Table 1 Risk factors, interleukin (IL) 1β 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
IL1β 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%)	
IL1β 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 IL1β –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 IL1β –511 polymorphism may be a result of either local or peripheral inflammation.

The increased local production of IL1β, more pronounced in TT carriers, may exaggerate the fragility of the aneurysm wall by stimulating the production of other proinflammatory molecules[1] 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.

A Slowik, A Borratynska, W Turaj, J Pera, T Dziedzic, D Wloch, A Szczudlik
Department of Neurology, Jagiellonian University, Krakow, Poland

M Betlej, T Krzyszkowski, R Czepko
Department of Neurosurgery, Jagiellonian University

Correspondence to: Dr Agnieszka Slowik, Department of Neurology, Jagiellonian University, 31-503 Krakow, Botaniczna 3, Poland; slowik@neuro.cm-uj.krakow.pl

doi: 10.1136/jnnp.2005.075457

Competing interests: none declared

References

1 **Mrak R**, Griffin WS. Interleukin-1, neuroinflammation and Alzheimer disease. *Neurobiol Aging* 2001;**22**:903–8.
2 **Nemetz A**, Nosti-Escanilla MP, Molnar T, *et al*. IL-1B gene polymorphisms influence the course and severity of inflammatory bowel disease. *Immunogenetics* 1999;**49**:527–31.
3 **Kataoka K**, Taneda M, Asai T, *et al*. Structural fragility and inflammatory response of ruptured cerebral aneurysms. A comparative study between ruptured and unruptured cerebral aneurysms. *Stroke* 1999;**30**:1396–401.
4 **Slowik A**, Borratynska A, Pera J, *et al*. II genotype of the angiotensin-converting enzyme gene increases the risk for subarachnoid hemorrhage from ruptured aneurysm. *Stroke* 2004;**35**:1594–7.
5 **Kanemoto K**, Kawasaki J, Miyamoto T, *et al*. Interleukin (IL)-1β, IL-1α, and IL-1 receptor antagonist gene polymorphism in patients with temporal lobe epilepsy. *Ann Neurol* 2000;**47**:571–4.

A novel sacsin 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 sacsin, 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

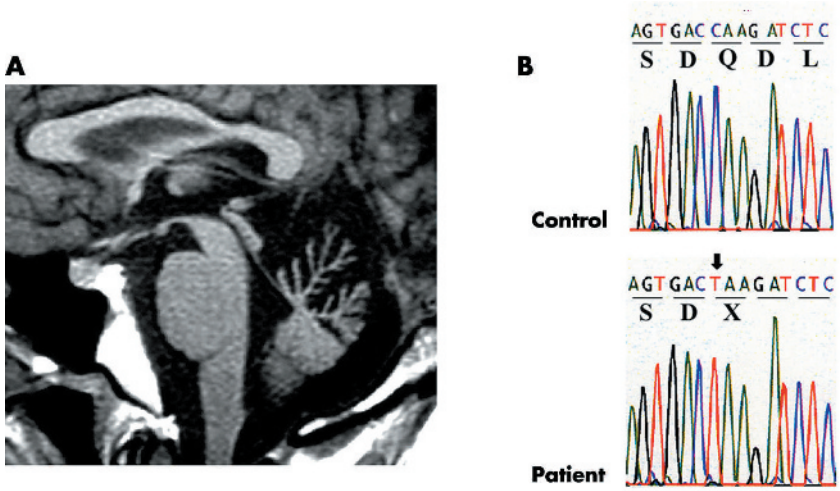

Figure 1 Brain magnetic resonance imaging and electropherogram of mutation. (A) Sagittal section of T1 weighted image showing marked vermian atrophy limited to the portion above the pyramis. (B) The arrow indicates a homozygous C→T transition at nt3774, leading to the nonsense mutation.

upward gaze. Fundoscopy was normal without hypermyelinated retinal fibres. She had a claw hand on the left and an ape hand on the right. Pes cavus was also present. Muscle weakness and atrophy were present in the distal limbs. The Romberg sign was positive and her gait was markedly spastic and ataxic, but she did not need any assistance in walking. Vibration sense was reduced in the distal extremities, but superficial sensation was maintained. Muscle stretch reflexes were increased in both knees, while the right radial and ulnar reflexes and both ankle reflexes were absent. The Babinski sign was positive on both sides. Her verbal IQ was 101, motor IQ 81, and total IQ 92, according to the Wechsler Adult Intelligence Scale–Revised.

