Received: 16 February 2021 | Revised: 8 November 2021 | Accepted: 17 December 2021

DOI: 10.1002/mgg3.1859

Molecular Genetics & Genomic Medicine Open Access WILEY

ORIGINAL ARTICLE

Neutrophil cytosolic factor 2 (*NCF2*) gene polymorphism is associated with juvenile-onset systemic lupus erythematosus, but probably not with other autoimmune rheumatic diseases in children

Ivan Y. Bakutenko[1] | **Irena D. Haurylchyk**[1] | **Natalia V. Nikitchenko**[1] | **Elena V. Sechko**[2] | **Inna A. Kozyro**[2] | **Alexei M. Tchitchko**[2] | **Galina M. Batyan**[2] | **Alexander V. Sukalo**[2] | **Nadezhda I. Ryabokon**[1]

[1]Laboratory of Molecular Basis of Genome Stability, Institute of Genetics and Cytology, National Academy of Sciences of Belarus, Minsk, Belarus

[2]1st Department of Childhood Diseases, Belarusian State Medical University, Minsk, Belarus

Correspondence
Nadezhda I. Ryabokon, Laboratory of Molecular Basis of Genome Stability, Institute of Genetics and Cytology, National Academy of Sciences of Belarus, 27 Akademicheskaya St., Minsk 220072, Republic of Belarus.
Email: n.ryabokon@igc.by

Funding information
This research was supported by The Research Technical Program "DNA Identification" (2017–2021), project number: 6.4C.

Abstract

Background: Genetic variations of neutrophil cytosolic factor 2 (NCF2), a subunit of NADPH oxidase, are usually associated with chronic granulomatous disease, and their relationship with autoimmune disorders through the defective NADPH oxidase function during phagocytosis is suggested. Our study aimed to explore whether there is an association between the non-synonymous single nucleotide polymorphism in the *NCF2* gene (rs17849502, NC_000001.11:g.183563445G>T) and the development of juvenile autoimmune rheumatic diseases.

Methods: In order to test this hypothesis, we conducted a pilot case–control study. In total, 709 children and adolescents, all Belarusians, were involved in the study including patients with juvenile-onset systemic lupus erythematosus (JSLE), juvenile idiopathic arthritis (JIA), Kawasaki disease (KD), and subjects without autoimmune and inflammatory diseases as the clinical control, as well as health newborns as the population control. Real-time polymerase chain reaction was used for genotyping.

Results: The minor T allele of *NCF2* occurred most frequently in patients with JSLE (OR = 2.60, 95% CI = 1.18–5.73, $p = 0.023$ as compared to the clinical control). In groups with JIA and KD, its frequency did not differ from the control. The TT genotype was only observed in 5.7% of patients with JSLE ($p = 0.007$), but not in other groups.

Conclusion: Therefore, our study suggested that *NCF2* rs17849502 polymorphism is a potential genetic risk factor for JSLE, while it is probably not for such autoimmune rheumatic diseases as JIA or KD.

KEYWORDS

genetic risk factor, juvenile idiopathic arthritis, juvenile-onset systemic lupus erythematosus, Kawasaki disease, *NCF2* gene

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.
© 2021 The Authors. *Molecular Genetics & Genomic Medicine* published by Wiley Periodicals LLC

1 | INTRODUCTION

Neutrophil cytosolic factor 2 (NCF2 or p67^{phox}) is a subunit of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, an enzyme that produces superoxide in the phagosomes of neutrophils and other phagocytic leukocytes for the neutralization (digestion) of foreign microorganisms and cellular debris, although evidence for *NCF2* (OMIM accession number: 608515) expression in other cells is also available (Bedard & Krause, 2007; Cachat et al., 2015). The leucocyte NADPH oxidase complex consists of multiple subunits, including two membranous (CYBA and CYBB) and three soluble (NCF1, NCF2, and NCF4), as well as small GTPase (Rac1/2) as an activator (Cachat et al., 2015). Deficiency in any of the above proteins may lead to chronic granulomatous disease (CGD), a primary immunodeficiency disorder with its usual onset in early childhood, which is characterized by a very low level of reactive oxygen species (ROS) in phagosomes due to the defects in NADPH oxidase activity, and as a result, abnormal cellular digestion in phagocytes, followed by the formation of granulomas in various organs resulting in recurrent infections, impaired inflammation and autoimmune disorders (Arnold & Heimall, 2017).

