A novel variant in *RUNX1* in a patient with refractory eosinophilic gastrointestinal disease and long-term clinical response to ketotifen

Ori Scott[a], Jenny Garkaby[a], Laura Abrego Fuentes[a], Chaim M. Roifman[a,b]*, and Jessica Willett Pachul[a]

ABSTRACT

Background: Eosinophilic gastrointestinal disease (EGID) is an umbrella term for a heterogeneous group of disorders affecting the GI tract. In contrast to the relatively common eosinophilic esophagitis (EoE), eosinophilic gastroenteritis (EGE) remains poorly understood in terms of both its pathophysiology and genetic etiology, while treatment options remain limited.

Aim: To expand the genotypic spectrum of EGE and describe our long-term experience of treatment with ketotifen.

Methods: Case report of a patient with EGE followed by our team for over 27 years.

Results: Our patient was diagnosed with EGE at the age of 4 years, accompanied by multiple other atopic manifestations and serum eosinophilia. He was later diagnosed with a heterozygous variant in *RUNX1*, a gene implicated in multi-lineage hematopoiesis, inhibition of Th2 polarization and T regulatory cell function. The patient has experienced long-term symptom improvement while treated with the mast cell stabilizing H1 antihistamine, ketotifen, with substantial symptomatic worsening after this agent was briefly stopped.

Conclusion: We expand the genotypic spectrum of EGID etiology to include mutations in *RUNX1*, and suggest ketotifen as a viable option for patients with treatment-refractory EGE.

Statement of novelty: This case reports on a possible novel genetic cause of EGID and describes long-term successful clinical management with ketotifen.

Introduction

Eosinophilic gastrointestinal disease (EGID) represents a wide spectrum of conditions marked by inflammatory eosinophilic infiltration of the GI tract, including eosinophilic esophagitis (EoE), gastroenteritis (EGE), and colitis (EoC). EoE is the most common form of EGID, with an estimated prevalence of 1:2000, while EGE remains rarer (estimated prevalence of 2–5:100 000), although this latter figure likely represents a substantial underestimation due to heterogeneity of symptoms and a lack of clear, standardized diagnostic criteria (Gonsalves 2019; Rossi et al. 2022; Dellon and Spergel 2022). In line with our incomplete clinical understanding of EGID, its genetic etiology has not been fully elucidated. Single nucleotide polymorphisms (SNPs) in genetic loci associated with barrier response to tissue damage and Th2 immunity have been implicated in

[a]Division of Immunology and Allergy, Department of Paediatrics, Hospital for Sick Children and University of Toronto, Toronto, ON; [b]The Canadian Centre for Primary Immunodeficiency and The Jeffrey Modell Research Laboratory for the Diagnosis of Primary Immunodeficiency, The Hospital for Sick Children, Toronto, ON

*Corresponding author: Chaim M. Roifman/chaim.roifman@sickkids.ca

Submitted 15 August 2022
Accepted 28 August 2022
Available online 30 August 2022

LymphoSign Journal 9:72–76 (2022)
dx.doi.org/10.14785/lymphosign-2022-0010

EoE, with notable examples including *CAPN14, STAT6*, and *TSLP* (Ryu et al. 2020). However, much less is known about the genetics of other forms of EGID.

Further to the above challenges in diagnosing EGID and establishing its etiology, therapeutic options for patients with EGID remain limited. In EoE patients, current approaches may involve both dietary and pharmaceutical interventions, including various degrees of elimination diets, proton pump inhibitors, steroids (topical and/or systemic), leukotriene receptor antagonists, mast cell stabilizers, and more recently biologic agents (Gonsalves 2019; Dellon and Spergel 2022). However, well-established and effective treatment protocols are still lacking for EGE.

The current report described our experience of caring for a patient with EGE over a period of 27 years. The patient was diagnosed with a variant in the *RUNX1* gene, potentially accounting for his presentation. He had substantial clinical and endoscopic improvement on ketotifen, with rapid symptom recurrence after treatment was briefly stopped.

Methods

Patient and blood samples

Data compiled prospectively and retrospectively from patient medical records were entered into the Canadian Centre for Primary Immunodeficiency Registry and Tissue Bank, which has been approved by the SickKids Research Ethics Board (protocol No. 1000005598). Patient and family members provided written informed consent.

Genetic diagnosis and sequencing confirmation

Genomic DNA was isolated from patient peripheral blood leukocytes using the Geneaid genomic DNA extraction kit (Geneaid Mini Kit; Sensi Capital Corp, Toronto, Ontario, Canada). The patient's mutation was identified via whole exome sequencing using Illumina HiSeq2500 GATK 1.1.28 sequencing platform, and confirmed by Sanger sequencing using DTCS Quick Kit on an automated sequencer (Beckman-Coulter CEQ 8000).

