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Review article

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Inborn errors of immunity: an expanding universe of disease and genetic architecture

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Abstract

Inborn errors of immunity (IEIs) are generally considered to be rare monogenic disorders of the immune system that cause immunodeficiency, autoinflammation, autoimmunity, allergy and/or cancer. Here, we discuss evidence that IEIs need not be rare disorders or exclusively affect the immune system. Namely, an increasing number of patients with IEIs present with severe dysregulations of the central nervous, digestive, renal or pulmonary systems. Current challenges in the diagnosis of IEIs that result from the segregated practice of specialized medicine could thus be mitigated, in part, by immunogenetic approaches. Starting with a brief historical overview of IEIs, we then discuss the technological advances that are facilitating the immunogenetic study of IEIs, progress in understanding disease penetrance in IEIs, the expanding universe of IEIs affecting distal organ systems and the future of genetic, biochemical and medical discoveries in this field.

Sections

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Introduction

Inborn errors of immunity (IEIs) are monogenic disorders of the immune system causing immunodeficiency, autoinflammation, autoimmunity, allergy and neoplasms. IEIs were traditionally considered to be rare diseases. However, new genetic defects underlying IEIs are being discovered at an unprecedented rate, and their associated clinical phenotypes are being ever more clearly defined, which is revealing the major health burden collectively posed by these diseases. The prevalence of IEIs is now estimated to be between 1 in 1,000 and 1 in 5,000 individuals[1]. However, these frequencies are probably an underestimate, and we speculate that the true prevalence may be as high as 1 in 500. Here, we consider four factors that may contribute to this underestimation of IEI prevalence, namely, the underutilization of next-generation sequencing (NGS); the ubiquitous but understudied concept of incomplete penetrance, which means that some mutation carriers have only partial disease or no disease at all; the increasing number of patients with IEIs presenting with clinical symptoms affecting distal organ systems (here defined as all organ systems excluding the blood and lymphatic system) but apparently not the immune system, who are therefore managed by neither immunologists nor geneticists; and the role of somatic mutations, which are probably the cause of many immune disorders with adult onset and about which our current knowledge is limited[2].

A detailed summary of all monogenic defects is comprehensively reported by the International Union of Immunological Societies (IUIS) Inborn Errors of Immunity Committee[3,4], and advances in gene therapy for IEIs have been well discussed in another recent review[5] and are not covered here. In this Review, we provide a short historical overview of IEIs and then discuss the current state of technological advancements in the field, followed by progress in our understanding of disease penetrance in IEIs and the expanding universe of IEIs, including those affecting distal organ systems. Finally, we consider the future avenues of scientific exploration for the coming decade.

Brief history of the discovery of IEIs

In this section, we briefly recall the events that led to the discovery of genetically determined immune disorders (Fig. 1) and highlight key technological innovations, before the advent of NGS, that enabled an understanding of the genetic and molecular basis of IEIs.

Early history

The notion of a relationship between genes and the immune system had already emerged in the first two decades of the twentieth century in the genetic theory of infectious disease, but this concept was not widely accepted at the time. In 1939, Karl Diehl and Otmar von Verschuer studied 205 pairs of twins in which one twin in each pair was a confirmed index case of tuberculosis[6]. They found that the probability of the twin of the index case developing manifest tuberculosis was 65% for monozygotic twins but 25% for dizygotic twins[6], which suggested that genetic factors could contribute to the tuberculosis risk. Indeed, we now know not only of genetic disorders that underlie tuberculosis in some patients[7] but also of many genetic variations that increase susceptibility to other infections, autoinflammation, severe allergy, autoimmunity and malignancy, which are collectively known as IEIs.

In 1952, Colonel Ogden Bruton, a paediatrician at the Walter Reed Army Hospital in the United States, saw an 8-year-old boy who had had 19 episodes of pneumonia over a period of 4 years[8]. Bruton suspected that any patient with such an extensive history of infections would have high antibody levels. However, to the surprise of both Bruton and his laboratory technician, who assumed their newly acquired electrophoretic apparatus was faulty, the fractionated serum from this patient was devoid of antibody-containing γ-globulin[9]. This discovery of agammaglobulinemia was confirmed later the same year, by Charles Janeway and colleagues in their report of two additional patients with the same condition[10]. This paved the way for the identification of genetic lesions, such as mutations in the *BTK* gene, causing agammaglobulinemia[11] and, arguably, was the starting point for the study of monogenic disorders of the immune system.

Probably the best-known case in medicine of a monogenic immune disorder is that of David Vetter (1971–1983), the so-called 'boy in the bubble', who had severe combined immunodeficiency (SCID) and lived in a sterile isolator from birth until his death at the age of 12 years; subsequent studies have identified 19 different causal gene defects for this condition[4].

Historical approaches to characterization

Primary immune deficiencies, which together with monogenic autoinflammatory diseases comprise IEIs, were initially genetically characterized mainly by the Sanger sequencing of DNA. This technique[12] has since been used in combination with several other genetic approaches — such as cytogenetics, linkage mapping, somatic cell fusion complementation[13–15], positional cloning and candidate gene investigations — to identify the causal genes underlying IEIs. Several examples of the use of these methods to identify IEI-associated genes in early studies are provided below.

In 1996, fluorescence in situ hybridization was used to investigate patients with Di George syndrome, leading to the discovery of a microdeletion on chromosome 22q11.2 that results in the failure of various organs, including the thymus, to develop normally[16]. Another genetic approach of linkage mapping with microsatellite markers was used to map X-linked SCID to the proximal long arm of the X chromosome[17–19]. Linkage analysis has often been coupled with positional cloning; for example, the identification of an interstitial deletion on Xp21 as the genetic lesion causing X-linked chronic granulomatous disease resulted from the combined use of cytogenetic tools, linkage mapping and positional cloning[20,21]. Positional cloning also led to the discovery of genetic defects in *BTK* as the cause of X-linked agammaglobulinemia[11] and in *WAS* as the driver of Wiskott–Aldrich syndrome[22].

Many mutations responsible for IEIs have also been identified using a candidate gene approach. This was the case for the identification of *RAG1* and *RAG2* mutations in patients with SCID who were B cell-deficient[23] and the discovery of *GATA2* mutation in patients with a syndrome consisting of monocytopenia, B cell and natural killer cell lymphopenia, and mycobacterial, fungal and viral infections[24].

Many of these early approaches to characterizing IEIs remain in use today in a refined form. For example, comparative genome hybridization, a competitive adaptation of fluorescence in situ hybridization, has been used in the diagnosis of genetic deficiencies involving large deletions (such as DOCK8 deficiency[25] or chronic granulomatous disease[26,27]). Similarly, the earlier use of microsatellite markers for autozygosity mapping[28,29], involving the detection of runs of homozygosity in the offspring of consanguineous unions, has been modified with the development of single-nucleotide polymorphism microarrays. Thus, these technologically innovative approaches of their time continue to aid our progress in understanding IEIs.

