Journal of Human Genetics (2019) 64:955–960
https://doi.org/10.1038/s10038-019-0631-3

BRIEF COMMUNICATION

Primary immunodeficiency with chronic enteropathy and developmental delay in a boy arising from a novel homozygous *RIPK1* variant

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Received: 20 April 2019 / Revised: 4 June 2019 / Accepted: 4 June 2019 / Published online: 18 June 2019

Abstract

Identification of genetic causes of primary monogenic immunodeficiencies would strengthen the current understanding of their immunopathology. Pathogenic variants in genes in association with tumor necrosis factor α (TNFα) signaling, including *OTULIN, TNFAIP3, RBCK1*, and *RNF31* cause human congenital autoinflammatory diseases with/without immunodeficiency. *RIPK1*, encoding a receptor interacting serine/threonine kinase 1, is present in protein complexes mediating signal transduction including TNF receptor 1. Biallelic loss-of-function variants in *RIPK1* were recently reported in individuals with primary immunodeficiency with intestinal bowel disease and arthritis. Here, we report a novel homozygous *RIPK1* variant in a boy with immunodeficiency and chronic enteropathy. Our patient exhibited severe motor delay and mild intellectual disability, which were previously unknown. The present results are expected to deepen the current understanding of clinical features based on *RIPK1* abnormalities.

Introduction

To date, numerous monogenic immunodeficiencies with neonatal to infantile onset have been reported [1–3]. Recently two independent groups described *RIPK1* variants in primary immunodeficiencies (OMIM # 618108) [4, 5]. RIPK1 is a key molecule to assess TNFα signaling in association with primary immunodeficiency, severe intestinal bowel disease (IBD) and arthritis, referred to as autosomal recessive immunodeficiency 57 [4]. Interestingly, one of the phenotypic features of *RIPK1* defect in humans was necroptosis (not apoptosis) due to increased phosphorylation of RIPK3 and MLKL, similar to the mouse RIP3 deficiency, suggesting abnormal cytokine responses with pleiotropic effects of *RIPK1* deficiency in multiple organs rather than sequential pathological changes from the immune to the digestive system [6–8]. Furthermore, *RIPK1* defect in humans reportedly results in various functional consequences in response to TNFα stimulation, including impairment of MAPK, NF-κß activation with multiple cytokine release reduction, and necroptosis with increasing interleukin 1ß (IL-1ß) release [4, 8]. These evidences suggest that *RIPK1* abnormalities may account for two primary

Supplementary information The online version of this article (https://doi.org/10.1038/s10038-019-0631-3) contains supplementary material, which is available to authorized users.

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phenotypes, i.e., immunodeficiency and autoinflammation, based on dysregulated cytokine release.

In this study, we report on a novel homozygous *RIPK1* variant in a patient from Brazilian consanguineous family. The patient shows unreported clinical features together with previously described immunodeficiency and an excessive inflammatory phenotype.

Material and methods

The proband (IV-4) was an 8-year-old Brazilian boy with immunodeficiency, chronic enteropathy, and severe growth delay. Whole-exome sequencing (WES) was performed for proband (IV-4) as previously reported (see also Supplementary Information) [9–15]. Sanger sequencing was performed to confirm the variant and its segregation. To verify the mutational pathogenicity, immunophenotyping of peripheral blood mononuclear cells by flow cytometry and reverse transcription (RT)-PCR were performed as previously described [4, 10, 12]. Detailed methods were described in Supplementary Information. The study protocol was approved by IRBs. Written informed consent was obtained from all participants.

