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Genetic and Clinical Features of Blau Syndrome among Chinese Patients with Uveitis

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Purpose: The American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology call for cautious interpretation of variants as causative of a monogenic disorder by stringent standards. We aimed to reclassify the pathogenicity of *nucleotide binding oligomerization domain containing 2* (*NOD2*) variants according to the ACMG guidelines and to characterize clinical features in patients whose ocular disease might actually be explained by Blau syndrome.

Design: Genetic analysis and descriptive study.

Participants: A total of 1003 unrelated healthy individuals and 3921 sporadic patients who presented with uveitis.

Methods: Whole-exome sequencing was performed on all healthy participants and 551 patients with uveitis, and targeted *NOD2* resequencing was performed on the remaining 3370 patients with uveitis. Pathogenicity for Blau syndrome was classified for *NOD2* variants identified by sequencing in study participants according to the ACMG guidelines. Clinical manifestations were compared among *NOD2* variants of different levels of classification.

Main Outcome Measures: Pathogenicity of variants.

Results: Eight *NOD2* gain-of-function mutations, p.R334W, p.R334Q, p.E383K, p.G481D, p.W490S, p.M513T, p.R587C, and p.N670K, were classified as pathogenic, and 66 patients (1.7%) with uveitis were diagnosed with Blau syndrome due to these mutations. Of 66 with Blau syndrome, anterior uveitis accounted for 39.4%, posterior uveitis for 9.1%, and panuveitis for 51.5%. A proportion of 21.2% of Blau syndrome presented as multifocal choroiditis, 48.5% had papillitis, and 74.2% showed retinal microvasculitis detected by fundus fluorescein angiography. Six *NOD2* variants, p.P268S, p.R311W, p.R471C, p.A612T, p.R702W, and p.V955I, were considered nonpathogenic for Blau syndrome and were identified in 96 patients with uveitis. The incidence of bilateral uveitis (86.4%), secondary glaucoma (47.0%), epiretinal membrane (7.6%), choroidal neovascularization (4.6%), retinal atrophy (10.6%), arthritis (69.7%), joint deformity (51.5%), and skin rash (40.9%) was higher in Blau syndrome than in patients with uveitis carrying non–Blau-causing *NOD2* variants. Patients with Blau syndrome permanently experienced overall poorer best-corrected visual acuity. Several rare *NOD2* mutations, p.I722L (2 cases), p.T476P (1 case), p.T476del (1 case), and p.R439H (1 case), were newly identified.

Conclusions: Pathogenic *NOD2* variants for Blau syndrome were limited to those gain-of-function mutations and were associated with a high risk for arthritis, skin rash, permanent visual loss, and ocular complications in patients with uveitis. *Ophthalmology 2022;■:1–8 © 2022 by the American Academy of Ophthalmology*

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Blau syndrome is an early-onset autoinflammatory disorder, typically characterized by a triad of uveitis, granulomatous polyarthritis, and skin lesions.[1] Its incidence was estimated to be 0.05 per 100 000 person-years.[2] This disease is well known as an autosomal-dominant disease, resulting from a mutation at a single genetic locus within *nucleotide binding oligomerization domain containing 2* (*NOD2*). *Nucleotide binding oligomerization domain containing 2* encodes a pattern recognition receptor that is responsible for innate sensing of bacterial products and thereby initiation of inflammatory response.[3] *Nucleotide binding oligomerization domain containing 2* gain-of-function mutations have been shown to induce a constitutive autoactivation of nuclear factor kappa B (NF-κB) proinflammatory cascades in Blau syndrome.[4,5]

Blau syndrome causes considerable ocular morbidity and blindness in children.[6] Despite extensive clinical evaluations, this disease is not easily recognized by ophthalmologists.[7] Its clinical presentation varies considerably. The typical triad may be absent in some patients, and extratriad manifestations, such as fever, panniculitis, and granulomatous lymphadenopathy, may yet be

