

Serum amyloid A serum concentrations and genotype do not explain low incidence of amyloidosis in Hyper-IgD syndrome

J. C. H. VAN DER HILST¹, J. P. H. DRENTH², E. J. BODAR¹, J. BIJZET³,
J. W. M. VAN DER MEER¹, A. SIMON¹, & CONTRIBUTING MEMBERS OF THE
INTERNATIONAL HIDS STUDY GROUP

¹Department of General Internal Medicine, ²Department of Gastroenterology, UMC St. Radboud, Nijmegen, The Netherlands, and ³Department of Rheumatology, University Hospital Groningen, Groningen, The Netherlands

Keywords: Periodic fever, Hyper-IgD syndrome, serum amyloid A, amyloidosis, SAA1 genotype

Abbreviations: AA = amyloid A; SAA = serum amyloid A; CRP = C reactive protein; MVK = mevalonate kinase; CINCA = Chronic Infantile Neurological Cutaneous and Articular syndrome; FCAS = familial cold auto-inflammatory syndrome; FMF = familial Mediterranean fever; HIDS = Hyper-IgD syndrome; MWS = Muckle–Wells syndrome; TRAPS = TNF receptor associated periodic syndrome

Abstract

Background. Hyper-IgD and periodic fever syndrome (HIDS) is an autosomal recessively inherited disorder characterized by recurrent episodes of fever and inflammation. Unlike other chronic inflammatory conditions, amyloidosis is very rare in HIDS. For deposition of amyloid of the AA type, high concentrations of SAA are a prerequisite, together with certain SAA1 gene polymorphisms. The SAA1.1 genotype predisposes for amyloidosis, while SAA1.5 genotype exerts a protective effect.

Aim of the study. To determine if SAA concentrations and SAA1 gene polymorphisms could explain the virtual absence of amyloidosis in HIDS patients.

Methods. We measured SAA and CRP concentrations in serum of 20 HIDS patients during an attack and during the asymptomatic phase. Genotype of SAA1 gene was determined in 60 HIDS patients.

Results. SAA serum concentrations during attacks were very high (median 205 mg/l; range 75–520 mg/l, normal < 3.1 mg/l). During attack-free periods 45% of patients still had elevated SAA concentrations. The distribution of the genotype of SAA1 gene in HIDS was similar to healthy controls (SAA1.1 0.41 vs. 0.50 $p=0.32$).

Conclusion. Patients with HIDS have high SAA during attacks and show sub-clinical inflammation when asymptomatic. The low incidence of amyloidosis cannot be explained by a predominance of non amyloidogenic SAA related genotypes.

Introduction

Reactive (AA) amyloidosis refers to the systemic deposition of insoluble fibrillar amyloid proteins in the extra-cellular space in a number of different organs, most notably the kidney. The first manifestation of the AA type amyloidosis is proteinuria, which progresses to nephrotic syndrome and finally renal failure [1]. The AA protein that forms the amyloid fibrils in type AA amyloidosis is a degradation product of serum amyloid A (SAA), an acute phase protein produced in response to inflammation. It is generally accepted that high SAA serum concentrations during a long period of time are a prerequisite for the development of AA amyloidosis [2]. AA type

amyloidosis has been described in chronic inflammatory disorders such as juvenile chronic arthritis, inflammatory bowel disease, and in hereditary periodic fever syndromes.

Hereditary periodic fever syndromes or auto-inflammatory syndromes are a group of genetic disorders characterized by recurrent episodes of fever with vigorous acute phase response separated by symptom-free intervals [3]. The best known representative of this group is familial Mediterranean fever (FMF), but over the last two decades five other disorders have been described: the Hyper-immunoglobulinemia D and periodic fever syndrome (HIDS), TNF-receptor-associated periodic syndrome (TRAPS), Muckle–Wells syndrome (MWS),

familial cold auto-inflammatory syndrome (FCAS), and lastly Chronic Infantile Neurological Cutaneous and Articular syndrome (CINCA).

