Atypical Manifestation of LRBA Deficiency with Predominant IBD-like Phenotype

Nina Kathrin Serwas, MSc,* Aydan Kansu, MD, † Elisangela Santos-Valente, MD,* Zarife Kuloğlu, MD, ‡ Arzu Demir, MD, † Aytaç Yaman, MD, † Laura Yaneth Gamez Diaz, MSc, † Reha Artan, MD, †* Ersein Sayar, MD, †* Arzu Ensari, MD,* Bodo Grimbacher, MD, †* and Kaan Boztug, MD**

Background: Inflammatory bowel diseases (IBDs) denote a heterogeneous group of disorders associated with an imbalance of gut microbiome and the immune system. Importance of the immune system in the gut is endorsed by the presence of IBD-like symptoms in several primary immunodeficiencies. A fraction of early-onset IBDs presenting with more severe disease course and incomplete response to conventional treatment is assumed to be inherited in a Mendelian fashion, as exemplified by the recent discovery of interleukin (IL)-10 (receptor) deficiency.

Methods: We analyzed a patient born to consanguineous parents suffering from severe intestinal manifestations since 6 months of age and later diagnosed as IBD. Eventually, she developed autoimmune manifestations including thyroiditis and type 1 diabetes at the age of 6 and 9 years, respectively. Combined single-nucleotide polymorphism array-based homozygosity mapping and exome sequencing was performed to identify the underlying genetic defect. Protein structural predictions were calculated using I-TASSER. Immunoblot was performed to assess protein expression. Flow cytometric analysis was applied to investigate B-cell subpopulations.

Results: We identified a homozygous missense mutation (p.Ile2824Pro) in lipopolysaccharide-responsive and beige-like anchor (LRBA) affecting the C-terminal WD40 domain of the protein. In contrast to previously published LRBA-deficient patients, the mutant protein was expressed at similar levels to healthy controls. Immunophenotyping of the index patient revealed normal B-cell subpopulations except increased CD21
d B cells.

Conclusions: We describe a patient with a novel missense mutation in LRBA who presented with IBD-like symptoms at early age, illustrating that LRBA deficiency should be considered in the differential diagnosis for IBD-like disease even in the absence of overt immunodeficiency.

Key Words: LRBA, inflammatory bowel disease, autoimmunity, exome sequencing

T he gastrointestinal tract represents the largest interface of the organism with the environment and is constantly confronted with foreign antigens and bacteria, which may elicit either beneficial or pathogenic effects. Whether the outcome is beneficial is determined by a variety of systems, including the immune system (reviewed in Ref. 1). Effector functions of the immune system in the gut are tightly regulated as inadequate activation may have destructive effects on the bowel (reviewed in Ref. 2). Inflammatory bowel diseases (IBDs) represent a group of diseases resulting from pathologically increased activation of host defense systems leading to severe inflammation and diarrhea (reviewed in Ref. 3). Conversely, primary immunodeficiency disorders including common variable immunodeficiencies (CVIDs) are also associated with IBD-like manifestations. It has been hypothesized that for IBDs, disease onset is both partially environmentally and partially genetically driven (reviewed in Ref. 5). Recent studies have identified mono- genetic causes of IBD, which may explain early disease onset in particular cases where the relative contribution of host genetics will arguably be the highest (reviewed in Ref. 6). For instance, mutations affecting the interleukin (IL) 10 (receptor), ADAM17, XIAP, and TERC1 genes have recently been identified as monogenic causes of very early-onset IBD (onset before 6 yr of age).

The diagnosis in these cases is difficult due to the unusual phenotype and lack of specific laboratory signs of intestinal inflammation (reviewed Ref. 6). A large proportion of patients with very early- or early-onset IBD (symptoms before 10 yr of age) remain...
molecularly unclassified.\textsuperscript{14,15} Early detection of such diseases and identification of the underlying causative genetic aberration(s) may improve the treatment strategies and enable further understanding of the pathogenic mechanisms underlying IBD.

