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The Ile198Thr and Ala379Val Variants of Plasmatic Paf-Acetylhydrolase Impair Catalytical Activities and Are Associated with Atopy and Asthma

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The platelet-activating factor (PAF) represents a phospholipid with complex biological functions, including involvement in inflammatory processes. The degrading enzyme PAF acetylhydrolase (PAFAH) represents a candidate for asthma and other atopic diseases. Two loss-of-function mutations of PAFAH are associated with severe asthma in Japanese individuals. Our aim was to look for further PAFAH variants in white populations, their possible association with atopic and asthmatic phenotypes, and their functional importance. We picked up three common variants in the PAFAH gene: Arg92His (exon 4), Ile198Thr (exon 7), and Ala379Val (exon 11). The known loss-of-function mutations were not seen. The variant allele Thr198 was found to be highly associated with total IgE concentrations in an atopic population ($P = .009$) and with "atopic asthma" in an asthmatic population ($P = .008$). The variant allele Val379 was found to be highly associated with "specific sensitization" in the atopic population ($P = .002$) and with "asthma" in the asthmatic population ($P = .003$). By use of recombinant PAFAH enzymes, the variant Val379 showed increased (14 μM) and Thr198 markedly increased (42 μM) K_M values compared to the wild type (7 μM); furthermore, *V*max of Val379 was highly increased (132%). Thr198 and Val379 influence plasmatic PAFAH toward lower substrate affinities and therefore are very likely to prolong the activities of PAF. At the same time, they are associated with an increased risk to develop asthma and atopy. Thus, two PAFAH variants seem to play a key role in atopic and asthmatic processes in Caucasian populations.

Introduction

The platelet-activating factor (PAF) represents a phospholipid with a variety of biological functions. Beside others, it is involved in the signaling and activation of proinflammatory cells such as platelets, neutrophils, and macrophages (Prescott et al. 1990) and also was shown to inhibit apoptosis of B cells (Toledano et al. 1999). Furthermore, it has been reported to alter vascular permeability and to induce hypertension, platelet-dependent bronchoconstriction, and smooth-muscle contraction (MIM 145500; MIM 208550) (Miwa et al. 1988). Therefore, PAF is involved in and associated with many inflammatory diseases including the allergic disorders (MIM 147050) (Averill et al. 1992). An increase in PAF levels has been shown during acute asthmatic attacks in children (Hsieh and Ng 1993).

The release and degradation of PAF is controlled by intracellular and plasmatic PAF-acetylhydrolases (PAFAH). PAFAH represent functional antagonists of PAF, and the coding genes count as important candidate genes for atopic diseases (MIM 147050) (Tjoelker et al. 1995*a*). The gene for the plasmatic PAFAH has been mapped to chromosome 6p21.2-p12 (Stafforini et al. 1996), originally assigned as the human leukocyte antigen region. Previously, linkage was found in this region between microsatellite markers and asthma and atopy phenotypes (Ober et al. 1998; Wjst et al. 1999). An acquired deficiency of plasmatic PAFAH (PAFAH) has been shown in patients with systemic lupus erythematosus (MIM 152700) and bronchial asthma (MIM 208550) (Tetta et al. 1990; Tsukioka et al. 1993). In addition, two common loss-of-function mutations in the plasmatic PAFAH gene (situated in exon 9) have been shown to be associated with severe asthma and stroke (Val279Phe [MIM 601367]) and various cardiovascular diseases (Gln281Arg [MIM 208060; MIM 600995]) (Hiramoto et al. 1997; Yamada and Yokota 1997; Yamada et al. 1998; Satoh et al. 1999; Stafforini et al. 1999*a*). To date, however, these loss-of-function mutations were found only in Japanese populations, not in white ones. Two further gene variants, Ile198Thr (exon 7) and Ala379Val (exon 11), have been described in the context of schizophrenia (MIM 181500) (Bell et

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al. 1997). Our interest was to investigate whether any variant of the PAFAH gene found in white populations would be associated with atopy or asthma and would impair the enzymatic activity.

