Published in final edited form as:

Curr Opin Immunol. 2014 October ; 0: 17–23. doi:10.1016/j.coi.2014.05.004.

Discovery of single-gene inborn errors of immunity by next generation sequencing

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Abstract

Many patients with clinical and laboratory evidence of primary immunodeficiency do not have a gene specific diagnosis. The use of next generation sequencing, particularly whole exome sequencing, has given us an extraordinarily powerful tool to identify the disease-causing genes in some of these patients. At least 34 new gene defects have been identified in the last 4 years. These findings document the striking heterogeneity of the phenotype in patients with mutations in the same gene. In some cases this can be attributed to loss-of-function mutations in some patients, but gain-of-function mutations in others. In addition, the surprisingly high frequency of autosomal dominant immunodeficiencies with variable penetrance, and *de novo* mutations in disorders with a severe phenotype has been unmasked.

Introduction

As many as a third of patients who are seen in an immunodeficiency clinic clearly have a defect in their ability to fight infection, but do not have a constellation of findings that has been well described in the literature. These patients present a challenge to our ability to predict outcomes and design appropriate therapy. In the last 3 years, genetic tools provided by next generation sequencing (NGS), particularly whole exome sequencing (WES), often coupled with linkage analysis, have given us a powerful method of determining the underlying genetic defects in many of these patients. As a result, there has been an explosion in the identification of new gene defects that result in immunodeficiency and new immunodeficient phenotypes for known gene defects (Table I). Of at least 34 new gene

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defects, 22 are autosomal recessive, 11 are autosomal dominant and one is X-linked recessive [1].

Most of these immunodeficiencies are rare but some occur frequently enough that they were reported by several groups almost simultaneously. The identification of these new genes provides an immediate improvement in genetic counselling for affected families and the potential for targeted therapy; however these discoveries also have major public health implications. The mutant genes highlight critical pathways that protect from infection and pathways that prevent the development and progression of both cancer and autoimmunity. In this review, we will discuss some of the most provocative insights provided by these gene discoveries.

Step by step WES

An efficient approach to WES was first described by Ng et al. in 2009 [2]. Since that time several protocols have been developed to perform WES [3] but one of the most popular involves the shearing of patient DNA into small fragments, adding adaptors to the ends of the sheared fragments, then selecting the coding regions by allowing a labelled “bait”, consisting of exons with flanking splice sites, to hybridize to the patient DNA. The labelled bait and the patient’s coding regions are then isolated and primers hybridizing to the adaptors are used for massively parallel sequencing. Each patient coding region must be sequenced 10 to 100 times to avoid confusing a polymerase or sequencing error with a DNA alteration and to identify heterozygous alterations. The sequencing data can be compared with over 10,000 genomes available in public databases to filter out high-frequency polymorphisms that are unlikely to be the cause of disease. In the rapidly evolving field of genetic technology, WES is gradually being replaced with whole genome sequencing which can provide better coverage of non-coding as well as coding exons, intronic sequences and regulatory regions.

Choosing immunodeficient patients for WES

Three types of patients have been particularly informative in NGS studies. Patients who are the products of consanguineous unions, or belong to isolated populations, are highly likely to be homozygous for alterations in the disease-causing gene. Filtering of the sequencing data to select homozygous variants not found in any database markedly reduces the number of potential targets for further study. Eighteen of the recently identified autosomal recessive immunodeficiencies were identified in this way. Examples of mutant genes found using this approach include *PIK3R1* [4], *MALT1* [5] and *ACT1* [6].

The second type of patients consists of groups of unrelated individuals who share a distinctive and rare phenotype. Filtering data to look for alterations that are found in two or more of these patients is also likely to be rewarding. This approach has documented heterozygous mutations in *GATA2* [7–10], *RSPA* [11] and *PIK3CD* [12,13]; and a homozygous mutation in *LRBA* [14–16].

The final group of patients consists of those who are not from consanguineous families or isolated populations, who have no family history of disease but who have the early onset of

a very rare, highly lethal disorder with a very unusual phenotype. These patients may have *de novo* mutations and WES of the patient and parents' DNA may reveal a mutation in the patient that is not seen in either parent. Mutations in the transcription factor E47, which is encoded by *TCF3*, and in the signal transduction molecule PLC γ 2 (encoded by *PLCG2*) were identified in this way [17,18]. It is interesting to note that WES has unmasked the relatively high incidence of autosomal dominant immunodeficiencies. Some of these gene defects are due to *de novo* mutations that are highly lethal and therefore rarely passed on from generation to generation. In 7 of the 11 recently identified autosomal dominant traits, at least one *de novo* mutation has been reported.