We here describe, for the first time, a patient with very early-onset intestinal manifestations and diagnosed as IBD later in her life in whom we identified a biallelic mutation affecting the CVID-related gene \textit{lipopolysaccharide-responsive and beige-like anchor (LRBA)}.

**MATERIAL AND METHODS**

**Patient**

The described study was performed according to the Helsinki Declaration and approved by the local ethics committee. All investigated individuals signed informed consent documents. The patient was treated at the Department of Pediatric Gastroenterology at Ankara University and at the Department of Pediatric Gastroenterology at Akdeniz University in Turkey.

**Immunohistochemistry**

Staining against CD3 was performed using an anti-CD3 antibody (DAKO, United Kingdom) combined with streptavidin–peroxidase method. Hemosiderin staining was used to detect iron deposition in hepatocytes (Prussian blue staining).

**Homozygosity Mapping**

Homozygous regions were mapped using Affymetrix 6.0 SNP arrays (Affymetrix, High Wycombe, United Kingdom) as previously described\textsuperscript{16} with minor modifications. In brief, genomic DNA was digested using the enzymes NspI and StyI (New England Biolabs, Frankfurt, Germany). Fragmented DNA was purified using Agencourt AMPure XP magnetic beads (Beckman Coulter, Vienna, Austria) and ligated to adapters, which were labeled and hybridized to the chips. Analysis was done using Genotyping console (Affymetrix) and the online tool homozygositymapper.org\textsuperscript{17} (accession date February 10, 2014).

**Exome Sequencing**

The index patient’s exome was sequenced applying the Nextera exome enrichment kit (Illumina, Eindhoven, the Netherlands) according to manufacturer’s recommendation. In brief, 50 ng of genomic DNA (gDNA) extracted from whole blood were subjected to transposase-based in vitro shotgun library preparation (tagmentation), which introduced adapters into the genomic DNA, while fragmenting it into 300 to 500 bp segments.\textsuperscript{18} The adapter sequence was used to amplify fragmented gDNA in a limited cycle polymerase chain reaction. The DNA was enriched for exonic fragments, which were also amplified. Clusters were generated on a cBot Cluster Generation System (Illumina) applying the SE cluster kit v3 (Illumina) and sequenced on an Illumina HiSeq 2000 (Illumina) applying 3-plexed 50 bp single-end sequencing. Sequences were demultiplexed and aligned to the human genome 19 with Burrows-Wheeler Aligner version 0.5.9. Insertion/deletion realignment, quality score recalibration, and variant calling were done applying the genome analysis toolkit version 1.6\textsuperscript{19}. Annotation of single nucleotide variants, insertion and deletions were performed with ANNOVAR.\textsuperscript{20} Common variants listed in dbSNP 137 were excluded from further analysis. Validation of the identified variants in LRBA was performed using conventional Sanger sequencing.

**FACS Analysis**

Peripheral blood monocytes were isolated from shipped blood samples after 2 days using Ficoll Paque PLUS (VWR International GmbH, Vienna, Austria) and stored in 90\% FBS (PAA Laboratories GmbH, Pasching, Austria) and 10\% dimethyl sulfoxide (Sigma-Aldrich Handels GmbH, Vienna, Austria) in liquid nitrogen. After thawing of peripheral blood monocytes, surface molecules were blocked in RPMI (PAA Laboratories GmbH) supplemented with 10\% FBS. Staining was performed 30 minutes on ice using the following antibodies: CD19-PerCP-Cy5.5 (eBioscience; Vienna, Austria); CD3-APC-H7; CD4-APC; CD8-V501; IgD-FITC; IgM-APC; CD27-Brilliant violet; CD38-PECy7; CD21-PE (all: Becton Dickinson Austria GmbH; Vienna, Austria); CD14-PECy7; CD56-PE (both: Beckman Coulter; Vienna, Austria). Cells were analyzed on a LSR Fortessa (Becton Dickinson Austria GmbH; Vienna, Austria).

