

# Thalidomide may be an effective medicine for Blau Syndrome

Blau Syndrome (BS) is a monogenic autoinflammatory disease caused by mutations in the nucleotide-binding domain region of the NOD2/CARD15 gene. We reported the case of a patient with a typical triad of rash, arthritis, and uveitis. Sanger sequencing indicated a NOD2 missense variant (c.1759 C > T, p.R587C) in the patient that was inherited from the mother. After treatment with methotrexate, a TNF- $\alpha$  inhibitor, and corticosteroids, the patient's clinical symptoms and inflammatory indicators remained uncontrolled. In the meantime, the patient experienced multiple side effects, such as hypertension and growth retardation attributed to prolonged use of corticosteroids. After treatment with thalidomide, the condition was controlled without recurrence or side effects, and corticosteroids were stopped as soon as possible. This report suggests that thalidomide may be an effective drug for BS treatment.

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**Keywords:** Blau syndrome • monogenic autoinflammatory disease • thalidomide

**Abbreviations:** BS: Blau Syndrome; CRP C: Reactive Protein; EOS Early-Onset Sarcoidosis; ESR Erythrocyte Sedimentation Rate; IKBK I: KB Kinase; MDP: Muramyl Dipeptide; MGCs: Multinucleated Giant Cells; NOD2: Nucleotide-binding oligomerization domain containing 2; RIP2: Receptor-Interacting Serine/Threonine protein Kinase2; TNF; Tumour Necrosis Factor; WBC: White Blood Cell

Submitted: 14 March 2021; Accepted: 24 March 2021; Published online: 30 March 2021

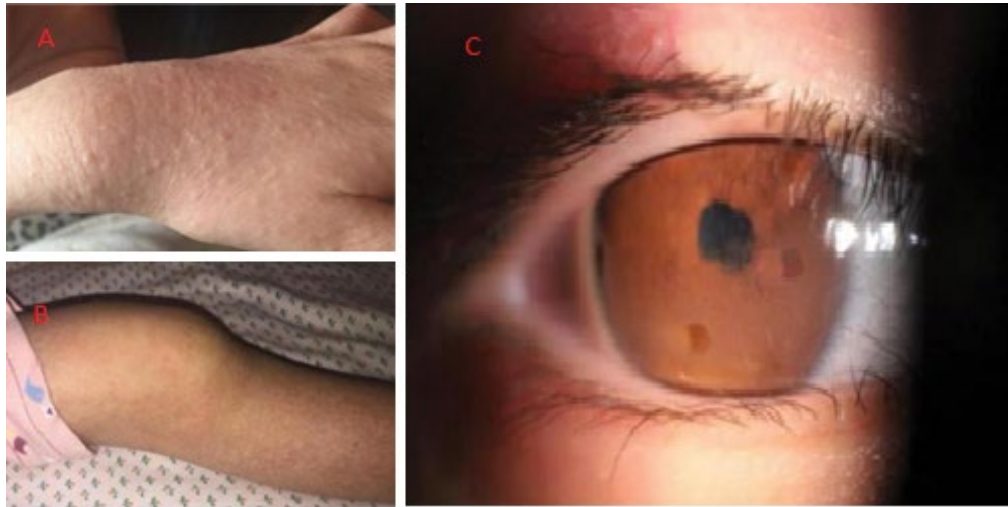
## Case Presentation and Method

The patient, a 10-year-old Chinese girl, developed papules on the trunk and limbs since the age of 4 months (Figure 1A). Her skin biopsy showed granulomatous dermatitis. At 3 years of age, she presented with a history of polyarthritis involving bilateral joints of the wrists, elbows, knees, and ankles (Figure 1B). All the joints manifested as boggy synovitis. She was diagnosed with juvenile idiopathic arthritis and treated with methotrexate with some benefit. The rash and arthritis were partially relieved. At the age of 4, she started complaining about the increasing loss of her eyesight. Ophthalmic examination revealed band keratopathy and partial posterior synechia of the iris of the eye (Figure 1C). She was diagnosed with uveitis. Based on the presence of persistent arthritis, rash and uveitis, Blau syndrome was suspected.

