ORIGINAL ARTICLE

Mutant Adenosine Deaminase 2 in a Polyarteritis Nodosa Vasculopathy

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ABSTRACT

BACKGROUND

Polyarteritis nodosa is a systemic necrotizing vasculitis with a pathogenesis that is poorly understood. We identified six families with multiple cases of systemic and cutaneous polyarteritis nodosa, consistent with autosomal recessive inheritance. In most cases, onset of the disease occurred during childhood.

METHODS

We carried out exome sequencing in persons from multiply affected families of Georgian Jewish or German ancestry. We performed targeted sequencing in additional family members and in unrelated affected persons, 3 of Georgian Jewish ancestry and 14 of Turkish ancestry. Mutations were assessed by testing their effect on enzymatic activity in serum specimens from patients, analysis of protein structure, expression in mammalian cells, and biophysical analysis of purified protein.

RESULTS

In all the families, vasculitis was caused by recessive mutations in *CECR1*, the gene encoding adenosine deaminase 2 (ADA2). All the Georgian Jewish patients were homozygous for a mutation encoding a Gly47Arg substitution, the German patients were compound heterozygous for Arg169Gln and Pro251Leu mutations, and one Turkish patient was compound heterozygous for Gly47Val and Trp264Ser mutations. In the endogamous Georgian Jewish population, the Gly47Arg carrier frequency was 0.102, which is consistent with the high prevalence of disease. The other mutations either were found in only one family member or patient or were extremely rare. ADA2 activity was significantly reduced in serum specimens from patients. Expression in human embryonic kidney 293T cells revealed low amounts of mutant secreted protein.

CONCLUSIONS

Recessive loss-of-function mutations of ADA2, a growth factor that is the major extracellular adenosine deaminase, can cause polyarteritis nodosa vasculopathy with highly varied clinical expression. (Funded by the Shaare Zedek Medical Center and others.)

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Polyarteritis nodosa, first described in 1866,[1] is a systemic necrotizing vasculitis that affects medium and small muscular arteries.[2,3] The ensuing tissue ischemia can affect any organ, including the skin, musculoskeletal system, kidneys, gastrointestinal tract, and the cardiovascular and nervous systems. Polyarteritis nodosa is usually diagnosed in middle age or later but can appear in childhood.[2,4,5] The diagnosis remains challenging despite classification criteria for adults[6] and children,[7] because polyarteritis nodosa frequently presents with nonspecific constitutional symptoms, and organ involvement and disease severity are highly varied. Polyarteritis nodosa is most often primary, but in adults it may be associated with infection (e.g., hepatitis B infection[8]) or hematologic cancer.[9] The cause of primary polyarteritis nodosa is unknown.

Among Israeli Jewish persons of Georgian Caucasus ancestry, pediatric polyarteritis nodosa, often familial, has been observed repeatedly[10] and may include both systemic and cutaneous features.[11] To determine the genetic basis of this disease, we undertook gene discovery using genomic sequencing to identify the underlying mutations and to characterize their functional effects.

METHODS

STUDY PARTICIPANTS

In Israel, we evaluated 19 patients of Georgian Jewish ancestry with features of polyarteritis nodosa (Fig. 1 and Table 1; and Table S1 in the Supplementary Appendix, available with the full text of this article at NEJM.org). Peripheral-blood samples for DNA extraction were obtained from 16 patients, all of whom provided written informed consent for participation in the study. Two patients had died (Patients B-III-3 and D-III-3); 1 provided written informed consent but did not provide a sample (Patient S-3).

Similar consent was obtained and evaluation and sampling were performed in a German family that had been referred for evaluation of polyarteritis nodosa and in 14 unrelated Turkish patients who had been referred with pediatric polyarteritis nodosa fulfilling Pediatric Rheumatology European Society (PRES)[7] criteria. In all the patients, the polyarteritis nodosa was diagnosed clinically on the basis of criteria from the PRES or the American College of Rheumatology (ACR).[6] Controls were 246 Georgian Jewish and 200 Turkish adults who consented to anonymous use of their DNA. Appropriate ethics-committee approvals were obtained.

GENOMIC STUDIES

Exome sequencing of genomic DNA was performed as described previously, with modifications, in Georgian Jewish Patients A-III-1, A-III-3, A-III-4, and B-III-2[12] and German Patients F-II-1 and F-II-5[13] (Fig. 2 and Table 1). All variants that were potentially damaging to protein function were identified. In Georgian Jewish patients, homozygous variants shared by Patients A-III-1, A-III-3, A-III-4, and B-III-2 were considered to be candidate disease alleles. In German patients, compound heterozygous or homozygous variants shared by Patients F-II-1 and F-II-5 were considered to be candidate disease alleles. Full exome data are available by request.