**Protein 3D Structure Modeling**

The protein model covering the beige and Chediak-Higashi (BEACH)–WD40 domain of LRBA (amino acids 2073–2863; NP_006717.2) was calculated using I-TASSER.\textsuperscript{21} Secondary structures were assigned with the program ICM-Browser (Molsoft LLC, San Diego, CA). Phylogenetic conservation analysis was performed using Polyphen-2 (version 2.2.2r398).\textsuperscript{22}

**Western Blot**

Patient granulocytes were isolated without applying density centrifugation with Ficoll Paque PLUS (VWR International GmbH; Vienna, Austria) and stored at $-80^\circ$C. Healthy donor granulocytes were isolated from fresh blood stimulated or not with 100 ng/mL LPS (Sigma-Aldrich, Handels GmbH) and directly lysed. Lysis was performed using RIPA buffer (1% NP40, 0.1% SDS, 0.5% sodium deoxycholate, 150 mM NaCl, and 10 mM Tris-HCl pH 7.5). 50 μg of protein were loaded on a gradient of 12% and 8% polyacrylamide gel and subjected to gel electrophoresis. Cell lysates were blotted onto an immobilon polyvinylidene difluoride membrane (Roche, Grenzach-Wyhlen, Germany) for 4 hours at 45 V and then stained with the primary antibodies anti-LRBA (Sigma-Aldrich Handels GmbH) and anti-tubulin (Abcam, Cambridge, United Kingdom) as well as the secondary anti-rabbit antibody coupled to horseradish peroxidase.

**RESULTS**

The index patient is a female born at term in 1998 to healthy consanguineous parents of Turkish origin. Her sister is healthy except for hypothyroidism without detectable autoantibodies (Fig. 1A).

Symptoms of nonmucoid and nonbloody diarrhea commenced at the age of 6 months (Fig. 1B). Persisting diarrhea was
accompanied by severe edema due to hypoalbuminemia prompting recurrent albumine infusions. Other routine laboratory tests were normal. Known causes of persistent diarrhea such as congenital lactase deficiency, congenital chloride diarrhea, microvillous inclusion disease, primary intestinal lymphangiectasia, and food-induced enteropathy had been excluded by clinical history and appropriate laboratory tests. At the age of 1.5 years, a diagnosis of celiac disease was considered after endoscopic biopsy revealed diffuse villous atrophy, crypt hyperplasia, and intraepithelial lymphocytosis in duodenum (see Fig., Supplemental Digital Content 1, http://links.lww.com/IBD/A645). However, introduction of gluten-free diet did not induce remission of the disease, thus making this diagnosis highly unlikely. In addition, tissue transglutaminase antibodies were normal (0.4 U/mL; reference value <10 U/mL), and screening for HLA DQ2 and DQ8 was negative when assessed later in her life.

At the age of 6 years, she was diagnosed with autoimmune thyroiditis based on the presence of autoantibodies, and thyroid hormone therapy was initiated. Three years later, the index patient presented with type 1 diabetes mellitus. From 9 to 11 years of age, the patient remained clinically stable despite some episodes of diarrhea, which did not require hospitalization.

At the age of 11 years, the clinical course deteriorated with increasing diarrhea and a weight loss of 15 kg within a year. When she was 12 years old, another endoscopy was performed also showing diffuse villous atrophy, crypt hyperplasia, and intraepithelial lymphocytosis in duodenum. In addition, a colonoscopy revealed crypt epithelium injury and regenerative inflammation in the histology examination. Reanalysis of this biopsy at the age of 13 years showed T-cell mediated epithelium destruction based on autoinflammatory processes (Fig. 1C).
Those findings suggested autoimmune enteropathy or IBD. Autoimmune polyglandular syndrome, IPEX-like syndrome, and mitochondrial disease were excluded through Sanger sequencing of AIRE, IL2RA, CD25, TYMP, and POLG genes, respectively. Steroid therapy to treat the deterioration of the intestinal manifestations was started and resulted in clinical improvement. After 3 months, recurrence of intense diarrhea motivated the addition of cyclosporine A (CsA) to her therapy. This combined therapy induced partial improvement and was continued for 5 months.

