGFR1*, *PROK2, KAL3/PROKR2*, *CHD7*, *NELF*, etc (regulates GnRH neuronal development & migration) or *LEP*, *LEPR, KISS1, KISS1R, TAC3, TACR3, PCSK1, DAX1, etc* (regulates GnRH secretion) or *GnRH1, GNRHR, etc* (regulates GnRH activity). Although mutation in a single gene is sufficient to cause IHH, cases are often predicted to result from the combination of multiple genetic (oligogenic/polygenic) abnormalities.[37] Other causes of IHH are Bardet–Biedl syndrome (*BBS1, BBS10, BBS12, BBS2, BBS4, BBS5, BBS7, BBS9, ARL6, MKKS, CEP290, SDCCAG8, etc*), Laurence-Moon syndrome (*PNPLA6*), Prader Willi Syndrome (15q11-13), etc.

The post-testicular (eugonadotropic hypogonadism) causes of infertility are mainly obstruction or malfunction of male genital ducts (vas deferens, seminal vesicles, ejaculatory ducts, etc) or varicocele. Major causes of obstructive azoospermia are cystic fibrosis and genital tract infection. Cystic fibrosis is caused by a mutation of the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene that encodes a chloride-conducting transmembrane channel. *CFTR* gene variants may present as male infertility associated with congenital absence of vas deferens (CAVD), either bilateral (CBAVD) or unilateral (CUAVD), and with or without urogenital anomalies. The prevalence of CAVD in infertile men is 1-2 %.[38] Other genetic associations with CAVD are mutations in *ADGRG2, SLC9A3, SCNN1B, CA12* and copy-number variations in *PANK2* genes.[39-42] It is often found that men with CBAVD have normal production of spermatozoa, but due to agenesis of the vas deferens, there are no sperms in the ejaculate. This suggests that men with CBAVD can attain fatherhood by using ART. However, there is always a chance of transmission of *CFTR*/other pathogenic variants to the offspring. Therefore, genetic counseling should be offered to such couples undergoing ART. Both partners should be screened for *CFTR* and other gene mutations to discuss the probability of having offspring with similar problems.[43] It is worth exploring *CFTR*/related genes' involvement in conditions like semen with high viscosity & high liquefaction time or conditions with thick/viscid/scant cervical mucus.[43]

The testicular (hypergonadotropic hypogonadism/primary testicular failure) factors frequently associated with spermatogenic failure are classified into four distinct subtypes, such as Sertoli cell only syndrome (SCOS; no germ cells), maturation arrest (MA; no elongated spermatids), hypo-spermatogenesis (HS; grossly reduced spermatogenesis) and testicular atrophy (TA; destroyed tubules structure, no spermatogenesis) according to histopathology/cytology findings. However, one may often find differences between testes (mixed group), for example, SCOS on one side and tubular fibrosis on the other or any combinations. In addition, a patient may present as complete germinal cell aplasia in some tubules, whereas complete spermatogenesis in adjacent tubules (focal germinal cell aplasia) or any combinations. Often, patients may present as oligospermia (hypo-spermatogenesis) initially and later over a period of a few years as azoospermia (SCOS/TA); hence diagnosis may change with time. SCOS (no germ cells in testes) is described by Castillo et al.[44] It is characterized by normal secondary sexual character, absence of germ cells in the seminiferous tubule, and near normal tubular architecture and supporting cells, tubules containing only Sertoli cells.[44] Sertoli cells control spermatogonial stem cell division and differentiation, and various imbalances cause SCOS testicular phenotype.[45] Rarely, in SCOS, the primordial germ cells either do not migrate or do not survive in the gonads. SCOS is a heterogenous disorder (chromosomal, CNV, monogenic, etc), and underlying causes remain primarily unclear in most. Maturation arrest is characterized by the interruption of normal germ cell development and differentiation, leading to failure to produce mature spermatozoa. Germ cells are near normal in numbers. It is classified into two distinct subtypes, early maturation arrest, where the development of germ cells does not advance beyond secondary spermatocytes, and late maturation arrest, where the development of germ cells does not reach beyond round spermatids.[46] Most cases of maturation arrest present as early maturation arrest at the primary spermatocyte level. Hypospermatogenesis is characterized by decreased spermatogenesis leading to a decrease in the numbers of spermatogonia, primary/secondary spermatocytes, spermatids & spermatozoa, i.e., all stages of spermatogenesis are present but decreased in number. The thickening of the walls of the seminiferous tubules and hyaline deposition on the basement membrane with the absence of germ cells and Sertoli cells characterizes tubular fibrosis.

Primary testicular failure (PTF) is the major cause of non-obstructive azoospermia and oligospermia.[47] The prevalence of primary testicular failure is 1% in all men and 10% in infertile men. Genetic causes of primary testicular failure are chromosomal abnormalities, Yq microdeletions, gene mutations (*SYCP3*), etc. Following the advent of newer genomic technologies like DNA microarray/array comparative genomic hybridization and genome sequencing (whole exome/whole genome), more causes like gene mutation and copy number variations (CNVs) are coming up.[48,49] Cryptorchidism (failure of testicular descent), an androgen-dependent process, is one of the major causes of spermatogenic failure. It is commonly seen with androgen resistance and defects in testosterone synthesis. Cryptorchidism can be unilateral or bilateral and in either case, is associated with impaired spermatogenesis and an increased risk of developing testicular tumors. Many genes, such as *AKT3, INSL3, GREAT, RAS, MAPK*, etc are linked with cryptorchidism.[50,51]

The chromosomal abnormalities & Yq microdeletion account for about 25% of cases of primary testicular failure, and screening them during the

evaluation of male infertility is essential.[52] Chromosomal abnormalities account for about 15% and Yq microdeletions in about 10% of cases of primary testicular failure.[53,54] Klinefelter Syndrome (47,XXY) & mosaic Klinefelter Syndrome (46,XY/47,XXY) is frequently associated with primary testicular failure.[55] Klinefelter Syndrome affects approximately 1 in 1000 male births and is characterized by an extra X chromosome, i.e., 47,XXY.[56] Although an extra X chromosome (47,XXY) is the most common form, some men with Klinefelter syndrome have a greater or lesser number of X chromosomes or mosaicism, e.g., 48,XXXY, 46,XY/47,XXY.[57] The phenotype varies with the number of extra X chromosomes and possibly the number of trinucleotides CAG repeats on the androgen receptor gene (a polymorphism). A longer CAG repeat sequence has been associated with taller stature, lower bone mineral density, gynecomastia, and shorter penile length.[58] Men with Klinefelter syndrome generally have small testes resulting from damage to seminiferous tubules and Leydig cells. FSH and LH serum concentrations are elevated, and testosterone levels are decreased to varying extents. Affected men have severely reduced sperm counts and are under-virilized.[57] Cryptorchidism is more common in men with Klinefelter syndrome and causes more severe testicular damage.[59] Other chromosomal abnormalities presenting as primary testicular failure are 45,X/46,XY mosaic, 46,XX sex-reversed male, 47,XYY or 48,XXYY, Yq deletion, dicentric Y (46,X,dicY), isochromosome Xq (46,iXqY), etc.[56,60,61] The 45,X/46,XY syndrome is characterized by short stature and some features of Turner syndrome.[62] Because the testes may be streaks, dysgenetic, or normal, the phenotype varies from female to male. In those with a streak and a dysgenetic testis (mixed gonadal dysgenesis), the risk of gonadoblastoma is increased, and gonadectomy is therefore indicated. Rarely 46, XX males resulting from translocation of the testis-determining gene (SRY) to an X chromosome may have Klinefelter syndrome phenotype, although the phenotype is variable. The phenotype may range from severe impairment of the external genitalia to a normal male phenotype with infertility.[61] It generally results from an unequal crossing over between the short arms of the sex chromosomes (X and Y). Most XX male presents with normal external male genitalia, soft small testes, gynecomastia, azoospermia, high gonadotropins, with low Anti Mullerian Hormone (AMH) and Inhibin B. Testicular fine needle aspiration cytology (FNAC) often show SCOS.[61] Another genetic factor involved with male infertility is Yq microdeletions.[63] Microdeletions of the long arm of the Y chromosome are now recognized as a common cause of primary testicular failure leading to severe oligospermia and azoospermia.[64] Most microdeletions are mapped to the Yq11 region (named azoospermia factor or AZF), which contains three regions, AZFa, AZFb, and AZFc. Deletions of the AZFa or AZFb typically cause azoospermia. Deletion in the AZFc region causes infertility of varying severity, ranging from oligospermia to azoospermia and the commonest microdeletions in humans.[65] The *DDX3Y* and *USP9Y* genes in the AZFa region have an essential role in spermatogenesis. Deletions of these genes are consistently observed with azoospermia.[66] Y chromosome microdeletions also have been identified in men with cryptorchidism, varicocele, and obstructions of the vas deferens.[67]

Sex chromosomes play a significant role in gonadal function as sex chromosomes contain many genes that are expressed in the gonads. In male meiosis, the human X and Y sex chromosomes pair in prophase I, thus ensuring that at anaphase I, each daughter cell receives one sex chromosome, either the X or the Y.[68] The X-Y pairing is made possible by a region of homology between the X and Y chromosomes at the tips of their short arms (Xp22.3 and Yp11.32) called pseudo autosomal region (PAR) 1 and long arms (Xq28 and Yq12) called PAR 2.[69,70] Aberrations in sex chromosome number or structure disrupt sex chromosome pairing during meiosis thus producing gametogenic failure.[71,72]