Clinical case

A male patient was first referred to our practice at the age of 4 years, and is still currently followed at the age of 31 years. He was first referred with a history of difficult-to-control EGID, other atopic features, and peripheral hypereosinophilia. His EGID presenting complaints included poor growth, abdominal pain, severe reflux, dysphagia, and vomiting, and he was identified on an upper endoscopy to have marked distal esophagitis with deep linear ulcers of the antrum extending into the duodenum. On biopsy, he had clusters and aggregates of eosinophils in the distal esophagus, stomach, and duodenum, with foci of blunted villi in the duodenum. Review of past medical history revealed that the patient had other atopic features including asthma and mild eczema, and he later developed allergic rhinitis. He had no history of recurrent or unusual infections, and no autoimmune or inflammatory features. He had received all childhood vaccines uneventfully. On review of family history, his parents were noted to be non-consanguineous of European descent. His father had a similar history of eosinophilic esophagitis/EGID leading to extensive scarring and fibrosis, as well as peripheral hypereosinophilia, anaphylaxis to insect venom, multiple food allergies, and Addison's disease. His mother was healthy. The patient's brother had a diagnosis of psoriasis. There was no family history of malignancy. No abnormalities were noted on physical exam with the exception of mild eczema. Initial laboratory evaluation revealed peripheral hypereosinophilia of 1.9×10^9 cells/L but otherwise normal complete blood count and differential, and, unexpectedly, hypogammaglobulinemia, with IgG of 5.1 g/L and normal IgA (0.8 g/L) and IgM (0.8 g/L). His specific vaccine responses at the time to polio, measles, mumps, rubella, and tetanus were all reactive.

In regard to managing the patient's EGID, initial treatments included a strict six-food elimination diet, multiple courses of systemic steroids, proton pump inhibitors, swallowed steroids, and a trial of montelukast, with minimal symptomatic improvement. Ultimately, substantial clinical relief was noted when he was treated with a combination of ketotifen and an extensive elimination diet. A repeat endoscopy and biopsy at the age of 12 years while on ketotifen showed some improvement in duodenal architecture and a reduction in eosinophilic infiltration of his esophagus, although gastric eosinophilia had not improved. At the age of 27, the patient discontinued his treatment of ketotifen due to daytime somnolence, headaches, and flushing. Shortly thereafter he began experiencing fatigue, shortness of breath and developed peripheral edema. Work-up identified profound anemia with a hemoglobin of 50 g/L, as well as hypoalbuminemia.

Repeat endoscopy showed extensive inflammation of his mid esophagus, gastric antrum, gastric body, and duodenum, and >15 eos/hpf in all the above regions. He was suspected to have protein-losing enteropathy precipitated by uncontrolled EGID. He was re-started on ketotifen and experienced a rapid improvement in his symptoms, with normalization of his hemoglobin and serum albumin.

At the age of 29, given persistence of ketotifen side-effects, the patient was trialed on Dupilumab, of which he received 2 infusions. However, following his second infusion he experienced a rise in his serum eosinophils from the usual range of $2-3 \times 10^9$ cells/L up to 6×10^9 cells/L, without any evidence of end-organ damage related to hypereosinophilia. He therefore did not receive any further Dupilumab infusions. He has resumed treatment with ketotifen and remains on an extensive elimination diet. On his most recent laboratory assessment, he was noted to have hypereosinophilia of 2.6×10^9 cells/L (back to his baseline) but otherwise normal complete blood counts and differential, with normal lymphocyte subsets. His IgG was again low at 2.76 g/L with normal IgA and IgM. He had protective specific vaccine titres to measles, mumps, and varicella, while rubella titres were equivocal. His anti-A isohemagglutinin titre was normal at 1:128.

Genetic evaluation

The patient was first assessed via an inborn errors of immunity panel, which did not reveal any variants that could explain his phenotype. He was subsequently evaluated using research whole exome sequencing and was found to have a novel heterozygous variant in the *RUNX1* gene, NM_001754: exon 9: c.T1270C (p.S424P). This variant was not identified in large population databases, and was predicted *in-silico* to be borderline/deleterious. It is expected to alter the RUNX1-Foxp3 interaction domain, therefore preventing inhibition by T regulatory cells. The variant has previously been reported and classified as a variant of unknown significance in ClinVar, with no patient phenotype reported (ClinVar allele ID 958947).

