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Genetic tests used in modern diagnosis of inborn errors of immunity and hematological diseases

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Abstract

High-throughput genetic testing plays a key role in the diagnosis of genetically determined diseases. Its application has significantly increased the diagnostic efficiency of many rare diseases, including inborn errors of immunity (IEI) and hematological diseases. Most of these patients are managed by pediatricians, immunologists, and hematologists. These specialists are often responsible for selecting the appropriate diagnostic strategy, interpreting the results, and making subsequent therapeutic decisions. In this context, cooperation with clinical geneticists is crucial.

This review is intended for physicians of various specialties involved in the care of patients with rare genetically determined immunological and hematological diseases.

The authors' intention was to provide an accessible presentation of genetic testing methods used in the diagnosis of IEI and congenital hematological diseases, together with guidance on selecting the most appropriate diagnostic approach. A diagnostic algorithm based on the patient's clinical phenotype is proposed, and key principles for interpreting genetic test results are discussed.

Key words: *next generation sequencing, inborn errors of immunity, microarray SNP, congenital hematological diseases.*

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Introduction

Modern diagnostics of genetically determined diseases, including inborn errors of immunity (IEI) and hematological disorders, increasingly rely on high-throughput techniques which detect both single nucleotide variants and copy number variations.

Sanger sequencing of selected genes is used to confirm variants identified by next-generation sequencing (NGS) and to study the segregation of identified variants among the patient's family members. It also enables identification of known pathogenic variants in diseases with very characteristic phenotypes.

Constitutional karyotyping is used to detect large chromosomal aberrations visible under a light microscope that produce distinctive clinical phenotypes (e.g., trisomy 21). However, it has limited utility in the diagnosis of IEI. Much more effective diagnostic tools are those capable of detecting smaller structural aberrations such as microdeletions and microduplications in the genome. If the phenotype is distinctive enough to suggest aberrations in specific genes, multiplex ligation-dependent probe amplification (MLPA) can be firstly used in diagnostics. In other situations, high-throughput techniques such as array comparative genomic hybridization (aCGH) and high-resolution single nucleotide polymorphism (SNP) arrays are more appropriate tools for detection of copy number variants [1].

In cases of suspected genetic disease, the best approach involves collaboration between physicians of various specialties and a clinical geneticist. In disorders associated

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with congenital malformations – often multiple – and/or dysmorphic features, patients are typically referred to clinical geneticists early in the disease course. A clinical geneticist prepares the diagnostic plan, selecting the type and sequence of molecular tests.

In contrast, in patients with IEI and hematological disorders who do not usually show congenital malformations and/or dysmorphia, decisions regarding diagnostic priorities and the use of specific genetic testing methods are made by clinical immunologists and hematologists. A major limitation in the selection of appropriate diagnostic methods is their costs and lack of adequate funding for genetic testing [2].

The aim of this publication is to briefly present an outline of the specifics of IEI and selected hematological diseases, methods used in modern genetic diagnostics of patients suspected of these conditions, as well as the principles of test selection and result interpretation. The manuscript was developed by a team of specialists in pediatrics, clinical immunology, pediatric hemato-oncology, clinical genetics, and laboratory medical genetics.

Rare diseases

There is no single, universally accepted definition of rare diseases. In Europe, a disease is considered rare if it affects fewer than 5 in 10,000 people; in the USA, the threshold is 8 in 10,000, and in Japan, 4 in 10,000 [3]. Approximately 75-80% of rare diseases are genetically determined. According to the Orphanet portal, around 8,000 rare diseases have currently been identified [4]. It is estimated that globally, about 300 million people live with a rare disease, accounting for approximately 6% of the world's population [3]. In Poland, the number of individuals affected is estimated to be between 2.5 and 3 million. Thanks to advances in medicine, approximately 50 new rare diseases are described each year.

Rare diseases occur across all areas of medicine. They may affect single organs or manifest as complex syndromes. Providing care for patients with rare diseases is one of the major challenges and priorities of modern medicine [5].

Inborn errors of immunity

Inborn errors of immunity represent a heterogeneous group of primary immunodeficiencies (PIDs) with a documented genetic basis. They are characterized by abnormalities in the functioning of the immune system, including impaired recognition and elimination of pathogens, as well as immune dysregulation. To date, more than 500 such disease entities have been described in the literature, and the list continues to grow, with several to a dozen new disorders being identified each year. The current report of the International Union of Immunological Societies (IUIS) includes 559 IEI [6, 7].

