

Circulation

Cardiovascular Genetics

American Heart Association 
Learn and Live

JOURNAL OF THE AMERICAN HEART ASSOCIATION

Frequent and Widespread Vascular Abnormalities in Human Signal Transducer and Activator of Transcription 3 Deficiency

Marie-Olivia Chandesris, Arshid Azarine, Kim-Thanh Ong, Soraya Taleb, Pierre Boutouyrie, Elie Mousseaux, Mélissa Romain, Erwan Bozec, Stéphane Laurent, Nathalie Boddaert, Caroline Thumerelle, Isabelle Tillie-Leblond, Cyrille Hoarau, Yvon Lebranchu, Nathalie Aladjidi, François Tron, Vincent Barlogis, Gérard Body, Marine Munzer, Roland Jaussaud, Felipe Suarez, Olivier Clément, Olivier Hermine, Alain Tedgui, Olivier Lortholary, Capucine Picard, Ziad Mallat and Alain Fischer

Circ Cardiovasc Genet 2012;5:25-34; originally published online November 14, 2011;

DOI: 10.1161/CIRCGENETICS.111.961235

Circulation: Cardiovascular Genetics is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75214

Copyright © 2012 American Heart Association. All rights reserved. Print ISSN: 1942-325X. Online ISSN: 1942-3268

The online version of this article, along with updated information and services, is located on the World Wide Web at:

<http://circgenetics.ahajournals.org/content/5/1/25.full>

Data Supplement (unedited) at:

<http://circgenetics.ahajournals.org/content/suppl/2011/11/14/CIRCGENETICS.111.961235.DC1.html>

Subscriptions: Information about subscribing to Circulation: Cardiovascular Genetics is online at <http://circgenetics.ahajournals.org/site/subscriptions/>

Permissions: Permissions & Rights Desk, Lippincott Williams & Wilkins, a division of Wolters Kluwer Health, 351 West Camden Street, Baltimore, MD 21201-2436. Phone: 410-528-4050. Fax: 410-528-8550. E-mail: journalpermissions@lww.com

Reprints: Information about reprints can be found online at <http://www.lww.com/reprints>

Frequent and Widespread Vascular Abnormalities in Human Signal Transducer and Activator of Transcription 3 Deficiency

Marie-Olivia Chandesris, MD*; Arshid Azarine, MD, MSc*; Kim-Thanh Ong, MD, PhD*; Soraya Taleb, PhD*; Pierre Boutouyrie, MD, PhD; Elie Mousseaux, MD, PhD; Mélissa Romain, MSc; Erwan Bozec, PhD; Stéphane Laurent, MD, PhD; Nathalie Boddaert, MD, PhD; Caroline Thumerelle, MD; Isabelle Tillie-Leblond, MD, PhD; Cyrille Hoarau, MD, PhD; Yvon Lebranchu, MD, PhD; Nathalie Aladjidi, MD; François Tron, MD, PhD; Vincent Barlogis, MD; Gérard Body, MD; Marine Munzer, MD; Roland Jaussaud, MD, PhD; Felipe Suarez, MD, PhD; Olivier Clément, MD, PhD; Olivier Hermine, MD, PhD; Alain Tedgui, PhD; Olivier Lortholary, MD, PhD; Capucine Picard, MD, PhD*; Ziad Mallat, MD, PhD*; Alain Fischer, MD, PhD**

Background—Signal transducer and activator of transcription 3 (STAT3) deficiency is responsible for autosomal dominant hyperimmunoglobulin E syndrome, characterized by recurrent bacterial and fungal infections, connective tissue abnormalities, hyperimmunoglobulin E, and Th17 lymphopenia. Although vascular abnormalities have been reported in some patients, the prevalence, characteristics, and etiology of these features have yet to be described.

Methods and Results—We prospectively screened 21 adult STAT3-deficient patients (median age: 26 years; range 17–44 years) for vascular abnormalities. We explored the entire arterial vasculature with whole-body magnetic resonance imaging angiography, coronary multislice computed tomography, and echo-tracking–based imaging specifically for the carotid arteries. We also assayed for serum biomarkers of inflammation and endothelial dysfunction. Finally, we studied murine models of aortic aneurysm in the presence and absence of inhibitors of STAT3-dependent signaling. Ninety-five percent of patients showed brain abnormalities (white matter hyperintensities, lacunar lesions suggestive of ischemic infarcts, and atrophy). We reported peripheral and brain artery abnormalities in 84% of the patients and detected coronary artery abnormalities in 50% of the patients. The most frequent vascular abnormalities were ectasia and aneurysm. The carotid intima-media thickness was markedly decreased, with a substantial increase in circumferential wall stress, indicating the occurrence of hypotrophic arterial remodeling in this STAT3-deficient population. Systemic inflammatory biomarker levels correlated poorly with the vascular phenotype. In vivo inhibition of STAT3 signaling or blockade of IL-17A resulted in a marked increase in aneurysm severity and fatal rupture in mouse models.

Conclusions—Vascular abnormalities are highly prevalent in patients with STAT3 deficiency. This feature is consistent with the greater susceptibility to vascular aneurysm observed after inhibition of STAT3-dependent signaling in mouse models. (*Circ Cardiovasc Genet.* 2012;5:25-34.)

Key Words: STAT3 ■ autosomal dominant hyper-IgE syndrome ■ primary immunodeficiency ■ vascular abnormalities ■ white matter hyperintensities

Received June 7, 2011; accepted November 1, 2011.

From the Hematology Department (M.-O.C., F.S., O.H.), Necker Children's Hospital, Assistance Publique Hôpitaux de Paris (AP-HP), Paris, France; Paris Descartes University (M.-O.C., F.S., O.H., O.L., C.P., Z.M., A.F.), Paris Cité Sorbonne, Necker Faculty of Medicine, Paris, France; Centre de référence des déficits immunitaires héréditaires (CEREDIH) (M.-O.C., F.S., O.H., O.L., C.P., A.F.), Necker Children's Hospital, AP-HP, Paris, France; Radiology Department (A.A., E.M., O.C.), Georges Pompidou European Hospital, AP-HP, Paris, France; Pharmacology Department (K.-T.O., P.B., E.B., S.L.), Georges Pompidou European Hospital, Paris Descartes University, AP-HP, Paris, France; Institut National de la Santé et de la Recherche Médicale (INSERM) U970 (S.T., M.R., A.T., Z.M.), Paris-Descartes University, Paris, France; INSERM U678 (E.M.), Georges Pompidou European Hospital, AP-HP, Paris, France; Pediatric Radiology Unit (N.B.), Necker Children's Hospital, AP-HP, Paris, France; INSERM U1000 (N.B.), Paris-Descartes University, Paris, France; Pediatric Pneumology Department (C.T.), Jeanne de Flandres Hospital, Lille, France; Pneumology Unit (I.T.-L.), Calmette Hospital, Lille, France; Immunology Unit (C.H., Y.L.), Tours Hospital, Tours, France; Pediatric Hemato-oncology Department (N.A.), Pellegrin Hospital, Bordeaux, France; Immunology Unit (F.T.), Rouen Hospital, Rouen, France; Pediatric Hemato-oncology Department (V.B.), Timone Hospital, Marseille, France; Pediatric Pneumology Department (G.B.), Châlons-en-Champagne Hospital, Châlons-en-Champagne, France; Pediatric Onco-Hematology Department (M.M.), Pediatric Hospital, and Internal Medicine Department (R.J.), Robert Debré Hospital, Reims, France; CNRS UMR8147 (O.H.), Paris Descartes University, Necker Medical Faculty, Necker, France; Department of Infectious Diseases and Tropical Medicine (O.L.), Necker-Enfants Malades Hospital and Institut Pasteur, CNRS, CNRS URA3012, AP-HP, Paris, France; Study Centre of Primary Immunodeficiencies (C.P.), Necker-Enfants Malades Hospital, AP-HP, Paris, France; Laboratory of Human Genetics of Infectious Diseases (C.P.), Necker Branch, INSERM U980, Paris, France; Department of Medicine (Z.M.), University of Cambridge, Cambridge, United Kingdom; Immuno-Hematology Pediatric Department (A.F.), Necker-Enfants Malades Hospital, AP-HP, Paris, France; INSERM U768 (A.F.), Necker-Enfants Malades Hospital, Paris, France.

*These authors contributed equally; **These authors contributed equally.

The online-only Data Supplement is available at <http://circgenetics.ahajournals.org/lookup/suppl/doi:10.1161/CIRCGENETICS.111.961235/-/DC1>.

Correspondence to Alain Fischer, Immuno-Hematology Pediatric Department/INSERM U768, Hôpital Necker-Enfants Malades, 149 rue de Sèvres, F-75015 Paris, France. E-mail alain.fischer@nck.aphp.fr

© 2011 American Heart Association, Inc.

Circ Cardiovasc Genet is available at <http://circgenetics.ahajournals.org>

DOI: 10.1161/CIRCGENETICS.111.961235

Signal transducer and activator of transcription 3 (STAT3) deficiency is a rare primary immunodeficiency that is referred to as an autosomal dominant hyperimmunoglobulin E syndrome (AD-HIES), caused by mutations in the different domains of STAT3.^{1–3} Its clinical features include dermatitis; staphylococcal cold abscesses of the skin; chronic mucocutaneous candidiasis; recurrent cyst-forming bacterial pneumonia; ear, nose and throat infections; and high immunoglobulin E levels, associated with impaired IL-17 production by T cells and defective Th17 differentiation.^{4–7} Recently, focal brain white matter hyperintensities (WMHs) (in the absence of associated arterial hypertension)⁸ and coronary artery abnormalities (ranging from tortuosity to ectatic dilation and focal aneurysms)^{9–13} have been described in patients with AD-HIES.

Clinical Perspective on p 34

Whole-body magnetic resonance angiography (WB-MRA) enables the noninvasive assessment of almost the entire arterial vasculature¹⁴ and can be combined with conventional brain and cardiac magnetic resonance imaging (MRI) to detect brain WMHs and screen for myocardial infarction. In the present study, WB-MRA was further combined with multislice computed tomography (MSCT) of the coronary arteries and ultrasound-based imaging of the carotid arteries to investigate the prevalence and type of vascular abnormalities in adult patients with STAT3 deficiency. To gain further insights into the mechanisms responsible for cardiovascular abnormalities in this setting, we measured circulating levels of cardiovascular risk biomarkers in the patients and examined the impact of alterations in STAT3-related pathways on the development and severity of vascular aneurysms in validated murine models.

Patients, Materials, and Methods

Patients

Twenty-one adult patients (over 16 years of age), registered in the national French primary immunodeficiency database (CEREDIH) as having a heterozygous STAT3 mutation, were enrolled in a French, nationwide, prospective, multicenter study performed between August 2009 and December 2010. The study was approved by the investigational review board at Necker Children's Hospital (Paris, France). All subjects gave their written informed consent. The French National Data Protection Commission authorized the use of a computer database to store personal information (CNIL authorization No. 908256).

The main features of each patient were recorded and scored using the National Institutes of Health scoring system: All the scores were then added together to yield an overall score. Scores over 40 indicate that the subject is likely to carry an AD-HIES phenotype, scores of 20 to 40 points are inconclusive, and scores below 20 points indicate that the subject is unlikely to have an AD-HIES phenotype.¹⁵

For the echo-tracking procedure, healthy control subjects (drawn from a reference database) were matched for gender, age, and mean blood pressure. When 2 or more control subjects were available for a given patient, we chose the person whose arterial parameters were closest to those of the patient.

Imaging

The imaging procedures included brain MRI, gadolinium-enhanced whole-body MRA, and late gadolinium enhancement cardiac MRI in a single session, followed by iodine-enhanced prospective cardiac MSCT, using a weight-based, low-dose, sequential, prospective protocol. See the online-only Data Supplement Appendix.^{16–23} for details of the protocols. Nineteen patients underwent all imaging procedures. MRI was not performed in P18 because of claustrophobia, and MSCT was not performed in P16 because of iodinated contrast agent-related anaphylaxis. Overall vascular lesion scores were calculated by adding up the number of arteries with major lesions. Symmetrical bed lesions were counted as 2 defects, and multiple lesions affecting first-, second- or third-order arteries were counted as a single defect. The overall brain lesion severity was scored by grading the WMHs in both periventricular and subcortical areas and the number of lacunar lesions (see the online-only Data Supplement Appendix).

Carotid Artery Ultrasound and Hemodynamic Measurements

Ultrasound scans were performed to determine the geometric and stiffness parameters of the common carotid arteries in the 21 patients and 21 age-, gender- and arterial blood pressure-matched healthy controls drawn from a reference database. The online-only Data Supplement Appendix²⁴ provides detailed protocols.

Blood Sample Assays

In addition to standard blood analysis, serum from patients with STAT3 deficiency and gender- and age-matched healthy blood donors was assayed for circulating levels of a panel of biomarkers related to inflammation, extracellular matrix remodeling, and endothelial dysfunction. The online-only Data Supplement Appendix details the procedures.

