Table 1 Odds ratios for Alzheimer's disease risk according to interaction of dopamine β -hydroxylase (DBH) genotypes with interleukin (IL) 1A (–889) and IL6 (–174) genotypes

Genotype	DBH (T/T)	Alzheimer	Controls	OR (95% CI)	p Value	OR (95% CI) [†]	p Value
IL1A (1/2+2/2)							
–	–	134 (50.3%)	158 (57.9%)	1 (reference)		1 (reference)	
–	+	10 (3.8%)	15 (5.5%)	0.79 (0.34 to 1.81)	0.571	0.73 (0.29 to 1.83)	0.498
+	–	112 (42.1%)	98 (35.9%)	1.35 (0.94 to 1.93)	0.100	1.34 (0.90 to 1.99)	0.153
+	+	10 (3.8%)	2 (0.7%)	5.90 (1.25 to 27.90)	0.011	2.93 (0.56 to 15.25)	0.203
Total		266	273				
IL6 (G/G)							
–	–	116 (47.9%)	121 (54.3%)	1 (reference)		1 (reference)	
–	+	8 (3.3%)	10 (4.5%)	0.83 (0.32 to 2.19)	0.713	0.54 (0.19 to 1.54)	0.249
+	–	106 (43.8%)	89 (39.9%)	1.24 (0.85 to 1.82)	0.263	1.36 (0.89 to 2.08)	0.154
+	+	12 (5.0%)	3 (1.3%)	4.17 (1.13 to 15.42)	0.020	5.66 (1.37 to 23.35)	0.017
Total		242	223				

*Crude odds ratio.

[†]Odds ratio adjusting by age, sex, and APOE genotype (using multiple logistic regression).CI, confidence interval; DBT (T/T) (–), dopamine β -hydroxylase C/C+T; IL1A (1/2+2/2) (–), interleukin 1A 1/1; IL6 (G/G) (–): interleukin 6 G/C+C/C; OR, odds ratio.

(SD) age at the time of study, 75.4 (9.1) years, range 50 to 98; mean age at onset, 71.6 (9.0) years, range 48 to 95) who met NINCDS/ADRDA criteria for probable Alzheimer's disease, and 273 controls (71% women; mean age 80.3 (7.8) years, range 63 to 100). The controls underwent a complete neurological and medical examination which showed that they were free from significant illness and had mini-mental state examination scores of 28 or more. Cases and controls were not related. The Alzheimer's disease and control samples were white and originated from a homogeneous population in a limited geographical area in northern Spain. Blood samples were taken after written informed consent had been obtained from the subjects or their representatives. The study was approved by the ethics committee of the University Hospital "Marqués de Valdecilla". (–1021) DBH,¹ (–889) IL1A,⁴ and (–174) IL6⁵ polymorphisms were determined as described previously (we used the Taqman assay to genotype DBH, as stated in the paper by Healy *et al.*¹), and all genotypes were in Hardy–Weinberg equilibrium. Association between dichotomous variables was analysed with the odds ratio, and 95% confidence intervals were estimated by the Cornfield method or the exact method. Probability (p) values were estimated by χ^2 or Fisher exact tests. Interrelations were analysed by stratification. Odds ratios for interaction of DBH with interleukins were adjusted for variables including age, sex, and APOE genotype; APOE was included as the APOE ϵ 4 allele is the main recognised genetic risk factor for sporadic Alzheimer's disease, and not because it might fit a "proinflammatory" hypothesis.

When the risk was considered for a single polymorphism, the presence of the DBH T/T genotype was not associated with Alzheimer's disease. However, on evaluation of the interactive effects of DBH and either (–889) IL1A or (–174) IL6 polymorphisms by stratification (table 1), the subjects carrying both the DBH T/T genotype and the (–889) IL1A allele 2 (1/2 and 2/2 genotypes) or the (–174) IL6 G/G genotype had a higher risk of developing Alzheimer's disease than subjects without these risk genotypes. We chose to look only at minor allele homozygous patients for DBH and interleukin genes, because the heterozygous alleles showed no association. An additional synergistic effect

of these three loci (IL1A and IL6 in conjunction with DBH) on the risk of Alzheimer's disease could not be analysed, as stratification of the sample resulted in rather small groups.