Material and Methods

Screening Population (*German*)

The screening for mutations was performed in a separate population of 50 unrelated individuals recruited randomly without regard to an atopic history. These were not included in any of the following statistical evaluations.

Atopic Population (*German*)

Individuals were recruited in the southwestern part of Germany as described elsewhere (Kruse et al. 1999). An atopic child served as the index patient.

The genotyping and association studies were performed in 260 atopic and nonatopic children (first-degree siblings from 105 families) ranging in age from 6 to 22 years (mean 15 years) who were recruited through two studies (Kruse et al. 1999). Of these children, 77% (199 individuals) were atopic according to the definition stated below, whereas 63% (162 individuals) showed total IgE concentrations >100 kU/liter, 73% (189 individuals) showed specific sensitization to common inhalant allergens, and 11% (29 individuals) were asthmatics. Of the families, 45% presented with at least one unaffected child. The determinations of allele frequencies, linkage disequilibrium, and estimates of Hardy-Weinberg equilibrium were performed in the 105 unrelated eldest children of the families.

Specific IgE was detected by enzyme-linked immunosorbent assays against two mixtures of grass pollens, *Dermatophagoides pteronyssinus, Dermatophagoides farinae,* cat dander, and birch pollens (Magic Lite, Chiron Diagnostics). The cut-off point for a positive test was 1.43 Magic Lite units (Kleine-Tebbe et al. 1992). Detectable IgE to at least one of the four allergens or a positive skin-prick test response to one of the same allergens was the criteria for "any specific sensitization." Measurement of total serum IgE was carried out by an enzyme allergosorbent test (Phadezym, Pharmacia). "Atopy" was defined by at least one positive specific IgE test or by an elevated total IgE concentration >100 kU/liter (Kjellman et al. 1976).

Approval

The collection of serum and DNA material, as well as the experiments, have been approved by the Ethical Commission of the University of Freiburg (June 12, 1994, and August 10, 1996).

Asthmatic Population (*British*)

Details of the selection of the case-control studies for the British population (300 unrelated individuals) were described elsewhere (Gao et al. 1998). With atopy defined as for the atopic population (see above), 150 individuals showed "atopic asthma," 31 were asthmatic without atopic traits, and 119 served as nonasthmatic controls (Gao et al. 1998). The phenotype "asthma" comprises a combination of all asthmatics (181 individuals). Hardy-Weinberg estimates and the determination of allele frequencies were performed separately from the atopic population.

All the asthmatic subjects had specialist physician–diagnosed asthma with (1) recurrent breathlessness and chest tightness requiring ongoing treatment, (2) physician-documented wheeze, and (3) documented labile airflow obstruction with variability in serial peak expiratory flow rates >30%. There were no heavy smokers (>350 pack per year) among the subjects. The phenotype "asthma" consisted of atopic and nonatopic asthmatic patients.

Amplification of Genomic DNA by PCR

DNA was extracted from peripheral blood leukocytes, following standard protocols, and was column purified (DNA midi kit, Qiagen; IsoQuick, Microprobe). We screened for polymorphisms in the entire gene of plasmatic PAFAH (Stafforini et al. 1996), using the appropriate primers to gain ~300-bp fragments (not all primers listed). To amplify the target DNA in the polymorphic regions we used five oligonucleotide primer pairs: exon 2, 5′- TTTCTTGATTTGTCAGCTTA-3′ and 5′-CAACTTCTTGGGGCCCACTTG-3′; exon 4, 5′-CGAAAATAGCTGCTGGAATA-3′ and 5′-AGTTCTTGTTGTTTTCAAGG-3′; exon 7, 5′-ATAAATAATTTTGCTTGTAT-3′ and 5′-TAGGAGCATAACTTGCCAGG-3′; exon 9, 5′-CTATAAATTTATATCATGCT-3′ and 5′-TTTACTATTCTCTTGCTTTAC-3′; and exon 11, 5′-TTTTAAATGTCTTGTTCTCT-3′ and 5′-GGTCATGAAAAAAATAGTTT-3′. PCR was carried out in a volume of 10 μl containing 30 ng DNA, 5 pmol of each primer, 0.06 U *Taq* polymerase (Pharmacia), and 2 mmol dNTP mix with the buffer recommended by the supplier. After denaturation for 5 min at 94°C, 35 cycles were carried out, each cycle consisting of 45 s at 94°C, 90 s at 53°C (exon 2 and exon 11)/ 55°C (exon 4 and exon 7)/ 50°C (exon 9) and 90 s at 72°C. The last synthesis step was extended to 10 min.