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noted in others.[8] Medical application of exome sequencing has seemingly made it possible to efficiently establish its diagnosis in patients with suspected disease by identification of a pathogenic *NOD2* mutant[9]; however, a missense variant that has been predicted to be damaging to the protein for which it encodes may not be necessarily implicated in a disease.[10] Informing such noncausal variant incorrectly as pathogenic for a monogenic disorder may lead to far-reaching negative consequences, such as excess treatment and lifestyle modifications to the patient, unnecessary genetic counseling and surveillance for the relatives, and additional mental and economic burden on the family. Therefore, a joint consensus recommendation was proposed by the American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology, which calls for cautious interpretation of variants as causative of a monogenic disorder by stringent standards.[10]

A number of case reports, case series, and cohort studies have described the clinical characteristics of Blau syndrome and its associations with several previously unreported *NOD2* variants.[8,9,11-14] We recognize that there is possibility of historical errors in reporting a benign *NOD2* variant as pathogenic; therefore, some ambiguous clinical findings might not be truly due to Blau syndrome itself. In this study, we performed exome sequencing and targeted *NOD2* resequencing in healthy individuals and patients who presented with uveitis. We reclassified the pathogenicity of *NOD2* variants according to the ACMG guidelines and characterized the clinical features in patients whose ocular disease might actually be explained by Blau syndrome.

Methods

Study Participants and Procedures

The study and informed consent procedures were approved by the Ethics Committee of The First Affiliated Hospital of Chongqing Medical University and complied with the provisions of the Declaration of Helsinki. Each participant or parents/legal guardian of minors provided written informed consent, and each child gave assent when appropriate. This study recruited 1003 unrelated healthy individuals and 3921 sporadic patients who presented with uveitis at The First Affiliated Hospital of Chongqing Medical University between April 2008 and September 2021. Genomic DNA was extracted from peripheral blood samples of each participant in accordance with standard protocols. Whole-exome sequencing was performed on DNA samples of all healthy participants and a total of 551 patients with uveitis, and targeted *NOD2* resequencing was performed on those of the remaining 3370 patients with uveitis. For whole-exome sequencing, whole exomes of DNA samples were captured and sequenced at a mean $\pm$ standard deviation of 74.8 $\pm$ 9.4 $\times$ depth on the Illumina HiSeq XTen platform, followed by targeted analysis to identify variants within *NOD2*. For targeted *NOD2* resequencing, library preparation was performed by multiplex polymerase chain reaction with primers designed to cover the *NOD2* gene exonic regions, and sequencing was carried out at a commercial sequencing service (Sangon) using the Illumina HiSeq XTen sequencers. Detailed clinical information was retrieved from patients' medical records involving complete data on outpatient encounters, inpatient care, and laboratory tests and auxiliary examinations that were ever performed at the study center. This study did not plan for active follow-up encounters. Patients would have subsequent clinic visits according to their medical needs. Clinical evaluations at each visit included slit-lamp microscopy, ophthalmoscopy, and measurement of visual acuity and intraocular pressure. Auxiliary examinations, such as fundus photography, OCT, and fundus fluorescein angiography, were performed on the basis of clinical needs.

Ascertainment of Pathogenic Variants

We classified the pathogenicity for Blau syndrome of *NOD2* variants identified by sequencing in our study participants according to recent standards and guidelines of the ACMG.[10] This guideline provided detailed criteria within a set of evidence categories that can be used to weight varying levels of support for a pathogenic or benign assertion. We used the Genome Aggregation Database that had aggregated 125 748 human exomes and 15 708 genomes to obtain the frequency of *NOD2* variants in large populations.[15] The *NOD2* sequence variants were predicted on protein function, where nonsense, frameshift, missense, microdeletions, microduplications, and splice-site variants would be interpreted.[10] We searched PubMed for literature in any language published up to November 16, 2021, using the terms "NOD2 variant" or "NOD2 mutation" or "CARD15 variant" or "CARD15 mutation." Using PubMed, we examined all identified *NOD2* variants for their initial disease association and segregation in available medical reports and searched for their functional data in biological literature. An NF-κB luciferase reporter assay showing an increased basal NF-κB activity by a *NOD2* mutant compared with the wild type was prioritized among functional evidence supportive of a deleterious effect related to Blau syndrome.[14,16,17] Classification was manually performed in close collaboration among clinicians, laboratory scientists, and bioinformaticians by inspecting the entire body of available evidence in aggregate, such as frequency, predictive, and functional data, and finally drawing a single conclusion. Standard terminology ("pathogenic," "likely pathogenic," "uncertain significance," "likely benign," and "benign") was used to describe the causative nature of variants specific for Blau syndrome.[10] The *NOD2* mutations classified as "pathogenic" or "likely pathogenic" would have $>$ 90% certainty of being independently explicative of Blau syndrome,[10] and we considered those as Blau syndrome-causing variants.

