

Original article

Phenotypic variability in patients with ADA2 deficiency due to identical homozygous R169Q mutations

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Abstract

Objective. To determine the genotype–phenotype association in patients with adenosine deaminase-2 (ADA2) deficiency due to identical homozygous R169Q mutations in *CECR1*.

Methods. We present a case series of nine ADA2-deficient patients with an identical homozygous R169Q mutation. Clinical and diagnostic data were collected and available MRI studies were reviewed. We performed genealogy and haplotype analyses and measured serum ADA2 activity. ADA2 activity values were correlated to clinical symptoms.

Results. Age of presentation differed widely between the nine presented patients (range: 0 months to 8 years). The main clinical manifestations were (hepto)splenomegaly (8/9), skin involvement (8/9) and neurological involvement (8/9, of whom 6 encountered stroke). Considerable variation was seen in type, frequency and intensity of other symptoms, which included aplastic anaemia, acute myeloid leukaemia and cutaneous ulcers. Common laboratory abnormalities included cytopenias and hypogammaglobulinaemia. ADA2 enzyme activity in patients was significantly decreased compared with healthy controls. ADA2 activity levels tended to be lower in patients with stroke compared with patients without stroke. Genealogical studies did not identify a common ancestor; however, based on allele frequency, a North-West European founder effect can be noted. Three patients underwent haematopoietic cell transplantation, after which ADA2 activity was restored and clinical symptoms resolved.

Conclusion. This case series revealed large phenotypic variability in patients with ADA2 deficiency though they were homozygous for the same R169Q mutation in *CECR1*. Disease modifiers, including epigenetic and environmental factors, thus seem important in determining the phenotype. Furthermore, haematopoietic cell transplantation appears promising for those patients with a severe clinical phenotype.

Key words: ADA2, *CECR1*, auto-inflammatory disease, vasculitis, early-onset stroke, livedo reticularis, genotype, phenotype

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Rheumatology key messages

- We observed large phenotypic variability in adenosine deaminase-2 deficiency due to homozygous R169Q mutations.
- Haematopoietic cell transplantation restored adenosine deaminase-2 levels and reverted clinical symptoms in adenosine deaminase-2 deficiency.

Introduction

Deficiency of adenosine deaminase-2 (ADA2) is a recently described autoinflammatory disorder with cutaneous inflammatory disease, febrile episodes, cytopenias, splenomegaly and early-onset stroke [1–3] caused by mutations in the cat eye syndrome chromosome region candidate 1 gene (*CECR1*). In two series of patients with both homozygous and compound heterozygous mutations in *CECR1*, a subset of patients expressed ulcerative skin disease as the predominant symptom, while others presented with lacunar brain infarctions as a distinctive feature. Age of onset and severity of symptoms varied considerably between patients, ranging from an intermitting and relatively mild course to haematopoietic abnormalities necessitating donor stem cell transplantation [4–6]. It is currently not well understood what substantiates these differences in clinical phenotype.

Contrary to the well-known clinical entity of ADA1 deficiency causing severe combined immunodeficiency, the pathogenesis of clinical features in ADA2 deficiency is not yet fully understood. Vascular integrity appears to be disrupted by unrevealed effects of the ADA2 deficiency on macrophage and neutrophil function [7, 8]. The majority of disease-causing mutations in *CECR1* reported so far are missense mutations, and the variable phenotype may partly be explained by the different affected domains of the protein and variations in residual enzyme level and activity pertaining to the reported missense mutations.

To date, in 9 out of 10 patients with ADA2 deficiency identified in The Netherlands and Belgium, the homozygous R169Q mutation was detected. In previously reported ADA2 patients, this mutation was only seen in a compound heterozygous form. We hypothesized that phenotypic variability would be limited in patients homozygous for an identical mutation, and that the R169Q mutation could be traced to a Dutch founder. Here, we present clinical and laboratory data in a case series of nine patients with an identical homozygous R169Q mutation in *CECR1* leading to ADA2 deficiency. We performed genealogical studies and haplotype analyses, measured residual ADA2 activity levels and related these values to the clinical phenotype. We finally report on the prescribed treatment regimens and their clinical outcome.

Methods**Design**

We describe a case series of nine patients with ADA2 deficiency due to R169Q mutations in *CECR1*.