At the age of 13 years, the patient presented with cachexia (21 kg, 130 cm), severe diarrhea, finger clubbing (Fig. 1D), and long-standing severe anal fissure and skin tags (Fig. 1E). The finger clubbing had not been previously noticed, whereas the anal fissures and skin tags had already been present for 2 to 3 years. It remains unclear whether the finger clubbing may have been related to therapy with CsA and/or the autoimmune thyroiditis (despite euthyroid state under therapy). Those clinical manifestations, together with the previously described colonoscopy results, suggested the diagnosis of IBD and prompted reinitiation of treatment with steroids and CsA.

After 30 days of combined therapy, the patient developed renal, respiratory and cardiac failure, bicytopenia, pleural effusion, pericardial effusion, ascites, splenomegaly, deranged coagulation tests, and direct hyperbilirubinemia. Additional laboratory investigations evidenced thrombotic microangiopathy and thrombocytopenia associated with multiple organ failure possibly associated with CsA treatment. In light of these severe reactions, the combined immunosuppressive therapy was immediately discontinued, with subsequent recovery of the patient. After this crisis, she showed lactate and ammonium elevations, pseudo-obstruction, and metabolic acidosis attacks intermittently. Magnetic resonance imaging revealed cerebral and cerebellar atrophy, whereas MR spectroscopy was normal. Electromyogram showed demyelinating polyneuropathy. Autoantibodies other than thyroid autoantibodies (ANA, AMA, ASMA, TTG IgA, ANCA, anti dsDNA, LKM) were negative.

The presence of the thrombocytopenia and anemia prompted bone marrow aspiration which showed no abnormalities, suggesting that the bicytopenia was caused by peripheral destruction. Liver biopsy revealed fibrosis in portal area, perportal fibrosis, perisinusoidal fibrosis, patchy cholestatic findings, and hemosiderosis (Fig. 2A).

The clinical picture continued to deteriorate with persistent diarrhea and lack of weight gain despite enteral and parenteral nutrition support. Additional invasive examinations could not be performed because of her poor clinical condition.

To exclude that her symptoms were caused by an underlying CVID, we performed extensive immunophenotyping. The patient, however, did not at any time presented with either recurrent/severe infections or serum reduction of specific immunoglobulin subtypes. B- and T-lymphocyte counts were also within normal range (Fig. 2B). The evaluation of specific subgroups of B lymphocytes revealed normal numbers of class-switched IgD and CD27+ B cells (Fig. 2B). Interestingly, she presented with increased numbers of CD21low B cells.
Giving the early disease onset, a monogenetic cause for the disease was suspected. As the patient was born to consanguineous parents, we assumed an autosomal recessive mode of inheritance. Thus, we performed homozygosity mapping using Affymetrix 6.0 Genotyping SNP arrays. Calculations using homozygosity mapper revealed 2 homozygous stretches with the maximal homozygosity score 1000 each on chromosomes 4 and 7 (Fig. 3A). The patient’s DNA was subjected to exome sequencing which revealed a total of 43,772,399 reads that could be mapped uniquely to the genome (98.38% of total reads), resulting in a mean coverage of 19 reads per base. Exome sequencing revealed 5 variants in 4 genes (Table 1) fulfilling the criteria of novel nonsense, missense, or splice-site variants located inside the homozygous candidate intervals, among them 2 single nucleotide exchanges of neighboring nucleotides in the gene encoding LPS-responsive and beige-like anchor protein (LRBA; NP_006717.2). The variants affecting adjacent nucleotide positions (c.A8470C; c.T8471C) in LRBA lead to an amino acid exchange within the C-terminus of the protein (p.Ile2824Pro). Both variants were validated using conventional Sanger sequencing and showed perfect segregation under the assumption of autosomal-recessive inheritance with full penetrance (Fig. 3B). The mutated residue Ile-2824 is highly conserved throughout vertebrate evolution (Fig. 3C). Protein 3D structure modeling of the C-terminal region (amino acids 2073-2863) predicted the exchanged amino acid to be located in one of the 5 WD40 domains at...
the C-terminus (Fig. 3D, E). Polyphen-2 calculations predicted the mutation as probably damaging with a score of 0.985 (maximum 1). Immunoblot analysis showed that the mutation in LRBA allowed for protein expression at a similar level as in a healthy control (Fig. 3F).