CECR1 (cat eye syndrome chromosome region, candidate 1; Online Mendelian Inheritance in Man [OMIM] database number, 607575) was identified as a candidate gene. We then used Sanger sequencing to sequence *CECR1* in the Turkish persons with polyarteritis nodosa and to genotype all available members of each family and ancestry-matched controls (Table S2 in the Supplementary Appendix).

ADENOSINE DEAMINASE 2 ACTIVITY

Adenosine deaminase 2 (ADA2) is encoded by *CECR1*, and its activity in serum is based on the deamination of adenosine to inosine. We assayed ADA2 activity using a commercial kit (Diazyme Laboratories).

TRANSFECTION, IMMUNOPRECIPITATION, AND WESTERN BLOTTING

Complementary DNA constructs of human nonmutant and mutant ADA2 were generated and transfected into human embryonic kidney 293T cells. Proteins in total cell extracts or immunoprecipitated from cell media were then detected by means of Western blotting.

PURIFICATION OF RECOMBINANT ADA2 PROTEINS

For the generation and purification of recombinant ADA2 proteins, a construct for inducible ADA2 expression in insect cells[14] was modified to introduce mutations. We generated stable lines of ADA2-expressing cells by transfecting

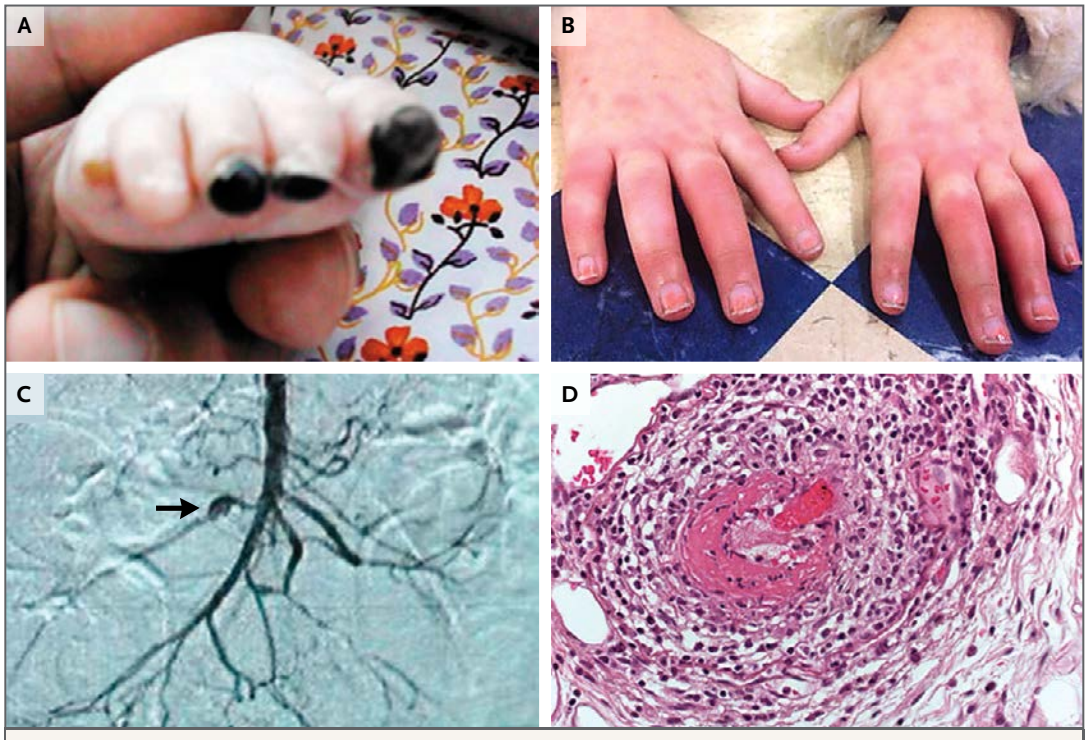


Figure 1. Clinical Features of Polyarteritis Nodosa Associated with Adenosine Deaminase 2 (ADA2) Mutations.