Experimental Models of Aortic Aneurysm and Rupture

We used validated murine models of angiotensin (Ang) II-induced aortic aneurysm and rupture.^{25,26} We treated the mice with either a cell-permeable STAT3 inhibitor that targets the STAT3-SH2 domain and prevents its association with upstream kinases (3 IP injections per week of 25 mg/kg static [Calbiochem]) or a mouse monoclonal antimouse IL-17A antibody (2 IP injections per week of 200 μ g per mouse), as previously described.²⁷ We also studied mice with severe reductions in STAT3-dependent signaling caused by transgenic overexpression of SOCS3 (Tg-SOCS3).^{28,29}

Statistic Analysis

Values were expressed as a percentage or the mean $\pm$ SEM, as appropriate. Statistical tests included the Mann-Whitney U, χ^2 , and Fisher exact tests. Multiple-group comparisons were performed with a general linear model analysis of variance. Kaplan-Meier survival curves were built and analyzed using a log-rank (Mantel-Cox) test. The threshold for statistical significance was set to $P < 0.05$.

Results

Patients

The patients' characteristics are summarized in Table 1. Twelve men and 9 women with a median age of 26 (range, 17 to 44) and a National Institutes of Health score over 40 (mean, 70.8; range, 52 to 86) were enrolled in the study. No acute, invasive infections were noted at the time of the imaging procedures, but we observed skin manifestations (n=4), chronic lower respiratory tract symptoms (n=4),

Table 1. Clinical Characteristics and Genotype Data for the 21 Patients With Signal Transducer and Activator of Transcription 3 (STAT3) Deficiency

Patient	Gender	Country of Origin	Age (y)	STAT3 Mutation	Domain	NIH Score	IgE Level (KU/l)	Previous Infections				Skin Signs			Developmental Signs				
								Skin	Lung	Deep Abscesses	ENT	Neonatal Rash	Dermatitis	Dysmorphia	Retained I Teeth	Bone Fracture	Osteopenia	Hyperextensibility	NHL
P1	M	France	37	V637M/wt	SH2	74	17 046	+	+	+	+	+	+	+	+	+	+	+	
P2	M	France	38	K642E/wt	SH2	66	4599	+	+	+	+	+	+	+	+	+		+	+
P3	F	France	26	V637M/wt	SH2	73	521	+	+	+	+		+	+	+	+		+	
P4	F	French West Indies	20	S668Y/wt	SH2	74	16 112	+	+	+	+	+	+	+	+	+			
P5	M	French West Indies	25	R382W/wt	DNA	84	22 000	+	+	+	+	+	+	+	+	+	+		
P6	M	France	23	R382W/wt	DNA	68	7892	+	+	+	+	+	+	+	+				+
P7	F	Pakistan	38	Vdel463/wt	DNA	79	27 920	+	+	+	+	+	+	+	+	+	+	+	
P8	M	France	30	Vdel463/wt	DNA	64	4703	+	+	+	+		+	+	+	+			
P9	F	France	45	I665N/wt	SH2	65	19 972	+	+	+	+	+	+	+	+	+			+
P10	F	France	22	R382W/wt	DNA	86	8463	+	+	+	+		+	+	+	+			
P11	M	France	19	N472D/wt	DNA	55	3990	+	+	+	+	+	+	+	+	+	+	+	
P12	F	Morocco	19	T714I/wt	TA	78	18 762	+	+	+	+	+	+	+	+	+	+	+	
P13	F	France	24	R382W/wt	DNA	73	4525	+	+	+	+	+	+	+	+	+	+	+	
P14	M	Algeria	18	R382W/wt	DNA	74	14 055	+	+	+	+		+	+	+	+	+		
P15	M	France	29	R382Q/wt	DNA	67	10 174	+	+	+	+		+	+	+	+	+		
P16	M	France	39	F384L/wt	DNA	72	803	+	+	+	+	+	+	+	+	+	+	+	
P17	F	France	28	R382W/wt	DNA	73	15 494	+	+	+	+		+	+	+	+			
P18	F	France	17	T620A/wt	SH2	52	4515	+	+	+	+		+	+	+				
P19	M	France	19	R382W/wt	DNA	37	26 677	+	+		+		+	+	+	+		+	
P20	M	France	19	K709E/wt	TA	77	1209	+	+		+	+	+	+	+	+	+		
P21	M	France	30	I568F/wt	Linker	41	11 228	+			+	+	+	+	+	+	+	+	

Age indicates age at the time of the study; DNA, DNA-binding domain; ENT, ear, nose, and throat infections; F, female; I teeth, primary teeth; M, male; NHL, non-Hodgkin lymphoma; P, patient; SH2, Src Homology 2; TA, transactivator; y, years; +, presence. Normal serum immunoglobulin E level is <150 KU/l.

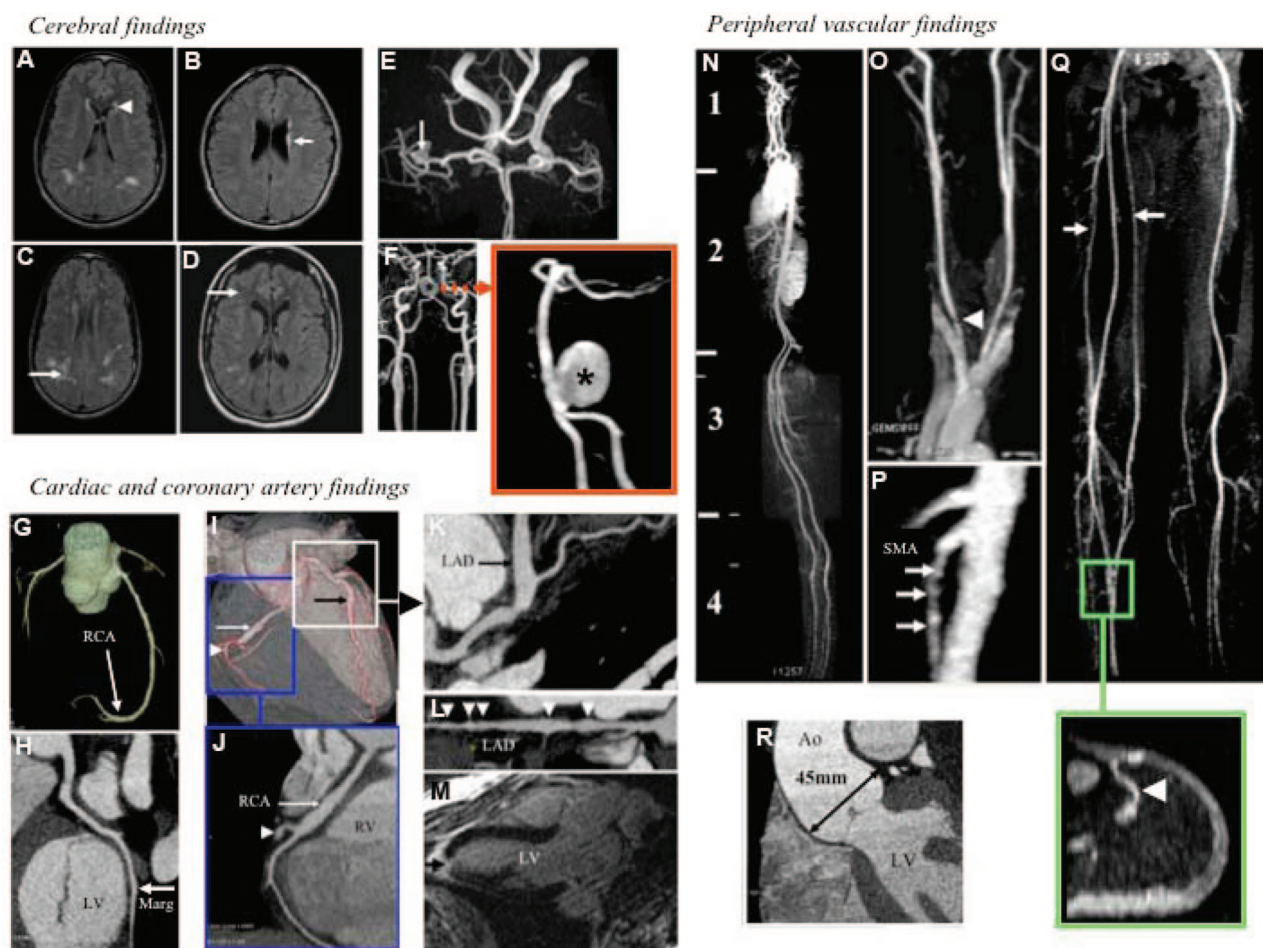


Figure 1. Brain, cardiac, and vascular imaging findings. **A–D** are axial flow-attenuated inversion recovery brain magnetic resonance images. **A** and **B** demonstrate the widespread presence of severe white matter (WM) lesions in P11, including parietal confluent WM hyperintensities (arrow). Note also the left frontal periventricular lacunar lesion (arrowhead). **C** shows a hyperintense periventricular lining (arrow), associated with mild cortical atrophy in P6. **D** demonstrates a moderate form of WM lesion, including numerous subcortical, frontal, punctuate lesions in P2 (arrow). **E** is a 3-dimensional (3D) time-of-flight magnetic resonance angiography image of the cerebral arteries in P2, showing a 7-mm right middle cerebral aneurysm (arrow). **F** is a 3D reconstruction from whole-body magnetic resonance angiography data, showing the head and neck vessels of P16 and demonstrating a 17×19-mm saccular aneurysm (asterisk) of the basilar trunk (more clearly visible in enlarged oblique sagittal view in the red outlined box). **G–L** are cardiac multislice computed tomography images. **G** shows a 3D reconstruction of the coronary arteries of P21, demonstrating distal right coronary artery (RCA) ectasia. Moderate dilation of the first marginal artery is seen in the same patient (**H**, a curved multiplanar image). Multiple coronary artery aneurysms and ectasia were found in P2 (**I**, 3D reconstruction; **J**, **K**, curved multiplanar reconstructions), including ectasia of the RCA (arrow), an aneurysm of the acute marginal branch of the RCA (arrowhead), and ectasia of the middle left anterior descending artery (LAD) (black arrow). A view of the whole left anterior descending lumen (**L**) shows 5 different dilated lesions located in the proximal, middle, and distal left anterior descending artery, whereas apical late gadolinium enhancement is suggestive of focal apical necrosis in P2 (**M**, late gadolinium enhancement-cardiac magnetic resonance, 3-chamber, long-axis view). **N** shows a 3D reconstruction (oblique sagittal view) from 4-step whole-body magnetic resonance angiography and depicting the entire arterial tree in P21. **O** is an enlarged frontal view of the neck vessels of P21, showing common carotid artery stenosis (arrowhead). **P** is an enlarged sagittal view of the abdominal region, showing lumen irregularities in the superior mesenteric artery in the same patient. **Q** shows an early venous return in the right leg in P15 (arrows) that is highly suggestive of arteriovenous fistula. The green outlined box shows an enlarged axial image of the right calf of P15, demonstrating a connection (via an arteriovenous fistula) between the posterior tibial artery and the great saphenous vein. **R** shows dilation of the sinus of Valsalva (measuring 45 mm) in P5 (multislice computed tomography reconstruction).

and chronic mucocutaneous candidiasis ($n=4$). Cardiovascular risk factors included active smoking ($n=5$), hypertension controlled with dihydropyridine therapy ($n=1$), and overweight ($n=4$). Incidental deep venous thrombosis was reported in 3 patients (P7, P16, and P17) and consisted of leg phlebitis ($n=3$; 6 deep vein thrombosis events) and pulmonary embolism ($n=2$; 3 pulmonary embolism events). None of the study patients had experienced or showed cardiac or neurological vascular events, migraine, or cognitive defects.

Central Nervous System Abnormalities

Brain abnormalities (Figure 1, Table 2, online-only Data Supplement Table I) were detected on MRI in 19 of the 20 patients (95%), with WMHs in 18 patients, lacunar lesions suggestive of ischemic infarcts in 3 patients, and atrophy in 11 patients (ventricle dilation/subcortical atrophy in 9 and/or cortical atrophy in 6). Brain vascular abnormalities were observed in 7 patients, including 4 with a cerebral artery aneurysm, 4 with arterial dilations and/or tortuosities, and 2 with a vascular agenesis. The WMHs were

Table 2. Imaging Findings in a Population of 21 Patients With Signal Transducer and Activator of Transcription 3 (STAT3) Deficiency Patients, Investigated With Cardiac MSCT, WB-MRA, Brain MRI, and Late Gadolinium-Enhanced (LGE) CMRI

Pts	Brain Findings			Extracoronary Vascular Findings				Cardiac and Coronary Artery Findings			
	WMH Score	Lacunar Lesion	Cerebral Atrophy	Aneurysm	Ectasia	Stenoses	Other Findings	CA Aneurysm	CA Ectasia	LGE	Other Findings
P1	Moderate	—	+	—	+	—	—	—	+	—	+
P2	Moderate	—	—	+	—	+	+	+	+	+	—
P3	Mild	—	—	—	—	+	+	—	—	—	—
P4		—	—	—	—	—	—	—	—	—	—
P5	Moderate	—	+	+	+	+	+	—	+	—	—
P6	Mild	—	+	—	—	—	—	+	—	—	+
P7	Mild	—	+	+	—	+	+	—	—	—	—
P8	Mild	—	—	+	+	—	+	+	—	+	—
P9	Severe	—	—	—	—	+	+	—	—	—	—
P10	Mild	—	—	—	+	+	+	—	—	—	+
P11	Severe	+	—	—	—	—	+	—	—	—	—
P12		—	—	—	—	—	—	—	—	+	—
P13	Severe	—	+	—	+	—	+	+	—	—	+
P14	Moderate	+	+	—	—	+	+	—	—	—	—
P15	Moderate	—	—	+	—	+	+	+	—	+	—
P16	Mild	—	+	+	+	+	+	No MSCT data		—	—
P17	Severe	—	—	+	—	+	+	—	—	—	—
P18				No MRI data				—	—		—
P19	Moderate	+	—	—	—	—	—	—	—	—	—
P20	Mild	—	—	—	—	+	+	—	—	—	—
P21	Moderate	—	—	—	—	+	—	—	+	—	+

CA indicates coronary artery; CMR, cardiac magnetic resonance imaging; LGE, late gadolinium enhancement; MRI, magnetic resonance imaging; MSCT, multislice computed tomography; P, patient; WB-MRA, whole-body magnetic resonance angiography; —, absence; +, presence.

