REVIEW

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Current Diagnostic Approaches in the Genetic Diagnosis of Disorders of Sex Development

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Abstract

Disorders of sex development (DSD) are a clinically and genetically highly heterogeneous group of congenital disorders. The most accurate and rapid diagnosis may be possible with a complementary multidisciplinary diagnostic approach, including comprehensive clinical, hormonal, and genetic investigations. Rapid and accurate diagnosis of DSD requires urgency in terms of gender selection and management of the case. Despite the genetic tests performed in current daily practice, the genetic cause is still not elucidated in a significant proportion of cases. Karyotype analysis can be used as a standard for sex chromosome identification. In addition, quantitative fluorescent-polymerase chain reaction or fluorescence *in situ* hybridization analysis can be used for faster and more cost-effective detection of the sex chromosome and *SRY* gene. Multiplex ligation-dependent probe amplification, single-gene sequence analysis, next-generation sequence analysis (NGSA), targeted NGSA, whole-exome sequencing, and whole-genome sequencing analyses can be performed according to preliminary diagnoses. Microarray analysis, including array comparative genomic hybridization and single nucleotide polymorphism array, should be performed in cases with syndromic findings and if no pathology is detected with other tests. In DSD cases, the use of optical genome mapping and techniques, which will probably be in daily practice in the near future, may be considered. In conclusion, the clinical and genetic diagnosis of DSD is difficult, and molecular genetic diagnosis is often not available. This has psychosocial and health implications for patients and their families. New genetic techniques, especially those targeting the whole genome, may provide a better understanding of DSD through the identification of little-known genetic causes. This review focuses on conventional genetic and next-generation genetic techniques used in the genetic diagnosis of DSD, as well as possible genetic diagnostic techniques and approaches that may be used in routine practice in the near future.

Keywords: Disorders of sex development, diagnostic approaches, genetic diagnosis

Introduction

Disorders of sex development (DSD) occur as a result of a disorder in one of the stages of sex development, especially in the first trimester due to incompatibility of chromosomes, gonads, or anatomical structure (1,2,3). DSDs are classified under three main headings: (i) sex chromosomal causes; (ii) 46, XY DSD; and (iii) 46, XX DSD (3,4). DSDs are observed in approximately 1 in 4,500-5,500 births and at least 50 different congenital urogenital differentiation anomalies have been defined to date (4,5). DSD is estimated to be more frequent in societies where consanguineous marriages are common. DSD is considered a medical, social, and forensic emergency in the neonatal and infancy periods because it involves many problems, especially in the first two years in affected cases, such as sexual identity development disorder, hormonal disorders, and psychosocial differences. Families ask questions just after birth about the clinical status and sex of the baby to the physicians who evaluate the newborn. Therefore, rapid, and accurate postnatal diagnosis of babies with DSD is very important (1,2,3,4,5).

DSDs are one of the most difficult groups of endocrine disorders to diagnose due to their genetic and clinical heterogeneity. Hence, a multidisciplinary approach is

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required from the very beginning of the evaluation of DSD patients, due to the highly complex molecular and hormonal etiological causes. Multiple genetic etiologies, ranging from missense single nucleotide variants (SNVs) to complete chromosome aneuploidies, have been demonstrated among the genetic causes of DSD. At least 75 genes have been associated with DSD in humans. With the genetic technologies developed in recent years, DSD cases can be diagnosed more quickly and accurately at lower costs (6,7,8).

Genetic Methods Used in the Diagnosis of DSD: Karyotype analysis and sex chromosome identification is the first step in the diagnostic approach to DSD. If sex chromosomal causes are identified by karyotype analysis, further analyses are not required. However, approximately 9% of cases have sex chromosome abnormalities, whereas higher-resolution molecular and molecular cytogenetic analyses are warranted in the majority of cases for genetic diagnosis (7,8,9). Cytogenetic and molecular diagnosis of biopsy tissues can rarely be performed when chromosomal anomalies, especially mosaicism, are considered.

Cytogenetic Investigation

Karyotype Analysis

Karyotype is the organization and classification of human chromosomes according to their size, shape, and banding patterns. In order to evaluate the organization and general morphology of human chromosomes by karyotype analysis, the cells to be used must be proliferated in culture. The most commonly used cells for karyotype analysis are white blood cells, particularly T lymphocytes. For cytogenetic analysis of these cells, "short-term culture", in which a peripheral blood sample is seeded in a tissue culture medium and then prepared to divide, is the most suitable method. After a few days, the dividing cells are arrested in mitosis using various chemicals that inhibit the mitotic cascade. In order to release the chromosomes, the cells are burst with a hypotonic solution added to the medium. Afterwards, they are made ready for staining by fixation and smearing (10). The work period is 1-2 weeks.

