

Coincidence of NOD2-Associated Autoinflammatory Disease (Yao Syndrome) and HCV Infection With Fatal Consequences *Interaction Between Genes and Environment*

To the Editor:

Autoinflammatory diseases are genetically determined disorders characterized by recurrent inflammatory episodes.^{1,2} In 2011, Yao and coworkers^{2,3} described a novel autoinflammatory disease that is now recognized under the name of NAID (NOD2-associated autoinflammatory disease) or Yao syndrome. It is characterized by periodic fever, dermatitis, polyarthritis, sicca-like symptoms, and gastrointestinal involvement. The syndrome appears to be caused by 2 mutations in the *NOD2* gene, *IVS8+158* and *Arg702Trp*. *NOD2* is a typical pattern-recognition receptor that binds degradation products from bacteria and viruses and induces inflammation.^{4,5} We have studied a patient with a chronic hepatitis C virus (HCV) infection who developed a systemic inflammatory response syndrome during treatment of the viral infection with ribavirin and interferon. The symptoms were typical of Yao syndrome, and the patient carried the 2 characteristic *NOD2* mutations. Although Yao syndrome is not a fatal disease,⁶ our patient developed progressive liver failure and died 14 years after onset of the autoinflammation. It is likely that the viral treatment had triggered the onset of the autoinflammatory syndrome. Thus, an interferon therapy should be avoided in patients with Yao syndrome. This is the second report of a patient with Yao syndrome from a hospital other than the Cleveland Clinic.⁷

DESCRIPTION OF THE PATIENT

In 2003, a patient presented at our clinic because of a systemic inflammatory response syndrome. It was known that he had an HCV infection, which was successfully controlled by treatment with ribavirin and interferon. However, shortly after start of the therapy, he began to complain about fever, night sweat, weight loss, fatigue, and joint pain. A thorough examination

for infectious, neoplastic, or autoimmune diseases was negative. Thus, the symptoms were judged to be of autoinflammatory origin, and the patient was successfully treated with prednisone over 1.5 years.

In February 2007, he had a relapse of the inflammatory syndrome with sicca-like symptoms, anemia, and nocturnal dyspnea. Treatment with the anti-TNF biologic adalimumab could stabilize the condition during the following year. Yet in January 2009, he had another relapse, which was accompanied by dermatitis. Because the symptoms were characteristic for IL-6 activity and because IL6 in serum was elevated, a therapy with the anti-IL6-receptor antibody tocilizumab was initiated. This treatment was highly effective; the acute phase response could be completely controlled by the antibody. The patient did not show any symptoms for a period of 5 years and was able to go traveling and skiing.

Unfortunately, the chronic liver disease proceeded during this period. Several liver biopsies were performed, which showed progression of fibrosis (F3 on the last biopsy), but Congo red staining for amyloidosis was negative. In 2013, the patient developed a nodular regenerative hyperplasia with portal hypertension. At this time, all symptoms of the autoinflammatory syndrome returned and were unresponsive to tocilizumab. The patient dramatically lost weight and required parenteral nutrition. In May 2017, he developed a *Candida* sepsis and died.

In 2016, when the inflammation could not anymore be controlled by tocilizumab, we set out to elucidate the molecular cause of the autoinflammatory syndrome and performed whole exome sequencing with genomic DNA from the patient's blood. A total of 33,034,680 sequence reads were obtained. Comparison with the reference genome GRCh37.75 revealed 41,379 polymorphisms. Sequence variants that did not occur in the reference library of humans with no medical impact (ftp://ftp.ncbi.nlm.nih.gov/pub/clinvar/vcf_GRCh37/) were further filtered for homozygous mutations, frame shifts, or stop codons. Furthermore, all genes that had previously been associated with autoinflammatory diseases,⁸ amyloidosis,⁹ and sarcoidosis¹⁰ were included. Finally, more than 600 genes were individually analyzed using the Integrative Genomics Viewer 2.3.92 (<http://www.broadinstitute.org/igv/>). This procedure led to the identification of 10 sequence variants that were of interest in the context of a patient with autoinflammatory syndrome

and liver fibrosis (Fig. A). All mutations were confirmed by Sanger cycle sequencing. At least 3 of the polymorphisms might have pathogenic consequences, namely, the *Arg702Trp* variant in *NOD2*, the *Met1Val* variant in *TLR8*, and the *Ala25Thr* variant in *CST3*. Additional sequencing of intron 8 from *NOD2* disclosed the pathogenic *IVS8+158* mutation,⁶ which finally led to the diagnosis of Yao syndrome. *TLR8* is known to occur in 2 alternatively spliced variants.¹¹ The hemizygous mutation *Met1Val* eliminates the start codon of transcript variant 1 (NM_016610) such that our patient was able to produce only transcript variant 2 (NM_138636). Cell culture experiments suggested that variant 2 induces significantly stronger cytokine production than variant 1.¹¹ *CST3* codes for the proteinase inhibitor cystatin C, which is a frequent constituent of amyloid plaques.¹² The homozygous *CST3* mutation of our patient substitutes Ala-25 by Thr at the signal peptide cleavage site. This Thr occurs within the tripeptide sequence Thr-Gly-Ser, which can function as acceptor site for O-glycosylation. The mutated protein does in fact become glycosylated as demonstrated by tandem mass spectrometry.¹³ Although the implications of this modification remain to be elucidated, the *Ala25Thr* mutation has been associated with Alzheimer disease and macular degeneration.¹² Together, the mutations may explain the autoinflammatory syndrome of our patient.