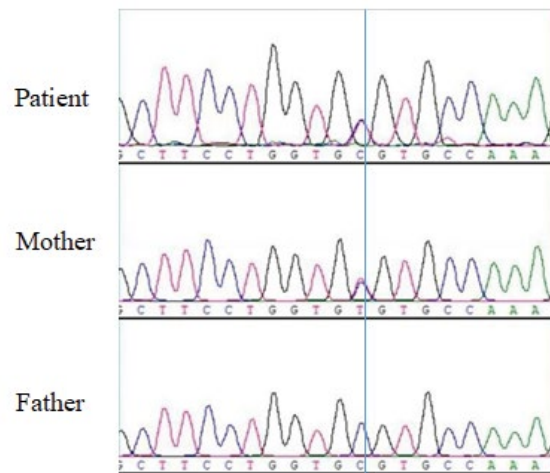
To confirm the diagnosis, DNA from the peripheral blood of the patient and parents was extracted, and Sanger sequencing was performed. The patient had a maternally inherited c.1759C>T, p.R587C mutation in the nucleotide-binding oligomerization domain containing 2 (NOD2) gene (Figure 2), which has been reported as a pathogenic

mutation. Therefore, our patient was diagnosed with Blau syndrome. However, the mother denied a similar medical history.

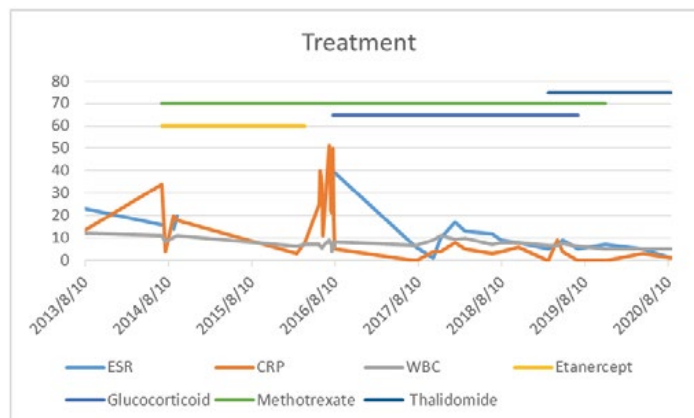
Her clinical symptoms had several recurrences, and her Erythrocyte Sedimentation Rate (ESR, 80 mm/hour at age 6 years) and C-reactive protein (CRP 50 mg/l) increased discontinuously over the years despite the various immunosuppressive therapies attempted, such as prednisolone, methotrexate, and etanercept (Figure 3). The long-term use of glucocorticoids caused severe side effects, such as refractory hypertension and growth retardation [9 years, height 125 cm (P3-P10), weight 24 kg (P10-P25)], even though the dose of glucocorticoids was reduced to 2.5 mg per day. She was treated with thalidomide at the age of 9 years. Glucocorticoids and methotrexate were stopped quickly due to their severe side effects and long-term application, respectively. Her rash and arthritis were in remission, and the inflammatory markers completely returned to the normal range for more than one year despite treatment with a single drug. Her height, weight, and blood pressure gradually returned to the normal range compared to children of the same age [10 years, height 137 cm (P25-P50), weight 32 kg (P50)]. Our patient was monitored



**Figure 1: The manifestations of the patient: A) papular rash; B) arthritis, with “boggy” synovitis of the knee; C) slit-lamp photograph of the eye, showing band keratopathy and partial posterior synechia of the iris.**



**Figure 2: Sanger sequencing of DNA from the patient and both parents shows the c.1759C>T, p.R587C heterozygous mutation in NOD2 in the patient and her mother.**



**Figure 3: Clinical courses of the patient. Thalidomide treatment was started at a dosage of 1 mg/kg/day (25mg/d), beginning in February 2019. The condition remained stable for 24 months despite thalidomide monotherapy**

with 3 monthly clinical examinations and no side-effects, such as peripheral neuropathy and sleepiness, were documented throughout the treatment with thalidomide.

## Discussion

We report here the case of a Chinese girl with BS that was uncontrollable with various immunosuppressive therapies that had a good response to thalidomide. Therefore, thalidomide can inhibit the inflammatory response and improve clinical symptoms in patients with Blau syndrome, with fewer adverse reactions.