Results

Detailed clinical information and data are summarized in Table 1, and S1, and Supplementary Information. The proband's parents (III-3 and III-4) were first-degree cousins (Fig. 1a). IV-4 had sepsis with ascites and hepatosplenomegaly immediately postpartum. Recurrent vomiting and persistent diarrhea occurred when he was 2 day and 6 months old, respectively. Gastroscopy at the age of 2 years revealed duodenitis and esophagitis, indicating IBD (Fig. 2 and Supplementary Information). At 4 years of age, he was diagnosed with chronic IBD by colonoscopy. He repeatedly experienced chronic cough, pneumonia, and bronchiolitis. At 5 years of age, he had disseminated varicella-zoster virus (VZV). Growth restriction was noted throughout his lifetime as his weight, length/height, and occipitofrontal circumference (OFC) were consistently below the 2.5th percentile. His overall development was delayed: social smile at the age of 2 months, meaningful words at 24 months, speaking sentences at 8 years, and head control at 36 months. He could not walk at the age of 8 years.

Whole-exome sequencing of IV-4 identified a novel homozygous *RIPK1* variant (NM_003804.5:c.636C>G:p.Tyr212*) (Fig. 1c). Sanger sequencing of the variant confirmed an autosomal recessive inheritance in this family (Fig. 1a, b). This variant is not present in our in-house Japanese control exomes database ($n = 575$) or other publicly available human variant databases. *RIPK1* mRNA levels were examined by RT-PCR using total RNA extracted from the patient-derived lymphoblastoid cells. *RIPK1* transcript was undetectable in IV-4, whereas *RIPK1* was expressed in an unrelated control individual. c.636C>G generates a premature stop codon, probably leading to nonsense-mediated mRNA decay (NMD). Moreover, the mutated transcript was significantly elevated after cycloheximide (CHX) treatment, supporting that c.636C>G:p.Tyr212* was indeed subjected to NMD (Fig. 1d).

Lymphocyte subsets were examined to clarify the patient's immunodeficiency. His immunological phenotype was characterized with a variable number of total T lymphocytes (CD3 +), CD4 + , CD8 + and persistent B lymphocytes (CD19 +), and NK cell (CD56 +) lymphopenia (Table S1). These data are concurrent with a medical history of infectious diseases such as disseminated VZV.

Discussion

In this study, we identified a novel *RIPK1* homozygous variant, c.636C>G:p.Tyr212*, in a Brazilian consanguineous family. The proband showed primary immunodeficiency, IBD, growth failure, and developmental delay.

Based on the clinical data of 12 reported patients and the present patient, primary immunodeficiency (13/13, 100%) and diarrhea (13/13, 100%) are consistently observed in *RIPK1* deficiency. All 13 affected individuals had infections, suggesting humoral immunodeficiency based on their clinical records. Four of six patients with severe CD4 + T cell lymphopenia of <500 cells/μL developed opportunistic infections, including disseminated VZV, deep-seated mycosis, and cytomegalovirus-associated esophagitis. This cutoff value may be useful for infectious disease control/management in *RIPK1* deficiency (Table 1). With regard to chronic autoinflammation, all 13 patients had gastrointestinal inflammatory lesions somewhere along the alimentary canal, and showed diarrhea (13/13, 100%) and colitis (12/12, 100%). Arthritis and skin lesions developed at later stages in four (31%) and five patients (38%), respectively. *RIPK1*-deficient patients had recurrent/persistent infections because of the primary immunodeficiency. Abnormal TNFα signaling under pathological conditions (i.e., recurrent/persistent infections lead to continuous activation of TNF receptor 1, Toll-like receptor 3 (TLR3), and TLR4 signaling, resulting in IL-1ß release) may modify these inflammatory phenotypes [4, 8]. All the patients displayed growth failure (13/13, 100%). Our patient displayed fetal growth restriction during pregnancy. Growth failure was also reported in *Ripk1*-deficient mice. Hence growth restriction should be highlighted