Karyotype analysis allows the detection of aneuploidy and/or mosaicism, and structural variants. In karyotype analysis, 20 metaphases are evaluated as standard in most laboratories. However, if necessary, especially if mosaic conditions are considered, up to 30-50 metaphases can be counted. In karyotyping with the standard G-banding, a total of approximately 400-550 bands can be visualized. This level of resolution allows the detection of deletions or duplications larger than approximately 5-10 Mb. The diagnostic efficiency in DSD is 15% and the majority of these are those with mosaic chromosome structure (6).

Molecular Cytogenetic Investigations

Fluorescence in situ Hybridization

The Fluorescence *in situ* hybridization (FISH) technique is a method that forms a bridge between molecular and cytogenetic examinations and is used for the detection of large and difficult to detect anomalies for molecular studies and small and undetectable anomalies for classical cytogenetic studies. It is used to investigate duplications larger than 500 kilobases (Kb) or deletions larger than 100 Kb. In this method, clones containing specific human DNA sequences, called "probes", are used to determine the presence or absence of the relevant region of the genome during metaphase or interphase. DNA probes can be prepared for an entire chromosome, only for a specific chromosome region or even only for gene-level targets. The probes are fluorescently labelled in different colors. They can be used to quickly determine the presence of abnormal chromosome numbers in clinical material or to detect chromosomal rearrangements. In FISH analysis, 100 or more metaphases allow 500 metaphases to be analyzed in an average-quality test. It provides more accurate information than karyotype analysis, especially in the detection and confirmation of structural and numerical chromosomal anomalies and mosaic conditions. Although FISH technology provides higher sensitivity and higher resolution than G-banding, it does not allow simultaneous analysis of all sex chromosomes and the whole genome (11).

Chromosomal Microarray

Copy number variations (CNVs) are one of the well-characterized causes of genetic diseases. Karyotype and microarray analyses are the gold standard methods for CNV detection. CNV is classically defined as changes greater than 1 Kb. The insertions, deletions, and duplications leading to CNVs are found throughout the genome in humans and affect approximately 12% of the human genome (12,13).

CNVs can occur at different frequencies in populations. When the frequency of CNV is more than 1%, it is called copy number polymorphism. In the general population, deletions are more common than duplications at rate of 2:1 (14). Deletions are more harmful than duplications because dosage-sensitive genes are unable to tolerate haploinsufficiency. CNVs containing more than one gene can have a wide spectrum of phenotypic effects due to the combined effect of different genes on a single phenotype or pleiotropic effects of a single gene on multiple phenotypes (6,7,13,14).

Chromosomal microarray is a molecular cytogenetic method that analyses CNVs in the DNA sequence across the whole genome. As a result of the combined use of molecular biological and robotic techniques, it is possible to perform gene expression analyses and genotyping of single nucleotide polymorphisms (SNPs) in cells with arrays obtained by adhering thousands of DNA fragments, each representing a specific gene on a glass matrix. The conventional cytogenetic methods frequently used in laboratories can detect chromosomal alterations of 5Mb (1 Mb = 1 million bases) or larger. With recent advances in chromosomal microarray analyses, the resolution limit of 5 Mb has decreased markedly (12).

Two frequently used types of microarray methods are array comparative genomic hybridization (aCGH) and single nucleotide polymorphism array (SNPa). CGH-based arrays measure the amount of genomic DNA. It compares the genomic DNA in a patient's sample with that in a normal control sample. Improvements in aCGH techniques have provided the opportunity to evaluate changes >1 Kb in size by comparing them with the reference genome (15). In SNPa, DNA probes derived from regions of a single base pair (BP) in the genome that show differences between individuals are used for CNV detection. It can determine the corresponding SNP genotype since each probe is present in SNPa (16). In addition, microarray method allows homozygosity mapping, detection of chimerism, uniparental disomies and inherited genetic identity, and diagnosis of polyploidy (15).

