European Journal of Human Genetics (2001) 9, 780–786

www.nature.com/ejhg

ARTICLE

Serum myeloperoxidase concentration in a healthy population: biological variations, familial resemblance and new genetic polymorphisms

Aline Hoy[1], David Trégouët[2], Brigitte Leininger-Muller[1], Odette Poirier[2], Mickaël Maurice[3], Catherine Sass[3], Gérard Siest[1], Laurence Tiret[2] and Sophie Visvikis*[,3]

[1]*Faculté de Pharmacie, INSERM Unité 525, Nancy, France;* [2]*Faculté de Médecine Pitié-Salpêtrière, INSERM Unité 525, Paris, France;* [3]*Centre de Médecine Préventive, INSERM Unité 525, Vandoeuvre-Les-Nancy, France*

Myeloperoxidase (MPO) has been involved in the pathogenesis of several diseases through excessive production of reactive oxygen species (ROS) as well as through its genetic polymorphism. The aims of this study were to identify the factors affecting MPO serum concentration, to study the familial resemblance of MPO levels and to investigate the association between newly described MPO polymorphisms as well as the G-463A one and MPO levels in a healthy population. MPO serum concentrations were measured by an enzymatic immuno-assay (EIA) in 82 healthy families of the STANISLAS Cohort and MPO genotype, determination was performed using PCR-restriction fragment length polymorphism or allele specific oligonucleotide assay. MPO concentrations were significantly higher in parents than in offspring. The factors affecting MPO levels were age, the number of white cells, smoking in fathers and oral contraceptive intake in mothers. They explain from 12.4% up to 35.9% of MPO variability in men and women, respectively. Family correlations of MPO concentrations were of similar magnitude. The -129A allele of a newly described G-129A substitution was significantly associated with decreased MPO levels, whereas the -463A allele was suggested to be associated with increased levels of lipid variables. In this study, we identified factors affecting MPO serum concentrations and showed that molecular variations of the gene have only a weak influence on MPO variability. In contrast, the association between the G-463A polymorphism and lipid levels would suggest a possible implication of MPO in the risk of cardiovascular diseases. These results have to be confirmed and further investigations will be conducted in that way. *European Journal of Human Genetics* (2001) **9,** 780–786.

Keywords: myeloperoxidase; serum; concentration; polymorphism; variation factors

Introduction

Myeloperoxidase (MPO, EC 1.11.1.7) is a glycoprotein present in azurophilic granules of polymorphonuclear neutrophils (PMNs).[1] In the presence of chloride ions and hydrogen peroxide produced by PMNs during their activation, MPO catalyses the formation of hypochlorous acid, a potent microbicidal agent. This enzymatic system plays an important role in human defense against micro-organisms.[2]

Even if neutrophils are responsible for the protection of the organism, they can also be harmful to host tissues, and diffusion of components of the MPO system often leads to tissue damage.[3] The oxidative processes implicated in the destruction of micro-organisms can act on adjacent host tissues and they are thus involved in the pathogenesis of various diseases associated with excessive leukocytes activation.

In acute inflammatory diseases like infections,[4] the stimulation of leukocytes leads to the release of MPO into the extracellular medium and its plasma concentration may

*Correspondence: S Visvikis, INSERM Unité 525 équipe 4, Centre de Médecine Préventive, 2 avenue du Doyen Jacques Parisot, 54 501 Vandoeuvre-Les-Nancy, France. Tel: +33 3 83 44 87 25; Fax: +33 3 83 44 87 21; E-mail: Sophie.Visvikis@cmp.u-nancy.fr
Received 20 March 2001; revised 8 June 2001; accepted 29 June 2001

therefore be considered as a specific index of leukocytes activation.

The implication of MPO has been especially reported in atherosclerosis where MPO has been suggested to oxidize low-density lipoproteins (LDLs) responsible for the initiation of the disease.[5] The detection of active MPO as well as specific markers of MPO-catalysed oxidation products in human atherosclerotic lesions supports this hypothesis.[6] This enzyme has also been involved in stroke where the measurement of its activity constitutes a marker of PMNs accumulation in the cerebral parenchymal tissue.[7] This infiltration of neutrophils is not devoid of side-effects. Indeed, experiments of neutrophils depletion have proved the implication of PMNs in brain swelling.[8] Involvement of MPO in ischaemic stroke is also supported by the work of Re *et al*,[9] who reported increased levels of markers of oxidative stress and more particularly of plasma MPO activity in patients with brain ischaemia. MPO is also involved in hypertension, one of the main risk factors for stroke,[10] as demonstrated in a recent study conducted in patients with essential hypertension who displayed an elevated leukocytes count and a faster release of superoxide anion from PMNs than in controls.[11]

