Gain-of-function *TLR7* and loss-of-function *A20* gene variants identify a novel pathway for Mendelian lupus and lupus nephritis

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**ABSTRACT**

Systemic lupus erythematosus (SLE) is a chronic and inflammatory autoimmune disease of unknown cause that may cause kidney disease, i.e. lupus nephritis. Within a wider trend towards an expanding field of genetic causes of kidney disease, two recent reports have emphasized the role of Mendelian autoimmune disorders in causing lupus nephritis both in children and in young adults. Loss-of-function (LOF) variants of Tumor Necrosis Factor Alpha-Induced Protein 3 (*TNFAIP3*) and gain of function (GOF) variants of Toll-like receptor 7 (*TLR7*) cause SLE and lupus nephritis. Interestingly, both genes regulate the same signaling route, as *A20*, the protein encoded by *TNFAIP3* inhibits NF-κB activation while *TLR7* promoted NF-κB activation. Moreover, *TNFAIP3* and *TLR7* variants are relatively frequent, potentially contributing to polygenic risk for lupus nephritis. Finally, they both may be expressed by kidney cells, potentially contributing to the severity of kidney injury in persons who have already developed autoimmunity. The fact that both genes regulate the same pathway may lead to novel therapeutic approaches targeting the shard molecular pathway.

**Keywords:** A20, HA20, inherited kidney disease, lupus nephritis, systemic lupus erythematosus, TLR7
Lupus nephritis

Systemic lupus erythematosus (SLE) is a chronic and inflammatory autoimmune disease of unknown cause, characterized by the loss of immune tolerance to nuclear self-antigens, B-cells hyperreactivity, and production of autoantibodies and inflammatory cytokines, resulting in damage to several tissues and organs and in increased morbidity and mortality. Lupus nephritis (LN) is one of the most common severe manifestations of SLE, as up to 60% of SLE patients develop LN, mainly people with juvenile-onset SLE. The incidence and severity of LN vary according to the geographical area, race/ethnicity, sex/age, and applied diagnostic criteria. LN is an immune-complex glomerulonephritis characterized by the development of proteinuria, hematuria, leukocyturia, and/or reduced kidney function. The course is characterized by relapses and remissions. LN may be the only initial manifestation of SLE. Classically, evidence of LN was one of the potential diagnostic criteria for SLE, including persistent proteinuria (>0.5g per 24 h) or the presence of urinary casts in the 1982 American College of Rheumatology SLE classification criteria or proteinuria >0.5g per 24 h or per gram of urinary creatinine or red blood cell casts in urinary sediment in the 2012 SLICC criteria. Kidney biopsy confirms the diagnosis of LN, assesses severity, and helps to predict outcomes and decide on treatment. The 2003 ISN/RPS LN classification and its 2018 update establish 6 histologic classes and activity and chronicity parameters of severity. The role of the kidney biopsy was recently highlighted in the 2019 EULAR-ACR classification criteria: a positive antinuclear antibody with proteinuria >0.5g per 24 h (or equivalent) and the presence of LN on kidney biopsy according to 2003 ISN/RPS classification, is sufficient to diagnose SLE.

The expanding field of genetic causes of kidney disease

Inherited kidney diseases (IKD) are more common than previously thought. They account for at least 10% of adult CKD cases. Prior to the increasing availability of genetic testing, many of these patients were incorrectly classified as having hypertensive nephropathy, CKD of unknown cause, or assigned a different cause of CKD. Contributing to the invisibility of IKDs, major registries only report autosomal dominant polycystic kidney disease (ADPKD) separately as cause of CKD, while all other causes of IKD are grouped under "other" or, if not diagnosed, in any other category. However, when all IKDs are grouped and reported together, we get a different perspective: IKDs are the third leading cause of kidney failure in Catalonia and the fourth in Madrid, Spain.
given the low uptake of genetic diagnostic tests even when they are freely available 16. A genetic basis for CKD was also identified for one of the most common causes of CKD, the so-called "Hypertensive Nephropathy", which in African Americans is usually an IKD, APOL1 variant nephropathy 17. Although there is no specific treatment available for most IKDs, a correct diagnosis may prevent unnecessary invasive procedures and treatments, and, as IKD can be directly attributed to the dysfunction of the responsible gene, this may lead to the design and development of specific therapies 15. Recent reports have also provided a genetic basis for some forms of LN 18.