Sequencing

Sequencing by the dideoxy chain termination method was performed on an automated sequencer (ALF DNA Analysis System, Pharmacia).

SSCP Analysis

For SSCP analysis (Orita et al. 1989), the amplified products were resolved on nondenaturing 10% or 12% polyacrylamide gels containing 10% glycerol at 20°C for 2 h. The gels were silver-stained as described elsewhere (Hiort et al. 1994). Sequencing was performed for all observed SSCP variants.

Restriction Fragment–Length Polymorphism Analysis

PCR for genotyping was performed with mutated primers introducing a restriction site for RFLP analysis in dependence on the respective genotype: Arg92His, 5′-CGAAAATAGCTGCTGGAATA-3′ and 5′-AGTTCTTGTTGTTTTCAAGG-3′; BclI-RFLP. Ile198Thr: 5′-ATAAATAATTTTGCTTGTAT-3′ and 5′-AGAGCCAAGACTTGTCAGCT-3′; PvuII-RFLP. *Ala*379Val: 5′-GGGAGACATAGATTCAACTG-3′ and 5′-GGTCATGAAAAAAATAGTTT-3′; PstI-RFLP (all from NEB). Restriction was performed in a volume of 10 μl containing 5 μl of the PCR-product and the buffer recommended by the supplier for 90 min at 37°C. The fragments were resolved on 12% polyacrylamide gels. Two known homozygote individuals and one heterozygote individual, as confirmed by sequencing, were included in each reaction. The genotyping was performed by two investigators who were unaware of the phenotypes.

Statistical Analysis

Atopic population.—The number of alleles for each polymorphism was counted in all 260 children of the study populations, as far as the genotype could be determined unequivocally (that is, in 98% of the individuals). To account for the individuals who were siblings and to adjust for age and sex of the study population, regression analysis has been applied. We performed logistic regression analysis and linear regression analysis for the explanation of the phenotypes "asthma," "atopy," and "specific sensitization to common inhalant allergens," treated as a dichotomic parameter, and "total serum IgE," treated as a continuous parameter on a logarithmic scale by the genotypes "presence of the polymorphic allele." Analysis followed the method of Zeger and Liang (1986) to account for the correlation between sibs. The average IgE concentration in individuals bearing one of the polymorphisms is calculated by 10 to the estimate. A P value of .009 was considered significant, to account for multiple testing.

Asthmatic population.—Contingency-table analysis, odds ratios, 95% confidence intervals, and significance values were estimated by computerized methods (SPSS, version 8). For both populations, the probability values were corrected for multiple comparison by multiplying the P values by number of loci compared (Bonferroni correction).

Linkage Disequilibrium

The linkage disequilibrium (Δ) was calculated separately in the atopic and asthmatic population according to the method of Mittal (1976). A t value calculated by Δ/standard error (SE) >1.96 was considered positive. Haplotype frequencies were estimated using the program TRANSMIT, version 2.5 (Clayton 1999; Clayton and Jones 1999).

Preparation of cDNA

mRNA was isolated from human whole blood by use of the RNeasy blood mini kit (Qiagen). The cDNA was synthesized by the "first-strand cDNA synthesis kit" (Amersham/Pharmacia).