Clinical manifestations of polyarteritis nodosa included digital necrosis of the toes in Patient B-III-3 (Panel A) and Raynaud's phenomenon and livedo reticularis in Patient B-III-6 (Panel B). Angiography of the celiac artery in Patient B-III-3 revealed an aneurysm (Panel C, arrow). Periarteritis, fibrinoid necrosis of the media, and destruction of the elastic laminae were revealed in a biopsy specimen of the superior mesenteric artery in Patient A-III-1 (Panel D, hematoxylin and eosin).

drosophila S2 cells with ADA2 expression constructs. Protein production was induced for 7 to 10 days, and ADA2 was purified from media, as described previously.[14]

STATISTICAL ANALYSIS

Analysis of variance was used to evaluate differences in ADA2 activity among serum samples, as well as differences in levels of ADA2 protein secreted into the media and in levels of ADA2 protein retained in cells after transfection with constructs of various ADA2 genotypes. All P values were two-sided.

RESULTS

CLINICAL FEATURES

The clinical features of the study participants are shown in Table 1 and Figure 1, and Table S1 and Figure S1 in the Supplementary Appendix. The 19 Georgian Jewish participants included 16 persons from 5 multiplex families (Fig. 2A, Families A through E) and 3 affected persons with no affected family members. The German participants included 4 persons, all of whom were siblings in a single family (Fig. 2A, Family F).

Of the 19 Georgian Jewish participants, 15 received a diagnosis of polyarteritis nodosa before 10 years of age; 6 received the diagnosis during infancy (≤1 year of age), of whom 4 had severe systemic disease. In contrast, 1 person (Patient D-III-2) had leg ulcers that first appeared when she was 59 years of age. Among the Georgian Jewish patients, all but 1 had cutaneous manifestations, most commonly livedo reticularis. Persons with severe disease had ischemia and necrosis of the fingers and toes. Visceral involvement, which was present in 10 of the 19 persons, most commonly resulted in gastrointestinal manifestations, followed by renal hypertension. Neurologic disease, which occurred in 8 of the 19 persons, affected the peripheral nervous system more commonly than the central nervous system. Magnetic resonance imaging of the brain re-

Table 1. Characteristics of Patients with Polyarteritis Nodosa (PAN).*

Patient No.	Sex	Age at Onset	Clinical Manifestation†								Imaging Result‡			Pathological Test Result§	Criteria for PAN¶
			Fever	Myalgia or Arthralgia	Cutaneous Feature				Visceral Feature		Neurologic Feature	Brain	Renal	Other		
					LR	*other*	*leg ulcer*	*GI*	*HTN or renal*	*testicular*						
Georgian Jewish familial cases																
A-III-1	M	2 mo	Yes		Yes	Yes		Yes	HTN				Infarct	Coronary-artery aneurysm	PAN-V	Yes
A-III-3	F	5 yr		Yes	Yes	Yes		Yes					Aneurysm, stenosis	Mesenteric-artery and hepatic-artery aneurysm and stenosis	LCV, pannic-ulitis	Yes
A-III-4	M	7 mo	Yes	Yes	Yes	Yes			HTN		CNS	Infarct	Aneurysm		LCV, PAN-V	Yes
B-III-1	F	3 yr	Yes	Yes	Yes	Yes					PNS	Normal			LCV	No
B-III-2	F	2 yr		Yes	Yes	Yes					PNS	Normal			LCV	No
B-III-3‖	F	2 mo	Yes			Yes		Yes			CNS	VH, aneurysm	Aneurysm	Celiac-artery aneurysm		Yes
B-III-6	F	2 mo	Yes	Yes	Yes	Yes		Yes				Normal				No
C-II-3	M	6 yr	Yes	Yes	Yes			Yes		Yes					PAN-V	Yes
C-II-4	M	9 yr			Yes											No
D-III-1	F	<10 yr	Yes	Yes	Yes	Yes	Yes								PAN-V	Yes
D-III-2	F	59 yr					Yes								LCV, pannic-ulitis	No
D-III-3‖	M	<10 yr	Yes	Yes	Yes	Yes										NA
D-IV-6	M	1 yr		Yes	Yes	Yes	Yes				PNS					No
D-V-1	M	4 yr	Yes	Yes	Yes	Yes				Yes					PAN-V	Yes
E-II-1	F	18 yr			Yes											No
E-II-2	F	1 yr	Yes					Yes	Both		PNS, CNS	Infarct	Aneurysm, infarct	SMA aneurysm and infarct	Inflammation	Yes