CNVs have been implicated in primary testicular failure and XY gonadal dysgenesis.[48,55,73] Our research finding indicates an association between CNVs of pseudoautosomal regions (PAR) 1, 2, and 3 (both deletion & duplication), AZFb, AZFc, AZFbc (both deletion & duplication), 9p12 (deletion containing *SPATA31A2-A5* gene), etc with testicular maturation arrest.[48]

About 300–600 genes are expressed in the male germline and, therefore, candidates for male infertility.[74,75] *CATSPER2, PLSCR2, SLC25A24,* and *SYT6* genes are involved in the last stage of spermatogenesis.[76] Normal male sexual differentiation and spermatogenesis require normal androgen production and androgen receptors. The androgen receptor plays an essential role in the differentiation of spermatids and their release from the seminiferous epithelium. The number of trinucleotides CAG repeats in exon 1 of the androgen receptor gene is inversely correlated with its transcriptional activity.[77] Men with spermatogenic disorders frequently have significantly longer CAG repeat lengths of androgen receptors.[78] Similarly, estrogen synthesis or action disorders are also associated with spermatogenesis defects. Impaired spermatogenesis has been observed in men lacking a functional estrogen receptor alpha.[79] In mice

inactivating mutation in the aromatase enzyme causes a spermatogenic defect.[80] *FSH* receptor gene mutation too affects spermatogenesis.[81] Men with myotonic dystrophy (an autosomal dominant disorder) also exhibit abnormal spermatogenesis.[82] Mutations in the *SYCP3* gene (involved in the regulation of the synapse between homologous chromosomes during meiosis) have been implicated as a potential cause of male infertility.[83] Similarly, *TEX11* mutations (X-linked gene, hemizygous) are known to cause meiotic arrest and azoospermia in males.[84] Other genes viz., *DAZL*, an autosomal homolog of the DAZ, deleted in azoospermia, *PRM1* and *PRM2* (protamine involved in chromatin compaction), *TNP1* and *TNP2* (transition nuclear proteins) and *USP26* (deubiquitinating enzyme family) are also associated with spermatogenic defects.[83,85]

Sperm chromosomal alterations are also highly prevalent in spermatogenic impairment.[7] Infertile males with oligo/astheno/ teratozoospermia but normal blood karyotype have a ten-fold increase of various chromosomal abnormalities in their sperms, such as diploidy, disomy, and nullisomy. Sperm FISH is commonly used to determine the proportion of aneuploidy in sperms of infertile men.[86] Testicular sperm from men with nonobstructive azoospermia display a higher rate of aneuploidy in spermatozoa than ejaculated sperms. Increased sperm aneuploidy increases the risk of IVF/ICSI failure and fetal aneuploidy. Indications for sperm FISH are repeated in vitro fertilization failure, oligospermia, nonobstructive azoospermia (testicular sperm), teratozoospermia, Klinefelter's syndrome (mosaic and non-mosaic), translocations, exposures to gonadotropins, chemotherapy, pesticides exposure, or suspected gonadal mosaicism (testes), etc. Testing of azoospermic factor (AZF) microdeletion has a prognostic impact on sperm extraction since no sperm can be retrieved in AZFa and AZFb, while there is a fair chance that viable sperm could be retrieved in AZFc. The link between epigenetics and male infertility involves protamine packaging of the sperm genome. Sperm chromatin compaction is increased twenty-fold compared with somatic cells following the replacement of 90–95% of histones in the genome by the highly negatively charged and arginine-rich nucleoproteins protamine. Integration of protamine 1 and protamine 2 into the sperm genome during the elongation phase of spermatogenesis occurs typically in a strictly controlled 1:1 fashion. Significant deviations in the ratio have been associated with alteration in motility, morphology, fertilization capacity, and increased DNA fragmentation.[87]

Male infertility due to functional defects of spermatozoa is teratozoospermia, asthenozoospermia, necrozoospermia, etc, involving defects in morphology, acrosome, motility, viability, capacitation, etc. Genetic defects leading to morphological defects (teratozoospermia) of sperms, in particular, round-headed sperm/sperm without acrosome (globozoospermia) are *DPY19L2*, *SPACA1*, *SPATA16*, *PICK1*, *GGN*, *AURKC*, *FAM71F1*, *GOPC*, *HRB*, *CSNK2A2*, *BS*, *ZPBP1*, *CCDC62*, *CCNB3*, etc.[88-93] Other genes that could also play a role in globozoospermia are *CFAP47*, *C2CD6*, *C7orf61*, *CCIN*, *DNH17*, *DNH6*, *PIWIL4*, *CHPT1*, etc.[92,93] Macrozoospermia (large-headed spermatozoa) is characterized by the presence of large-headed multi-flagellar spermatozoa. Macrozoospermia is often associated with abnormal genomic DNA in the form of diploidy (2n), triploidy (3n), tetraploidy (4n), and DNA fragmentation.[94,95] Genetic defect associated with macrozoospermia is *AURKC* genes.[96] Sperm FISH analysis should be advised to estimate the aneuploidy rate to assess the feasibility and the success of ICSI.[97] Genes linked with acephalic spermatozoa are *SUN5*, *PMFBP1*, *TSGA10*, *DNAH6*, *BRDT*, *etc.*[92,98-104] Multiple morphological abnormalities of the flagella (MMAF) are characterized by a combination of absent, short, coiled, bent, and irregular-caliber flagella.[105] MMAF is a common cause of male infertility. MMAF-associated genes are *AK7*, *ARMC2*, *CEP135*, *CFAP43*, *CFAP44*, *CFAP47*, *CFAP58*, *CFAP65*, *CFAP69*, *CFAP70*, *CFAP91*, *CFAP251*, *DNAH1*, *DNAH2*, *DNAH6*, *DNAH8*, *DNAH10*, *DNAH17*, *DZIP1*, *FSIP2*, *QRICH2*, *SPEF2*, *TTC21A*, *TTC29*, etc.[106-110] Genetic causes of sperm motility disorder (asthenozoospermia) are chromosomal abnormalities and specific mutations of nuclear and mitochondrial genes. Genetic causes of asthenozoospermia are mutations in *CFAP47*, *DNAH17*, *TBL1XR1* (disrupted the histone-to-protamine transition), *CREM* (cAMP responsive element modulator), etc genes.[111-113] Genes involved with severe necrozoospermia (>80% dead sperms) are *BMPR1B*, *PDHA2*, *PGPEP1*, *PREP*, *CAPZA3*, *ZFP174*, etc.[114-116] Some studies have linked necrozoospermia with *PKD1 & PKD2* genes (polycystic kidney disease) causing dilatations of the genital tract resulting in stagnation of sperm cells.[117,118] Genes involved in the spermiation process are *ASB17*, *ARPC1B*, *EVL*, *PICALM*, *EEA1*, *STX5A*, *CAPG*, etc.[119-122] These are involved in cytoskeleton remodeling, endocytosis, completion of spermiation, etc. Genetic defects of sperm leading to oocyte activation failure (fertilization failure) are *PLCζ*, *SEPTIN12*, etc genes.[123-125]

Female Infertility

The common underlying factors of female infertility are ovulatory disorders (polycystic ovary syndrome, premature ovarian insufficiency, premature ovarian failure, etc), hyperprolactinemia, hypothalamic amenorrhea (mainly following caloric restriction or excessive exercise), endometriosis, tubal blockage, uterine abnormalities, etc.[126] Common genetic causes of female infertility are sex chromosome abnormality (Turner or mosaic Turner, 47,XXX, 46,XX/46,XY, etc), structural anomalies of the X chromosome such as terminal and interstitial deletions, fragile X premutation (*FMR1*), *FOXL2* mutations (blepharophimosis–ptosis-epicanthus inversus), galactosemia (*GLAT* mutations), *POLG* mutations (mitochondrial disease), adrenal hyperplasia, etc.[127] Several CNVs have also been implicated in female infertility, mainly with premature ovarian insufficiency/ failure, XY gonadal dysgenesis, and Mayer Rokitansky Kuster Hauser syndrome.[73,128,129]

Polycystic ovary syndrome

Polycystic ovary syndrome (PCOS) is the most common reproductive endocrine disorder in women of reproductive age. PCOS is characterized by hyperandrogenism, ovulatory dysfunction, and polycystic ovary morphology.[130] The best available biomarker associated with PCOS is AMH.[131] Environmental factors-like bisphenol A, AGEs, and endogenous factors like kisspeptin and melatonin have strong associations with PCOS.[132] PCOS is a complex heterogeneous disorder with genetic susceptibility besides environmental & epigenetic influences.[132] The genetic influence of PCOS is supported by its twins and family clusters.[133,134] Often, a higher 17-OH progesterone level is associated with PCOS, indicating an enzymatic defect in steroid biosynthesis.[135,136]