Array CGH and SNPa are methods that can detect submicroscopic genome imbalance and CNV as small as 10 Kb throughout the whole genome. In cases with normal karyotype analysis, especially syndromic DSD, aCGH and/or SNPa analysis should be performed in the absence of other known molecular causes. Microarray analysis offers a highly efficient and powerful whole genome screening opportunity instead of many diagnostic tests used in the identification of DSDs. Microarray method is also recommended as a first-line test, especially in syndromic cases with multiple congenital anomalies (6,7,17).

Molecular Genetic Investigations

Quantitative Fluorescent-polymerase Chain Reaction

Quantitative fluorescent-polymerase chain reaction (QF-PCR) is a method for the rapid identification of chromosomes 13, 18, and 21, each a major cause of numerical anoploidy in humans, as well as of the X and Y chromosomes and the *SRY* (Sex-Determining Region Y) gene by short tandem repeat analysis. The advantages of this method over other methods, including FISH, are that it is faster, more reliable, less costly, and requires less material. However, it should be kept in mind that especially mosaic conditions may not be detected by QF-PCR technique (18).

Multiplex Ligation-dependent Probe Amplification

Multiplex ligation-dependent probe amplification (MLPA) is a multiplex polymerase chain reaction-based technique that can detect dose changes for more than 50 regions in the genomic DNA or RNA sequence. It can distinguish even a SNV on the genome and is widely used in genetic laboratories as a simple, fast, low-cost, practical technique, unlike microarray and SNPa methods. MLPA can be used in the diagnosis of single gene diseases in which deletions/duplications are frequently seen as disease-causing changes (mutations), or in the diagnosis of diseases in which large deletions/duplications are suspected but appear normal after screening for SNVs. Array-CGH is another method that is suitable for detecting deletions and duplications. However, aCGH is an expensive method and MLPA should be preferred if deletion/duplication is being investigated in a known region or in a known gene(s). In addition to deletion/duplication analyses, it may provide preliminary information about the number of chromosomes and detect aneuploidies with probes arranged according to certain regions of the chromosomes. Limitations of this method are that it is not suitable for the detection of balanced translocations and point mutations, is vulnerable to contamination, and has lower resolution due to being targeted (usually single gene). MLPA is an analysis method designed to be limited to a single gene or gene groups. If more than one gene/gene groups are considered in the preliminary diagnosis, each gene needs to be analyzed separately, which leads to an increase in the analysis time and cost. Chromosomal microarray methods should be used when deletion/duplication of more than one gene/gene groups is considered (19,20).

Single Gene Sequence Analysis

DNA sequence analyses or sequencing methods are used to determine DNA primary (basic) structures. DNA sequence analysis has provided a lot of knowledge about genetic control mechanisms and gene structure. In order to understand the mechanisms related to the appearance and treatment processes of hereditary diseases, it is necessary to elucidate the gene regions that affect the disease under investigation. In this respect, DNA sequence analyses are the most important factor in determining the path to be followed at the beginning and during the course of the treatment process.

With conventional Sanger sequence analysis, short sequence reads (maximum 1000-1200 BP) can be performed and

each gene is analyzed individually in sequence according to the preliminary diagnosis. While this process provides highly reliable results, it is time consuming and costly. It should be kept in mind that gene dosage imbalances and large deletion and duplication mutations cannot be detected by Sanger sequence analysis. Therefore, Sanger sequence analysis is not a practical approach for routine use in cases where a high number of genes or large genes need to be analyzed. Large gene deletions and duplications constitute an important part of the molecular defect in DSD. For this reason, gene-specific MLPA, aCGH or SNPa analyses should be performed in cases where mutations cannot be detected by Sanger array analysis (6,7,8,9,21).

Next Generation Sequence Analysis

Next generation sequence analysis (NGSA) is based on enzymatically cutting DNA, creating a database with a large number of DNA fragments, and reproducing these DNA fragments. With parallel sequencing, millions of small DNA fragments are sequenced simultaneously, ensuring that each base in the genome is read more than once and variations can be detected more accurately. The main steps of the system can be summarized as follows: obtaining biological material to be studied; isolation of genomic DNA from the obtained biological materials; then selecting the target regions in the isolated DNA; creating a DNA database by cutting the DNA with an enzymatic reaction; reproducing the DNA fragments that make up the database; sequencing the DNA fragments; generation of raw data after sequencing; mapping against a reference sequence; identification and interpretation of possible changes; verification and segregation analysis by Sanger sequencing or NGS; identification of candidate pathogenic changes; and finally reporting of these data (22).