Statistical Analysis

Skewed descriptive data are presented as median and interquartile range and were compared with the use of the Mann−Whitney *U* test. Categorical data are presented as numbers as well as proportions and were compared with the Fisher exact test. Odds ratios were calculated in 2 $\times$ 2 contingency tables. A generalized linear model was constructed to estimate the best-corrected visual acuity (scores for the logarithm of the minimum angle of resolution [logMAR]) by using repeated measurements at months 0, 6 $\pm$ 2, 12 $\pm$ 2, 18 $\pm$ 2, and 24 $\pm$ 3, where group (case or control), time, and their interaction term were included as the covariates in the regression model. For nonnumerical visual acuities, the following denotations were used as previously described[18]: Finger count was assigned as 1.7 logMAR, hand movement as 2.0 logMAR, light perception as 2.3 logMAR, and no light perception as 3.0 logMAR. No imputation techniques were used for missing data, as the generalized linear model regression systematically predicted the null value. All tests performed were 2 sided. Given the descriptive nature of the study, all analyses were exploratory. Statistical analyses were performed with IBM SPSS Statistics, version 22.0.

Results

NOD2 Variants

In this study, we classified a total of 8 *NOD2* variants, p.R334W, p.R334Q, p.E383K, p.G481D, p.W490S, p.M513T, p.R587C, and p.N670K, as pathogenic for Blau syndrome (Table 1). These Blau syndrome-causing variants were identified in 66 of 3921 patients (1.7%) and were explicative of the patient's uveitis. A diagnosis of Blau syndrome was established in these 66 patients. None of these variants were present in 1003 healthy participants. All of these pathogenic variants have been reported in cases with Blau syndrome in the medical literature[4,14,16,19] and had been shown to robustly increase the NF-κB activity in functional studies.[14,16,17,19]

Six *NOD2* variants, p.P268S, p.R311W, p.R471C, p.A612T, p.R702W, and p.V955I, previously reported in association with Blau syndrome,[11-13,16,20] were eventually classified as benign for Blau syndrome in our study (Table 1). These variants showed a similar frequency between our uveitis patients and healthy individuals. The allele frequency of p.P268S, p.R702W, and p.V955I was even higher than 1% in the Genome Aggregation Database, which was greater than expected for Blau syndrome. Functional studies suggested that all these variants were unable to promote the NF-κB activation response.[17,21] We concluded that these *NOD2* variants were not disease-causing variants for Blau syndrome in our patients and their ocular disease could not be independently explained by these variations.

We identified 3 *NOD2* variants, p.E383Q, p.H370Y, and p.H603R (Table 1), that were ever reported to be associated with Blau syndrome[13,22,23] and classified them as uncertain significance after reviewing all available evidence because the criteria for benign and pathogenic were unmet or contradictory. There was also a lack of functional data on these 3 variants. We additionally identified 6 rare *NOD2* variants as uncertain significance, p.I722L, p.A661P, p.Q902K, p.T476P, p.T476del, and p.R439H, of which none had been reported previously.