In addition to CGD, a number of studies provide evidence that changes in the *NCF2* gene sequence may be associated with the development of lupus and lupus-like diseases (Jordan & Baxter, 2020), less commonly with inflammatory bowel disease (O'Neill et al., 2015), coeliac disease (Gutierrez-Achury et al., 2016), systemic sclerosis (Márquez et al., 2018) and juvenile idiopathic arthritis (JIA). The latter was found in a patient with CGD (AlKhater, 2019). All these diseases are autoimmune and are characterized by impaired immunological tolerance. This condition leads to tissue and organ damage as a result of autoantibodies production and chronic inflammation. The aforementioned relationship underlies the hypothesis that the nature of certain autoimmune disorders may be partially associated with insufficient ROS production by the NADPH oxidase complex (O'Neill et al., 2015).

A known missense variant in the *NCF2* gene is rs17849502 single nucleotide polymorphism (NC_000001.11:g.183563445G>T), which leads to the substitution of a histidine residue with glutamine (H389Q) in the NCF2 protein. This substitution reduces the affinity of NCF2 for the Vav1 protein, causes a decrease in activity of the NADPH oxidase in response to stimulation by the signaling pathway using Vav1 and may be a genetic risk factor for both adult and juvenile-onset systemic lupus erythematosus (Jacob et al., 2012).

Taking into account the above considerations, we have focused in this pilot study on the relationship between the rs17849502 polymorphism and a risk of pediatric autoimmune rheumatic diseases, such as juvenile systemic lupus erythematosus (JSLE), JIA, and Kawasaki disease (KD). These are multifactorial diseases caused by both genetic and environmental factors, including infection attacks. Most of their forms are characterized by multi-tissue damage and disease severity (McCurdy & Parsa, 2021; Singh et al., 2018; Smith et al., 2019). We designed a case-control study for association analysis and our finding highlighted the importance of *NCF2* polymorphism in JSLE pathogenesis and suggested that this substitution does not possibly increase susceptibility to JIA and KD.

2 | METHODS

2.1 | Patient and control groups

A total of 709 children and adolescents under 16 years at the disease onset were recruited into the study. All participants of the study were of European origin, namely, Belarusians based on the questionnaires completed by the parents. Among them were 35 patients with JSLE, 200 patients with JIA, 49 with KD, and 368 subjects as the clinical control. In 2012–2020, they were consecutively enrolled in the 2nd City Children's Clinical Hospital (Minsk) and the City Children's Infectious Clinical Hospital (Minsk), both are the clinical bases of the 1st Department of Childhood Diseases, the Belarusian State Medical University (Minsk). All subjects were unrelated individuals. JSLE, JIA, or KD were diagnosed in accordance with the international criteria (Aringer et al., 2019; McCrindle et al., 2017; Petty et al., 2004). Patients with a history of other autoimmune diseases or a malignancy were not included in the study. Moreover, monogenic JSLE was not expected in our study, since there were no patients with the familial cases of autoimmune diseases and there was only one patient with an early-onset manifestation of JSLE (4 years old) as the clinical criteria for monogenic lupus (Demirkaya et al., 2020). The subjects admitted to the cardiological department of the 2nd City Children's Clinical Hospital with blood pressure dysregulation (mostly) or/and heart arrhythmia (more rarely), but without an articular pathology, or autoimmune, acute and chronic inflammatory diseases and with no familial history of any autoimmune disease were included in the clinical control group. The population control consisting of 57 disease-free newborns was also included in the study. The DNA samples for this group were collected by the Laboratory of Ecological Genetics and Biotechnology and kindly provided from the Republican DNA Bank of a Human, Animals, Plants, and Microorganisms (the Institute of Genetics and Cytology, NAS of Belarus).

Demographic characteristics of the study groups are listed in Table 1.

2.2 | DNA extraction and genotyping

Peripheral venous blood samples were obtained in tubes containing ethylenediaminetetraacetic acid (Sarstedt). Total genomic DNA was extracted from blood samples using the standard phenol-chloroform protocol as described previously (Sambrook & Russell, 2006). Before genotyping, DNA samples were stored at −20 and −86°C for short and long periods respectively.

The samples were genotyped by real-time polymerase chain reaction (PCR) using the Applied Biosystems QuantStudio 5 Real-Time PCR System. Primers and fluorescent probes were designed using the Beacon Designer Free Edition (http://www.premierbiosoft.com/qOligo/Oligo.jsp?PID=1) and are shown in Table 2. The primers and probes were checked for specificity using the Primer BLAST Tool at the NCBI website (http://www.ncbi.nlm.nih.gov/tools/primer-blast/) and were produced by the custom oligonucleotide synthesis service (Primetech ALC). The PCR conditions were as follows: 95°C for 5 minutes followed by 40 cycles at 95°C for 15 seconds, 63°C for 30 seconds, and elongation at 72°C for 20 seconds. The presence of different variants of investigated rs17849502 was confirmed by the Sanger sequencing method using the ABI Prism 3500 automatic sequencer (Applied Biosystems).