BS (MIM #186580) is a rare, dominantly inherited autoinflammatory disorder associated with gain-of-function mutations in the nucleotide-binding oligomerization domain containing 2 (NOD2) gene. BS is characterized by the clinical triad of granulomatous dermatitis, arthritis, and childhood-onset recurrent uveitis [1]. The diagnosis of Blau syndrome is difficult to make according to the clinical manifestations. Some patients are wrongly diagnosed with systemic juvenile idiopathic arthritis at the beginning of the disease [2]. Therefore, genetic testing is necessary for the diagnosis of Blau syndrome. The disease is caused by mutations in the NOD2 gene, mapped on chromosome 16q12, encoding the cytosolic NOD2 protein, one of the key molecules in the regulation of innate immunity. The inheritance pattern is autosomal dominant. BS and Early-Onset Sarcoidosis (EOS) have been identified as familial and sporadic phenotypes of the same disease [3]. The patient in this report has granulomatous dermatitis, arthritis, and childhood-onset recurrent uveitis. Gene sequencing showed a NOD2 missense variant (c.1759C>T; p.R587C) inherited from her mother, which has been reported in previous families [2,4]. However, the mother has no clinical manifestations. In 2009, Frank T. Saulsbury et al reported a family carrying the E383K mutation in NOD2. The proband had typical clinical symptoms of BS, but his aunt, father, and three younger brothers with the same mutation had no clinical manifestations [5]. In 2016, Jun Harada et al reported a child with the E383K pathogenic mutation in NOD2, which was inherited from the father, but the father had no clinical symptoms [6]. The authors suggested that the E383K heterozygous variant in NOD2 has incomplete penetrance. Our results indicate that incomplete penetrance may not be variant-specific and may be a characteristic of the disease.

BS is a progressive disease that can lead to blindness [4]. Therefore, early treatment is necessary to reduce the incidence of sequelae. Due to the low incidence of Blau syndrome and the lack of treatment studies with large samples, a specific treatment scheme has not been established. According to small-sample studies, high-dose glucocorticoids can be used to control symptoms in the acute phase of BS and EOS, and low-dose glucocorticoids can help to control uveitis and joint symptoms in stable periods. Immunosuppressants such as methotrexate, azathioprine, cyclosporine, and mycophenolate mofetil can be added in patients who are refractory to glucocorticoid therapy [7,8].

Therefore, anti-Tumor Necrosis Factor (TNF)- $\alpha$  inhibitors [9,10] or Interleukin (IL)-1 inhibitors [2] combined with corticosteroids and/or other immunosuppressants have been used to treat Blau syndrome. However, there are some reports indicating that these biologics may be ineffective [11].

Thalidomide was mainly used for sedation and as an antiemetic in the clinic in the 1950s [12]. In 1965, the successful treatment of the skin lesions of patients with leprosy with thalidomide suggested that thalidomide had potential anti-inflammatory effects. At present, thalidomide can be used in the treatment of rheumatoid arthritis [13], Behcet's disease [14], inflammatory bowel disease [15], lupus erythematosus [16], stomatitis [17], and so on. Thalidomide has a significant effect on the improvement of skin rash and mucosal ulcers [18]. The protein encoded by NOD2 is a pattern recognition receptor in the cytoplasm and is an intracellular bacterial sensor protein. This protein recognizes the oligomerization of Muramyl Dipeptide (MDP) and interacts with receptor-interacting serine/threonine-protein kinase 2 (RIP2) to activate RIP2 phosphorylation, leading to the activation of NF-kappa B; inducing the production of proinflammatory cytokines, chemokines, and adhesion molecules; protecting the host from infection, and participating in the regulation of inflammation [19]. The plasma levels of IL-1 $\beta$ , IL-6, and TNF- $\alpha$  in patients with BS have been reported to be significantly higher than those in healthy controls [2]. Increased basal NF-kappa B activity could have a central role in the activation of monocytes and the release of proinflammatory cytokines in EOS. Studies have found that thalidomide has a strong immunomodulatory effect and TNF- $\alpha$  effect. In contrast to biological agents that bind TNF- $\alpha$  directly, thalidomide can inhibit the synthesis of TNF- $\alpha$  [20] and promote the degradation of TNF- $\alpha$  mRNA [21]. On the other hand, thalidomide can block the activation of NF-kappa B by inhibiting the activity of I-kB kinase (IKK) in the TNF- $\alpha$ /NF-kappa B signaling pathway [22]. In 2010, Japanese scholar Kozo Yasui et al. found that thalidomide dramatically improved the symptoms of early-onset sarcoidosis/Blau syndrome. In that study, thalidomide downregulated NF-kappa B signaling by inhibiting IKK to further inhibit the formation of Multinucleated Giant Cells (MGCs) and osteoclasts, which are important in the development and maintenance of sarcoidal granulomatous lesions [23]. The condition of our patient with BS was also controlled after treatment with thalidomide. Therefore, thalidomide can effectively inhibit severe inflammatory reactions in patients with NOD2-related diseases.