Patients

We included all patients (n=9) diagnosed with ADA2 deficiency due to homozygous R169Q mutations in *CECR1* until March 2014 in one of the following hospitals: Leiden University Medical Center (n=1), Academic Medical Center Amsterdam (n=2), University Medical Center Groningen (n=1), University Medical Center Utrecht (n=3) and University Hospitals in Leuven, Belgium (n=2). This study is part of a larger research project on immunodeficiencies and inflammatory diseases. The larger research project was approved by the local ethical committee (Medical Ethical Board of the UMC Utrecht) and written informed consent was given by all subjects and/or caregivers according to the Declaration of Helsinki; this current study did not require separate ethical approval.

Clinical data collection

Based on initial presentations on the previously described phenotype [1, 3], a predefined set of clinical data on disease course, genetic studies, laboratory values, radiological imaging studies and treatments was collected from clinical files. Age-adjusted reference values for T and B cells were taken from the study of van Gent *et al.* [9].

Gene sequencing

DNA was isolated from peripheral blood mononuclear cells. Protein coding exons of the *CECR1* gene (NM 00128225.1) were evaluated using Sanger sequencing. Primer sequences are available upon request.

Haplotype analysis

The proband from each family was genotyped using BlueGnome (Illumina, San Diego, California, USA) CytoSNP-850K arrays following the manufacturer's protocol. Haplotype and homozygosity (ROH) analyses were performed using Nexus software.

Genealogy

Of the five Dutch families in this cohort, extended pedigrees of nine generations were studied using Aldfaer version 4.2 (www.aldfaer.net) to investigate possible common ancestry.

Measuring ADA2 enzyme activity

For measurement of ADA2 activity we modified the procedure of an ADA activity assay (Diazyme, Poway, CA, USA) by adding erythro-9-(2-hydroxy-3-nonyl)adenine (Sigma-Aldrich, Zwijndrecht, Netherlands) for inhibition of ADA1. Measurement was performed in a Spectramax M2 plate reader at 37°C and 550 nm at different time intervals (3–15 min). ADA2 activity levels were determined

using controls and calibrators (Diazyme, Poway, CA, USA) and expressed as units/millilitre where 1 U is defined as the amount of enzyme that generates 1 μmol of inosine from adenosine per minute. The inter- and intra-assay percentage coefficients of variation were both 14. Six anonymized adult volunteers served as healthy controls.

Radiological studies

All patients underwent one or more MRI scans of the brain during the course of their disease, as part of diagnostic evaluation. Of each patient, all available MRIs were re-assessed by one observer (K.B.). The type, localization and total number of lesions during the entire observed disease course were assessed.

Statistics

Mann-Whitney *U* tests were performed to compare median ADA2 function between patients and healthy controls, and between patients with and without stroke. IBM SPSS Statistics 21 was used for data analysis.

Results

Patients

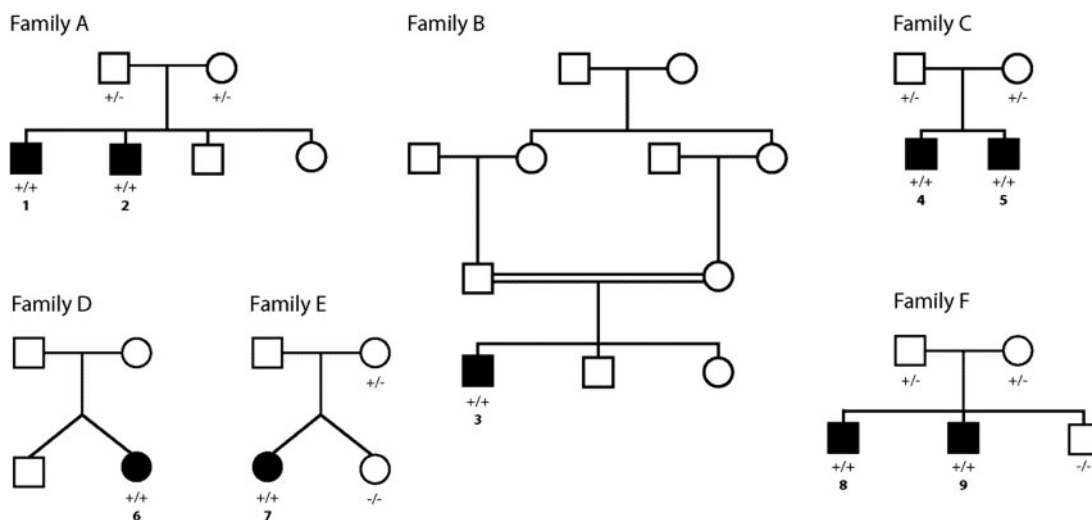
Nine patients from six families were included in this study. Family pedigrees are shown in Fig. 1. All patients are of Caucasian descent, except for patients 8 and 9, who are the offspring of a Caucasian mother and a father of Moroccan descent. All known first degree family members of patients 1–9 were noted to be healthy, except for the parents of patients 1 and 2, who are both affected by insulin-dependent diabetes mellitus, and the twin sister of patient 7, who suffers from MCTD.