Georgian Jewish simplex cases																
S-1	F	28 yr	Yes	Yes		Yes	Yes		HTN		PNS				Granulomatous panniculitis	Yes
S-2	F	2 yr				Yes					PNS	Normal			Vasculitis	No
S-3‖	M	16 yr		Yes		Yes	Yes		HTN	Yes			Aneurysm		PAN-V	Yes
Turkish simplex case																
T-1	M	10 yr	Yes	Yes	Yes	Yes		Yes	HTN				Aneurysm, stenosis, infarct	Mesenteric-artery aneurysm and stenosis	Necrotizing panniculitis	Yes
German familial cases																
F-II-1	F	1 yr	Yes	Yes	Yes				HTN		PNS, CNS	Infarct		Retinal-artery stenosis		Yes
F-II-2	F	12 yr									PNS, CNS	Infarct				NA
F-II-3	M	1 yr		Yes		Yes					PNS	Infarct			LCV	ND
F-II-5	M	9 yr		Yes	Yes	Yes					PNS	Normal				NA
All cases																
Total no. of patients	M:11; F:13	—	13	16	16	16	5	7	7	3	PNS: 10; CNS: 5					12

* F denotes female, M male, NA not available, and ND not determined.

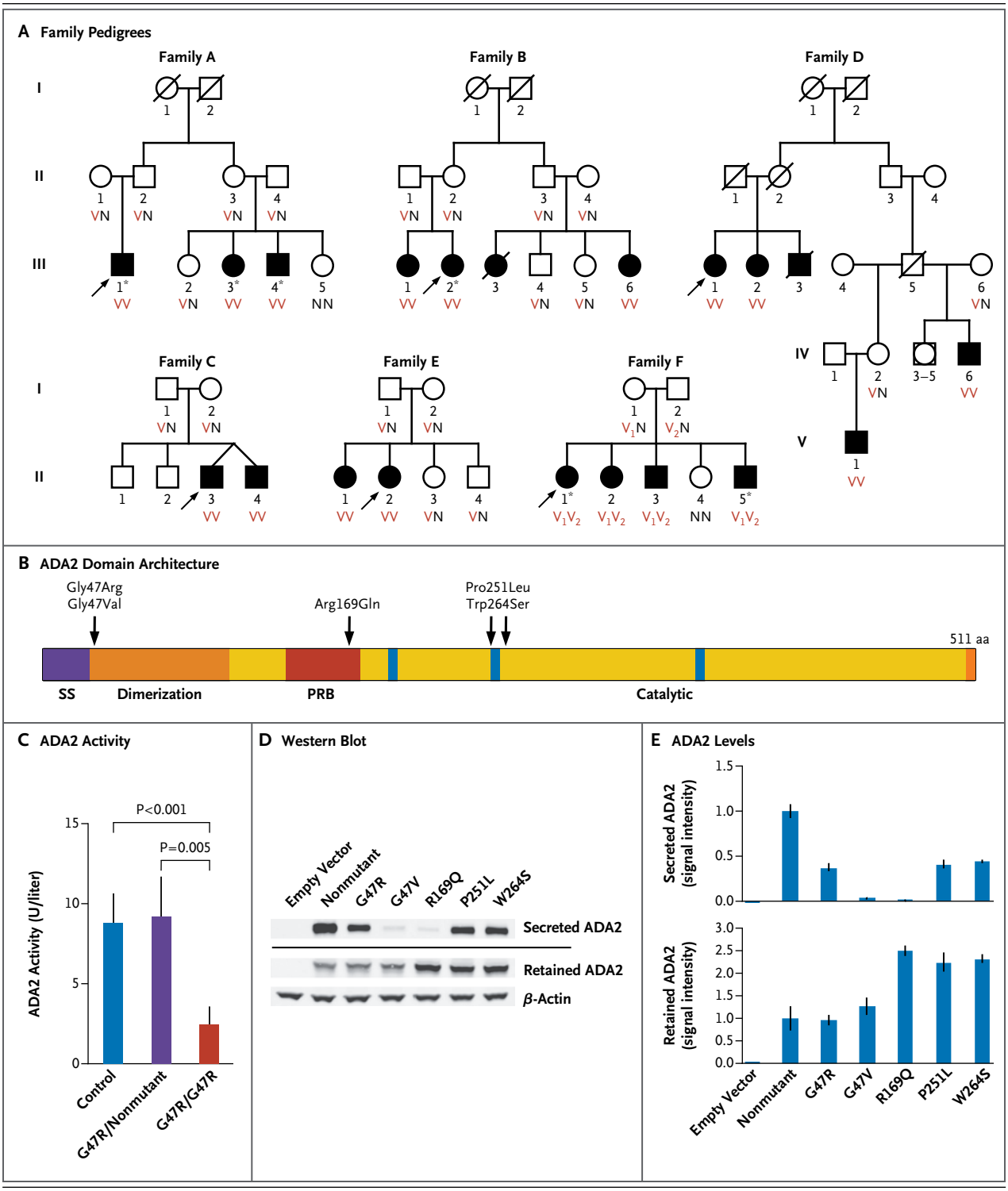
† Clinical manifestations included the following: constitutional manifestations such as fever; musculoskeletal manifestations such as myalgia or arthralgia; cutaneous manifestations such as livedo reticularis (LR), leg ulcers, Raynaud's phenomenon, skin manifestations (e.g., nodules, purpura, and erythema nodosum), oral aphthae, and digital necrosis (other); visceral manifestations such as gastrointestinal (GI) symptoms, renal proteinuria and hematuria, hypertension (HTN), and testicular pain; and neurologic manifestations in the peripheral nervous system (PNS) and central nervous system (CNS).