Next-generation sequencing for IEIs

The now widespread availability of NGS methods, including exome sequencing, whole-genome sequencing (WGS) and targeted gene

panels, has rapidly increased the identification of monogenic defects underlying IEIs in recent years. In the past 3 years alone, 55 novel monogenic defects have been documented[4]. Exome sequencing was first used in this context to identify STIM1 deficiency in a child with Kaposi sarcoma in 2010 (ref. 30). Although exome sequencing alone is the main tool used for the discovery of genetic lesions in IEIs, its combination with genome-wide linkage analysis has also often been used to discover novel causative variants. For example, this combination was used to characterize deficiency of the interferon-stimulated gene *ISG15* as a genetic aetiology of type I interferonopathy and Mendelian susceptibility to mycobacterial disease (MSMD)[31,32], and heterozygous deletions of *PLCG2*, which encodes a signalling molecule regulating cytosolic calcium levels, in 27 patients with cold-induced urticaria, immunodeficiency and autoimmunity[33]. Exome sequencing can also be coupled with the genome-wide analysis of copy number variations to identify structural abnormalities. Two patients with a fatal disorder characterized by immunodeficiency, autoinflammation and amylopectinosis were found to be compound heterozygous for a large deletion and a nonsense mutation in *HOIL1* (ref. 34), which encodes a protein that is involved in conjugating cytokine receptor signalling complexes with components of the IKK complex to activate NF-κB signalling.

Notably, the diagnosis of IEIs can also be improved by reanalysing NGS data. This approach is particularly powerful when additional relatives are added to a case, the proband of interest has an evolving phenotype that merits reinvestigation and/or there is improved knowledge of disease–gene associations that allows for the re-classification of variants[35,36]. Increasingly, NGS is leading to the identification of many variants of unknown significance. Most of these have not been functionally evaluated, owing to the effort, time and cost of carrying out such assessments. Thus, although the increasing amount of data from NGS studies provides greater statistical power to discerning genotype–phenotype relationships, it will only be possible to document true causality with concerted funding and dedicated effort for functional investigations.

Advances in NGS are also providing us with the basis for re-evaluating the prevalence of IEIs and are revealing that IEIs do not necessarily result from rare genetic variants. Typically, the causal genetic lesions of IEIs range from private to ultra-rare (<1 in 10,000) to rare (<1 in 1,000) in frequency (Fig. 2). However, more common genetic variants (~1 in 100) have been reported to cause IEIs. For example, the P1104A variant of *TYK2*, which is a risk allele for tuberculosis and MSMD in homozygosity, is present in the heterozygous state in ~1 in 20 individuals of European ancestries[37]. High-frequency null alleles of the interferon receptor subunits *IFNAR1* and *IFNAR2*, which confer susceptibility to life-threatening viral infection or disease caused by live-attenuated viral vaccines, have been reported in two isolated populations from the Pacific and Arctic regions, respectively[38,39]. The c.1156G>T variant of *IFNAR1* has an estimated frequency of 1 in 80 individuals from Western Polynesia[38], and the c.157T>C variant of *IFNAR2* is present in ~1 in 30 Inuit[39]. Notably, the susceptibility phenotype conferred by these variants of *IFNAR1* and *IFNAR2* is only apparent in individuals who are exposed to viral infections, despite the inborn nature of these defects (see later). These findings suggest that negative selection against the c.1156G>T variant of *IFNAR1* and the c.157T>C variant of *IFNAR2* by endemic viral infections has not occurred in these isolated geographic regions in recent millennia. Taking these findings into account, the concept of IEIs as rare disorders must now be expanded to encompass these and other common genetic variants, particularly when assessing variant frequencies in specific ancestral groups.

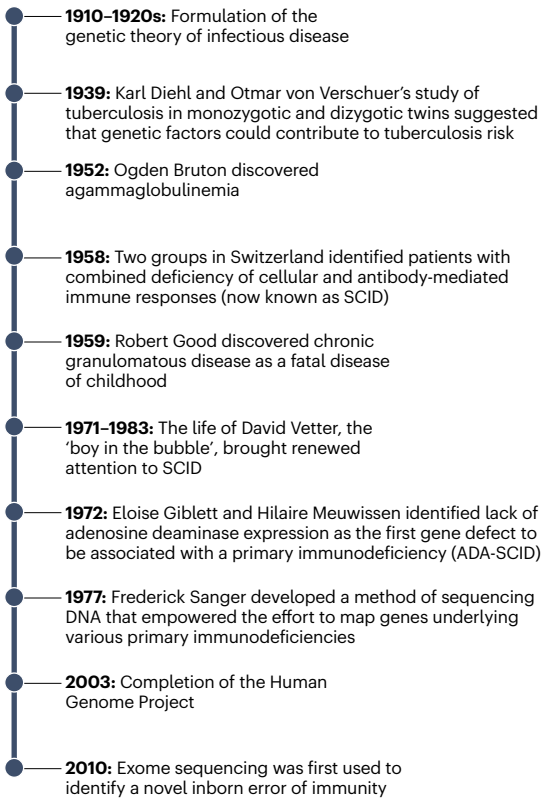

Fig. 1 | Ten key historical events that advanced the study of inborn errors of immunity. ADA-SCID, adenosine deaminase-deficient severe combined immunodeficiency; SCID, severe combined immunodeficiency.

The discovery of more common genetic variants underlying IEIs can be accelerated by building and increasing access to biobanks covering large and diverse cohorts. For example, the UK Biobank, which contains genetic and linked health information for half a million participants in the United Kingdom, has proved valuable in studies of genetic aetiology for common diseases and traits. However, this database, similar to other large genetic information repositories, contains mainly data from individuals of European ancestries[40–42]. This lack of diversity in genomic research data is hampering efforts to learn more about the genetic variants underlying IEIs in global populations. It is crucial that non-white, non-European populations should be adequately represented in the curation of large genetic databases to ensure that these groups can partake of the known and unknown future benefits of research. To this aim, the Mount Sinai Million Health Discoveries Program aims to use the diverse population of patients managed in the New York City health system for the genetic sequencing of one million individuals over the next 5 years. Similar efforts elsewhere to promote the inclusion of non-white, non-European populations in the expansion of biobanks need to be unwaveringly pursued.