The first IEI described was epidermodysplasia verruciformis, reported in 1946, although for many years it was considered an infectious skin disease and not linked to congenital immune dysfunction [8].

X-linked agammaglobulinemia (XLA), described in 1952 by the military physician Ogden Bruton, is considered to be the first classical primary immunodeficiency, currently known as Bruton's agammaglobulinemia [9].

The current IUIS classification identifies 10 groups of IEIs. In Poland, as in other European countries, humoral immune deficiencies are the most prevalent. The clinical presentation of IEIs includes two major phenotypes: infectious and those resulting from immune dysregulation. The latter may manifest as autoimmune or autoinflammatory diseases, allergic conditions, non-malignant lymphoproliferation, and malignancies [10, 11].

One of the greatest challenges for patients with IEIs is delayed or missed diagnosis. It is estimated that in Europe, approximately 80% of patients with IEI remain undiagnosed [12].

Therefore, from a diagnostic perspective, two elements are crucial: effective selection of patients with a possible inborn error of immunity and ensuring access to diagnostic procedures that can confirm the initial diagnosis. A well-collected and interpreted family and personal history should allow physicians of any specialty to identify patients potentially affected by IEI. In practice, however, this is not straightforward [13].

Ten warning signs of primary immunodeficiency in children and adults, promoted for many years by the Jeffrey Model Foundation, focus primarily on identifying patients with a dominant infectious phenotype. For this reason, current warning signs have been expanded to include autoimmune manifestations, allergies, non-malignant lymphoproliferation, and malignancies [10, 11, 14].

Additionally, artificial intelligence (AI) algorithms may significantly improve diagnostic accuracy, although they require access to reliable electronic health data [15-18]. One of these is the early identification of individuals with possible IEI through the use of IT tools that aid physicians in clinical decision-making (clinical decision support systems – CDSS) [19]. Another tool is the Exomiser computer program, which combines patient phenotypes defined according to the Human Phenotype Ontology (HOF) with the results of genetic analysis. The final result is the identification of gene variants that may be responsible for the disease (variant prioritization) [20]. One of the most significant diagnostic challenges is the early identification of severe combined immunodeficiency (SCID). In the absence of a positive family history, the only effective strategy is newborn screening (NBS). In Poland, such screening was temporarily conducted as part of a pilot program in the West Pomeranian Voivodeship [21]. NBS is currently being implemented in Poland. It is being used nationwide as a pilot program

prior to its final implementation into the national screening program [22].

According to estimates, in 2024, more than 2,500,000 newborns in Europe were expected to undergo screening for SCID and severe T-cell lymphopenia [23]. Early diagnosis of IEI is critical for implementing optimal treatment, which determines the quality and longevity of life for many patients [24, 25]. This is particularly evident in SCID, where early treatment with allo-HCT, before 3 months of age, offers an 80% chance of complete recovery, with no recurrence for the rest of the patients' lives. With delayed diagnosis, this figure decreases significantly [26]. According to various data sources, the diagnostic delay for common variable immunodeficiency (CVID) in children in Poland averages 1.8 years, and approximately 6 years in adults, which is consistent with findings from other countries [27-30]. In the United States, the median time from symptom onset to IEI diagnosis is 4.7 years, which contributes to the occurrence of complications and a reduced quality of life for patients [31].

Congenital hematological disorders

Selected congenital hematological disorders are classified as rare diseases. They represent a heterogeneous group of conditions resulting from pathogenic variants in various genes, as well as from genomic imbalance. These diseases may present as congenital single-lineage or multilineage cytopenias, coagulation disorders, and pathological lymphoproliferation (both non-malignant and malignant). This publication focuses exclusively on hematological symptoms and diseases that may either be manifestations of IEI or represent independent hematological disorders. Coagulation factor deficiencies of plasma origin are not included, as they constitute a distinct clinical entity and are not typically considered as associated with IEI. Hematological disorders include thrombocytopenia and anemia resulting from structural or functional defects of blood cells. An exception is Wiskott-Aldrich syndrome. Some congenital bone marrow failure syndromes are also classified as hematological diseases, for example Diamond-Blackfan anemia. Congenital neutropenia and bone marrow failure syndromes presenting with neutropenia and/or lymphopenia are, by definition, categorized both as hematological diseases and as IEIs. Thrombocytopenias are more difficult to classify, as they may be symptoms of inborn immunodeficiencies or purely hematological conditions. Immune-mediated multilineage cytopenias (such as Evans syndrome) are considered warning signs of IEI, although a genetic basis of these diseases is not always identified. Congenital non-malignant and malignant lymphoproliferative disorders typically result from immune dysregulation and are categorized as manifestations of IEI. Differentiating symptoms caused by other mechanisms, often associated with either hematological or immunological diseases, can be challenging. An example is lymphoid organ enlargement in certain storage diseases [32].