Other findings refer to vascular lumen irregularities, tortuosities, dissections, or arteriovenous fistulae.

P16 did not undergo MSCT because of iodinated contrast agent-related anaphylaxis, and P18 did not undergo MRI because of claustrophobia.

variously located in the frontal lobes (100% of affected cases), parietal lobes (37.5%), and/or temporal lobes (19%). Seven patients had mild WMH lesions, 7 had moderate lesions, and 4 had severe lesions. In a given patient, the WMH lesions tended to be equally disseminated between the periventricular and subcortical areas (Figure 1A to 1D). In 2 patients, asymptomatic aneurysms of the right middle cerebral artery (in P2) and the basilar trunk (in P16) prompted us to recommend intravascular coil embolization (Figure 1E and 1F). Unfortunately, P16 died from aneurysm rupture 1 month after diagnosis and before the endovascular procedure could be performed.

Cardiac and Arterial Lesions

Cardiac and coronary artery abnormalities (Table 2, online-only Data Supplement Table II) were detected in 10 of the 20 patients. Thirty coronary artery dilations were detected by MSCT in 8 patients, including 7 coronary artery ectasias in 4 patients, 16 coronary artery aneurysms in 5 patients, and moderate coronary artery dilations in 4 patients (Figure 1G to 1L). Only 1 instance of mild stenosis was detected. It is noteworthy that 4 focal areas of late gadolinium enhancement were observed at the apex of the left ventricle in 4 patients by cardiac MRI; this is

highly suggestive of small myocardial infarcts (Figure 1M). In 3 of these cases, MSCT (Figure 1L) detected left main or left anterior descending aneurysms. A 4-step WB-MRA protocol (Figure 1N) detected peripheral vascular abnormalities (Table 2, online-only Data Supplement Table III) in 17 of the 20 patients (85%), with artery ectasia in 6 patients (1 per patient), 9 artery aneurysms in 7 patients (including 4 aneurysms located in the brain), 5 artery stenoses >50% in 4 patients, 9 artery stenoses <50% in 7 patients, 1 dissection, and 1 arteriovenous fistula (Figure 1O to 1R). Minor artery abnormalities were also detected (10 tortuous arteries in 7 patients and 15 artery lumen irregularities in 8 patients). Atherosclerosis in these patients was mild or absent.

Echo-Tracking Findings

No stenoses, plaques, ectasia, or aneurysms were observed in the common carotid arteries; however, the study patients had a markedly thinner intima-media thickness (IMT) and a marginally greater internal carotid diameter, relative to healthy controls (Table 2, Figure 2A). The extent of the reduction in IMT varied from one patient to another but averaged $-86 \mu\text{m}$ (a 17% reduction, $P=0.004$). As a consequence, the wall cross-sectional area was 15% lower

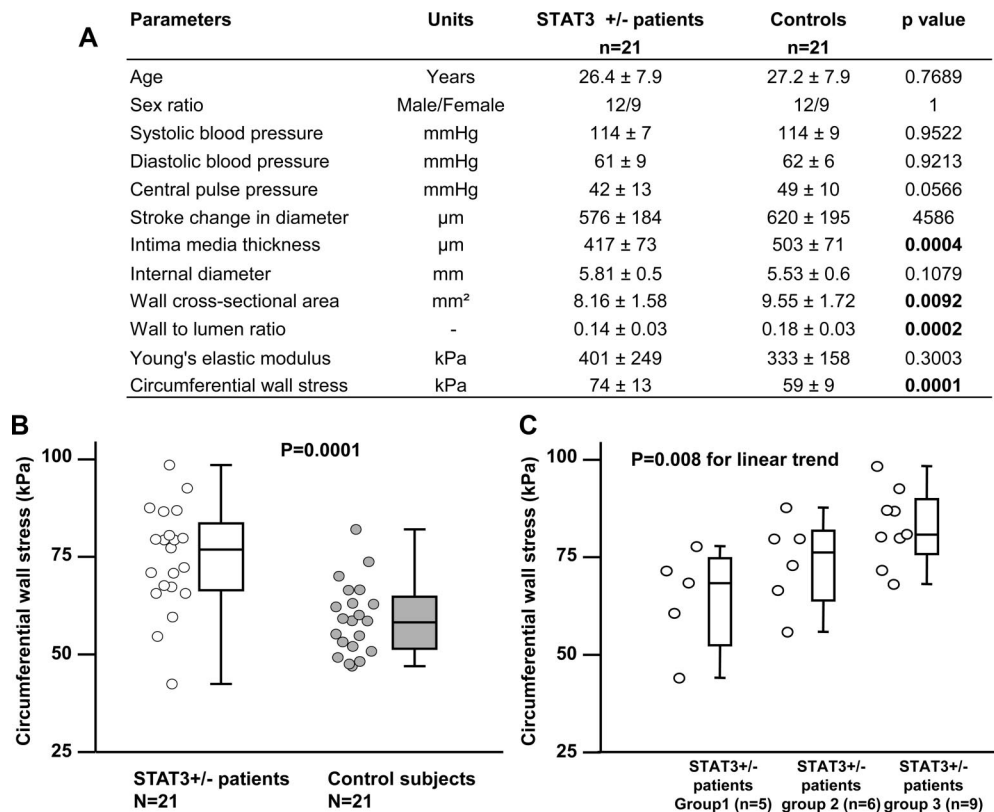


Figure 2. Echo-tracking findings. **A**, Common carotid artery parameters in 21 patients with signal transducer and activator of transcription 3 (STAT3) deficiency and age-, gender- and blood-pressure-matched healthy controls. **B**, Circumferential wall stress values (calculated with the Lamé equation [mean blood pressure $\times$ internal diameter/2 $\times$ intima-media thickness]) in patients with STAT3 deficiency and healthy controls. **C**, Values of circumferential wall stress according to the severity of arterial lesions detected in magnetic resonance angiography and multislice computer tomography scans and the severity of central nervous system lesions (white matter hyperintensities and silent brain infarctions). The scoring system was as follows: group 1 patients with STAT3 deficiency are those with moderate brain and vascular abnormalities; group 2 patients with STAT3 deficiency are those with moderate brain abnormalities and severe vascular abnormalities; group 3 patients with STAT3 deficiency are those with severe brain and vascular abnormalities. Median scores were used as cut-off values. It is noteworthy that none of the patients was in the upper category for central nervous system lesions and the lower category for vascular lesions.

in patients with STAT3 deficiency than in controls (8.1 ± 1.6 versus 9.6 ± 1.7 mm², respectively; $P=0.0092$), and the wall-to-lumen ratio was 21% lower ($P=0.002$). Because arterial blood pressure did not differ significantly when comparing patients and controls, circumferential wall stress was significantly ($P=0.001$) higher (by 26%) in patients than in control subjects (Figure 2B). Patients and controls did not differ significantly in terms of central blood pressure parameters (pulse pressure and augmentation index), carotid stiffness indexes, and aortic pulse wave velocity (Figure 2A). No significant association between carotid IMT and markers of disease severity (ie, the National Institutes of Health score) was found. Circumferential wall stress level was significantly and positively associated with (1) the number of affected arterial territories identified on imaging (increase for each additional affected artery territory: 2.5 kPa, 95% CI [0.2 to 4.8], $P=0.03$) and (2) the severity of cerebral lesions ($P=0.04$) (online-only Data Supplement Figure I). The total R^2 was 0.42. Circumferential wall stress rose in a stepwise manner with both vascular and cerebral lesion severity (Figure 2C, $P=0.008$).

In summary, it was found that virtually all patients had vascular abnormalities associated with WMH. These lesions

were mostly arterial dilations that often led to ectasia and aneurysms in both the coronary arteries and the periphery. The presence of abnormalities correlated with the observation of higher circumferential wall stress and a thinner IMT, as determined by echo-tracking.

Laboratory Test Data

The patients with STAT3-deficiency had normal standard blood parameters, with the exception of hypereosinophilia ($n=13$), hyper- α -2 globulinemia ($n=12$), and elevated fibrinogen ($n=4$). Serum lipid, glucose, and uric acid levels were in the normal range. Patients and controls did not significantly differ in terms of circulating levels of inflammatory cytokines. Levels of soluble vascular cell adhesion molecule-1 and of matrix remodeling markers (matrix metalloproteinase-1, tissue inhibitor of metalloproteinase-1 and plasminogen activator inhibitor-1) were higher in patients than in controls, with the exception of lower levels of matrix metalloproteinase-3 (online-only Data Supplement Table IV). Circulating levels of plasminogen activator inhibitor-1 and IL-1 α were inversely correlated with carotid IMT in female patients with STAT3 deficiency ($r=-0.73$, $P=0.045$).

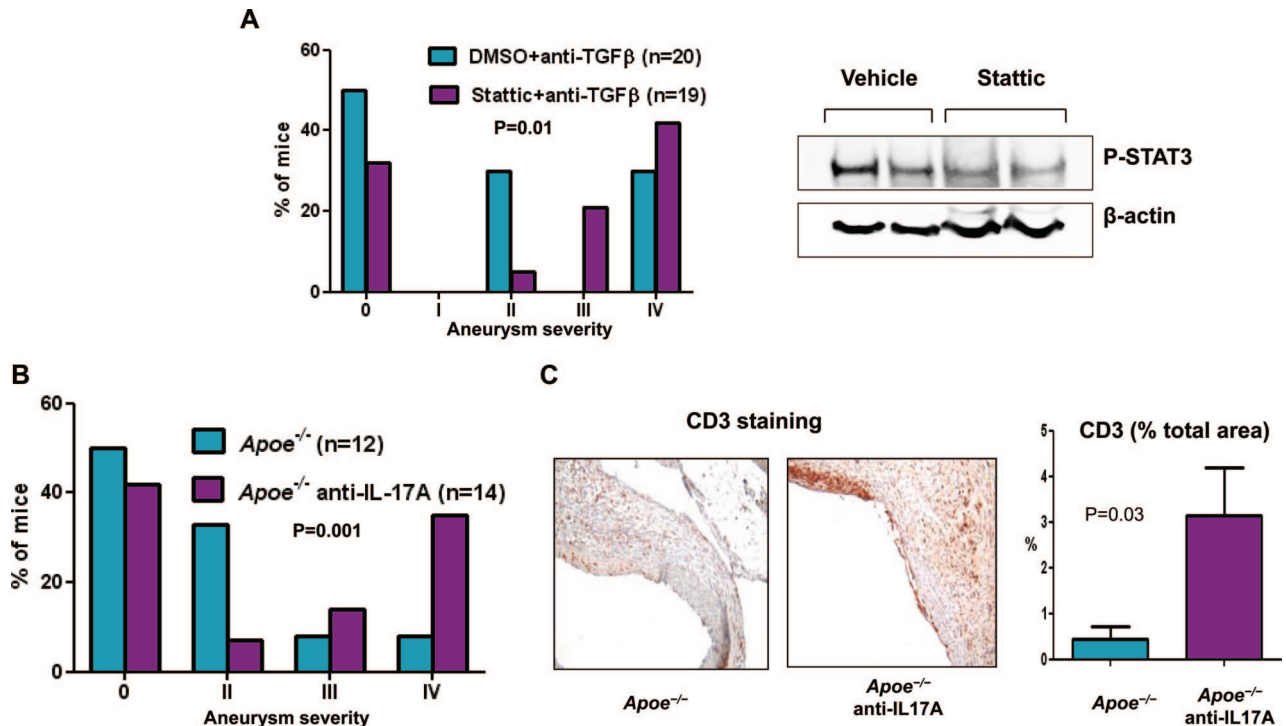


Figure 3. Signal transducer and activator of transcription 3 (STAT3) inhibition and IL-17 neutralization each increase the occurrence and severity of aneurysms in mice treated with angiotensin II. **A**, The left-hand panel depicts increasing levels of aortic aneurysm severity (from 0, no aneurysms, to IV, aneurysm rupture; see the Methods section) in C57Bl/6 mice treated with either a specific STAT3 inhibitor (stattic) or vehicle (dimethyl sulfoxide) in the angiotensin II + anti-TGF- β model of aneurysm formation. **B**, The right-hand panel shows a reduction in STAT3 phosphorylation in splenocytes from mice treated with stattic (versus dimethyl sulfoxide). **C**, Levels of aortic aneurysm severity in male APOE^{-/-} mice treated with either a neutralizing anti-IL17A or a control antibody in the angiotensin II model of aneurysm formation. The right-hand panels evidence the increased vascular infiltration of CD3⁺ T cells following the inhibition of IL17.