Discussion

We report on a patient with EGE who was found to harbour a variant in the *RUNX1* gene, coding for Runt-related transcription factor 1 (RUNX1), also known as acute myeloid leukemia 1 (AML1). RUNX1 plays a variety of roles in multi-lineage hematopoietic development, differentiation of myeloid and lymphoid cells, and its interaction with Foxp3 is essential for carrying out the suppressive role of T regulatory cells (Tregs) (Ono et al.2007; Cohen 2009). Additionally, RUNX1 inhibits differentiation of naïve CD4+ T cells into Th2 via repression of GATA3 (Komine et al. 2003). Somatic mutations in *RUNX1* have been implicated in the development of various hematopoietic malignancies, and are also commonly seen in cases of systemic mastocytosis (Holmes et al. 2014; Schwaab et al. 2020; Di Giacomo et al.2022). Heterozygous germline mutations in *RUNX1* have been associated with familial platelet disorder with associated myeloid malignancy (OMIM: 601399), a disorder marked by incomplete penetrance and broad phenotypic spectrum (Schmit et al. 2015; Kanagal-Shamanna et al. 2017; Simon et al. 2020; Li et al. 2021; Tang et al. 2022). Our patient has never displayed any abnormalities in platelet number or size; rather, his presentation is of systemic and gastrointestinal hypereosinophilia. Notably, bone marrow eosinophilia has been identified in 6 of 11 patients with germline *RUNX1* mutations in one case series (Kanagal-Shamanna et al. 2017), while eosinophilic leukemia was reported in another study in a patient with a germline *RUNX1* mutation (Tang et al. 2022). The potential mechanism of eosinophilia may relate to loss-of-function of RUNX1, leading to reduced inhibition of GATA3 and therefore excessive Th2 polarization.

Our patient's mutation has not been reported as causing familial platelet disorder with associated myeloid malignancy; however, it is possible that mutations affecting different amino acid residues of *RUNX1* may lead to a variable phenotype. In this regard, a recent study identified that while some *RUNX1* mutations caused haploinsufficiency others resulted in a dominant negative effect with differential transcriptional outputs (Li et al. 2021). The current mutation, Ser424Pro, affects the transcriptional activation domain at the site of Foxp3 interaction and it is possible therefore that it results in decreased Treg inhibitory capacity, resulting in a "Tregopathy", although this will require molecular functional validation. The patient presented with unexpected hypogammaglobulinemia, though his specific vaccine responses have been intact. Work in an experimental model of *runx1* loss-of-function in zebrafish revealed a humoral immunodeficiency, with failure of V(D)J recombination in B cells but not in T cells.

However, no human evidence is available to date to suggest an equivalent phenotype.

Our patient showed substantial clinical improvement while on treatment with ketotifen, and minimal response to all other treatments. To date, there are no standardized treatment protocols for patients with EGE. A number of ongoing clinical trials have investigated the potential use of biologic agents in EGID, with one of the most promising agents being the IL-4 receptor alpha chain antagonist, Dupilumab, currently approved in the United States for treatment of EoE (Dellon and Spergel 2022). However, evidence for use of biologics in EGE specifically is still lacking. Our patient showed a dramatic rise in serum eosinophils when treated with Dupilumab leading to treatment discontinuation, suggesting that we still do not fully understand the pathophysiology of EGE or how it differs from that of EoE. Other trials in EGE are ongoing for Lirentelmab (Siglec-8 antagonist) and Benralizumab (IL-5 receptor antagonist) (Dellon and Spergel 2022). In regard to evidence for ketotifen use in EGE, and in particular the long-term use of this agent, evidence is still scarce beyond our report of using this agent in 6 patients with EGE in 1991 (Melamed and Roifman 1991). Short-term ketotifen use for a period of 30 days has been reported to result in resolution of acute EGE symptoms, with a patient in clinical remission up to 10 months later (Bolukbas et al. 2004). Another report described a 44-year-old patient with difficult to control EGE who had been treated with ketotifen for over 20 years with excellent symptomatic relief, although histologic findings were suggestive of continued inflammation of his upper GI tract despite the patient being asymptomatic (Freeman 2019). This raises the importance of a combination of periodic clinical and endoscopic surveillance of EGE patients, regardless of treatment modality. While treatment with ketotifen may be a compelling option given the experience of our center and others, a systematic trial assessing the effectiveness and long-term impact of this agent in patients with EGID is warranted. Further points which would require more systematic large-scale evaluation include optimization of treatment dose and standardization of treatment response assessment.