‡ Imaging of the brain was performed by means of computed tomography or magnetic resonance imaging, and imaging of other organs by means of angiography. Findings included aneurysm, infarct, stenosis, and ventricular hemorrhage (VH). Arteries involved were coronary, retinal, celiac, hepatic, mesenteric, and superior mesenteric (SMA).

§ Histopathological analysis was performed by means of skin biopsy, except as follows: Patients A-III-1 and E-II-2 underwent biopsy of the ileum, and Patient S-3 biopsy of the kidney. Findings included PAN-related vasculitis (PAN-V) inflammation, panniculitis (granulomatous or necrotizing), vasculitis, and leukocytoclastic vasculitis (LCV).

¶ Criteria for PAN were assessed according to criteria from the Pediatric Rheumatology European Society and the European League against Rheumatism[7] for an onset before 18 years of age and according to the American College of Rheumatology[6] criteria for an onset on or after 18 years of age.

‖ Patient did not provide a peripheral-blood sample for DNA extraction.



vealed infarcts and ventricular hemorrhage. Angiographic findings included aneurysms and stenoses of abdominal arteries (mesenteric, celiac, hepatic, and renal) and renal-cortex infarcts. Necrotizing arterial vasculitis characteristic of polyarteritis nodosa was found in specimens from 4 of 10 skin biopsies performed (Fig. S1 in the Supplementary Appendix); nonspecific leukocytoclastic vasculitis or panniculitis was seen in the remainder of the specimens.

Figure 2 (facing page). Missense Mutations of ADA2 in Families with Polyarteritis Nodosa, with Reduced Extracellular ADA2 Protein.

Panel A shows pedigrees of families with polyarteritis nodosa. Solid symbols indicate persons with vasculitis, open symbols unaffected persons, arrows probands, asterisks persons evaluated by means of exome sequencing, and slashes deceased persons. Variant alleles are indicated by V, V_1, and V_2, and nonmutated alleles by N. In Georgian Jewish Families A through E, affected persons are homozygous for the mutation Gly47Arg (indicated by VV). In German Family F, affected persons are compound heterozygous for the mutations Arg169Gln (V_1) and Pro251Leu (V_2). Panel B shows the domain architecture of the ADA2 protein and the location of the ADA2 residues altered by the mutations. ADA2 domains are the signal sequence (SS; purple), dimerization domain (orange), putative receptor-binding domain (PRB; red), and catalytic domain (yellow). The catalytic domain includes regions that are specific to ADA2 and absent from ADA1 (blue). Panel C shows that the ADA2 activity in the serum of five persons who were homozygous for Gly47Arg is significantly less than that in the six who were heterozygous for Gly47Arg ($P=0.005$) and that of four controls without the mutation ($P<0.001$). Panel D shows the levels of ADA2 protein secreted (top) into the media of and retained (bottom) within transfected human embryonic kidney 293T cells. Panel E indicates levels of secreted and retained ADA2 protein on the basis of the Western blots in Panel D. The signal intensity was normalized to that of the nonmutant construct. Levels of secreted ADA2 are lower for all mutant proteins than for the nonmutant protein ($P<0.01$). As compared with nonmutant ADA2 and the other mutants, the level of ADA2 protein retained in cell lysates was higher for Arg169Gln ($P=0.005$), Pro251Leu ($P=0.005$), and Trp264Ser ($P=0.008$). Values in Panels C and E are means from three independent experiments; error bars indicate 1 SD.