Phenotypic Heterogeneity

One of the most striking features of recently identified genetic defects is the marked heterogeneity in phenotype caused by mutations in the same gene. In some instances, this is because different mutations in the same gene have very different effects on protein function. One of the first examples of this consists of the mutations in *WASP*, the gene responsible for the classic X-linked recessive immunodeficiency Wiskott Aldrich syndrome. Null or inactivating mutations in this gene result in thrombocytopenia, eczema and recurrent infections due to both T cell and B cell dysfunction [19]. By contrast, activating mutations, which disable the auto-inhibitory domain of WASP, cause neutropenia with no obvious defects in platelets or unusual susceptibility to infection [20]. More recent studies related to *CARD11*, a scaffold protein required for activation of NF- κ B through the classical pathway, demonstrate a similar phenomenon. Homozygous null mutations in *CARD11* cause a form of severe combined immunodeficiency characterized by the early onset of opportunistic, bacterial and viral infections in patients with normal numbers of T cells and B cells but poor lymphocyte function [21,22]. By contrast, some heterozygous amino acid substitutions result in splenomegaly, increased B cell numbers and susceptibility to leukemia due to spontaneous *CARD11* aggregation and constitutive NF- κ B activation [23]. Mutations in *STAT1* represent another example of the contrast between inactivating and activating mutations. Heterozygous inactivating mutations in *STAT1*, mutations that inhibit phosphorylation of critical tyrosine 701 or DNA binding of phosphorylated STAT1, result in decreased response to IFN γ stimulation and increased susceptibility to atypical mycobacterial infections [24]. However, a different set of heterozygous mutations in *STAT1* impair nuclear dephosphorylation of tyrosine 701, thus enhancing transcriptional activity. This results in T cells with a shift in cytokine synthesis with decreased IL-17 production, and an increased vulnerability to mucocutaneous candidiasis and a broader array of infections [25,26]. A third set of alterations in *STAT1* sequence have a minimal effect when present in heterozygous individuals but are associated with life threatening susceptibility to viral, fungal and mycobacterial infections in infants who are homozygous for the mutation [27].

The heterozygous forms of *CARD11* and *STAT1* dysfunction highlight fact that the autosomal dominant forms of immunodeficiency may cause disease by a dominant activating effect (*STAT1*, *CARD11*, *PLCG2* and *PIK3CD*), a dominant negative effect (*STAT1*, *TWEAK*, and *TCF3*), or they may result in haploinsufficiency (*GATA2* and *RPSA*).

It has long been understood that hypomorphic mutations, mutations that decrease but do not ablate the function of a gene product, may result in a milder phenotype or delayed onset of disease. The various mutations in *RAG1* are an excellent example of this. Null mutations result in classic SCID characterized by a complete absence of T cells and B cells [28]; however some amino acid substitutions in this gene are associated with Omenn syndrome [29], granulomatous disease [30], autoimmune manifestations [31], or most recently, inflammatory osteomyelitis [32]. The variability in clinical phenotype cannot always be explained by the different functional consequences of the mutation. The first identified patient with a mutation in *TYK2*, a member of the JAK family of tyrosine kinases, was described as having hyper-IgE syndrome with skin lesions, elevated serum IgE and viral and fungal infections as well as BCG disease [33]. WES allowed the detection of a second patient, the product of a consanguineous union. This patient had BCG disease and brucellosis but did not have skin lesions or elevated serum IgE [34]. Both patients had small deletions in *TYK2*, and an absence of protein on western blot.

The same mutation in the same gene, even in members of the same family, may be associated with very different clinical findings. Activating mutations in *PIK3CD*, which encodes the p110 catalytic component of the predominant PI3 kinase isoform in leukocytes, are associated with recurrent sino-pulmonary infections, lymphadenopathy, splenomegaly, progressive lung disease, chronic EBV and CMV infection, lymphoma, elevated serum IgM, and poor antibody production in some affected patients, but other family members may not be recognized to have immunodeficiency prior to genetic studies [12,13]. T cells from these patients show an activated phenotype, increased phosphorylation of AKT and enhanced activation induced cell death. Similarly, mutations in the transcription factor *GATA2* are associated with an increased incidence of disseminated warts, atypical mycobacterial infections, myelodysplasia, leukemia, lymphedema, chronic pulmonary disease and hearing loss [7–10]. Laboratory findings include reduced or absent monocytes, B cells, NK cells and dendritic cells, bone marrow hypoplasia and increased expression of Flt-3 ligand. Several syndromes that were thought to be unrelated: 1) familial myelodysplasia/AML; 2) dendritic cell, monocyte, B cell and NK cell deficiency (DCML); 3) decreased monocytes with increased susceptibility to atypical mycobacterial infection (MonoMAC); and autosomal dominant deafness, lymphedema and myelodysplasia (Emberger syndrome) were all found to be due to mutations in *GATA2* that result in an impressively variable phenotype [35].

