Genetic testing in pediatric endocrine pathology

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Abstract

In genetic endocrine diseases, genetic testing is necessary for a precise diagnosis, which will provide a better knowledge of the evolution and prognosis and also indicate the adequate therapy, targeting the precise etiopathogenesis of the disease. Genetic testing in endocrinology is often based on classical cytogenetic techniques, molecular cytogenetic analysis or molecular biology techniques. Genetic testing in disorders of sex development includes the karyotype and SRY gene analysis and depending on the presence of associated clinical signs and on the observations at paraclinical examination, these tests will be followed by chromosomal array techniques and NGS sequencing. In short stature, the decision to perform a genetic test is taken depending on clinical, paraclinical and imaging signs. In case of a short stature associated with a low weight/length for gestational age, genetic testing is proposed to evaluate a Russell-Silver syndrome or if the short stature is associated with other clinical signs (e.g. intellectual disability), chromosomal analysis by microarray is proposed. If the short stature is disproportionate, it is indicated to perform a next generation sequencing (NGS) of a panel of genes involved in skeletal dysplasia. If an endocrine cause for short stature is observed at the hormonal evaluation, it is indicated to test a panel of genes involved in these pathways. In genetic obesity, depending on clinical signs associated to obesity, it will be a more targeted genetic testing. If obesity is associated with intellectual disability or other nonspecific neurological changes, a chromosomal analysis by microarray will be indicated. If monogenic obesity is suspected, NGS testing will be indicated (as genes panel or whole exome or genome analysis). Genetic testing in endocrine diseases brings an etiological diagnosis, but a favorable cost-benefit ratio derives from an adequate indication of these tests, generally proposed in expert centers for rare endocrine diseases.

Keywords: genetic testing, disorders of sex development, short stature, obesity

Introduction

Genetic endocrine diseases are represented by over 250 different clinical entities [1]. Genetic testing is usually recommended for a precise diagnosis of a patient with a suggestive clinical picture for this pathology. This diagnosis implies a better knowledge of the evolution and prognosis, an adequate therapy, targeting the precise etiopathogenesis of the disease. Genetic testing is useful not only for the affected patient, it also has an impact on the family, a precise etiology of a disease guiding to the research of the family members at risk of developing the same pathology, respecting some specific ethical norms [2,3]. It also involves an adequate evaluation, at the appropriate time, and in case of future descendants of these patients, by exposing the reproductive options, the correct evaluation by prenatal diagnostic techniques, thus allowing an adequate prophylaxis or treatment of these pathologies. In the case of certain endocrine genetic diseases, knowing the etiology of an index case, allows a presymptomatic diagnosis in other family members, thus proposing adequate prophylaxis, such as thyroidectomy in the case of carriers of pathogenic variants of *MEN2* gene.

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Table I. Genetic tests used in the evaluation of genetic endocrine disorders [2-5].

Genetic test	Evaluated DNA regions	Advantages	Limitation
Karyotype	Chromosomal abnormalities, numerical or structural, up to 5Mb	Genomic analysis Detection of balanced and unbalanced chromosomal abnormalities, of mosaicism	Less resolutive Up to 5Mb
FISH technique	Copy number variants detection (CNVs), up to 100 kb	Better resolution Mosaicism detection	Targeted assay
MLPA technique	CNVs up to 100Kb	Better resolution	Limited evaluation of genomic regions (max 50-60)
Chromosomal array techniques (single nucleotide polymorphism-SNP array or comparative genomic hybridization, CGH array)	CNVs up to 1 kb	Genomic analysis Good resolution Mosaicism detection Genotype evaluation (SNP array) Parental disomy evaluation (SNP array)	Does not allow to determine the balanced rearrangements
Whole genome imaging	Chromosomal abnormalities, up to 150 kb	Genomic analysis Good resolution Detection of balanced and unbalanced chromosomal abnormalities	Less resolutive than microarray techniques Fresh samples
PCR-derived techniques	Single nucleotide variants (SNVs) detection	Targeted, less expensive	Targeted testing of hotspot variants
Sanger sequencing	SNVs detection	Economic and robust techniques Applicable for monogenic diseases, with specific phenotype, lacking genetic heterogeneity	It is not applicable to pathologies with genetic heterogeneity not applicable for larger genes
NGS sequencing (genes panel, exome, genome)	SNVs, indels, CNVs detection	Allows the analysis of genomic regions, depending on the application	CNVs detection is difficult Incidental findings Variants of unknown significance (VUSs)
Methylation specific testing (MS-MLPA, array techniques, NGS)	Detects differentially methylated regions	can detect parental disomy, imprinting abnormalities	The various causes of aberrant methylation cannot always be highlighted