Our patient experienced no side-effects throughout the treatment with thalidomide. Peripheral neuropathy is a common and severe side effect of thalidomide treatment, presenting as a sensory, painful neuropathy [24]. It may improve after thalidomide dosage-reduction or discontinuation. Thalidomide-induced peripheral neuropathy has been documented in children. The prevalence ranges from 1% to 70% [25,26]. The risk factors for thalidomide neuropathy include daily dosage, duration

of drug exposure, cumulative dosage. A prospective study of 135 dermatologic patients treated with thalidomide identified the daily dosage as the main risk factor and found that the risk of neuropathy seems to be negligible for dosages less than 25 mg per day, whatever the duration of therapy [27]. But another study in the group of children shown that long-term application for >10 months and cumulative dosages at >20 gm of thalidomide were likely to increase the risk of peripheral neuropathy [28]. Our patient was monitored with 3 monthly clinical examinations without neurologic examination and she had no neuropathic symptoms, weakness, or sensory loss 24 months after treatment with a dosage of 25 mg per day, after a cumulative dosage of 18 gm of thalidomide. So the daily dosage of 25 mg daily is probably safe. Longer follow-up is needed to monitor the side effects of thalidomide.

## Conclusion

In summary, we reported the case of a patient with BS whose treatment with glucocorticoids, methotrexate, and biologics was ineffective. Thalidomide contributed to the control of inflammation in the patient. Therefore, thalidomide can inhibit the inflammatory response and improve clinical symptoms in patients with BS, with fewer adverse reactions. For patients with refractory BS, thalidomide use can be attempted, but at present, studies about the treatment of BS

with thalidomide are few, and more research is needed to evaluate the efficacy and side effects of thalidomide.

## Author Contributions

WW1 contributed to the analysis of data and writing of the original draft. WW2 and LQZ contributed to the performing of Sanger sequencing. WDL contributed to the collection of raw clinical data. SJW contributed to the examination of the patient's eyes. HMS contributed to conceptualization, editing, funding acquisition, and supervision. Both authors approved the final version of the manuscript.

## Funding/Support

Supported by Beijing Natural Science Foundation (L202050), CAMS Innovation Fund for Medical Sciences (CIFMS)(2016-I2M-1-008), Public Welfare Scientific Research Project of China (201402012) and The National Key Research and Development Program of China (2016YFC0901500).

## Conflicts of Interest

The authors state no conflict of interest. No financial or non-financial benefits have been received or will be received from any party related directly or indirectly to the subject of this article..

**Executive summary**

Blau Syndrome (BS) is a monogenic autoinflammatory disease caused by mutations in the nucleotide-binding domain region of the NOD2/CARD15 gene. We reported the case of a patient with a typical triad of rash, arthritis, and uveitis. Sanger sequencing indicated a NOD2 missense variant (c.1759 C >T, p.R587C) in the patient that was inherited from the mother. After treatment with methotrexate, a TNF- $\alpha$  inhibitor, and corticosteroids, the patient's clinical symptoms and inflammatory indicators remained uncontrolled. In the meantime, the patient experienced multiple side effects, such as hypertension and growth retardation attributed to prolonged use of corticosteroids. After treatment with thalidomide, the condition was controlled without recurrence or side effects, and corticosteroids were stopped as soon as possible. This report suggests that thalidomide may be an effective drug for BS treatment.

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