**Genetic causes of lupus**

SLE is recognized as a polygenic autoimmune disease. The strong genetic component in SLE is estimated to be 66% of heritability in twin studies 19. Over the last decades, genome-wide association studies (GWAS) have identified over a hundred SLE susceptibility loci 20. The proportion of phenotypic variances explained by variants in HLA is 2.6%21 and non-HLA 38% 22. Additionally, more than 30 genes causing monogenic forms of SLE or SLE-like syndromes have been reported 23–25 (Table 1). Among them, deficiencies of complement factors such as C1q, C4A, B, and C2 confer a high disease susceptibility 26. 90% of people with C1q deficiency develop a lupus-like phenotype 27. TREX1 variants are also associated to monogenic diseases, such as familial chilblain lupus 1, a cutaneous form of SLE, and Aicardi-Goutieres syndrome, an inflammatory encephalopathy that shares features with SLE. 26.

The type I interferon (IFN) system also plays a major role in SLE pathogenesis 28. GWAS studies have reported associations with type I IFN-induced genes, and several monogenic lupus or lupus-like diseases are associated with interferonopathy (e.g. IFIH1, TNFAIP3, RNASEH2A, RNASEH2B, IRF7). IFN-alpha-induced genes are overexpressed in the peripheral blood of 60-80% of patients with SLE 29,30.

The HLA region is a strong predictor of genetic risk, predominately HLA class II [e.g. HLA-DR2 (HR 1.2) and HLA-DR3 (2.4)] loci related to T cell-dependent antibody responses 31–33. Other predisposing genes involve those encoding lymphocyte signaling molecules that regulate the activation or suppression of T- or B-cell activity or survival, such as PTPN22, OX40L and PD1 34–37.

Lastly, in recent years, evidence suggests the role of genetic factors in both disease susceptibility and on different disease phenotypes 38. In this regard, ITGAM and FCGR2A variants have been associated
with susceptibility to skin involvement, while *ITGAM, HLDR2, and STAT4* are associated with kidney disease \(^3^9\).

Loss-of-function (LOF) variants of Tumor Necrosis Factor Alpha-Induced Protein 3 (*TNFAIP3*) and gain of function (GOF) variants of Toll-like receptor 7 (*TLR7*) were recently reported to underlie LN in children and adults in the Clinical Kidney Journal and Nature, respectively \(^1^8\). \(^2^5\).

### A20, HA20 and lupus nephritis

A20 is a negative regulator of inflammation encoded by the *TNFAIP3* gene \(^4^0\). A20 inhibits NF-κB signaling and restricts the interferon regulatory factor (IRF) pathway and autophagy \(^4^1\) (Figure 1). Haploinsufficiency A20 (HA20; OMIM 616744) is an autosomal-dominant monogenic disease caused by heterozygous LOF *TNFAIP3* variants and is characterized by early-onset systemic inflammation in multiple organs \(^4^0\). Although phenotypes may vary according to specific *TNFAIP3* variants \(^4^2\), the major phenotype is Behcet-like symptoms. In a literature review of clinical manifestations, the most common symptoms were oral ulcers (70%), recurrent fever (42%), gastrointestinal ulcers (40%), skin lesions (38%), genital ulcers (36%), musculoskeletal disorders (34%) and autoimmune thyroid disorder (19%). Ocular involvement, vasculitis, atrophic gastritis, kidney or liver injury, recurrent respiratory tract infection, interstitial lung disease or dental anomaly were found in less than 10% of the patients \(^4^2\). The diverse clinical manifestations may result from variable penetrance as well as from the interaction with other genes and the environment.

In this issue of *ckj*, Zhang et al. report that HA20 is a cause of biopsy-proven LN with both early and late onset in males and females in three families with different *TNFAIP3* variants \(^1^8\).

A male patient had late-onset (age 29 years) SLE with multi-organ involvement: alopecia, arthralgia, nephrotic proteinuria, thrombocytopenia, hypocomplementemia and positive autoantibodies. Additionally, atopy-like clinical manifestations and high IgE levels were present. A novel heterozygous variant c.634+2T>C in the *TNFAIP3* gene affected mRNA splicing and created a frameshift mutation that removed both the Ovarian TUmor (OTU) domain and all Zinc Finger (ZnF) domains. Family members with the same genetic variant had milder involvement, including oral ulcers with or without duodenal ulcers, skin rashes, anemia and allergic history, illustrating the clinical variability even within the same family \(^4^3\). The other two patients were girls with an early onset (3-year-old). One had recurrent fever, autoimmune haemolytic anaemia, hepatosplenomegaly, lymphadenopathy, acute cutaneous lupus,
serositis, cardiovascular compromise, mild growth retardation, and kidney injury. A deletion of exons 7-8 in the TNFAIP3 gene resulted in loss of both OUT and ZnF domains. The other girl also had recurrent fever, autoimmune haemolytic anaemia, hepatosplenomegaly, lymphadenopathy and kidney injury, and additionally had oral ulcers and musculoskeletal involvement. A c.1300_1301delinsTA (p.A434*) TNFAIP3 variant disrupted the ZnF coding region. This report adds TNFAIP3 variants to the list of causes of Mendelian LN.s. Although mainly found in children, this report illustrates that monogenic LN.s may be found in adults.