On electroencephalographic study, background activity was 8–9 Hz, and diffuse high amplitude slow waves appeared continuously during and after hyperventilation. Electronystagmography showed marked impairment of optokinetic nystagmus and defective visual suppression of caloric nystagmus. Smooth ocular pursuit was affected, while saccade velocity appeared intact. On nerve conduction studies, distal motor latencies increased, and the amplitude of compound muscle action potential was low, especially in the lower limbs. In contrast, motor conduction velocities were spared except in the right ulnar and left median nerves. Sensory nerve action potentials were not evoked in any of the limbs. Magnetic resonance imaging revealed marked atrophy of the cerebellar vermis limited to the portion above the pyramis (fig 1A).

Gene analysis

After informed consent was obtained, genomic DNA was extracted from the patient's leucocytes. Using 30 appropriate primer pairs, the coding exon of the sacsin gene was amplified by polymerase chain reaction (PCR). PCR fragments were sequenced directly on an automated sequencer (ABI PRISM 3100, Applied Biosystems, Foster City, USA). Sequence analysis revealed a homozygous C→T transition at position 3774 (fig 1B), which results in substitution of glutamine 1198 for a stop codon (Q1198X). This mutation was absent in 100 chromosomes of the normal controls.

Comment

We report the 19th sacsin mutation identified to date. Interestingly, all mutations and families show a one to one correspondence, and the same mutation has not been reported among different families. Most mutations are of homozygous nonsense and frameshift type leading to an early stop codon, which suggests loss of function of sacsin as the cause of the disease.[3 4]

The clinical variation in ARSACS has been pointed out, especially in age of onset, retinal myelination, and mental disturbance.[3 4] French Canadian patients show gait disturbance when they begin walking, have prominent retinal hypermyelination, and show no mental disturbance.[1] Although our mutation led to an earlier stop codon than mutations in Quebec, our patient had a milder phenotype, with an older age at onset of ataxia and no retinal hypermyelination. In 8534 deletion A, which resulted in the same early stop codon as 8585 deletion T in Quebec, there was dementia but no retinal hypermyelination.[4] From these observations, we suggest that the clinical variation in ARSACS is not influenced by the loss of function of sacsin.

Apart for the variations mentioned above, all ARSACS patients have rather uniform clinical features. They have unsteadiness on walking in childhood and became chair bound in adulthood. There is spastic ataxia, and most patients have brisk patellar tendon reflexes, a positive Babinski sign bilaterally, nystagmus, and dysarthria. Pes cavus and disturbed deep sensation are also common. Progression of peripheral neuropathy probably results in the absence of the Achilles tendon reflex and distal muscle atrophy in the course of the disease. Patients with T2902C lack spasticity and brisk patellar tendon reflexes,[5] which suggests that the progressive peripheral neuropathy in ARSACS may occasionally mask pyramidal signs.

It is interesting that clinical uniformity arose from mutations distributed widely in the 13299 base pair coding region of sacsin. All frameshift and nonsense mutations in the sacsin gene are located at the N-terminal side of a leucine zipper motif at the C-terminal side, dnaJ motif, and HEPN domain.[3] Loss of these domains may lead to loss of critical function of sacsin and result in similar phenotypes. Although missense mutations have these domains, phenotypes resemble frameshift and nonsense mutations. As the predicted structure in T2902C,[5] missense mutations may lead to a conformational change of sacsin, which causes malfunction of the critical domains. Another possibility is that missense mutations may make sacsin a misfolded protein, which rapidly leads to degradation. We need to examine the degradation speed of the sacsin protein in ARSACS patients. Further studies of the critical domains of sacsin and the conformational change by mutations will clarify the mechanism of ARSACS.

Electronic database information

The nucleotide and the amino acid positions are based on the transcript GenBank accession number NM_014363 and NP_055178.

Acknowledgements

We thank Yuki Watanabe and Takako Sasaki for their technical assistance.

S Okawa, M Sugawara, S Watanabe, I Toyoshima
Department of Neurology, Akita University School of Medicine, Akita, Japan