Clinical presentation

The mean age of presentation with first symptoms was 2.6 years (range: 0 months to 8 years). The presenting symptom was different for all patients (including laboratory abnormalities, neurological symptoms or inflammatory diseases), as was the initial clinical diagnosis before the ADA2 deficiency was recognized in 2014 (Table 1). Common clinical findings during the course of the disease included (hepato)splenomegaly (8/9), skin involvement (8/9), neurological involvement (8/9) and to a lesser extent gastrointestinal complaints (6/9); however other clinical symptoms varied considerably between patients over the course of their disease (supplementary Fig. S1, available at *Rheumatology* Online). Symptoms noted less frequently included growth hormone deficiency (patients 1 and 2), cutaneous lesions diagnosed as acute myeloid leukaemia (AML) for which systemic chemotherapy was given, as well as hypertension and cardiomyopathy (patient 6). A single episode of acute liver failure with spontaneous recovery was seen in patient 7. Despite the fact that hypogammaglobulinaemia (8/9 patients) and cytopenias (9/9 patients) were seen frequently, only 3/9 patients encountered recurrent infections. An overview of clinical symptoms is shown in Table 1 and in supplementary Table S1, available at *Rheumatology* Online.

Neurological symptoms were noted in 8/9 patients (Table 1), of whom two only experienced a single episode with headache or vomiting, one in the context of a jugular vein thrombosis that led to hydrocephalus, the other concurrent with a pineal gland bleeding due to severe thrombocytopenia following allogeneic haematopoietic cell transplantation (HCT). Six patients had more than one episode of clinical neurological involvement. Symptoms mainly consisted of cranial nerve deficits (hearing loss, vertigo, optic nerve atrophy, oculomotor disturbances due to documented third, fourth or sixth nerve

Fig. 1 Pedigrees



Pedigrees of the nine patients are shown, indicating R169Q mutation carriers. Affected patients are numbered.

TABLE 1 Clinical manifestations and treatments observed in patients with ADA2 deficiency due to homozygous R169Q mutations in CECR1

	1	2	3	4	5	6	7	8	9	No. /total no.
Current age, years	13	16	50	22	20	9	13	9	4	—
Age at presentation	1 year	0 months	6 years	3 years	1 year	9 months	8 years	6 months	5 months	—
Presenting symptom	VZV infection complicated with diplopia, vertigo and deafness	Pancytopenia, (hepato) splenomegaly	Splenomegaly, hemolytic anemia and thrombocytopenia	Acute behavioural regression	Strabismus and ataxia	AML related chloroma	Thalamic stroke, hypogammaglobulinemia	Complicated RSV infection, hypogammaglobulinaemia	Profound Coombs negative anaemia	—
Predominant symptom	Arthralgias	Refractory anaemia	Ulcerative skin disease	Cerebral stroke, skin inflammation	Cerebral involvement	Skin + Brain involvement	Recurrent diplopia	Autoimmune cytopenia, lymphoproliferation	Viral infections (herpes-viridae)	—
Initial clinical diagnosis	XLP-like disease	Atypical Diamond Blackfan anaemia	Storage disease	Cerebral stroke, stroke eci, cutaneous PAN	Cerebral stroke eci	Atypical cutaneous AML	Recurrent stroke	Autoimmune HLH ALPS like	CID with lymphoproliferation and autoimmunity	—
No. of clinical episodes with neurological symptoms (no. of documented lesions (stroke) by neuroimaging)	4 (5)	1 (0)	0 (0)	5 (2)	3 (3)	3 (3)	5 (2)	1 (1)	2 (2)	8/9 (6/9)
Systemic/rheumatic symptoms										
Recurrent fever (non-infectious)	x					x	x			7/9
Recurrent infections				x					x	3/9
Lymphoproliferation	x							x	x	3/9
Lymphadenopathy	x		x					x	x	4/9
Arthralgia	x						x			3/9
Oral aphthae	x						x			3/9
Treatment		x								8/9
Acetylsalicylic acid, 38 mg	x		x	x				x		4/9
Immunoglobulin supplementation	x									5/9
Corticosteroids (systemic)	x		x	x				x		6/9
Azathioprin	x		x					x		5/9
TNF- α inhibition, etanercept/adalimumab	x		x	x						4/9
HCT		x						x		3/9