Our understanding of the functional consequences of a mutation may be complicated by the fact that the same mutation may have different effects in different cell lines. Mutations in *HOIL-1* (encoded by *RBCK1*), a component of the linear ubiquitin assembly complex (LUBAC) that is required for normal NEMO function, result in decreased NF- κ B activation in response to IL-1 β in B cells and fibroblasts but increased NF- κ B activation in response to the same cytokine in monocytes [36]. This suggests that caution should be used in interpreting the results of functional assays that are used to determine the consequences of a particular mutation.

Even in immunodeficiencies for which a phenotype/genotype association has been described, the correlation is not strong enough to predict clinical outcome. Modifying genetic factors, environmental or infectious exposures, or even the age at which

environmental or infectious exposures occur, may influence the clinical findings. It is notable that the laboratory phenotype in patients with a mutation in a specific gene, for example *RAG1*, *PLCG2* or *GATA2*, is generally more consistent than the physical signs and symptoms [37–39].

Murine Models

Although murine models have been very useful in highlighting proteins that are essential for particular pathways and in suggesting appropriate gene candidates for some clinical disorders, mutations in some genes result in a different phenotype in humans compared to mice. Mutations in several genes required for B cell development, including the recently described defect in *PIK3RI* [4], result in an earlier and more severe block in B cell differentiation in humans compared to mice [40]. The reverse is true for mutations in *IL-7* receptor, which are associated with absent B cells in the mouse [41] but normal numbers of B cells in humans [42]. *IKK α* , *IKK β* , and *NEMO* form a critical complex required for canonical *NF κ -B* pathway activation. Null mutations in *IKK β* (encoded by *IKBKB*) are lethal in mice [43,44] but cause a SCID-like disorder characterized by normal numbers of T cells and B cells in patients [45]. Heterozygous loss of function mutations in the ribosomal protein *RPSA* are associated with isolated congenital asplenia in humans but heterozygous null mutations in the same gene have no obvious phenotype in mice [11].

In some ways, murine models resemble descriptions of a single patient with a genetic defect. The mice share a genetic background and environment that do not elicit the range of effects that may be seen in unrelated patients who have mutations in the same gene. The types of exposures experienced by patients who live in the community are more variable than those provided in a laboratory setting and give a more accurate picture of the gene function.

Future Directions

Many of the recently described genetic defects provide satisfying answers to our diagnostic dilemmas but most of them also stimulate us with new questions. Some of the mutant genes are widely expressed but the phenotype is quite narrow. Examples include *STAT1* [25,26], *PIK3RI* [4] and *TCF3* [17]. Is the protein redundant in other cell lineages? Is regulation of protein modification, protein stability or potential binding partners different in different cell lines? For some of the recently reported genetic disorders, the mechanism by which the mutant gene causes the phenotype is completely unknown. WES revealed heterozygous loss of function mutations in the broadly expressed ribosomal processing gene *RPSA* in 18 patients from 8 kindreds with isolated congenital asplenia [11]. It is not clear if the ribosomal protein is required for the processing of particular transcripts that are required for development or survival of the splenic anlage or whether *RPSA* has additional functions unrelated to the ribosome.

Careful analysis of the disease genes reported here helps clarify the pathways that are required for normal immune function and, by so doing, enhances our understanding of more common disorders, including autoimmunity and cancer. In the case of *PIK3CD*, identification of the mutant gene suggests potential treatment modalities [12,13]. More targeted therapies are likely to be forthcoming.

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Highlights

- Next generation sequencing has resulted in an explosion of new immunodeficiency genes (85)
- Some clinical heterogeneity is explained by gain versus loss of function mutations (82)
- The same mutation in the same gene may be associated with very different phenotypes (83)
- A surprising number of newly defined disorders are the result of de novo mutations (83)
- Mutant genes can result in different phenotypes in mice versus humans (69)

Table 1Novel PID genotypes or phenotypes discovered exclusively or partly by NGS^a.