STAT3 Signaling and Susceptibility to Vascular Aneurysm in Mouse Models

In vivo inhibition of STAT3 with a small molecule (stattic) that targets the SH2 domain led to a significant ($P=0.01$) increase in the severity of abdominal aortic aneurysms in a validated Ang II- and TGF- β -dependent C57Bl/6 mouse model²⁶ when compared with treatment with vehicle (Figure 3A). This finding suggests that STAT3 signaling has a critical role in the maintenance of vessel integrity. Accordingly, transgenic SOCS3-overexpressing mice that exhibit reduced STAT3 phosphorylation had more severe aneurysms and more fatal vessel ruptures than C57Bl/6 wild-type mice ($P<0.0001$) in the same Ang II-infusion model²⁶ (online-only Data Supplement Figure II). Because impaired Th17 differentiation is a hallmark of STAT3 deficiency,^{4,6,7} we also addressed the role of IL-17A in aneurysm formation. Administration of a neutralizing antimouse IL-17A antibody significantly increased aneurysm severity in response to Ang II infusion, compared with mice treated with control immunoglobulin G ($P=0.001$). Following IL-17A neutralization, we observed more intense vascular inflammation and greater infiltration of CD3⁺ T lymphocytes; this observation further supports a vascular protective role for IL-17 signaling (Figure 3B).

Discussion

The present report describes the systematic, whole-body screening of patients with STAT3 deficiency for vascular

abnormalities. We also sought to identify a mechanistic relationship between this type of abnormality and alterations in STAT3-dependent signaling. Importantly, we found that adult patients with STAT3 deficiency frequently exhibit brain hyperintensities (suggestive of small-vessel alterations) and have an abnormally high prevalence of medium- and large-artery dilations throughout the vascular tree. Analysis of the mechanical properties of the common carotid arteries revealed hypotrophic remodeling of the carotid artery wall, which is consistent with defective mechanotransduction and susceptibility to aneurysm formation.

Brain abnormalities have already been observed in 36 AD-HIES patients for whom STAT3 mutation status data were not reported.^{1,8} The WMHs varied greatly in severity and ranged from isolated, punctuate spots to multifocal, confluent lesions, with a periventricular distribution in many cases, as seen in other WM diseases and notably leukoaraiosis.³⁰ Leukoaraiosis is observed in elderly subjects and is considered to be a small-vessel disease resulting from a widespread, chronic brain hypoperfusion, with disruption of the blood-brain barrier. The fact that small lacunae were also observed further evokes a primary vascular origin. Thus, the hypothesis of an inflammatory or immune disease of the central nervous system appears to be less probable. Moreover, it is striking to note the absence of vascular risk factors in the present cohort of

patients with STAT3 deficiency (with the exception of smoking in 25% of the group).^{21,30}

We also identified vascular ectasia, aneurysms, irregularities, and stenoses in brain, peripheral, and coronary arteries. Coronary artery abnormalities (notably aneurysms) have been previously reported in patients with AD-HIES, including a recently studied group of 26 STAT3-deficient subjects.^{9,10,12,13} Cerebral vascular events (stroke, retinal occlusion, and internal carotid aneurysm) were first reported in 3 patients with AD-HIES,¹ and 5 other cases with lacunar infarction were subsequently identified.⁸ In the present cohort (in which all the patients had genetically confirmed STAT3 deficiency), it is noteworthy that all anatomic areas appear to be potentially affected, with a trend toward more damage in small- and medium-diameter vessels. These findings prompt us to conclude that patients with STAT3 deficiency are prone to developing a systemic, vascular disease, regardless of the presence or absence of vascular risk factors and, apparently, infectious complications; however, a potential infectious etiology should always be screened for, since 3 cases of mycotic aneurysm have been reported.^{8,31,32}

The results of common carotid artery hemodynamic measurements revealed an abnormally low IMT and a marked greater circumferential wall stress in patients with STAT3 deficiency; these findings suggest a defect in tensile stress mechanotransduction and a subsequent propensity for arterial dilation and tortuosity in many arterial sites. These data are reminiscent of the inherited Ehlers-Danlos vascular-type syndrome,^{23,33} in which hyperextensibility with scoliosis and other musculoskeletal disorders are major features, a strikingly similar situation to STAT3-deficiency. The vascular abnormalities observed here in patients with STAT3 deficiency may, therefore, result from a systemic connective tissue disorder that includes arterial fragility. The correlation between greater circumferential wall stress and (1) the extent of peripheral arterial lesions and (2) the severity of brain lesions suggests a causal link in the occurrence of vascular lesions such as ectasia and aneurysm. Lastly, this situation contrasts with the greater IMT observed in vascular diseases associated with infections or atheroma.^{34–37}

The animal model studies reported here strongly suggest that alterations in STAT3-dependent signaling are directly involved in the vascular phenotype of patients with STAT3 deficiency. Although impaired IL-17 production by T cells is the most important consequence of defective STAT3-dependent transcription in patients, the specific role of IL-17 remains to be clearly defined. The murine data evidenced the aggravation of aneurysm formation following neutralization of IL-17A and thus suggest that Th17 deficiency has an important role in mediating the vascular phenotype; however, other IL-17-unrelated STAT3-dependent pathways may also be involved. Further studies based on a mouse model with dominant-negative mutations of STAT3 should be able to confirm or negate this hypothesis and help identify the specific cell-type(s) responsible for the vascular phenotype.

The frequency and severity of vascular abnormalities associated with STAT3 deficiency observed here have clinical implications and likely justify the development of screening, surveillance, and therapeutic strategies. Two cohort members were found to require endovascular treatment for a brain aneurysm; unfortunately, 1 died before the procedure. Moreover, STAT3 deficiency appears as an emerging, hereditary cause of brain small-vessel disease whose long-term repercussions remain to be determined. In the literature, WM lesions have been associated with subtle neurological deficits (memory decline, cognitive disability, and depressive symptoms) that may initially go unnoticed by both patients and physicians.^{21,38,39} Systematic screening for memory and cognitive dysfunctions could thus be offered with a view to define their prognostic significance. Despite the absence of any evidence for effective, preventive strategies, there may be a case for primary prophylaxis of WMH progression, ischemic strokes, vascular leukoencephalopathy, and aneurysm rupture by evicting vascular risk factors and initiating antithrombotic and antihypertensive treatments, if needed. On the basis of the murine data, the use of an Ang II receptor antagonist could legitimately be investigated. This type of preventive measure will have to be carefully evaluated, given the risk of internal hemorrhage associated with the use of antithrombotic agents.⁴⁰

In conclusion, adult patients with a STAT3 deficiency were found to have a high prevalence of vascular abnormalities throughout the arterial tree. These abnormalities were characterized by hypotrophic remodeling of the artery wall, increased circumferential stress, and enhanced susceptibility to dilation and aneurysm formation. Some of these aneurysms affect heart and brain vessels and might incur a high vascular risk. Animal model studies strongly suggest a direct role for STAT3-dependent signaling in the vascular phenotype and should facilitate the identification of specific molecular targets for disease modulation.

Acknowledgments

We thank the patients and their families for their trust and cooperation. We also thank members of INSERM U768, GHMI-INSERM U980, and the Immunohematology Unit at Necker Hospital for helpful discussions. We thank Dr Fanny Lanternier, MD; Dr Caroline Charlier, MD, PhD; and Dr Blandine Denis, MD, from the department of infectious diseases and Tropical Medicine at Necker Hospital. We thank Dr Isabelle Melki, MD, for clinical help with describing the patients. We thank Corinne Jacques, AS; Chantal Harre, AS; Stéphanie N'daga, AS; and Aminata Diabate, AS; for excellent technical assistance. We thank Dr Nizar Mahlaoui, MD, MSc, for maintenance of the CEREDIH database.

Sources of Funding

Institut National de la Santé et de la Recherche Médicale (INSERM) unit U768 (Alain Fischer) received INSERM core funding and an advanced European Research Council grant. The Laboratory of Human Genetics of Infectious Diseases received funding from the March of Dimes, the Dana Foundation, and INSERM. Ziad Mallat received a fellowship from the French National Research Agency (Agence Nationale de la Recherche).