Functional Studies

Wild-type cDNA, coding for the mature protein, was amplified by PCR with primers introducing a *Kpn*I (Pharmacia) restriction site at the 5′ and 3′ ends of the coding gene (primers 5′-GCGCGGTACCATACAAGT-ACTGATGGCTGC-3′ and 5′-GCGCGGTACCCTAAT-TGTATTTCTCTATTCC-3′). The fragment was cloned into a bacterial expression vector (pQE; Qiagen) and transformed into *Escherichia coli* (*E. coli*) BL21DE3 pLysS. Positive clones were confirmed by sequencing and by expression analysis according to the supplier's protocols. In the system used, six histidine residues are added to the protein at the N-terminus in order to facilitate purification by affinity-chromatography (Ni-NTA resin, Qiagen).

PCR and primers bearing the polymorphic base pairs were used to produce a total of nine different mutant enzymes: wild type; *His*92 (PAF4); *Thr*198 (PAF7); *Val*379 (PAF11); PAF 4/7; PAF 4/11; PAF 7/11; PAF 4/7/11. The loss-of-function mutation *Phe*279 served as a control. The mutant constructs were confirmed by sequencing.

Kinetic Studies

Enzyme characterizations were performed with equal amounts of purified proteins (according to BioRad Protein assay, BioRad), using the standard enzymatic test (substrate: acetyl-^{3}H-PAF) and recommended reagents (Stafforini et al. 1990). K_M and Vmax were measured in three parallel reactions with increasing substrate concentrations (1/10/50/100 μM), and mean values were calculated. Data was fitted to the Michaelis-Menten equation by nonlinear regression with the program Enzfitter (Biosoft). For substrate inhibition assays we used substrate concentrations of 0.1–1 mM. The results of all

Table 1

Allele Frequencies of Common Polymorphisms in Human Plasmatic PAFAH

	Frequency in Population (%)			
	Atopic (German)		Asthmatic (British)	
Polymorphism	Nonatopic (n = 33)	Atopic (n = 72)	Nonatopic (n = 150)	Atopic (n = 150)
His92	24.1	25.4	23.2	23.4
Thr198	.8	3.7	7.6	11.7
Val379	10.3	21.9	15.2	21.6

kinetic studies are means of at least three independent experiments.

Results

Polymorphisms

Screening for common polymorphisms in the PAFAH gene was performed by SSCP analysis. Twelve PCR-products covered the coding gene as well as the adjacent splice sites. We could not detect the two "Japanese" loss-of-function mutations Val279Phe and Gln281Arg in our white populations; however, we picked up the variants Arg92His (exon 4; position 275; G→A), Ile198Thr (exon 7; position 593; T→C) and Ala379Val (exon 11; position 1136; T→C). In addition, we localized a new, not-yet-described conservative polymorphism in exon 2 that is found at nucleotide position −11 relative to the "ATG" start site, C→T. No polymorphisms were found in the other exons tested.

Allele Frequencies

We determined the frequencies of the three alleles that differed from the published genotype in all groups of individuals, as shown in table 1. All variants were in Hardy-Weinberg equilibrium with both populations (German and British) calculated separately (data not shown). The noncoding polymorphism in exon 2 occurred in <3% of the alleles and was not further investigated.

Linkage Disequilibrium

Linkage disequilibrium between the three pairs of variants was tested in the atopic and the asthmatic population separately, as shown in table 2.

Association Studies

Atopic population.—The polymorphism Ile198Thr showed association with atopic phenotypes, which was highly significant for "total serum IgE" concentrations (P = .0087) (table 3). The variant allele Thr198 was associated with elevated total IgE concentrations. Regarding the parameter estimate, the expected IgE level in the presence of Thr198 was 2.4 times higher than the level in wild-type individuals. The variant allele Val379 also showed association with atopic phenotypes. The most prominent significance was seen for "specific sensitization" (P = .0017; table 3).

Asthmatic population.—The variant allele Thr198 showed significant association with the phenotype "atopic asthma" (P = .008; table 4). The variant Val379 was significantly associated with "asthma" (P = .003; table 4).