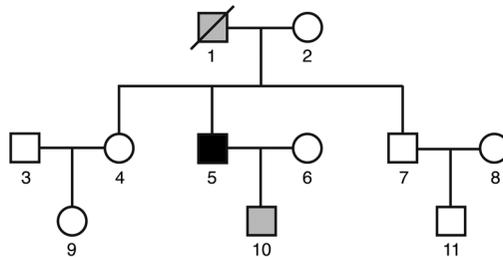
The pedigree of the patient's family (Figs. B–C) suggested that the genetic predisposition for Yao syndrome was inherited from the father who died at 58 years as a weak, ailing person. The wife of our patient was negative for the potentially pathogenic *NOD2* and *CST3* mutations, but the son was positive for all the mutations in *NOD2*, *TLR8*, and *CST3*. Thus, he must have inherited the pathogenic *NOD2* mutations from his father, which rules out the possibility of compound heterozygosity.

DISCUSSION

Yao syndrome is a polygenic disease that requires a specific trigger.^{2,3} To our knowledge, this is the first case of Yao syndrome in the context of HCV infection. Based on the patient chart, the HCV infection was acquired during a blood transfusion in 1985 and persisted for more than 15 years as an unnoticed chronic disease. It is likely that the HCV infection contributed to the

Gene	Location	DNA	Protein	Allele	Variant	Frequency
NOD2	16:50,745,926	c.2104C>T	p.R702W	+/-	rs2066844	T=0.022 (ExAC)
NOD2	16:50,744,624	c.802C>T	p.P268S	+/-	rs2066842	T=0.184 (ExAC)
NOD2	16:50,756,774	IVS8+158		+/-	rs5743289	T=0.051 (1000G)
NOD2	16:50,756,881	IVS8+265		+/-	rs2076756	G=0.097 (1000G)
MEFV	16:3,293,922	c.1272A>T	p.D424E	+/-	rs1231123	A=0.394 (ExAC)
IFIH1	2:163,124,051	c.2836G>A	p.A946T	+/-	rs1990760	G=0.495 (ExAC)
IFIH1	2:163,128,824	c.2528A>G	p.H843R	+/-	rs3747517	A=0.323 (ExAC)
TLR8	X:12,924,826	c.1A>G	p.M1V	+0	rs3764880	G=0.357 (ExAC)
CST3	20:23,618,427	c.73G>A	p.A25T	+/+	rs1064039	A=0.254 (ExAC)
SAA1	11:18,290,859	c.209T>C	p.A70V	+/-	rs1136743	C=0.478 (ExAC)
SAA1	11:18,290,874	c.224T>C	p.V75A	+/-	rs1136747	T=0.458 (ExAC)
FGA	4:155,507,590	c.991A>G	p.T331A	+/-	rs6050	G=0.287 (ExAC)

A



B

Gene	Mutation	Patient 5	Wife 6	Son 10
NOD2	Pro268Ser	+/-	-/-	+/-
NOD2	Arg702Trp	+/-	-/-	+/-
NOD2	IVS8+158	+/-	-/-	+/-
NOD2	IVS8+265	+/-	+/-	+/+
TLR8	Met1Val	+0	+/+	+0
CST3	Ala25Thr	+/+	-/-	+/-

C

FIGURE. Sequence variants of the patient with autoinflammatory syndrome. A, Whole exome sequencing revealed 10 variants that are of interest in the context of the disorder of the patient. Sanger sequencing of intron 8 from *NOD2* revealed the potentially pathogenic variant *IVS8+158* that finally led to the diagnosis of Yao syndrome. B, Pedigree of the patient's family. Overt Yao syndrome is observed only with the index patient (5). His father (1) was known as a weak, ailing person, who died at the age of 58 years. C, Sequence variants identified in the family. The index patient (5) and his son (10) carry the *NOD2* mutations Arg702Trp and *IVS8+158*, the *TLR8* mutation, as well as the *CST3* mutation.

outbreak of Yao syndrome. However, the actual trigger might have been the ribavirin/interferon therapy, because this therapy immediately preceded the outbreak of the autoinflammatory symptoms. This fact is of particular interest because interferon has been associated with several newly discovered autoinflammatory disorders,¹⁴ implying that interferon should be avoided in patients with Yao syndrome. In fact, our patient carried 2 common polymorphisms in *IFIH1* (Fig. A), which have been shown to augment the levels of interferon and to increase resistance to viral infection, but at the cost of a propensity for interferon-mediated autoinflammation.¹⁴ Although the virus had been completely eliminated as verified on multiple occasions, the patient developed a chronic hepatopathy after completion of the HCV therapy. This suggests that the Yao syndrome might have been involved in the onset of the fibrosing liver disease. Thus, it might have been the fatal coincidence of HCV infection

and Yao syndrome that finally led to liver failure and the death of our patient.

Our thorough genetic analysis leads to several take-home messages:

- A genetic predisposition for an autoinflammatory disease may remain silent until an exogenous trigger activates the pathway.
- Autoinflammatory diseases are often polygenetic, and several mutations may act in concert.
- An anticytokine therapy to neutralize IL-6 may result in complete clinical remission. However, it bears the risk of progression of a subclinical fibrosing liver disease.

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