References

1. Grimbacher B, Holland SM, Gallin JI, Greenberg F, Hill SC, Malech HL, Miller JA, O'Connell AC, Puck JM. Hyper-IgE syndrome with recurrent infections — an autosomal dominant multisystem disorder. *N Engl J Med*. 1999;340:692–702.
2. Minegishi Y, Saito M, Tsuchiya S, Tsuge I, Takada H, Hara T, Kawamura N, Ariga T, Pasic S, Stojkovic O, Metin A, Karasuyama H. Dominant-negative mutations in the DNA-binding domain of STAT3 cause hyper-IgE syndrome. *Nature*. 2007;448:1058–1062.
3. Holland SM, DeLeo FR, Elloumi HZ, Hsu AP, Uzel G, Brodsky N, Freeman AF, Demidowich A, Davis J, Turner ML, Anderson VL, Darnell DN, Welch PA, Kuhns DB, Frucht DM, Malech HL, Gallin JI, Kobayashi SD, Whitney AR, Voyich JM, Musser JM, Woellner C, Schäffer AA, Puck JM, Grimbacher B. STAT3 mutations in the hyper-IgE syndrome. *N Engl J Med*. 2007;357:1608–1619.
4. Ma CS, Chew GY, Simpson N, Priyadarshi A, Wong M, Grimbacher B, Fulcher DA, Tangye SG, Cook MC. Deficiency of Th17 cells in hyper IgE syndrome due to mutations in STAT3. *J Exp Med*. 2008;205:1551–1557.
5. Minegishi Y, Saito M, Nagasawa M, Takada H, Hara T, Tsuchiya S, Agematsu K, Yamada M, Kawamura N, Ariga T, Tsuge I, Karasuyama H. Molecular explanation for the contradiction between systemic Th17 defect and localized bacterial infection in hyper-IgE syndrome. *J Exp Med*. 2009;206:1291–1301.
6. Milner JD, Brechley JM, Laurence A, Freeman AF, Hill BJ, Elias KM, Kanno Y, Spalding C, Elloumi HZ, Paulson ML, Davis J, Hsu A, Asher AI, O'Shea J, Holland SM, Paul WE, Douek DC. Impaired T(H)17 cell differentiation in subjects with autosomal dominant hyper-IgE syndrome. *Nature*. 2008;452:773–776.
7. de Beaucoudrey L, Puel A, Filipe-Santos O, Cobat A, Ghandil P, Chrabieh M, Feinberg J, von Bernuth H, Samarina A, Jannié L, Fieschi C, Stéphan JL, Boileau C, Lyonnet S, Jondeau G, Cormier-Daire V, Le Merrer M, Hoarau C, Lebranchu Y, Lortholary O, Chandesris MO, Tron F, Gambineri E, Bianchi L, Rodriguez-Gallego C, Zitnik SE, Vasconcelos J, Guedes M, Vitor AB, Marodi L, Chapel H, Reid B, Roifman C, Nadal D, Reichenbach J, Caragol I, Garty BZ, Dogu F, Camcioglu Y, Güle S, Sanal O, Fischer A, Abel L, Stockinger B, Picard C, Casanova JL. Mutations in STAT3 and IL12RB1 impair the development of human IL-17-producing T cells. *J Exp Med*. 2008;205:1543–1550.
8. Freeman AF, Collura-Burke CJ, Patronas NJ, Ilcus LS, Darnell D, Davis J, Puck JM, Holland SM. Brain abnormalities in patients with hyperimmunoglobulin E syndrome. *Pediatrics*. 2007;119:e1121–e1125.
9. Ling JC, Freeman AF, Gharib AM, Arai AE, Lederman RJ, Rosing DR, Holland SM. Coronary artery aneurysms in patients with hyper IgE recurrent infection syndrome. *Clin Immunol*. 2007;122:255–258.
10. Young TY, Jerome D, Gupta S. Hyperimmunoglobulinemia E syndrome associated with coronary artery aneurysms: deficiency of central memory CD4+ T cells and expansion of effector memory CD4+ T cells. *Ann Allergy Asthma Immunol*. 2007;98:389–392.
11. Alomar-Melero E, Martin TD, Janelle GM, Peng YG. An unusual giant right coronary artery aneurysm resembles an intracardiac mass. *Anesth Analg*. 2008;107:1161–1162.
12. Gharib AM, Pettigrew RI, Elagha A, Hsu A, Welch P, Holland SM, Freeman AF. Coronary abnormalities in hyper-IgE recurrent infection syndrome: depiction at coronary MDCT angiography. *AJR Am J Roentgenol*. 2009;193:W478–W481.
13. Freeman AF, Avila EM, Shaw PA, Davis J, Hsu AP, Welch P, Matta JR, Hadigan C, Pettigrew RI, Holland SM, Gharib AM. Coronary artery abnormalities in Hyper-IgE syndrome. *J Clin Immunol*. 2011;31:338–345.
14. Lehrke S, Egenlauf B, Steen H, Lossnitzer D, Korosoglou G, Merten C, Ivandic BT, Giannitsis E, Katus HA. Prediction of coronary artery disease by a systemic atherosclerosis score index derived from whole-body MR angiography. *J Cardiovasc Magn Reson*. 2009;11:36.
15. Grimbacher B, Schäffer A, Holland SM, Davis J, Gallin JI, Malech HL, Atkinson TP, Belohradsky BH, Buckley RH, Cossu F, Español T, Garty BZ, Matamoros N, Myers LA, Nelson RP, Ochs HD, Renner ED, Wellinghausen N, Puck JM. Genetic linkage of hyper-IgE syndrome to chromosome 4. *Am J Hum Genet*. 1999;65:735–744.
16. Fazekas F, Chawluk JB, Alavi A, Hurtig HI, Zimmerman RA. MR signal abnormalities at 1.5 T in Alzheimer's dementia and normal aging. *AJR Am J Roentgenol*. 1987;149:351–356.
17. Austen WG, Edwards JE, Frye RL, Gensini GG, Gott VL, Griffith LS, McGoon DC, Murphy ML, Roe BB. A reporting system on patients evaluated for coronary artery disease. Report of the Ad Hoc Committee for Grading of Coronary Artery Disease, Council on Cardiovascular Surgery, American Heart Association. *Circulation*. 1975;51:5–40.
18. Díaz-Zamudio M, Bacilio-Pérez U, Herrera-Zarza MC, Meave-González A, Alexanderson-Rosas E, Zambrana-Balta GF, Kimura-Hayama ET. Coronary artery aneurysms and ectasia: role of coronary CT angiography. *Radiographics*. 2009;29:1939–1954.
19. Satran A, Bart BA, Henry CR, Murad MB, Talukdar S, Satran D, Henry TD. Increased prevalence of coronary artery aneurysms among cocaine users. *Circulation*. 2005;111:2424–2429.
20. Pahlavan PS, Niroomand F. Coronary artery aneurysm: a review. *Clin Cardiol*. 2006;29:439–443.
21. de Groot JC, de Leeuw FE, Oudkerk M, van Gijn J, Hofman A, Jolles J, Breteler MM. Cerebral white matter lesions and cognitive function: the Rotterdam Scan Study. *Ann Neurol*. 2000;47:145–151.
22. Kim KW, MacFall JR, Payne ME. Classification of white matter lesions on magnetic resonance imaging in elderly persons. *Biol Psychiatry*. 2008;64:273–280.
23. Yan AT, Shayne AJ, Brown KA, Gupta SN, Chan CW, Luu TM, Di Carli MF, Reynolds HG, Stevenson WG, Kwong RY. Characterization of the peri-infarct zone by contrast-enhanced cardiac magnetic resonance imaging is a powerful predictor of post-myocardial infarction mortality. *Circulation*. 2006;114:32–39.
24. Ong KT, Perdu J, De Backer J, Bozec E, Collignon P, Emmerich J, Faure AL, Fiessinger JN, Germain DP, Georgesco G, Hulot JS, De Paep A, Plauchu H, Jeunemaitre X, Laurent S, Boutouyrie P. Effect of celioprolol on prevention of cardiovascular events in vascular Ehlers-Danlos syndrome: a prospective randomised, open, blinded-endpoints trial. *Lancet*. 2010;376:1476–1484.
25. Daugherty A, Manning MW, Cassis LA. Angiotensin II promotes atherosclerotic lesions and aneurysms in apolipoprotein E-deficient mice. *J Clin Invest*. 2000;105:1605–1612.
26. Wang Y, Ait-Oufella H, Herbin O, Bonnin P, Ramkhalawon B, Taleb S, Huang J, Offenstadt G, Combadière C, Rénia L, Johnson JL, Tharaux PL, Tedgui A, Mallat Z. TGF-beta activity protects against inflammatory aortic aneurysm progression and complications in angiotensin II-infused mice. *J Clin Invest*. 2010;120:422–432.
27. Taleb S, Romain M, Ramkhalawon B, Uyttenhove C, Pasterkamp G, Herbin O, Esposito B, Perez N, Yasukawa H, Van Snick J, Yoshimura A, Tedgui A, Mallat Z. Loss of SOCS3 expression in T cells reveals a regulatory role for interleukin-17 in atherosclerosis. *J Exp Med*. 2009;206:2067–2077.
28. Seki Y, Inoue H, Nagata N, Hayashi K, Fukuyama S, Matsumoto K, Komine O, Hamano S, Himeno K, Inagaki-Ohara K, Cacialano N, O'Garra A, Oshida T, Saito H, Johnston JA, Yoshimura A, Kubo M. SOCS-3 regulates onset and maintenance of T(H)2-mediated allergic responses. *Nat Med*. 2003;9:1047–1054.
29. Tanaka K, Ichihama K, Hashimoto M, Yoshida H, Takimoto T, Takaesu G, Torisu T, Hanada T, Yasukawa H, Fukuyama S, Inoue H, Nakanishi Y, Kobayashi T, Yoshimura A. Loss of suppressor of cytokine signaling 1 in helper T cells leads to defective Th17 differentiation by enhancing antagonistic effects of IFN-gamma on STAT3 and Smads. *J Immunol*. 2008;180:3746–3756.
30. Van der Knaap MS, Valk J. *Magnetic Resonance of Myelination and Myelin Disorders*. Third Ed. New York, NY: Springer; 2005:759–766.
31. Connolly B, Manson D, Khattak S, Burrows P. Bronchial artery aneurysm in hyperimmunoglobulinemia E syndrome. *Pediatr Radiol*. 1994;24:592–593.
32. el Noor IB, Venugopalan P, Johnston WJ, Froude JR. Ventricular aneurysm and myocarditis in a child with the hyperimmunoglobulin E syndrome. *Eur Heart J*. 1995;16:714–715.
33. Boutouyrie P, Germain DP, Fiessinger JN, Laloux B, Perdu J, Laurent S. Increased carotid wall stress in vascular Ehlers-Danlos syndrome. *Circulation*. 2004;109:1530–1535.
34. Cao JJ, Arnold AM, Manolio TA, Polak JF, Psaty BM, Hirsch CH, Kuller LH, Cushman M. Association of carotid artery intima-media thickness, plaques, and C-reactive protein with future cardiovascular disease and all-cause mortality: the Cardiovascular Health Study. *Circulation*. 2007;116:32–38.
35. Kumeda Y, Inaba M, Goto H, Nagata M, Henmi Y, Furumitsu Y, Ishimura E, Inui K, Yutani Y, Miki T, Shoji T, Nishizawa Y. Increased thickness of the arterial intima-media detected by ultrasonography in

- patients with rheumatoid arthritis. *Arthritis Rheum*. 2002;46:1489–1497.
36. Seth S, Goyal NK, Jagia P, Gulati G, Karthikeyan G, Sharma S, Talwar KK. Carotid intima-medial thickness as a marker of disease activity in Takayasu's arteritis. *Int J Cardiol*. 2006;108:385–390.
 37. Cheung YF, Wong SJ, Ho MH. Relationship between carotid intima-media thickness and arterial stiffness in children after Kawasaki disease. *Arch Dis Child*. 2007;92:43–47.
 38. Vermeer SE, Longstreth WT Jr, Koudstaal PJ. Silent brain infarcts: a systematic review. *Lancet Neurol*. 2007;6:611–619.
 39. DeBette S, Markus HS. The clinical importance of white matter hyperintensities on brain magnetic resonance imaging: systematic review and meta-analysis. *BMJ*. 2010;341:c3666.
 40. Freeman AF, Kleiner DE, Nadiminti H, Davis J, Quezado M, Anderson V, Puck JM, Holland SM. Causes of death in hyper-IgE syndrome. *J Allergy Clin Immunol*. 2007;119:1234–1240.

CLINICAL PERSPECTIVE

STAT3 deficiency is a rare autosomal dominant primary immune deficiency in which vascular complications have been reported. We performed a systematic, prospective assessment of the entire vascular tree in 21 adult patients with STAT3 deficiency by using whole-body magnetic resonance imaging angiography, coronary multislice computed tomography, and echo-tracking-based imaging of the carotid arteries. We found that vascular abnormalities involved arteries of all sizes and were extremely frequent and widespread, with lesions of the peripheral and brain arteries detected in more than 80% of cases and lesions of the coronary arteries seen in 50% of cases. Most of the lesions were vascular dilations (ectasia and aneurysm), and some were immediately life-threatening, as illustrated by the death of a 35-year-old patient from intracranial bleeding (caused by the rupture of a giant basilar artery aneurysm before the scheduled endovascular procedure). In accordance with these structural abnormalities, we report the occurrence of hypotrophic arterial remodeling in this population, as demonstrated by a marked decrease in the carotid intima-media thickness and a substantial increase in circumferential wall stress. Lastly, we present data from murine models showing that inhibition of STAT3-dependent signaling significantly increases the susceptibility to vascular aneurysm and fatal rupture. In conclusion, our data strongly suggest that STAT3 deficiency in humans is mechanistically linked to the vascular phenotype. We consider that patients with STAT3 deficiency should be closely monitored for their susceptibility to aneurysm formation and rupture and, accordingly, should receive appropriate preventive care.

SUPPLEMENTAL MATERIAL

Frequent and widespread vascular abnormalities in human STAT3 deficiency.

Supplementary imaging methods:

Magnetic resonance imaging:

P18 did not undergo MRI because of claustrophobia. Magnetic resonance imaging was performed on a 1.5 T scanner system (Excite HDx, General Electric Medical Systems, Waukesha, WI). Brain imaging was first achieved using a neurovascular coil, by acquiring a single series of axial fluid-attenuated inversion recovery (FLAIR) images, followed by an axial multi-slab three-dimensional time-of-flight (3D-TOF) non-enhanced intracranial MR angiography. WB-MRA was then performed in four steps by using a neurovascular coil for station 1 (head and neck) and a body coil for stations 2 (chest and abdomen), 3 (pelvis and thighs) and 4 (calves) (**Figure 1N**) during administration of a total dose of 0.2 mmol/kg of gadoterate meglumine (Dotarem® from Guerbet, Villepinte, France). The neurovascular coil was then replaced with a cardiac-phased array coil to screen for small, asymptomatic myocardial infarcts (as previously described [16]) by using conventional 2D late gadolinium enhanced imaging (LGE) 10 minutes after the initial gadolinium bolus injection.

Cardiac multislice computed tomography (MSCT) imaging:

P16 did not undergo MSCT because of iodinated contrast agent-related anaphylaxis. All cardiac MSCT scans were performed on a 64-row system (LightSpeed VCT, GE Healthcare, Milwaukee, Wisconsin, USA) during intravenous (IV) injection of 1.5 mL/kg of 350 mg iodine per mL contrast medium (iobitridol from Guerbet, France) at a flow rate of

5 mL/s. A low X-ray dose sequential protocol was applied by using prospectively electrocardiography-gated acquisition of 0.625 mm-thick slices. This led to a mean radiation exposure of 159 mGy.cm dose-length product (range: 97-272), corresponding to an effective dose of 2.7 mSv (range: 1.6-4.6). To decrease the heart rate, all patients but P12 (an asthma sufferer) received an IV injection of 5 mg of atenolol 10 minutes before initiation of the MSCT scan.

All original and post-processed MSCT and MRA images were analyzed on a dedicated workstation. In cases of vessel dilation, we estimated the maximum cross-sectional diameter of the lesion and that of the adjacent normal segment. All potential lesions were considered in a consensus analysis by at least two radiologists with expertise in cardiovascular and brain imaging (AA, NB, EM). Peripheral and coronary arteries were divided into 58 and 17 segments, respectively [17]. An arterial dilation exceeding 1.5 times the diameter of a normal arterial segment was classified as an aneurysm if it involved less than 50% of the total length of the segment and as an artery ectasia if it involved more than 50% of the total length of the segment. When the entire length of a peripheral artery was dilated, it was classified as an artery ectasia when the maximum diameter of the artery exceeded the diameter of the contralateral artery by a factor of 1.5 [18-20]. Furthermore, moderate focal artery dilation, abnormal arterial tortuosity and lumen irregularities were considered in cases of dilation of between 1.0 and 1.5 times the adjacent artery segment. Arterial stenosis was qualified as a reduction of more than 50% or less than 50% in the lumen diameter. Brain MR images were classified in a consensus analysis by two experts (AA, NB). As previously reported [16,21,22], WMHs were rated visually on separately axial FLAIR images for periventricular and subcortical areas and graded as mild, moderate and severe as a function of their size (i.e. punctuate versus confluent) and number. Cortical atrophy, ventricular dilation and lacunar lesions were also

identified. In a consensus analysis by two experts (AA, EM), late gadolinium enhancement (LGE) on CMRI was defined as the presence of myocardial hyper-intensity in two different cardiac views [23].

Supplementary methods for carotid artery ultrasound and hemodynamic measurements:

Measurements were performed in a dedicated, air-conditioned room. Blood pressure was measured using a Colin oscillometric device (Press-Mate 8800 from Omron, Rosny, France). Central pulse pressure (cPP) was measured using a Sphygmocor device (Atcor Medical, Sydney, Australia). The right common carotid artery was measured with a high-precision echotracking device (the Artlab system from Esaote PIE Medical, Maastricht, Netherlands), as previously described [24]. The following parameters were measured or calculated: intima media thickness (IMT), diameter (internal (Di) and external (De)), wall to lumen ratio ($2IMT/Di$), distensibility ($Dist=dD/Di*cPP$), elastic modulus ($Einc=[3(1+Di^2/(De^2-Di^2))]/Dist$) and circumferential wall stress ($CWS=Di*MBP/2*IMT$).