**TLR7**

TLR7 is an intracellular receptor mainly expressed in B cells and plasmacytoid dendritic cells (pDCs) that recognizes pathogen-associated molecular patterns. Overexpression of TLR7 results in more severe autoimmune responses and incidence of lupus-like disease. TLR7 is encoded by the X chromosome. Although female cells randomly inactivate one of the two X chromosomes, 15-23% of X-linked human genes escape X chromosome inactivation and both alleles are simultaneously expressed. Female B cells with biallelically expressed TLR7 have an increased susceptibility to TLR7-dependent autoimmune syndromes. In this regard, female pDCs produce more IFN-α than male pDCs upon stimulation with synthetic ligands or ssRNA that selectively activate TLR7. Furthermore, increased IFN-α is related to exogenous and endogenous estrogens. Indeed, a TLR7-dependent, IFN-independent immune activation has been proposed to be sufficient to accelerate SLE. Additionally, TLR7 stimulation activates the proinflammatory transcription factor NF-κB, that links TLR7 and A20 on opposite sides of the same pathway. TLR7 gene dosage also contributed to accelerating autoimmunity when a cluster of at least 16 X-linked genes is duplicated and translocated to the Y chromosome in mice (Yaa-chromosome, Y linked autoimmune accelerator). Finally, in a Mexican population, an increased copy number of TLR7 was associated with an increased risk of pediatric SLE-like.

Brown et al. have now described female patients with SLE and GOF TLR7 variants. A 7-year-old patient had a de novo TLR7 p.Tyr264His (Y264H) missense variant. Whole exome sequencing of additional patients with SLE identified TLR7 p.Arg28Gly (R28G) in a young female with mucosal and hematological involvement and TLR7 p.Phe507Leu (F507L) in a pediatric patient with optic neuritis. To explore the impact of TLR7 gene variants, Brown et al. overexpressed TLR7Y264H, TLR7R28G and TLR7F507L in cultured RAW264.7 macrophages and found that these variants caused NF-κB activation.
Moreover, they demonstrated that TLR7 Y264H could cause SLE, as CRISPR-Cas9 editing into C57BL/6 mice caused splenomegaly, decreased survival, development of antinuclear antibodies (ANA), thrombocytopenia, and proliferative glomerulonephritis with mesangial electron-dense deposits and increased mesangial cellularity in male or female mice carrying one or two alleles. Lymphoid cells infiltrated the liver, salivary glands, and pancreas and mice displayed increased levels of IFNγ, IL-6, IL-10 and TNF. These findings are consistent with previous studies and confirm the role of excess TLR7 activity in the pathogenesis of SLE, including LN. This has clear therapeutic implications. In this regard, an intravenous Toll-like Receptor Inhibitory Peptide 1 (IP1) decreased albuminuria, kidney inflammation, and mRNA expression downstream of TLR7 or TLR9 in MRL/lpr mice with SLE. However, preclinical results of therapeutic interventions may be more solid if they are confirmed at multiple sites.

Pathophysiological and clinical impact

The unstoppable advance of kidney genetics is now expanding into immune-mediated kidney disease. TNFAIP3 and TLR7 should be added to the list of genes to be assessed in the evaluation of patients with LN. Interestingly, both genes regulate the same signaling route (Figure 1) and gene variants are relatively frequent (Table 2). Although most of the more common gene variants are labeled benign, this means that they are not associated with Mendelian inherited disease, but they might contribute to polygenic risk scores, and this should be explored (Figure 2). Furthermore, the identification of individual contributors to the pathogenesis of SLE and LN will allow to develop new targeted therapies. Moreover, although studies on their role in SLE have focused on the driving events of LN (i.e. autoimmunity), both genes are also expressed by kidney parenchymal cells. Thus, a potential role in kidney injury, independent from the presence of autoimmunity, including a specific role in LN once autoimmunity develops, should be explored. In this regard, both TNFAIP3 and TLR7 may be differentially expressed in kidney parenchymal cells in the course of kidney injury (Figure 3).