2.3 | Statistical analysis

Data analysis was performed using SNPassoc package (Gonzalez et al., 2007) for R v.4.0.0 and SciStat web calculators (https://www.scistat.com/statisticaltests/). Deviation from the Hardy-Weinberg equilibrium, as well as the allele and genotype distributions between the cases and the control were analyzed using the chi-square test. An association between genetic polymorphism and a susceptibility to disease was assessed by the odds ratio (OR) with a corresponding 95% confidence interval (95% CI) computed with the likelihood ratio test, and OR calculations were adjusted for sex. The value of $p < 0.05$ was considered statistically significant. The statistical power was also calculated using the pwr package for R v.4.0.0 to detect possible differences among the case and control groups.

3 | RESULTS

Table 3 summarizes basic data on the rs17849502 allele and genotype distribution in the groups under study. We did not find any differences ($p < 0.05$) in allele and genotype frequencies between the population and clinical controls that allowed us to use the latter in the case-control comparison. Furthermore, our results did not show a significant deviation from the Hardy-Weinberg equilibrium ($p < 0.05$) and demonstrated that the minor T

TABLE 1 Demographic characteristics of case and control groups

Group or subgroup	Number of patients	Females, %	Age at study, mean ± SD, year	Age at disease onset, mean ± SD, year
Clinical control	368	43.5	14.4 ± 2.5	—
Population control	57	50.9	Newborns	—
JSLE	35	88.6	13.4 ± 2.8	11.6 ± 3.2
including JSLE forms				
with lupus nephritis	27	88.9	13.6 ± 2.5	11.5 ± 3.0
without lupus nephritis	8	87.5	12.6 ± 4.2	12.0 ± 4.1
JIA	200	64.5	9.8 ± 4.9	6.8 ± 4.9
including JIA subtypes				
Systemic	20	50.0	6.8 ± 5.0	4.3 ± 4.5
Oligoarthritis	117	68.4	8.9 ± 4.7	5.7 ± 4.5
Polyarthritis, RF positive	4	75.0	15.0 ± 0.0	13.1 ± 1.3
Polyarthritis, RF negative	31	64.5	11.0 ± 4.2	7.0 ± 3.7
Psoriatic arthritis	2	100	16.0 ± 1.4	15.5 ± 0.7
Enthesitis-related arthritis	13	23.1	15.1 ± 2.1	14.1 ± 2.0
Undifferentiated arthritis	13	84.6	11.3 ± 4.6	10.2 ± 4.1
KD	49	20.4	3.1 ± 2.7	2.5 ± 2.4

Abbreviations: JIA, juvenile idiopathic arthritis; JSLE, juvenile systemic lupus erythematosus; KD, Kawasaki diseases; RF, rheumatoid factor; SD, standard deviation.

TABLE 2 Sequences of primers and probes used for genotyping

Oligo name	Sequence (5′−3′)
Fw	AGCACAAGGTTCCCACTGTA
Rv	CCGGGACATGGTGTCTAAGA
Probe G	FAM-TCAGCTTAGTGTGTTCCAGCC-BHQ1
Probe T	ROX-TCAGCTTAGTTTGTTCCAGCC-BHQ2

Abbreviations: Fw, forward primer; Rv, reverse primer.

allele frequency in the control groups (3.5% and 4.9%) was similar to that of other European populations (3.6%−6.8%) (The 1000 Genomes Project Consortium, 2015). At the same time, the T allele frequency was significantly higher in the patients with JSLE (OR = 2.60, 95% CI = 1.18–5.73, $p = 0.023$) when compared with the clinical control, but not in other groups with autoimmune diseases, including JIA and KD. Moreover, a tendency for an opposite (i.e., protective) effect of the T allele in the patients with JIA may be expected (Table 3).

We also found that the TT genotype was rare in the children of the Belarusian population and occurred only in the JSLE group ($p = 0.007$ as compared to the clinical control group). The GT genotype, as well as GT + TT genotypes, did not demonstrate any difference in terms of prevalence between the patient and control groups ($p < 0.05$). However, there was a tendency for a greater distribution of GT + TT genotypes (OR = 2.30, 95% CI = 0.93–5.67, $p = 0.084$) in the JSLE group.

For the log-additive allelic (T vs. G) model and the preset $\alpha = 0.05$ (one-sided), the statistical power to detect an association between the rs17849502 variant and JSLE (i.e., to reject a null hypothesis of genetic association) was equal to 83.8% that achieves the adequate statistical power value of 80%. Regarding other groups of patients, the statistical power values were very low, about 21.9% and 9.8% for the association with JIA and KD, respectively, which means a large risk of false-negative results.