Supplementary methods for biomarker analysis:

Total IgE, glucose, lipid and uric acid levels, blood cell counts, hemostatic and inflammatory (serum protein electrophoresis, fibrinogen, C-reactive protein) parameters were measured in all patients. Furthermore, serum from STAT3-deficient patients and from gender- and age-matched healthy blood donors was sampled in order to assess the circulating levels of a panel of biomarkers related to inflammation, extracellular matrix remodeling and endothelial dysfunction (high-sensitivity C-reactive protein (hsCRP), IL-1 α , IL-1 β , IL-4, IL-5, IL-6, IL-9, IL-10, IL-12p70, IL-13, IL-18, IL-22, IL-23, RANTES (regulated upon activation, normal T-cell expressed, and secreted), tumor necrosis factor (TNF) - α , TNF- β ,

matrix metalloproteinase (MMP) -1, MMP-2, MMP-3, MMP-8, MMP-9, tissue inhibitor of MMP-1 (TIMP-1), plasminogen activator inhibitor-1 (PAI-1), soluble vascular cell adhesion molecule-1 (sVCAM-1), using FlowCytomix technology (Bender MedSystems), Fluorokine® MultiAnalyte Profiling kits (R&D Systems for MMP-1, MMP-2, MMP-3, MMP-8), an immunonephelometric assay for hsCRP (Siemens) and/or high-sensitivity ELISA assays (R & D Systems for sVCAM-1).

Supplementary Table 1: Brain abnormality screening results in a cohort of 20 STAT3-deficient patients explored with brain MRI.

Patient	White matter hyperintensities		Lacunar lesion	Cortical atrophy	Ventricle dilation	Brain lesion score
	Periventricular	Subcortical				
P1	++ / Linear / Frontal	++ / Punctate / Frontal	-	Present	Present	Moderate
P2	++ / Punctate / Frontal & Parietal	++ / Punctate & Confluent / Frontal & Parietal	-	-	Present	Moderate
P3	-	+ / Punctate & Confluent / Frontal	-	-	Present	Mild
P4	-	-	-	-	Present	Mild
P5	++ / Punctate / Frontal & Parietal	++ / Punctate / Frontal & Parietal	-	Present	Present	Moderate
P6	+ / Linear & Confluent / Frontal & Parietal	++ / Punctate / Frontal	-	Present	-	Mild
P7	+ / Punctiform / Frontal & Parietal	++ / Punctate / Frontal	-	Present	-	Moderate
P8	+ / Punctiform / Frontal	-	-	-	-	Mild
P9	+++ / Confluent / Frontal & Parietal	+++ / Confluent / Frontal & Parietal	-	-	-	Severe
P10	-	+ / Punctate / Frontal & Temporal	-	-	-	Mild
P11	+++ / Confluent / Frontal	+++ / Confluent / Frontal & Parietal	1 lesion / Periventricular / Frontal	-	-	Severe
P12	-	-	-	-	-	Absence
P13	+ / Punctiform / Frontal	+++ / Punctate & Confluent / Frontal & Parietal	-	Present	Present	Severe
P14	++ / Linear / Frontal	++ / Punctate / Frontal	1 lesion / Cortical / Frontal	Present	Present	Severe
P15	++ / Linear, Frontal	++ / Punctate & Confluent / Frontal	-	-	Present	Moderate
P16	+ / Punctate / Frontal	+ / Punctate / Frontal	-	-	Present	Mild
P17	++ / Punctiform / Frontal	+++ / Punctate / Frontal & Temporal	-	-	-	Severe
P19	++ / Linear / Frontal	++ / Punctate / Frontal	1 lesion / Cortical / Frontal	-	-	Severe
P20	-	+ / Punctate / Frontal	-	-	Present	Mild
P21	++ / Punctiform & Linear / Frontal	++ / Punctiform / Frontal, Temporal & Parietal	-	-	-	Moderate

Abbreviations: MRI: magnetic resonance imaging; P: patient; WMHs: white matter hyperintensities; 0: absence.

Notes:

- The intensity of WMHs was expressed by using 1 to 3 cross (+), depending on the size (ranging from punctuate to confluent) and the number of the lesions: - for no WMH lesions; + for mild, with one to 3 lesions; ++ for moderate, with more than 3 lesions and/or a few confluent foci; +++ for severe, with large confluent WMHs.
- The overall brain lesion severity score was rated as follows: absence, mild, moderate or severe.
- P18 did not undergo MRI because of claustrophobia.

Supplementary Table 2: Cardiac and coronary artery abnormality screening results in 10 of the 20 STAT3-deficient patients investigated with cardiac MSCT and late gadolinium enhanced CMRI.

Pt	CA aneurysm (maximum diameter, in mm)	CA ectasia (maximum diameter in mm)	Late gadolinium enhancement (LGE)	Other findings
P1		Distal RCA		Moderate dilation of distal first marginal branch < 50% ostial proximal LAD stenosis
P2	Acute marginal segment of RCA (5) Distal Cx (4) Proximal LAD (7) 3 lesions of distal LAD (6.5; 6; 5.5) 2 lesions of first marginal (5.5; 5.5)	Mid RCA (7) Distal RCA (4) Mid LAD (8)	Apical (Segment 17)	
P5		LM CA (7)		
P6	Distal Cx (3.5) Distal LAD (5)			Moderate dilation of mid and distal LAD
P8	LM CA (6) Mid Cx (3.5) First marginal branch (2.5)		Apical (Segment 17)	
P10				Bicuspid aortic valve with raphe Membranous VSD
P12			Apical (Segment 17)	
P13	Proximal LAD (5)			Moderate dilation of distal RCA and mid LAD
P15	Mid LAD (4.5)		Apical (Segment 17)	
P21		LM CA (5.5) Distal RCA (5.5)		Moderate dilation of mid Cx and mid RCA

Mis en forme : Portugais
(Brésil)

Abbreviations: CA: coronary artery; CMR: cardiac magnetic resonance; Cx: circumflex; LAD: left anterior descending coronary artery; LGE: late gadolinium enhancement; LM CA: left main coronary artery; mm: millimeters; MSCT: multislice computed tomography; P/Pt: patient; R: right; RCA: right coronary artery; VSD: ventricular septal defect.

Notes:

- P16 did not undergo cardiac MSCT because of iodinated contrast agent-related anaphylaxis.
- No cardiac or coronary artery abnormalities were detected in P3, P4, P7, P9, P11, P14, P17, P18, P19 and P20.
- The coronary imaging results in P12 were influenced by a contra-indication to beta-blockers (due to asthma) and thus an insufficient decrease in heart rate.
- In P18, only 5 coronary segments could be analyzed, due to artifacts.

Supplementary Table 3: Extracoronary vascular abnormalities in 17 of the 20 STAT3-deficient patients investigated with WB-MRA.

Pt	Artery aneurysm (maximal diameter in mm)	Artery ectasia (maximal diameter in mm)	Artery stenosis	Lumen irregularities	Other findings
P1		R int carotid (7.5)			
P2	R mid cerebral (7)		> 50% R common carotid	R vertebral	
P3			< 50% ostial celiac trunk short	R and L vertebral	Tortuous and early bifurcation of L ant cerebral , Tortuous R and L vertebral
P5	Valsalva sinus (45) Pulmonary (31)	Celiac trunk (9)	< 50% R Ext iliac	L subclavian L vertebral Int carotid, SMA	Local dissection of L subclavian
P7	Proximal SMA (10)		< 50% R superficial femoral	R vertebral A	Absence of post tibial
P8	Proximal SMA (12)	Superficial femoral (8)		R vertebral (V4) L vertebral (V3)	Tortuous R and L int carotid
P9			< 50% ostial L and R vertebral		Tortuous R int carotid
P10		R common iliac (11)	< 50% L vertebral		2 L mid cerebral
P11					Tortuous R int carotid
P12					Jugular asymmetry with enlarged R jugular vein and many collaterals
P13		Celiac trunk (9)			Agenesis of L post communicating Tortuous R int carotid
P14			> 50% R subclavian	R and L vertebral	
P15	Ostial enlargement of L ant cerebral (3)		< 50% ostial R vertebral	R vertebral (V4)	Arteriovenous fistula between R post tibial artery and greater saphenous vein
P16	Pre-stenotic R hepatic (6.5) Basilar trunk (19 x 17)	Upper branch of the R fibular (5.5)	> 50% R hepatic		Tortuous R int carotid Azygos continuation of the inf vena cava
P17	At the junction of L ant communicating and ant cerebral (2)		< 50% long sub-bulbar R int carotid		Early venous return of the L ovarian vein Agenesis of the L proximal ant cerebral
P20			> 50% L vertebral (V2) > 50% celiac trunk	L vertebral (V1) R vertebral (V3)	Highly tortuous R int carotid (loop)
P21			< 50% L common carotid < 50% celiac trunk		

Abbreviations: Ant: anterior; ext: external; inf: inferior; int: internal; L: left; mm: millimeters; P/Pt: patient; post: posterior; R: right; SMA: superior mesenteric artery; V: vertebral artery segment; WB-MRA: whole-body magnetic resonance angiography; > greater than; < less than.

Notes:

- P18 did not undergo MRA because of claustrophobia.
- No extracoronary artery abnormalities were detected in P4, P6 and P19.
- P15: The fistula between the right posterior tibial artery and the internal saphenous vein was confirmed using Doppler ultrasound.

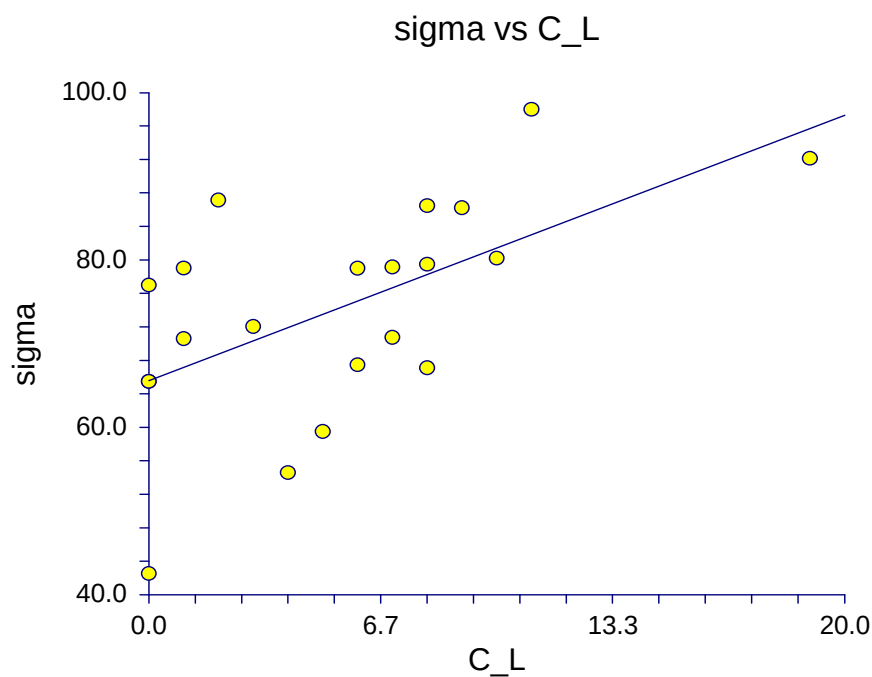
Supplementary Table 4: Biomarkers related to inflammation, extracellular matrix remodeling and endothelial dysfunction

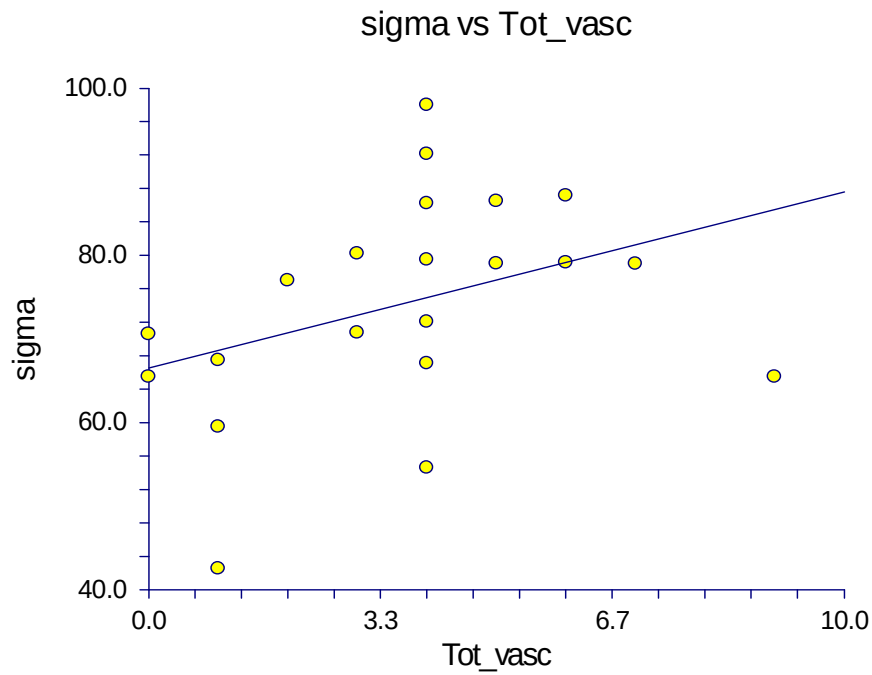
Data are given as the mean $\pm$ SEM; * $p < 0.05$; † in mg/l.