At least 1 patient from each multiplex family and all unrelated patients had received a clinical diagnosis of polyarteritis nodosa (see the Supplementary Appendix). Of the 15 patients with pediatric onset (<18 years of age) and sufficient information, 9 fulfilled the PRES criteria, including at least 1 person from each multiplex family, and 6 did not. Among 3 patients with adult onset, 1 fulfilled the ACR criteria and 2 did not, but these 2 had relatives who fulfilled the ACR criteria.

In the German family, two siblings had received a diagnosis during infancy and the other two by 12 years of age. All four affected siblings had peripheral neuropathy, and symptomatic or subclinical cerebral infarctions were present in three. Skin manifestations, arthralgia, and myalgia were also present, but other visceral organs were unaffected. Of the two fully evaluated siblings in the German family, one fulfilled the PRES criteria; the status of the other could not be determined. Patient T-1, who was of Turkish ancestry (Table 1, and Table S1 in the Supplementary Appendix), had livedo reticularis, hypertension, renal aneurysms, and necrotizing vasculitis.

Overall, disease severity was highly varied, even within families. A total of 4 patients had mild disease limited to the skin, with no constitutional symptoms, but 13 patients had severe, often visceral, polyarteritis nodosa, which was fatal in 2 of the patients (Patients B-III-3 and D-III-3, who died at 9 months and 31 years of age, respectively). A total of 20 patients received systemic therapy (Table S1 in the Supplementary Appendix). Ten severely affected patients had a clinically significant therapeutic response to anti–tumor necrosis factor (TNF) drugs, including 2 patients who had life-threatening disease despite maximal doses of cyclophosphamide.

GENE DISCOVERY

We postulated a recessive model for disease inheritance. In the exome sequences of Georgian Jewish participants, one putatively damaging variant was homozygous in all four affected persons in whom exome sequencing of genomic DNA was performed: chromosome 22:17,690,429 C→T, corresponding to *CECR1* c.139G→A and leading to a Gly47Arg amino acid substitution (National Center for Biotechnology Information reference sequence number, NM_001282225.1) (Fig. S2 in the Supplementary Appendix). *CECR1* encodes the ADA2 protein (Fig. 2B). Glycine at residue 47 of ADA2 is conserved in all sequenced species (Fig. S3 in the Supplementary Appendix), and substitution with arginine is predicted to be highly damaging (Polymorphism Phenotyping, version 2 [Polyphen-2] score,[15] 1.000).

All the Georgian Jewish patients were homozygous for the Gly47Arg mutation, and all their unaffected relatives were either heterozygous for the mutation or did not carry it (Fig. 2A, and Fig. S2 in the Supplementary Appendix). This mutation was not present in 864 other in-house exome sequences or in more than 7500 exome sequences present in public databases.[16,17]

Using population-genetics principles, we assessed the likelihood that the Gly47Arg substitution causes polyarteritis nodosa in the persons we analyzed. Of 246 unrelated Georgian Jewish controls, 25 were heterozygous and none were homozygous for this variant, yielding an esti-

mated carrier frequency of 0.102 in this population. Of the 16 genotyped Georgian Jewish patients, all were homozygous for the mutation. The probability of homozygosity at this allele by chance among these 16 Georgian Jewish patients, taking into account the allele frequency in the population and the pedigree structures, is 3.8×10^{-20} (see the Supplementary Appendix).

In the German family (Fig. 2A, Family F), all affected siblings were compound heterozygous for damaging variants in only one gene, *CECR1*. The mutations encoded Arg169Gln (c.506G→A; chromosome 22:17,687,997 C→T) and Pro251Leu (c.752C→T; chromosome 22:17,684,454 G→A). Each parent was heterozygous for one allele, and an unaffected sister did not have a mutation at either site (Fig. 2A, and Fig. S2 in the Supplementary Appendix). The Polyphen-2 scores were 1.000 for Arg169Gln and 0.989 for Pro251Leu. Among 4300 controls of European ancestry with data in a public database, 7 carried Arg169Gln and 1 carried Pro251Leu, corresponding to allele frequencies of 0.0008 and 0.0001, respectively.[16]