A key warning sign for IEI is the coexistence of cytopenia with severe infections and/or signs of lymphoproliferation. These patients are usually referred to hematologists, who should consider the possibility of IEI during the diagnostic process [33, 34].

The role of genetic testing in the diagnosis of IEI and congenital hematological disorders

The diagnostic workup of IEI and congenital hematological disorders may involve various genetic testing methods, including Sanger sequencing, NGS such as targeted gene panels (targeted NGS – tNGS), or whole exome sequencing (WES). Additionally, array-based technologies such as aCGH and single nucleotide polymorphism arrays (SNP-array) are used. Other techniques, such as MLPA or classical cytogenetic methods, including constitutional karyotyping and fluorescence *in situ* hybridization (FISH), play a marginal role in the diagnosis of IEI.

Sanger sequencing

Until recently, Sanger sequencing was the primary method used for gene sequencing [35]. It allows for the identification of variants within a small gene region, typically spanning a few hundred nucleotides, in a single test. This method is currently employed in targeted studies when a specific variant is suspected to cause a highly defined phenotype [36]. Sanger sequencing is also used to confirm variants identified through NGS or to verify the inheritance pattern of a given variant within the patient's family [37]. A key application of this method is determining whether two heterozygous variants identified in a patient are in *cis* (on the same allele) or in *trans* (on opposite alleles) configurations. Only in the latter case can the diagnosis of an autosomal recessive disorder be established in the patient; in the former case, the individual is considered a carrier of the variant [38].

Next-generation sequencing

Next-generation sequencing is a diagnostic method that enables the simultaneous analysis of the structure of multiple genes [39]. While whole genome sequencing is technically possible, it is rarely used in clinical practice. For diagnostic purposes, sequencing is typically performed on the coding regions of the genome, known as exons, and may include selected genes (tNGS) or the whole coding region (WES). WES analyses only about 1.5% of the entire genome, covering over 20,000 nuclear genes and the entire mitochondrial genome. However, it is important to note

that WES provides only a theoretical view of the whole exome. In practice, depending on the laboratory methodology, approximately 75-85% of exons are successfully sequenced. Certain genomic regions, including tandem repeats, pseudogenes, and splice sites, may be technically difficult or even impossible to sequence. Therefore, WES does not guarantee the identification of a genetic defect in every patient with a suspected genetic disorder. An alternative to WES is the use of targeted gene panels, which may include from a few to several thousand genes. In the diagnostics of IEI and congenital hematological disorders, NGS panels are selected based on the patient's phenotype or designed to include all known genes associated with the relevant disease group [40]. The advantage of targeted NGS is its high sensitivity, as the panels are typically designed to cover all relevant regions, including non-coding sequences that may be important for disease pathogenesis [41]. Additionally, tNGS studies typically yield a higher read depth from the sequenced genomic regions, which allows for more sensitive analysis, as well as the identification of potential mosaicism or detection of somatic variants resulting from clonal hematopoiesis associated with certain congenital bone marrow dysfunctions. It is also important to consider phenocopies of PIDs, which are caused by somatic mutations and require deep sequencing, a capability provided by tNGS [42]. A drawback of targeted panels is the lack of information about variants in genes not included in the panel. As a result, retesting may be required after a few years as new genes are discovered and knowledge about the pathogenesis of specific disorders evolves.

Constitutional karyotype

A constitutional karyotype is a cytogenetic test used to assess and analyze the number and structure of chromosomes within the chromosomal complement. When evaluating structural abnormalities, this method enables the detection of changes larger than 10 Mb visible under a light microscope.

As a result, its significance in modern genetic diagnostics is increasingly limited. In the diagnosis of IEI and hematological disorders, this method has very restricted applicability. The only clear indication is a highly characteristic patient phenotype, such as features suggestive of trisomy 21 (Down syndrome) [43].