The involvement of MPO in inflammatory diseases has been supported by genetic studies reporting an association with the G-463A polymorphism located in the promoter region of the MPO gene. The -463G allele creates a SP1 transcription factor binding site which increases the promoter activity in transfection assays.[12] The *GG* genotype has been associated with increased MPO expression in myeloid leukaemia cells.[13] This genotype has also been related to acute promyelocytic leukaemia,[13] earlier onset of Alzheimer's disease (AD)[14] and multiple sclerosis (MS)[15] in women, while the *A* allele has been reported to be associated with a decreased risk of lung cancer.[16] Given the growing implication of MPO in several diseases, it seems justified to hypothesise that MPO could constitute a marker of cardiovascular diseases. However, the assessment of MPO as a biological marker requires the knowledge of its biological variations, which are poorly documented. Only one study showed an increase in MPO enzymatic activity in isolated murine neutrophils during development.[17] Others reported differences with gender[18] and smoking,[19,20] by measuring the mean peroxidasic index (MPXI) of neutrophils by an automated haematological system. MPO can also be quantified by EIA but, to our knowledge, biological variations of serum MPO concentrations have never been investigated so far.

The aims of this study were to identify the factors affecting MPO serum concentrations measured by EIA, to study the familial resemblance of MPO levels and to investigate the association between polymorphisms of the MPO gene and MPO levels in a sub-sample of the STANISLAS Cohort. For this purpose, a molecular screening of the gene was first performed to identify all common polymorphisms in the coding and regulatory sequences of the gene. The newly identified polymorphisms as well as the G-463A polymorphism were then tested for the association with MPO serum concentrations.

Materials and methods

Subjects and data collection

Families were recruited between October 1993 and June 1994 in the Center for Preventive Medicine in Nancy (France) as a sub-sample of the 1000 families of the STANISLAS Cohort.[21] The study sample included 82 nuclear families of European origin volunteering for a free health examination and having signed a statement of informed consent. Families were composed of both natural parents ($n=2\times82$) and at least two offspring ($n=188$) aged more than 6 years. The data collection was obtained by physical examination, functional tests, screening of blood constituents, life-style description and socio-professional questionnaire.

Preparation of blood samples and analysis

Blood was collected by venipuncture (after overnight fasting) in Vacutainer® Tubes containing a gel for serum separation and EDTA for buffy coat preparation. The sera and buffy coat were aliquoted and frozen in liquid nitrogen until analysis. White blood cell counts were performed on Coulter MAXM analyzer (Beckman Coulter, France). Total cholesterol, triglycerides and glucose were measured using standard enzymatic techniques (Merck, Germany) automated on AU5000 (Olympus, Japan). HDL-cholesterol level was quantified using enzymatic measurement in supernatant obtained following precipitation with $MgCl_2$/phosphotungstate, on a Cobas-Mira Centrifugal Analyzer (Roche Diagnostic Systems, France). LDL-cholesterol level was calculated according to the Friedewald equation. Serum apoA-I and apoB were determined by immunonephelometry on Behring Nephelometer Analyzer. Serum MPO concentrations were measured with the MPO-EIA kit (Bioxytech, France). Briefly, the amount of MPO in each sample was measured enzymatically after addition of p-nitrophenyl phosphate by colorimetric reading at a wavelength of 405 nm. A standard curve was generated using human purified MPO. The assay sensitivity was 1.5 ng/ml and the results were expressed in nanograms per milliliter of serum. Intra-individual day-to-day variability was 5%.

Screening of the MPO gene for detection of polymorphisms

The molecular screening of the MPO gene was performed by comparing 190 chromosomes from 95 unrelated patients selected from the extensions of the ECTIM (Etude Cas-Témoin sur l'Infarctus du Myocarde) study.[22] Each of the 95 patients had suffered a myocardial infarction (MI) at a young age ($\leqslant$60 years for males and $\leqslant$65 years for females)

according to the MONICA diagnostic category I over the study period (December 1988 – June 1991) and had a parental history of MI at a young age (same criteria as above). This sample size provided approximately 100% power to detect alleles with a frequency >0.05 and a 85% power to detect alleles with a frequency of 0.01. Genomic DNA was prepared from white blood cells by phenol extraction. DNA-sequence variations were identified by PCR/SSCP and followed by sequencing, as previously described.[23] The entire exonic sequence and the intronic sequences flanking exons, 369 bp in the 5′ region and 42 bp in the 3′UTR region of the MPO gene were explored.

Genotyping of the MPO polymorphisms in the STANISLAS sample

New polymorphisms were genotyped in all subjects of the STANISLAS sample using allele specific oligonucleotides[24] (ASOs). All informations for genotyping polymorphisms (PCR primers, probes, conditions of hybridation and amplification) can be found at our Internet site (http://genecanvas.idf.inserm.fr). The G-463A polymorphism was amplified by polymerase chain reaction-restriction fragment length polymorphism (PCR – RFLP) with few modifications of the method described by London *et al*.[16]

Statistical analysis

Statistical analysis was performed with the SAS software (SAS Institute Inc., USA). As individuals within a family are not independent, statistical analyses were based on the estimating equation (EE) technique,[25,26] using the SAS GENMOD procedure with a repeated statement.