Targeted Next Generation Sequence Analysis Panels

With targeted NGSA panels, a large number of genes can be sequenced simultaneously in a shorter time for diseases with genetic heterogeneity in their etiology, such as DSD (23). The results can be more easily analyzed in a targeted NGSA than in clinical exome or genome analysis, as fewer variants will be identified. Thus, NGSA panels provide faster results compared to clinical exome and genome analyses. Due to genetically highly heterogeneous causes, targeted NGSA panels are very fast, highly successful and economical for molecular diagnosis in DSD cases (24).

Whole Exome Sequencing

Whole exome sequencing (WES) is a high-resolution technology with a relatively high diagnostic success rate that allows simultaneous analysis of the coding regions of more than 20,000 known genes in the human genome (25,26). WES is currently the most common technological approach used to analyze the protein-coding part of the human genome. Although WES covers only 1-1.5% of the human genome, even this small portion of the genome contains approximately 85% of the known mutations that cause disease. However, it can elucidate the genetic origin of diseases in only 25-40% of cases (27). This rate is higher than that obtained by more classical methods such as karyotype and chromosomal microarray (15-20%) (22). Although WES is a powerful diagnostic tool, it should be recognized that it is not the best diagnostic approach for all clinical indications and is the most important step in establishing the necessary relationships between clinical findings and the phenotype variants (28).

Whole Genome Sequencing

While single gene analyses, panel tests, and microarray analysis examine known variants in a previously identified gene, WES analysis only examines exon regions encoding functional proteins. In whole genome sequencing (WGS), all coded and non-coded regions of all genes in the human genome are sequenced. Thus, nucleotide changes that can cause genetically complex diseases can be fully analyzed. WGS enables the comprehensive identification of many variants simultaneously in a single gene analysis. Today, numerous clinical studies have revealed that non-coding sequence variants also play a critical role in the diagnosis of diseases. While 85% of information can be provided with WES, detailed information about the genome is provided by looking at non-coding variants, deletions, duplications, and CNVs encoded in "WGS" (27).

Clinical information and phenotypic characteristics of the patient are very important in the diagnostic success of WGS. If the clinical information is given in detail, it will be easier to find the relevant gene variant among thousands of genes. A very large variant database is also required for more successful phenotype-genotype matching and faster determination of the patient's diagnosis (29).

Diagnostic Approach for Disorders of Sex Development

Rapid and accurate diagnosis of DSD is urgent in terms of sex selection and management of the case in neonates. Incorrect and delayed diagnosis in the early period may cause serious and sometimes irreversible medical, anatomical, and psycho-social problems for the child and his/her family. The difficulty in diagnosis and the long duration of the diagnosis make the management of the case challenging for healthcare professionals and increase medical expenditures. For all these reasons, early, accurate, and rapid diagnosis is

very important in DSD cases. Currently, diagnosis of DSD cases takes a long time with classical hormonal and genetic analyses. One of the most important problems in the diagnosis of DSD is the high genetic heterogeneity (1,2,3,4). The first step in the diagnostic approach in DSD cases requires the determination of the patient's sex chromosome and the presence of the *SRY* gene. The gold standard test for sex chromosome determination is karyotype analysis. However, since the determination of sex chromosomes by karyotype analysis is quite time consuming, QF-PCR or FISH analysis can be used for rapid sex chromosome determination. FISH and QF-PCR analyses are also used to identify the presence of the *SRY* gene (3,4,6,7,9).

Following the determination of sex chromosomes and evaluation of the presence of the SRY gene, further molecular genetic analyses are required for the preliminary diagnosis based on clinical and hormonal findings. With the widely used Sanger sequence analysis, short sequence (maximum 1,000-1,200 BP) readings can be performed, and each gene is analyzed separately in sequence for preliminary diagnosis. This procedure is very time consuming and costly. In addition, large deletion and duplication mutations cannot be detected by Sanger sequence analysis. Large gene deletions and duplications constitute an important part of molecular defects in DSD. MLPA analysis specific to the relevant gene should be performed in patients who are thought to have a mutation in a specific gene according to the preliminary diagnosis but in whom mutation cannot be detected by Sanger sequence analysis (6,7,8,9).

Algorithms in current diagnostic flowcharts are often time-consuming, costly, and unsuitable to accurately and rapidly diagnose DSD, which is considered one of the endocrine urgencies.