Type AA amyloidosis is a frequent complication of most hereditary periodic fever syndromes. For example, it was found in up to 60% of FMF patients before the advent of colchicine treatment [4], and it has been reported in 25% of TRAPS patients [5,6], and in up to 35% of patients with MWS [7]. Although the prevalence of amyloidosis among the hereditary periodic fever syndromes is high, current data indicate that it is a very rare event in HIDS. HIDS is an autosomal recessively inherited disorder characterized by recurrent attacks of fever, bilateral cervical lymphadenopathy, and by abdominal pain and diarrhoea. In its classical form, it is caused by mevalonate kinase (MVK) gene mutations that lead to a deficiency of mevalonate kinase, a central enzyme to the isoprenoid metabolism. HIDS earned its name because patients have markedly elevated serum concentrations of polyclonal IgD [8,9]. Patients with HIDS seem to have the pre-requisites for development of amyloidosis. It is a chronic inflammatory condition; symptoms of HIDS start typically in the first year of life, patients suffer from frequent attacks and during attacks they exhibit a vigorous acute-phase response. Despite a thorough 20-year follow-up, there are only 2 documented cases among 92 patients with genetically proven HIDS held at The International Hyper-IgD syndrome Registry (www.HIDS.net), of which one has recently been published [10].

In view of these facts, the low incidence of amyloidosis in HIDS patients is remarkable.

A prolonged high plasma level of SAA in chronic inflammation is considered necessary for deposition of AA proteins in tissues. However, a high concentration of SAA alone is not sufficient for the development of reactive type AA amyloidosis. SAA1 gene polymorphisms have been identified as additional risk factors. The presence of 2 single nucleotide polymorphisms within exon 3 of the SAA1 gene defines 3 haplotypes (1.1, 1.3, 1.5) [11]. In Caucasians, the 1.1 allele exhibits a pro-amyloid phenotype, while 1.5 allele seems to protect [12,13].

The present study was performed to investigate whether SAA concentrations and genotype prevalence could explain the low incidence of amyloidosis in HIDS.

Patients and methods

Cohort study

Twenty HIDS patients known in our clinic were enrolled in this first part of our study (Table I). All

patients carried MVK gene mutations. Sampling of plasma and serum was performed at two time points during a 6-month period, during fever attack and during remission. A fever attack was defined as (1) raised body temperature ($\geq 38^{\circ}\text{C}$), (2) at least one of the following symptoms and signs: lymphadenopathy, abdominal pain, arthritis, and/or skin rash and (3) no clinical indications for the presence of infection. Remission was defined as the absence of symptoms for at least 1 month. No medication was allowed during the study period. Close follow-up of the patients did exclude bacterial and/or viral infections during the course of the investigation. We sampled for CRP, SAA, serum creatinine, and performed urinalysis. SAA and CRP were measured with enzyme linked immunosorbent assays [14] (normal SAA < 4.2 mg/l; normal CRP < 3.7 mg/l; detection limit for both assays is 0.001 mg/l).

SAA genotype study

We collected DNA samples from 60 HIDS patients from the Nijmegen HIDS registry. All patients had MVK gene mutations. DNA was extracted by standard methods and we included 50 healthy Dutch blood donors as controls. SAA1 genotypes were determined by polymerase chain reaction followed by restriction fragment length polymorphism analysis as described elsewhere [15]. We established the presence of 2 single-nucleotide polymorphisms within exon 3 of the SAA1 gene that define 3 haplotypes corresponding to SAA1.1 (Val52, Ala57), SAA1.3 (Ala52, Ala57), and SAA1.5 (Ala52, Val57).

The ethical committee of our institution approved the study protocol, and all patients gave informed consent.

Statistical analysis

The statistical significance of differences between groups was calculated either by Chi-square test for categorical data or Fisher's exact test where appropriate. The distribution of each allele frequency among the control population was tested whether it fitted the Hardy-Weinberg equilibrium.

Results

Cohort study

The distribution of the 20 patients in the cohort with respect to MVK genotypes, gender, age, and duration of disease is presented in Table I. Renal function was normal in all patients (mean serum creatinine 64 $\mu\text{mol/l}$, range 53–86) and none had proteinuria, the first sign of nephropathic amyloidosis. During attacks, all HIDS patients exhibited high concentra-

Table I. Characteristics of 20 HIDS patients in the cohort study.