Serum MPO concentrations were adjusted on age separately in each group of relatives (fathers, mothers, sons and daughters) and additionally on smoking in fathers and on oral contraception in mothers. Pearson correlation coefficients were calculated between MPO concentrations and biological and anthropometric variables in each group of relatives, and were tested by means of the EE technique. Family correlations for MPO concentrations were estimated by using a maximum likelihood method.[27]

Hardy-Weinberg equilibrium for each polymorphism of the MPO gene was tested in parents by a χ^2 test with one df, and pairwise linkage disequilibrium coefficients D′ between polymorphisms were estimated using a log-linear model analysis.[28] Haplotype frequencies were estimated using the Arlequin package.[29] Associations between polymorphisms of the MPO gene and serum MPO concentrations or lipid levels were investigated by linear regression analysis. In case an association is suggested, a TDT-type analysis was further applied to confirm that the observed association was really due to linkage disequilibrium and not to other uncontrolled phenomenon. Such analysis was performed using the extension of the quantitative TDT model of Allison[30] we recently proposed.[31] Statistical significance was taken at $P<0.05$.

Results

General characteristics of the studied population are given in Table 1.

Serum MPO levels

MPO levels were significantly higher in parents (mean: 46.3 ng/ml; range: [5.4 – 141.6]) than in offspring (mean: 37.4 ng/ml; range: [6.0 – 132.1]) ($P<10^{-3}$), as well as in fathers as compared to mothers ($P<0.05$). The same trend according to gender was observed in offspring, although not reaching significance. Figure 1 shows the variation of MPO concentrations according to age in each group of relatives. In offspring of both sexes, MPO concentrations increased with age up to 15 – 18 years and then decreased to reach a level close to that measured in younger parents (<40 years). Age explained 10.5% and 15.5% of MPO variability in sons and daughters, respectively. In fathers, MPO levels slightly increased with age while in mothers, the increase was steeper. Age explained 2% and 15.7% of MPO variability in fathers and mothers, respectively.

In fathers, MPO levels were higher in smokers than in non-smokers (59.7 ng/ml versus 48.8 ng/ml, $P<0.02$). The percentage of variability additionally explained by smoking was 6.3% in fathers. In mothers, MPO levels were increased by oral contraception uptake (50.3 ng/ml versus 40.1 ng/ml, P=0.03). The percentage of variability additionally explained by oral contraception was 4.7% in mothers. In further analyses, MPO levels were adjusted on age (and age^2 when necessary) in all classes of relatives, smoking in fathers and oral contraception in mothers.

Correlation coefficients of MPO levels with biological and anthropometric variables were estimated for each class of relatives. As expected, MPO levels strongly correlated with white cell counts, the correlation being especially high in mothers and sons (ρ=0.17, ρ= 0.42, ρ= 0.43, ρ= 0.24 in fathers, mothers, sons and daughters, respectively). There was no significant correlation with any of the other parameters studied. The total percentage of MPO variability explained by age, smoking, oral contraception and white cell counts was 12.4, 35.9, 26.8 and 20.5% in fathers, mothers, sons and daughters, respectively.

There was a significant familial resemblance of MPO levels (ρ_{FM}=0.29 $\pm$ 0.10 between spouses, ρ_{PO}=0.26 $\pm$ 0.06 between parent and offspring, and ρ_{SS}=0.32 $\pm$ 0.08 between offspring; χ^2= 4.72 with 3 df, $P<10^{-3}$). The three familial correlation coefficients were not significantly different (χ^2=0.7 with 2 df, P=0.70), and they were not modified by the adjustment on white cell counts.

MPO gene polymorphisms

Five new polymorphisms were identified by molecular screening of the MPO gene: a G/A substitution located at position -129 upstream from the transcription start site (G-129A), abolishing a SP1 site; three substitutions in intronic regions, at positions -13 in 5′ of exon 2 (C-13/in1T), +27 in 3′