Advances in next-generation sequencing

Common single-nucleotide polymorphisms with weak phenotypic effects can be revealed by genome-wide association studies, whereas rare genetic variants with highly penetrant and deleterious phenotypes can be characterized by NGS and Sanger sequencing. However, there remains a little explored zone between the two, corresponding

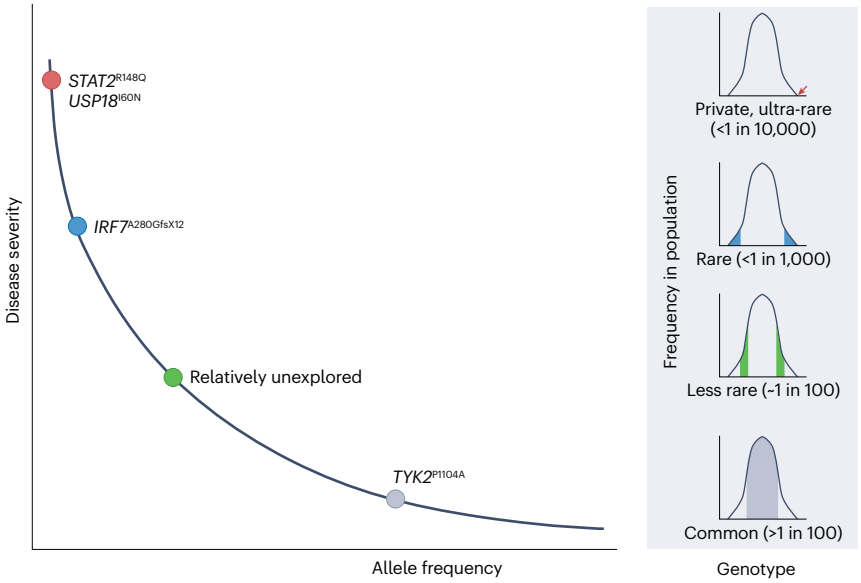

Fig. 2 | The relationship between allele frequency and disease severity for causal genetic lesions of inborn errors of immunity. The classic view is that private, ultra-rare and rare gene variants (such as variants of *STAT2*, *USP18* and *IRF7*) cause severe disease, whereas common gene variants (such as variants of *TYK2*) cause mild disease. The notion that less rare variants may cause inborn errors of immunity (IEIs) remains relatively unexplored, and advances in next-generation sequencing (NGS) are likely to uncover new variants belonging to this category. Examples of IEI gene variants that are common (*TYK2*^{P1104A}; ~1 in 20 individuals of European ancestries)[37], rare (*IRF7*^{A280GfsX12}; ~1 in 5,000 or ~1 in 1,400 individuals of Swedish or Finnish ancestries, respectively)[136], ultra-rare (*USP18*^{I60N}; ~1 in 250,000 individuals)[137] or private (*STAT2*^{R148Q})[138] are indicated.

to more common variants with incompletely penetrant phenotypes, which could give rise to the discovery of additional IEIs or to differential diagnoses for genes underlying IEIs through genetic lesions with different properties. However, for this to become a reality, many more individuals with milder disease will need to be sequenced owing to the anticipated reduced penetrance of these conditions. WGS is superior to exome sequencing for the identification of a broader range of variant types, including copy number variants, and provides more uniform coverage of exons and increased coverage of intronic and intergenic regions[43]. However, WGS is still markedly more expensive than exome sequencing and requires more complex data analysis. Importantly, the inclusion of non-coding regions of the genome in WGS will markedly increase the number of variants of unknown significance that are identified. Thus, determining the causal effects of candidate variants will require rigorous experimental testing for functional validation. More detailed discussions of the advantages and disadvantages of the various NGS approaches that are currently in use are presented in other recent reviews[43–45]. As NGS becomes increasingly commonplace in research, diagnostic and commercial settings, we anticipate its application to the general population and that data sharing to facilitate personalized healthcare and to improve public health will become more common. This would have many advantages in terms of the preventive focus of modern medicine and for the early detection of diseases, but also raises many ethical and privacy-related issues that will require careful consideration and legislative frameworks.

Penetrance and expressivity of IEIs

It is challenging to match the clinical presentations of a disease with a specific genetic lesion, as genetic mutations frequently segregate imperfectly with disease traits. Individuals with disease-associated genotypes may have no clinical manifestations of disease, a phenomenon known as incomplete penetrance. Moreover, individuals with disease symptoms may have heterogeneous phenotypes of varying severity, a phenomenon known as variable expressivity. The factors accounting for disease penetrance and expressivity may be external (environmental) or internal ((epi)genetic)[46].

Environmental factors

Some of the best-known examples of environmental modifiers of the penetrance of IEIs are seen in the context of susceptibility to infection. A susceptibility phenotype is only apparent if an individual encounters the specific pathogen against which the patient's immune defences have been compromised since birth. Patients presenting with mycobacterial disease after *Mycobacterium bovis* bacillus Calmette–Guérin (BCG) vaccination characteristically have impaired interferon-γ-dependent immunity[47]. These individuals often do not develop disease if they are not vaccinated with BCG or are not naturally exposed to mycobacteria, providing clear evidence that microbial exposure triggers the manifestations of the immune defect.

Another example of an environmental trigger that modulates IEI penetrance is exposure to DNA damage-inducing radiotherapy or chemotherapy, as documented in patients with deficiency of DNA ligase 4 (LIG4)[48,49]. LIG4 is a component of the non-homologous end-joining pathway of double-stranded DNA break repair that is required in T cell and B cell development. Individuals with *LIG4* mutations are radiosensitive, resulting in alterations in T cell and B cell counts and hypogammaglobulinemia, but are asymptomatic until treated with chemotherapy or radiotherapy. In these contexts, the genetic defect is necessary but not sufficient for disease manifestation.

Genetic penetrance

Differences in penetrance and expressivity of IEIs can also result from the severity of the genetic alteration, which determines the extent of the molecular defect and, ultimately, the probability of disease. There is, therefore, a sort of hierarchy of the variants of a given gene, and of the different causal genes for a particular disease. Variant hierarchy refers to the ranking of variations in a gene according to the degree of molecular dysfunction that they confer (which may or may not correlate with the amount of gene product), which ultimately determines the tendency for clinical manifestation of disease. The principle of variant hierarchy is demonstrated in three groups of patients with mutations of *STAT1*, an essential mediator of interferon signalling (Fig. 3a). Notably, even variants of a similar nature — for example, missense variants

corresponding to the coiled-coil domain of STAT1 — can underlie any one of the three types of IEI affecting human STAT1-dependent immune responses[50]. The first type of IEI consists of patients with autosomal recessive complete STAT1 deficiency[51–53], leading to lethal intracellular bacterial and viral infections with full penetrance. The second type consists of patients with autosomal recessive partial STAT1 deficiency[54–56], characterized by impaired, but not abolished, interferon signalling that gives rise to less penetrant and milder forms of intracellular bacterial and viral disease. Individuals in the third group have autosomal dominant *STAT1* deficiency[57–60], leading to even milder mycobacterial disease that is incompletely penetrant. No overt susceptibility to viral infections is observed in this group. A similar hierarchy of variants is observed for IFNGR1 deficiency and the penetrance of mycobacterial disease[47,61,62]. Thus, different lesions in a single gene can have marked differences not only in phenotype but also in disease penetrance.