Characteristics of 20 patients		
Gender (male:female)	11:9	
Age (mean (range))	29.5 (13–50)	
Disease duration (mean)	28.4	
Mutations in mvk gene	V377I/unknown	5
	V377I/H20P	4
	V377I/I268T	4
	V377I/W62X	2
	P167L/I268T	2
	V377I/G309S	1
	P167L/G202R	1
	V377I/V377I	1

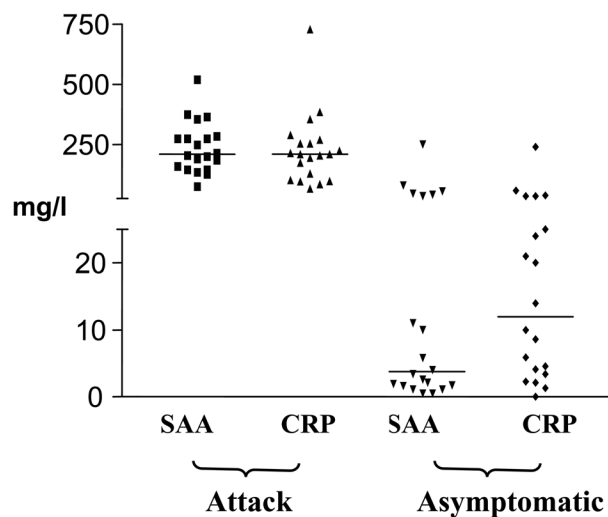


Figure 1. SAA and CRP concentrations during an attack and when asymptomatic in 20 HIDS patients. Thick bar represents the median. SAA normal < 4.2 mg/l; CRP normal < 3.7 mg/l.

tions of SAA (median 205 mg/l; range 75–520 mg/l) and CRP (210 mg/l; 67–385 mg/l) (Figure 1). During the asymptomatic stage, 9 of the 20 patients (45%) still had SAA concentrations above normal. SAA concentrations during attacks and symptom-free intervals did not correlate with age, gender or type of MVK mutation. Also CRP was elevated in the majority of patients (10 mg/l; 0.1–240 mg/l).

Genotype study

Figure 2 displays the SAA1 genotype of 60 HIDS patients and 50 healthy controls. The distribution of alleles fitted the Hardy–Weinberg equilibrium. There was no difference in distribution of amyloidogenic (1.1) and protective (1.5) genotypes between patients and controls ($P=0.28$). Neither the concentrations of SAA nor the SAA/CRP ratio was significantly correlated with one of the SAA1 genotypes.

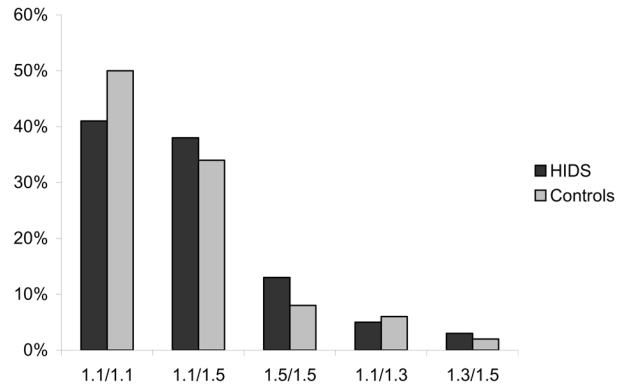


Figure 2. Genotype of SAA1 gene in 60 patients and 50 controls.

Discussion

Despite strongly elevated SAA concentrations during attacks and persistently raised SAA concentrations inbetween attacks, HIDS patients do not show a high incidence of amyloidosis.

From the available data in the literature we could calculate that the incidence of developing amyloidosis is approximately 1.8/100 patient years in untreated FMF [16]. This is 20 times higher than the incidence of amyloidosis in HIDS (0.09/100 patient years). Although median SAA concentrations in FMF are in some studies reported to be higher than those we found in HIDS, this does not seem to be sufficient explanation [17]. Even in systemic juvenile chronic arthritis, a condition that tends to regress during life, the incidence of amyloidosis is 5–7 times higher after 15–29 years of follow up (0.50–0.65 cases/100 patient years) [18,19].

