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Somatic NOD2 mosaicism in Blau syndrome

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To the Editor:

Blau syndrome (BS) is a dominant inherited autoinflammatory disease caused by gain-of-function *NOD2* mutations and characterized by the triad of granulomatous arthritis, dermatitis, and uveitis.¹ We identified a 17-year-old patient with a clinical diagnosis of BS (see pedigree in Fig 1, A, and a complete clinical description in this article's Online Repository at www.jacionline.org). Sanger *NOD2* sequencing using DNA from total leukocytes did not detect disease-causing mutations (Fig 1, B). A careful analysis of Sanger chromatograms revealed at c.1001 position (Ref Seq: NM_022162.1) a very small peak of adenine associated with a subtle decrease in the wild-type allele (Fig 1, C). This potential c.1001G>A transition might provoke the appearance of the p.Arg334Gln *NOD2* variant, which is a well-known BS-causing mutation.^{1–4} A selective allele amplification was reasonably ruled out by the heterozygous c.802C>T transition in the same amplicon (Fig 1, D), which is a common polymorphism of the gene (rs2066842; allele frequency of 13% in the 1000 Genomes Project Phase 1). These data suggested the presence of a somatic *NOD2* mosaicism. To address this issue, we performed targeted deep *NOD2* sequencing using DNA extracted from hematological and nonhematological tissues (see this article's Methods section in the Online Repository at www.jacionline.org). These analyses detected the p.Arg334Gln *NOD2* mutation in all analyzed samples, with variable frequencies (4.9% to 11.0%) depending on the cells' origin (Table I). The *NOD2* mutation was not detected in the patient's parents, confirming its *de novo* nature.

The patient's phenotype was compared with those detected in patients with BS carrying germline *NOD2* mutations. Differences were detected only in the joint involvement, which

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included the number of affected joints (oligoarthritis in our patient in place of polyarthritis in most patients with germline mutations), the type of affected joints (absence of involvement of small joints in our patient), and the absence of tenosynovitis and joint deformities in our patient (see Table E1 in this article's Online Repository at www.jacionline.org).

Genetic mosaicism describes an individual with 2 or more cell types with different genotypes that derive from the same zygote. This implies the occurrence of *de novo*, postzygotic mutational event(s) that may occur either during embryogenesis or after birth.⁵ Somatic mosaicism has been extensively studied in cancer, but its role in Mendelian inherited diseases has scarcely been reported. The arrival of next-generation sequencing technologies is providing evidence of its relevance in this field. Among the monogenic autoinflammatory diseases, somatic mosaicism has been well established in cryopyrinopathies and in stimulator of interferon genes-associated vasculopathy with onset in infancy.^{6–8} In the present work, the use of next-generation sequencing technologies enabled us to detect a well-known *NOD2* mutation as a somatic mutation at low frequencies in all analyzed tissues. To our knowledge, this evidence represents the first description of somatic *NOD2* mosaicism in the pathogenesis of BS, thus expanding the number of monogenic diseases in which this mechanism can play an important role. This evidence also enabled us to confirm the BS diagnosis and support the idea that a *de novo* mutational event affecting the *NOD2* gene occurred in this patient, probably at an early stage during embryogenesis because tissues from different embryonal layers are equally affected.

The involvement of somatic *NOD2* mosaicism in BS may have relevant clinical consequences. During the past few years, the clinical diversity of BS has increased as the number of diagnosed patients has increased. Thus, we currently know that some patients never developed some of the symptoms of the classical triad, whereas other patients developed symptoms beyond the triad.^{4,9} The phenotype of the patient described here resembles that detected in patients with BS affected exclusively with the triad, although differences in the articular involvement should be highlighted. Our patient developed seronegative oligoarthritis affecting exclusively large joints, with no tenosynovitis or joint deformities, whereas most patients with BS carrying germline *NOD2* mutations suffered from polyarthritis (95% in the International BS Registry), always associated with tenosynovitis, involving both large and small joints, which often progressed to joint deformities (42.8% in the Spanish BS cohort).^{1,4,9} It could be speculated that the apparent less severe articular disease observed in our patient could be a direct consequence of the low-level somatic mosaicism. The description of the joint disease in novel patients carrying somatic *NOD2* mosaicism will probably confirm or invalidate our observation.