The principle of gene hierarchy (differences between genes in relation to the necessity of their function in a particular pathway) is demonstrated by comparing the consequences of complete STAT1 deficiency, which is fully penetrant, with those of complete TYK2 deficiency (Fig. 3b). TYK2 lies upstream of STAT1 in the signalling pathway downstream of interferon receptors. It also signals downstream of many other cytokine receptors, including the IL-6 receptor, where its activity does not induce activation of STAT1. The complete loss of TYK2 results in a disease with incomplete penetrance, as demonstrated by reports of an absence of viral infections, despite documented exposure, in three of ten individuals with TYK2 deficiency[63–66]. Similar gene hierarchy is noted for *ISG15* and *USP18*, which encode negative regulators of type I interferon signalling. Whereas complete deficiency of USP18 results in fully penetrant, lethal severe interferonopathy[67], complete ISG15 deficiency leads to non-life-threatening interferonopathy involving infectious, neurological and dermatological features[31,32,68]. Gene hierarchy is also evidenced in autosomal recessive complete IFNGR1 deficiency, which leads to completely penetrant MSMD by 5 years of age, compared with autosomal recessive complete IL-12RB1 deficiency, which results in MSMD in only 50% of individuals by 40 years of age[69]. These findings suggest that the nature of the genetic lesion and the identity of the gene within the affected biological pathway influence disease penetrance.

In addition, the site of a mutation in a particular gene can predict, on its own, the penetrance and severity of some IEIs. For example, among missense *RPSA* mutations underlying congenital asplenia, those with incomplete penetrance were found to be located structurally close together[70]. Evaluation of missense mutations in *FAS*, which are a genetic aetiology of autoimmune lymphoproliferative syndrome, showed that variations affecting the intracellular death domain were highly penetrant[71] whereas mutations of the extracellular domain had reduced penetrance. These examples demonstrate that penetrance can be a function of the location of a mutation in a gene.

Epigenetic factors and genetic modifiers

Other putative mechanisms underlying the incomplete penetrance and expressivity of IEIs are thought to involve epigenetic and genetic modifiers. The impact of epigenetic modifications on disease penetrance can be seen from the study of a single pair of monozygotic twins discordant

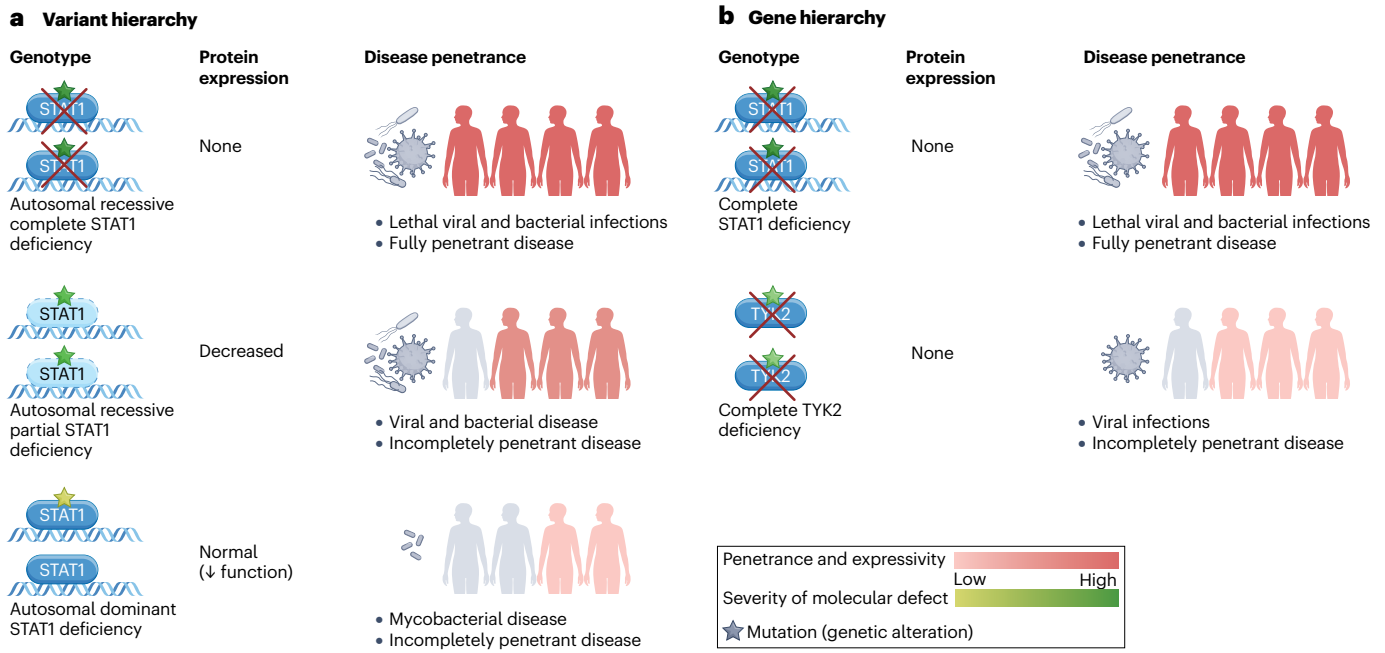

Fig. 3 | A hierarchy of genetic alterations influences the penetrance and expressivity of inborn errors of immunity. a, Variant hierarchy demonstrated by the consequences of various alterations in *STAT1* as evidenced in three groups of patients. Homozygous mutation leading to autosomal recessive complete STAT1 deficiency results in lethal viral and bacterial infections with full penetrance. Biallelic mutation resulting in autosomal recessive partial STAT1 deficiency manifests as incompletely penetrant and milder intracellular bacterial and viral disease. Autosomal dominant STAT1 deficiency, characterized by normal STAT1 levels but hypomorphic function, does not lead to disease or results in only mild mycobacterial disease. **b**, Gene hierarchy illustrated by comparing complete loss of STAT1 protein with complete loss of TYK2 protein, both of which function in the signalling pathway downstream of interferon receptors. In contrast to complete STAT1 deficiency, the absence of TYK2 leads to viral infections only in some exposed individuals.