Our study demonstrates that the low incidence of amyloidosis is not explained by the distribution of SAA1 genotype: The amyloidogenic SAA 1.1 genotype being present in 41% of patients. The risk of developing amyloidosis with the SAA1.1 genotype in an autoinflammatory condition such as FMF is strongly elevated [12,13]. The odds ratio of acquiring amyloidosis in FMF patients with SAA1.1 genotype was 2.99 [12]. In other inflammatory disorders associated with type AA amyloidosis such as juvenile chronic arthritis, the SAA1.1 genotype was detected in 80.5% of cases complicated by amyloidosis compared to 12.5% in cases free from amyloidosis [20]. Recently Terai et al. found similar results in Finnish patients with rheumatoid arthritis [21].

The question is how to explain the protection against amyloidosis in HIDS. In classical HIDS, the defect concerns mevalonate kinase (MVK), the enzyme phosphorylating mevalonate.

Mevalonate is the precursor of isoprenoid groups that are incorporated into an array of end-products,

such as ubiquinone, Rho, Ras, haem A, and dolichol [22]. One possibility to explain the decreased incidence of amyloidosis is that the excess of mevalonate might influence amyloidogenesis in a still obscure way. A more attractive hypothesis concerns a possible shortage of metabolites downstream, interfering with isoprenylation of proteins that protect against amyloidosis [23]. A variety of regulating proteins, like Ras, are dependent on isoprenylation to be activated. Decreased isoprenylation of these proteins could interfere with amyloidogenesis on different levels. For example, the expression of lysosomal proteases has been shown to be regulated by Ras [24]. Since lysosomal SAA degradation is seen as a central process in amyloidosis, decreased isoprenylation could influence this process. Furthermore, a role for matrix metalloproteinases (MMP) could be envisaged. MMPs have been shown to degrade SAA and amyloid fibrils [25] and MMP expression is regulated by isoprenylated proteins [26]. Another possibility is that the decreased isoprenoid metabolism alters HDL cholesterol to which SAA is bound as an apolipoprotein. This could interfere with SAA uptake in macrophages, leading to decreased processing of SAA to AA proteins. If the latter hypotheses are true it might be expected that treatment with HMG-CoA reductase inhibitors would interfere with amyloidogenesis.

More investigation in this area is needed to find out whether these drugs are effective in prevention and perhaps treatment of secondary amyloidosis.

In conclusion, although HIDS patients have all the prerequisites for developing amyloidosis, including high SAA concentrations and normal distribution of *SAA1* genotype, amyloidosis is only a rare complication. Thus, the causative defect in HIDS, mevalonate kinase deficiency, seems to interfere with amyloidogenesis.

Acknowledgments

We thank Hans Scheffer and Christa van Velzen from the Department of Human Genetics, University Medical Center St. Radboud, Nijmegen, The Netherlands for providing control DNA samples. Anna Simon is a recipient of a Dutch organization for Scientific Research Fellowship for Clinical Investigators (NWO nr. 920-03-116).