Table 1 Characteristics of the population

	Fathers (n = 82)	*Mothers* (n = 82)	P	*Sons* (n = 92)	*Daughters* (n = 96)	P
Age, years	42.1 (4.4)	40.3 (3.9)	<.001	12.9 (3.8)	13.8 (3.9)	ns
Alcohol consumption, g/day	22.2 (25.3)	4.67 (8.83)	<.001	1.00 (7.28)	0.18 (1.19)	ns
Current smokers, %	32.9	18.3	<.01	5.4	13.5	ns
BMI, kg/m^2	24.9 (3.0)	23.3 (3.8)	<.001	18.7 (3.0)	19.1 (3.3)	ns
WHR	0.89 (0.06)	0.76 (0.06)	<.001	0.81 (0.04)	0.74 (0.05)	<.001
Total cholesterol, g/l	2.33 (0.44)	2.14 (0.37)	<.01	1.78 (0.33)	1.88 (0.32)	.05
HDL cholesterol, g/l	0.49 (0.14)	0.62 (0.18)	<.001	0.52 (0.15)	0.55 (0.14)	ns
LDL cholesterol, g/l	1.62 (0.39)	1.37 (0.36)	<.001	1.16 (0.28)	1.20 (0.29)	ns
Triglycerides, g/l*	1.01 (0.53)	0.76 (0.46)	<.001	0.56 (0.24)	0.61 (0.41)	ns
Glucose, mmol/l	5.35 (0.55)	4.91 (0.36)	<.001	4.98 (0.40)	4.88 (0.36)	ns
apoA-I, mmol/l	1.55 (0.24)	1.72 (0.28)	<.001	1.46 (0.23)	1.54 (0.23)	<.01
apoB, mmol/l	1.14 (0.28)	0.98 (0.25)	<.001	0.81 (0.22)	0.85 (0.18)	ns
White cells, $\times 10^3/\mu$l	7.30 (1.99)	7.18 (1.93)	ns	7.04 (1.88)	7.37 (1.78)	ns
MPO, ng/ml	49.7 (27.6)	42.8 (20.7)	<.05	39.3 (24.9)	35.6 (17.3)	ns

ns indicates not significant. Values are mean (SD). *Tests are performed on log-transformed values.

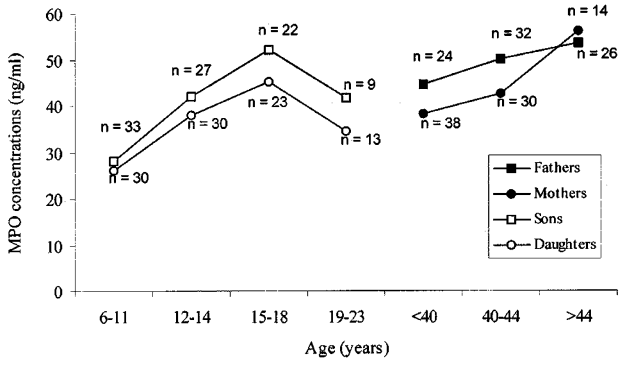

Figure 1 Variations of MPO levels according to age and sex.

of exon 11(G+27/in11A), and −6 in 5′ of exon 12 (A-6/in11C); one synonymous polymorphism affecting the amino acid 711 (C/T→Thr711Thr). All details on these polymorphisms can be found at our web site.

Four polymorphisms were studied in the STANISLAS sample: the G-129A, C-13/in1T and A-6/in11C polymorphisms, and the G-463A polymorphism, already described.[12] No mutant was detected for the Thr711Thr polymorphism. The G+27/in11A polymorphism was not genotyped because of lack of DNA. Genotyping success rate ranged from 97.1% for the A-6/in11C polymorphism to 100% for the G-463A polymorphism.

Allele frequencies and pairwise linkage disequilibrium coefficients between polymorphisms of the MPO gene are given in Table 2. All genotype distributions were compatible with Hardy-Weinberg equilibrium. The G-129A polymorphism was rather rare (minor allele frequency 0.07), other allele frequencies being in the range 0.15–0.25. All polymorphisms were in strong linkage disequilibrium one with each other, except for G-463A and A-6/in11C, which were the most distant. This pattern of linkage disequilibrium resulted in the observation of only six haplotypes amongst the 16 possible (data not shown).

Association between MPO gene polymorphisms and serum MPO levels

In the whole population, the G-129A polymorphism was significantly associated with serum MPO concentrations, the *A* allele being associated with lower levels (Table 3). The G-129A polymorphism explained 2.6% of the variance in the entire population, after adjustment for class of relatives, age, smoking and oral contraception. The association was maintained after further adjustment on white cell counts and the R^2 was unmodified. Similar observations were made when considering parents and offspring separately. In offspring, mean MPO levels were 27.4 ng/ml in *GA* heterozygotes versus 38.9 ng/ml in *GG* homozygotes (R^2=3.6%; $P<10^{-2}$) whereas the difference did not reach significance in parents (39.9 ng/ml versus 47.2 ng/ml, *P*=0.10). The significant effect of the *A* allele was further confirmed by the TDT-type analysis (*P*=0.047). No relationship was observed with any of the other polymorphisms.

Association between MPO gene polymorphisms and lipid variables

In the whole population, the G-463A polymorphism was significantly associated with apoB (R^2=3.5%), total cholesterol (R^2=3.3%), LDL-cholesterol (R^2=2.6%) and triglyceride levels (R^2=4.3%), the *A* allele being associated with increased levels of these parameters. The same trends were observed in parents and offspring, although associations were stronger in offspring. There was no significant association with HDL-cholesterol (Table 4). Unfortunately, the associations between the G-463A polymorphism and lipid parameters were not confirmed by the TDT-type analysis (*P*-value ranging from 0.19 to 0.38). None of the other polymorphisms was associated with lipid parameters.