4 | DISCUSSION

Our preliminary case-control study aimed to identify if there is a relationship between the rs17849502 polymorphism of the neutrophil cytosolic factor 2 (*NCF2*) gene and the development of juvenile autoimmune rheumatic diseases. We hereby demonstrated a significant association between the minor T allele of rs17849502 and a risk of JSLE, but in case of other diseases (JIA and KD), such an association was not observed. We also evaluated that the TT genotype may be specific for JSLE with a strong level of significance ($p = 0.007$), and its frequency in the Belarusian patients who suffered from JSLE is about

TABLE 3 Associations of the rs17849502 allele and genotypes with juvenile autoimmune diseases (adjusted for sex)

	Population control	Clinical control	JSLE			JIA			KD		
Allele and genotypes	***n* (%)**	***n* (%)**	***n* (%)**	**OR (95% CI)**	***P***	***n* (%)**	**OR (95% CI)**	***p***	***n* (%)**	**OR [95% CI]**	***p***
T	4 (3.5)	36 (4.9)	**10 (14.3)**	**2.60 (1.18–5.73)**	**0.023**	15 (3.8)	0.67 (0.35–1.26)	0.205	4 (4.1)	0.85 (0.29–2.54)	0.769
TT	0 (0.0)	0 (0.0)	**2 (5.7)**	-	**0.007**	0 (0.0)	—	—	0 (0.0)	—	—
GT	4 (7.0)	36 (9.8)	6 (17.1)	1.62 (0.61–4.33)	0.353	15 (7.5)	0.67 (0.35–1.26)	0.205	4 (8.2)	0.85 (0.29–2.54)	0.769
TT + GT	4 (7.0)	36 (9.8)	8 (22.9)	2.30 (0.93–5.67)	0.084	15 (7.5)	0.67 (0. 35–1.26)	0.205	4 (8.2)	0.85 (0.29–2.54)	0.769

Note: A case-control comparison was drawn between the case groups and the clinical control. Statistically significant data ($p < 0.05$) are highlighted in bold.

Abbreviations: 95% CI, 95% confidence interval; JIA, juvenile idiopathic arthritis; JSLE, juvenile systemic lupus erythematosus; KD, Kawasaki diseases; *n*, number of patients; OR, odds ratio.

5.7%. We similarly observed that the frequencies of the rs17849502 T allele in the groups of children of the Belarusian population (14.3% of JSLE cases vs. 4.9% in the clinical control) are close to those observed in children of European Americans (19.6% of JSLE cases vs. 5.6% in the control; Jacob et al., 2012).

Previously published data suggested that the rs17849502 T allele is strongly associated with the development of adult-onset systemic lupus erythematosus (SLE) in different ethnic groups (Armstrong et al., 2015; Jacob et al., 2012; Kim-Howard et al., 2014; Reid et al., 2020). Wherein, the T allele frequency was reported to be 2.1%, 3.5%, and 5.0% in the controls and 8%, 6.9%, and 12.0% in the case groups of African Americans, Hispanic ancestry, and European Americans, respectively, with ORs between 2.0 and 2.8 (Jacob et al., 2012; Kim-Howard et al., 2014). As regards JSLE, this association was described as strong in European Americans and weak in other American ethnic groups (Jacob et al., 2012). Moreover, a higher value of OR was found by Jacob et al., 2012 in juveniles (4.1) as compared to adults (2.4).

Despite such promising results with regard to the JSLE risk assessment, and also detailed functional characteristics of rs17849502 as a risk factor of JSLE (Jacob et al., 2012), we did not find other studies, including population ones, that investigated an association between rs17849502 and JSLE. Thus, the results of our study carried out in one of the European populations may prove the importance of *NCF2* rs17849502 single nucleotide polymorphism as a risk factor of JSLE. The results also suggested that rs17849502 polymorphism does not possibly increase a risk for the development of other severe rheumatic diseases in children (JIA and KD).

Taking into account the data on the amino acid change (H389Q) in the NCF2 protein at the rs17849502 G>T substitution in the association with SLE (Jacob et al., 2012), we suggest that this genetic variant may be classified as strongly pathogenic (class PS1), according to the recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (Richards et al., 2015). Accumulating evidence has indicated the functional importance of the rs17849502 nucleotide substitution. At the molecular level, the homozygous H389Q substitution (TT genotype) may lead to an ~50% reduction in the ROS production by NADPH oxidase as experiments with transformed cells show (Jacob et al., 2012).