ALPS: autoimmune lymphoproliferative syndrome; AML: acute myeloid leukaemia; CID: combined immunodeficiency; HCT: haematopoietic cell transplantation; HLH: hemophagocytic lymphohistiocytosis; PAN: polyarteritis nodosa; RSV: respiratory syncytial virus; TNF- α : tumour necrosis factor α ; XLP-disease: X-linked lymphoproliferative disease; VZV: varicella zoster virus; x: represents the symptom is present.

palsy), other oculomotor signs (not further specified or consistent with brainstem, cerebellar or thalamic lesions), focal sensorimotor signs, ataxia or seizures (supplementary Table S2, available at *Rheumatology* Online). In most patients, clinical signs were concordant with imaging findings, although imaging was not performed in all clinical episodes, or new MRI lesions were documented. Over the course of their disease, all nine patients underwent one or multiple MR scans of the brain, the findings of which are summarized in supplementary Table S2, available at *Rheumatology* Online. Two patients had an intracranial haemorrhagic lesion. All other MRI abnormalities were characterized by small fluid-attenuated inversion recovery (FLAIR) hyperintensities, consistent with deep lacunar infarcts, most often in the thalamus or brain stem. Abnormal contrast enhancement was not seen in any of the patients to whom gadolinium was administered, either in the acute or chronic phase.

Laboratory values

Lymphocytopenia and granulocytopenia were eventually noted in all patients, but at initial presentation 2/9 patients had normal values. Thrombocytopenia was noted in 3/9 patients (Table 2). Laboratory evaluations further showed low (<2 s.d. for age) IgG, IgA and IgM in 8/9 patients. Low total CD19⁺ B cell counts were detected in 7/9 patients. Stroke was noted in 6/9 patients, whereas none of these patients had abnormal humoral clotting tests (PT, aPTT).

ADA2 enzyme activity

Median (95% CI) ADA2 enzyme activity was found to be significantly lower in ADA2 deficiency patients compared with healthy controls [0.6 (0.10–2.50) vs 5.4 (3.41–7.10), $P=0.001$, Fig. 2A]. When comparing patients with and without stroke, we noted that median ADA2 activity levels were significantly lower in patients with stroke [0.1 (0.10–0.70) IU/l compared with patients without stroke [1.50 (0.70–2.50) IU/l, $P=0.036$]. Patient 8 was included in the category of patients without stroke, as his pineal gland bleeding was likely caused by thrombocytopenia post-HCT, instead of the ADA2 deficiency itself.

Normal levels of ADA2 activity were found in patients who had undergone HCT at least 6 months before current testing (patients 2, 6 and 8). Patients 6 and 8 had pre- and post-HCT samples available, and these showed resolution of abnormal ADA2 activity within weeks after allogeneic HCT (Fig. 2B).

Founder analysis

Genealogical analysis was performed to determine whether the c.506G > A p.(R169Q) mutation could be traced to a common Dutch founder. Consanguinity was detected after six generations in family A and two generations in family B. Family D is linked to family B, with a common ancestor after seven generations. Family D is also linked to family C with a different common ancestor after six generations.