NGSA is widely used in genetic research laboratories and clinical diagnostic centers. Whole genome, whole exome, or targeted gene analyses can be performed with NGSA. NGSA provides important advantages in the diagnosis of genetic heterogeneous diseases like DSD (30,31). Genes responsible for the etiology can be studied simultaneously with NGSA and thus, results can be obtained much more easily and in a shorter time compared to Sanger sequence analysis. It was shown by Özen et al. (24) that 45% of cases were diagnosed molecularly with a targeted NGSA gene panel in 46, XY DSD cases, the diagnostic time could be reduced to three days and the diagnostic cost was one-third of the conventional diagnostic approach. Despite all these advantages, the NGSA method has some limitations. Especially in cases where the reading depth is low, it may result in sequence errors and misalignment. It is not possible to detect large deletion or insertion mutations, triple nucleotide repeat regions, and some CNVs with NGSA due to short reads. This situation interferes with the holistic approach to the diagnosis of DSD with NGSA. Additional molecular analyses, such as MLPA or microarray analysis, are needed to demonstrate large deletions and duplications (30,31). Table 1 shows the characteristics of current and future genetic technologies used for the diagnosis of DSD (6).

The Approach to Be Followed in Genetic Diagnosis

Depending on the history, findings on physical examination, family history of DSD or reproductive problems, chromosomal sex, initial hormonal evaluation, presence of associated malformations, presence of functional testis or Müllerian structures, locally preferred or available genetic testing facilities, diagnostic pathways for the genetic diagnosis of DSD can be designed. The clinical and genetic diagnosis flowcharts for 46, XX DSD and 46, XY DSD are presented in Figures 1, 2.

In general, a preliminary diagnosis of DSD subgroup is first made by physical examination and hormonal evaluation, followed by sex chromosome identification. According to these preliminary diagnoses, targeted gene panels based on NGSA, WES and then WGS analyses can be performed. However, chromosomal microarray analysis should also be performed, especially in cases with syndromic findings and if no pathology is detected with other tests. In an infant with atypical external genital appearance, the presence of palpable gonads, the status of Müllerian structures, initial chromosome analyses, and hormonal evaluations may determine the genetic test to be selected according to the preliminary diagnosis. Currently, a multidisciplinary diagnostic approach is recommended from the beginning in genetically heterogeneous diseases like DSD.

Most genetic laboratories follow the American College of Medical Genetics and Genomics guidelines and use standard terminology ['pathogenic', 'likely pathogenic', 'variants of uncertain significance (VUS)', 'likely benign', and 'benign'] for the interpretation of variants obtained from sequence analysis of genes causing Mendelian inherited diseases.

Currently, the general recommendation is to report variants categorized as 'pathogenic', 'likely pathogenic', and 'VUS' in the gene(s) related to the patient phenotype (32). A multidisciplinary team consisting of pediatric endocrinology, genetics, and clinical biochemistry specialists is required in the evaluation of genetic results, especially "VUS" obtained from targeted gene panel and/or whole exome/genome sequencing and microarray technologies in cases related to DSD other than chromosome disorders. After the evaluation

Table 1. Characteristics and utilisation fields of genetic tests used in the diagnosis of DSD

Methodology	Operation time	Identifiable variants					Resolution	Diagnostic efficiency
		Aneuploidy and/or mosaicism	CNV	Encoded SNV	Uncoded SNV	Structural variant		
Clinically feasible methods								
Karyotype	1-4 weeks		X	X	X		> 5 Mb	15 % (mostly mosaics)
Interphase FISH (X, Y or SRY markers)	< 3 days		X	X	X	X	Inapplicable	Rapid gender decision
Microarray	2-3 weeks			X	X	X	< 50 Kb	15 %
Single-gene analysis or gene panel		X	X		X	X	SNV: 1 nucleotide indels: < 50 BP	Panel dependent
Whole exome sequencing		X	X		X	X	SNV: 1 nucleotide indels: < 50 BP	30-45 %
Whole genome sequencing		X	+ Requires verification			+ Requires verification	SNV: 1 nucleotide	At least 30-45 %
Preclinical, future methods								
Optical genome mapping				X	X		Structural variant: > 500 BP	?
"Long read" sequencing	?	X		X	X		Structural variant: 50 BP	?