Genetic alterations (pathogenic/likely pathogenic variants) associated with PCOS are *INSR, IRS1, GHRL, LDLR, MC4R, ADIPOQ, UCP1, UCP2, UCP3, FTO, PCSK9, FBN3, NEIL2, FDFT1, PCSK9, CYP11, CYP17, CYP21, HSD17, STAR, POR, AKR1C3, AMH, AMHR2, INHBA, AR, SHBG, LHR, FSHR, FSH β, SRD5A, GATA4, THADA, YAP1, ERBB2, DENND1A, FEM1B, FDFT1, NEIL2, TCF7L2*, etc.[132] We are working on PCOS for several years and our initial whole exome sequencing results identify pathogenic/likely pathogenic/novel variants in obesity and insulin-related genes like *UCP1* (c.680C>T), *UCP2* (c.262C>T), *IRS1* (c.2674A>G) and *GHRL* (c.214C>A, n=5) in PCOS patients with high BMI and high fasting insulin level and steroid biosynthesis pathway genes like *CYP21A2* (c.1174G>A, c.955C>T, c.428T>A), *STAR* (c.158G>T), *POR* (c.1000G>A, c.751G>A), *HSD17B6* (c.118G>A) and *AKR1C3* (c.613T>G) in phenotype A/D PCOS with normal BMI, & insulin level.[137,138] We have also detected pathogenic & likely pathogenic variants for *AMH, AMHR2, INHBA, AR, SHBG, LHR, FSHR, FSHβ, SRD5A, GATA4, THADA, YAP1, ERBB2, DENND1A, FEM1B, FDFT1, NEIL2, TCF7L2, INSR, LDLR, MC4R, ADIPOQ, UCP3, FTO, PCSK9, THADA, FBN3, NEIL2, FDFT1, PCSK9, CYP11, CYP17*, etc genes in PCOS WES study (ongoing study). These genes can be categorized as metabolic, steroid biosynthesis, gonadal function, and others. We have also observed multiple pathogenic/likely pathogenic variants of more than one gene in many PCOS cases, thus indicating oligogenic/polygenic etiology in most (ongoing study). Other genes frequently associated with PCOS are *C9orf3, DENND1A, ERBB3/RAB5, TOX3, SRD5A2, SRD5A1, HMGA2, THADA, SOD2, ERRB4, YAP1, GATA4/NEIL2, ZBTB16, FSH-β, FTO, SIRT1*, etc.[139-141] PCOS-linked genes listed in the OMIM database are *PCOS1, FOXL2, CAPN10, SHBG, AKR1C3, FBN3, GATA6, SRD5A1, SRD5A2, AR, SULT2A1, H6PD, 17beta-HSD3, INS, INSR, IGF2, IRDN, IL18, ADIPOQ, AMH, LHB, FSHR, CYP19A1, CYP11A1, CYP17A1, HSD11B1, HSD3B2, STAR, CORTRD1*, etc. Reddy et al. reported CYP11A1 (tttta)(n) repeat polymorphism as a potential molecular marker for PCOS risk.[142] Higher prevalence has been reported in Turkey (33%), France (23%), Portugal (18%), Greece (9%), etc.[136,143,144] Other genes for which association with PCOS has been replicated include *MC2R/ARMC5, StAR, POR, MRAP, FBN3, HSD17B6, INSIG2, TCF7L2, MC4R, POMC, ACVR2A, FEM1B, FTO, ADIPOQ, LHCGR, FSHR, THADA, DENND1A, YAP1, RAB5B, SUOX, ERBB4, FSHB, RAD50, aKRR1*, etc.[145-148] During the last few years, growing evidence is pouring on etiopathogenetic associations of *AMH* gene/receptors with PCOS rather than being merely a marker.[149] In vitro experiment on granulosa cells from the ovary of anovulatory PCOS shows 75 folds higher production of AMH compared to granulosa cells of normal ovaries, indicating an intrinsic dysregulation of the granulosa cells.[150] This is supported by the finding of AMH and AMHR (AMHR2) pathogenic variants with PCOS.[140]

The role of epigenetics in PCOS is supported by studies on animals where intrauterine exposure to testosterone, DHT, bisphenol A, etc induces PCOS phenotype in the female offspring.[151,152] During development, adverse prenatal conditions may influence persistent epigenetic changes like imprinting of genes or increased or decreased

levels of DNA methylation on CpG sites, which can lead to under or overexpression of genes and alteration of molecular pathways, which may lead to a risk of development of PCOS later part of life.[153] Epigenetic alteration of various genes, ncRNAs, lncRNA, miRNA, etc linked with PCOS are *LHCGR, YAP1, FOXO3, CYP19A, CYP11B2, PPARGC1A, PPARG, INSR, IRS1, GHRL, ADIPOQ, FTO, LEPR, TWIST1, CCL2, GnRH, NF kappa, TNF, AGE/RAGE, AMPK, ESR1, PROLACTIN, PGR, CASP3, CASP8, CASP6, CASP7, CASP9,* miR-93/*GLUT4*, miR-320/*ERK1/2*, miR-21/*LATS1*, lncRNA/H19, lncRNA/SRA, lncRNA/GAS5, miRNA21, miRNA93, miRNA320, etc.[139,154] Promoter hypomethylation of *YAP1* in ovarian granulosa cells promotes the *YAP1* expression, which also plays a crucial role in the pathogenesis of PCOS.[155]

Premature ovarian failure/premature ovarian insufficiency

Premature ovarian failure (POF)/premature ovarian insufficiency (POI) is characterized by hypergonadotropic hypoestrogenic amenorrhea in women under the age of 40 years. The clinical spectrum is divided into occult, biochemical, and overt stages. The overt stage, often known as POF, denotes total ovarian function loss.[156] POF is a very diverse disorder with many different etiologies, and genetic etiology contributes to about 25% of cases.[157] The female sex chromosomes, i.e., chromosome X, are crucial for normal ovarian function. The chromosome X abnormality is frequently associated with premature ovarian failure. The aberrations include either whole or partial X-chromosome deletions (45,XO; 47XXX; 45,X/46,XX; etc), duplications, or translocations. Turner syndrome (45,XO) with loss of X-chromosome or mosaic Turner syndrome (45,XO/46,XX) are the most common genetic etiology associated with POF. Loss of both q arm (isodisomy Xp) or p arm (isodisomy Xq) manifest in gonadal dysfunction.[158] Two dominant genetic pathways involved in the pathophysiology of POF have reduced gene dosage and poor DNA repair. Many genes involved in meiosis and DNA repair have been shown to affect ovarian reserve and thus produce ovarian failure by reducing the pool of primordial follicles, increasing ovarian follicle atresia, or preventing follicle maturation.[159,160] Over the years, various other genes have also been linked to the pathogenesis of POF, of which FMR1 gene premutation is one such gene.[161-163] In the presence of FMR1 gene premutation, the relative risk of POF is expected to be about 16%.[164] The prevalence of FMR1 gene premutation varies within different populations. FMR1 premutation is present in approximately 11%-13% of cases of POF, although another study suggests that only 2% of POF cases have FMR1 premutation.[165,166] About 29 CGG repeats of the FMR gene are the most common trinucleotide repeats observed in the general population, while women with mid-range repeats (70-90) have been found to be at the highest risk for the development of POF/POI.[167] The mechanisms by which fragile X-associated POF develops are due to the impairment of primordial follicle development and survival.[168] Two loci on Xq22-q26 (*HS6ST2, E2F, GPC3, etc*) and Xq27-q28 (*DIAPH2, XPNPEP2, DACH2*, etc) containing several essential genes appear to be critical for premature ovarian failure.[169,170] The *DIAPH2* gene is expressed in the ovary and is involved in oogenesis by affecting cell divisions.[171] Another critical locus on Xq13.3-q22 (*DIAPH2, DACH2, POF1B*, etc) also contains several vital genes linked with premature ovarian failure.[172] The *DACH2* gene is essential in oogenesis to differentiate somatic follicle cells into polar cells. It is crucial for developing mammalian gonads, and its alteration in humans may be a risk factor for POF.[173] The *POF1B* gene is also involved in early ovarian development, and its aberrations are associated with POF.[173] Genes such as *FOXL2, NR5A1, NOBOX, FIGLA, BMP15, GDF9, AR, INHA,* etc genes are associated with POF.[174-179] Other potential genes proposed to be associated with POF are *MCM8, BRSK1, ADAMTS19, PTHB1, HS6ST2*, etc.[180-182]

In the last several years, research using whole genome screening methods (microarray) on POF cases has generated important information on copy number variations (CNVs). Aboura et al identified five potential candidate CNVs containing genes involved in reproductive disease (*DNAH5 and NAIP*), reproductive endocrinology (*DUSP22 and NUPR1*), and folliculogenesis (*AKT1*) in both sporadic and familial POF cases.[183] Another CNV study identified various essential genes on meiosis (*PLCB1, RB1CC1, and MAP4K4*), DNA repair (*RBBP8*), and folliculogenesis (*IMMP2L, FER1L6, and MEIG1*).[128] McGuire et al also discovered novel autosomal CNVs involved in DNA double-strand break repair during meiosis (*SYCE1*) and mitotic cell progression (*CPEB1*).[184]

Various studies using NGS identified mutations in several genes like *BMP15, CYP17A1, CYP19A1, DIAPH2, FIGLA, FOXL2, FSHB, FSHR, GDF9, NOBOX, NR5A1, POF1B, PSMC3IP, STAR, WNT4, LHCGR, POR, ADAMTS1, ADAMTS19, BMP4-7, BMPR1B, BMPR2, CDKN1B, CITED2, DMC1, ESR1, ESR2, FOXL2, FOXO3, FOXO4, FSHB, FST, FZD4, GJA1, GJA4, GNRH1, GNRHR, INHA, INHBA, INHBB, INHBC, KISS1, KISS1R, KIT, KITLG, LHB,*

LEPR, LHX8, MSH4, MSH5, NALP5, NANOS2, NANOS3, NBN, NTF4, PGR, PGRMC1, PIK3CA, POU1F1, POU5F1, PRL, PRLR, PROP1, PTEN, PTGER2, PTGS2, PTX3, RSPO1, SMAD1-5, SOD1, etc and studies in consanguineous families with familial POF have identified some pathogenic genes in *STAG3, HFM1, MCM8, MCM9*, etc genes.[185-187] Interestingly, these genes are also implicated in DNA replication and repair, meiosis, and chromosome stability, further supporting the importance of these pathways in idiopathic ovarian failure pathogenesis. Although the exact mechanism of how defects in DNA repair pathways and genomic instability contribute toward ovarian failure is unknown, likely, an accumulation of DNA damage and chromosomal instability in the ovary would lead to accelerated follicle atresia, therefore predisposing women to ovarian failure. A targeted NGS study reported an association between mutations in the *ADAMTS19* and *BMPR2* genes with ovarian failure pathogenesis in 12 unrelated idiopathic ovarian failure women.[188] *ADAMTS19* was previously reported with ovarian failure, and defects in *BMPR2* may disrupt the downstream signaling of its ligands, BMP-15 and GDF-9, therefore disrupting folliculogenesis and contributing to POF pathogenesis.[182,188]