No common ancestor for all families could be detected by genealogy. Haplotype analysis showed a small

overlapping haplotype (run of homozygosity; ROH) of 49.3 kb defined by 26 single nucleotide polymorphisms (SNPs) (Fig. 3), suggestive of a common but ancient ancestral mutation. Based on the minor allele frequencies (MAFs) of the mutation in different populations, a Northern European founder effect can be noted: the mutation has been detected once in 500 Dutch controls [10], in European populations the MAF was $\sim 1:1500$, and the mutation was not observed in Latin American, and East and South Asian populations (<http://exac.broadinstitute.org>).

Treatment

In the time frame before a definitive diagnosis was made, five out of nine patients received empiric systemic immunosuppressive treatments at any point during their disease episodes for their different inflammatory diseases (Table 1). For ulcerative skin disease reported in patient 3, immunosuppressive therapy with topical and systemic corticosteroids did not prove effective, while a remarkable response on skin ulcerations was seen upon treatment with an anti-TNF- α agent (adalimumab). For rheumatic systemic inflammatory sequelae (including arthralgias, arthritis and fever), only limited effect of treatment was seen after NSAID and systemic corticosteroid treatment; anti-TNF- α treatment was prescribed in two additional patients and was effective for these complaints. One patient received IL-1 receptor blocking therapy (anakinra), which proved effective for systemic inflammatory symptoms. After diagnosis of stroke was made, three out of six patients (who did not undergo HCT) were prescribed antiplatelet aggregation therapy with low dose acetylsalicylic acid (38 mg/day). Whilst using this prophylaxis, one out of three patients had a recurrence of stroke. Five out of nine patients receive immunoglobulin prophylaxis for hypogammaglobulinaemia. Using this medication, only sporadic infections were seen.

Three out of nine patients underwent HCT for the following reasons: refractory pure red cell aplasia and associated iron overload (patient 2), pancytopenia and absence of B cells after AML treatment (patient 6) and corticosteroid-dependent autoimmune thrombocytopenia causing growth failure (patient 8). Allogeneic haematopoietic stem cell transplantation (HCT) proved to be effective on complete resolution of skin symptoms, splenomegaly, cytopenias and systemic inflammation in 3/3 patients. Complications after HCT included non-engraftment (for which a second HCT was performed) in patient 2 and veno-occlusive disease in patient 8. Patient 8 also suffered from a pineal gland haemorrhage 36 days post-HCT during severe thrombocytopenia ($2 \times 10^9/l$). During further follow up, transplanted patients kept normal blood counts and absence of inflammatory disease associations, including neurological symptoms.

Discussion

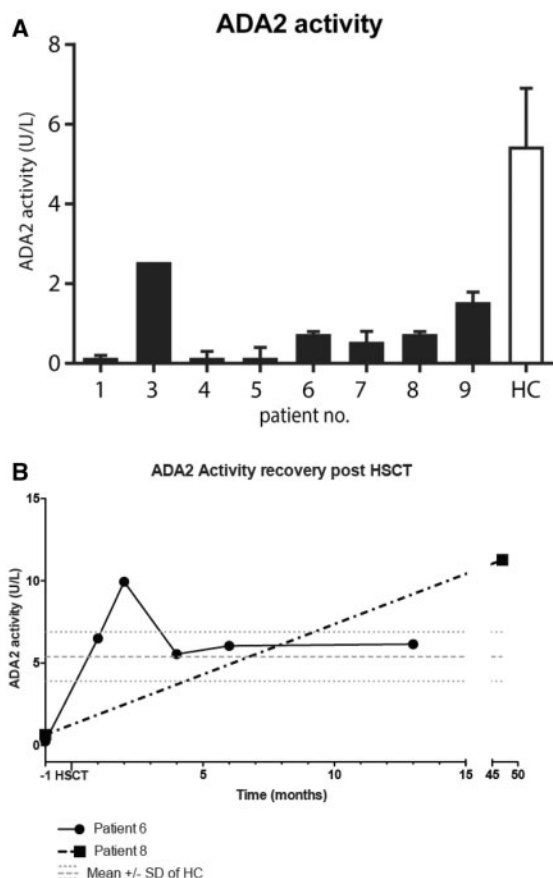
We describe the clinical and laboratory findings in nine patients with ADA2 deficiency due to identical homozygous mutations (R169Q) in *CECR1*. We observed