BP: base pair, CNV: copy number variation, FISH: fluorescence *in situ* hybridization, Kb: kilobase, Mb: million bases, SNV: single nucleotide variant, SRY: sex-determining region Y, DSD: disorders of sex development

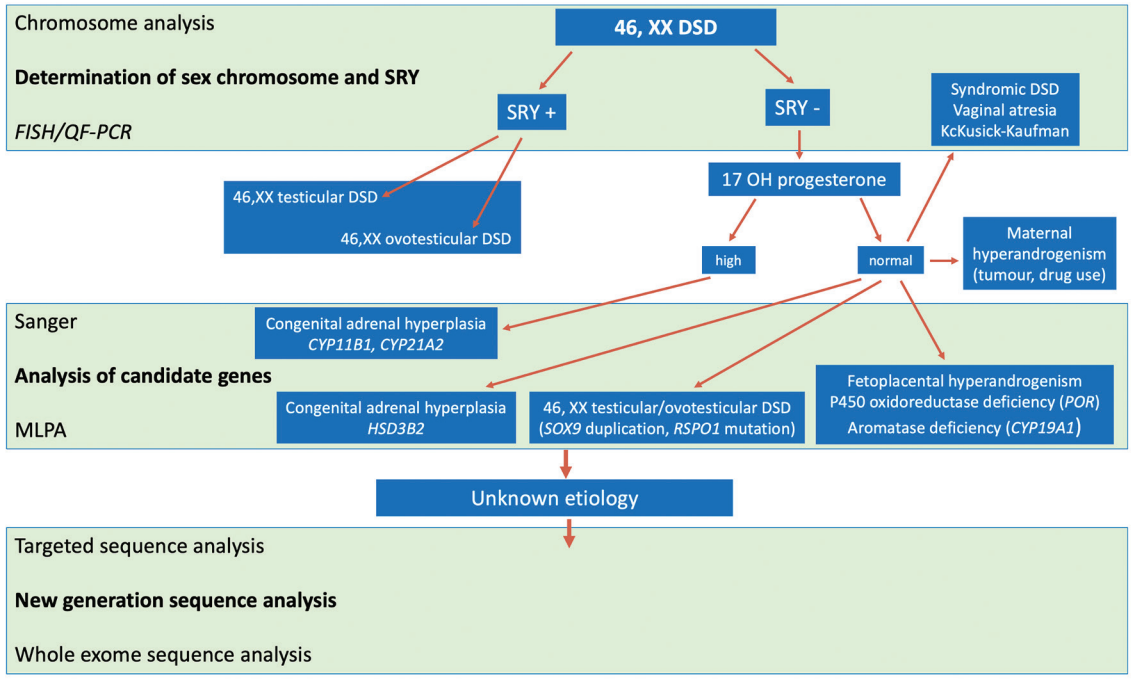

Figure 1. Genetic-based diagnostic approach pathway in 46 XX, DSD

CYP11B1: 11β-hydroxylase, CYP19A1: cytochrome P450 aromatase, CYP21A2: 21α-hydroxylase, DSD: disorders of sex development, FISH: fluorescence in situ hybridization, HSD3B2: 3-beta hydroxysteroid dehydrogenase 2, MLPA: multiplex ligation-dependent probe amplification, QF-PCR: quantitative fluorescent-polymerase chain reaction, RSPO1: R-spondin1, SOX9: SRY-box transcription factor 9