**DISCUSSION**

Recently identified monogenic forms of IBD such as IL-10 (receptor) deficiency,\(^7,^8\) XIAP deficiency,\(^10\) TTC7A deficiency,\(^11\) and ADAM17 deficiency\(^12\) are associated with very early and severe onset of the disease. These patients often do not respond well to conventional therapy (reviewed in Ref. 6).

Here, we describe a female patient whose main symptom was very early-onset and treatment-resistant nonmucoid, nonbloody diarrhea. She developed signs of autoimmunity, such as thyroiditis, at the age of 6 and diabetes mellitus type 1 at the age of 9 years. Histological analysis of duodenal biopsies revealed T-cell mediated epithelial destruction, thus enabling a diagnosis of IBD at the age of 13 years. Retrospectively, the diagnosis of early-onset IBD could be considered if early supporting evidence of intestinal inflammation had been available. However, as she was under treatment at a rural hospital until the age of 13, such evidence was not available.

Exome sequencing covering more than 98% of coding genomic region revealed no variants or mutations with minor allele frequency of less than 1% in the IL10, IL10RA, IL10RB, XIAP, ADAM17, or TTC7A genes, respectively. Further genes related to polyglandular autoimmune syndrome or other diseases that might explain the phenotype of the patient were also not recovered by combined homozygosity mapping and exome sequencing. Heterozygous mutations were excluded from the analysis as they would lead to symmetrical inheritance of the disorder. Homozygous missense variants in the 4 genes, ABCE1, LRBA, SCIN, and DNAH11, were identified (Table 1). ABCE1 encodes the protein adenosine triphosphate-binding cassette subfamily E member 1, which is a negative regulator of RNAse L.\(^23\) SCIN encodes the protein adseverin, which is an actin capping and serving protein.\(^24\) Deleterious mutations in DNAH11 are causative for situs inversus totalis.\(^25\) Regarding the index patient, none of these 3 genes can be easily linked to her phenotype. Mutations in the fourth gene LRBA have been recently identified as a cause of a CVID associated with autoimmunity and IBD-like disease,\(^26–^28\) which prompted us to further investigations.

LRBA was first identified as a lipopolysaccharide responsive gene in B cells and macrophages whose protein structure is similar to the lysosomal-trafficking regulator LYST.\(^29\) Both LYST and LRBA belong to the group of BEACH-domain-containing proteins, which consists of 9 human proteins (reviewed in Ref. 30). Apart from mutations in LRBA, 3 other BEACH-domain-containing genes have been implicated in autosomal recessive Mendelian disorders. Homozygous LYST mutations lead to Chediak–Higashi syndrome;\(^31\) homozygous Neurobeachin-like 2 mutations result in gray platelet syndrome;\(^25–^34\) and biallelic WD repeat domain 81 mutations result in a cerebellar ataxia, mental retardation, and dysequilibrium syndrome.\(^35\) Two of these Mendelian disorders, namely Chediak–Higashi syndrome and LRBA deficiency, result in reduced immune functions.

The functional role of BEACH-domain-containing proteins remains elusive. It has been speculated that these proteins are involved in membrane dynamics and vesicular transport (reviewed in Ref. 30). LRBA has been shown to colocalize with lysosomes, ER, and the Golgi complex, respectively.\(^36\) Furthermore, it has been implicated as a negative regulator of apoptosis as it is overexpressed in several cancers.\(^36\) This is in concordance with the increased apoptosis in B cells, which has been described in LRBA-deficient patients.\(^37\) Elevated cell death might be due to defective autophagy.\(^37\) Interestingly, our patient did not show reduced numbers of B cells (Fig. 2B). Whether autophagy is affected in the index patient could not be determined.