We conclude that EGID is a broad-spectrum group of disorders, with EGE possibly having distinct genetic etiology and pathophysiology as well as differential treatment response compared with the more common EoE. While several genome-wide association studies have identified various risk loci associated with EoE (Kottyan et al. 2021; Chang et al.2022), enabling identification genes and risk variants (Ryu et al. 2020), there is a substantial lag in our knowledge of genetic predisposition to other EGID such as EGE. To date, no clear risk loci have been identified in association with forms of EGID other than EoE. We suggest that mutations in *RUNX1,* previously associated with other hematologic abnormalities such as eosinophilia and malignancy, may also underlie a presentation of tissue eosinophilia such as EGID. We further suggest that in refractory EGE cases, the mast cell stabilizer H1 antihistamine ketotifen may be considered as a therapeutic option.

Ongoing surveillance of patients is warranted to assess for clinical symptoms and histologic evidence of disease.

REFERENCES

Bolukbas, F.F., Bolukbas, C., Uzunkoy, A., Baba, F., Horoz, M., and Ozturk, E. 2004. Case report: a dramatic response to ketotifen in a case of eosinophilic gastroenteritis mimicking abdominal emergency. Dig. Dis. Sci. **49**(11–12): 1782–1785. PMID: 15628703. doi: 10.1007/s10620-004-9570-7.

Chang, X., March, M., Mentch, F., Nguyen, K., Glessner, J., Qu, H., Liu, Y., Furuta, G., Aceves, S., Gonsalves, N., Nadeau, K., Cianferoni, A., Spergel, J., Sleiman, P., and Hakonarson, H. 2022. A genome-wide association meta-analysis identifies new eosinophilic esophagitis loci. J. Allergy Clin. Immunol. **149**(3): 988–998. PMID: 34506852. doi: 10.1016/j.jaci.2021.08.018.

Cohen, M.M. Jr. 2009. Perspectives on RUNX genes: an update. Am. J. Med. Genet. A. **149A**(12): 2629–2646. PMID: 19830829. doi: 10.1002/ajmg.a.33021.

Dellon, E.S., and Spergel, J.M. 2022. Biologics in eosinophilic gastrointestinal diseases. Ann. Allergy Asthma Immunol. **20**: S1081-1206(22)00536-1. PMID: 35738437. doi: 10.1016/j.anai.2022.06.015.

Di Giacomo, D., Quintini, M., Pierini, V., Pellanera, F., La Starza, R., Gorello, P., Matteucci, C., Crescenzi, B., Fiumara, P.F., Veltroni, M., Borlenghi, E., Albano, F., Forghieri, F., Maccaferri, M., Bettelli, F., Luppi, M., Cuneo, A., Rossi, G., and Mecucci, C. 2022. Genomic and clinical findings in myeloid neoplasms with PDGFRB rearrangement. Ann. Hematol. **101**(2): 297–307. PMID: 34859285. doi: 10.1007/s00277-021-04712-8.

Freeman, H.J. 2009. Longstanding eosinophilic gastroenteritis of more than 20 years. Can. J. Gastroenterol. **23**(9): 632–634. PMID: 19816628. doi: 10.1155/2009/565293.

Gonsalves, N. 2019. Eosinophilic gastrointestinal disorders. Clin. Rev. Allergy Immunol. **57**(2): 272–885. PMID: 30903439. doi: 10.1007/s12016-019-08732-1.

Holmes, A., Coviello, J., and Velagaleti, G. 2014. Acute multilineage (B/myeloid) leukemia with RUNX1 duplication/amplification and hypereosinophilia. Eur. J. Haematol. **93**(5): 449–452. PMID: 24912843.

Kanagal-Shamanna, R., Loghavi, S., DiNardo, C.D., Medeiros, L.J., Garcia-Manero, G., Jabbour, E., Routbort, M.J., Luthra, R., Bueso-Ramos, C.E., and Khoury, J.D. 2017. Bone marrow pathologic abnormalities in familial platelet disorder with propensity for myeloid malignancy and germline RUNX1 mutation. Haematologica. **102**(10): 1661–1670. PMID: 28659335. doi: 10.3324/haematol.2017.167726.

Komine, O., Hayashi, K., Natsume, W., Watanabe, T., Seki, Y., Seki, N., Yagi, R., Sukzuki, W., Tamauchi, H., Hozumi, K., Habu, S., Kubo, M., and Satake, M. 2003. The Runx1 transcription factor inhibits the differentiation of naive CD4+ T Cells into the Th2 Lineage by repressing GATA3 expression. J Exp Med. **198**(1): 51–61. PMID: 12835475. doi: 10.1084/jem.20021200.