A study of the *NCF2* gene's role using a mouse model showed that the knockout of a gene alone does not cause a lupus-like disorder. However, the *NCF2* knockout leads to the accelerated development of symptoms and a more severe course of the disease in the lines of lupus-prone mice (Jacob et al., 2017). This may explain why the prevalence of the rs17849502 risk allele among SLE and JSLE patients is not very high.

As regards SLE, the macrophages of patients with SLE show a decrease in the efficiency of efferocytosis, that is phagocytosis of apoptotic bodies (Gaipl et al., 2007; Hahn et al., 2019), including at the level of the phagocytosis-related genes expression (Majai et al., 2014). A similar phenomenon is observed in murine macrophages isolated from CGD animals (Bagaitkar et al., 2018). Moreover, there is evidence that the NADPH oxidase is involved in the formation of patterns of major histocompatibility complex class II-restricted epitopic repertoires by controlling phagosome acidification (Allan et al., 2014; Bagaitkar et al., 2018). The pathogenetic mechanism associated with a decrease in the ROS production by neutrophils and the development of SLE is more likely to be a deficient clearance of apoptotic cells due to a decrease in the efferocytosis efficiency and impaired digestion of apoptotic debris (Hahn et al., 2019). In lupus-prone genetic background, this may lead to the production of autoantibodies and chronic inflammation, which are characteristic of SLE.

Failure in the process of efferocytosis is not only associated with the development of SLE (Abdolmaleki et al., 2018; Boada-Romero et al., 2020; Tajbakhsh et al., 2020) and related, or SLE-like disorders (Boada-Romero et al., 2020; Tajbakhsh et al., 2020), but also with other autoimmune diseases such as rheumatoid arthritis, type I diabetes, multiple sclerosis, autoimmune lymphoproliferative syndrome, ulcerative colitis, Crohn's diseases, and autoimmune uveitis (reviewed in Abdolmaleki et al., 2018; Boada-Romero et al., 2020). In this regard, it is obviously possible to continue the above list of diseases by including JSLE, which is also characterized by increased apoptosis rates (Midgley et al., 2009), aside from JIA or KD in which the processes related to the formation or removal of apoptotic bodies have not been well studied yet and possibly due to the lack of obvious involvement in the pathogenesis of these diseases. The latter may explain the reason why *NCF2* rs17849502 polymorphism is unlikely to have any association with JIA and KD.

The discussed mechanism unravels the pathogenesis only for a small fraction of patients with JSLE (at least about 5.7% with the homozygous H389Q substitution in the present work). This is in line with the genetic heterogeneity of JSLE, but on other hand, this does not exclude the involvement of *NCF2* rs17849502 polymorphism in other signal pathways, and therefore further thought and examination are required.

There is a limitation to the present study, namely the small groups of patients. Since the Belarusian population is relatively small, it is difficult to expect the formation of large study groups of patients with juvenile rheumatic diseases. Nevertheless, as regards data on the rs17849502 T allele in the JSLE patients,

our study reached *p* value less than a preset threshold of 0.05, and thus a null hypothesis is rejected and the likelihood of type I error (false-positive results) is low. Due to small groups under study, the statistical power might be expected to be low. Nevertheless, for the data on JSLE, the achieved power level was higher than the conventional threshold of 80% that may contribute to good sensitivity and reproducibility of the results obtained. In this context and taking into account the previously obtained data on European Americans with JSLE (Jacob et al., 2012) we may suggest that the reproducibility of our study can be estimated as the fifth-class association according to the Better Associations for Disease and Genes (BADGE) system (Manly, 2005). However, as regards the data on JIA and KD, showing inadequate statistical power, the possibility of a type II error cannot be ruled out. Anyway, the results of this study are of exploratory character and may stimulate further investigation.

5 | CONCLUSIONS

As far as we know, our study is the first one to identify that the rs17849502 polymorphism of the *NCF2* gene may be a genetic risk factor of JSLE in the European population. The results of our pilot work suggest that a minor T allele and a likely homozygous H389Q substitution (TT genotype) are associated with JSLE, but probably not with JIA and KD, and additional studies of pediatric populations are required to power these suggestions.

ACKNOWLEDGMENTS

The authors thank the parents and children for their participation in the study; the researchers of the Laboratory of Molecular Basis of Genome Stability (the Institute of Genetics and Cytology, NAS of Belarus) for their assistance in building up the DNA collection in 2012–2017; the researchers of the Laboratory of Ecological Genetics and Biotechnology (the Institute of Genetics and Cytology, NAS of Belarus) for providing the DNA sample collection of newborns as the population control (stored in the Republican DNA Bank of a Human, Animals, Plants and Microorganisms, the Institute of Genetics and Cytology, NAS of Belarus).