Our findings suggest, for the first time, that DBH allelic variations interact synergistically with the IL1A and IL6 polymorphisms on the development of Alzheimer's disease. The (–889) IL1A allele 2 and (–174) IL6 G allele have a functional effect; they are associated with increased production of IL1A and IL6, respectively, and could confer increased risk for Alzheimer's disease.^[4, 5] probably by inducing an increase in cytokine mediated inflammation in the Alzheimer brain. Recent data implicate a role for noradrenaline in attenuating the cortical inflammatory response to A β protein: in control animals, A β induced IL1 and IL6 expression in microglial cells, and following noradrenaline depletion by systemic treatment of animals with the selective noradrenergic neurotoxin DSP4, there was a robust increase and prolonged expression of both IL1 and IL6.³ In the light of recent findings that inflammatory processes contribute to Alzheimer's disease pathogenesis, it is tempting to speculate that loss of noradrenaline mediated anti-inflammatory protection associated with the (–1021) DBH T/T genotype might increase the risk of Alzheimer's disease by exacerbating inflammatory events linked to IL1 and IL6 polymorphisms. Our sample size had 70% power to detect an odds ratio of 5 or greater for a risk effect of IL1A interacting with DBH, and 63% power to detect an odds ratio of 4 or greater for a risk effect of IL6 interacting with DBH. This synergistic interaction needs to be confirmed by additional studies using different sets of patients and control subjects.

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Interleukin 1 β –511 C/T polymorphism and risk of aneurysmal subarachnoid haemorrhage

Production of the proinflammatory cytokine interleukin 1 β (IL1 β) increases in several acute or chronic diseases.¹ The IL1 β gene is a member of the IL1 gene family clustered on chromosome 2.¹ A C/T polymorphism in the promoter region of the IL1 β gene at position –511 influences the protein production, with the highest levels in T allele carriers.²

Data suggest that inflammation plays a role in the pathogenesis of subarachnoid haemorrhage (SAH) from ruptured aneurysm.³ We hypothesise that individuals genetically predisposed to an exaggerated cytokine production may be at risk of aneurysmal SAH. We studied the significance of IL1 β –511 C/T polymorphism in patients with aneurysmal SAH and in healthy controls.

We included 231 unrelated patients with aneurysmal SAH, of a total of 401 patients with SAH admitted to the Institute of

Table 1 Risk factors, interleukin (IL) 1 β genotypes and alleles in patients with aneurysmal subarachnoid haemorrhage and their controls

	Controls (n = 231)	Cases (n = 231)	p Value
Age (years) (mean (SD))	50.4 (12.4)	49.9 (12.7%)	*0.66
Female	132 (57.1%)	132 (57.1%)	†1.00
Hypertension	80 (34.6%)	125 (54.1%)	†<0.00001
Smoking	98 (42.4%)	147 (63.6%)	†<0.00001
Excessive alcohol intake	10 (4.3%)	35 (15.2%)	†0.0001
IL1 β genotype			
CC	111 (48.1%)	100 (43.3%)	†CC v CT+TT, p=0.3
CT	106 (45.8%)	99 (42.85)	†CC+CT v TT, p=0.006
TT	14 (6.1%)	32 (13.9%)	
IL1 β alleles			
T allele frequency	134 (29.0%)	163 (35.3%)	†C v T, p=0.041

Values are n (%) unless stated otherwise.

*Student *t* test.

† χ^2 test.

Neurology, Krakow, Poland between 2003 and 2005. Patients with dissecting and fusiform aneurysms (n = 10), arteriovenous malformations (n = 30), SAH of unknown origin (n = 39), those who were comatose on admission (n = 51), and those who did not agree to participate in the study (n = 40) were excluded.

The diagnosis of aneurysmal SAH was confirmed by cranial computed tomography or lumbar puncture, or both, and in each case by digital subtraction angiography. We also included 231 unrelated controls, matched for age (± 1 years) and sex with the patients, without any stroke history. Both the cases and the controls were white and came from southern Poland. Before inclusion in the study all participants gave their informed consent. The study was approved by the university ethics committee and was carried out in accordance with the Helsinki Declaration of 1975, as revised in 1983.