Hyperprolactinemia

Prolactin is a 23 kDa polypeptide hormone that has more than 300 functions. The prolactin gene is 10Kb in size, localized on chromosome 6, and comprises five exons and four introns. Two independent promoter regions regulate prolactin gene transcription; a proximal 5000 bp region regulates the expression of pituitary prolactin, and an upstream promoter region regulates the expression of extra-pituitary prolactin.[189] Prolactin receptor, a transmembrane protein, belongs to class 1 of the cytokine receptor superfamily.[190] It is synthesized and secreted mainly by the anterior pituitary lactotroph cells. The extra pituitary sources of prolactin include skin fibroblasts, spleen, thymus, breast, prostate, etc. Prolactin is critical to the mammary gland's development during pregnancy, lactation establishment, and milk production. Prolactin maintains the corpus luteum and stimulates ovarian production of progesterone required for the maintenance of pregnancy. Prolactin also plays a significant role in reproductive and parental behavior. Besides its known reproductive functions, prolactin also has a stimulatory role in immune reactions, growth and development, and homeostasis. Prolactin increases immune cell proliferation and the production of cytokines.[191] The presence of abnormally high prolactin levels (>25ng/mL) in the blood is called hyperprolactinemia. Physiological hyperprolactinemia occurs during pregnancy and lactation. It may also be caused by pathologic conditions such as pituitary adenoma, drugs, or secondary to chronic kidney disease/liver disease besides idiopathic.[192] Hyperprolactinemia may manifest as galactorrhoea, menstrual irregularity, erectile dysfunction, loss of libido, decreased bone mineral density, osteoporosis, etc. Hyperprolactinemia is one of the leading causes of infertility. The genetic/genomic cause of hyperprolactinemia can be abnormalities in prolactin (*PRL*) or prolactin receptor (*PRLR*) genes. A germline, loss-of-function mutation in the prolactin receptor (*PRLR*) gene results in prolactin insensitivity in familial hyperprolactinemia.[193-195] In our ongoing study, we have observed CNVs containing genes *HCCS* (11p15.4) gain and *OLFML1* (Xp22.2) loss with cases of hyperprolactinemia due to a pituitary adenoma (*HCCS*) and idiopathic (*OLFML1*) group (unpublished). The *HCCS* gene has a role in the mitochondrial respiratory chain and programmed cell death, whereas the *OLFML1* gene is involved in cell proliferation. Also, the *STAT5* gene, which is involved in downstream signal transduction of prolactin, is a regulator of *OLFML1*gene. Our preliminary work on epigenomic analysis in hyperprolactinemia demonstrated significantly enriched MHC-related protein classes and cellular components. MHC classes of proteins are also involved in immune tolerance during pregnancy. The prolactin hormone plays a role in reproduction and autoimmunity and regulates via epigenetic modifications. Thus, the increased prolactin during pregnancy might regulate MHC I expression by epigenetic alterations and maintain immune tolerance. Studies on methylation changes in pregnant women are expected to provide insight into the mechanisms involved in immune tolerance during pregnancy, a state of physiological hyperprolactinemia. This will help explain the mechanisms underlying disorders of immune tolerance during pregnancy. To receive the semi-allogenic fetus, the maternal immune system undergoes multiple processes of immune adaption.[196] The immune cell types and cytokine profiles change during different gestational stages. If this coordination is disrupted, it can lead to several pregnancy complications, such as recurrent pregnancy loss, preeclampsia, and intrauterine growth restriction.[197,198]

Endometriosis

Endometriosis is the presence of endometrial tissue outside the endometrial layer of the uterine cavity and is associated with pelvic pain and infertility. It is commonly found in the pelvis and ovary and

affects at least 10% of women at reproductive age and about 50% with infertility.[199] This often runs in families with two or more affected sibs.[200] Endometriosis is an inflammatory estrogen-dependent disorder.[201] It is characterized by inflammation, excessive production of estrogens, and progesterone resistance.[202] Others categorized this as a complex disease influenced by genetic, epigenetic, and environmental factors.[203] Endometriosis is also considered a neoplastic disease, more precisely, a benign metastatic disease closely linked to cancer but with limited malignant transformation, likely by systematic repression of the genes involved in the cell cycle and regulation of the HOX genes.[202] Although endometriosis is a benign disorder, the process by which endometrial cells attach and invade surfaces shares features of malignant neoplasm. Other features shared by endometriosis and cancer are the ability to evade apoptosis, the stem cell-like ability, and angiogenic potential.[204] Endometriotic cells have all the characteristics of neoplastic cells, viz., uncontrolled proliferation, invasion, and metastasis at the macro level, whereas aneuploidy, copy number variations (20q13.33 duplication), and mutations in epithelial component as well as epigenomic changes in stromal component at the molecular level and still not categorized as neoplastic condition, at least low grade locally invasive category.[205] High estradiol levels are observed in endometriotic tissue, one of the primary mechanisms through high stromal aromatase activity. Also, deficient methylation of the ERβ promoter results in overexpression of ERβ in endometriotic stromal cells, suppressed progesterone receptors, and increased cyclo-oxygenase-2 levels contributing to progesterone resistance and inflammation.[206] The only difference with most tumors with endometriosis is a severe inflammatory reaction (probably due to cyclical extravasation of blood, extravasated blood is highly irritant, leading to inflammation) which is suppressed in tumors. The genetic basis of endometriosis is supported by the experimental development of peritoneal endometriosis in mice through conditional activation of the K-ras oncogene in endometrial cells and deposited into the peritoneum.[207] Up-regulation of the antiapoptotic gene BCL-2 and genomic alteration in endometrial cells of affected women also supports the neoplastic/genetic basis of endometriosis.[208] Genomic alterations in endometriotic implants using aCGH have been described.[209,210] Furthermore, loss of heterozygosity and somatic mutation of the tumor suppressor gene *PTEN*, p53, *ARID1A, APC, KRAS,* etc are frequently documented in endometriosis.[211,212] Increasing evidence is coming on epigenetic dysregulation of endometriotic stromal cells in women with endometriosis.[213] DNA methylation of promoters of genes whose products are critical for normal endometrial progesterone response has been reported in endometriosis resulting in progesterone resistance.[214]

Early pregnancy losses

Fecundity in humans, in comparison to other mammals, is relatively low. This is due to an enormous frequency of abnormalities in oogenesis and spermatogenesis. About 30%-50% of oocytes and 8-16% of sperms are chromosomally abnormal.[215,216] This results in as high as 70% abnormal conception leading to pregnancy losses, either before, during, or after implantation.[217] Later on, in clinically recognized pregnancies, 15-20% of conceptuses are lost as spontaneous abortion.[218]

Early pregnancy loss can be caused by chromosomal & genetic errors, anatomical uterine defects, maternal autoimmune disorders, endocrine dysfunction (hypothyroidism, hyperprolactinemia, PCOS, etc), endometrial dysfunction (as with uterine fibroid, endometriosis, etc), and possibly alloimmune factors. However, genetic factors are the most common causes of pregnancy wastage.[219] Genetic abnormalities predisposing to early pregnancy loss are chromosomal abnormalities, copy number variations, single-gene mutations, and others. Consanguinity, which is common in various parts of the world, significantly affects reproductive outcomes due to an increase in inherited autosomal recessive disorders, particularly inborn errors of metabolism and congenital malformations.[220] The majority of early pregnancy wastage is caused by abnormalities of chromosome number, which is considered to be responsible for 50-60% of cases.[221] The prevalence of chromosomal or sub-chromosomal, including CNV, in spontaneous abortion using aCGH could be much higher than reported with conventional cytogenetics.[222,223] European Society for Human Reproduction and Embryology (ESHRE) guideline defines recurrent pregnancy loss (RPL) as the loss of two or more pregnancies, whereas the Royal College of Obstetricians and Gynecologists (RCOG) as at least three consecutive miscarriages before 24 weeks of gestation.[224,225] Frequency of abnormal karyotypes in products of conception from women with recurrent spontaneous abortion compared with women without recurrent spontaneous abortion using conventional cytogenetics is contradicting. Some reported no differences, whereas others reported as infrequent (8% to 18%) and decreased with the number of previous abortions.[226-228] Cytogenetic studies have reported a frequency of chromosomal