Types of genetic tests

Genetic testing in endocrinology is often based, depending on the clinical context, on classical cytogenetic techniques (karyotype) or molecular cytogenetic analysis (fluorescent *in situ* hybridisation technique - FISH, chromosomal array techniques, multiplex ligation-dependent probe amplification techniques - MLPA) and on molecular biology techniques (polymerase chain reaction techniques-PCR, Sanger sequencing, next generation sequencing techniques, NGS). These tests are described in table I.

Genetic testing in disorders of sex development and other reproductive disorders

The evocative clinical phenotype is most often represented by: abnormalities of the external genitalia in the newborn or infant (isolated clitoral hypertrophy, isolated posterior hypospadias, bilateral cryptorchidism / testicular ectopia, unilateral cryptorchidism / testicular ectopia and hypospadias or micropenis) or abnormalities of the external genitalia at puberty by virilization at the time of reactivation of the gonadotropic axis (in case of type 2 5-α reductase deficiency or ovotestis), puberty delay (in case of gonadal dysgenesis in Turner syndrome, Klinefelter syndrome or 45,X/46,XY) or primary amenorrhea (in case of complete androgen insensitivity syndrome) [6gonadal or anatomical sex is atypical. DSD 46,XX are mainly represented by congenital adrenal hyperplasia (HCS].

Paraclinical examinations should include: hormonal determinations (17hydroxy progesterone, dehydroepiandrosterone, delta4 androstenedione, testosterone, dihydrotestosterone, anti-Mullerian hormone), imaging morphological evaluation of the gonads and internal genitals (ultrasonography to detect Mullerian derivatives, pelvic MRI or sometimes, gonadal biopsy) genetics (karyotype and SRY gene analysis by PCR or FISH technique). Depending on the presence of associated clinical signs and paraclinical examination, it is decided to perform chromosomal array techniques (if there are associated clinical signs, such as intellectual disability) and

NGS sequencing (if a 21 hydroxylase deficiency has been ruled out and there are no chromosomal abnormalities or deletion of the *SRY* gene).

Genetic testing in congenital adrenal hyperplasia - 21 hydroxylase deficiency

The clinical phenotype in 21 hydroxylase deficiency is represented by salt wasting forms, simple virilizing forms or non-classical forms, with late onset.

Genetic testing is indicated if 17-hydroxy progesterone has high values, the upper threshold being set at 6 nmol/l, sometimes it is necessary to stimulate with synthetic ACTH with the subsequent determination of 17 hydroxy progesterone values (especially if 17-hydroxy progesterone is between 6 and 30 nmol/l) [7]. Genetic testing is based on Sanger sequencing of the *CYP21A2* gene and MLPA techniques for deletions / duplications detection.

Genetic testing in short stature

Growth is a complex multifactorial process, in which genetic (over 80% contribution) and environmental factors contribute [8]. More than 700 genes involved in the growth process are known, among them, one is the SHOX gene, with major effect, influencing the height by about 20 cm, the other genes, taken individually having a minor but additive effect, together significantly influencing the final height. Short stature is defined as a height below 2DS compared to the average.

The decision to perform certain genetic tests is taken depending on clinical, paraclinical and imaging signs [9]. Thus, in the case of a short stature associated with a low weight / length for gestational age, genetic testing is proposed to evaluate a Russell-Silver syndrome. If the short stature is associated with other clinical signs (e.g. intellectual disability), chromosomal analysis by microarray is proposed. If the short stature is disproportionate (associated with abnormal upper / lower segment ratio or a modified upper limb amplitude) it is indicated to perform an NGS sequencing of a panel of genes involved in skeletal dysplasia. If a certain type of skeletal dysplasia is evident on clinical examination, such as achondroplasia, targeted genetic testing is proposed, in this case by PCR techniques. If there are radiological skeletal changes suggestive of SHOX gene abnormalities, evaluation of this gene is indicated by classical sequencing and MLPA techniques. If an endocrine cause for short stature is observed at the hormonal evaluation, in this case proportionate and with bone age delay, it is indicated to test a panel of genes involved in these pathways (GH deficiency, GH resistance, thyroid or adrenal pathology).