We collected demographic data and risk factor profiles for all subjects, as described elsewhere.⁴ The procedure for genotyping the polymorphism studied has already been reported.⁵

Data on continuous characteristics are expressed as means (SD) and on categorical characteristics as per cent values or absolute numbers. Comparisons between groups were made with the χ^2 test or Student's *t* test. A probability (p) value of <0.05 was considered significant. Hardy-Weinberg equilibrium was tested for by the χ^2 method. The association of the polymorphism studied with the risk of aneurysmal SAH was investigated by logistic regression analysis. For multivariate risk predictors, adjusted odds ratios (OR) and 95% confidence intervals (CI) are reported. The calculations were undertaken using the commercial statistical package "STATISTICA for Windows", v.6.0 (StatSoft Inc, Tulsa, Oklahoma, USA).

Genotype frequencies in the patients and controls were in Hardy-Weinberg equilibrium. The TT genotype was more often present in patients with aneurysmal SAH than in their controls. There was no difference between the studied groups when the frequencies of CC genotypes were compared with CT and TT genotypes taken together. The T allele was overrepresented in the cases. Patients with aneurysmal SAH presented significantly more often with hypertension,

smoking, and excessive alcohol intake (table 1).

Logistic regression analysis showed that the TT genotype (OR = 1.98 (95% CI, 1.001 to 3.99), p = 0.049), hypertension (OR = 2.58 (1.69 to 3.94), p = 0.00001), smoking (OR = 2.11 (1.42 to 3.17), p = 0.0002), and excessive alcohol intake (OR = 3.56 (1.62 to 7.8), p = 0.001) independently affected the risk for aneurysmal SAH.

We found that the TT genotype of IL1 β – 511 C/T polymorphism increased the risk of aneurysmal SAH in a Polish population. The p value in the multivariate analysis, in contrast to the univariate analysis, is at the limit of significance, what may reflect the correlation between hypertension and the polymorphism studied (TT carriers in the control group were more likely to have hypertension than the other genotype carriers (57.1% v 33.2%, p<0.05)).

It is suggested that inflammation is involved in the pathogenesis of ruptured intracranial aneurysms. The endothelium of ruptured aneurysms is invaded with macrophages and leucocytes.³ It is possible that inflammation is not only a direct response to bleeding after aneurysm rupture, but could have existed before the rupture. Inflammatory and immunological reactions are also present in unruptured aneurysms.³ The increased risk of aneurysmal SAH in TT carriers of the IL1 β – 511 polymorphism may be a result of either local or peripheral inflammation.

The increased local production of IL1 β , more pronounced in TT carriers, may exaggerate the fragility of the aneurysm wall by stimulating the production of other proinflammatory molecules¹ or by a decrease in collagen production, which has been shown to occur in the aneurysm wall.³ It cannot be excluded that chronic systemic inflammation, which is more pronounced in TT carriers, increases the risk of aneurysmal SAH. However, this has not been examined so far in this type of stroke.

Genetic association studies have an important risk of false positive findings. Our results should be replicated in an independent population to confirm the association.

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A novel saccin mutation in a Japanese woman showing clinical uniformity of autosomal recessive spastic ataxia of Charlevoix-Saguenay

Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) was originally described among French Canadians in the Charlevoix-Saguenay-Lac-Saint-Jean region of Quebec (OMIM 270550).¹ The gene responsible for ARSACS was identified as saccin, and frameshift (8585 deletion T, 2805X) and nonsense (C7245T, R2355X) mutations were reported in Quebec.² Recently, patients with other mutations have been described in countries elsewhere.³ These showed not only the spastic ataxia with peripheral neuropathy recognised in Quebec, but some additional features as well.³ Here we describe a female Japanese ARSACS patient with a novel nonsense mutation (C3774T, Q1198X), which resulted in a shorter truncated protein than those of the French Canadian patients. We report the details of her clinical and genetic data, and discuss the correlation between mutations and phenotypes in ARSACS.

The patient was a 39 year old woman who first walked at 12 months. Her gait was normal in early childhood. A spastic gait started at nine years of age, but she made no complaint about it for many years. After the age of 35 she complained of unsteadiness in her gait and clumsiness in her hands. Her gait disturbance progressed and she visited our clinic at the age of 37.

Consanguinity was not identified in her parents. Family members (parents and one brother) and other relatives have no evident neurological disorder. She had slurred and scanning speech. Horizontal gaze-evoked nystagmus was observed, and downward nystagmus occasionally appeared in the frontal eye position and with horizontal and