FISH

Fluorescence *in situ* hybridization is a technique that enables the detection of specific genomic regions using DNA probes that are complementary to the target sequence. One of the advantages of this method is its ability to detect microdeletions (indicated by the absence of one signal) or microduplications (indicated by a triple signal). It should be noted, however, that when performing an analysis using FISH, the target region for probe hybridization must be precisely selected. In the diagnosis of IEI and congenital hematological disorders, the utility of this method is limited. For example, it can be used for the identification of a 22q11.2 deletion syndrome in a child with the classical phenotype of DiGeorge syndrome. Nevertheless, a similar phenotype may be seen in other genetic syndromes, and therefore more comprehensive diagnostic methods offer greater accuracy and effectiveness [44].

MLPA

Multiplex ligation-dependent probe amplification is a technique that allows for the simultaneous analysis of several dozen specific DNA fragments to detect microdeletions or microduplications. It is a targeted test and therefore can only be used when the patient presents with a specific and well-defined phenotype that may be associated with a limited number of microdeletion or microduplication variants in one or several genes. For example, a patient with a phenotype suggestive of DiGeorge syndrome can be more effectively diagnosed using MLPA than FISH, as it can detect not only the common 22q11.2 deletion but also other copy-number changes within the analyzed genomic regions [45].

aCGH

Array comparative genomic hybridization is a method that enables the detection of genomic imbalances across the entire human genome, specifically copy number variations (CNVs) such as microdeletions or microduplications ranging from approximately 100 to 200 kilobase pairs (kbp) in a single test. This technique is increasingly being used as a first-line diagnostic tool in the evaluation of children with developmental anomalies, autism spectrum disorders, as well as in prenatal diagnostics. It can also be useful in diagnosing immunodeficiencies in children who present with congenital anomaly syndromes or other associated health conditions, such as autism. Examples of disorders that can be detected using this method include the previously mentioned DiGeorge syndrome, 11q23 deletion (Jacobsen syndrome), 10p13-p14 deletion (DiGeorge syndrome type 2, DGS2), and 14q32 deletion (associated with antibody deficiencies) [46-48].

SNP array

A highly sensitive method for detecting CNVs, including deletions and duplications affecting individual exons, is the single nucleotide polymorphism array (SNP array). These high-resolution arrays not only allow for the identification of imbalanced abnormalities – copy number variations, including those affecting individual exons – but also, thanks to the presence of polymorphic probes (SNPs),

enable the detection of copy-neutral regions of loss of heterozygosity (LOH). This is particularly relevant in disorders involving genomic imprinting. In the diagnosis of IEI, high-resolution SNP arrays can identify intragenic deletions or duplications of single exons that would not be detected by lower resolution aCGH testing [49].

Despite its many advantages, the SNP-array technique also has drawbacks, such as its inability to detect balanced chromosomal alterations or low mosaicism (< 20%).

Emerging techniques in molecular diagnostics of IEI

In spite of the wide range of methods used in molecular diagnostics, a significant number of IEI patients still do not receive a genetic diagnosis. Therefore, modern solutions are being used, such as those enabling single-cell sequencing or focusing on long DNA fragments, primarily optical genome mapping (OGM) and long read sequencing (LRS). Optical genome mapping is a technique that allows for the detection of structural changes and complex rearrangements that cannot be detected with NGS or SNP arrays, such as balanced translocations or inversions. This can be achieved by comparing specifically tagged very long DNA fragments (100 kbp-1 Mbp) with reference sequences, enabling the identification of changes with a resolution of up to 500 bp [50]. This method is already widely used in the diagnosis of leukemia and other hematological malignancies, but it is becoming increasingly important in detecting the causes of IEI [49]. Another technique that offers new possibilities in IEI diagnosis is single-cell sequencing. Immune system cells form very diverse populations, so identifying their unique genetic profile can provide new information, crucial for understanding the genetic basis of the disease. Furthermore, this technique allows for the detection of mosaicism at a very low level, with the highest possible single-cell resolution, which makes it particularly useful in the diagnosis of phenocopies of IEI [51]. However, long-read sequencing is emerging as a highly promising tool in genetic diagnostics. Compared with conventional short-read sequencing approaches, such as WES and whole-genome sequencing (WGS), it generates substantially longer sequence reads, enabling more accurate analysis of genomic regions that are difficult to assess using short-read technologies. These include repetitive regions, tandem repeat expansions, and loci containing highly homologous pseudogenes, often referred to as genomic "dead zones" [52]. LRS enables comprehensive analysis of both coding and non-coding regions of the genome. In addition to detecting single-nucleotide variants (SNVs), it facilitates the identification of structural variants and, in some platforms, DNA methylation abnormalities [53].