Functional Studies

These studies were performed using purified preparations of recombinant enzymes; an example is shown in figure 1. We detected a K_M of 7 μM for the wild-type enzyme and a Vmax of 180 μmol/min × mg. The data presented from the kinetic studies are mean values; av-

Table 2

Linkage Disequilibrium between the Variants in Both Populations

Population	His92/Thr198 (Δ/SE)	Thr198/Val379 (Δ/SE)	His92/Val379 (Δ/SE)
Atopic (German)	1.93	2.22[a]	3.04[a]
Asthmatic (British)	1.01	2.24[a]	6.41[b]

Note.—Linkage disequilibrium (Δ) was tested according to the method of Mittal (1976). A t value calculated by Δ/SE > 1.96 was considered positive. Substantial linkage disequilibrium was seen for Thr198/Val379 and His92/Val379 in both populations.

[a] $P < .05$.

[b] $P < .01$.

Table 3

Association Studies of Common Variants in Plasmatic PAFAH with Atopy and Asthma—Atopic Population (German)

	Atopy			Specific Sensitization			log (IgE)		
Polymorphism	Odds Ratio	95% CI	*P* Value	Odds Ratio	95% CI	*P* Value	Parameter Estimate	95% CI	*P* Value
His92	1.01	.54/1.88	.98	.98	.54/1.81	.97	−.04	.14/−.22	.66
Thr198	4.89	.79/30.1	.087	5.84	1.02/33.36	.047	.38	.098/.67	.0087
Val379	2.42	1.13/5.15	.022	3.25	1.56/6.78	.0017	.14	.033/.33	.11

Note.—Calculations for the phenotype "atopy" were performed with 199 affected and 61 control individuals, for the phenotype specific sensitization with 189 affected and 71 control individuals and for the phenotype " total serum IgE (log IgE)" with 162 affected and 98 control individuals. Analysis followed the method of Zeger and Liang (1986) to account for the correlation between sibs. The average IgE concentration in individuals bearing one of the polymorphisms is calculated by 10 to the estimate. A *P* value of .009 was considered significant to account for multiple testing. Significant associations (underlined) were seen for Thr198 (log IgE) and Val379 (specific sensitization).

erage standard errors in all experiments were 1–2 μM for K_M and +/− 20 μmol/min × mg for *V*max.

The presence of the variant Val379 revealed a two-times increased K_M value (14 μM) (table 5). The variant Thr198 showed a six-fold higher K_M value (42 μM). This effect was also seen in almost all combined mutated enzyme forms bearing this polymorphism (table 5). *V*max values were increased for the Val379 variant (132%) (table 5). As expected from the literature, the enzyme form bearing the polymorphism Val279Phe was almost inactive and served as a control in activity tests. Compared to the wild-type enzyme, lower substrate concentrations were necessary to inhibit the activities of the recombinant enzyme Thr198, and markedly lower concentrations inhibited His92 and Val379 (fig. 2).

Discussion

Search for Polymorphisms and Association Studies

The aim of this study was to test for common polymorphisms in plasmatic PAFAH in white populations and their potential association with asthmatic or atopic phenotypes. This seemed reasonable regarding the involvement of PAF in the attraction of eosinophils and lymphocytes as the basis of IgE mediated responses (Simon et al. 1994; Bartemes et al. 1999) and inflammation and bronchoconstriction as the additional basis of bronchial asthma (Miwa et al. 1988; Prescott et al. 1990). Furthermore, an association of two loss-of-function mutations of PAFAH with asthma severity has been known (Stafforini et al. 1999*a*). However, these latter two mutations are known only from Japanese individuals (Satoh et al. 1999; Stafforini et al. 1999*a*) and also had not been detected in a previous study screening a British population (Bell et al. 1997).

