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Role of NOD2 Pathway Genes in Sarcoidosis Cases with Clinical Characteristics of Blau Syndrome

To the Editor:

Blau syndrome, a rare autosomal dominant disorder, presents in early childhood with granulomatous arthritis, dermatitis, and uveitis (1). Blau has been strongly associated with mutations in the *NOD2* gene (nucleotide-binding oligomerization domain–containing protein 2) (2), a member of the NOD-like receptor family involved in innate immunity. Blau shares similarities in phenotype and histological findings (presence of noncaseating granulomas) with sarcoidosis, another multisystemic granulomatous disorder. However, it differs in mode of inheritance, onset age, and pattern of organ involvement. Sarcoidosis rarely presents in childhood (3) and involves the lungs in more than 90% of cases, although any organ may be affected (e.g., bone/joints, skin) (3).

Despite these similarities, studies of patients with adult sarcoidosis have failed to find an association with NOD2 (4). In this study, we assess the role of genes within the NOD2 pathway in patients with adult sarcoidosis exhibiting similar pattern of organ involvement to Blau (eye, skin, bone/joint).

Methods

Analysis was performed independently for European American (EA) and African American (AA) sarcoidosis cases. AA cases (n = 1273) were compiled from the Sarcoidosis Genetic Analysis study population (5) and the National Heart, Lung, and Blood Institute's ACCESS (A Case Controlled Etiologic Study of Sarcoidosis) study (6), and a cohort from the Detroit Henry Ford Health System. EA cases (n = 442) were obtained from the ACCESS study. Analysis of each cohort involved the comparison of cases positive for both skin and bone/joint involvement to those negative for both. Using EMMAX (Efficient Mixed-Model Association eXpedited) (7), single-marker sex-adjusted association tests were performed for the single-nucleotide polymorphisms (SNPs) in a number of genes selected from the NOD2 pathway (Figure 1). The NOD2 pathway has a total of 30 components, but our analysis excluded genes encoding pathway byproducts (e.g., chemokines), leaving 23 genes (covering 7,615 SNPs for AA and 2,612 for EA). The effective number of independent tests (N_{eff}) was estimated by computing the number of linkage disequilibrium (LD) blocks (LD threshold: $r^2 = 0.5$) in our SNP data, and Bonferroni correction was used to adjust for multiple testing. Thresholds for suggestive associations in each analysis were chosen on the basis of the expectation of a single false-positive association per analysis.

Results and Discussion

No significant associations were seen in *NOD2* in both AA and EA analyses. For EA, we observed novel significant associations in four variants within the *TAB2* gene. These variants were in complete LD ($r^2 = 1$). For AA, we observed a significant genetic association in a *MAPK13*, a gene encoding p38-δ, one of the four isoforms of the p38 MAP kinase.

Table 1 provides information on the SNPs, and Figure 1 provides a graphical overview of the pathway components harboring significant and/or suggestive variants in either cohort.

The four perfectly correlated SNPs identified in the EA cohort analysis reside in a large haplotype block within the *TAB2* (TAK1-binding protein 2) gene. In the AA cohort, we also discovered a suggestive association in the *TAB2* gene. Although there is no overlap between the *TAB2* SNPs identified in either cohort, the association of this gene in both race groups suggests its potential relevance to the subphenotype of interest and provides evidence of "gene-level replication." Our AA analysis also revealed suggestive associations for SNPs in *TAB1* (TAK1-binding protein 1), a functional counterpart of *TAB2*. *TAB1* and *TAB2* bind to *TAK1* to form a complex that functions as a single unit. These results suggest a key role for the TAK1–TAB1–TAB2 complex in the intracellular signaling cascade initiated by NOD2. The complex is known to be involved in macrophage (a component of granulomas) activation, and deletion of TAB1/TAB2 results in macrophage death (8). Studies have also shown this complex plays regulatory roles in the epidermal and intestinal epithelium (9) and in joint and cartilage development (10).

We also observed a suggestive associated variant in *TNFAIP3* (tumor necrosis factor α–induced protein 3), a gene encoding A20, a ubiquitin-editing enzyme that has been shown to inhibit NF-κB activation as well as TNF-mediated apoptosis.

All variants showing significant/suggestive associations were located in noncoding regions. Functional annotation using HaploReg and RegulomeDB revealed enrichment for transcription factor binding sites, enhancer/promoter histone modification, and DNase hypersensitivity in different cell types.

This study is the first to fully explore the NOD2 signaling pathway in search of downstream components that might play a role in the granulomatous inflammation common to Blau and sarcoidosis. Our findings will guide further investigations into the mechanisms leading to Blau-like manifestations of sarcoidosis. Although we further confirm that the *NOD2* gene itself is not a contributor to sarcoidosis, we do provide evidence indicating the NOD2 pathway is involved. ■