Clinical Features

Clinical manifestations of 66 patients with Blau syndrome are summarized according to specific disease-causing *NOD2* variants (Table 2). Of our patients with Blau syndrome, 57.6% were female, and the median age of visit at our department was 13 years (Table 3). A total of 18 patients (27.3%) with Blau syndrome reported a family history of uveitis, arthritis, or skin lesions in their first-degree relatives (Table 3). Bilateral uveitis was present in 86.4% of patients with Blau syndrome. The anterior segment of eyes (90.9%) was more commonly affected than the posterior segment (60.6%), and anterior uveitis accounted for 39.4% of the cases, posterior uveitis for 9.1%, and panuveitis for 51.5%. A granulomatous uveitis as evidenced by the presence of mutton-fat keratic precipitates or the record of this sign in their history was observed in 70% of patients. A proportion of 21.2% of Blau syndrome presented a multifocal choroiditis, and 48.5% had a papillitis manifesting as swelling of the optic disc (Fig S1, available at www.aaojournal.org). Among 31 cases examined by means of fundus fluorescein angiography, 23 (74.2%) of them showed retinal microvasculitis characterized by diffuse vascular leakage (Fig S2, available at www.aaojournal.org) in association with or without hyperfluorescence of optic disc and cystoid macular edema (Fig S3, available at www.aaojournal.org).

Variants p.R334W and p.R334Q were the most common *NOD2* mutations identified, where 38 patients (57.6%) carried a p.R334W mutation and 17 patients (25.8%) carried a p.R334Q (Table 2). Patients with p.R334W or p.R334Q predominantly had a panuveitis (54.5%), band keratopathy (52.7%), complicated cataract (67.3%), secondary glaucoma (49.1%), arthritis (69.1%), and joint deformity (50.9%). In addition, p.E383K, p.W490S, p.M513T, and p.N670K were extremely rare, each of which was identified in only 1 patient among our study population (Table 2). All the patients with 1 of these 4 rare mutations had arthritis and uveitis. Patients with p.E383K or p.W490S had a severe ocular disease, presenting a panuveitis in association with complicated cataract and secondary glaucoma, whereas patients with p.M513T or p.N670K seemed to show a mild ocular disease with anterior uveitis but more total systemic involvements including arthritis, joint deformity, and skin rash (Table 2). Regardless of which specific pathogenic mutation was inherited, patients with Blau syndrome frequently developed at least 1 ocular complication, including band keratopathy, complicated cataract, and secondary glaucoma, and 1 systemic involvement, including arthritis, joint deformity, and skin rash (Table 2).

Comparisons of clinical manifestations between patients carrying disease-causing *NOD2* mutations (Blau syndrome) and those uveitis patients carrying non—Blau syndrome-causing *NOD2* variants (controls) are shown in Table 3. Patients with Blau syndrome (27.3%) had a higher proportion of a positive family history than the controls (1.0%). The incidence of bilateral uveitis (86.4% vs. 69.8%), secondary glaucoma (47.0% vs. 26.0%), epiretinal membrane (7.6% vs. 1.0%), choroidal neovascularization (4.6% vs. 0%), and retinal atrophy (10.6% vs. 1.0%) in Blau syndrome was higher than in the controls. Comparably, patients with Blau syndrome dynamically presented overall poorer best-corrected visual acuity than the controls, which hardly showed any improvement during a 24-month observation (Fig 1). Systemic involvements, including arthritis (69.7%), joint deformity (51.5%), and skin rash (40.9%), were predominantly present in Blau syndrome, whereas these manifestations were rarely seen (<10%) in the controls (Table 3).

Novel Mutations

Among 6 *NOD2* variants that we reported here, p.A661P and p.Q902K presented a lower frequency in patients with uveitis than in our healthy participants, suggesting that these 2 variants would likely be reclassified as "likely benign" or even "benign" for Blau syndrome when evidence from