Clinical presentation of the disease and the choice of diagnostic method

The clinical presentation of IEI and congenital hematological disorders is often non-specific. Therefore, establishing an accurate diagnosis cannot rely solely on medical history, physical examination, and standard laboratory tests. In many cases, genetic testing is the only way to reach a definitive diagnosis [54, 55].

It is crucial to plan the diagnostic process in a way that prioritizes the most effective and cost-efficient methods. When a patient presents with a highly characteristic phenotype strongly suggestive of a pathogenic variant in a specific gene, Sanger sequencing should be performed. An example of such a condition is Nijmegen syndrome, whose phenotype includes microcephaly, short stature, characteristic facial dysmorphic features, and combined immunodeficiency. Over 95% of patients with this syndrome harbor the same homozygous founder mutation in the *NBN* gene, characterized by a 5-nucleotide deletion at position c.657_661; hence, Sanger sequencing is considered the most appropriate method for its detection. Single-gene sequencing is also applicable in patients with a known genetic disorder identified in the family, or in parents of a patient with an identified variant/variants to determine the mode of inheritance – whether the variant is inherited or occurred *de novo* [56]. However, a clearly defined phenotype is rare in patients with IEI or congenital hematological disorders. Therefore, high-throughput methods are more commonly used. In patients presenting with immune deficiency or suspected congenital hematological disease without accompanying developmental anomalies, diagnostic evaluation typically begins with NGS-based testing, aiming to identify pathogenic single-nucleotide variants. Currently, this includes either targeted gene panels (tNGS) or WES [57]. If the genetic etiology of the disease cannot be determined or reveals a single pathogenic variant in an autosomal recessive disease, microarray analysis should be considered. In more complex cases – those involving immune and/or hematological symptoms alongside congenital anomalies – genomic imbalance is investigated first. If this yields no definitive result, or only a single pathogenic variant is identified in an autosomal recessive condition, NGS testing should follow. In congenital bone marrow failure syndromes, hematologic abnormalities are often accompanied by developmental anomalies. These disorders may be caused by point mutations, CNVs, or both. In such cases, diagnostics usually start with NGS, followed by microarray analysis if no diagnosis is achieved. A well-structured genetic diagnostic strategy currently allows for a definitive diagnosis in 25-50% of tested individuals. The percentage of patients with a genetic diagnosis of IEI depends on many factors, including the criteria for molecular testing eligibility (all patients with a recognized phenotype suggesting immunodeficiency, pa-

tients with a positive family history, and dysmorphic features). It is therefore not surprising that in one study, the range of positive IEI diagnoses ranged from 10% to 70% [58]. In another study, the average positive result from the analysis of 29 different cohorts was 42% [59]. Interesting results were presented in a Turkish study, in which the diagnosis rate was 41%. However, in this population, many patients were related to each other. For unrelated patients, the diagnosis rate was 25% [60].

It should be assumed that the diagnostic yield is higher when testing is strictly limited to well-selected patients (e.g., those with a positive family history or accompanying dysmorphic features or congenital anomalies). A less restrictive approach lowers the percentage of positive diagnoses but ultimately identifies a greater number of affected individuals by testing a broader population. The pursuit of higher diagnostic accuracy in genetically determined diseases is driving the adoption of increasingly advanced techniques in clinical practice, such as optical genome mapping and long read sequencing mentioned above [61, 62].

Figure 1 presents a proposed diagnostic algorithm based on family history and clinical phenotype.

The importance of genetic testing

A diagnosis of a genetically determined disease is crucial for the well-being of the patient, their family, and the healthcare system. It may shorten or end the patient's diagnostic odyssey, facilitate more targeted clinical management, and reduce the need for unnecessary investigations and ineffective treatments. It enables proper care planning for the patient and their family and provides access to genetic counseling. The diagnostic process tends to be shorter in patients with a proven genetic basis for their condition [29]. In the context of IEI and congenital hematological disorders, a genetic diagnosis helps iden-