abnormalities as high as 60% to as low as 5% in recurrent spontaneous abortions.[227,229] With molecular cytogenomics technologies, more and more sub-chromosomal & segmental gains/losses (CNV) were detected in as many as 50% of cases.[228] Investigations of RPL products using the microarray technique (aCGH) provide a role of copy number variations (segmental aneuploidy) in more than 50% of cases.[230,231] Chromosome 16 and X abnormalities (aneuploidy or CNVs) were most frequently associated with RPL.[230] Duplications of 16q24.3 and 16p13.3 loci were observed more frequently in RPL.[230] CNVs of chromosome X, 22, 8, and 4 were also common.[230] Next-generation sequencing (NGS) using whole exome, whole genome, or gene panels is now used to identify cryptic balanced/ unbalanced chromosome rearrangements, copy number variations (CNVs), and pathogenic variants in couples with RPL as well as abortuses.[231-233] Genes linked with RPL are *DYNC2H1, ALOX15, KIF14, RYR1, GLE1*, etc, and these gene's functional pathways linked with cell division, cilia function, chemotaxis, protease activity, protein modification, immune response, etc.[231-233] Compound heterozygous mutations in DYNC2H1 and ALOX15 genes in miscarriages from families with RPL were reported by Qiao et al.[233] *DYNC2H1* plays a role in cilia biogenesis and is associated with fetal lethality. *ALOX15* is expressed in the placenta and is associated with placental function, oxidative stress response, inflammation, and angiogenesis. Another important gene in RPL, mainly with recurrent hydatidiform mole and to a lesser extent with RPL, particularly with missed abortions/anembryonic pregnancies, is *NLRP7*.[234] *NLRP7* gene mutations account for a recurrent hydatidiform mole in about 50% of cases.[235,236] *NLRP7* gene mutations result in defective oocyte development and embryo development, leading to RPL.[237,238] Rare non-synonymous variants (NSVs) or heterozygous mutations in *the NLRP7* gene may confer genetic susceptibility for RPL.[237] Other genes involved with RPL (hydatidiform mole, miscarriage, early developmental arrest, etc) are *KHDC3L, PADI6, NLRP2, NLRP5, TLE6, DNAH11, CCNO, etc.*[238-242] Some studies have implicated immunological abnormalities and thrombophilic aberration as possible mechanisms in RPL. Inherited antithrombin III or factor V or combinations deficiency due to homozygous or heterozygous mutations of *SERPINC1/FVL* (factor V Leiden) genes may be associated with the increased risk for miscarriage and fetal demise besides preeclampsia.[243,244]

The sex ratio in early spontaneous abortions is skewed towards females, which could be monosomy X, imprinted paternal X chromosome, or failure of X chromosome inactivation.[228,245,246] One of the possible mechanisms related to altered sex ratio in early pregnancy losses could relate to disturbed epigenetic processes (namely, X-inactivation or genomic imprinting) in early embryos. Some idiopathic RPL are related to aberrations of epigenetic genome reprogramming in the parent's gametes or early embryos.[247] Whether preimplantation sexing and male embryo transfer can prevent another abortion in alloimmune/idiopathic RPL or recurrent molar pregnancy is worth exploring.

Congenital malformation

Congenital malformations are structural defects due to abnormal embryonic or fetal development. It affects different organ systems and is etiologically heterogeneous. Congenital malformations could be multifactorial (both genetics and environmental contribution), genetic (chromosomal or copy number variations or monogenic), or predominantly environmental (teratogenic exposure). Environmental exposures cause about 3% of all birth defects, and an interaction between genes and the environment may cause another 25% or more. The remaining cases seem to be genetic/genomic etiology.[248,249] Congenital malformations are often repeated in a subsequent pregnancy. The incidence is expected to increase due to an increase in maternal age at conception and contamination of our food chain with toxic chemicals. The prevalence of major congenital malformation at birth varies between 1-5%.[250] It is estimated that each year 7.9 million children are born worldwide with major birth defects. Among them, at least 3.3 million dies before the age of 5 years, and 3.2 million survive with a disability.[251] Congenital malformation is a global problem, but its impact is more severe in low and middle-income countries, where the conditions for prevention, treatment, and rehabilitation are more complicated.[252] World Health Assembly stressed the importance of addressing birth defects and committed to reducing the load. This could be achieved through primary preventive measures (periconceptional multivitamins or folic acid treatment or rubella vaccination or carefully prescribing medication in early pregnancy, preimplantation genetic diagnosis, etc) or secondary preventive measures (prenatal detection and selective termination of affected fetus). First/early second-trimester non-invasive prenatal screening (for chromosomal/CNV abnormalities) and second trimester (18-20 weeks) fetal scan provides an opportunity for early detection of fetal malformations and elective termination of pregnancy. Level 2 ultrasound scan is an essential

tool in present-day obstetrics practice, and this helps in the early diagnosis of fetal malformations, although not all malformations are detectable. Routine ultrasound screening for fetal malformations is justifiable because they are relatively standard, and the overall detection rate in a tertiary care unit is over 60%.[253] The detection rate of fetal malformations can be improved by an efficient, systematic approach of fetal malformation scan using an integrative anomaly chart.[254] Now, prenatal fetal echocardiography, 3D ultrasonography, and ultrafast magnetic resonance imaging (MRI) are frequently used as an adjunct to ultrasound for the characterization of fetal malformations (cardiac defect, facial dysmorphology, open fetal defect, CNS defect, skeletal defect, genitourinary defect, etc). Prenatal detection of fetal malformations also helps healthcare providers plan for birth in the appropriate place to avail special postnatal/neonatal care for the malformed fetus, including diagnosis, management, and counseling.[255] The most challenging area in fetal malformation is an accurate diagnosis, proper counseling, and early prediction and prevention. For this, it is essential to have an etiologic diagnosis that is expected to be primarily genetic/genomic. However, the cause of malformation is unknown in the majority of cases.[255] Despite deciphering the Human Genome, challenges in the diagnosis & management of developmental defects remain the same. The challenges are understanding developmental defects' pathophysiology and resolute phenotype & genotype heterogeneity. Hence, it is essential to have detailed clinical information and suitable biological materials (biorepository) as initiators and later screening through high throughput omics platforms.[256] It is hoped that the data generated in this field in the coming years and later integrated into system biology will be of excellent service to answer underlying etiology, pathophysiology, early prediction, and prevention.

Genomic abnormalities are a significant cause of a large proportion of congenital malformations. The copy number variations (microdeletion/microduplication syndromes) are genomic disorders characterized by small and variable chromosomal hemizygous deletion/duplication (<5 mb) in which generally many genes are involved. It is primarily spontaneous (de novo). They are frequently associated with multiple congenital anomalies and developmental delays.[257,258] The phenotype is mainly due to an imbalance of genes in the critical interval. The common microdeletion/microduplication syndromes are DiGeorge/Velocardiofacial (22q11.2), Prader-Willi/ Angelman (15q11-13), William (7q11.23), Smith-Magenis (17p11.2), Hereditary Neuropathy with Liability to Pressure Palsy/Charcot–Marie–Tooth syndrome type 1A (17p11.2), Beckwith-Wiedemann syndrome (11p15), Potocki-Lupski syndrome (17p11.2), *etc.* FISH, MLPA, quantitative fluorescent polymerase chain reaction (QFPCR), aCGH/SNP microarray, and NGS are used for genetic diagnosis of CNVs disorders. G-banded karyotyping has limited resolution, hence being unable to detect genomic changes of less than 5 Mb and hence cannot be used to detect CNV disorders.[257,258] In addition, most rearrangements of the ends of the chromosomes (telomere or sub-telomere) are too small to be seen using conventional cytogenetic techniques. A DNA microarray (SNP microarray) identifies not only CNVs (microdeletions or microduplications) but also any unbalanced chromosomal abnormalities (trisomy, triploidy, partial aneuploidy, and mosaicism) as well as uniparental disomy disorders.[12] Microdeletion/ microduplication syndrome cases are frequently associated with second/more hits (deletion or duplication) elsewhere in the genome.[12] The DNA microarray (SNP microarray) is a superior technique and covers the whole genome hence should be used as the first step for evaluating multiple malformations. It is essential in clinically doubtful cases, which is often evident in the early weeks of life when dysmorphic features are challenging to recognize, in particular sick neonates on life support. The DNA microarray (SNP microarray) seems superior to NGS for CNV detection as bioinformatic pipelines for analyzing NGS data are still evolving. The DNA microarray provides the highest chance of making a diagnosis, sparing the patient unnecessary diagnostic testing from many places and saving crucial times.[12,259]

An etiologic diagnosis is essential to provide accurate reproductive counseling and, most importantly, prevention and targeted management in the future. Therefore, research is necessary for this field to identify the underlying etiology of congenital malformation. Research with families affected by several pregnancies is essential as these will likely provide positive genetic/genomic associations. Studying (morphologic, genomic, epigenomic, proteomic, etc) embryos from the period when most developmental defects arise (early embryo) will be an essential step towards understanding and perhaps eventually predicting and preventing fetal abnormality in the future. Malformed fetal repository (tissue, organ, blood, etc, from affected parents & unaffected family members), as well as clinical details of the family (at least 3 generation pedigree), is the first step

towards achieving this goal. When a fetal developmental defect is identified in pregnancy, the parents should be referred to a tertiary ultrasound unit for targeted assessment (level 2 ultrasound), and attempt must be tried to detect other anomalies and underlying etiology. Every step should be ensured for prenatal diagnosis from parental & fetal testing, even if it involves invasive testing. Then parents should be offered reproductive genetic counseling and encouraged to complete fetal autopsy and biorepositories in case of termination or perinatal death. Hence, understanding the reproductive genetics of major developmental disorders is essential for today's perinatal care specialists.[256] And finally, but most importantly, families with multiple affected should be referred to a genetic center to find out the underlying genetic/genomic cause which is most likely to contribute to a better future in this field.