Genetic testing in obesity

Genetic obesity is generally suggested when obesity is associated with short stature, skeletal abnormalities (brachymetacarpia/brachymetatarsia), hypogonadism and other endocrinopathies, hypotonia and eating disorders in infants, global developmental delay/intellectual disability, ophthalmologic abnormalities, hearing loss, language delay, epilepsy, behavioral disorders, dysmorphic syndrome, malformation of internal organs [10].

Depending on these morbid associations, a specific etiological diagnosis is sometimes specifically suggested, and genetic testing is targeted. Thus, in case of a patient with morbid obesity, in whom there is a history of hypotonia and eating disorders at infant age, targeted testing for Prader-Willi syndrome is indicated (methylation analysis). Obesity associated with short stature and IV-V brachymetatarsia may indicate type 1A pseudohypoparathyroidism. Obesity associated with other clinical signs (intellectual disability and other nonspecific neurological changes) indicates chromosomal testing by the SNP array for the detection of pathogenic CNVs [11] both in cases of syndromic obesity as well as in cases of isolated or syndromic DD/ID. However, more data are needed to further elucidate the link between the two. The aim of this pangenomic study was to use single nucleotide polymorphism (SNP). If this test does not allow a diagnosis, NGS testing of a genes panel is proposed, known to be involved in obesity or whole exome/genome testing, to highlight the aetiology of monogenic obesity.

”Genotype first” or ”Phenotype first”

Genetic testing follows the actual trend in genomic medicine, taking into account the existing technological possibilities, which allow the establishment of an accurate diagnosis, at lower costs. One of the biggest advances in recent years has been the high-throughput sequencing of a large genomic region, a panel of genes, the exome or even the genome, thus increasingly influencing medical thinking for a better precision in diagnosis. However, these analyses cannot replace the clinical evaluation part, this being necessary in the interpretation of genomic data. The phenotypic expression must always be well evaluated, by noting all the clinical signs observed in a patient, using a standardized clinical terminology (Human Phenotype Ontology-HPO terms), in order to obtain a deep phenotypic analysis. Genotypic evaluation is more often based on NGS analysis, the analysis of genomic data being made by bioinformatics study, then on a comparison with existing genomic data in various databases including data on population frequencies (eg. Exac, GnomAD, EVS, dbSNP), conservation scores (eg. GERP, phyloP), in silico prediction of genetic variants (e.g. MutationAssessor, MutationTaster, Polyphen-2, SIFT), genes (OMIM, RefSeq), splice-site prediction, clinical evidence (eg. ClinVar, UniProt), data from the literature (eg. HGMD). The interpretation of genomic sequencing results considers all these data present in several platforms, but also considers the patient’s

phenotype, which, if well described, contributes decisively to establishing the correct genomic diagnosis. We often find in the literature the terms of the diagnosis approach starting from the phenotype study, the so-called "phenotype first" approach or starting from the genotype, the so-called reverse genetics or "genotype first" approach. Both types of approaches are correct, the choice of one or the other depends on the clinical context, thus, in a situation where the clinical phenotype is very specific and allows a specific diagnosis, genetic testing is targeted and often involves choosing a less expensive testing technology (e.g. diagnosis of achondroplasia is based on PCR techniques). On the other hand, sometimes a patient presents a nonspecific phenotypic change, such as a disproportionate short stature, but without evocative signs, and in this situation, we prefer to test a panel of genes associated with skeletal dysplasia and so the approach is rather reverse genetics or "genotype first" approach.

In conclusion, genetic testing in endocrine diseases brings an etiological diagnosis, but a favorable cost-benefit ratio derives from an adequate indication of these tests, generally proposed in expert centers for rare endocrine diseases. Establishing an accurate diagnosis is indispensable for the understanding of a molecular mechanism for a disease, thus choosing a precision therapy.

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