Sporadic patients with BS with negative *NOD2* analysis have been previously reported.^{1,3} This scenario is similar to that previously described for cryopyrinopathies, in which the use of different genetic technologies finally detected somatic *NLRP3* mosaicism.^{6,7} The evidence shown here supports the possibility that “mutation-negative” patients with BS could actually be carriers of somatic *NOD2* mosaicism, and its definitive diagnosis probably relies on the use of novel genetic technologies. In our experience, targeted deep sequencing is probably the most efficient method to identify low-level mosaicism and will be the

recommended method to address this issue in future studies. In addition, it could be hypothesized that the candidates in whom to evaluate the presence of somatic *NOD2* mosaicism had probably been previously diagnosed with juvenile idiopathic arthritis (JIA), especially in the forms of systemic-onset and seronegative polyarthritis and oligoarthritis. Future studies with selected candidates from these JIA subgroups will provide evidence regarding the validity of this hypothesis.

The tissue distribution of the mosaicism could vary between different patients and is often difficult to analyze comprehensively. The potential transmission of the somatic mutation to the offspring relies on its presence in the germ cells. In the case of patients with BS with germline *NOD2* mutation, this probability is 50%. In our patient, it was not possible to calculate this probability because, for ethical reasons, we were unable to analyze the degree of gonadal mosaicism. In the case that the vertical transmission occurred, the *NOD2* mutation will be present at the first zygote. Consequently, the child will carry a germline mutation and it is conceivable that his or her phenotype will be even more severe than that observed in patients carrying a somatic mutation.

In conclusion, the evidence described here shows for the first time the involvement of somatic *NOD2* mosaicism in the pathogenesis of BS. This opens the possibility that this genetic mechanism underlies additional patients previously diagnosed as BS or as JIA. Because of the potential clinical consequences, further studies are needed to confirm this hypothesis.

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FIG 1.

A, Family's pedigree. **B**, Genomic organization of the *NOD2* gene (top) and scheme of the pair of primers designed to analyze the exon 4 (below) (green arrows represent the pair primers used for PCR amplification and Sanger sequencing, while white and red arrows represent those primers designed for targeted deep sequencing surrounding the c.1001 *NOD2* nucleotide position). **C**, Sanger sense and antisense *NOD2* chromatograms from a healthy control (left boxes), from a patient with BS carrying the germline p.Arg334Gln *NOD2* mutation (middle boxes), and from the patient described here (right boxes). Gray arrows show the c.1001 position. **D**, Sanger sense and antisense chromatograms from the patient described here showing the heterozygous c.802C>T transition (gray arrows) in the PCR amplicon used for Sanger sequencing.

TABLE I

Summary of *NOD2* genotyping performed in different family members

Individual	Sample	Cell sorting purity (%)	Sanger <i>NOD2</i> genotype	Targeted deep <i>NOD2</i> sequencing		
				Amino acid exchange	Mutated allele frequency (%)	Coverage (mean/range)
Patient	Peripheral blood	—	WT/WT	p.Arg334Gln	7.7*	3572/2837–3866*
	Neutrophils (CD15 ⁺)	98.2	WT/WT	p.Arg334Gln	7.3 [†]	2908/2469–3145 [†]
	Monocytes (CD14 ⁺)	98.6	WT/WT	p.Arg334Gln	11.0 [†]	1978/1959–1989 [†]
	T cells (CD3 ⁺)	99.5	WT/WT	p.Arg334Gln	8.3 [†]	3756/3483–4000 [†]
	B cells (CD19 ⁺)	98.0	WT/WT	p.Arg334Gln	8.2 [†]	3054/1834–4310 [†]
	Mucosal swab	—	WT/WT	p.Arg334Gln	4.9 [†]	3359/2333–4018 [†]
	Urine	—	WT/WT	p.Arg334Gln	7.4 [†]	3306/2691–3642 [†]
	Skin	—	WT/WT	p.Arg334Gln	7.0 [†]	2788/2386–3076 [†]
Father	Peripheral blood	—	WT/WT	—	0 [†]	5206/5192–5219 [†]
Mother	Peripheral blood	—	WT/WT	—	0 [†]	4449/4365–4532 [†]
Germline mutation	Peripheral blood	—	p.Arg334Gln/WT	p.Arg334Gln	51.8 [†]	2217/2097–2337 [†]

WT, Wild type.

* Data collected from 6 independent experiments.

[†] Data collected from 3 independent experiments.