References

1. Grateau G. The relation between familial Mediterranean fever and amyloidosis. *Curr Opin Rheumatol* 2000;12:61–64.
2. Gillmore JD, Lovat LB, Persey MR, Pepys MB, Hawkins PN. Amyloid load and clinical outcome in AA amyloidosis in relation to circulating concentration of serum amyloid A protein. *Lancet* 2001;358:24–29.
3. Drenth JPH, van der Meer JWM. Hereditary periodic fever. *N Engl J Med* 2001;345:1748–1757.
4. Gafni J, Ravid M, Sohar E. The role of amyloidosis in familial Mediterranean fever. A population study. *Isr J Med Sci* 1968;4:995–999.
5. McDermott MF. Autosomal dominant recurrent fevers. Clinical and genetic aspects. *Rev Rhum Engl Ed* 1999;66:484–491.
6. Aganna E, Hawkins PN, Ozen S, Pettersson T, Bybee A, McKee SA, et al. Allelic variants in genes associated with hereditary periodic fever syndromes as susceptibility factors for reactive systemic AA amyloidosis. *Genes Immun* 2004;5:289–293.
7. Muckle TJ. The ‘Muckle–Wells’ syndrome. *Br J Dermatol* 1979;100:87–92.
8. Drenth JP, van Deuren M, van dV, Schalkwijk CG, van der Meer JW. Cytokine activation during attacks of the hyperimmunoglobulinemia D and periodic fever syndrome. *Blood* 1995;85:3586–3593.
9. Drenth JPH, Cuisset L, Grateau G, Vasseur C, van de Velde-Visser SD, de Jong JG, et al. Mutations in the gene encoding mevalonate kinase cause hyper-IgD and periodic fever syndrome. International Hyper-IgD Study Group. *Nat Genet* 1999;22:178–181.
10. Obici L, Manno C, Muda AO, Picco P, D’Osualdo A, Palladini G, Avanzini MA, Torres D, Marciano S, Merlini G. First report of reactive (AA) amyloidosis in a patient with hyperimmunoglobulinemia D with periodic fever syndrome. *Arthritis Rheum* 2004;50:2966–2969.
11. Moriguchi M, Terai C, Kaneko H, Koseki Y, Kajiyama H, Uesato M, et al. A novel single-nucleotide polymorphism at the 5'-flanking region of *SAA1* associated with risk of type AA amyloidosis secondary to rheumatoid arthritis. *Arthritis Rheum* 2001;44:1266–1272.
12. Gershoni-Baruch R, Brik R, Zacks N, Shinawi M, Lidar M, Livneh A. The contribution of genotypes at the *MEFV* and *SAA1* loci to amyloidosis and disease severity in patients with familial Mediterranean fever. *Arthritis Rheum* 2003;48:1149–1155.
13. Cazeneuve C, Ajrapetyan H, Papin S, Roudot-Thoraval F, Genevieve D, Mndjoyan E, et al. Identification of *MEFV*-independent modifying genetic factors for familial Mediterranean fever. *Am J Hum Genet* 2000;67:1136–1143.
14. Hazenberg BP, Limburg PC, Bijzet J, van Rijswijk MH. A quantitative method for detecting deposits of amyloid A protein in aspirated fat tissue of patients with arthritis. *Ann Rheum Dis* 1999;58:96–102.
15. Yamada T, Wada A, Itoh Y, Itoh K. Serum amyloid A1 alleles and plasma concentrations of serum amyloid A. *Amyloid; Int J Exp Clin Invest* 1999;6:199–204.
16. Sohar E, Gafni J, Pras M, Heller H. Familial Mediterranean fever. A survey of 470 cases and review of the literature. *Am J Med* 1967;43:227–253.
17. Duzova A, Bakalloglu A, Besbas N, Topaloglu R, Ozen S, Ozaltin F, et al. Role of A-SAA in monitoring subclinical inflammation and in colchicine dosage in familial Mediterranean fever. *Clin Exp Rheumatol* 2003;21:509–514.
18. Packham JC, Hall MA. Long-term follow-up of 246 adults with juvenile idiopathic arthritis: functional outcome. *Rheumatology (Oxford)* 2002;41:1428–1435.
19. Schnitzer TJ, Ansell BM. Amyloidosis in juvenile chronic polyarthritis. *Arthritis Rheum* 1977;20:245–252.

20. Booth DR, Booth SE, Gillmore JD, Hawkins PN, Pepys MB. SAA1 alleles as risk factors in reactive systemic AA amyloidosis. *Amyloid; Int J Exp Clin Invest* 1998;5:262–265.
21. Terai C, Kaneko H, Moriguchi M, Koseki Y, Kajiyama H, Kamatani N, et al. SAA1 gene analysis in Finnish patients with AA amyloidosis. Xth International congress on amyloid and amyloidosis. April 18–22, 2004, Tours, France.
22. Goldstein JL, Brown MS. Regulation of the mevalonate pathway. *Nature* 1990;343:425–430.
23. van der Hilst JC, Simon A, Drenth JP. Molecular mechanisms of amyloidosis. *N Engl J Med* 2003;349:1872–1873.
24. Collette J, Ulku AS, Der CJ, Jones A, Erickson AH. Enhanced cathepsin L expression is mediated by different Ras effector pathways in fibroblasts and epithelial cells. *Int J Cancer* 2004;112:190–199.
25. Stix B, Kahne T, Sletten K, Raynes J, Roessner A, Rocken C. Proteolysis of AA amyloid fibril proteins by matrix metalloproteinases-1, -2, and -3. *Am J Pathol* 2001;159:561–570.
26. Westermarck J, Kahari VM. Regulation of matrix metalloproteinase expression in tumor invasion. *FASEB J* 1999;13:781–792.