Table 1. Characteristics of *NOD2* Variants

NOD2 Variant	Allele Frequency in Patients with Uveitis (N = 3921)	Allele Frequency in Healthy Individuals (N = 1003)	Historical Allele Frequency Data in General Population (Genome Aggregation Database)	Functional Data (Effect on NF-κB Activity)
Blau syndrome–causing variant				
c.1000C>T (p.R334W)	0.01	0	NA	Promotional effect
c.1001G>A (p.R334Q)	0.004	0	NA	Promotional effect
c.1147G>A (p.E383K)	0.0003	0	NA	Promotional effect
c.1442G>A (p.G481D)	0.0005	0	NA	Promotional effect
c.1469G>C (p.W490S)	0.0003	0	NA	Promotional effect
c.1538T>C (p.M513T)	0.0003	0	NA	Promotional effect
c.1759C>T (p.R587C)	0.001	0	0.00003	Promotional effect
c.2010C>G (p.N670K)	0.0003	0	NA	Promotional effect
Non–Blau syndrome-causing variant				
c.802C>T (p.P268S)	0.01	0.01	0.2	No promotional effect
c.931C>T (p.R311W)	0.005	0.002	0.0006	No promotional effect
c.1411C>T (p.R471C)	0.04	0.03	0.001	No promotional effect
c.1834G>A (p.A612T)	0.003	0.001	0.0006	No promotional effect
c.2104C>T (p.R702W)	0.0003	0	0.03	No promotional effect
c.2863G>A (p.V955I)	0.001	0.003	0.06	No promotional effect
NOD2 variant of uncertain significance				
c.1147G>C (p.E383Q)	0.0003	0	NA	NA
c.1108C>T (p.H370Y)	0.002	0.002	0.0001	NA
c.1808A>G (p.H603R)	0.0008	0	NA	NA
c.2164A>C (p.I722L)*	0.0005	0	NA	NA
c.1981G>C (p.A661P)*	0.003	0.006	0.00007	NA
c.2704C>A (p.Q902K)*	0.01	0.02	0.0004	NA
c.1426A>C (p.T476P)*	0.0003	0	NA	NA
c.1427_1429delCCT (p.T476del)*	0.0003	0	NA	NA
c.1316G>A (p.R439H)*	0.0003	0	NA	NA

NA = not available; NF-κB = nuclear factor kappa B; *NOD2* = *nucleotide binding oligomerization domain containing 2*.
*Mutations newly reported in this study.

functional studies or pedigree analyses were reinforced (Table 1). Other novel *NOD2* mutations, p.I722L (2 cases), p.T476P (1 case), p.T476del (1 case), and p.R439H (1 case), were not shown in healthy individuals. Clinical manifestations of these 5 cases are shown in Table 4. Anterior uveitis was present in all 5 patients. Arthritis was shown in 4 patients (p.I722L, p.T476P, p.T476del, or p.R439H). In addition, 3 patients (p.I722L, p.T476del, or p.R439H) had a carpal cyst, and 1 patient (p.I722L) had a conjunctiva cyst that had been present before the cataract phacoemulsification surgery in the right eye (Fig S4, available at www.aaojournal.org).

Discussion

In this study, we reclassified the pathogenicity of *NOD2* variants and determined 8 rare mutations, p.R334W, p.R334Q, p.E383K, p.G481D, p.W490S, p.M513T, p.R587C, and p.N670K, as pathogenic for Blau syndrome. Our study suggested that several *NOD2* variants (p.P268S, p.R311W, p.R471C, p.A612T, p.R702W, and p.V955I), of which frequency was higher than expected for Blau syndrome and which failed to cause NF-κB response autoactivation, had previously been misclassified in suspected patients undergoing genetic testing.[11-13,16,19] Compared with those carrying non–disease-causing *NOD2* variants, uveitis patients with Blau syndrome-causing mutations were more likely to develop arthritis, joint deformity, and skin rash and had a worse visual acuity comparably along with more frequent ocular complications. True manifestations of pathogenic mutations seem to be more severe than previously considered.

The *NOD2* gain-of-function mutation is a known mechanism of Blau syndrome, which biologically causes an aberrant autoactivation of NF-κB signaling and results in a