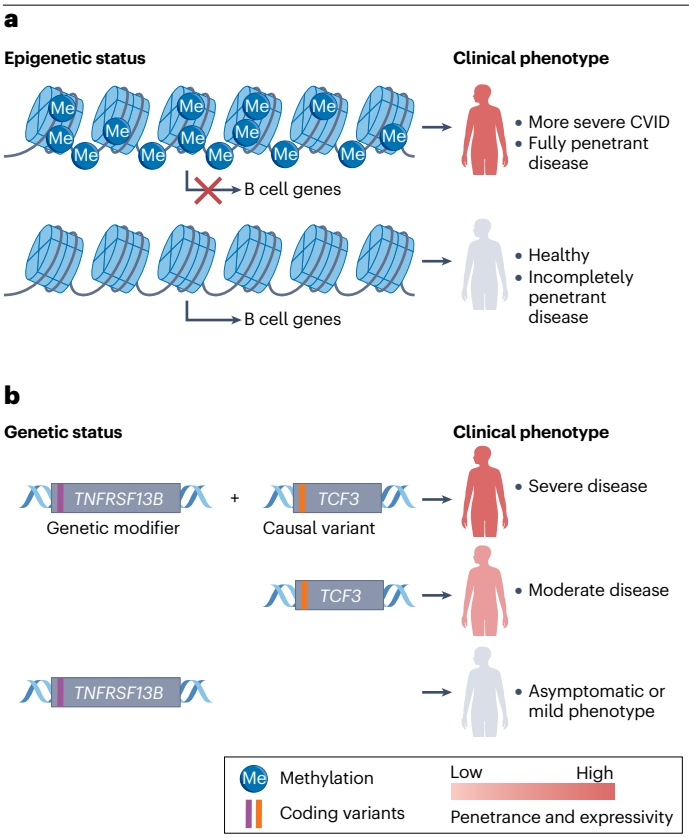

Fig. 4 | Epigenetic and genetic modifiers determine the penetrance and expressivity of inborn errors of immunity. a, The discordant phenotypes of common variable immunodeficiency (CVID) observed in patients with different methylation status of B cell genes. Hypermethylation of B cell genes is associated with a more penetrant and severe form of CVID. **b**, The effect of a gene variant that modifies disease outcome in the presence of a risk allele on a different gene in CVID. Common mutations in *TNFRSF13B* are not causative of CVID. However, the presence of a common *TNFRSF13B* mutation in an individual with a rare *TCF3* mutation that causes CVID has been reported to drive a more severe form of the disease.

for common variable immunodeficiency (CVID), which revealed marked differences between the twins in terms of the hypermethylation of crucial B cell genes, such as *PIK3CD*, *BCL2L1*, *RPS6KB2*, *TCF3* and *KCNN4* (ref. 72) (Fig. 4a). An inability to demethylate, and therefore upregulate the expression of, key genes during the transition of naive B cells to memory B cells is also well documented in larger cohorts of patients with CVID[72,73].

The impact of genetic modifiers on disease penetrance can also be seen in the context of CVID (Fig. 4b). *TNFRSF13B* variants do not cause disease themselves but are identified as risk factors in 10% of patients with CVID; they are also found in 1–2% of the general population[74]. In one family carrying a *TNFRSF13B* mutation, the proband also had a de novo *TCF3* mutation and presented with severe CVID and the autoimmune disease systemic lupus erythematosus[75]. The proband's brother, who was homozygous for the *TNFRSF13B* mutation but negative for the *TCF3* mutation, was in good health and had only a mild immune phenotype, whereas the proband's son, who inherited the *TCF3* mutation but not the *TNFRSF13B* mutation, had a partial clinical phenotype[75]. The mechanism by which this probable gene modifier (*TNFRSF13B* mutation) affects disease outcome is not yet well understood and the concept of gene modifiers in general merits more thorough investigation.

Mosaicism

Another possible cause of incomplete clinical penetrance for IEIs is mosaicism, which occurs when a mutation arises post-zygotically and two or more cell populations with different genotypes are present in the same individual. Mosaicism in patients with primary immune deficiencies has been known since the 1990s[76,77] but only recently has started to be documented in more detail. In a large, systematic analysis of 128 families with primary immune deficiencies, the frequency of mosaicism was estimated at 23.4%[78]. In this study, ten families had parental gonosomal mosaicism, of which eight families were clinically asymptomatic. In the other two families, the parents were mildly affected whereas their offspring with germline mutations were severely affected[78] (Fig. 5a). Interestingly, revertant mosaicism can also underlie mild presentations or an absence of IEI-related clinical disease[79] (Fig. 5b). The somatic reversion of mutations, in the form of either genetic alterations that restore the wild-type sequence or second-site mutations affecting another site within the protein, has been documented to underlie incompletely penetrant clinical disease in several IEIs, including those caused by defects of *WASP*[80,81], *ADA*[82], *IL2RG*[83] and *LAD1* (ref. 84). Another class of somatically caused disorders of immune dysregulation, which can perhaps be referred to as genetic errors of immunity rather than inborn errors, are lymphoproliferative disorders such as non-malignant forms of histiocytosis and VEXAS (vacuoles, E1 enzyme, X-linked, autoinflammatory, somatic syndrome)[2]. Somatic mosaicism of *NLRP3* (refs. 85–90) and *TLR8* (ref. 91) have also been documented as primary mechanisms of monogenic IEIs.

Although sporadic gene mutations that lead to disease can occur early in development, the likelihood of somatic mosaicism increases with age and can drive adult-onset disease presentation. Adult humans have an estimated 1×10^{16} somatic mutations[92], with every nucleotide site, including those of IEI-associated genes, being mutated in thousands of cells. The vast majority (~99%) of somatic variants are tolerated[93], but older individuals have an unavoidable accumulation of somatic mutations with deleterious consequences. However, very few adult-onset IEIs that are likely to be caused by mosaicism have been reported as compared with IEIs of germline origin[4]. This gap in our understanding of the factors that set the threshold for non-deleterious somatic mutations requires further investigation.

Other less widely studied factors

Several host-extrinsic and genome-intrinsic factors that might affect the penetrance and expressivity of IEIs remain less well explored. These factors include the microbiome, transcriptional adaptation, non-coding modifier alleles and monoallelic expression. Infectious microorganisms have been identified as drivers of disease in IEIs, but less effort has been devoted to understanding the relevance of commensal microbial species to IEIs. Notably, a few studies have investigated the correlation between disturbances of the bacterial microbiota and systemic inflammation in IgA deficiency[94], CVID[95,96] and X-linked agammaglobulinemia[97]. For example, reduced bacterial diversity and increased dysbiosis were reported in patients with more severe cases of CVID[95,96]. Nevertheless, a mechanistic link between alterations in the gut microbiota and immunodeficiency is currently lacking. It remains unclear whether specific members of the microbiota

regulate the penetrance of IEIs or whether the observed changes in the microbiota are a consequence of immune system dysregulation. Alterations to the size and composition of the microbiota owing to the use of antibiotics may reveal the otherwise masked impact of the microbiota on IEI penetrance.

More than a century ago, ~25% of the human population would have died from an infectious disease[98], whereas more than 99% of individuals who are infected now survive owing to improvements in sanitation, vaccination and antibiotics. It could be argued that it is now possible to study genetic susceptibility to infectious diseases only in the most extreme cases, whereas intrinsically common variants probably influenced our immune architecture in the past in a way that is impossible to study today. The use of immunosuppressants in clinical practice (in patients with inflammatory diseases and recipients of transplants) may reveal genetic susceptibilities that can be detected only in this immunosuppressed state. This could provide an ideal context in which to identify genetic susceptibilities to specific microorganisms, such as the reactivation of human polyomavirus 2 in only some of the many recipients of transplants who are seropositive for this virus.