Among 14 unrelated Turkish children with polyarteritis nodosa who fulfilled PRES criteria, 1 child (Patient T-1; Table 1) was compound heterozygous for two damaging variants in *CECR1*: one encoding Gly47Val (c.140G→T; chromosome 22:17,690,428C→A) and the other encoding Trp264Ser (c.791G→C, chromosome 22:17,672,663C→G) (Fig. S2 in the Supplementary Appendix). Each parent was heterozygous for one of these variants. Gly47Val alters the same codon as the allele in the Georgian Jewish patients. The Polyphen-2 scores were 1.000 for both Gly47Val and Trp264Ser, and neither variant was present in 200 Turkish controls or in more than 7500 exome sequences present in public databases.[16,17]

ANALYSIS OF PROTEIN STRUCTURE

CECR1 encodes ADA2, a 511-amino-acid protein (Fig. 2B) that is a secreted homodimer highly expressed in plasma. ADA2 is responsible for extracellular degradation of adenosine and has been implicated in the regulation of the proliferation of activated T cells and macrophages and in the differentiation of monocytes to macrophages.[18] The sites of the mutations observed in patients with polyarteritis nodosa are highly conserved (Fig. S3 in the Supplementary Appendix). Analysis of the three-dimensional protein structures of ADA2 suggests that Gly47Arg and Gly47Val may affect the stability of homodimers or their individual subunits (Fig. S4A in the Supplementary Appendix), that Arg169Gln may alter the receptor-binding domain (Fig. S4B in the Supplementary Appendix), and that Pro251Leu and Trp264His are likely to affect the active site of the enzyme (Fig. S4C in the Supplementary Appendix).

ADA2 ACTIVITY IN PATIENT SERUM AND EXPRESSION IN MAMMALIAN CELLS

Analysis of serum samples from five patients who were homozygous for the Gly47Arg mutation showed that ADA2 activity was reduced by a factor of more than four, as compared with controls ($P<0.001$) (Fig. 2C). In six heterozygous carriers of Gly47Arg, the ADA2 activity in serum specimens was similar to that in control specimens, which is consistent with the absence of disease in carriers. In a serum sample from one patient who was compound heterozygous for the Arg169Gln and Pro251Leu mutations, ADA2 activity was even more severely compromised.

ADA2 was expressed exogenously in mammalian cells, with nonmutant and mutant constructs expressed at similar levels (Fig. S5 in the Supplementary Appendix). In transfected cells, secreted ADA2 levels in media were significantly lower in the case of the mutant proteins than in the case of the nonmutant protein; ADA2 was barely detectable in cells expressing the Gly47Val or Arg169Gln mutation (Fig. 2D and 2E). Analysis of cell lysates from the same experiments indicated elevated amounts of retained intracellular Arg169Gln, Pro251Leu, and Trp264Ser mutant proteins, as compared with nonmutant ADA2. Overall, the proportion of total ADA2 secreted into the media was significantly lower in the case of all the mutant proteins than in the case of the nonmutant protein (Table S3 in the Supplementary Appendix).

Lower proportions of mutant versus nonmutant ADA2 detected in the media could be due to impaired secretion, the altered stability of the mutant proteins, or both. To examine whether the ADA2 mutations affected structural properties of the protein, recombinant ADA2 and the Gly47Arg, Gly47Val, and Trp264Ser mutants were expressed in drosophila S2 cells and then purified from cell media.[14] As seen in mammalian cells, the levels of mutant ADA2 proteins were substantially lower than the level of nonmutant ADA2

protein in the S2-cell media (Fig. S6 in the Supplementary Appendix). Only Trp264Ser yielded sufficient protein for analysis. Biophysical analysis of Trp264Ser by means of circular dichroism revealed reduced helical content, indicating less stable secondary structure and reduced thermostability (Fig. S7A and S7B in the Supplementary Appendix). Increased binding of the fluorescent dye 8-anilino-1-naphthalenesulfonic acid indicated partial unfolding of this mutant protein (Fig. S7C in the Supplementary Appendix).

DISCUSSION

Our results indicate that vasculopathy overlapping polyarteritis nodosa can be caused by reduced activity of ADA2 owing to recessive mutations in the ADA2-encoding gene *CECR1*. This vasculitis is characterized by highly varied age at onset, severity, and organ involvement, even within families and among patients with the same mutations. Manifestations range from severe or fatal systemic vasculitis or multiple strokes in children to limited cutaneous manifestations in middle-age persons. The condition may thus be influenced by the specific mutations and by modifying factors (environmental, genetic, or both).