Table 1 Phenotypic landscape of RIPK1 deficiency

		Truncating variants			Missense variants					Microdeletion	
		Current case	Cuchet-Lourenço et al. [1]		Li et al. [2]					Cuchet-Lourenço et al. [1]	
Family ID			Family B	Family A	Family C	Family D	Family F	Family A	Family B and E	Family C	P/T
Nucleotide change		c.636C>G	c.688A>C	c.867_870delTTTA	c.1278C>A	c.954delG	c.1802G>A	c.1844T>C	c.1934C>T	c.460–133_688 +1702del	
Amino acid change		p.Tyr212*	p. Asn230Hisfs*14	p.Tyr289*	p.Tyr426*	p. Met318Ilefs*194	p.Cys601Tyr	p. Ile615Thr	p.Thr645Met	p.?	
Number of patients		1	1	2	1	1	3	1	2	1	
Sex		Male	Female	Male	Female	Female	Male/Female	Male	Female	Female	
Ethnicity		Brazilian	Arab	Pakistani	Caucasian	Arab	North African	Caucasian	Arab	Arab	
Country		Brazil	Saudi Arabia	UK	Germany	Saudi Arabia	Algeria	Poland	Kuwait/Israel	Saudi Arabia	
Consanguinity		+	+	+	+	+	+	NA	+	+	
Age of onset		1 Day	Soon after birth	1 Month	1 Day	1 Day	20 Days/ 3 month/ 6 months	6 Months	1 Month/ 1 day	4 Months	
Age of diagnosis		7 Years	NA	NA	Post mortem	Post mortem	3 Years/ 5 years 5 months/ 10 years 6 months	3 Years 6 months	3 Years 1 month/ 6 years 10 months	NA	
Immunodeficiency	Cellular immunodeficiency	+	+	+	+	NA	NA	NA	NA	+	6/7
	Humoral Immunodeficiency	+	+	+	+	+	+	+	+	+	13/13
	Hepatosplenomegaly	+	NA	+	+	NA	–	+	NA	NA	5/8
Chronic active inflammation	Abdominal pain	–	NA	NA	+	+	+	+	–/+	NA	7/9
	Vomiting	+	NA	+	NA	NA	NA	NA	NA	NA	3/3
	Diarrhea	+	+	+	+	+	+	+	+	+	13/13
	Oral lesions	–	+	+	–	–	+	+	–/+	NA	8/12
	Esophagitis	+	NA	NA	NA	+	–	+	NA	NA	3/6
	Gastritis	+	+	+	NA	+	–	+	NA	NA	6/9
	Colitis	+	+	+	+	+	+	+	NA/+	+	12/12
	Perianal disease	–	+	+	–	+	+	+	+	+	11/13
	Skin lesion	–	NA	+	+	–	–	+	–	NA	5/12
	Arthritis	–	+	+	NA	NA	NA	NA	+/NA	+	4/5
	Growth failure	+	+	+	+	+	+	+	+	+	13/13
	Imbalance of electrolyte	+	NA	NA	NA	NA	+ 2/3	NA	NA	NA	4/5

P/T total number of reported patients with symptoms/total number of patients regardless of symptoms, NA not available