So far, only a limited number of patients with LRBA deficiency have been published.\(^26–^28\) Interestingly, all previously described patients bear mutations that lead to absence of protein expression.\(^26–^28\) One of the identified patients presented with a missense mutation in LRBA located inside the WD40 domains (p.Ile2657Ser) similar to the index patient (p.Ile2824Pro).\(^27\) The reason why the mutation p.Ile2657Ser results in an absent protein in contrast to the mutation p.Ile2824Pro might be explained by the different location of the amino acids in the protein. Protein 3D structure modeling of the BEACH-WD40 domain of LRBA revealed that the amino acid Ile-2657 is located in close proximity to the BEACH domain (Fig. 3D), which might potentially be crucial for protein stability. However, the amino acid Ile-2824 is located between the last 2 β-sheets of the WD40 β-propeller (Fig. 3D) and does not

**TABLE 1. Identification of 5 Variants Within the Homozygous Intervals**

| Gene      | Function | Nucleotide | Amino Acid | Chromosome | Position | Reference | Observed |
|-----------|----------|------------|------------|------------|----------|-----------|----------|
| ABCE1     | SNV      | c.727G     | p.243A     | 4          | 146033407| C         | G        |
| LRBA      | SNV      | c.8471C    | p.1284T    | 4          | 151199035| A         | G        |
| LRBA      | SNV      | c.8470C    | p.1284L    | 4          | 151199036| T         | G        |
| SCIN      | SNV      | c.274T     | p.192F     | 7          | 12666242 | A         | T        |
| DNAH11    | SNV      | c.3200G    | p.1067R    | 4          | 21640493 | A         | G        |

www.ibdjournal.org | 45

Downloaded from https://academic.oup.com/ibdjournal/article-abstract/21/1/40/4604228 by guest on 15 March 2020

Copyright © 2014 Crohn’s & Colitis Foundation of America, Inc. Unauthorized reproduction of this article is prohibited.
significantly influence the stability of the protein, as it is still detectable (Fig. 3F). Because there is currently no simple test for intact function of LRBA protein, we cannot formally assess whether the mutation described here allows for residual protein function.

LRBA-deficient patients present with heterogeneity of clinical symptoms with no clear genotype to phenotype correlation. Common features of LRBA-deficient patients are quantitative and/or qualitative B-cell defects as well as autoimmunity.26,28 Nine of 11 published patients to date present with autoimmune IBD-like manifestations.26,27 Another common feature is recurrence of pulmonary infections. The patient described here differs from those previously described because her leading symptom was a potential IBD-like disease, starting at the age of 6 months and diagnosed as IBD when she was 13 years old. Further autoimmune features only manifested later in her life.

B-cell phenotyping at the age of 14 years revealed increased numbers of CD21<sup>hi</sup> B cells. This subgroup of B cells has been associated with autoimmunity in patients suffering from CVID27 but has not been reported for LRBA deficiency to date. Overall B-cell numbers and numbers of class-switched B cells were not affected in the index patient, and she did not show any pulmonary complications. Also in contrast to other LRBA-deficient patients,26,27 the index patient does not fulfill the formal criteria for a CVID diagnosis.38,39

To our knowledge, this is the first LRBA-mutant patient presenting exclusively with gastrointestinal symptoms in the first few months of life. Only one previously reported patient bears some similarities to the patient described here as he also initially showed nonbloody diarrhea.26 The patient however presented in addition with autoimmunity and Epstein–Barr virus-associated lymphoproliferative disease very early in his life.26 We speculate that the differences in phenotype might be due to a hypomorphic nature of the variant in LRBA, as the missense mutation identified may allow for residual function of the corresponding gene product, although we cannot formally rule out the effects of the other variants found and of hidden intronic variants, which are not covered by exome sequencing.

Taken together, we describe for the first time a patient with a missense mutation in LRBA allowing for detectable protein expression (and potentially residual function) presenting exclusively with gastrointestinal manifestations at very young age, later diagnosed as IBD. LRBA deficiency thus represents an important molecular differential diagnosis for severe persisting IBD-like disease and related conditions.