T Imota
Division of Neurology, Akita City Hospital

Correspondence to: Dr Satoshi Okawa, Department of Neurology, Akita University School of Medicine, 1-1-1 Hondo, Akita, 010-8543, Japan; sato@doc.med.akita-u.ac.jp

doi: 10.1136/jnnp.2005.077297

Competing interests: none declared

References

1 **Bouchard JP**, Barbeau A, Bouchard R, *et al.* Autosomal recessive spastic ataxia of Charlevoix-Saguenay. *Can J Neurol Sci* 1978;**5**:61–9.
2 **Engert JC**, Berube P, Mercier J, *et al.* ARSACS, a spastic ataxia common in northeastern Quebec, is caused by mutations in a new gene encoding an 11.5-kb ORF. *Nat Genet* 2000;**24**:120–5.
3 **Gomez CM**. ARSACS goes global. *Neurology* 2004;**62**:10–11.
4 **Hara K**, Onodera O, Endo M, *et al.* Sacsin-related autosomal recessive ataxia without

prominent retinal myelinated fibers in Japan. *Mov Disord* 2005;**20**:380–2.
5 **Shimazaki H**, Takiyama Y, Sakoe K, *et al*. A phenotype without spasticity in sacsin-related ataxia. *Neurology* 2005;**64**:2129–31.

Qualitative evidence of anti-Ri specific intrathecal antibody synthesis and quantification of anti-Ri antibodies in serial samples from a patient with anti-Ri syndrome

Anti-Ri associated paraneoplastic neurological syndrome was initially described in patients suffering from breast or lung malignancy and presenting with opsoclonus, myoclonus, and ataxia.[1] Since anti-Ri antibodies have been reported to react both with central neuronal cells and with tumour tissue of patients with anti-Ri antibodies and breast cancer,[1] they are believed to reflect an autoimmune process.

Case report

Ten years after treatment for breast cancer, a 66 year old woman presented with complete horizontal gaze palsy to the right, jaw opening and neck dystonia, and slight ataxia of the upper extremities. No opsoclonus or myoclonus was observed. Four months previously a relapsing tumour in the lymph nodes of the right axilla had been treated with extirpation and radiotherapy; afterwards the patient was discharged on tamoxifen. MRI of the brain was normal. CSF cell count and protein were normal and no malignant cells were observed. Increased IgG index (1.1) indicated intrathecal antibody synthesis. Anti-Ri serum antibodies were detected by immunoblot with recombinant Ri protein. Apart from the gaze palsy, the patient improved after high dose treatment with methylprednisolone for 3 weeks. However, despite concurrent immunosuppressive treatment with cortisone and azathioprine, the patient developed truncal instability, slight appendicular ataxia, cervical dystonia, and severe tetraspasticity and became wheelchair bound. MRI of the cervical myelon revealed signs suggestive of myelopathy. Repeated, extensive searches found no hint of tumour relapse. Therapy with cyclophosphamide (700 mg/m^2 with a 6 week gap between courses) resulted in improvement of symptoms. The patient is able to walk some steps with help and jaw opening dystonia has also improved, but the gaze palsy is unchanged.

Isoelectric focusing and affinity blotting were performed as previously described.[2] Briefly, serum and CSF pairs were adjusted to equal IgG concentrations of 20 mg/l. Furthermore, we applied the patient's serum in serial concentrations of total IgG of 40–2560 mg/l. Focused antibodies were blotted onto nitrocellulose membranes which had been previously loaded (50 µg/10 cm^2) with recombinant Ri antigen (constructed by standard methods[3] in a baculovirus expression system). Bound antibodies were detected with peroxidase conjugated goat anti-human IgG (Dianova, Hamburg, Germany) diluted 1:1000. As controls, six CSF/serum pairs from patients with paraneoplastic neurological syndromes (PNS) other than anti-Ri syndrome and intrathecal synthesis of total IgG were investigated (anti-CV2 syndrome, anti-Hu syndrome, and anti-Yo syndrome).

ELISA detection of anti-Ri IgG serum antibodies was performed by standard methods described elsewhere.[4] Briefly, plates were coated with recombinant Ri antigen (20 µg/ml) and incubated with the patient's sera, diluted 1:1600. Bound anti-Ri IgG antibodies were detected by peroxidase conjugated goat anti-human IgG antibodies (Dianova), diluted 1:5000. Sera of 31 patients with neurological symptoms not compatible with PNS were investigated as controls.

Discussion

Detection of oligoclonal bands of total IgG exclusively in CSF and not in the corresponding serum is taken to indicate an intrathecal inflammatory process. In previous studies we provided qualitative evidence of anti-HuD and anti-Yo specific intrathecal antibody synthesis by demonstrating specific oligoclonal bands in CSF.[2] Using this approach, we now investigated a serum/CSF pair from a patient with an atypical anti-Ri syndrome. We found anti-Ri specific oligoclonal bands exclusively in CSF (fig 1B) but not in the corresponding equilibrated serum of the patient with Ri-syndrome. Weaker and less frequent oligoclonal bands were detected in the patient's serum with higher concentrations of total IgG (160–2560 mg/l).