Discussion

In the present study, we have evidenced the main factors influencing MPO serum concentrations in a healthy popula-

tion. The familial feature of our population also allowed us to study the familial resemblance of MPO concentration. Moreover, to date, this is the first report investigating the association between MPO polymorphisms and its serum concentration.

The first step of this work consisted of screening the whole regulatory and coding sequences of the MPO gene for polymorphisms. Five polymorphisms were detected, one in the 5′ flanking regions of the gene, three in its intronic flanking regions and one in its coding sequence. These observations were then compared to the information provided in public databases (http//www.ncbi.nlm.nih.gov, http://hgbase.cgr.ki.se, http://archive.uwcm.ac.uk/). None of the five polymorphisms identified in our screening nor the previously described G-463A polymorphism were found in these databases. Other polymorphisms were, however, available in these databases but not identified in our study. Three of them were located in regions we did not explore (two A/T substitutions in intron 7 at nucleotide 6747 and 6750, and one C/T substitution in the 3′ region at nucleotide 13700). Four additional non-synonymous polymorphisms (A/G→Tyr173Cys; T/C→Met251Thr; C/T→Arg569Trp, A/G→Ile717Val) were also found in these databases. The first three of these nonsense mutations were only observed in patients suffering from hereditary myeloperoxidase deficiency.[32–34] As regard the Ile717Val polymorphism described by Wang *et al*,[35] it cannot be ruled out that this polymorphism is not a real one since many polymorphisms described in public databases have still not be confirmed.

Table 2 Allele frequencies and pairwise linkage disequilibrium coefficients between polymorphisms of the MPO gene in parents ($n=164$)

Polymorphism	*Allele frequency*	*G-129A*	*C-13/in1T±D′*	*A-6/in11C*
G-463A	.773/.227	-1.00^{ns}	$+1.00^{***}$	$+.12^{ns}$
G-129A	.935/.065		-1.00^{ns}	-1.00^{*}
C-13/in1T	.845/.155			-1.00^{**}
A-6/in11C	.804/.196			

ns: not significant. *$P<0.05$; **$P<0.01$; ***$P<0.001$.

Table 3 Association between polymorphisms of the MPO gene and MPO serum levels in the whole population

Polymorphism	*Genotype*			*Test*
G-463A	GG	GA	AA	
	$n=204$	$n=131$	$n=16$	
	42.8 (23.3)	39.5 (18.6)	42.0 (27.3)	$P=0.42$
G-129A	GG	GA	AA	
	$n=302$	$n=43$	$n=1$	
	42.7 (22.5)	33.0 (14.8)	18.4	$P=0.002$
C-13/in1T	CC	CT	TT	
	$n=244$	$n=97$	$n=6$	
	42.3 (22.9)	38.9 (18.0)	47.3 (34.8)	$P=0.31$
A-6/in11C	AA	AC	CC	
	$n=221$	$n=110$	$n=13$	
	40.3 (22.2)	44.1 (21.1)	37.9 (25.9)	$P=0.25$

Means (SE) adjusted for class of relatives, age, smoking in men, and oral contraception in women. For the G-129A polymorphism, the *AA* homozygote was pooled with *GA* heterozygotes for testing.

In the literature, quantification of MPO is often reported as the measurement of MPO enzymatic activity in neutrophils *via* the MPXI calculated on an automated haematological analyser. EIA-determined serum MPO concentrations represent circulating levels of MPO released from neutrophils. Except exercise practice[36] and interferon treatment,[37] no data about variations of serum MPO levels measured by EIA have been reported.

The main factors influencing MPO serum concentration which we evidenced in our work were age, the number of white cells, tobacco consumption in fathers and oral contraceptive intake in mothers. These observations remained significant even after adjustment on white cell counts.

MPO serum concentrations were significantly higher in parents than in offspring which would suggest an increase in MPO levels during the development. These results were consistent with the work of Mohacsi *et al*[38] who reported an increase in MPO release from the PMNs during the aging process. Other authors demonstrated reduced neutrophil function and thus enzyme release in elderly or middle-aged

Table 4 Association between the G-463A polymorphism of the MPO gene and lipid parameters

	All			*Parents*			*Offspring*		
	GG n = *204*	*GA* n = *131*	*AA* n = *16*	*GG* n = *96*	*GA* n = *60*	*AA* n = *8*	*GG* n = *108*	*GA* n = *71*	*AA* n = *8*
apoB	0.92 (0.25)	0.95 (0.28) $P<10^{-3}$	1.10 (0.26)	1.04 (0.26)	1.05 (0.31) $P=0.04$	1.23 (0.29)	0.80 (0.19)	0.85 (0.21) $P=0.003$	0.96 (0.11)
Total Cholesterol	1.98 (0.41)	2.07 (0.44) $P<10^{-3}$	2.22 (0.32)	2.19 (0.37)	2.29 (0.48) $P=0.07$	2.38 (0.36)	1.78 (0.33)	1.88 (0.31) $P<10^{-3}$	2.07 (0.17)
Triglycerides*	0.69 (0.42)	0.75 (0.45) $P<10^{-2}$	1.03 (0.74)	0.86 (0.49)	0.84 (0.45) $P=0.02$	1.43 (0.88)	0.53 (0.25)	0.67 (0.44) $P=0.01$	0.63 (0.23)
LDL-cholesterol	1.30 (0.36)	1.34 (0.40) $P<10^{-2}$	1.52 (0.27)	1.47 (0.36)	1.52 (0.45) $P=0.06$	1.64 (0.30)	1.15 (0.29)	1.19 (0.28) $P=0.004$	1.40 (0.19)
HDL-cholesterol	0.54 (0.16)	0.56 (0.16) $P=0.09$	0.50 (0.12)	0.55 (0.19)	0.57 (0.17) $P=0.005$	0.45 (0.14)	0.52 (0.14)	0.55 (0.16) $P=0.53$	0.54 (0.09)