REFERENCES

1. Furness JB, Rivera LR, Cho HJ, et al. The gut as a sensory organ. Nat Rev Gastroenterol Hepatol. 2013;10:729–740.
2. DuPont AW, DuPont HL. The intestinal microbiota and chronic disorders of the gut. Nat Rev Gastroenterol Hepatol. 2011;8:523–531.
3. Xavier RJ, Podolsky DK. Unravelling the pathogenesis of inflammatory bowel disease. Nature. 2007;448:427–434.
4. Agarwal S, Smerkala P, Harpaz N, et al. Characterization of immunologic defects in patients with common variable immunodeficiency (CVID) with intestinal disease. Inflamm Bowel Dis. 2011;17:251–259.
5. Khor B, Gardet A, Xavier RJ. Genetics and pathogenesis of inflammatory bowel disease. Nature. 2011;474:307–317.
6. Uhlig HH, Schwert T, Koletzko S, et al. The diagnostic approach to mono- genic very early onset inflammatory bowel disease. Gastroenterology. 2014; 147:990–1007.
7. Glocker EO, Kotlarz D, Boztug K, et al. Inflammatory bowel disease and mutations affecting the interleukin-10 receptor. N Engl J Med. 2009;361: 2033–2045.
8. Glocker EO, Frede N, Perro M, et al. Infant colitis–it’s in the genes. Lancet. 2010;376:1272.
9. Blaydon DC, Biancheri P, Di W-L, et al. Inflammatory skin and bowel disease linked to ADAM17 deletion. N Engl J Med. 2011;365:1502–1508.
10. Worthey EA, Mayer AN, Syverson GD, et al. Making a definitive diagnosis: successful clinical application of whole exome sequencing in a child with intractable inflammatory bowel disease. Genet Med. 2011;13:255–262.
11. Aftzur Y, Gao C, Mastropolo LA, et al. Mutations in Tetratricopeptide repeat domain 7A result in a severe form of very early onset inflammatory bowel disease. Gastroenterology. 2014;146:1028–1039.
12. Muise AM, Snapper SB, Kugathasan S. The age of gene discovery in very early onset inflammatory bowel disease. Gastroenterology. 2012;143:285–288.
13. Levine A, Griffiths A, Markowitz J, et al. Pediatric modification of the Montreal classification for inflammatory bowel disease: the Paris classification. Inflamm Bowel Dis. 2011;17:1314–1321.
14. Christodoulou K, Wiskin AE, Gibson J, et al. Next generation exome sequencing of paediatric inflammatory bowel disease patients identifies rare and novel variants in candidate genes. Gut. 2013;62:977–984.
15. Maisawa S, Sasaki M, Ida S, et al. Characteristics of inflammatory bowel disease with an onset before eight years of age: a multicenter epidemiological survey in Japan. J Gastroenterol Hepatol. 2013;28:499–504.
16. Salzer E, Santos-Valente E, Klaver S, et al. B cell deficiency and severe autoimmunity caused by deficiency of protein kinase C delta. Blood. 2013;121:3112–3116.
17. Seelow D, Schuelke M, Hildebrandt F, et al. HomozygosityMapper—an interactive approach to homozygosity mapping. Nucleic Acids Res. 2009; 37:W593–W599.
18. Caruccio N. Preparation of next-generation sequencing libraries using Nextera technology: simultaneous DNA fragmentation and adaptor tagging by in vitro transposition. Methods Mol Biol. 2011;733:241–255.
19. McKenna A, Hanna M, Banks E, et al. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res. 2010;20:1297–1303.
20. Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res. 2010;38:e164.
21. Roy A, Kucukural A, Zhang Y. I-TASSER: a unified platform for automated protein structure and function prediction. Nat Protoc. 2010;5: 725–738.
22. Adzhubei I, Jordan DM, Sunyaev SR. Predicting functional effect of human missense mutations using PolyPhen-2. Curr Protoc Hum Genet. 2013;76:7.20.1–7.20.41.
23. Biskup C, Martinand C, Silhol M, et al. Cloning and characterization of a RNase L inhibitor. A new component of the interferon-regulated 2-5A pathway. J Biol Chem. 1995;270:13308–13317.
24. Chumnamrtpa S, Lee WL, Nag S, et al. The crystal structure of the C-terminus of actinavin reveals the actin-binding interface. Proc Natl Acad Sci U S A. 2009;106:13719–13724.
25. Bartoloni L, Blouin JL, Pan Y, et al. Mutations in the DNAH11 (axonomal heavy chain dynein type 11) gene cause one form of situs inversus totalis and most likely primary ciliary dyskinesia. Proc Natl Acad Sci U S A. 2002;99:10282–10286.
26. Alangari A, Alsultan A, Adly N, et al. LPS-responsive beige-like anchor (LRBA) gene mutation in a family with inflammatory bowel disease and combined immunodeficiency. J Allergy Clin Immunol. 2012;130:481 e2–488 e2.
27. Lopez-Herrera G, Tampella G, Pan-Hammarstrom Q, et al. Deleterious mutations in LRBA are associated with a syndrome of immune deficiency and autoimmunity. Am J Hum Genet. 2012;90:986–1001.
28. Burns SO, Zenner HL, Plagnol V, et al. LRBA gene deletion in a patient presenting with autoimmunity without hypogammaglobulinemia. *J Allergy Clin Immunol.* 2012;130:1428–1432.