Although ADA2-associated disease is exceptionally common among persons of Georgian Jewish ancestry, it is probably still underdiagnosed in that community, given the high rate of mutation carriers (10%). In this and other populations, underdiagnosis is explained at least in part by clinical variability: mild cases were often recognized in patients only after severe disease developed in a relative. This problem can now be addressed by means of genetic diagnosis.

The high rate of polyarteritis nodosa in the historically endogamous Georgian Jewish population facilitated the discovery of *CECR1* as the critical gene, but, as indicated by the presence of the mutations in German and Turkish patients, *CECR1* mutations leading to polyarteritis nodosa are not limited to a particular ethnic group. Future *CECR1* sequencing in patients with similar manifestations may reveal the full mutational and phenotypic spectrum of ADA2-associated vasculitis.

There are no unequivocal diagnostic criteria for many vasculitides,[19,20] and clinicopathological classification criteria are not entirely sensitive.[6,7] Diagnoses that are based on analysis of tissue samples and angiographic findings increase specificity but are organ-dependent, and invasive procedures are not always feasible. Biochemical or genetic disease markers, as exemplified by antineutrophil cytoplasmic antibodies in small-vessel and medium-vessel vasculitis, can substantially improve diagnostic discrimination. The identification of mutations altering ADA2 as one cause of polyarteritis nodosa is an initial step in the gene-based definition of disease and may contribute to molecular classification of the vasculitides.

A role for ADA2 is consistent with the nature of polyarteritis nodosa as an immune disorder and suggests new perspectives with regard to its pathogenesis. ADA2 is both the major extracellular adenosine deaminase and an adenosine deaminase–related growth factor. In humans, the irreversible degradation of adenosine to inosine and deoxyadenosine to deoxyinosine is catalyzed by intracellular ADA1 and extracellular ADA2. Recessive ADA1 mutations are a well-known cause of severe combined immune deficiency.[21,22] In the patients described here, however, ADA2 deficiency manifested as increased vascular inflammation without clinically apparent immune deficiency. This observation may reflect the complexity of the role of adenosine in the inflammatory response. Whereas adenosine signaling dampens the inflammatory response in acute disease states, especially in ischemia and hypoxia, chronically elevated levels of adenosine may promote tissue injury and fibrosis by prolonging inflammation.[23] Further investigation may determine whether constitutionally reduced ADA2 activity leads to vasculitis by affecting the adenosine inflammatory-response pathway.

ADA2-associated vasculopathy may also be related to impairment of the activity of ADA2 as a growth factor, which is important both in the immune system and in early development. In the immune system, ADA2 is secreted by monocytes differentiating into dendritic cells and macrophages, leading to macrophage proliferation by costimulation of monocyte-induced CD4+ T-cell proliferation.[17] This role is unique to ADA2, as compared with ADA1, and is independent of the catalytic activity of ADA2. Genomic duplications that include *CECR1*, thus leading to the overexpression of ADA2, cause the cat-eye syndrome, a congenital malformation disorder affecting the eye, heart, and kidney. Overexpression of

CECR1 in mice, which have no orthologue of the human gene,[24] recapitulates these developmental anomalies.[25]

We speculate that manifestations of ADA2 deficiency reflect impairment of both its catalytic and growth-factor activities. The combination of arterial aneurysms and livedo reticularis is characteristic not only of polyarteritis nodosa, an inflammatory disease, but also of familial thoracic aneurysms (OMIM database number, 611788), a structural disease of the arterial wall caused by mutations in *ACTA2*, the smooth-muscle alpha actin, a major component of the contractile apparatus.[26] The noninflammatory occlusive vasculopathy caused by this structural mutation leads to both livedo reticularis and susceptibility to ischemic stroke.[27] Polyarteritis nodosa is a vasculitis that also includes structural arterial abnormalities, and the hypothesis that ADA2 deficiency leads to both vasculopathy and vasculitis can now be investigated directly, with the use of cellular and animal models.

In conclusion, we found that vasculopathy overlapping polyarteritis nodosa can be caused by a single-gene defect. We suggest that treatment with anti-TNF agents should be considered, especially in severe cases of ADA2-associated vasculitis. Our results also suggest that investigation of ADA2-replacement therapy (e.g., with the use of fresh-frozen plasma) may be warranted.

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APPENDIX

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