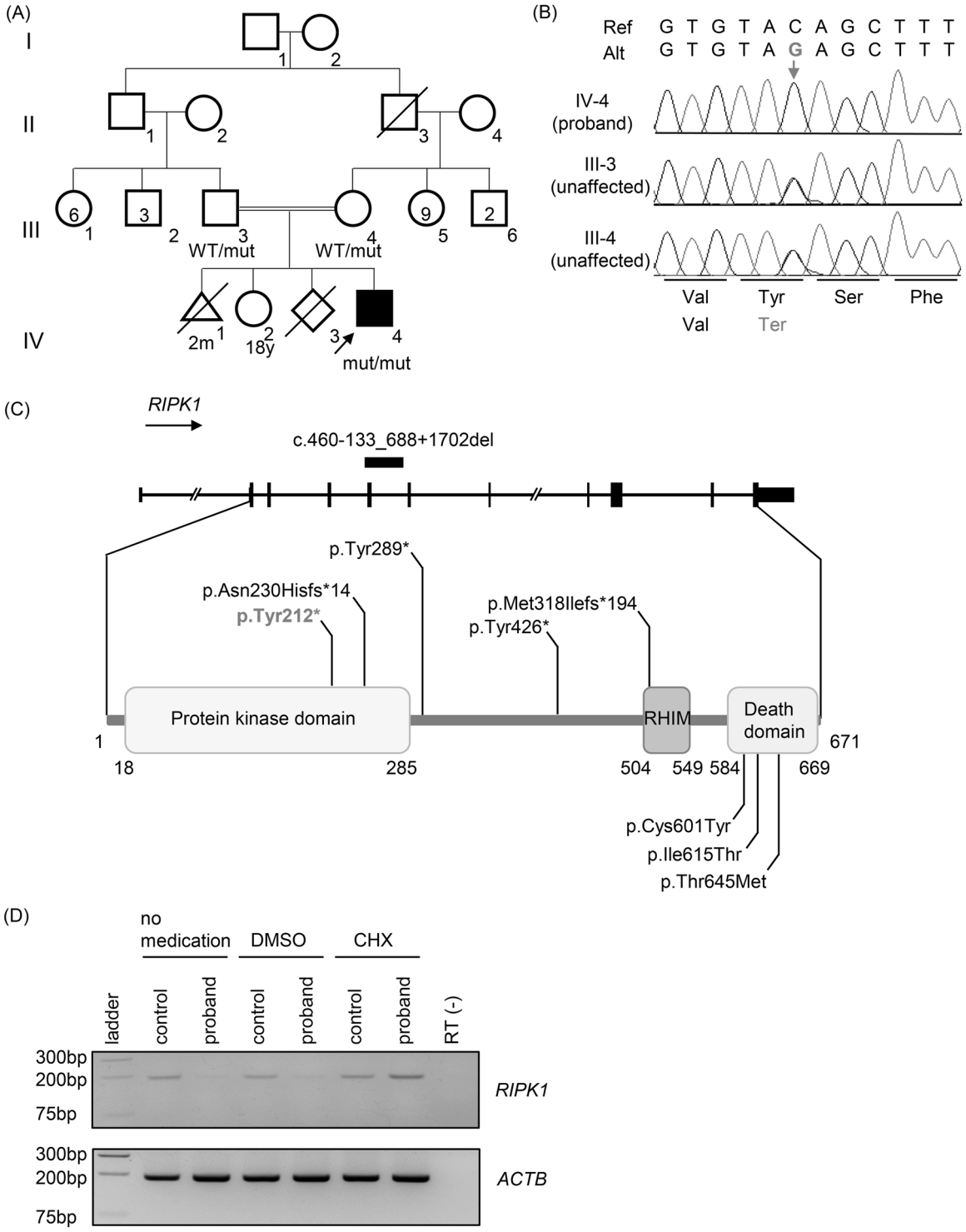

Fig. 1 Familial pedigree and genetic study. **a** Familial pedigree of primary immunodeficiency with IBD, growth failure, and developmental delay. WT/mut and mut/mut refer to a heterozygous and homozygous *RIPK1* variant, respectively. An arrow indicating the proband assessed by WES. **b** Electropherograms of samples from family members with a heterozygous or a homozygous *RIPK1* variant. Red arrow indicates the position of *RIPK1* variant. **c** A schematic representation of the *RIPK1* gene (upper) and protein structure (lower). The RIPK1 protein (amino acid residues 1–671) comprises protein kinase domain (18–285), intermediate domain with RIP homotypic interaction motif (RHIM, 504–549) and death domain (584–669). A small deletion is shown above the *RIPK1* gene. Pathogenic truncate and missense variants are shown above and below the RIPK1 protein, respectively. A novel variant identified herein is depicted in red. **d** *RIPK1* transcript analysis. RT-PCR was performed using the total RNA of IV-4 and an unrelated control individual as a template. Total RNA was extracted from the patient's lymphoblastoid cells treated with dimethyl sulfoxide (DMSO) or cycloheximide (CHX). RT (−), no reverse transcription