Table 1 showing important genes implicated with DSD

Disorder/phenotype	Gene	Cytogenetics	Inheritance
46,XY sex reversal 1	*SRY*	Yp11.2	YL
46,XY sex reversal 2	*NR0B1*	Xp21.2	XL
46,XYsex reversal 3/POF7	*NR5A1*	9q33.3	AD
46,XY sex reversal 4/ gonadal dysgenesis and XY sex reversal	*DMRT1/DMRT2*	9p24.3	MDS
46,XY sex reversal 5	*CBX2*	17q25.3	AR
46,XY sex reversal 6	*MAP3K1*	5q11.2	AD
46,XY sex reversal 7	*DHH*	12q13.12	AR
46,XY sex reversal 8	*AKR1C2/AKR1C4*	10p15.1	AR
46,XY sex reversal 9	*ZFPM2*	8q23.1	AD
46,XY sex reversal 10	regulatory region (XYSR) -640 to -607 kb upstream of the *SOX9* gene	17q24	AD
Adrenal insufficiency, with 46,XY sex reversal	*CYP11A1*	15q24.1	AR
Palmoplantar hyperkeratosis and true hermaphroditism (SRY negative XX sex reversal)	*RSPO1*	1p34.3	AR
Testicular anomalies with or without congenital heart disease	*GATA4*	8p23.1	AD
Cytochrome B5, type A (microsomal)	*CYB5A*	18q22.3	AR
Mitogen-activated protein kinase kinase kinase 4	*MAP3K4*	*6q26*	?
Zinc finger protein, X-linked	*ZFX*	*Xp22.11*	XL
Lim homeobox gene 9	*LHX9*	*1q31.3*	
Denys-drash syndrome/ meacham syndrome	*WT1*	11p13	AD
Androgen insensitivity syndrome; AIS	*AR*	Xq12	XLR
Additional sex combs-like 1	*ASXL1*	20q11.21	AD
Smith-lemli-opitz syndrome	*DHCR7*	11q13.4	AR
Aarskog-scott syndrome	*FGD1*	Xp11.22	XLR
Persistent Mullerian duct syndrome, type II	*AMHR2*	12q13.13	AR
Persistent Mullerian duct syndrome, type I	*AMH*	19p13.3	AR

Lysine-specific demethylase 6B	*KDM6B*	*17p13.1*	
Steroidogenic acute regulatory protein	*STAR*	*8p11.23*	AR
Transformer 2, drosophila, homolog of, alpha	*TRA2A*	*7p15.3*	
Lysine-specific demethylase 3A	*KDM3A*	*2p11.2*	
Precocious puberty, male-limited	*LHCGR*	2p16.3	AD
46,XX sex reversal 1 (XX male, sry-positive)	*SRY*	Yp11.2	
46,XX sex reversal 2	heterozygous duplication or triplication of a 68-kb regulatory region (XXSR) -584 to -516 kb upstream of the *SOX9* gene	17q24.3-q25.1	AD
46,XX sex reversal 3	*SOX3* (SRY-BOX 3)	Xq27.1	XL
46,XX sex reversal 4	*NR5A1*	9q33.3	AD
Serkal syndrome/ Mullerian aplasia and hyperandrogenism	*WNT4*	1p36.12	AR/ AD
CYP17A1 (steroid 17-hydroxylase /17,20-lyase)	*CYP17A1*	10q24.32	AR
BPES type I (with premature ovarian failure)	*FOXL2*	3q22.3	AD
Mccune-albright syndrome	*GNAS1*	20q13.32	postzygotic somatic mutation
Gonadotropin-releasing hormone 1	*GNRH1*	8p21.2	AR
Estrogen receptor 2 (ovarian dysgenesis 8)	*ESR2*	14q23.2-q23.3	AD
Short stature homeobox	*SHOX*	Xp22.33	
Estrogen receptor 1	*ESR1*	6q25.1-q25.2	AR/AD
Genitopalatocardiac syndrome			
Ethanolamine kinase 2	*ETNK2*	*1q32.1*	
Doublesex- and mab3-related transcription factor A1	*DMRTA1*	*9p21.3*	
Ovo-like 1	*OVOL1*	*11q13.1*	
Ring Finger Protein 1	*RING1*	*6p21.32*	
Others	*CTNNBIP1, FGF9, etc*		
True Hermaphrodite (XX; SRY -ve)	*GJA1, GJB2, WNT4, RSPO1, etc*		

Differences or Disorders of Sex Development (DSD)

Differences or Disorder of Sex Development & Determination (DSD) is a developmental abnormality in which the determination and differentiation of chromosomal, gonadal, and phenotypic/anatomical sex are abnormal.[260] Chromosomal sex is determined at fertilization and influences the differentiation of the gonads. The differentiation of the gonads determines the development of internal and external genitalia, i.e., phenotypic/anatomic sex. Both male and female gonads and genitalia differentiate from the same structures along the urogenital ridge. About four weeks after fertilization, primordial germ cells migrate from the yolk sac to the urogenital ridge. The urogenital ridge is also a precursor for granulosa-theca or Sertoli - Leydig cells.[261] Gonadal development is regulated by the temporospatial expression of many different genes with critical dosage effects (**Table 1**), and disruption of this complex process can result in atypical sex development.[262] The indifferent gonads to differentiate into testes require the expression of

the *SRY, SOX9, SF1, WT1, GLI1, AMH, CYP17A1,* etc genes, and into ovaries require an expression of the *WNT4, RSPO1, FOXL2, LIN28, WNT2B*, *ETV5*, *etc* genes.[263-265] At about six weeks of embryonic life, the Mullerian ducts appear adjacent to the Wolffian ducts. Anti-Mullerian Hormone (AMH), secreted by Sertoli cells of the testis, promotes regression of Mullerian ducts, and testosterone, produced from Leydig cells of the testis, promotes differentiation of the Wolffian ducts. Genes involved in Müllerian duct and wolffian ducts development are *AMH, AMHR2, HOXA10, HOXA11, HOXA13, LHX1, TCF2, WNT4, WNT1, PAX2, PAX8, LIM1, EMX2, RAR, AR, IGF, EGF, FGF, etc.*[266-272] Testosterone is converted to dihydrotestosterone by the enzyme 5 alpha-reductase that masculinizes external genitalia. The degree of masculinization is determined by the amount of fetal androgen present and the ability of the tissues to respond to the androgens, i.e., androgen receptors. Defects in any part of this pathway will result in genital ambiguity such as under-virilization of an XY individual, virilization of an XX individual, or rarely gonadal differentiation. AMH controls regression of the Mullerian duct in males. Incomplete embryonic regression of the Mullerian ducts in males could result from inappropriate synthesis and action of AMH.[273] Mullerian ducts remnants are seen frequently in 46,XY DSD and 46,XY gonadal dysgenesis.[274,275] Androgen insensitivity syndrome (AIS) is a heterogeneous group of defects in the androgen receptor, resulting in varying degrees of defective masculinization in 46,XY individuals. Ambiguous genitalia in 46,XX females is caused by early antenatal exposure to androgen from fetal adrenals, fetal aromatase deficiency, maternal androgen-producing tumors, maternal exogenous androgen exposure, *WNT4* mutation, or as associations.[276-279] The most typical cause of 46,XX DSD is virilizing congenital adrenal hyperplasia (CAH) due to 21 hydroxylase deficiency.[280] CAHs are disorders of steroidogenesis that mainly involve 21-hydroxylase (*CYP21A2*) and 11-hydroxylase (*CYP11A1, CYP11B1, CUP11B2, etc*) deficiencies of the adrenal glands and 3-beta-hydroxysteroid dehydrogenase and P450-oxidoreductase of the gonads.[281] Here, the affected female is typically born with varying degrees of ambiguity of external genitalia, including occasional complete masculinization of the external genitalia and feminine internal genitalia.[276] Also, aromatase deficiency (*CYP19A1* gene), mainly involving gonads, causes partial virilization of 46,XX fetuses due to an altered testosterone/estrogen ratio.[281,282] Patients with ovotesticular DSD must have well-developed ovarian, and testicular tissues proved on histological examination. The chromosome analysis in ovotesticular DSD could reveal a karyotype of 46,XX/46,XY or 46,XY or 46,XX. Cases of gonadal dysgenesis present as female phenotype, normal to tall stature, bilateral dysgenetic gonads, sexual infantilism with primary amenorrhea, absent breast development, eunuchoid habitus, and a 46,XY karyotype.[283] The internal genital structures are female with bilateral fallopian tubes, a uterus, and a vagina (infantile). In some cases of 46,XY gonadal dysgenesis is associated with mutations in key players of testis-determination, i.e., *SRY, SOX9, MAP3K1, NR5A1*, etc.[284] Mutations of the *SRY* gene account for about 10–20% of cases. Mixed gonadal dysgenesis is when one gonad displays complete development (immature ovary or testis) and the other gonad is a streak. Most patients with this condition have testicular tissue on one side and a streak gonad on the other. Some of the variants of *NR5A1* are inherited in an autosomal dominant fashion, with incomplete penetrance and variable expressivity. If a fertile parent is heterozygous, they will pass the variant to 50% of their offspring; offspring who are XX are at risk for testicular or ovotesticular DSD.[285]