pg/ml	CONTROL	STAT3-deficient
IL-2	156.3 $\pm$ 41.8	138.4 $\pm$ 23.2
IL-10	51.4 $\pm$ 12.6	60.0 $\pm$ 11.8
IL-9	134.6 $\pm$ 78.4	25.4 $\pm$ 6.1
IL-22	36.0 $\pm$ 9.6	130.1 $\pm$ 51.7*
IL-6	11.4 $\pm$ 2.5	27.7 $\pm$ 19.3
IL-13	152.1 $\pm$ 24.1	141.8 $\pm$ 11.9
IL-4	295.0 $\pm$ 35.4	331.6 $\pm$ 28.1
IL-5	1602.5 $\pm$ 296.6	1009.6 $\pm$ 161.3*
IL-1 β	79.2 $\pm$ 13.1	98.6 $\pm$ 17.3
IL-1 α	23.5 $\pm$ 9.9	10.4 $\pm$ 2.1*
IL-23	2389.7 $\pm$ 507.6	3125.6 $\pm$ 593.3
IL-17A	125.2 $\pm$ 27.3	120.2 $\pm$ 19.7
TNF- α	44.5 $\pm$ 8.1	53.4 $\pm$ 8.8
TNF- β	85.6 $\pm$ 24.1	66.7 $\pm$ 15.6
IL-12p70	98.0 $\pm$ 36.0	145.9 $\pm$ 54.7
IFN- γ	245.6 $\pm$ 75.7	318.1 $\pm$ 63.9
RANTES	124759.0 $\pm$ 9414.7	132888.3 $\pm$ 26625.9
IL-18	346.2 $\pm$ 24.5	418.6 $\pm$ 52.9
MMP1	113.45 $\pm$ 19.07	170.89 $\pm$ 24.06*
MMP2	20025.7 $\pm$ 796.6	19892.2 $\pm$ 778.2
MMP3	560.40 $\pm$ 64.75	283.90 $\pm$ 45.56*
MMP8	1081.9 $\pm$ 250.6	1732.4 $\pm$ 372.4
MMP9	295.9 $\pm$ 84.3	329.1 $\pm$ 76.2
TIMP-1	55.0 $\pm$ 19.1	163.4 $\pm$ 49.5*
CRP $_{us}$ †	5.8 $\pm$ 2.7	4.7 $\pm$ 2.2
PAI-1	1.8 $\pm$ 1.2	14.8 $\pm$ 5.1*
VCAM-1s	52281.2 $\pm$ 6447.3	82718.7 $\pm$ 1101.4*

Supplementary Figure 1:

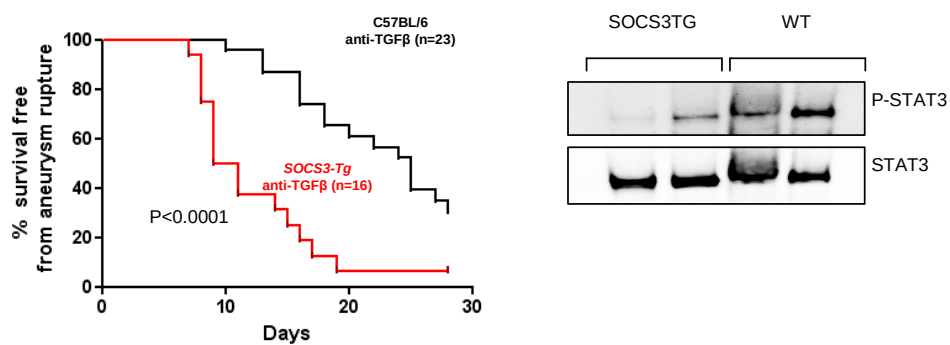
Scatter plots showing that the circumferential wall stress level was significantly and positively associated with the severity of cerebral lesions ($p=0.04$) and the number of affected arterial territories identified on imaging (increase for each additional affected artery territory: 2.5 kPa, 95% CI [0.2 to 4.8], $p=0.03$). A Spearman rank test, yielding R values of 0.641 ($p=0.0017$) for the severity of the cerebral lesions and 0.442 ($p=0.045$) for the number of arterial territories was used followed by a bivariate analysis using a robust regression procedure. The total R square is derived from the bivariate analysis.





Supplementary Figure 2:

Over-expression of SOCS3 increases the prevalence and severity of aortic aneurysm in mice. Left-hand panel: Kaplan-Meier curves for aortic-rupture-free survival in wild type (WT) and SOCS3-Tg mice using the Ang II + anti-TGF β model. The right-hand panel shows a reduction in STAT3 phosphorylation in the spleen of SOCS3-Tg mice.



SUPPLEMENTAL MATERIAL

Frequent and widespread vascular abnormalities in human STAT3 deficiency.

Supplementary imaging methods:

Magnetic resonance imaging:

P18 did not undergo MRI because of claustrophobia. Magnetic resonance imaging was performed on a 1.5 T scanner system (Excite HDx, General Electric Medical Systems, Waukesha, WI). Brain imaging was first achieved using a neurovascular coil, by acquiring a single series of axial fluid-attenuated inversion recovery (FLAIR) images, followed by an axial multi-slab three-dimensional time-of-flight (3D-TOF) non-enhanced intracranial MR angiography. WB-MRA was then performed in four steps by using a neurovascular coil for station 1 (head and neck) and a body coil for stations 2 (chest and abdomen), 3 (pelvis and thighs) and 4 (calves) (**Figure 1N**) during administration of a total dose of 0.2 mmol/kg of gadoterate meglumine (Dotarem® from Guerbet, Villepinte, France). The neurovascular coil was then replaced with a cardiac-phased array coil to screen for small, asymptomatic myocardial infarcts (as previously described [16]) by using conventional 2D late gadolinium enhanced imaging (LGE) 10 minutes after the initial gadolinium bolus injection.

Cardiac multislice computed tomography (MSCT) imaging:

P16 did not undergo MSCT because of iodinated contrast agent-related anaphylaxis. All cardiac MSCT scans were performed on a 64-row system (LightSpeed VCT, GE Healthcare, Milwaukee, Wisconsin, USA) during intravenous (IV) injection of 1.5 mL/kg of 350 mg iodine per mL contrast medium (iobitridol from Guerbet, France) at a flow rate of

5 mL/s. A low X-ray dose sequential protocol was applied by using prospectively electrocardiography-gated acquisition of 0.625 mm-thick slices. This led to a mean radiation exposure of 159 mGy.cm dose-length product (range: 97-272), corresponding to an effective dose of 2.7 mSv (range: 1.6-4.6). To decrease the heart rate, all patients but P12 (an asthma sufferer) received an IV injection of 5 mg of atenolol 10 minutes before initiation of the MSCT scan.

All original and post-processed MSCT and MRA images were analyzed on a dedicated workstation. In cases of vessel dilation, we estimated the maximum cross-sectional diameter of the lesion and that of the adjacent normal segment. All potential lesions were considered in a consensus analysis by at least two radiologists with expertise in cardiovascular and brain imaging (AA, NB, EM). Peripheral and coronary arteries were divided into 58 and 17 segments, respectively [17]. An arterial dilation exceeding 1.5 times the diameter of a normal arterial segment was classified as an aneurysm if it involved less than 50% of the total length of the segment and as an artery ectasia if it involved more than 50% of the total length of the segment. When the entire length of a peripheral artery was dilated, it was classified as an artery ectasia when the maximum diameter of the artery exceeded the diameter of the contralateral artery by a factor of 1.5 [18-20]. Furthermore, moderate focal artery dilation, abnormal arterial tortuosity and lumen irregularities were considered in cases of dilation of between 1.0 and 1.5 times the adjacent artery segment. Arterial stenosis was qualified as a reduction of more than 50% or less than 50% in the lumen diameter. Brain MR images were classified in a consensus analysis by two experts (AA, NB). As previously reported [16,21,22], WMHs were rated visually on separately axial FLAIR images for periventricular and subcortical areas and graded as mild, moderate and severe as a function of their size (i.e. punctuate versus confluent) and number. Cortical atrophy, ventricular dilation and lacunar lesions were also

identified. In a consensus analysis by two experts (AA, EM), late gadolinium enhancement (LGE) on CMRI was defined as the presence of myocardial hyper-intensity in two different cardiac views [23].

Supplementary methods for carotid artery ultrasound and hemodynamic measurements:

Measurements were performed in a dedicated, air-conditioned room. Blood pressure was measured using a Colin oscillometric device (Press-Mate 8800 from Omron, Rosny, France). Central pulse pressure (cPP) was measured using a Sphygmocor device (Atcor Medical, Sydney, Australia). The right common carotid artery was measured with a high-precision echotracking device (the Artlab system from Esaote PIE Medical, Maastricht, Netherlands), as previously described [24]. The following parameters were measured or calculated: intima media thickness (IMT), diameter (internal (Di) and external (De)), wall to lumen ratio ($2IMT/Di$), distensibility ($Dist=dD/Di*cPP$), elastic modulus ($Einc=[3(1+Di^2/(De^2-Di^2))]/Dist$) and circumferential wall stress ($CWS=Di*MBP/2*IMT$).

Supplementary methods for biomarker analysis:

Total IgE, glucose, lipid and uric acid levels, blood cell counts, hemostatic and inflammatory (serum protein electrophoresis, fibrinogen, C-reactive protein) parameters were measured in all patients. Furthermore, serum from STAT3-deficient patients and from gender- and age-matched healthy blood donors was sampled in order to assess the circulating levels of a panel of biomarkers related to inflammation, extracellular matrix remodeling and endothelial dysfunction (high-sensitivity C-reactive protein (hsCRP), IL-1 α , IL-1 β , IL-4, IL-5, IL-6, IL-9, IL-10, IL-12p70, IL-13, IL-18, IL-22, IL-23, RANTES (regulated upon activation, normal T-cell expressed, and secreted), tumor necrosis factor (TNF) - α , TNF- β ,

matrix metalloproteinase (MMP) -1, MMP-2, MMP-3, MMP-8, MMP-9, tissue inhibitor of MMP-1 (TIMP-1), plasminogen activator inhibitor-1 (PAI-1), soluble vascular cell adhesion molecule-1 (sVCAM-1), using FlowCytomix technology (Bender MedSystems), Fluorokine® MultiAnalyte Profiling kits (R&D Systems for MMP-1, MMP-2, MMP-3, MMP-8), an immunonephelometric assay for hsCRP (Siemens) and/or high-sensitivity ELISA assays (R & D Systems for sVCAM-1).

Supplementary Table 1: Brain abnormality screening results in a cohort of 20 STAT3-deficient patients explored with brain MRI.

Patient	White matter hyperintensities		Lacunar lesion	Cortical atrophy	Ventricle dilation	Brain lesion score
	Periventricular	Subcortical				
P1	++ / Linear / Frontal	++ / Punctate / Frontal	-	Present	Present	Moderate
P2	++ / Punctate/ Frontal & Parietal	++ / Punctate & Confluent / Frontal & Parietal	-	-	Present	Moderate
P3	-	+ / Punctate & Confluent / Frontal	-	-	Present	Mild
P4	-	-	-	-	Present	Mild
P5	++ / Punctate / Frontal & Parietal	++ / Punctate / Frontal & Parietal	-	Present	Present	Moderate
P6	+ / Linear & Confluent / Frontal & Parietal	++ / Punctate / Frontal	-	Present	-	Mild
P7	+ / Punctiform / Frontal & Parietal	++ / Punctate / Frontal	-	Present	-	Moderate
P8	+ / Punctiform / Frontal	-	-	-	-	Mild
P9	+++ / Confluent / Frontal & Parietal	+++ / Confluent / Frontal & Parietal	-	-	-	Severe
P10	-	+ / Punctate / Frontal & Temporal	-	-	-	Mild
P11	+++ / Confluent / Frontal	+++ / Confluent / Frontal & Parietal	1 lesion / Periventricular / Frontal	-	-	Severe
P12	-	-	-	-	-	Absence
P13	+ / Punctiform / Frontal	+++ / Punctate & Confluent / Frontal & Parietal	-	Present	Present	Severe
P14	++ / Linear / Frontal	++ / Punctate / Frontal	1 lesion / Cortical / Frontal	Present	Present	Severe
P15	++ / Linear, Frontal	++ / Punctate & Confluent / Frontal	-	-	Present	Moderate
P16	+ / Punctate / Frontal	+ / Punctate / Frontal	-	-	Present	Mild
P17	++ / Punctiform / Frontal	+++ / Punctate / Frontal & Temporal	-	-	-	Severe
P19	++ / Linear / Frontal	++ / Punctate / Frontal	1 lesion / Cortical / Frontal	-	-	Severe
P20	-	+ / Punctate / Frontal	-	-	Present	Mild
P21	++ / Punctiform & Linear / Frontal	++ / Punctiform / Frontal, Temporal & Parietal	-	-	-	Moderate

Abbreviations: MRI: magnetic resonance imaging; P: patient; WMHs: white matter hyperintensities; 0: absence.