We observed clear negative results in control serum/CSF pairs of six patients suffering from clinically and serologically unambiguous non-Ri paraneoplastic neurological syndromes, confirming the high specificity of the affinity blot. In a previous study,[5] a disproportionately high concentration of anti-Ri antibodies in the CSF compared to serum in most patients was revealed by semi-quantitative methods. These authors suggested intrathecal production of paraneoplastic neuronal autoantibodies as the most likely explanation for the elevated CSF/serum ratios. In our present study, we confirm this assumption of intrathecal anti-Ri specific autoantibody synthesis with qualitative data. Using ELISA, the CSF specific anti-Ri index was 5.6, strongly indicating intrathecal anti-Ri specific antibody synthesis by a semi-quantitative method and confirming our qualitative results. In conclusion, these data provide further evidence that anti-Ri specific antibodies are produced by B cell clones in the central nervous system.

Usually the term opsoclonus-myoclonus syndrome (OMS) is used to describe a paraneoplastic syndrome associated with anti-Ri antibodies.[1] Opsoclonus and myoclonus were never observed in our patient. Other studies on larger groups of anti-Ri patients described a wide spectrum of multifocal disorders; Pittock[1] reported on four patients with jaw opening dystonia, as seen in the patient featured in this report. These widespread clinical findings reflect the broad distribution of the Ri antigen in the central nervous system.[3]

In the present study, quantification of circulating anti-Ri antibodies over a period of 28 months with ELISA revealed a clear decrease, together with improvement in paraneoplastic neurological symptoms during treatment with cortisone and azathioprine (fig 1A). Later deterioration due to myelopathy was not reflected by an increase in anti-Ri antibody titre, possibly due to immediate immunosuppressive therapy with cyclophosphamide. These data are in rough agreement with previous findings describing a correlation between the concentration of anti-Hu antibodies and improvement in paraneoplastic neurological symptoms.[4] Unfortunately, no serum sample from the time before the onset of neurological symptoms was available. The decline in Ri-specific antibody levels as well as the clinical

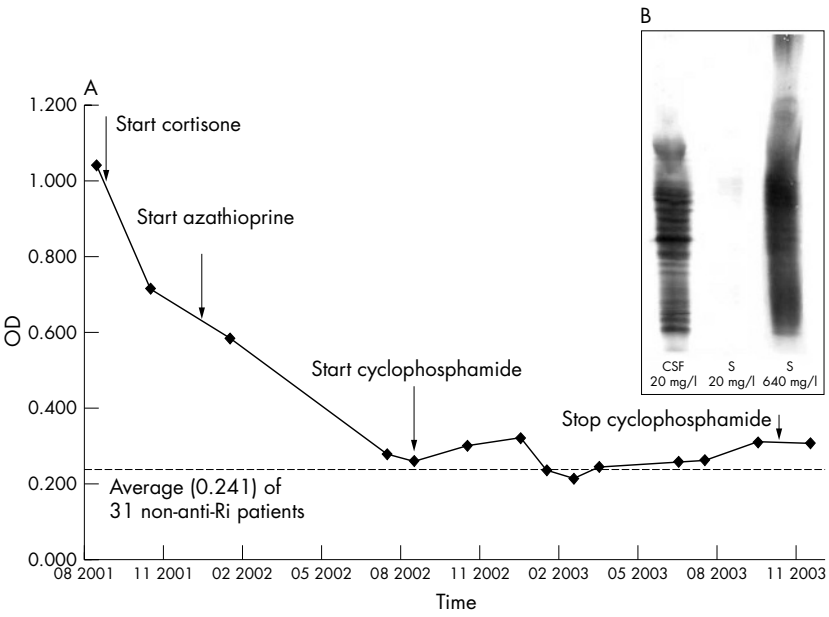

Figure 1 (A) Decrease in anti-Ri antibody concentration in serial serum samples (dilution 1:1600), spanning an observation period of 28 months from diagnosis of anti-Ri syndrome to the end of immunosuppressive treatment. Antibody concentration is shown as optical density (OD) in ELISA employing recombinant Ri protein as antigen. Line indicates average OD of controls (31 non anti-Ri patients). (B) Results of affinity blotting after isoelectric focusing of CSF/serum pair (total IgG: 20 mg/l) demonstrating anti-Ri specific oligoclonal bands exclusively in CSF but not in the patient's serum. A blot of the patient's serum with a concentration of total IgG of 640 mg/l revealed only weak and less frequent oligoclonal bands. CSF, cerebrospinal fluid; S, serum; pH range: bottom pH 10, top pH 3.