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

ETHICAL COMPLIANCE

The present case-control study was approved by the Ethics Committee of the Belarusian State Medical University. Parents of all the participants signed informed consent forms in accordance with the ethical principles of the Declaration of Helsinki.

ORCID

Ivan Y. Bakutenko https://orcid.org/0000-0002-4672-1124
Nadezhda I. Ryabokon https://orcid.org/0000-0002-4820-9722

REFERENCES

Abdolmaleki, F., Farahani, N., Gheibi Hayat, S. M., Pirro, M., Bianconi, V., Barreto, G. E., & Sahebkar, A. (2018). The Role of efferocytosis in autoimmune diseases. *Frontiers in Immunology*, *9*, 1645. https://doi.org/10.3389/fimmu.2018.01645

AlKhater, S. (2019). A novel mutation in NCF2 resulting in very-early-onset colitis and juvenile idiopathic arthritis in a patient with chronic granulomatous disease. *Allergy, Asthma & Clinical Immunology*, *15*(1), 68. https://doi.org/10.1186/s13223-019-0386-6

Allan, E. R. O., Tailor, P., Balce, D. R., Pirzadeh, P., McKenna, N. T., Renaux, B., Warren, A. L., Jirik, F. R., & Yates, R. M. (2014). NADPH oxidase modifies patterns of MHC class II–restricted epitopic repertoires through redox control of antigen processing. *The Journal of Immunology*, *192*(11), 4989–5001. https://doi.org/10.4049/jimmunol.1302896

Aringer, M., Costenbader, K., Daikh, D., Brinks, R., Mosca, M., Ramsey-Goldman, R., Smolen, J. S., Wofsy, D., Boumpas, D. T., Kamen, D. L., Jayne, D., Cervera, R., Costedoat-Chalumeau, N., Diamond, B., Gladman, D. D., Hahn, B., Hiepe, F., Jacobsen, S., Khanna, D., ... Johnson, S. R. (2019). 2019 European League Against Rheumatism/American College of Rheumatology classification criteria for systemic lupus erythematosus. *Annals of the Rheumatic Diseases*, *78*(9), 1151–1159. https://doi.org/10.1136/annrheumdis-2018-214819

Armstrong, D. L., Eisenstein, M., Zidovetzki, R., & Jacob, C. O. (2015). Systemic lupus erythematosus-associated neutrophil cytosolic factor 2 mutation affects the structure of NADPH oxidase complex. *Journal of Biological Chemistry*, *290*(20), 12595–12602. https://doi.org/10.1074/jbc.M115.639021

Arnold, D. E., & Heimall, J. R. (2017). A review of chronic granulomatous disease. *Advances in Therapy*, *34*(12), 2543–2557. https://doi.org/10.1007/s12325-017-0636-2

Bagaitkar, J., Huang, J., Zeng, M. Y., Pech, N. K., Monlish, D. A., Perez-Zapata, L. J., Miralda, I., Schuettpelz, L. G., & Dinauer, M. C. (2018). NADPH oxidase activation regulates apoptotic neutrophil clearance by murine macrophages. *Blood*, *131*(21), 2367–2378. https://doi.org/10.1182/blood-2017-09-809004

Bedard, K., & Krause, K.-H. (2007). The NOX family of ROS-generating NADPH oxidases: Physiology and pathophysiology. *Physiological Reviews*, *87*(1), 245–313. https://doi.org/10.1152/physrev.00044.2005

Boada-Romero, E., Martinez, J., Heckmann, B. L., & Green, D. R. (2020). The clearance of dead cells by efferocytosis. *Nature Reviews Molecular Cell Biology*, *21*(7), 398–414. https://doi.org/10.1038/s41580-020-0232-1

Cachat, J., Deffert, C., Hugues, S., & Krause, K.-H. (2015). Phagocyte NADPH oxidase and specific immunity. *Clinical Science*, *128*(10), 635–648. https://doi.org/10.1042/CS20140635

Demirkaya, E., Sahin, S., Romano, M., Zhou, Q., & Aksentijevich, I. (2020). New horizons in the genetic etiology of systemic lupus erythematosus and lupus-like disease: Monogenic lupus and beyond. *Journal of Clinical Medicine*, *9*(3), 712. https://doi.org/10.3390/jcm9030712

Gaipl, U. S., Munoz, L. E., Grossmayer, G., Lauber, K., Franz, S., Sarter, K., Voll, R. E., Winkler, T., Kuhn, A., Kalden, J., Kern, P., & Herrmann, M. (2007). Clearance deficiency and systemic lupus erythematosus (SLE). *Journal of Autoimmunity*, *28*(2–3), 114–121. https://doi.org/10.1016/j.jaut.2007.02.005