TABLE 2 Laboratory results

Patient no.	1	2	3	4	5	6	7	8	9
Haemoglobin ^a (normal range per age group), mmol/l	6.3 (8.6–10.7)	4.9 (7.4–8.7)	8.3 (8.6–10.7)	8.1 (8.6–10.7)	7.7 (7.4–8.7)	4.3 (7.4–8.7)	8.1 (7.4–9.6)	8.8 (7.4–8.7)	9.7 (7.4–8.7)
Thrombocytes, 150–450 × 10 ⁹ /l	39	365	402	179	270	120	153	25	300
Leukocytes ^a (normal range per age group), 10 ⁹ /l	1.1 (4.5–13)	7.8 (5.5–15.5)	9.8 (4–10)	4.3 (4–10)	0.9 (4–10)	2.7 (5.5–15.5)	3.1 (4.5–13)	2.02 (5.5–15.5)	9.08 (5.5–15.5)
Neutrophils ^a (normal range per age group), 10 ⁹ /l	0.39 (1.8–8)	0.78 (1.5–8.5)	6.87 (1.6–8.3)	2.78 (1.6–8.3)	0.33 (1.6–8.3)	0.9 (1.5–8.5)	2.25 (1.8–8)	0.3 (1.5–8.5)	6.4 (1.5–8.5)
Lymphocytes ^a (normal range per age group), 10 ⁹ /l	0.48 (1.2–5.2)	5.61 (2.0–8.0)	1.78 (0.8–4.0)	0.57 (0.8–4.0)	0.43 (0.8–4.0)	22.9 (2.0–8.0)	0.51 (1.2–5.2)	1 (2.0–8.0)	8.35 (2.0–8.0)
Immunoglobulins									
IgM, 0.28–2.4 g/l	0.19	0.3	0.71	<0.03	<0.03	0.09	<0.1	0.09	0.22
IgG, 5.2–15.6 g/l	2.36	3.6	9.89	4.2	2.2	3.54	3.6	<1	2.77
IgA, 0.7–3.6 g/l	0.11	0.6	2.7	0.08	0.09	0.34	0.2	<0.07	0.7
Antibodies									
ANA	Neg	—	Neg	Neg	Neg	Pos	Neg	Neg	Neg
ANCA	Neg	—	Neg	Neg	—	Pos	Neg	Neg	Neg
aPL	Neg	—	Neg	—	—	—	Neg	—	Neg

‘—’ indicates no data available. Other laboratory results available in supplementary Table S3, available at *Rheumatology* Online. ^aTypical value, neg: negative; pos: positive.

Fig. 2 ADA2 enzyme function



(A) Functioning ADA2 enzyme in ADA2 deficient patients compared with healthy controls. For patient 2, no pre-haematopoietic cell transplantation (HCT) sample was available. Median ADA2 activity is significantly lower in ADA2 mutated patients compared with controls [(0.6 (0.10–2.50) vs 5.4 (3.41–7.10), $P=0.001$]. ADA2 activity was higher in patients that did not experience stroke (patients 3, 8 and 9) compared with patients with stroke [(1.50 (0.70–2.50) vs 0.1 (0.10–0.70) IU/l, $P=0.036$]. **(B)** Recovery of ADA2 enzyme function after haematopoietic stem cell transplantation in patients 6 and 8. ADA2: adenosine deaminase-2.

significant clinical heterogeneity within this case series. The small common haplotype and the MAFs of the mutation were suggestive of a North European founder effect. Common disease manifestations included neurological manifestations, splenomegaly, cytopenias and hypogammaglobulinaemia. Lower residual ADA2 activity was detected in patients with stroke, in contrast with higher residual ADA2 activity levels in patients with cutaneous symptoms only.