Means (SE) adjusted for class of relatives, alcohol consumption in men, and oral contraception in women. *Tests are performed on log-transformed values but untransformed values are shown.

individuals[39] and this would explain why old people are more prone to infections. Higher MPO levels were measured in fathers as compared to mothers. The same trend was observed in offspring, although not reaching significance.

In addition to the effect of age, smoking increased MPO levels in fathers for which the highest tobacco consumption is usually observed. Beyond an effect of smoking on leukocytes count,[40] Pitzer *et al*[41] evidenced leukocytes activation in smokers. This is consistent with findings of Jay *et al*[42] who demonstrated an effect of nicotine on superoxide anion generation by human neutrophils. The effect of smoking on neutrophils MPO activity has also been measured by the MPXI but the results were controversial.[19,20]

In mothers, the intake of oral contraceptive increased the level of circulating MPO. A recent study demonstrated that in women having menopause and being treated with hormone replacement, the intracellular activity and the amount of MPO released increased significantly compared to the level at the time of starting the treatment.[43] This work is in accordance with another study where women treated with high oestradiol levels display increased plasma MPO concentrations.[44] In addition, Jansson[45] demonstrated *in vitro* that the presence of oestrogen led to the release of MPO from non activated PMNs.

The pattern of family resemblance characterized by correlations of similar magnitude between spouses and between biological relatives was more compatible with the existence of common environmental factors influencing MPO variability rather than a strong impact of genetic factors. However, the G-129A polymorphism was shown to explain 2.6% of the MPO variability in the whole population, the *A* allele being associated with lower MPO serum concentrations. The significant effect of the *A* allele was then confirmed by a TDT-type analysis suggesting that the observed association was not spurious. Due to its location in the promoter region of the MPO gene, the G-129A polymorphism may be involved in the alteration of the transcriptional rate of the gene. Further investigations will permit us to determine whether this polymorphism had a functional role or was only a marker in linkage disequilibrium with a still unidentified MPO functional variant.

Another finding of this study is that subjects carrying the *A* allele of the G-463A polymorphism display higher levels of triglycerides, total cholesterol, LDL-cholesterol and apoB which are known risk factors for atherosclerosis, than subjects with the *G* allele. These results would suggest that this polymorphism could modulate the risk of cardiovascular diseases. However, the TDT-type analyses failed to confirm the results suggested by the association analyses. Therefore, it cannot be ruled out that the observed associations between the G-463A polymorphism and lipid parameters were due to random fluctuations and/or uncontrolled phenomenon. Nevertheless, it must be stressed that, based on the sample size calculations we recently developed for TDT-type analysis,[31] the power we actually had to confirm the observed additive effect of this polymorphism by a TDT-type analysis was as low as 50%. The hypothesis of an implication of MPO in cardiovascular diseases could however be reinforced by a recent report which showed that MPO deficiency was protective against such diseases.[46] In the literature, the G-463A polymorphism has been related to several diseases like AD[14] and MS[15] where the *GG* genotype has been associated with higher incidence of disease in women. More recently, Reynolds *et al*[47] showed that the *A* allele was associated with an increased risk for AD in men carrying the $\varepsilon 4$ allele of apolipoprotein E (apoE). This allele is usually linked to an increased cholesterol level in blood and constitutes a well known risk factor for cardiovascular diseases.[48]

In conclusion, we evidenced the main factors affecting MPO serum concentrations in a healthy population. Molecular variations of the gene have only a weak influence on MPO concentrations. However, the G-463A polymorphism was found to be associated with lipid levels which would suggest a possible implication of MPO in the risk of cardiovascular diseases. These results have to be confirmed and further investigations will be conducted in that way.