Gonzalez, J. R., Armengol, L., Sole, X., Guino, E., Mercader, J. M., Estivill, X., & Moreno, V. (2007). SNPassoc: An R package to perform whole genome association studies. *Bioinformatics*, *23*(5), 654–655. https://doi.org/10.1093/bioinformatics/btm025

Gutierrez-Achury, J., Zorro, M. M., Ricaño-Ponce, I., Zhernakova, D. V., Diogo, D., Raychaudhuri, S., Franke, L., Trynka, G., Wijmenga, C., & Zhernakova, A. (2016). Functional implications of disease-specific variants in loci jointly associated with coeliac disease and rheumatoid arthritis. *Human Molecular Genetics*, *25*(1), 180–190. https://doi.org/10.1093/hmg/ddv455

Hahn, J., Euler, M., Kilgus, E., Kienhöfer, D., Stoof, J., Knopf, J., Hahn, M., Harrer, T., Hultqvist, M., Olofsson, P., Mokhir, A., Holmdahl, R., Herrmann, M., Schett, G., Muñoz, L. E., & Hoffmann, M. H. (2019). NOX2 mediates quiescent handling of dead cell remnants in phagocytes. *Redox Biology*, *26*, 101279. https://doi.org/10.1016/j.redox.2019.101279

Jacob, C. O., Eisenstein, M., Dinauer, M. C., Ming, W., Liu, Q., John, S., Quismorio, F. P., Reiff, A., Myones, B. L., Kaufman, K. M., McCurdy, D., Harley, J. B., Silverman, E., Kimberly, R. P., Vyse, T. J., Gaffney, P. M., Moser, K. L., Klein-Gitelman, M., Wagner-Weiner, L., ... Zidovetzki, R. (2012). Lupus-associated causal mutation in neutrophil cytosolic factor 2 (NCF2) brings unique insights to the structure and function of NADPH oxidase. *Proceedings of the National Academy of Sciences*, *109*(2), E59–E67. https://doi.org/10.1073/pnas.1113251108

Jacob, C. O., Yu, N., Yoo, D.-G., Perez-Zapata, L. J., Barbu, E. A., Kaplan, M. J., Purmalek, M., Pingel, J. T., Idol, R. A., & Dinauer, M. C. (2017). Haploinsufficiency of NADPH oxidase subunit neutrophil cytosolic factor 2 is sufficient to accelerate full-blown lupus in NZM 2328 mice: Accelerated lupus in NCF-2-deficient mice. *Arthritis & Rheumatology*, *69*(8), 1647–1660. https://doi.org/10.1002/art.40141

Jordan, M., & Baxter, A. (2020). Genetic predisposition, humans. In N. R. Rose, & I. R. Mackay (Eds.), *The autoimmune diseases* (6th ed., pp. 383–418). Academic Press.

Kim-Howard, X., Sun, C., Molineros, J. E., Maiti, A. K., Chandru, H., Adler, A., Wiley, G. B., Kaufman, K. M., Kottyan, L., Guthridge, J. M., Rasmussen, A., Kelly, J., Sánchez, E., Raj, P., Li, Q.-Z., Bang, S.-Y., Lee, H.-S., Kim, T.-H., Kang, Y. M., ... Nath, S. K. (2014). Allelic heterogeneity in NCF2 associated with systemic lupus erythematosus (SLE) susceptibility across four ethnic populations. *Human Molecular Genetics*, *23*(6), 1656–1668. https://doi.org/10.1093/hmg/ddt532

Majai, G., Kiss, E., Tarr, T., Zahuczky, G., Hartman, Z., Szegedi, G., & Fésüs, L. (2014). Decreased apopto-phagocytic gene expression in the macrophages of systemic lupus erythematosus patients. *Lupus*, *23*(2), 133–145. https://doi.org/10.1177/0961203313511557

Manly, K. F. (2005). Reliability of statistical associations between genes and disease. *Immunogenetics*, *57*(8), 549–558. https://doi.org/10.1007/s00251-005-0025-x

Márquez, A., Kerick, M., Zhernakova, A., Gutierrez-Achury, J., Chen, W.-M., Onengut-Gumuscu, S., González-Álvaro, I., Rodriguez-Rodriguez, L., Rios-Fernández, R., González-Gay, M. A., Mayes, M. D., Raychaudhuri, S., Rich, S. S., Wijmenga, C., & Martín, J. (2018). Meta-analysis of Immunochip data of four autoimmune diseases reveals novel single-disease and cross-phenotype associations. *Genome Medicine*, *10*(1), 1–13. https://doi.org/10.1186/s13073-018-0604-8