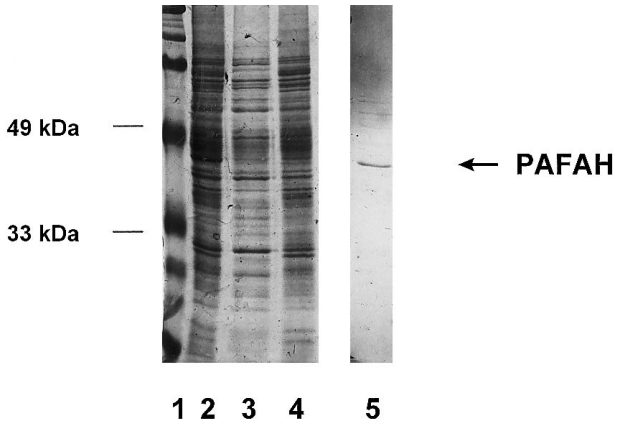

Figure 1 Purification of pPAFAH by affinity chromatography on Ni-NTA columns, shown for the wild-type enzyme. *Lane 1,* Molecular weight standard. *Lane 2,* Crude cell extract. *Lane 3,* Flow-through. *Lane 4,* Wash. *Lane 5,* Purified enzyme.

All mutations found had already been known from the literature, and all three of them had been used in an association study to schizophrenia (Bell et al. 1997). The polymorphism preceding the ATG start codon at position −11 is described here for the first time. Possible functional implications have not yet been tested.

With respect to atopy, association studies were applied in a population of children and young adults aged 6–22 years to optimize the discrimination between affected and nonaffected individuals. This age group represents the most reliable test population, eliminating the possibility of sensitization being not yet or no longer detectable. The atopic population was recruited through an atopic index individual, without regard to clinical symptoms.

To elucidate the influence of the PAFAH variants on clinical asthma, the typing was repeated in a British population recruited through asthmatic index patients. In our studies, the most prominent effect was seen for the variant allele Thr198, which was associated with all phenotypes of "atopy" in the German population, whereby the highest significance was found for total IgE levels (the presence of the allele leading to 2.4 times higher IgE levels; see table 3). The same accounts for the British population, where Thr198 was found to be highly associated with the phenotype "atopic asthma" (table 4).

Table 4

Association Studies of Common Variants in Plasmatic PAFAH with Atopy and Asthma—Asthmatic Population (British)

	Atopic Asthma			Asthma		
Polymorphism	Odds Ratio	95% CI	*P* Value	Odds Ratio	95% CI	*P* Value
His92	1.15	.71/1.76	.64	1.11	.69/1.77	.68
Thr198	2.26	1.22/4.15	.008	1.49	.81/2.76	.20
Val379	1.67	1.02/2.71	.038	2.20	1.32/3.70	.003

Note.—Calculations for the phenotype "atopic asthma" were performed with 150 affected and 150 control individuals, for the phenotype "asthma" with 181 affected and 119 control individuals. Analysis was performed with the SPSS program (version 8) involving a Bonferroni correction (see Material and Methods). A *P* value of .009 was considered significant to account for multiple testing. Significant associations (underlined) were seen for Thr198 (atopic asthma) and Val379 (asthma).

(Again, the presence of the allele leading to a 2.3-times-higher odds ratio.) Likewise, the variant allele Val379 was associated with "atopy" in the German population (table 3), showing the most prominent effects with "specific sensitization" and with "asthma" in the British population, including patients suffering from atopic and intrinsic asthma (table 4). The importance of the two variants is stressed by the finding that the allele frequencies are much higher in affected than in nonaffected individuals in both populations (table 1). The polymorphism Arg92His does not seem to be associated with atopy or asthma.

One should bear in mind that substantial linkage disequilibrium between the variants has already been published (Bell et al. 1997) and was shown in this study also (table 2). That this was most prominent between His92 and Val 379 but not between His92 and Thr198 was observed employing different statistical approaches and might be due only to the low frequency of the Thr198 allele. It might, however, also reflect a functional link between His92 and Val379, with the presence of both of them influencing the phenotype. This would be equivalent to the findings on decreased *V*max for His92/Val379 (PAF 4/11), as shown in table 5. A similar phenomenon of potential functional links has been described for IL4Rα, FcεRIβ and IL-4 variants (Adra et al. 1999; Suzuki et al. 1999; Shirakawa et al. 2000). We suspected at least Thr198 and Val379 having an influence on enzyme characteristics. In order to prove our assumptions, functional studies were applied.