References

1 Bainton DF, Ullyot JL, Farquhar MG: The development of neutrophilic polymorphonuclear leukocytes in human bone marrow. Origin and content of azurophil and specific granules. *J Exp Med* 1971; **134**: 907–934.
2 Klebanoff SJ: Myeloperoxidase: contribution to the microbicidal activity of intact leukocytes. *Science* 1970; **169**: 1095–1097.
3 Elneihoum AM, Falke P, Hedblad B, Lindgärde F, Ohlsson K: Leukocyte activation in atherosclerosis: correlation with risk factors. *Atherosclerosis* 1997; **131**: 79–84.
4 Tischendorf FW, Brattig NW, Büttner DW, Pieper A, Lintzel M: Serum levels of eosinophil cationic protein, eosinophil-derived neurotoxin and myeloperoxidase in infections with filariae and schistosomes. *Acta Tropica* 1996; **62**: 171–182.
5 Heinecke JW: Mechanisms of oxidative damage of low density lipoprotein in human atherosclerosis. *Curr Opinion Lip* 1997; **8**: 268–274.
6 Hazen SL, Heinecke JW: 3-chlorotyrosine, a specific marker of myeloperoxidase-catalyzed oxidation, is markedly elevated in low density lipoproteins isolated from human atherosclerotic intima. *J Clin Invest* 1997; **99**: 2075–2081.
7 Barone FC, Hillegas LM, Price WJ *et al*: Polymorphonuclear leukocyte infiltration into cerebral focal ischemic tissue: myeloperoxidase activity assay and histologic verification. *J Neurosci Res* 1991; **29**: 336–345.
8 Hudome S, Palmer C, Roberts RL, Mauger D, Housman C, Towfighi J: The role of neutrophils in the production of hypoxic-ischemic brain injury in the neonatal rat. *Pediatr Res* 1997; **41**: 607–616.
9 Re G, Azzimondi G, Bassein L, Vaona I, Guarnieri C: Plasma lipoperoxidative markers in ischaemic stroke suggest brain embolism. *Eur J Emerg Med* 1997; **4**: 5–9.
10 MacMahon S, Peto R, Cutler J *et al*: Blood pressure, stroke, and coronary heart disease. Part 1, prolonged differences in blood pressure: prospective observational studies corrected for the regression dilution bias. *Lancet* 1990; **335**: 765–774.
11 Kristal B, Shurtz-Swirski R, Manaster J *et al*: Participation of peripheral polymorphonuclear leukocytes in the oxidative stress and inflammation in patients with essential hypertension. *Am J Hypertension* 1998; **11**: 921–928.