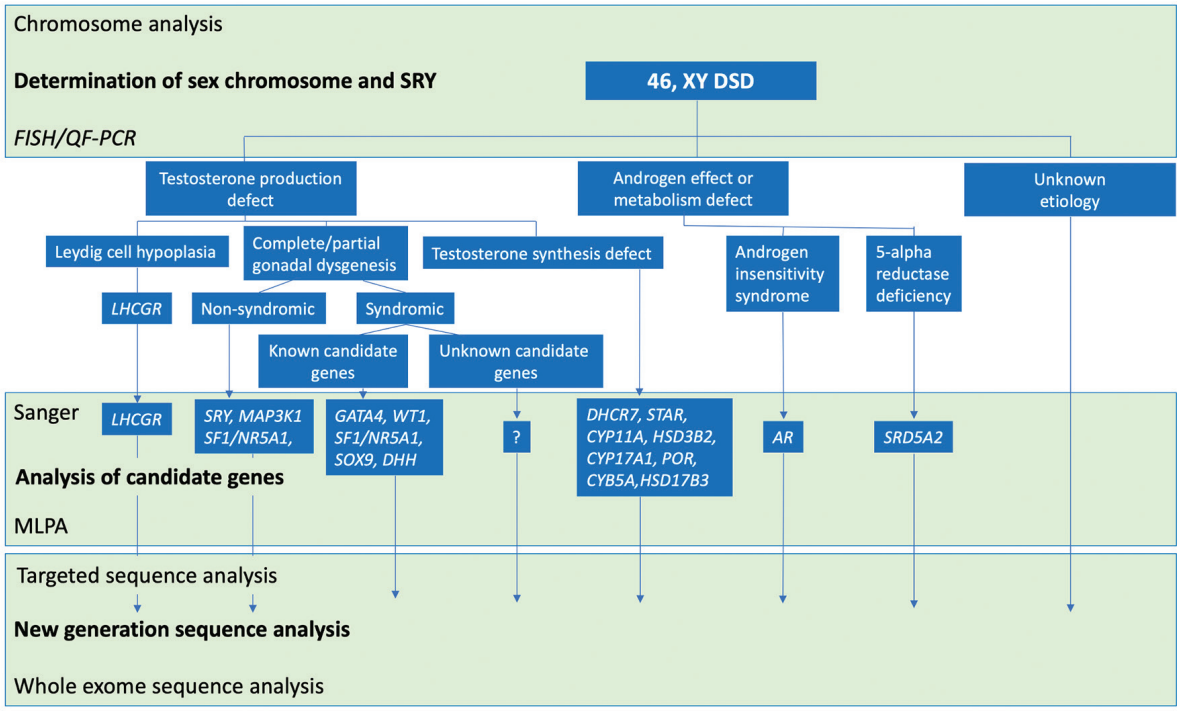

Figure 2. Genetic-based diagnostic approach pathway in 46 XY, DSD

AR: androgen receptor, CYB5A: cytochrome b5 type A, CYP11A1: P450 side-chain cleavage, CYP17A1: cytochrome P450, family 17, subfamily A, DHCR7: 7-dehydrocholesterol reductase, DHH: desert hedgehog signaling molecule, DSD: disorders of sex development, FISH: fluorescence in situ hybridization, GATA4: GATA binding protein 4, HSD17B3: 17β-hydroxysteroid dehydrogenase 3, HSD3B2: 3-beta hydroxysteroid dehydrogenase 2, LHCGR: luteinizing hormone/choriogonadotropin receptor, MAP3K1: mitogen-activated protein kinase 1, MLPA: multiplex ligation-dependent probe amplification POR: cytochrome P450 oxidoreductase, QF-PCR: quantitative fluorescent-polymerase chain reaction, SF1/NR5A1: steroidogenic factor 1, SOX9: SRY-box transcription factor 9, SRD5A2: steroid 5 alpha-reductase 2, STAR: steroidogenic acute regulatory protein, SRY: sex-determining region Y, WT1: WT1 transcription factor