In general, we observed a variable phenotype dominated by cutaneous and neurological symptoms. Rheumatological and inflammatory symptoms were present in the majority of patients, but they showed

considerable inter-patient variability in frequency and intensity. Severity of disease course also differed strongly: several patients had disease-free intervals lasting for years, while others ($n=3$) had severe or progressive disease that required HCT. The phenotypes associated with the R169Q mutation correspond to the phenotypes previously described in patients with other mutations in *CECR1* [1, 3]. It was noted that the patients affected by the homozygous R169Q mutation appear to experience less visceral pathology compared with those with other mutations; no renal problems or testicular pain were reported in our patient group. Furthermore, the spectrum of cutaneous pathology was variable. In previously published literature, the livedo reticularis/livedoid skin pathology phenotype predominated [1, 3]. Most of our patients presented with transient rashes or aspecific skin nodules, while digital necrosis or purpura were not observed. Patient 3 was the only patient of this series of R169Q affected patients that was free of neurological problems and did have the severe livedoid vasculopathy that was previously described [1] with painful ulcers on extremities and trunk. Patient 9, in whom no brain lesions were detected, suffered from ulcerating disease in the intestinal tract.

Several mutations in different domains of the protein in ADA2 deficiency have been described. The location of the mutation, affecting dimerization, receptor binding or the catalytic domain, may contribute to the variation in clinical phenotype. The p.G47R mutation in *CECR1* seems the most prominent mutation causing polyarteritis nodosa [1]. Indeed the patients homozygous for this mutation included in the study of Zhou *et al.* [3] met the criteria for polyarteritis nodosa. Our study demonstrates that even the R169Q mutation can cause disease with a highly variable clinical phenotype, where even within families, clinical differences could be noted.

Several causes may give rise to the clinical heterogeneity in patients with identical R169 mutations. The activity of ADA2 was clearly diminished in all patients as compared with controls (Fig. 2a); however, large differences in enzyme activity were noted within our patient population. Stroke occurred predominantly in the patient group with lowest ADA2 activity levels, suggesting that residual ADA2 activity levels are important in clinical phenotype; a larger series is, however, needed to further confirm this relation. ADA2 binds to different cell types via proteoglycans and yet-unknown receptors [11], and functional differences in these receptors might play a role in the differences in clinical outcome. ADA2 is known to promote macrophage differentiation [7], and ADA2-deficient patients seem to enhance differentiation of macrophage and monocyte subsets toward proinflammatory cells [3]. It can thus be speculated that other monocyte and macrophage regulating mechanisms (than ADA2) have an important contribution to the clinical outcome in ADA2-deficient patients. These differences could either be environmental, such as differences in exposure to inflammatory agents, or could have a genetic cause. Next to functional differences in genes implicated in inflammatory

pathways, functional differences in genes playing a role in vascular integrity, especially in the small vessels of patients with ADA2 deficiency, could also explain the difference in clinical outcome.

The fact that the R169Q mutation is as yet the most commonly identified mutation in ADA2 deficiency patients in the Netherlands and Belgium suggests a common founder. Using genealogy, the mutation could not be traced back to a common Dutch founder. This is in line with the small overlapping homozygous region in *CECR1* surrounding the R169Q mutation suggesting a mutation that occurred many generations ago. We cannot exclude, however, that the mutation occurred on multiple occasions. This was described previously for the p.G47R mutation in *CECR1*, which was detected in different haplotypes [3]. The MAFs of the mutation were, however, suggestive of a north-west European founder effect. Based on a carrier frequency of ~1:500, it may be expected that the number of ADA2 patients based on this mutation is small; however, it is likely that more patients will be diagnosed carrying the same homozygous mutation.

Treatment of patients with ADA2 deficiency due to homozygous R169Q mutations should in our opinion be focused on the predominant symptom, and should include a consideration of stroke prophylaxis. In our patients, ulcerative skin disease, as well as systemic