29. Wang JW, Howson J, Haller E, et al. Identification of a novel lipopolysaccharide-inducible gene with key features of both A kinase anchor proteins and chs1/beige proteins. *J Immunol.* 2001;166:4586–4595.

30. Cullinane AR, Schaffer AA, Huizing M. The BEACH is hot: a LYST of emerging roles for BEACH-domain containing proteins in human disease. *Traffic.* 2013;14:749–766.

31. Nagle DL, Karim MA, Woolf EA, et al. Identification and mutation analysis of the complete gene for Chediak-Higashi syndrome. *Nat Genet.* 1996;14:307–311.

32. Albers CA, Cvejic A, Favier R, et al. Exome sequencing identifies NBEAL2 as the causative gene for gray platelet syndrome. *Nat Genet.* 2011;43:735–737.

33. Gunay-Aygun M, Falik-Zaacai TC, Vilboux T, et al. NBEAL2 is mutated in gray platelet syndrome and is required for biogenesis of platelet alpha-granules. *Nat Genet.* 2011;43:732–734.

34. Kahr WH, Hinckley J, Li L, et al. Mutations in NBEAL2, encoding a BEACH protein, cause gray platelet syndrome. *Nat Genet.* 2011;43:738–740.

35. Gulsuner S, Tekinay AB, Doerschner K, et al. Homozygosity mapping and targeted genomic sequencing reveal the gene responsible for cerebellar hypoplasia and quadrupedal locomotion in a consanguineous kindred. *Genome Res.* 2011;21:1995–2003.

36. Wang JW, Gamsby JJ, Highfill SL, et al. Deregulated expression of LRBA facilitates cancer cell growth. *Oncogene.* 2004;23:4089–4097.

37. Warnatz K, Wehr C, Drager R, et al. Expansion of CD19(hi)CD21(lo/neg) B cells in common variable immunodeficiency (CVID) patients with autoimmune cytopenia. *Immunobiology.* 2002;206:502–513.

38. Conley ME, Notarangelo LD, Etzioni A. Diagnostic criteria for primary immunodeficiencies. Representing PAGID (Pan-American group for immunodeficiency) and ESID (European Society for immunoodeficiencies). *Clin Immunol.* 1999;93:190–197.

39. Ameratunga R, Woon ST, Gillis D, et al. New diagnostic criteria for common variable immune deficiency (CVID), which may assist with decisions to treat with intravenous or subcutaneous immunoglobulin. *Clin Exp Immunol.* 2013;174:203–211.