12 Piedrafita FJ, Molander RB, Vansant G, Orlova EA, Pfahl M, Reynolds WF: An Alu element in the myeloperoxidase promoter contains a composite SP1-thyroid hormone-retinoic acid response element. *J Biol Chem* 1996; **271**: 14412 – 14420.
13 Reynolds WF, Chang E, Douer D, Ball ED, Kanda V: An allelic association implicates myeloperoxidase in the etiology of acute promyelocytic leukemia. *Blood* 1997; **90**: 2730 – 2737.
14 Reynolds WF, Rhees J, Maciejewski D *et al*: Myeloperoxidase polymorphism is associated with gender specific risk for Alzheimer's disease. *Exp Neurol* 1999; **155**: 31 – 41.
15 Nagra RM, Becher B, Tourtelotte WW *et al*: Immunohistochemical and genetic evidence of myeloperoxidase involvement in multiple sclerosis. *J Neuroimmunol* 1997; **78**: 97 – 107.
16 London SJ, Lehman TA, Taylor JA: Myeloperoxidase genetic polymorphism and lung cancer risk. *Cancer Res* 1997; **57**: 5001 – 5003.
17 Christensen RD, Rothstein G: Neutrophil MPO concentration: changes with development and during bacterial infection. *Pediatr Res* 1985; **19**: 1278 – 1282.
18 Kabutomori O, Yanagihara T, Iwatana Y, Kawarazaki A, Kabuomori M: Sex difference in MPO activity of neutrophils. *Am J Hematol* 1999; **60**: 312 – 313.
19 Bain BJ, Gresham N, Addison G: High neutrophil MPO content in smokers. *Blood* 1992; **80**: 845 – 846.
20 Dash S, Sen S, Behera D: High neutrophil MPO activity in smokers. *Blood* 1991; **77**: 1619.
21 Siest G, Visvikis S, Herbeth B *et al*: Objectives, design and recruitment of a familial and longitudinal cohort for studying gene-environment interactions in the field of cardiovascular risk: the Stanislas Cohort. *Clin Chem Lab Med* 1998; **36**: 35 – 42.
22 Donger C, Georges JL, Nicaud V *et al*: New polymorphisms in the interleukin-10 gene. Relationships to myocardial infarction. *Eur J Clin Invest* 2000; **30**: 1 – 7.
23 Cambien F, Poirier O, Nicaud V *et al*: Sequence diversity in 36 candidate genes for cardiovascular disorders. *Am J Hum Genet* 1999; **65**: 183 – 191.
24 Saiki RK, Bugawan TL, Horn GT, Mullis KB, Horn HA: Analysis of enzymatically amplified beta-globin and HLA-DQA alpha DNA with allele-specific oligonucleotide probes. *Nature* 1986; **324**: 163 – 166.
25 Liang KY, Zeger SL: Longitudinal data analysis using generalized linear models. *Biometrika* 1986; **73**: 13 – 22.
26 Trégouët DA, Ducimetière P, Tiret L: Testing association between candidate-gene markers and phenotype in related individuals, by use of estimating equations. *Am J Hum Genet* 1997; **61**: 189 – 199.
27 Donner A, Koval JJ: A multivariate analysis of family data. *Am J Epidemiol* 1981; **114**: 149 – 154.
28 Tiret L, Amouyel P, Rakotovao R, Cambien F, Ducimetière P: Testing for association between disease and linked marker loci: a log-linear model analysis. *Am J Hum Genet* 1991; **48**: 926 – 934.
29 Schneider S, Roessli D, Excouffier L: Arlequin ver. 2.000: A software for population genetics data analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland, 2000.
30 Allison DB: Transmission disequilibrium tests for quantitative traits. *Am J Hum Genet* 1997; **60**: 676 – 690.
31 Trégouët DA, Pallaud C, Sass C, Visvikis S, Tiret L: Sample size calculations for classical association and TDT-type methods using family data. *Ann Hum Genet* 2001; **65**: 293 – 312.
32 DeLeo FR, Goedken M, McCormick SJ, Nauseef WM: A novel form of hereditary myeloperoxidase deficiency linked to endoplasmic reticulum/proteasome degradation. *J Clin Invest* 1998; **101**: 2900 – 2909.
33 Romano M, Dri P, Dadalt L, Patriarca P, Baralle FE: Biochemical and molecular characterization of hereditary myeloperoxidase deficiency. *Blood* 1997; **90**: 4126 – 4134.
34 Nauseef WM, Brigham S, Cogley M: Hereditary myeloperoxidase deficiency due to a missense mutation of arginine 569 to tryptophan. *J Biol Chem* 1994; **269**: 1212 – 1216.
35 Wang DG, Fan JB, Siao CJ *et al*: Large-scale identification, mapping and genotyping of single nucleotide polymorphisms in the human genome. *Science* 1998; **280**: 1077 – 1082.
36 Bury TB, Pirnay F: Effect of prolonged exercise on neutrophil myeloperoxidase secretion. *Int J Sports Med* 1995; **16**: 410 – 412.
37 Nakamuta M, Ohashi M, Fukutomi T, Tanabe Y, Hiroshige K, Nawata H: Rise of plasma myeloperoxidase during interferon therapy. *J Gastroenterol Hepatol* 1995; **10**: 277 – 280.
38 Mohacsi A, Kozlovszky B, Kiss I, Seres I, Fulop T: Neutrophils obtained from obliterative atherosclerotic patients exhibit enhanced resting respiratory burst and increased degranulation in response to various stimuli. *Biochim Biophys Acta* 1996; **1316**: 210 – 216.
39 Suzuki K, Swenson C, Sasagawa S *et al*: Age-related decline in lysosomal enzyme release from polymorphonuclear leukocytes after N-formyl-methionyl-leucyl-phenylalanine stimulation. *Exp Hematol* 1983; **11**: 1005 – 1013.
40 Van Eeden SF, Hogg JC: The response of human bone marrow to chronic cigarette smoking. *Eur Respir J* 2000; **15**: 915 – 921.
41 Pitzer JE, Del Zoppo GJ, Schmid-Schönbein GW: Neutrophil activation in smokers. *Biorheology* 1996; **33**: 45 – 58.
42 Jay M, Kojima S, Gillepsie MN: Nicotine potentiates superoxide anion generation by human neutrophils. *Toxicol Appl Pharmacol* 1986; **86**: 484 – 487.
43 Bekesi G, Kakucs R, Varbiro S *et al*: In vitro effects of different steroid hormones on superoxide anion production of human neutrophil granulocytes. *Steroids* 2000; **65**: 889 – 894.
44 Santanam N, Shern-Brewer R, McClatchey R *et al*: Estradiol as an antioxidant : incompatible with its physiological concentrations and function. *J Lipid Res* 1998; **39**: 2111 – 2118.
45 Jansson G: Oestrogen-induced enhancement of myeloperoxidase activity in human polymorphonuclear leukocytes-a possible cause of oxidative stress in inflammatory cells. *Free Radic Res Commun* 1991; **14**: 195 – 208.
46 Kutter D, Devaquet P, Vanderstocken G, Paulus JM, Marchal V, Gothot A: Consequences of total and subtotal MPO deficiency: risk or benefit? *Acta Haematol* 2000; **104**: 10 – 15.
47 Reynolds WF, Hiltunen M, Pirskanen M *et al*: MPO and apoE ε4 polymorphisms interact to increase risk for AD in finnish males. *Neurology* 2000; **55**: 1284 – 1290.
48 Utermann G, Kindermann I, Kaffarnik H, Steinmetz A: Apolipoprotein E phenotypes and hyperlipidemia. *Hum Genet* 1984; **65**: 232 – 236.