Genetic diagnosis is vital for appropriately managing a patient with DSD and counseling of patient/parents regarding recurrence, prevention (prenatal/preimplantation diagnosis), and screening of family members. Commonly involved genes are *AMH, AMHR2, AR, HSD17B3, HSD3B2, MAMLD1, NR5A1, SRD5A2, WT1,* etc.[286] This is very important in XY DSD where the diagnostic yield is low.[287] *MAMLD1* (mastermind-like domain containing 1) on the X chromosome is one of the causative genes for 46,XY DSD but the same variants are also often shared by unaffected individuals in the family.[288] Hence, it is proposed that *MAMLD1* variants lead to DSD in combination with other genetic (involved in DSD) abnormalities.[289-291] Moreover, finding from genomic screening through massive parallel sequencing (NGS) approaches indicate that many DSD phenotypes might be only explained by oligogenic inheritance rather than monogenic.[292] Oligogenic inheritance represents an intermediate between monogenic inheritance, in which a trait is determined by a single causative gene, and polygenic inheritance, in which many genes and often environmental factors influence a trait. A few genes cause oligogenic inheritance. Although many of these cases are monogenic, the expression of the phenotype is also influenced by genetic modifiers.[293] Our experience with NGS screening on 46,XY as well as 46,XX sex reversal adults indicate involvement of multiple modifiers with/without other pathogenic genes (manuscript

under preparation). The WES in one of 46,XY sex-reversed females (with low testosterone, estradiol, cortisol, SRY+, AZF+) detected *CYP11A1* (pathogenic variants) along with *NR5A1, AKR1C2, AKR1C4, ZFX, NR0B1, WT1*, etc modifiers. In contrast, in another 46,XY sex reversal females with virilization at puberty (with lower normal male range testosterone, low estradiol, high FSH, normal cortisol, SRY+, AZF+) detected *MAP3K1* (pathogenic variants) along with *NR5A1, AKR1C2, AKR1C4, ZFX, NR0B1, GATA4, DMRT1, SOX9, WT1*, etc modifier genes involvement (ongoing unpublished study). The mutation of *MAP3K1* gene is known with 46,XY DSD with complete gonadal dysgenesis.[294] The mutation of *the MAP3K1* gene may interfere with interaction with the *RHOA* gene and contributes to complete gonadal dysgenesis.[294] Finally, the interpretation of the genetic diagnosis requires an understanding of the wide range of conditions like monogenic, oligogenic, polygenic, CNVs, etc that are associated with DSD, its inheritance pattern, and utilization of genetic information for reproductive genetic counseling besides the prevention of the disorder in the family.

Reproductive Cancer

Cancer is a genomic disorder. The first evidence of genetic involvement in cancer came from identifying the Philadelphia chromosome in chronic myeloid leukemia.[295] Now, we can create neoplastic cells in the laboratory through the genetic manipulation of normal cells.[296] Ectopic expression of three genes viz., *hTERT,* simian virus 40 large-T oncoprotein & *H-RAS* can result in the tumorigenic conversion of normal human epithelial and fibroblast cells.[296] A critical conclusion from in vitro tumorigenic conversion is that there are not infinite molecular changes separating early cancer cells from normal cells but that tumor development is a finite process, requiring only three genes in the initial stage. However, in the advanced stage cell, genetic changes are chaotic. Neoplastic transformation is initiated by a genetic abnormality occurring during normal cell division. This initial event provides a platform for other genetic lesions to develop, and when the proper combination of genetic lesions accumulates in the cell, it becomes neoplastic. Neoplastic cells possess numerous genetic abnormalities such as chromosomal/ sub-chromosomal aneuploidy, polyploidy, rearrangements, deletions, duplication, amplifications, etc &/or genomic alterations such as mutations, small deletions & small insertions, etc &/or epigenomic alterations like promoter/euchromatin hypermethylation, heterochromatin deacetylation, etc. These genomic changes are multi steps processes resulting from failure in checkpoints of cell cycle regulation. The hallmark of cancer cells is genetic instability, which generates genetic diversity. These lead to proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, activating invasion, metastasis, and more prolonged survival. In the advanced stage of cancer, all tumor cells are different as their genetic alterations are unstable/dynamic. Identification and testing recurrent genetic abnormality in cancers can assist in diagnosis, prognosis, and selecting appropriate treatment (targeted therapy viz., a tyrosine kinase inhibitor for *ABR/BCL* fusion-positive chronic myeloid leukemia, *Her2/neu* receptor blocker/ trastuzumab in *Her2/neu* positive breast cancers, anti-C-*MET*-targeted therapy C-*MET* positive gastric carcinoma, etc).[297] Genomic changes should disappear (normal newer cells with the death of neoplastic cells) following the completion of successful treatment. Hence, genomic markers should be looked upon during treatment and the follow-up period to declare complete remission or relapse.[298] However, despite extensive information on cancer genomics, very little is progressed in cancer survival and is mainly due to late diagnosis as survival is primarily related to early detection (downstaging).[299]

Previous techniques, such as FISH or PCR provided limited information on cancer cells.[297,299,300] However, presently, global genomic approaches, viz., next-generation sequencing and DNA microarray provides complete information on genomic alterations (characterization of point mutations and structural alterations including copy number variations, aneuploidy, loss of heterozygosity, etc) in cancer cells. Advance-stage cancer cells may accumulate 100s of CNVs and millions of gene variants besides epigenetic changes, mainly in stromal cells, i.e., an infinite number of cellular genomic changes. With the disease progression, the accumulation of genetic changes increases in frequency, type, and size of the abnormalities. The complete genome sequences of many cancer types are obtained through the cancer genome project, providing us with a comprehensive view of cancer-related genomic changes. Cancer genome analysis will provide our understanding of cancer biology. It will likely prompt newer medical approaches, i.e., predictive (identifying at-risk individuals long before disease development) and preventive (reducing the likelihood of disease) medicine. Soon genetic profile of tumors will be characterized as well as rationalized personalized cancer therapy. However, we also realize that the high genomic variability & dynamic nature of tumor cells and

difficulties distinguishing driver mutations (confer growth advantage and metastasis) from passenger mutations (do not confer growth advantage) are severe obstacles to success against cancer. Targeting the driver mutations will impact tumor growth while targeting passenger mutations will have no effect. It is, therefore, crucial to differentiate driver from passenger mutations to clearly identify the targets of interest and avoid the administration of unnecessary, costly, and potentially toxic treatment. In addition, some passenger mutations may be deleterious to cancer cells; hence, targeting them will have harmful effects. Furthermore, mutations in non-coding regions of the genome (usually untested) also contribute to cancer development and progression and are likely to create additional complexity.[301]

Massive information on most cancers at genomic, epigenomic, proteomic, etc levels are available now in public databases. Integrating these data into meaningful manners will require enormous work in the coming years. For this, we need a convergence of system biology approaches.[302] This will allow our current reactive medical policy (treating when a patient is sick) to turn into a predictive, preventive & personalized medical approach (predicting, downstaging & early treatment) that will also be cost-effective. Cancer genomics will guide us to predict, prevent, early detect, and early manage, including pharmacogenomics, to prevent drug toxicity-related death arising from cancer chemotherapy or radiotherapy as part of personalized medicine. Even prevention can be achieved through preimplantation genetic diagnosis, for example, PGD for adenomatous polyposis coli (APC-gene-related colonic carcinoma prevention).[303]

Another area of reproductive genetics is reproductive cancers which need special attention as reproductive cancers are increasing (some could be due to excessive use of hormones in ART), appearing in significantly younger ages (in particular ovarian; 35% cases below 25 years), poor prognosis due to late diagnosis (ovarian epithelial cancer), following exposure (estrogen) in fetal life (testicular dysgenesis syndrome, adenocarcinoma uterus/vagina, etc) or adult life (hormone replacement therapy with breast/uterine cancer) and often familial (breast, ovary, endometrium). Germline mutations are responsible for familial cancer, viz., BRCA mutation in familial breast, uterine & ovarian cancer. In the current genome sequencing era, clinical cancer diagnosis is transforming.[304] This technology has enabled the discovery of genes implicated in cancer risk and created opportunities to develop more precise and early tumor detection, even at the preimplantation/prenatal stage. The growing possibility of cancer prediction, prevention, and early precision treatment seems to be a reality.

Reproductive Genetic Counselling

Reproductive counseling is professional assistance to an individual and/or a couple for the prediction, prevention, and personalized reproductive options. This includes the risk of recurrence of a disease (in particular genetic), how to prevent the disorder, and the alternatives. This also provides information on fertility preservation, assisted reproductive technology, and third-party reproduction. It requires knowledge of genetics & genomics of reproductive disorders, reproductive technologies to manage reproductive disorders, dangers of transmitting genetic disorders to gametes/offspring, and how to prevent transmissions of genetic diseases.[305] Understanding genetics and genomics is essential for reproductive specialists, particularly in the molecular medicine era with evolving high throughput genomic/omics technologies.[28,32,306] It is also necessary to know reproductive genetic counseling, particularly knowledge on risk and how to prevent genetic disorders in offspring.[305] This is essential as this will protect reproductive specialists from medico-legal consequences due to failure to avoid genetic disorders in offspring. As scientific knowledge and medicine advance, so do the public's expectations. Genetic and genomic testing is now commonplace in all specialties. Therefore, genetic counseling is progressively becoming integral to reproductive medicine practice.[28,305,307] Historically, at-risk couples were given information regarding their reproductive chances of producing an affected offspring. The couple then had the option of either taking the opportunity or not reproducing at all. The advent of preimplantation & prenatal diagnosis has allowed these couples to have unaffected offspring, even for familial cancers.[303] This has immensely increased the scope of genetic counseling, especially those requiring assisted reproductive technologies (ART), as these may increase the transmission of genetic disorders in offspring. Infertility is a high-risk situation for genetic abnormality in offspring. Hence all precautions should be discussed to prevent transmission. Reproductive genetic counseling is also essential to avoid future risks of medicolegal problems (lawsuits).