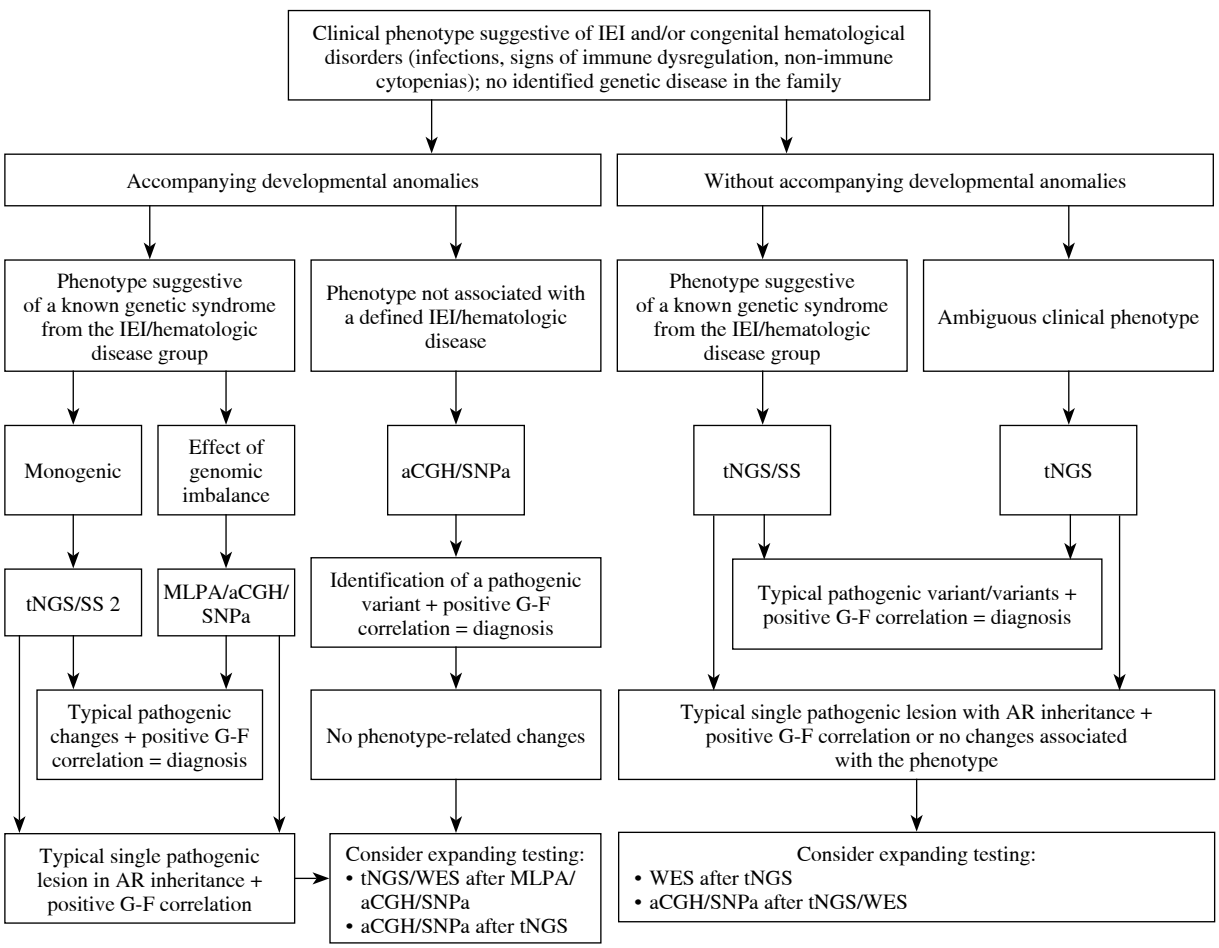

Fig. 1. Exemplary genetic testing algorithm for patients with suspected inborn errors of immunity (IEI) and/or congenital hematological disease

aCGH – array comparative genomic hybridization, AR – autosomal recessive inheritance, IEI – inborn errors of immunity, correlation G-F – genotype-phenotype correlation, MLPA – multiplex ligation-dependent probe amplification, SS – Sanger sequencing, SNPa – single nucleotide polymorphism array, tNGS – targeted next generation sequencing, WES – whole exome sequencing

tify patients who are eligible for hematopoietic stem cell transplantation (HSCT) [24, 63] – for example, in cases of SCID, chronic granulomatous disease, congenital hemophagocytic syndromes, or selected congenital bone marrow failure syndromes. It also enables the use of enzyme replacement therapies (e.g., SCID due to adenosine deaminase deficiency) and disease-modifying treatments, such as Janus kinase inhibitors for patients with gain-of-function *STAT1* variations, or abatacept for those with congenital *LRBA* or *CTLA4* deficiencies [64, 65]. The identification of chromosomal instability syndromes, such as Nijmegen breakage syndrome, ataxia-telangiectasia, or Fanconi anemia, prompts the implementation of radiation protection measures. Because nearly all IEIs are associated with an increased risk of early malignant transformation, every patient requires ongoing cancer surveillance for early detection of malignancies [66, 67].