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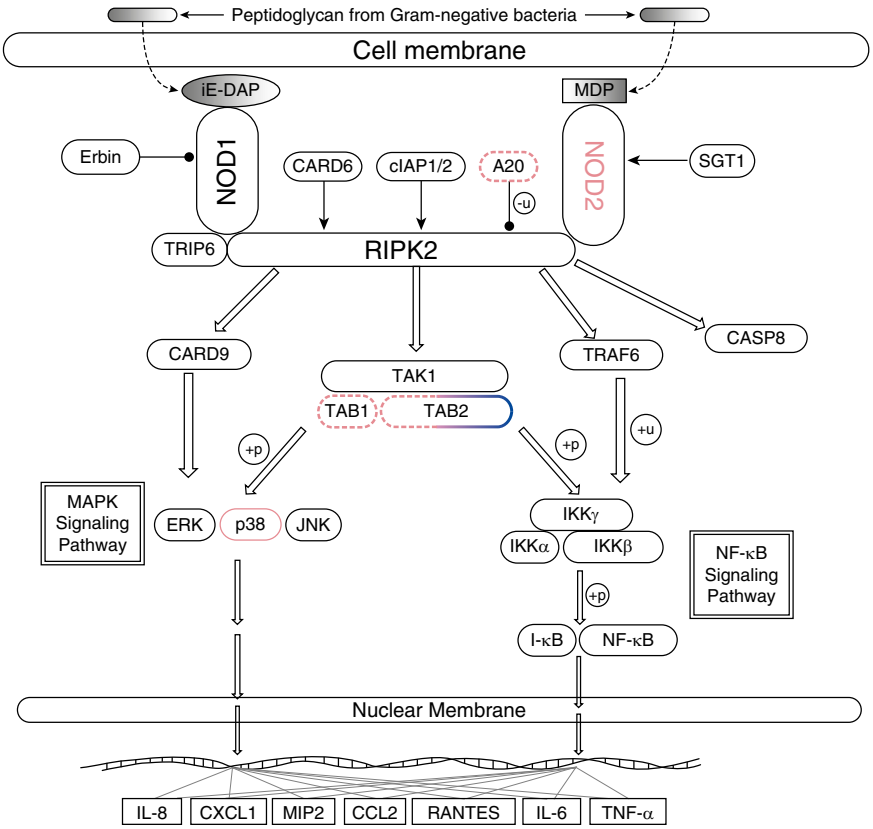

Figure 1. NOD2 pathway. Genes found to harbor significant/suggestive single-nucleotide polymorphism (SNPs) are highlighted with *pink* (for African Americans) or *blue* (for European Americans) borders. *Dashed borders* are used to denote genes with only suggestive SNPs. *Lines with circular tips* denote inhibitory effects. A20 = TNFAIP3 (tumor necrosis factor-α–induced protein 3); MAPK = mitogen-activated protein kinase; NOD2 = nucleotide-binding oligomerization domain–containing protein 2; p38 = p38 MAPK; TAB = TAK1-binding protein.

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Table 1. NOD2 Pathway SNPs Exhibiting Significant or Suggestive Associations with a Blau Syndrome–like Phenotype in Adult Sarcoidosis Cases

Cohort	Chromosome	Gene	SNP	Position (hg19)	Reference/Alternate Allele	*P* Value
European American	6	*TAB2*	rs76778446	149646830	C/T	1.30×10^{-5}*
			rs79995379	149650461	A/G	
			rs111447766	149689465	A/G	
			rs111576955	149697332	C/T	
African American	6	*TAB2*	rs9404026	149616799	C/G	1.91×10^{-4}
		MAPK13	rs138268427	36109934	G/A	8.45×10^{-7}*
	22	*TAB1*	rs35506409	39812207	C/G	4.71×10^{-5}
			rs34804656	39827556	G/A	5.37×10^{-4}

Definition of abbreviations: MAPK13 = mitogen-activated protein kinase 13; NOD2 = nucleotide-binding oligomerization domain–containing protein 2; SNP = single-nucleotide polymorphism; TAB = TAK1-binding protein.
*Statistically significant *P* values.

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Middle East Respiratory Syndrome: A Concern

To the Editor:

I found the recent editorial on Middle East respiratory syndrome (MERS) to be quite interesting (1). Hui and Peiris report that "while the spread of MERS-[coronavirus] infection to Korea, China, and Thailand has posed challenges to the clinicians and hospital administrators, this has also provided an opportunity to try to tackle many unanswered questions." In fact, as is the case with many new emerging diseases, lack of knowledge of diagnosis, treatment, and prevention is common.

However, there are other considerations. First, the occurrence of the disease in new settings in East and Southeast Asia highlights the importance of international disease control. Although there are many international policies that address disease control, the problem still exists. In the first period of the emergence of MERS in 2012, the main focus was usually on the Hajj pilgrims (2). However, the present problem in Asia is not related to the Hajj pilgrimage at all (2, 3). The importance of having a single standard in the disease surveillance system should be discussed. Second, the phenomenon of "silent" MERS infection also should be mentioned (4–6). Silent infection can be difficult to detect and may be an important route of transmission for this disease. ■

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Reply

From the Authors:

We thank Dr. Wiwanitkit for his comments on our editorial (1), which mainly addressed the risk factors of nosocomial outbreaks and knowledge gaps in our current understanding of the Middle East respiratory syndrome coronavirus (MERS-CoV) infection after the World Health Organization (WHO) mission to the Republic of Korea in June 2015. The first case of MERS-CoV infection was confirmed in the Republic of Korea on May 20, 2015, and there was export of a case to China on May 26, 2015, as a result of inadequate implementation of a quarantine protocol and suboptimal public health surveillance in the initial period (2). As the WHO Emergency Committee called for all countries on June 16, 2015, to be prepared for the possibility of outbreaks of disease (3), and the WHO Regional Office for South East Asia urged countries in the region to step up their vigil and review preparedness to respond to the disease, Thailand became the 26th country to have a confirmed case of MERS-CoV disease on June 18, 2015, in a traveler from the Middle East region (4). It is therefore essential for all countries to enhance surveillance for severe acute respiratory infections such as MERS, focus on early diagnosis and isolation, and step up infection control and prevention procedures in healthcare facilities.

Outbreaks of MERS in different geographic settings may pose special challenges. Although the Hajj pilgrimage in Saudi Arabia has not been shown to increase the risk for MERS-CoV infection over the last few years (5–8), active public health surveillance of the pilgrims is needed to deal with any infectious diseases that may arise from mass gathering events.

Asymptomatic contacts including healthcare workers with positive polymerase chain reaction for MER-CoV have been detected (9, 10), including one case with positive polymerase chain reaction for up to 35 days (11). However,