Kottyan, L.C., Trimarchi, M.P., Lu, X., Caldwell, J.M., Maddox, A., Parameswaran, S., Lape, M., D'Mello, R.J., Bonfield, M., Ballaban, A., Mukkada, V., Putnam, P.E., Abonia, P., Morgenstern, N.B-B, Eapen, A.A., Wen, T., Weirauch, M.T., and Rothenberg, M.E. 2021. Replication and meta-analyses nominate numerous eosinophilic esophagitis risk genes. J. Allergy Clin. Immunol. **147**(1):255–266. PMID: 33446330. doi: 10.1016/j.jaci.2020.10.018.

Li, Y., Yang, W., Devidas, M., Winter, S.S., Kesserwan, C., Yang, W., Dunsmore, K.P., Smith, C., Qian, M., Zhao, X., Zhang, R., Gastier-Foster, J.M., Raetz, E.A., Carroll, W.L., Li, C., Liu, P.P., Rabin, K.R., Sanda, T., Mullighan, C.G., Nichols, K.E., Evans, W.E., Pui, C.H., Hunger, S.P., Teachey, D.T., Relling, M.V., Loh, M.L., and Yang, J.J. 2021. Germline RUNX1 variation and predisposition to childhood acute lymphoblastic leukemia. J. Clin. Invest. **131**(17): e147898. PMID: 34166225. doi: 10.1172/JCI147898.

Melamed, I., and Roifman, C.M. 1991. Benefit of ketotifen in patients with eosinophilic gastroenteritis. Am. J. Med. **90**(3): 310–314. PMID: 2003512.

Ono, M., Yaguchi, H., Ohkura, N., Kitabayashi, I., Nagamura, Y., Nomura, T., Miyachi, Y., Tsukada, T., and Sakaguchi, S. 2007. Foxp3 controls regulatory T-cell function by interacting with AML1/Runx1. Nature. **446**(7136): 685–689. PMID: 17377532. doi: 10.1038/nature05673.

Rossi, C.M., Lenti, M.V., Merli, S., Licari, A., Votto, M., Marseglia, G.L., and Sabatino, A.D. 2022. Primary eosinophilic gastrointestinal disorders and allergy: clinical and therapeutic implications. Clin Transl Allergy [Internet]. [cited 2022 Jul 7]; **12**(5). doi: 10.1002/clt2.12146.

Ryu, S., Lee, K.H., Tizaoui, K., Terrazzino, S., Cargnin, S., Effenberger, M., Shin, J.I., and Kronbichler, A. 2020. Pathogenesis of eosinophilic esophagitis: a comprehensive review of the genetic and molecular aspects. Int J Mol Sci. **21**(19):7253. PMID: 33008138. doi: 10.3390/ijms21197253.

Schmit, J.M., Turner, D.J., Hromas, R.A., Wingard, J.R., Brown, R.A., Li, Y., Li, M.M., Slayton, W.B., and Cogle, C.R. 2015. Two novel RUNX1 mutations in a patient with congenital thrombocytopenia that evolved into a high grade myelodysplastic syndrome. Leuk Res Rep. **4**(1): 24–27. PMID: 25893166. doi: 10.1016/j.lrr.2015.03.002.

Schwaab, J., Cabral do O Hartmann, N., , Naumann, N., Jawhar, M., Weiß, C., Metzgeroth, G., Schmid, A., Lübke, J., Reiter, L., Fabarius, A., Cross, N.C.P., Sotlar, K., Valent, P., Kluin-Nelemans, H.C., Hofmann, W-K., Horny, H-P., Panse, J., and Reiter, A. 2020. Importance of adequate diagnostic workup for correct diagnosis of advanced systemic mastocytosis. J. Allergy Clin. Immunol. Pract. **8**(9): 3121–3127.e1. PMID: 32422371. doi: 10.1016/j.jaip.2020.05.005.

Simon, L., Spinella, J.F., Yao, C.Y., Lavallée, V.P., Boivin, I., Boucher, G., Audemard, E., Bordeleau, M-E., Lemieux, S., Hébert, J., and Sauvageau, G. 2020. High frequency of germline RUNX1 mutations in patients with RUNX1-mutated AML. Blood. **135**(21): 1882–1886. PMID: 32315381. doi: 10.1182/blood.2019003357.

Tang, K.H., Li, S., Khodadadi-Jamayran, A., Jen, J., Han, H., Guidry, K., Chen, T., Hao, Y., Fedele, C., Zebala, J.A., Maeda, D.Y., Christensen, J.G., Olson, P., Athanas, A., Loomis, C.A., Tsirigos, A., Wong, K-K., and Neel, B.G. 2022. Combined inhibition of SHP2 and CXCR1/2 promotes antitumor T-cell response in NSCLC. Cancer Discov. **12**(1): 47–61. PMID: 34353854. doi: 10.1158/2159-8290.CD-21-0369.