Notes:

- The intensity of WMHs was expressed by using 1 to 3 cross (+), depending on the size (ranging from punctuate to confluent) and the number of the lesions: - for no WMH lesions; + for mild, with one to 3 lesions; ++ for moderate, with more than 3 lesions and/or a few confluent foci; +++ for severe, with large confluent WMHs.
- The overall brain lesion severity score was rated as follows: absence, mild, moderate or severe.
- P18 did not undergo MRI because of claustrophobia.

Supplementary Table 2: Cardiac and coronary artery abnormality screening results in 10 of the 20 STAT3-deficient patients investigated with cardiac MSCT and late gadolinium enhanced CMRI.

Pt	CA aneurysm (maximum diameter, in mm)	CA ectasia (maximum diameter in mm)	Late gadolinium enhancement (LGE)	Other findings
P1		Distal RCA		Moderate dilation of distal first marginal branch < 50% ostial proximal LAD stenosis
P2	Acute marginal segment of RCA (5) Distal Cx (4) Proximal LAD (7) 3 lesions of distal LAD (6.5; 6; 5.5) 2 lesions of first marginal (5.5; 5.5)	Mid RCA (7) Distal RCA (4) Mid LAD (8)	Apical (Segment 17)	
P5		LM CA (7)		
P6	Distal Cx (3.5) Distal LAD (5)			Moderate dilation of mid and distal LAD
P8	LM CA (6) Mid Cx (3.5) First marginal branch (2.5)		Apical (Segment 17)	
P10				Bicuspid aortic valve with raphe Membranous VSD
P12			Apical (Segment 17)	
P13	Proximal LAD (5)			Moderate dilation of distal RCA and mid LAD
P15	Mid LAD (4.5)		Apical (Segment 17)	
P21		LM CA (5.5) Distal RCA (5.5)		Moderate dilation of mid Cx and mid RCA

Mis en forme : Portugais
(Brésil)

Abbreviations: CA: coronary artery; CMR: cardiac magnetic resonance; Cx: circumflex; LAD: left anterior descending coronary artery; LGE: late gadolinium enhancement; LM CA: left main coronary artery; mm: millimeters; MSCT: multislice computed tomography; P/Pt: patient; R: right; RCA: right coronary artery; VSD: ventricular septal defect.

Notes:

- P16 did not undergo cardiac MSCT because of iodinated contrast agent-related anaphylaxis.
- No cardiac or coronary artery abnormalities were detected in P3, P4, P7, P9, P11, P14, P17, P18, P19 and P20.
- The coronary imaging results in P12 were influenced by a contra-indication to beta-blockers (due to asthma) and thus an insufficient decrease in heart rate.
- In P18, only 5 coronary segments could be analyzed, due to artifacts.

Supplementary Table 3: Extracoronary vascular abnormalities in 17 of the 20 STAT3-deficient patients investigated with WB-MRA.

Pt	Artery aneurysm (maximal diameter in mm)	Artery ectasia (maximal diameter in mm)	Artery stenosis	Lumen irregularities	Other findings
P1		R int carotid (7.5)			
P2	R mid cerebral (7)		> 50% R common carotid	R vertebral	
P3			< 50% ostial celiac trunk short	R and L vertebral	Tortuous and early bifurcation of L ant cerebral , Tortuous R and L vertebral
P5	Valsalva sinus (45) Pulmonary (31)	Celiac trunk (9)	< 50% R Ext iliac	L subclavian L vertebral Int carotid, SMA	Local dissection of L subclavian
P7	Proximal SMA (10)		< 50% R superficial femoral	R vertebral A	Absence of post tibial
P8	Proximal SMA (12)	Superficial femoral (8)		R vertebral (V4) L vertebral (V3)	Tortuous R and L int carotid
P9			< 50% ostial L and R vertebral		Tortuous R int carotid
P10		R common iliac (11)	< 50% L vertebral		2 L mid cerebral
P11					Tortuous R int carotid
P12					Jugular asymmetry with enlarged R jugular vein and many collaterals
P13		Celiac trunk (9)			Agenesis of L post communicating Tortuous R int carotid
P14			> 50% R subclavian	R and L vertebral	
P15	Ostial enlargement of L ant cerebral (3)		< 50% ostial R vertebral	R vertebral (V4)	Arteriovenous fistula between R post tibial artery and greater saphenous vein
P16	Pre-stenotic R hepatic (6.5) Basilar trunk (19 x 17)	Upper branch of the R fibular (5.5)	> 50% R hepatic		Tortuous R int carotid Azygos continuation of the inf vena cava
P17	At the junction of L ant communicating and ant cerebral (2)		< 50% long sub-bulbar R int carotid		Early venous return of the L ovarian vein Agenesis of the L proximal ant cerebral
P20			> 50% L vertebral (V2) > 50% celiac trunk	L vertebral (V1) R vertebral (V3)	Highly tortuous R int carotid (loop)
P21			< 50% L common carotid < 50% celiac trunk		

Abbreviations: Ant: anterior; ext: external; inf: inferior; int: internal; L: left; mm: millimeters; P/Pt: patient; post: posterior; R: right; SMA: superior mesenteric artery; V: vertebral artery segment; WB-MRA: whole-body magnetic resonance angiography; > greater than; < less than.

Notes:

- P18 did not undergo MRA because of claustrophobia.
- No extracoronary artery abnormalities were detected in P4, P6 and P19.
- P15: The fistula between the right posterior tibial artery and the internal saphenous vein was confirmed using Doppler ultrasound.

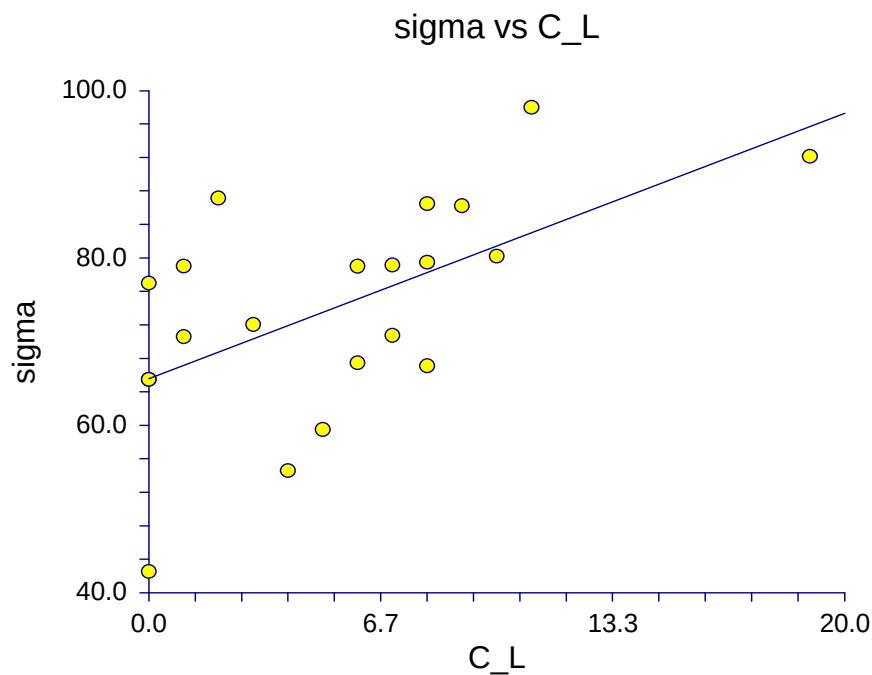
Supplementary Table 4: Biomarkers related to inflammation, extracellular matrix remodeling and endothelial dysfunction

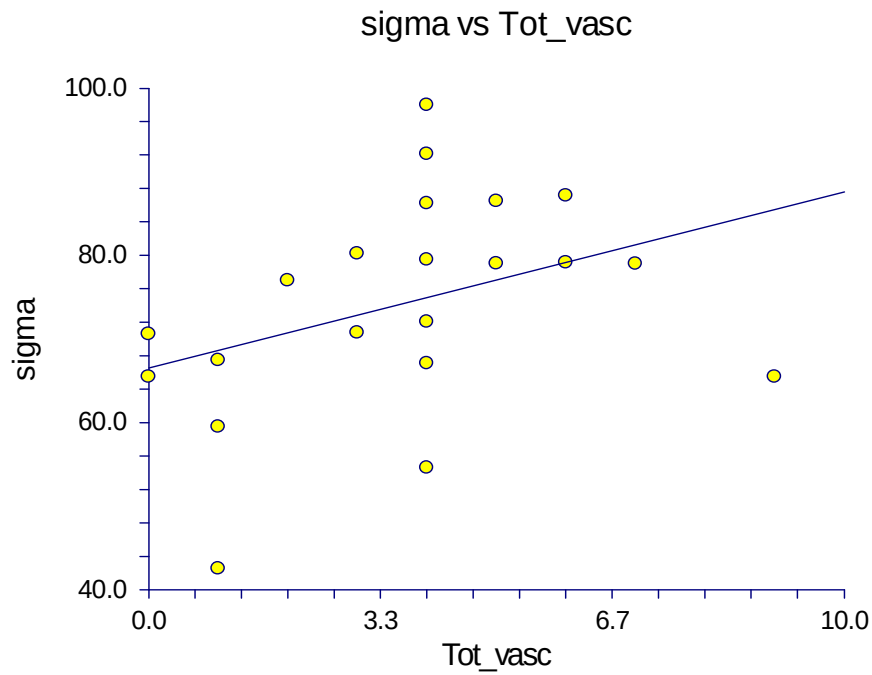
Data are given as the mean $\pm$ SEM; *p<0.05; † in mg/l.

pg/ml	CONTROL	STAT3-deficient
IL-2	156.3 $\pm$ 41.8	138.4 $\pm$ 23.2
IL-10	51.4 $\pm$ 12.6	60.0 $\pm$ 11.8
IL-9	134.6 $\pm$ 78.4	25.4 $\pm$ 6.1
IL-22	36.0 $\pm$ 9.6	130.1 $\pm$ 51.7*
IL-6	11.4 $\pm$ 2.5	27.7 $\pm$ 19.3
IL-13	152.1 $\pm$ 24.1	141.8 $\pm$ 11.9
IL-4	295.0 $\pm$ 35.4	331.6 $\pm$ 28.1
IL-5	1602.5 $\pm$ 296.6	1009.6 $\pm$ 161.3*
IL-1 β	79.2 $\pm$ 13.1	98.6 $\pm$ 17.3
IL-1 α	23.5 $\pm$ 9.9	10.4 $\pm$ 2.1*
IL-23	2389.7 $\pm$ 507.6	3125.6 $\pm$ 593.3
IL-17A	125.2 $\pm$ 27.3	120.2 $\pm$ 19.7
TNF- α	44.5 $\pm$ 8.1	53.4 $\pm$ 8.8
TNF- β	85.6 $\pm$ 24.1	66.7 $\pm$ 15.6
IL-12p70	98.0 $\pm$ 36.0	145.9 $\pm$ 54.7
IFN- γ	245.6 $\pm$ 75.7	318.1 $\pm$ 63.9
RANTES	124759.0 $\pm$ 9414.7	132888.3 $\pm$ 26625.9
IL-18	346.2 $\pm$ 24.5	418.6 $\pm$ 52.9
MMP1	113.45 $\pm$ 19.07	170.89 $\pm$ 24.06*
MMP2	20025.7 $\pm$ 796.6	19892.2 $\pm$ 778.2
MMP3	560.40 $\pm$ 64.75	283.90 $\pm$ 45.56*
MMP8	1081.9 $\pm$ 250.6	1732.4 $\pm$ 372.4
MMP9	295.9 $\pm$ 84.3	329.1 $\pm$ 76.2
TIMP-1	55.0 $\pm$ 19.1	163.4 $\pm$ 49.5*
CRP $_{us}$ †	5.8 $\pm$ 2.7	4.7 $\pm$ 2.2
PAI-1	1.8 $\pm$ 1.2	14.8 $\pm$ 5.1*
VCAM-1s	52281.2 $\pm$ 6447.3	82718.7 $\pm$ 1101.4*

Supplementary Figure 1:

Scatter plots showing that the circumferential wall stress level was significantly and positively associated with the severity of cerebral lesions ($p=0.04$) and the number of affected arterial territories identified on imaging (increase for each additional affected artery territory: 2.5 kPa, 95% CI [0.2 to 4.8], $p=0.03$). A Spearman rank test, yielding R values of 0.641 ($p=0.0017$) for the severity of the cerebral lesions and 0.442 ($p=0.045$) for the number of arterial territories was used followed by a bivariate analysis using a robust regression procedure. The total R square is derived from the bivariate analysis.





Supplementary Figure 2:

Over-expression of SOCS3 increases the prevalence and severity of aortic aneurysm in mice. Left-hand panel: Kaplan-Meier curves for aortic-rupture-free survival in wild type (WT) and SOCS3-Tg mice using the Ang II + anti-TGF β model. The right-hand panel shows a reduction in STAT3 phosphorylation in the spleen of SOCS3-Tg mice.