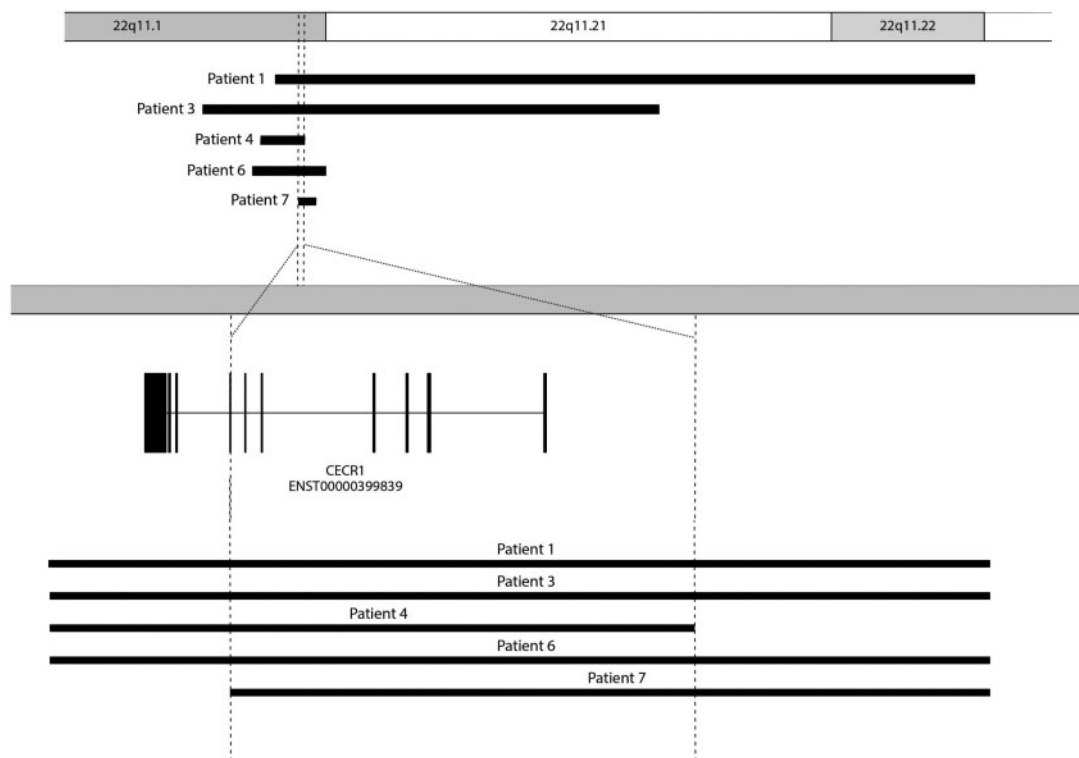
inflammatory and rheumatic symptoms, seemed most responsive to TNF- α blocking agents. Systemic corticosteroid treatment was only of limited benefit in our patients. No recurrence of stroke was seen in 2/2 patients treated with TNF- α blocking agents. It is currently not known whether prophylactic use of acetylsalicylic acid is effective in prevention of stroke. HCT proved effective in all three patients, but careful risk and benefit analysis concurrent with a HCT procedure is necessary on a per patient basis.

The fact that ADA2 deficiency can present with many different clinical symptoms necessitates awareness of this diagnosis in many different patient categories, including those with unexplained rheumatic symptoms, early onset stroke and unexplained cytopenias. In this cohort, splenomegaly and low IgM were seen in almost all patients. Screening of the disease can be performed by measuring ADA2 enzyme function; however, a definitive diagnosis can only be made by genetic investigation.

This study describes nine patients with identical R169Q mutations in *CECR1*. Although the largest series with this mutations so far, this relatively small patient number necessitates reconfirmation of our findings in larger series. Especially the relation between residual ADA2 activity and stroke risk may have clinical consequences, and should thus be studied in further detail.

In conclusion, we describe the clinical and laboratory phenotype of nine patients with a homozygous R169Q

Fig. 3 Haplotypes



Haplotypes were determined for families A–E. Each bar indicates the homozygous region on 22q11 in a patient of each family. The lower part of the figure shows a close-up of the region around the *CECR1* gene.

mutation in *CECR1*. In our population, neurological and cutaneous pathology dominated the phenotype, on a background of inflammation and mild immunodeficiency manifesting as ulcerations and various rheumatological problems. A large variation was seen in type, frequency and intensity of symptoms. This suggests that factors other than the *CECR1* mutations are important in determining clinical course. Interestingly, although the cohort is small, a possible relation between disease severity and ADA2 function was observed: no brain infarcts were observed in the two patients with highest residual ADA2 function. Lastly, the disease resolved in the three patients who underwent HCT, which thus appears promising for patients with severe clinical manifestations. Further research is necessary to elucidate other factors determining the clinical course.

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Supplementary data

Supplementary data are available at *Rheumatology* Online.

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