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Table 1. Examples of genes whose variants are associated with human SLE and impact of the gene variants on the activity of the protein product (modified from 25)

<table>
<thead>
<tr>
<th>SLE predisposition resulting from functional deficiency in protein product</th>
<th>SLE predisposition resulting from excess activity of protein product</th>
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<tbody>
<tr>
<td>TNFAIP3</td>
<td>TLR7 respectively 25.</td>
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<tr>
<td>C1QA</td>
<td>TMEM173</td>
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<tr>
<td>C1QB</td>
<td>TNFSF6</td>
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<td>C1QC</td>
<td>IFIH1</td>
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<td>STAT4</td>
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<td>Gene</td>
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<tr>
<td>TNFAIP3</td>
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<td></td>
<td>c.296-15_296-13delCCT</td>
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<td>p.Phe127Cys</td>
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<td>p.Asn102Ser</td>
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<td>p.Ala448Val</td>
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<td>p.Val222Asp</td>
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Table 2. Allele distribution for gnomAD variants for TNFAIP3 and TLR7. Gene variants with an allele frequency higher than 1 in 1000 are shown. Gene changes are shown in or within 75 base pairs of a coding exon. Range of allele frequency refers to allele frequencies for different ethnicities.

HGVS: Human Genome Variation Society; VEP: Variant Effect Predictor
Legends to figures

Figure 1. Loss of function TNFAIP3 variants, gain-of-function TLR7 variants, and lupus nephritis. B lymphocytes are central players in SLE. Degradation of guanosine triphosphate (GTP) from processed single-stranded RNA (ssRNA) yields guanosine and 2’,3’-cyclophosphate guanosine monophosphate (cGMP) that are recognized by the BCR, and ssRNA is endocytosed complexed with BCR and delivered to endosomes, thus allowing ssRNA interaction with TLR7. TLR7 then recruits the adaptor Myd88 and signals to activate NF-κB and IRF7, leading to synthesis and secretion of cytokines (yellow, green, red dots) and type I interferon (T1-INF, blue dots). The TNFAIP3 gene encodes the A20 protein, a negative regulator of NF-κB (and of NF-κB association with IRF7), contributing to B cell tolerance. Gain-of-function (GOF) variants of TLR7 break B cell tolerance, leading to SLE, as exemplified by Y264H, F507L, and R28G. TLR7 GOF variants may disrupt B cell tolerance resulting in increased proliferation.
of autoimmune B cells, antigen presentation to autoreactive T cells, differentiation to plasma cells, and cytokine production. Cytokines contribute to inflammation while T1-INF endocrine or paracrine (from activated DCs) loops decisively contribute to loss of tolerance. The introduction of the TLR7-Y264H variant in mice resulted in SLE characterized by autoimmunity, tissue injury, and inflammation, including lupus nephritis and this was prevented by Myd88 deficiency. Also recently, loss-of-function (LOF) mutations in TNFAIP3 decisively contribute to loss of tolerance and LN. BCR: B cell receptor, IFNAR: interferon-α/β receptor, ssRNA: single stranded RNA.

Figure 2. TNFAIP3 and TLR7 gene variants and SLE/lupus nephritis. TNFAIP3 encodes A20, an inhibitor of the proinflammatory transcription factor NF-κB that is activated by TLR7. This means that A20 and TLR7 can be traced to the same intracellular signaling pathway and have the potential for clinically relevant interactions. Several severe loss-of-function (LOF) TNFAIP3 variants cause Mendelian SLE/lupus nephritis as do several gain-of-function (GOF) TLR7 gene variants. These variants with a severe impact on function are generally associated with severe, early onset disease, but this may represent the tip of the iceberg, as milder variants may exist that cause late onset disease that has not yet being characterized, just as familial hypocholesteremia was initially identified in patients having coronary artery disease in childhood. Additionally, even milder gene variants, with the potential to be present in genetic databases from the general population (table 1) and that are considered benign when present isolated, may contribute to polygenic risk for more classical forms of SLE/lupus nephritis, especially when associated with risk variants of the other gene.
Figure 3. TNFAIP3 and TLR7 mRNA expression in human control and diabetic kidney tissue. Images from [62] with permission [63,64].