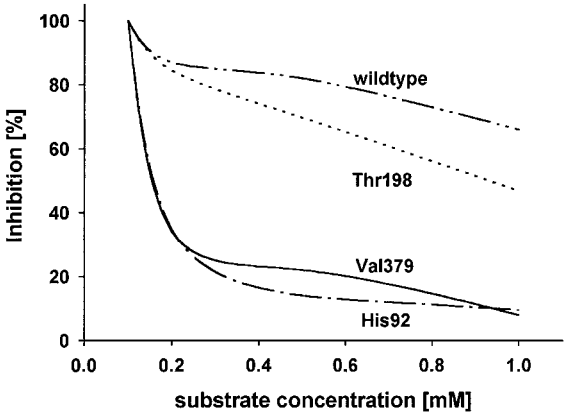

Figure 2 Substrate inhibition assay with substrate concentrations of 0.1–1 mM. Inhibition is shown in percent of the maximum activity of the enzyme forms (= mean values of three independent experiments). All polymorphic enzyme forms showed an increased substrate inhibition compared to the wild type (wt). Dash-point-dash line: wt; dash line: Arg92His (PAF 4); dotted line: Ile198Thr (PAF 7); straight line: Ala379Val (PAF 11).

Functional Studies

These studies were performed with different variant-combinations of recombinant enzymes. Previously, it has been shown that cloning and expression of plasmatic PAFAH in *E. coli* does not alter the overall enzyme structure and activity (Tjoelker et al. 1995*b*).

Our kinetic experiments revealed a K_M of 7 μM for the wild-type enzyme and a *V*max of 180 μmol/min × mg. These values are comparable to the ones found for purified PAFAH from LDL, namely 12 μM and 170 μmol/min × mg (Tew et al. 1996). Slight alterations might be due to the additional six histidine molecules in our expression system or to the different purification procedure.

Compared to the wild-type enzyme the molecular activity (*V*max) was increased for Val379 (table 5), which means that the turnover of substrate is increased. PAFAH possessing Thr198 alone revealed no reduction in activity. This corresponds exactly to findings that were recently made by Stafforini et al. (1999*a*). The combinations of variants showed different effects concerning *V*max (table 5), probably because of different influences on enzyme conformation and the active site.

Table 5

Kinetic Studies Performed with Recombinant Enzymes of PAFAH

	Parameter	
Protein	K_M (μM)	*V*max (%)
Wild type	7	100
His92 (PAF4)	9	108
Thr198 (PAF7)	42	100
Val379 (PAF11)	14	132
PAF 4/7	11	128
PAF 4/11	8	80
PAF 7/11	41	120
PAF 4/7/11	50	105
Phe279	Inactive	Inactive

Note.—Kinetic studies were performed with equal amounts of nine different recombinant proteins produced by PCR (Vector pQE [Qiagen]; expressed in *E.coli* BL21DE3pLysS). Proteins were affinity purified. Enzymatic tests were performed by means of the standard test (Stafforini et al. 1990), and the substrate acetyl-^{3}H-PAF. K_M and *V*max were measured in three parallel reactions with increasing substrate concentrations (1/10/50/100 μM). Mean values were calculated. The protein Phe279 (loss of function mutation) served as a control. Data were fitted to the Michaelis-Menten equation by nonlinear regression with the program Enzfitter (Biosoft). *V*max values are shown as percentage of the wild-type level (wild type = 100%). Thr198 and Val379 (alone and in combined forms) showed highly increased K_M, Val379 a markedly increased *V*max.