Table 2. Clinical Manifestations of Patients with Blau Syndrome

Clinical Manifestation	Overall	p.R334W	p.R334Q	p.E383K	p.G481D	p.W490S	p.M513T	p.R587C	p.N670K
No. of patients	66	38	17	1	2	1	1	5	1
Uveitis classification									
Anterior uveitis, no. (%)	26 (39.4)	12 (31.6)	8 (47.1)	0 (0)	1 (50)	0 (0)	1 (100)	3 (60)	1 (100)
Posterior uveitis, no. (%)	6 (9.1)	3 (7.9)	2 (11.8)	0 (0)	1 (50)	0 (0)	0 (0)	0 (0)	0 (0)
Panuveitis, no. (%)	34 (51.5)	23 (60.5)	7 (41.2)	1 (100)	0 (0)	1 (100)	0 (0)	2 (40)	0 (0)
Common ocular complications									
Band keratopathy, no. (%)	34 (51.5)	21 (55.3)	8 (47.1)	0 (0)	1 (50.0)	0 (0)	1 (100)	2 (40.0)	1 (100)
Complicated cataract, no. (%)	45 (68.2)	24 (63.2)	13 (76.5)	1 (100)	1 (50.0)	1 (100)	0 (0)	5 (100)	0 (0)
Secondary glaucoma, no. (%)	31 (47.0)	20 (52.6)	7 (41.2)	1 (100)	0 (0)	1 (100)	0 (0)	2 (40.0)	0 (0)
Systemic involvement									
Arthritis, no. (%)	46 (69.7)	26 (68.4)	12 (70.6)	1 (100)	2 (100)	1 (100)	1 (100)	2 (40.0)	1 (100)
Joint deformity, no. (%)	34 (51.5)	19 (50.0)	9 (52.9)	1 (100)	2 (100)	0 (0)	1 (100)	1 (20.0)	1 (100)
Skin rash, no. (%)	27 (40.9)	15 (39.5)	7 (41.2)	0 (0)	2 (100)	1 (100)	1 (100)	0 (0)	1 (100)

persistent induction of proinflammatory cytokines, including interferon-γ, interleukin-6, and interleukin-17.[4,5,24] Such systemic, chronic inflammatory condition may lead to a number of consequences, particularly notable extraocular manifestations, frequent ocular complications, and permanent visual loss,[6,14,25,26] which is largely in line with our clinical findings. Despite the location at different loci within *NOD2*, the gain-of-function mutations produce identical biological effects, present similar clinical features, and have the same causative nature characterized by an autosomal dominant mode. Thus, the symptoms and signs due to diverse gain-of-function mutations within *NOD2* should be considered as a single disease,[16] Blau syndrome, and correct diagnosis largely relies on identification of such mutations. On the contrary, the diagnosis of Blau syndrome may be ruled out by detecting several *NOD2*

Table 3. Comparisons of Patients with or without Disease-Causing *NOD2* Variants

	Patients with Blau Syndrome (Carrying Blau Syndrome–Causing Variants)	Patients with Uveitis (Carrying Non–Blau Syndrome-Causing Variants)	OR (95% CI)†	*P* Value
NOD2 variants	p.R334W, p.R334Q, p.E383K, p.G481D, p.W490S, p.M513T, p.R587C, and p.N670K	p.P268S, p.R311W, p.R471C, p.A612T, p.R702W, and p.V955I	–	–
No. of patients	66	96	–	–
Age of visit, year			–	0.1097*
Median	13	11	–	
Interquartile range	7–23.5	8–14	–	
Female, no. (%)	38 (57.6)	46 (47.9)	1.48 (0.80–2.79)	0.2639‡
Positive family history, no. (%)§	18 (27.3)	1 (1.0)	35.63 (5.78–376.40)	<0.0001‡
Both eye affected, no. (%)	57 (86.4)	67 (69.8)	2.74 (1.24–6.53)	0.0150‡
Intraocular inflammation involvement				
Anterior segment, no. (%)	60 (90.9)	87 (90.6)	1.03 (0.36–3.11)	0.9999‡
Posterior segment, no. (%)	40 (60.6)	66 (68.8)	0.70 (0.36–1.38)	0.3156‡
Ocular complications				
Band keratopathy, no. (%)	34 (51.5)	41 (42.7)	1.43 (0.77–2.67)	0.3361‡
Complicated cataract, no. (%)	45 (68.2)	56 (58.3)	1.53 (0.79–2.93)	0.2486‡
Secondary glaucoma, no. (%)	31 (47.0)	25 (26.0)	2.52 (1.28–4.82)	0.0073‡
Macular edema, no. (%)	7 (10.6)	8 (8.3)	1.31 (0.49–3.91)	0.7836‡
Epiretinal membrane, no. (%)	5 (7.6)	1 (1.0)	7.79 (1.02–92.57)	0.0415‡
Choroidal neovascularization, no. (%)	3 (4.6)	0 (0)	–	0.0658‡
Retinal atrophy, no. (%)	7 (10.6)	1 (1.0)	11.27 (1.89–128.10)	0.0082‡
Systemic involvement				
Arthritis, no. (%)	46 (69.7)	6 (6.3)	34.50 (12.55–88.70)	<0.0001‡
Joint deformity, no. (%)	34 (51.5)	1 (1.0)	100.9 (17.40–1042)	<0.0001‡
Skin rash, no. (%)	27 (40.9)	6 (6.25)	10.38 (4.19–26.08)	<0.0001‡