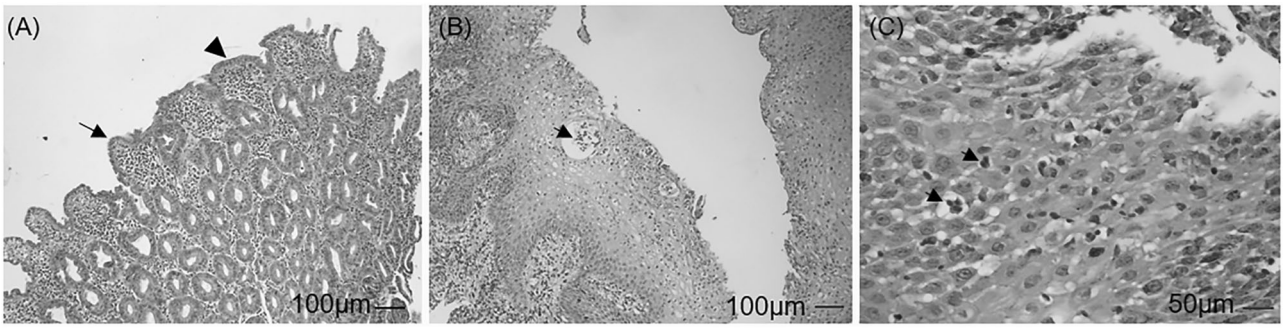

Fig. 2 Pathological findings of chronic inflammation in the digestive tract. Duodenal and esophageal biopsy (**a**–**c**). Chronic duodenitis, with villus atrophy (arrowhead) and few intraepithelial lymphocytes (arrow), hematoxylin and eosin staining, 200 × (**a**). Chronic esophagitis, with intraepithelial neutrophils, eosinophils and lymphocytes (arrows), hematoxylin and eosin staining, 200 × (**b**). Esophageal biopsy. Chronic esophagitis, with intraepithelial neutrophils, eosinophils and lymphocytes (arrows), hematoxylin and eosin staining, 200 × (**c**)

as a specific feature of *RIPK1* deficiency. Unexpectedly, our patient displayed severe motor delay and mild intellectual disability, both of which were not documented previously. In WES data, no pathogenic single nucleotide variants and copy number variations associated with motor delay and intellectual disabilities were noted. According to the GTEx (https://gtexportal.org/home/gene/ENSG00000137275.9) protein expression, *RIPK1* is expressed in multiple organs including those in the CNS (central nervous system), thus indicating *RIPK1* involvement in the CNS (Supplementary Information). Li et al. [5] reported that two patients showed tetany, with a suspicion of electrolyte imbalance. Concurrently, our patient presented hypocalcemia and hypokalemia, thus necessitating electrolyte monitoring in *RIPK1* deficiency [5].

IL-1ß inhibitors or other immunosuppressive agents may effectively ameliorate systemic inflammation in *RIPK1* deficiency as in IBD-like diseases [1, 16, 17]. However, recurrent infections are persistent even after medication. Thus, those treatments have conflicting effects for the infection control. Accordingly, hematopoietic stem cell transplantation (HSCT) in the early phase may be effective and even curative because it resolves both excessive inflammation and immune-deficiency. We emphasize that genetic diagnosis in the early phase using WES is important, such that patients may have an adequate therapeutic opportunity such as HSCT.

Acknowledgements This work was supported by AMED under the grant numbers JP18ek0109280, JP18dm0107090, JP18ek0109301, JP18ek0109348, and JP18kk020501; JSPS KAKENHI grant numbers JP17H01539, JP16H05160, JP16H05357, JP16H06254, JP17K10080, JP 17K15630, and JP17H06994; the Takeda Science Foundation; and the Ichiro Kanehara Foundation for the Promotion of Medical Science and Medical Care. We also thank N. Watanabe, T. Miyama, M. Sato, and K. Takabe for their technical assistance. We would like to thank Editage (www.editage.jp) for English language editing. We thank all patients and their families for their participation in this study.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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