Severe genetic mutations do not always lead to disease; indeed, they may putatively be offset by a phenomenon known as transcriptional adaptation. Transcriptional adaptation was first documented as a compensatory response mechanism activated by frameshift and nonsense mutations[99,100]. Two groups studying zebrafish embryos and mouse cell lines showed that nonsense-mediated decay triggers the upregulation of homologous genes for functional compensation[99,100]. This compensatory mechanism may account for incomplete penetrance of IEIs in asymptomatic carriers of nonsense mutations, although it remains unknown whether such a mechanism of transcriptional adaptation operates in humans.

Non-coding modifier alleles are another factor that has been relatively little explored but might account for the variability of IEI penetrance. Exome sequencing has accelerated the identification of rare IEI-causing protein-coding variants. By contrast, only a small number of non-coding variants have been implicated in IEIs[101]. With continuing improvements in WGS approaches, it is likely that increasing numbers of both causal variants and modifier alleles — that regulate the expression of other pathogenic mutations in *cis* or in *trans* and, thus, influence disease penetrance — will be discovered in non-coding regions. One study reported an index patient who was compound heterozygous for a rare coding sequence variant and a common variant in the non-coding regulatory region of *PTPN2* (ref. 102). The index patient presented with CVID, whereas his mother, who had only the rare variant in the coding region, presented with autoimmunity but not immunodeficiency[102]. These observations have not been confirmed by biochemical studies, but they suggest that future studies should investigate the possibility that there are many more non-coding variants with epistatic effects on pathogenic variants of protein-coding regions in IEIs.

Finally, some of the incomplete penetrance of IEIs may be explained by monoallelic expression. In a similar manner to random X chromosome inactivation, somatic cell lineages may be committed to the expression of a single allele of certain autosomal genes. As a result, even though the DNA sequence is the same in all the somatic cells of the individual, some cells in heterozygous individuals express predominantly either the reference allele or the alternative allele[103–105], leading to discrepancies between genotype and 'transcriptotype'[106] that remain fixed over time[104,107]. Although they require further investigation, findings suggest that stochastic allele choice can be a contributing factor in disease aetiology. Analysis of human clonal neural stem cells derived from the central nervous system showed evidence for autosomal monoallelic expression of ~2% of genes[105], many of which are known neurodevelopmental genes. The same genes were also reported to be over-represented among candidate risk genes for schizophrenia and autism[108]. Careful testing is required to determine whether monoallelic expression accounts for some of the incomplete penetrance of IEIs, but one study has already provided the first demonstration of allelic bias in a patient with a *JAK1* gain-of-function mutation[106]. Continued improvements in single-cell RNA-sequencing technology and lineage tracing will be required for detailed studies of the monoallelic expression of IEI-associated genes.

Effects of IEIs on distal organs

Early in their study, when only a small number of IEIs had been identified, it was relatively simple to classify these as defects of specific components of the immune system — B cells, T cells, neutrophils, monocytes or complement. Now that almost 500 IEIs have been discovered, including an increasing number with multisystem manifestations, we need to reconsider the best ways of detecting, naming and studying immune gene dysfunctions that predominantly cause clinical manifestations in distal organs (organ systems other than the blood and lymphatic

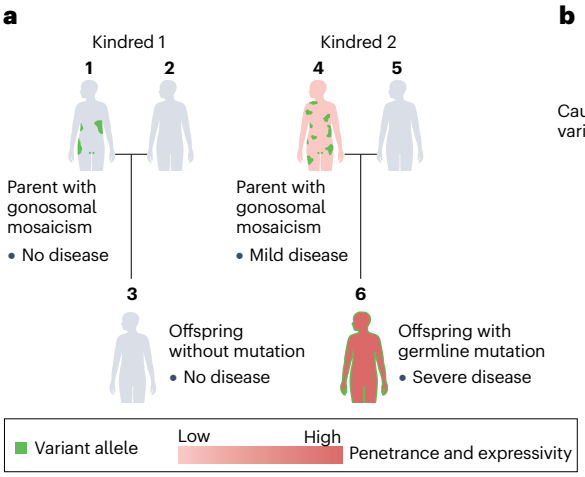

Fig. 5 | Effects of mosaicism on the penetrance and expressivity of inborn errors of immunity. **a**, Parental gonosomal mosaicism can be associated with no (individual 1) or mild (individual 4) clinical manifestation of disease. Incomplete penetrance might be attributed to differences in the frequency of the mutant allele and/or its tissue distribution, both of which remain to be proven experimentally. This is contrasted with a fully penetrant and severe phenotype in the offspring of individual 4 (indicated as individual 6), resulting from a germline mutation. Although individual 1 has the potential for transmission of the disease-causing variant, in this example the genetic variant is not passed down to individual 3, who is thus healthy. **b**, Incomplete penetrance could be explained by restoration of the wild-type sequence in some individuals, termed revertant mosaicism.

Glossary

Agammaglobulinemia
A rare disorder characterized by complete or near-complete lack of serum antibodies and circulating B cells owing to early termination of B cell development.

Aicardi–Goutières syndrome
(AGS). A rare type I interferonopathy that affects the brain, immune system and skin.

Common variable immunodeficiency
(CVID). The most common form of primary immunodeficiency characterized by antibody deficiency, increased susceptibility to infection, autoimmune manifestations and impaired vaccine responses.

Chronic granulomatous disease
A rare condition, mainly affecting phagocytic cells of the immune system, that is characterized by an increased susceptibility to bacterial and fungal infections, as well as the development of granulomas.

Di George syndrome
A primary immune deficiency associated with susceptibility to infections, owing to an absent or poorly developed thymus.

Genetic theory of infectious disease
A theory proposing that the genetic background of the host is a determinant of resistance or susceptibility to a given microorganism.

Incomplete penetrance
The occurrence of individuals having a disease-causing mutation who develop partial or no disease.

Monoallelic expression
The maintenance of expression of an autosomal gene from a single allele in a somatic cell over time.

Nonsense-mediated decay
A mechanism to reduce errors in gene expression by eliminating mRNA transcripts that contain premature stop codons.

Primary immune deficiencies
A varied group of disorders that result from genetic defects that impair the development and/or function of the immune system, mostly presenting as severe recurrent infections and occasionally with an increased incidence of autoimmunity and malignancies.

Type I interferonopathy
An inherited disorder involving a central role for dysregulation of the type I interferon pathway in disease pathogenesis.

Severe combined immunodeficiency
(SCID). A very rare life-threatening genetic disorder in which there is combined absence of T cell and B cell function.