CI = confidence interval; *NOD2* = *nucleotide binding oligomerization domain containing 2*; OR = odds ratio; – = not applicable.
*Mann–Whitney *U* test.
†Those with non–disease-causing *NOD2* mutations were used as a reference.
‡Fisher exact test.
§Family history was considered positive if any first-degree relative had uveitis, arthritis, or skin lesions. The family history was self-reported.

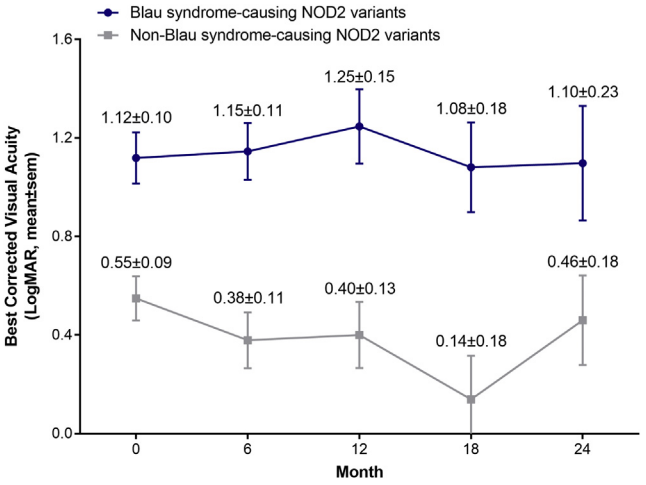

Figure 1. Best-corrected visual acuity in patients with or without disease-causing *nucleotide binding oligomerization domain containing 2* (*NOD2*) variants. The best-corrected visual acuity is shown as scores for the logarithm of the minimum angle of resolution (logMAR), with higher values indicating poorer vision. The following logMAR denotations were used for nonnumerical visual acuities: finger count = 1.7 logMAR, hand movement = 2.0 logMAR, light perception = 2.3 logMAR, and no light perception = 3.0 logMAR. Best-corrected visual acuity was analyzed in a generalized linear model with data of uveitis affected eye for those with unilateral uveitis and with data of right eye for those with bilateral uveitis. SEM = standard error of the mean.

loss-of-function variants that demonstrate impaired NF-κB signaling transduction in early immune response to pathogens and have been interpreted as nonpathogenic for Blau syndrome in our study.[17,27] Evidence suggested that these loss-of-function variants might be at least partially involved in the development of complex or polygenic disorders, especially conferring susceptibility to Crohn's disease, Yao syndrome, necrotizing enterocolitis, or focal intestinal perforation possibly through distinct mechanisms.[28–34] We recognize that the terms "pathogenic," "likely pathogenic," and "benign" are not appropriate to describe these variants in this context that addresses the relevance to a monogenic disorder.[10]

Because of the diagnostic boundaries of Blau syndrome, we were able to characterize the typical ocular features of the disease in patients who presented with uveitis. Consistent with previous reports,[6,25,35] uveitis in the anterior segment may be characterized by mutton-fat keratic precipitates, aqueous flare, or cells, whereas inflammation in the posterior segment may manifest as multifocal choroiditis, papillitis, or retinal microvasculitis. In addition, we observed an overall poor visual outcome of Blau syndrome, probably due to chronic posterior segment inflammation and its irreversible complications, such as secondary glaucoma, epiretinal membrane, choroidal neovascularization, and retinal atrophy. Our findings suggest the need for close and active surveillance of retinal and choroid lesions, if necessary, by virtue of fundus fluorescein angiography or OCT, as well as early intervention for posterior segment inflammation.[6]