Gene (Protein)	Inheritance	Phenotype	Reference
2010:			
<i>STIM1</i>	AR ^b	Kaposi sarcoma	[46]
<i>FADD</i>	AR ^b	Infections, auto-immunity	[47]
2011:			
<i>MAGT1</i>	XR	T cell deficiency	[48]
<i>ZBTB24 (ICF2)</i>	AR ^b	B cell deficiency, dysmorphism	[49]
<i>STAT1</i>	AD ^c (GOF)	Candidiasis, auto-immune thyroiditis	[25,26]
<i>GATA2</i>	AD	Infections, cytopenias and leukemia	[7–10]
2012:			
<i>RHOH</i>	AR ^b	T cell deficiency	[50]
<i>CARD11</i>	AD (GOF)	B lymphocytosis	[23]
<i>MCM4</i>	AR ^b	NK cell and adrenal deficiency	[51,52]
<i>POLE</i> (POLε1)	AR ^b	Bacterial infections, dysmorphism	[53]
<i>TBK1</i>	AD	Herpes simplex encephalitis	[54]
<i>CARD14</i>	AD ^c (GOF)	Psoriasis and related conditions	[55–57]
<i>PLCG2</i> (PLCγ2)	AD ^c (GOF)	Infections, autoimmunity, cold uricaria	[18,38]
<i>PIK3R1</i> (p85)	AR ^b	Absent B cells, agammaglobulinemia	[4]
<i>ADAR</i>	AR ^b	Aicardi-Goutières Syndrome	[58]
<i>ISG15</i>	AR ^b	Mycobacterial disease	[59]
<i>RBCK1</i> (HOIL1)	AR ^b	Infections, autoinflammation	[36]
<i>CD27</i>	AR ^b	Epstein-Barr virus disease	[60]
<i>LRBA</i>	AR ^b	Infections, autoimmunity	[14–16]
2013:			
<i>MALT1</i>	AR ^b	T cell deficiency	[5]
<i>IL21R</i>	AR ^b	T and B cell deficiency	[61]
<i>CARD11</i>	AR ^b (LOF)	T cell deficiency	[21,22]
<i>TNFRSF4</i> (OX40)	AR ^b	Kaposi sarcoma	[62]
<i>TTC7A</i>	AR ^b	T cell deficiency, intestinal atresia	[63,64]
<i>TRAF3IP2</i> (ACT1)	AR ^b	Candidiasis	[6]
<i>TCF3</i> (E47)	AD ^c	Absent B cells, agammaglobulinemia	[17]
<i>TNFSF12</i> (TWEAK)	AD	B cell deficiency	[65]
<i>NFKB2</i> (p105/p52)	AD ^c	B cell and adrenal insufficiency	[66]
<i>PRKCD</i> (PKCδ)	AR ^b	Autoimmunity	[67–69]
<i>PIK3CD</i> (p110δ)	AD ^c (GOF)	T and B cell deficiency	[12]
<i>RPSA</i>	AD ^c	Isolated congenital asplenia	[11]

Gene (Protein)	Inheritance	Phenotype	Reference
<i>VPS45</i>	AR ^b	Neutropenia, enlarged kidneys	[70, 71]
<i>RTEL1</i>	AR	Pancytopenia, hypogammaglobulinemia	[72, 73]
<i>IKKB</i> (IKK β)	AR ^b	Various infections, hypogammaglobulinemia	[45, 74]

Abbreviations: AR = autosomal recessive; AD = autosomal dominant; XR = X-linked recessive; GOF = gain of function; LOF = loss of function

^a. The genes listed are novel genotypes underlying PIDS, with one exception (STIM1 deficiency in a child with Kaposi sarcoma, which was the first use of WES for PIDS). NGS was used alone, or with linkage, or after a candidate gene was found mutated to exclude other lesions, in at least one study per gene defect. In most, but not all cases, WES was employed.

^b. At least one family is consanguineous.

^c. At least one mutation is *de novo*.

7–10**

References 7–10 indicate that the clinical phenotype in patients with heterozygous mutations in the transcription factor GATA2 is highly variable but includes adult onset mycobacterial and fungal infections, disseminated warts, myelodysplasia and leukemia, deafness and lymphedema. Penetrance is incomplete.

12–13**

References 12 and 13 show that heterozygous activating mutations in PIK3CD, which encodes the hematopoietic specific p110 δ catalytic domain of PI3 kinase, cause a syndrome of recurrent sino-pulmonary infections, EBV or CMV viremia, elevated serum IgM. Lymphocytes from the patients demonstrate increased activation induced cell death and an exhausted phenotype.

14–16**

Genetic studies reported in references 14, 15 and 16 show that mutations in LRBA (LPS responsive beige-like anchor) result in increased susceptibility to infection and autoimmune disease, particularly thrombocytopenia and enteropathy. The mechanisms by which this broadly expressed gene cause disease are not yet known.

26–27**

References 26 and 27 show that patients with heterozygous gain-of-function mutations in STAT1 display chronic mucocutaneous candidiasis (CMC) and auto-immune thyroiditis. Their cells are hyper-responsive to STAT1-dependent signals triggered by IFNs and IL-27. This results in impaired development of IL-17-producing T cells, underlying CMC.