Wiskott–Aldrich syndrome
A rare X-linked recessive immunodeficiency that is characterized by abnormal bleeding resulting from a reduced number of platelets in the blood.

system) rather than systemic effects on the immune system. Some immune genes, such as those encoding the type I interferon receptor, are expressed by most, if not all, cell types, which gives them the ability to regulate the immune response. Immune genes expressed in non-immune tissues could also mediate functions that are not typically considered as immune activity but mutation of which could lead to the activation of the immune system. For example, Aicardi–Goutières syndrome (AGS), an IEI that has primarily neurological manifestations, can result from mutations in genes encoding nucleases. The defective removal of endogenously produced nucleic acids by these nucleases in AGS results in activation of the immune system[109,110].

Investigations of immune cell abnormalities have yielded many discoveries in the context of IEIs and will continue to do so. However, assuming that all IEIs will necessarily have clinically evident immunological phenotypes impedes our ability to detect these defects. Epidermodysplasia verruciformis provides a clear example of this. In 1946, epidermodysplasia verruciformis was characterized as an autosomal recessive disease presenting as disseminated warts[111,112]. In the absence of a detectable immunological phenotype, the disease was considered to be a dermatological condition. It was subsequently shown that patients with epidermodysplasia verruciformis have biallelic loss-of-function mutations of *EVER1* and *EVER2* (ref. 113) and that EVER1 and EVER2 form a complex that is essential for keratinocyte-intrinsic immunity to β-papillomaviruses[114], thus categorizing the disease as an IEI. Similarly, there is evidence that intestinal epithelial cells[115–119], pulmonary epithelial cells[120] and cortical neurons and oligodendrocytes[121] have cell-autonomous mechanisms to protect against enteric viruses, influenza virus and herpes simplex virus, respectively, which suggests that genetic mutations could affect immunity in specific, non-haematopoietic cell types. Although many of the distal organ manifestations of IEIs can be linked to defects in circulating and tissue-resident leukocytes, some IEIs cannot readily be explained by inborn variants of genes operating in these cells. Thus, genetic studies should be expanded to 'non-traditional' IEIs, of which the type I interferonopathies and very early-onset inflammatory bowel disease are classic examples.

Type I interferonopathies

Many IEIs with primary features manifesting in distal organs, including the skin and the central nervous system, have been described. Most type I interferonopathies fall into this category, including AGS[4]. AGS is a clinically heterogeneous disease caused by mutations of ten different genes (*ADAR*, *IFIH1*, *RNASEH2A*, *RNASEH2B*, *RNASEH2C*, *SAMHD1*, *TREX1*, *DNASE2*, *LSM11* and *RNU7-1*)[4,122]. AGS has phenotypic overlap with both the sequelae of congenital infection and systemic lupus erythematosus. Although they have no indication of viral infection, patients with AGS develop early-onset encephalopathy with intracranial calcifications, white matter changes, cerebral atrophy and bilateral striatal necrosis that are evident upon neuroimaging[122]. Epileptic seizures occur with a variable frequency but are also a common feature of AGS. These central nervous system symptoms require that patients are primarily under the care of neurologists. Another main site of disease manifestation is the skin (35% of cases)[122]; patients have chilblain-like lesions on their fingers, toes or auricles that worsen in cold weather[123].

Another interferonopathy with primary symptoms in distal organs — in this case, the skin and lungs — is STING-associated vasculopathy with infantile onset (SAVI). SAVI is caused by gain-of-function mutations in *STING1*, which encodes a cytosolic sensor of double-stranded DNA. *STING1* gain-of-function mutations lead to increased activation of the type I interferon pathway through JAK–STAT signalling, and treatment of lymphocytes from patients with SAVI with JAK1/JAK2 inhibitors has been shown to reduce the constitutive phosphorylation of STAT1 (ref. 124). Children with SAVI typically have dilation of cutaneous blood vessels on cold-sensitive areas such as the nose and cheeks[123]. Respiratory symptoms in SAVI are generally non-specific, such as chronic cough, exertional dyspnoea and coughing up blood[125]. More than 75% of patients eventually present with interstitial lung disease[126], and lung involvement leads to high levels of

morbidity and mortality in patients with SAVI[127]. One study reported that 25 of the 53 patients evaluated had radiological or histological evidence of lung fibrosis[126].

Gastrointestinal diseases

There are also several monogenic defects that predominantly affect the gut, such as very early-onset inflammatory bowel disease resulting from mutation of genes including *IL10RA*, *IL10RB*[128], *TTC7A*[129], *IL21* (ref. 130) and *TRIM2* (ref. 131). Immune gene defects that disrupt the sensing of bacteria — for example, biallelic loss-of-function mutations of *ALPI* — can trigger very early-onset inflammatory bowel disease[132]. In response to the large amounts of lipopolysaccharide (LPS) released by the microbiota in the intestinal lumen and to limit LPS-induced inflammatory signalling, intestinal epithelial cells produce the alkaline phosphatase ALPI for the catalytic detoxification of LPS. In the absence of ALPI, patients have early-onset severe diarrhoea, weight loss, recurrent abdominal pain and rectal bleeding[132]. Patients generally have normal serum immunoglobulin levels and no extra-gastrointestinal manifestations have been reported. Initial screens of patients using targeted panels of genes that are known to be associated with intestinal disorders identified no potentially damaging variants[132]. As this disorder primarily involves the gastrointestinal system, it can easily be overlooked as an IEI during diagnosis.

Thus, in the absence of overt immunological symptoms or when patients with multisystem manifestations are managed primarily by non-immunologists, it may not be evident that an IEI is the cause of disease. This is illustrated by the example of a patient with persistent and severe renal, dermatological, gastrointestinal and growth disturbance-related symptoms, but with a largely normal distribution of immune cells and immunoglobulin levels within normal limits, who was managed by a team of nephrologists, dermatologists and gastroenterologists[106]. When she was eventually referred to an Undiagnosed Disease Program (UDP), the genetic defect underlying the patient's symptoms was discovered to be a de novo *JAK1* mutation. Furthermore, in an unpublished study carried out within the UDP at Mount Sinai Hospital in New York, we investigated 100 patients who were referred to the UDP irrespective of clinical presentation and for whom genetic factors were suspected to contribute to symptoms. Underlying defects of immune genes were detected in 21% of the cases that were solved. Remarkably, none of these patients was initially seen or referred by a clinical immunologist or infectious disease specialist. This finding and others suggest that immunogenetic approaches could facilitate the recognition of IEIs that may seem to have little to do with immunology and could mitigate current diagnostic challenges resulting from the fragmented practice of medical specialities.

Future directions in IEI research

IEIs have traditionally been thought of as disorders of the immune system resulting from germline mutations. These disorders were initially assumed to follow a Mendelian pattern of inheritance with complete penetrance. Today, the discovery and study of nearly 500 IEIs, mainly facilitated by advances in NGS, has advanced our knowledge of the factors external and internal to the host that influence disease penetrance and expressivity. Furthermore, a growing subset of IEIs that manifest primarily in distal organ systems, without obvious symptoms in the immune system, has been documented. In this section, we consider the future outlook for the field. We propose that use of reverse genetics approaches and the study of somatic genetics will be major new directions in the next decade.