The enzyme bearing the variant Thr198 showed a six-fold higher K_M value, Val379 revealed a two-fold higher K_M (table 5), which means a decreased substrate affinity. This correlates with the association studies for Thr198 indicating total IgE levels being increased by a factor of 2.4 (positive parameter estimate of 0.38; table 3). In both cases, PAF is degraded more slowly, and, consequently, the signal transduction is prolonged. Finally, elevated PAF concentrations might result in prolonged B cell survival (Toledano et al. 1999) and, consequently, higher IgE levels or development of asthmatic symptoms. An increase in PAF levels during acute asthmatic attacks has been shown previously (Hsieh and Ng 1993). The striking K_M effect was also seen in the combined mutant-enzyme forms Thr198/Val379 and His92/Thr198/Val379. An only slightly increased K_M was seen for the form His92/Thr198, which might be explained by conformational neutralization of the Thr198 influence (*cis* effect). Presumably, individuals with two different alleles (His92 and Thr198) will show an addition of influences of the polymorphisms (*trans* effect) different from the His92/Thr198 effect (*cis* effect). On the whole, His92, not being associated with any phenotype tested, showed wild-type enzyme characteristics regarding K_M and *V*max.

Substrate inhibition assays indicated alterations for the variants His92, Thr198, and Val379. The enzyme forms revealed an increased substrate inhibition compared to the wild type, which would imply that lower substrate concentrations are needed first to reach *V*max and then again to inhibit the activity. The most striking effects are seen for His92 and Val379. However, strictly speaking, these results can be considered only from an academic point of view, which means showing mere differences in enzyme characteristics of the different variants in vivo. The range of substrate concentrations used for this assay are far above physiological concentrations of PAF measured in plasma (Stafforini et al. 1990). We postulate conformational changes in the protein structure in case of any polymorphic status, and, at least for the variants Thr198 and Val379, possible influences on the active site leading to the altered enzyme characteristics.

As indicated, we could not find an association of His92 with IgE concentrations, although we saw functional effects—for instance, in substrate inhibition assays. One should bear in mind that all associations studied were performed in individuals with complex genotypes regarding the PAFAH gene and that association testing for any variant also covers adjacent variants as long as they are in linkage disequilibrium. Arg92His has been shown to be in such linkage disequilibrium with Ile198Thr as well as Ala379Val (see above). Negative association results might therefore reflect linkage rather than missing functional effects, especially in the combined enzyme forms (table 5).

The polymorphism Ile198Thr in human plasmatic PAFAH is located near tyrosine 205, which has been proposed to be important for LDL binding (Stafforini et al. 1999*b*). The same accounts for the Trp115 and Leu116, which are in the vicinity of Arg92His.

PAFAH circulates in a complex with LDL and HDL in human plasma. These lipoproteins determine its catalytic activity in vivo. The LDL-associated form of PAFAH is responsible for all of the PAF hydrolysis (Stafforini et al. 1999*b*). Therefore, it might well be that especially Ile198Thr alters LDL binding and consequently influences PAF degradation. Further studies on the three-dimensional structure of plasmatic PAFAH might help to test for this hypothesis. Ala379Val is situated in the vicinity of His351, one of the amino acids—along with Ser273 and Asp296—belonging to the catalytic triad characteristic of phospholipases (Tjoelker et al. 1995*b*), so the variant might influence enzymatic activities.

In conclusion, Thr198 and Val379, the two common variants of the plasmatic PAFAH, have been shown to

be of functional importance, which parallels their association with atopy and asthma. The basis of these findings might be found in the influence of PAF on IgE regulation as well as on inflammatory processes and bronchoconstriction. This would also explain the previously described associations of these variants with other inflammatory diseases. Finally, a suspected direct role of human plasmatic PAFAH in the pathogenicity of atopy and asthma in whites has been strengthened and is not restricted to the presence of the two known loss-of-function mutations.

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nim.nih.gov/Omim (for hypertension [MIM 145500], asthma [MIM 208550], atopic IgE responsiveness [MIM 147050], lupus erythematosus [MIM 152700], cerebrovascular accident [MIM 601367], arteriosclerosis [MIM 208060], nephrotic syndrome [MIM 600995], and schizophrenia [MIM 181500])

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