Reproductive genetic disorders could be chromosomal, monogenic (Mendelian inheritance) or polygenic, multifactorial (many genes interacting with environmental factors) or genomic disorders (copy number variations), or epigenomic disorders or mitochondrial.[32,306] Dominant monogenic disorder

is caused by one mutated gene and has a 50% chance of transmitting the mutated gene to offspring. The autosomal recessive monogenic disorder is caused by two mutated genes on two homologous chromosomes. Carriers have only one mutated gene (heterozygote) and are usually asymptomatic. When both parents are carriers, there is a 1 in 4 (25%) chance of having an affected child. A single mutated gene causes the X-linked dominant monogenic disorder on the X chromosome. Males and females are both affected. However, males are more severely affected than females. Offspring of either sex have a 50% chance of inheriting the disorder from affected females. The situation differs for affected males whose daughters will always inherit the gene and sons will never. The X-linked recessive disorder is caused by a single mutated gene located on the X chromosome in males. Males are affected, and females are usually carriers (asymptomatic) or mildly symptomatic. Female carriers have a 50% chance of transmitting the mutated gene to their offspring. Half of the sons will be affected, and half of the daughters will be carriers. Oligogenic disorders are caused by more than one gene, commonly a few genes. This is commonly seen with infertility as well as DSD. Polygenic/multifactorial disorders are caused by an interaction of many genes and many exogenous (environmental) factors. The inheritance pattern is complex and the risk of transmitting the disease is less than in monogenic disorders and difficult to predict recurrence risk in offspring. Genomic disorders are a group of diseases resulting from genomic rearrangements, such as insertions, deletions, duplications, inversions, etc. It is commonly evident with cancer, malformation, and early pregnancy loss, including the preimplantation stage.[8,11,12,228] The mechanisms by which rearrangements contribute to various phenotypes (microdeletion/ duplication syndromes, schizophrenia, autism, infertility, autoimmune disease, neurodegenerative disorders, cancer, etc) are diverse and include gene dosage alterations, gene disruption, gene fusion, position effects, mutations, etc.[308] Epigenetic changes can be inherited across cell divisions or generations and can have a profound effect on an individual's phenotype. Mitochondrial disorders are associated with defects in mitochondrial DNA. These defects can either be inherited from the mother (sperm do not contribute mitochondrial DNA at fertilization) or acquired through somatic mutation with age. Males do not transmit the disease, and all offspring of affected mothers will have the disease.

Genetic counseling is a communication process concerning the occurrence and the risks of the recurrence of genetic disorders within a family. This aims to provide the patient with a clear and comprehensive understanding, possible options and to facilitate rational decisions.[309] This should be non-directive & non-biased manner. However, this may be modified in exceptional circumstances and may be more proactive. The ideal genetic counseling should aim at the medical, psychological, and social events so that couples can make appropriate decisions. The non-directive decision-making may sometimes prove unattainable, and hence one may rely on shared decision-making. A genetic counselor should be sensitive to diagnosis, particularly infertility, as it may be associated with social stigma, inferiority complex, familial disharmony/ anxiety, and ethics of various treatment options.

Genetic counseling is always required when genetic risks are related to the cause of infertility. There are several options for genetic counseling for infertility, viz., avoiding having an affected child, having no children, having no genetic testing, having prenatal/preimplantation genetic diagnosis, or conception using donor gametes, or adoption. For prenatal diagnosis (PND)/ preimplantation genetic diagnosis (PGD) or use of donor gamete, genetic counseling should be provided by trained professionals. Counseling should begin with a thorough history, including both partners' medical, social, reproductive, and genetic histories. In situations where both partners are known to carry genetic defects (causing infertility), there can be a high chance of transmitting the disease to the child. In some countries, the law may govern these matters, but, in the absence of law, this type of conflict makes the doctor's role very difficult. In this situation, the future child's interests should take precedence over the interests of a couple. Indications of genetic counseling are the advanced age of the couple, a parent with a known genetic disorder/carrier (chromosomal, Yq microdeletion, cystic fibrosis, etc) before offering assisted reproduction, donor gamete use, etc. Pre-ART counseling is essential in the identification of risk factors, disease states & potential teratogens and prompts the elimination of teratogens as well as to discuss preventive measures through carrier screening, preimplantation screening, and prenatal screening. The patient's ethnicity, medical history, and genetic family history are crucial elements in this evaluation.

Preconception is the optimal time to review the importance of preventive measures for transmitting genetic disorders in offspring. It also provides the opportunity to address the risks associated with environmental hazards and medications and the general risk of a congenital anomaly or chromosomal abnormality associated with advanced parental age. Preconception counseling

clinic is progressively becoming an integral part of modern reproductive care. This should cover risks associated with advanced age, ethnicity, an individual with balanced chromosomal translocations, risk of fetal malformations related to drugs/ radiation exposure, etc. Y chromosome microdeletion analysis should routinely be offered to all men with severe oligozoospermia or azoospermia. If infertility is secondary to AZF microdeletion, all male offspring will inherit that micro deleted Y chromosome and experience infertility or sterility, whereas no female will have the defect. The couple may choose for PGD to have a normal daughter. However, suppose the couple elects to have an AZF micro-deleted son. In that case, they must be aware of accumulating knowledge/interventions that may help their son preserve or optimize any future fertility-related problem.

Similarly, cystic fibrosis transmembrane reductase (*CFTR*) mutations have implications for clinical infertility practice. When the male partner has congenital bilateral agenesis of vas deferens (CBAVD), it is also essential to test the female partner for CFTR mutations. Suppose she is also found to be a carrier. In that case, there must be very careful consideration about whether the couple wishes to proceed with ICSI using the husband's sperm as the chance of a baby with cystic fibrosis will be 25% if he is heterozygous or 50% if one partner is homozygous & another partner is heterozygous or have PGD to avoid an affected offspring. Careful genetic counseling should also be offered to female partners carrying FMR 1 permutation, as premutation predisposes to further expansion of the triplet repeat in the germ line. PGD/PND for fragile X syndrome should be offered in these cases.

ART procedures related to genetic counseling

There is an increased risk of transmitting genetic defects to the offspring from ART procedures. Genetic counseling should be a crucial step before the ART procedure. There is a fourfold increase in the incidence of sex chromosomal abnormalities, a threefold increase in the incidence of structural chromosomal defects, and a sixfold increase in the incidence of imprinting defects in babies conceived by ART/ICSI.[310,311] This may be due to retrieving epigenetically immature germ cells from the testes besides in vitro condition and mechanical insults. There has been concern about chromosomal, genetic, and developmental abnormalities in children born after ICSI. Available data so far has shown that there is a small but definite increased risk of chromosomal abnormality, in particular, sex chromosome abnormalities, major congenital malformations, etc.[312] Counselling processes should cover adverse effects of ART, viz., risk of a significant birth defect, epigenetic and imprinting disorders, etc.[313,314] Imprinted genes play critical roles in embryonic growth and later after birth behavior.[314] Epigenetic changes affect transcriptional activity and control developmental plasticity, including cell-type-specific gene expression.[315,316] Studies on animal models have established that environmental factors, such as ovulation induction, culture medium composition, and/or embryo manipulation, etc affect epigenome and impact the conceptus, including birth weight. Animal models, such as mice and cows, commonly suffer from the so-called large-offspring syndrome with ART.[317] Genomic imprinting usually occurs during gametogenesis, which may not be completed early in the round spermatid stage. Genomic imprinting is a process through which alleles of given genes are expressed in a parent-of-origin-specific manner. Genes that are subject to imprinting often play critical roles in embryonic development. In humans, several defects in imprinted genes are linked to syndromes such as Beckwith Wiedemann, Prader Willi, Angelman, and Silver Russell. Studies suggest a possible link between ART and genomic imprinting disorders.[318] Cryopreservation, a technique commonly used for gamete/embryo storage, also affects gene expression, telomere length, replication senescence, plasma/nuclear membranes, chromatin condensation, and chromosomal aneuploidy.[319,320] Gametes or gonad cryopreservation before cancer treatment should be investigated for possible hereditary etiology of cancer & risk of passing mutated genes leading to hereditary cancer in offspring. Similarly, when donors are chosen for oocyte or sperm donation, one should evaluate family history and appropriate genetic tests to prevent any transmission of genetic defect to the offspring.

Conclusion and future perspective

Genetic and genomic factors are greatly responsible for most reproductive disorders, and gradually becoming part of the modern medical management of reproductive disorders. With continued research using high throughput platforms on reproductive disorders, extensive information on genetic/genomic etiology is coming each day, including its complexity (oligogenic/ polygenic/multifactorial) with most diseases. However, the genetic association of many cases is yet to be identified. The massive information generated should guide reproductive counseling and be used to predict & prevent the disorder in the next generation. Advances in reproductive technology and reproductive genetics should be

used to manage (predict, prevent, and treat) reproductive disorders. Recent progress in *in vitro* gamete development initiated a new era of gametogenesis in a dish and personalized infertility treatment in the coming years. Rapid dissemination of information has affected daily reproductive care so much that understanding human reproductive genetics is essential for all reproductive specialists, in particular, to know the risks of a genetic disorder and how to prevent it. The ideal time to apply genetics & genomics should be from gametogenesis to the peri-conception period so that prediction and/or prevention (primary and/or secondary) is possible. This knowledge will protect reproductive specialists from medico-legal consequences following failure to prevent a genetic disorder in offspring. Once genomic screening technologies are in use, high-risk groups may be identified before the development of the disease, and appropriate measures may be started before the pathology is too late in the near future. Cases like Klinefelter syndrome, turner syndrome, Yq microdeletion, premature ovarian failure, etc where reproductive pathology manifests gradually after puberty, may benefit in the future through predictive genomic medicine practice (e.g., gonad/ gamete cryopreservation & later when required in vitro/in vivo gametogenesis). Reproductive counseling along with preventive measures like gonad/gamete cryopreservation & use later when required through *in vitro or in vivo* gametogenesis would be of much help to young cancer patients wanting a child in the future as their survival rate is increasing. With a better understanding of the underlying cause of most reproductive disorders and continued advances in genomics and epigenomics, it is likely to have personalized medicine to predict, prevent and manage reproductive disorders in coming years.

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