Variants in the inflammatory *IL6* and *MPO* genes modulate stroke susceptibility through main effects and gene-gene interactions

H Manso[1,2,3], T Krug[2,4], J Sobral[2,4], I Albergaria[1], G Gaspar[1], JM Ferro[5], SA Oliveira[2,4], AM Vicente[1,2,3]

1 – Instituto Nacional de Saúde Dr. Ricardo Jorge, Lisbon, Portugal 2 – Instituto Gulbenkian de Ciência, Oeiras, Portugal, 3 – Center for Biodiversity, Functional & Integrative Genomics, 4 – Instituto de Medicina Molecular, Lisbon, Portugal, 5 – Serviço de Neurologia, Hospital de Santa Maria, Lisbon, Portugal

INTRODUCTION

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 equally 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.

METHODS

➢ 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 Sequence Detector 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 (mRS≤1 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 Haploview (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).

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 0.556, 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).

Table 1. Demographic and clinical characteristics of stroke patients.

Characteristic	Controls	Patients	*P* *
Age, mean±SD (yrs)	62.9±6.8	52.2±9.1	$<10^{-4}$
Gender (male), n/N (%)	247/530 (46.6)	428/672 (63.7)	$<10^{-4}$
Stroke Type, n/N (%)			
Ischemic stroke	–	551/672 (82.0)	–
Hemorrhagic stroke	–	111/672 (16.5)	–
Unknow type of stroke	–	10/672 (1.5)	–
Stroke Risk Factors, n/N (%)			
Hypertension (>85-140 mmHg)	193/513 (37.6)	369/601 (61.4)	$<10^{-4}$
Diabetes	59/501 (11.8)	102/628 (16.2)	0.033
Hypercholesterolemia (cholesterol >200 mg/dL)	328/520 (63.1)	385/623 (61.8)	0.657
Smoking status	147/512 (28.7)	308/660 (46.7)	$<10^{-4}$
Drinking status	218/505 (43.2)	388/662 (58.6)	$<10^{-4}$

*Mann-Whitney test or χ^2 test. SD – standard deviation, yrs – years.

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.

Gene	SNP	Genotype	Genotype frequency		OR [95% CI]	*P*
			Controls, n (%)	Cases, n (%)		
IL6	**rs2069837**					
		A/A	365 (76.4)	461 (81.2)	0.66 [0.50-0.89]	**0.005**†
		G/A	105 (22.0)	102 (18.0)		
		G/G	8 (1.7)	5 (0.9)		
IL6	**rs2069861**					
		C/C	442 (91.9)	497 (86.9)	1.74 [1.15-2.63]	**0.007**†
		T/C	39 (8.1)	70 (12.2)		
		T/T	0 (0.0)	5 (0.9)		
MPO	**rs8178406**					
		T/T	151 (31.4)	221 (38.9)	0.78 [0.65-0.95]	**0.011**†
		T/C	254 (52.8)	262 (46.1)		
		C/C	76 (15.8)	85 (15.0)		

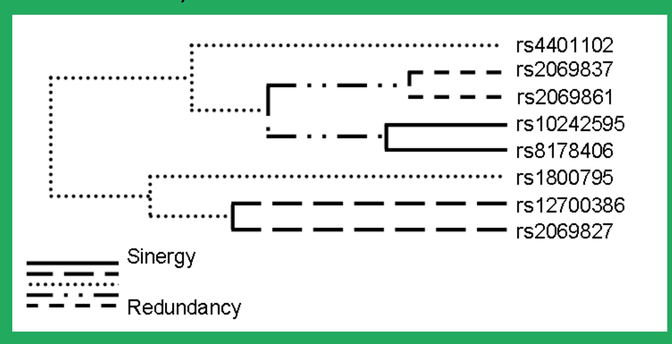
CI – Confidence Interval
*OR [95% CI] and *P* for the log-additive genetic model after adjustment for significant covariates (gender, history of hypertension, diabetes, smoking status).
†Significant result after Bonferroni correction.

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

Genes	Best model				CVC	TBA	*P**
	SNP1	SNP2	SNP3	SNP4			
IL1B _TNF	rs1143643(*IL1B*)	rs16944(*IL1B*)	rs2071590(*TNF*)	–	8/10	0.517	0.487
IL6 _TNF	rs10242595(*IL6*)	rs909253(*TNF*)	–	–	10/10	0.549	0.054
IL6 _IL1B	rs2069837(*IL6*)	rs10242595(*IL6*)	rs1143643(*IL1B*)	–	5/10	0.541	0.145
MPO _TNF	rs2071590(*TNF*)	rs8178406(*MPO*)	–	–	9/10	0.547	0.060
IL6 _MPO	rs10242595(*IL6*)	rs8178406(*MPO*)	–	–	9/10	0.556	**0.031**
MPO _IL1B	rs1143643(*IL1B*)	rs16944(*IL1B*)	rs8178406(*MPO*)	rs4401102(*MPO*)	9/10	0.538	0.160
IL1B _IL6 _TNF_MPO	rs10242595(*IL6*)	rs1143643(*IL1B*)	rs8178406(*MPO*)	rs4401102(*MPO*)	8/10	0.527	0.323

*1000 permutations *P*.
CVC – Cross Validation Consistency, TBA – Testing Balanced Accuracy.

Figure 1 – Interaction dendrogram for the *IL6* and *MPO* polymorphisms in stroke susceptibility. The length of the dendrogram branch that connects two polymorphisms indicates the strength of interaction (the shorter the branch, the stronger is the interaction).



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

1. Rodríguez-Yáñez M, Castillo J. (2008) Role of inflammatory markers in brain ischemia. Curr Opin Neurol 21:353-357
2. Wang Q, Tang XN, Yenari MA. (2007) The inflammatory response in stroke. J Neuroimmunol 184:53-68
3. Bastard JP, Maachi M, Lagathu C, Kim MJ, Caron M, Vidal H, Capeau J, Feve B. (2006) Recent advances in the relationship between obesity, inflammation, and insulin resistance. Eur Cytokine Netw 17:4-12
4. Hansson GK, Libby P. (2006) The immune response in atherosclerosis: a double-edged sword. Nat Rev Immunol 6:508-519
5. Moutsopoulos NM, Madianos PN. (2006) Low-grade inflammation in chronic infectious diseases: paradigm of periodontal infections. Ann N Y Acad Sci 1088:251-264
6. Correale J, Villa A. (2004) The neuroprotective role of inflammation in nervous system injuries. J Neurol 251:1304-1316
7. Lakhan SE, Kirchgessner A, Hofer M. (2009) Inflammatory mechanisms in ischemic stroke: therapeutic approaches. J Transl Med 7:97
8. Lucas SM, Rothwell NJ, Gibson RM. (2006) The role of inflammation in CNS injury and disease. Br J Pharmacol 147 Suppl 1:S232-240

Acknowledgments: This work was supported by the grant PTDC/SAU-GMG/64426/2006, Fundação para a Ciência e Tecnologia (FCT). Helena Manso and Tiago Krug are supported by an FCT fellowship.