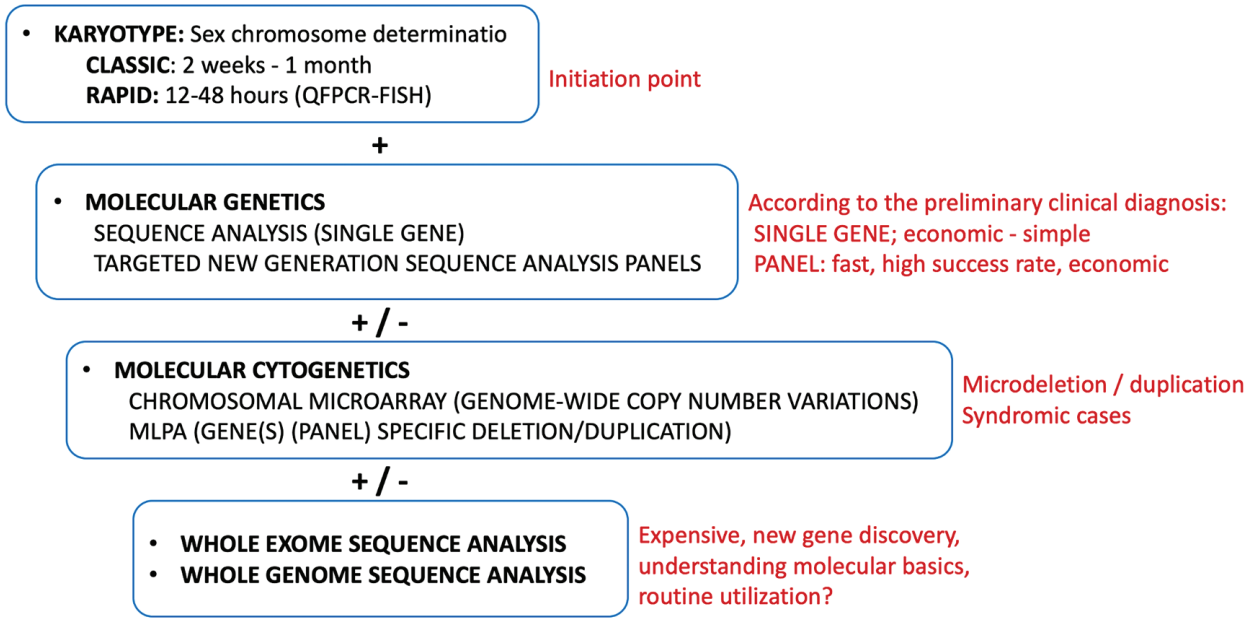

Figure 3. Characteristics of genetic tests used in the diagnosis of DSD

FISH: fluorescence in situ hybridization, QF-PCR: quantitative fluorescent-polymerase chain reaction, DSD: disorders of sex development

by the team, second-line endocrine tests and *in silico* and/or *in vitro* functional analyses should be planned to assess the association of any "VUS" with the disease.

Figure 3 shows the main characteristics of genetic tests used in the genetic diagnosis of DSD.

Conclusion

The first step in cases with suspected DSD is to determine the presence of sex chromosome and the *SRY* gene. Karyotype analysis can be used as a standard for sex chromosome detection. In addition, QF-PCR or FISH analysis can be used to detect the sex chromosome and *SRY* gene more rapidly and cost-effectively. However, it should be kept in mind that QF-PCR analysis cannot show mosaic conditions.

Microarray analysis (array-CGH and SNPa) showing high-resolution CNV throughout the WGS can be added to the first-line genetic tests, especially in DSDs with additional malformations, syndromic cases, and those in whom variants cannot be detected with other genetic tests.

MLPA can be used in the diagnosis of single gene diseases in which deletion/duplication is frequently seen as a disease-causing variation, or in the diagnosis of diseases in which a large deletion/duplication is suspected but was apparently negative after screening for SNVs. MLPA should be preferred if deletion/duplication is suspected in a gene(s) known to be associated with a DSD. Furthermore, in addition to deletion/duplication analyses, it may be possible to obtain preliminary information about the number of chromosomes and detect aneuploidies with probes arranged according to some regions of chromosomes.

In all newborns and small infants presenting with ambiguous external genitalia, potentially life-threatening acute adrenal insufficiency (e.g. forms of congenital adrenal hyperplasia such as 21-hydroxylase, 11ß-hydroxylase or 3ß-hydroxysteroid dehydrogenase deficiencies) should be urgently excluded. For this purpose, if a preliminary diagnosis can be established by first-line hormonal analyses and steroid profile measurement in urine and/or plasma together with history and clinical findings, Sanger sequence analysis can be promptly performed. In addition, targeted gene panels or WES analyses can be performed in these cases according to the clinical preliminary diagnosis and the facilities of the local genetic laboratory. In all other cases, clinical phenotyping, biochemical/hormonal analyses, and genetic tests should be planned simultaneously, through a multidisciplinary team. To confirm the cause of monogenic familial DSD, a simple and cost-effective gene and variant-specific Sanger sequence analysis can be performed. Moreover, a targeted gene panel of suspect genes or WES should preferably be used to analyze candidate genes in DSD cases with a highly heterogeneous genetic cause. WES is currently used for the investigation of new DSD genes, in cases where an oligogenic/polygenic basis of DSD is suspected, and for further research.

Despite the genetic tests performed in current daily practice, the genetic cause is still not elucidated in a significant proportion of cases. In these cases, the use of optical genome mapping and techniques, which will probably be in daily practice in the near future, may be considered.

Footnotes

Authorship Contributions

Design: Deniz Özalp Kızılay, Samim Özen, Literature Search: Deniz Özalp Kızılay, Samim Özen, Writing: Deniz Özalp Kızılay, Samim Özen.

Conflict of Interest: One author of this article, Samim Özen, is a member of the Editorial Board of the Journal of Clinical Research in Pediatric Endocrinology. However, he did not involved in any stage of the editorial decision of the manuscript. The editors who evaluated this manuscript are from different institutions. The other author declared no conflict of interest.

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