Reverse genetics approaches

The cost of NGS is falling rapidly, and this is leading to an exponential growth of genomic biobanks, which collectively contain sequencing information from millions of individuals. Typically, once an affected individual presents with clinical symptoms of disease suspected to be owing to a genetic defect, NGS is used to identify the genetic variants present in that individual and large reference databases are consulted to assess the frequency of the identified variants in the general population. This phenotype-first, forward genetics approach is likely to remain crucial to the investigation of undiagnosed rare IEIs, but the growth in genomic biobanks also provides an opportunity to achieve diagnosis through the use of reverse genetics, an approach that remains relatively rare in the field of IEI research (Fig. 6). In this approach, the genotype is used to determine the disease-associated phenotype. This approach would be particularly useful for IEIs associated with milder disease in the population or those that have heterogeneous presentations that make diagnosis more challenging. A reverse genetics approach can potentially aid diagnosis and make it possible to propose interventions in situations in which this was not previously possible, going beyond the familial genetic counselling that is typically provided for individuals with rare diseases. For example, mutations in *UBA1* were previously reported to underlie VEXAS[2], an often fatal, treatment-refractory inflammatory syndrome. In a retrospective observational study of exome data from 163,096 individuals in a single regional health system in Pennsylvania, USA, 11 individuals were identified to be carriers of *UBA1* variations[133]. Only six of these carrier individuals had previously reported clinical features of VEXAS, and the others contributed to the phenotypic expansion of VEXAS, a phenomenon that is often noted in genetic disorders[133]. This study illustrates how reverse genetics

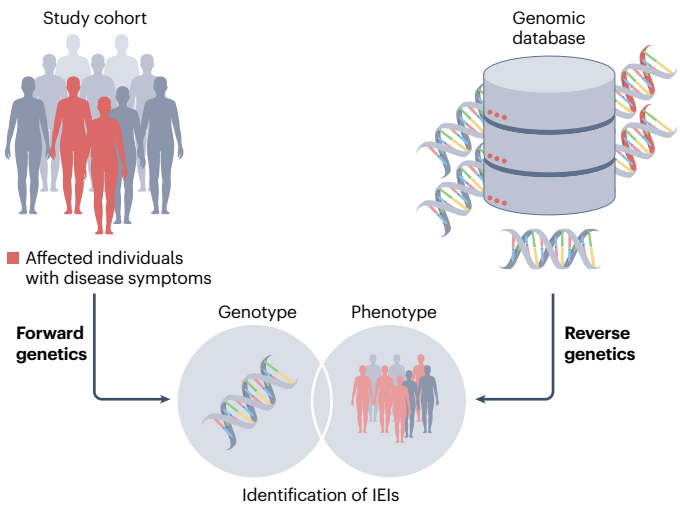

Fig. 6 | The promise of reverse genetics approaches for the identification of undiagnosed inborn errors of immunity. The contrasting use of forward and reverse genetics approaches in the identification of inborn errors of immunity (IEIs). Typically, when affected individuals who are suspected to have genetic defects present with disease symptoms, next-generation sequencing and large reference databases are used to identify underlying genetic variants, which is referred to as a phenotype-first, forward genetics approach. Genomic biobanks also enable diagnosis through a genotype-first approach, termed reverse genetics. A reverse genetics approach is likely to enable the identification of more common IEIs with milder manifestations, define the true prevalence of IEIs in the general population and broaden the phenotypic spectrums of known IEIs.

approaches can help to define the general population prevalence and phenotypic spectrum of a given IEI. A genotype-first approach can also connect seemingly unrelated syndromes to lead to disease diagnosis, thereby overcoming the limitations of recognizing discrete phenotypes.

In addition to new ways of using existing databases, the study of IEIs would also benefit from the construction of more ethnically diverse genomic databases. Efforts are currently being made to counteract the underrepresentation of non-white ethnic groups in genomic databases — such as the All of Us Research Program in the United States and the African Genome Variation Project — and we expect to see more such initiatives launched in the next decade. Such efforts have the potential to reveal the genes and variants contributing to disease in the global population. We must continue moving in this direction to build equity in the understanding of the genetic architecture of all for the benefit of all.

Somatic genetics

The IUIS classification of primary monogenic disorders includes a category for phenocopies of IEIs, which includes seven conditions resulting from somatic mutations[4]. As discussed above, there is growing evidence that somatic mosaicism is a primary cause of IEIs. Testing for some forms of somatic mosaicism can be difficult. In cases of suspected mosaicism, in addition to exome sequencing and WGS, quantitative PCR techniques such as droplet digital PCR can efficiently detect and quantify mosaicism. The limit of detection depends not only on the proportion of the allele present but also on the type and breadth of tissues analysed. In efforts to identify the genetic cause of a new IEI, it is often unclear which cell type is affected by the variant, and several somatic mutations have been shown to be detectable only in specific immune cell types[2,134,135]. There are probably many more cell type-specific mutations in both the haematopoietic and non-haematopoietic compartments[106]. In the coming years, the field of IEI research could adopt a similar approach to that of cancer biologists. Genetic testing for variants driving carcinogenesis involves comparing tumour cells with non-tumour samples from the same individual. In the case of individuals suspected of having genetic disorders of the immune system, DNA could be collected from the ectoderm (buccal swabs), mesoderm (peripheral blood mononuclear cells) and endoderm (epithelial lining of gastrointestinal tract) for a comparative analysis of mosaicism across the different tissues. Furthermore, the likelihood of detecting cell type-specific mutations can also be increased by sorting cell subtypes, such as different types of immune cell, before sequencing, to narrow down the possible subsets having the variant allele.

We believe that the study of somatic mutations is likely to provide explanations for many adult-onset manifestations of IEIs. If the expected role of somatic mutations is confirmed, then it might be more appropriate to use the term genetic error of immunity rather than IEI to encompass a broadening of the underlying concept. In that case, we will need to adapt our language and practices to reflect the expanding scope of the field as we continue to make new biological discoveries. Finally, we ought to incorporate artificial intelligence in the discovery of novel causative variants. Only with increased computational power will we be able to study the compounding effects of two, three or four distinct variants on human health.

Conclusions

The immune system is proving to have a much more far-reaching role in disease than previously imagined. Increasing numbers of patients are presenting with effects of disease on distal organ systems and are being managed by non-immunologists despite the (often unknown) presence of genetic defects of the immune system in these patients. There is, therefore, likely to be a severe underdiagnosis of IEIs, and we must now reach out and educate specialists from other disciplines, encouraging them to use immunogenetic approaches to disease diagnosis, which may partially unite the diverse clinical specialties.

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Author contributions

Both authors contributed equally to all aspects of the article.

Competing interests

D.B. is a founder and part owner of Lab11 Therapeutics. Y.T.A. declares no competing interests.

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