Difficulties in genetic test interpretation

Interpreting genetic test results requires substantial expertise and experience. An incorrectly interpreted result can lead to a misdiagnosis, either diagnosing a condition that is not present or failing to diagnose a condition that truly exists. Accurate interpretation requires knowledge of the disease's inheritance pattern and the clinical significance of identified single-nucleotide variants and/or genomic imbalances. For single nucleotide variations, interpretation is generally straightforward when variants are clearly pathogenic, likely pathogenic, benign, or likely benign (i.e., clinically insignificant). However, approximately 50% of all identified variants fall into the category of variants of unknown significance (VUS). These are variants for which pathogenicity cannot be definitively determined, especially if they have not been previously reported in genetic databases and are *de novo* changes. If a rare variant is not inherited from either parent, the likelihood of its pathogenicity increases [68].

In the case of VUS, functional studies are often necessary to determine their impact. Unfortunately, access to such studies is limited for most genes and is often associated with high costs. Genetic test reports should include information on whether the detected variant affects a gene known to be involved in the pathogenesis of a disease or syndrome and specify the mode of inheritance. Such data can be retrieved from genomic databases. Identifying a single heterozygous variant in a gene associated with autosomal recessive inheritance does not confirm causality. Further diagnostics are needed to rule out the presence of other types of aberrations, such as microdeletion involving the second allele. This decision should be made by the ordering physician and/or a clinical geneticist after evaluating the phenotype–genotype correlation. If the patient's clinical picture matches the known consequences of a pathogenic or likely pathogenic variant, additional genetic testing is warranted. However, if the phenotype does not align with the identified variant's known effects, the variant should be considered a carrier finding only [54]. When two heterozygous variants are found in a patient, parental testing is necessary to determine whether they are located on the same chromosome (in *cis*) or on opposite homologous chromosomes (in *trans*). Only variants occurring in trans support a diagnosis of an autosomal recessive disorder [38].

In autosomal dominant disorders, a major challenge is variable expressivity and incomplete penetrance. This means that within the same family carrying the same variant, some individuals may be asymptomatic, while others are affected. Among affected individuals, symptoms may differ in type and severity. If a patient inherits a variant from an asymptomatic parent, this does not rule out the variant's pathogenicity. When laboratory findings support the clinical consequences of the variant, it strengthens the phenotype-genotype correlation. Unfortunately, confirming this often requires complex studies, including laboratory or animal models, which are difficult to perform in routine clinical practice [69].

Due to the complexity of correlating a patient's phenotype with their genetic findings, close collaboration between diagnosticians and clinicians is essential. Access to highly specialized functional assays, sometimes available only in select laboratories worldwide, is often required. International collaboration plays a key role in resolving these cases.

It is important to remember that the diagnosis of all rare diseases, including IEI, extends beyond defining the genetic basis of the disease. Changes at the level of RNA, proteins, and small molecules (metabolites) in cells, tissues, and organs are also important. Therefore, considerable research is currently focused on proteomics and metabolomics. Multi-omics technologies are also becoming increasingly common in transcriptomics. However, the clinical application of these technologies is currently significantly limited [70].

Table 1 presents examples of patients with clinical phenotype analysis, the correlation with their genetic test results, and conclusions regarding causality and further clinical decisions.

Summary

It is difficult to imagine modern diagnostics of genetically determined diseases without access to advanced genetic testing. It is essential to use these tools appropriately, selecting tests tailored to the patient's clinical needs, and ensuring accurate interpretation of the results. Establishing a precise diagnosis of a genetic disorder can bring the diagnostic odyssey to an end and enable the implementation of targeted medical interventions, including providing genetic counseling to the family. This leads to a significant

Table 1. Diagnostic decisions based on phenotype and genotype correlation – examples

