Stroke pathophysiology is regulated by a combination of environmental, life-style and unclear genetic risk factors. There is substantial evidence that inflammation within the CNS contributes to stroke risk, and known clinical risk factors for stroke, like atherosclerosis, diabetes, obesity, hypertension, and peripheral infection, are associated with an elevated systemic inflammatory profile [1-5]. The inflammatory response is of major importance in the recovery and healing processes after stroke [6-8]. In this study we tested the genetic association of major inflammatory players IL1B (2q14), IL6 (7p21), TNF (6p21.3) and MPO (17q23.1) with stroke susceptibility and with stroke outcome at three months after the stroke event. The apparent complexity of the inflammatory mechanisms in stroke, and the multiplicity of players involved suggest a concerted process, in which implicated molecules interact to tightly regulate each other. We therefore examined both independent gene effects and the occurrence of gene-gene interactions among the tested inflammatory genes in stroke risk and stroke recovery.

- Single Nucleotide Polymorphisms (SNPs) covering the IL1B (N=3), IL6 (N=6), TNF (N=3) and MPO (N=2) genes and their 5kb flanking regions were genotyped using the Sequenom MassARRAY system (Sequenom, San Diego, USA) or the ABI PRISM 7900HT Sequencing Detection System (Applied Biosystems, Foster City).
- The population sample consisted of 672 patients and 530 controls. A subset of 546 patients were assessed for outcome three months after a stroke event, using the modified Rankin Scale (mRS); patients were classified for good or poor recovery (mRS1 or mRS>1, respectively).
- SNPs were tested for association with stroke by logistic regression (using the SNPassoc package of the R software), adjusting for significant demographic, clinical and life-style risk factors and/or stroke severity parameters for stroke outcome. Haplotype association analysis was performed with Haplopv7 (4.0 version). Bonferroni correction for multiple testing was used to correct significant SNP and haplotype associations.
- Testing for genetic interactions in association with stroke susceptibility and outcome was performed using the multifactor-dimensionality reduction (MDR) method (v2.0, beta 7.2).

**INTRODUCTION**

We provide evidence for a main effect of the IL6 and MPO genes, as well as an epistatic gene interaction effect between these two genes, in stroke susceptibility. Our genetic findings thus support previous evidence from other research areas for a role of inflammatory molecules in stroke.

As the associated IL6 SNPs are not in LD with the widely tested IL6 functional SNP rs1800795, and we don’t replicate this association, they likely signal a different IL6 causative variant.

We provide novel evidence for the association of the MPO gene with stroke, an effect that was largely driven by the ischemic stroke subset.

The synergistic interaction between IL6 and MPO illustrates the complexity of the inflammatory processes in stroke, suggesting that stroke risk is modulated by various genetic factors and by non-linear gene-gene interactions; our finding is in agreement with a previous in vitro functional study, showing that enzymatically inactive MPO induced IL-6 secretion in a dose and time-dependent manner by endothelial cells.

The complex interplay between genetic background, clinical and life-style factors and the environment may ultimately regulate the onset, acute phase and outcome of stroke. We present supporting evidence for a role of the IL6 and MPO inflammatory genes in stroke susceptibility, modulated by main gene effects together with clinical and life-style factors as well as by gene-gene interactions. Our findings are compatible and strengthen previous genetic and biological observations, highlighting the need of further functional studies, particularly in view of the possible utility of IL-6 as a diagnostic/prognostic stroke biomarker.

**METHODS**

**RESULTS**

Population sample demographic and clinical characteristics are presented in Table 1.

Two contiguous SNPs in the IL6 gene were associated with stroke susceptibility under a log-additive model (Table 2), after adjusting for covariates significant in the multivariate analysis model – gender, hypertension, diabetes and smoking status. These associations with stroke susceptibility remained significant after Bonferroni correction for multiple testing, highlighting gene variants with low to moderate effect in stroke risk.

One SNP in the MPO gene was significantly associated with stroke susceptibility and this association survived Bonferroni correction (Table 2). Interestingly, restricting the analysis to ischemic patients showed a more significant association (corrected P=0.006).

None of the IL1B and TNF SNPs were associated with stroke susceptibility in this sample.

An epistatic interaction between the IL6 and MPO genes was identified in association with stroke susceptibility (Table 3). The most significant interaction model was a two-marker combination between rs10242595 in IL6 and rs8178406 in MPO, showing a moderately increased TBA of 0.566, thus correctly classifying 55.6% of the individuals tested, but a high CVC of 9/10, i.e the model was selected 9 times out of 10 cross validation subsets, and a global OR=1.69 (95%CI=1.31-2.19). Interaction between these two markers shows a positive information gain (Figure 1), indicating a nonlinear, synergistic relationship between the IL6 (rs10242595) and the MPO (rs8178406) genes (i.e. epistasis).

In the subset of 546 patients assessed for stroke outcome at three months, using the modified Rankin Scale (mRS), we found only one IL6 haplotype associated with stroke outcome (corrected P=0.024).

**DISCUSSION & CONCLUSIONS**

- We provide evidence for a main effect of the IL6 and MPO genes, as well as an epistatic gene interaction effect between these two genes, in stroke susceptibility. Our genetic findings thus support previous evidence from other research areas for a role of inflammatory molecules in stroke.
- As the associated IL6 SNPs are not in LD with the widely tested IL6 functional SNP rs1800795, and we don’t replicate this association, they likely signal a different IL6 causative variant.
- We provide novel evidence for the association of the MPO gene with stroke, an effect that was largely driven by the ischemic stroke subset.
- The synergistic interaction between IL6 and MPO illustrates the complexity of the inflammatory processes in stroke, suggesting that stroke risk is modulated by various genetic factors and by non-linear gene-gene interactions; our finding is in agreement with a previous in vitro functional study, showing that enzymatically inactive MPO induced IL-6 secretion in a dose and time-dependent manner by endothelial cells.
- The complex interplay between genetic background, clinical and life-style factors and the environment may ultimately regulate the onset, acute phase and outcome of stroke. We present supporting evidence for a role of the IL6 and MPO inflammatory genes in stroke susceptibility, modulated by main gene effects together with clinical and life-style factors as well as by gene-gene interactions. Our findings are compatible and strengthen previous genetic and biological observations, highlighting the need of further functional studies, particularly in view of the possible utility of IL-6 as a diagnostic/prognostic stroke biomarker.

**REFERENCES**

Table 1. Demographic and clinical characteristics of stroke patients.

Table 2. SNP genotype frequency distribution and association with stroke. Results were adjusted for significant covariates. Odds Ratio (OR) >1 indicates increased probability of having a stroke for the carriers of the minor allele.

Table 3. Gene X Gene interaction models obtained using the multifactor-dimensionality reduction (MDR) method in stroke susceptibility;