Our study provides several clinical implications. First, although Blau syndrome may be characterized by frequent ocular complications and systemic involvements, most of these manifestations were lack of specificity. Therefore, genetic testing would be introduced to assist the diagnosis of the disease in clinical practice. Second, our study reinforces the idea that only *NOD2* gain-of-function mutations are responsible for Blau syndrome. Identification of a rare, novel, or de novo *NOD2* mutation does not necessarily mean this disease, and clinician and laboratory scientists may further need to differentiate the pathogenic nature of the variation from others that are incidental findings or benign. Third, assertions of pathogenicity of variants must be cautiously made on sufficient evidence in accordance with stringent standards[10] and should be explained carefully to families to avoid irrevocable medical decision, such as gene therapy or pregnancy termination, during patient counseling and management.

Table 4. Ocular and Systemic Manifestations of Novel *NOD2* Mutations

NOD2 Mutation	Intraocular Inflammation Involvement		Common Ocular Complication			Systemic Involvement			Additional Ocular Manifestation	Additional Systemic Manifestation
	Anterior	*Posterior*	*Band Keratopathy*	*Complicated Cataract*	*Secondary Glaucoma*	*Arthritis*	*Skin rash*	*Joint Deformity*		
p.I722L	Yes	Yes	Yes	No	No	Yes	No	No	Multifocal choroiditis, choroidal neovascularization and cystoid macular edema	Carpal cyst and conjunctiva cyst
p.I722L	Yes	Yes	No	No	No	No	No	No	Retinal vasculitis	–
p.T476P	Yes	No	Yes	Yes	Yes	Yes	Yes	No	–	–
p.T476del	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Choroidal neovascularization	Carpal cyst and knee cyst
p.R439H	Yes	No	Yes	Yes	No	Yes	No	Yes	–	Carpal cyst

NOD2 = nucleotide binding oligomerization domain containing 2; – = not applicable.

Study Limitations

This study has some limitations. First, our study determined the pathogenicity of several *NOD2* variants identified from 4924 participants and could not cover the entire spectrum of natural *NOD2* variants. Second, we classified these *NOD2* variants based on available evidence in current literature, and the results may need to be modified as evidence on variants evolves. Third, we realize that some of clinical findings may be due not only to the disease itself but also to treatment or patient compliance and that Blau syndrome is individually rare. Our sample size might not be powered enough to address confounders and compare clinical features in detail, and we only described notable traits of the disease based on the descriptive nature of this study. Fourth, this study was conducted by ophthalmologists and mainly focused on uveitis. Those patients without uveitis have not been included in the study. In addition, our study only enrolled Chinese participants, and the study population did not cover all regions of China. Therefore, we were unable to investigate the geographic or ethnic relevance of *NOD2* variants in our study population, and the clinical manifestations reported may be varied with different geographic areas and ethnic groups.

In conclusion, Blau syndrome—causing *NOD2* variants were those gain-of-function mutations biologically causing NF-κB response autoactivation and clinically associated with a high incidence of arthritis, skin rash, visual loss, and ocular complications due to uveitis. Our study calls for cautious interpretation of a *NOD2* variant detected in genetic testing as causative of a patient's signs and symptoms to avoid adverse consequences to the patient's entire family in medical care.

Footnotes and Disclosures

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HUMAN SUBJECTS: Human subjects were included in this study. The study and informed consent procedures were approved by the Ethics Committee of The First Affiliated Hospital of Chongqing Medical University and complied with the provisions of the Declaration of Helsinki. Each participant or parents/legal guardian of minors provided written informed consent, and each child gave assent when appropriate.

No animal subjects were used in this study.

Author Contributions

Conception and design: Zhong

Data collection: Zhong, Ding, Su, Liao, Zhu, Deng, Li, Du, Yang

Analysis and interpretation: Zhong, Liao

Obtained funding: Yang

Overall responsibility: Zhong, Gao, Yang

Abbreviations and Acronyms:
ACMG = American College of Medical Genetics and Genomics; **logMAR** = logarithm of the minimum angle of resolution; **NF-κB** = nuclear factor kappa B; ***NOD2*** = *nucleotide binding oligomerization domain containing 2*.

Keywords:
Blau syndrome, Genetic Variant, NOD2, Uveitis.

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