McCrindle, B. W., Rowley, A. H., Newburger, J. W., Burns, J. C., & Bolger, A. F. (2017). Diagnosis, treatment, and long-term management of Kawasaki disease: A scientific statement for health professionals from the American Heart Association. *Circulation*, *135*(17), 927–999. https://doi.org/10.1161/CIR.0000000000000484

McCurdy, D., & Parsa, M. F. (2021). Updates in juvenile idiopathic arthritis. *Advances in Pediatrics*, *68*, 143–170. https://doi.org/10.1016/j.yapd.2021.05.014

Midgley, A., McLaren, Z., Moots, R. J., Edwards, S. W., & Beresford, M. W. (2009). The role of neutrophil apoptosis in juvenile-onset systemic lupus erythematosus. *Arthritis and Rheumatism*, *60*(8), 2390–2401. https://doi.org/10.1002/art.24634

O'Neill, S., Brault, J., Stasia, M.-J., & Knaus, U. G. (2015). Genetic disorders coupled to ROS deficiency. *Redox Biology*, *6*, 135–156. https://doi.org/10.1016/j.redox.2015.07.009

Petty, R. E., Southwood, T. R., Manners, P., Baum, J., Glass, D. N., Goldenberg, J., He, X., Maldonado-Cocco, J., Orozco-Alcala, J., Prieur, A.-M., Suarez-Almazor, M. E., & Woo, P.; International League of Associations for Rheumatology (2004). International League of Associations for Rheumatology classification of juvenile idiopathic arthritis: Second revision, Edmonton, 2001. *The Journal of Rheumatology*, *31*(2), 390–392.

Reid, S., Alexsson, A., Frodlund, M., Morris, D., Sandling, J. K., Bolin, K., Svenungsson, E., Jönsen, A., Bengtsson, C., Gunnarsson, I., Illescas Rodriguez, V., Bengtsson, A., Arve, S., Rantapää-Dahlqvist, S., Eloranta, M.-L., Syvänen, A.-C., Sjöwall, C., Vyse, T. J., Rönnblom, L., & Leonard, D. (2020). High genetic risk score is associated with early disease onset, damage accrual and decreased survival in systemic lupus erythematosus. *Annals of the Rheumatic Diseases*, *79*(3), 363–369. https://doi.org/10.1136/annrheumdis-2019-216227

Richards, S., Aziz, N., Bale, S., Bick, D., Das, S., Gastier-Foster, J., Grody, W. W., Hegde, M., Lyon, E., Spector, E., Voelkerding, K., & Rehm, H. L. (2015). Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology; on behalf of the ACMG Laboratory Quality Assurance Committee. *Genetics in Medicine*, *17*(5), 405–423. https://doi.org/10.1038/gim.2015.30

Sambrook, J., & Russell, D. W. (2006). Isolation of high-molecular-weight DNA from mammalian cells using proteinase K and phenol. *Cold Spring Harbor Protocols*, *2006*(1), pdb.prot4036. https://doi.org/10.1101/pdb.prot4036

Singh, S., Jindal, A. K., & Pilania, R. K. (2018). Diagnosis of Kawasaki disease. *International Journal of Rheumatic Diseases*, *21*(1), 36–44. https://doi.org/10.1111/1756-185X.13224

Smith, E. M. D., Lythgoe, H., Midgley, A., Beresford, M. W., & Hedrich, C. M. (2019). Juvenile-onset systemic lupus erythematosus: Update on clinical presentation, pathophysiology and treatment options. *Clinical Immunology*, *209*, 108274. https://doi.org/10.1016/j.clim.2019.108274

Tajbakhsh, A., Rezaee, M., Barreto, G. E., Moallem, S. A., Henney, N. C., & Sahebkar, A. (2020). The role of nuclear factors as "Find-Me"/alarmin signals and immunostimulation in defective efferocytosis and related disorders. *International Immunopharmacology*, *80*, 106134. https://doi.org/10.1016/j.intimp.2019.106134

The 1000 Genomes Project Consortium (2015). A global reference for human genetic variation. *Nature*, *526*(7571), 68–74. https://doi.org/10.1038/nature15393

How to cite this article: Bakutenko, I. Y., Haurylchyk, I. D., Nikitchenko, N. V., Sechko, E. V., Kozyro, I. A., Tchitchko, A. M., Batyan, G. M., Sukalo, A. V., & Ryabokon, N. I. (2022). Neutrophil cytosolic factor 2 (*NCF2*) gene polymorphism is associated with juvenile-onset systemic lupus erythematosus, but probably not with other autoimmune rheumatic diseases in children. *Molecular Genetics & Genomic Medicine*, 10, e1859. https://doi.org/10.1002/mgg3.1859