Clinical phenotype	Genetic test	Genetic test result	Genotype/phenotype correlation	Diagnostic decision
A male pediatric patient with thrombocytopenia, decreased MPV, recurrent infections, atopic dermatitis Clinical diagnosis: suspected Wiskott-Aldrich syndrome	Immunology/hematology NGS panel including genes associated with IEI and thrombocytopenia – analysis for Wiskott-Aldrich syndrome	Pathogenic hemizygous variant in the *WAS* gene	Clinical and genetic phenotype consistent	Diagnosis of Wiskott-Aldrich syndrome Maternal testing for carrier status of the pathogenic variant recommended for genetic counseling
A male pediatric patient with recurrent respiratory tract infections, agammaglobulinemia, absence of B cells in lymphocyte subpopulation analysis, absence of BTK expression Clinical diagnosis: X-linked agammaglobulinemia	Immunology NGS panel including genes associated with IEI – analysis focused on agammaglobulinemia-related genes	Rare hemizygous variant of unknown significance (VUS) in the *BTK* gene, previously unreported	The clinical phenotype clearly indicates X-linked agammaglobulinemia The described variant should be considered pathogenic – confirmed by functional testing – absence of *BTK* expression	The described variant should be considered pathogenic – confirmation *via* functional testing – absence of *BTK* expression. Diagnosis of X-linked agammaglobulinemia (XLA) Maternal testing for variant carrier status recommended for genetic counseling Reporting of identified *BTK* gene variant associated with XLA phenotype
14-year-old male patient with symptoms of immune dysregulation (lymphadenopathy, splenomegaly, autoimmune cytopenias), hypogammaglobulinemia	Immunology NGS panel including genes associated with IEI – analysis focused on immune dysregulation syndromes and hypogammaglobulinemia	Potentially pathogenic heterozygous variant in the *TNFRSF13B* gene encoding the TACI protein	Clinical phenotype correlates with genotype, but the same variant was found in the asymptomatic father	Diagnosis of common variable immunodeficiency (CVID) based on potentially pathogenic *TACI* variant possible Incomplete gene penetrance means the father may currently be asymptomatic Immunological care recommended for asymptomatic father
13-year-old female patient diagnosed with cancer (ALL) Family history: multiple family members on the mother's side with cancer	NGS panel for cancer predisposition	Two pathogenic heterozygous variants in the *ATM* gene, described in patients with ataxia-telangiectasia	Phenotype does not correlate with genotype – no signs of ataxia telangiectasia. Literature reports patients with late-onset symptoms	Parental testing: both variants inherited from the mother – located on one allele No basis for diagnosis of AT Possible genetic predisposition to cancer in a carrier of pathogenic *ATM* variants
4-year-old male patient with recurrent lower respiratory tract infections, hypogammaglobulinemia, no abnormalities in lymphocyte subpopulations	Immunology NGS panel including genes associated with IEI	Two heterozygous variants of unknown significance (VUS) in a gene associated with osteogenesis imperfecta	Child's phenotype does not correlate with genotype	No basis for diagnosis of osteogenesis imperfecta No basis for diagnosis of inborn error of immunity Further observation of the child – consider transient hypogammaglobulinemia of infancy If infectious symptoms progress or other clinical signs of IEI appear, consider extending genetic diagnostics

Table 1. Cont.

Clinical phenotype	Genetic test	Genetic test result	Genotype/phenotype correlation	Diagnostic decision
2-year-old female patient with positive family history: mother with chronic splenomegaly and childhood episode of thrombocytopenia; child presents with splenomegaly, thrombocytopenia, autoimmune hemolytic anemia, neutropenia, elevated vitamin B12, and hypergammaglobulinemia Clinical diagnosis: autoimmune lymphoproliferative syndrome (ALPS)	Immunology NGS panel including genes associated with IEI	Pathogenic heterozygous variant in the *FAS* gene	Clinical phenotype correlates with genotype	Diagnosis of autoimmune lymphoproliferative syndrome Child to be placed under specialist immunological care, treatment with mTOR inhibitor Sanger testing of the mother for the *FAS* variant found in the child – confirmation and diagnosis of ALPS – referral for long-term adult immunological care
Male patient, 3 months old, cow's milk protein allergy, thrombocytopenia, active CMV infection, lymphopenia, neutropenia; SCID T-B-NK+ phenotype in subpopulation analysis	Immunology NGS panel including genes associated with IEI	Heterozygous potentially pathogenic variant in *CARD11* gene associated with autosomal dominant IEI Heterozygous VUS in LRBA gene Heterozygous VUS in POLD1 gene	Clinical phenotype correlates with variant in *CARD11* gene with AD inheritance pattern Single heterozygous VUS in *LRBA* gene does not support IEI diagnosis on this basis VUS in *POLD1* gene not related to phenotype	Diagnosis of SCID T-B-NK+ based on potentially pathogenic variant in *CARD11* gene The child was deemed eligible for allo-HCT

improvement in the quality of life for both the patient and their family. Moreover, it is of great importance from the perspective of healthcare system organization. Early diagnosis of a genetic disorder can be – and often is – more cost-effective for the healthcare system. These potential benefits should be considered when planning and funding genetic diagnostic services in Poland [2, 13].

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