Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoenцеphalopathy (CADASIL) is a type of hereditary stroke and dementia. More than 90% of patients with CADASIL have mutations in the Notch3 gene. All mutations either create or destroy a cysteine residue in the epidermal growth factor-like repeats. In addition, five polymorphisms, which lead to amino acid substitutions, have been identified within the Notch3 coding sequence. However, whether these polymorphisms affect Notch signalling or are involved in cerebrovascular diseases is unknown. In the present study, we investigated a possible association between a T6746C polymorphism in the Notch3 coding region and the occurrence of symptomatic ischaemic cerebrovascular disease (CVD) was investigated. Two hundred and thirty five patients with CVD, as confirmed by brain CT or MRI, and 315 age and sex matched control subjects were analyzed for genotype frequencies of the T6746C polymorphism in the Notch3 gene. The genotype distributions in patients with CVD, C/C, 14.0%; C/T, 45.5%; and T/T, 40.4%; controls, C/C, 14.3%; C/T, 47.9%; T/T, 37.8%. The Japanese population has a higher C allele frequency of the T6746C polymorphism than European populations. There was no significant difference between the T6746C polymorphism in patients with CVD and controls ($\chi^2$=0.414, p=0.813). This was confirmed by the results of multiple logistic regression analysis including established risk factors ($\chi^2$=4.65, p=0.311). In conclusion, the results indicate that T6746C polymorphism in the intracellular domain of the Notch3 gene is not associated with an increased risk for CVD.

**Abbreviations:** CADASIL, Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoenencephalopathy; CVD, cerebrovascular disease; EGF, epidermal growth factor; TIA, transient ischaemic attack; PCR, polymerase chain reaction.
Polymorphism analysis
To analyze the T6746C polymorphism of Notch3, the polymerase chain reaction (PCR) was carried out as described previously. Amplification of a 203 bp fragment of the Notch3 gene was performed with the 5’ primer 5’-CTTACCTGG CAGTCCCAAGG-3’ and 3’ primer 5’-AGTGGCAGTGGCT GGGCTAG-3’. The PCR consisted of 1 cycle of 15 minutes at 80°C and 4.5 minutes at 94°C, 43 cycles of 1 minute at 94°C, 1 minute at 65°C, and 45 seconds at 72°C, followed by 7 minutes at 72°C in a Gene Amp PCR system 2400 (Perkin Elmer, Foster City, CA, USA). The PCR product (6 µl) was cleaved with 0.5 U of Mwo I restriction enzyme (New England Biolabs, Beverly, MA, USA). Digestion of the PCR products yielded bands of 203 bp in TT homozygotes, 158 bp in CC homozygotes, and both bands in heterozygotes.

Statistical analysis
The differences in genotype frequencies and other risk factors were analyzed by the χ² test. Mean age in the two groups and the allele frequency were compared by Student’s t test. Multiple logistic regression methods were used to control for possible confounding factors. All statistical analyses were performed using Statview software (ver 5.0 for windows, SAS Institute, CA, USA).

RESULTS
Table 1 summarises the clinical features of the patients with CVD and the control subjects studied. There were no significant differences in age or sex between the two groups. The risk factors hypertension, diabetes mellitus, and smoking were significantly more common in the patients with CVD. Although the frequency of a family history of stroke was higher in patients with CVD, the difference between the groups was not statistically significant.

Polymorphism analysis
Table 2 summarises the allele and genotype frequencies of the Notch3 polymorphism in patients with CVD and controls. The genotype distributions and allelic frequencies of the T6746C polymorphism in Notch3 were not significantly different between patients with CVD and controls (14.0% were C/C, 45.5% were C/T, and 40.4% were T/T). This distribution was not significantly different from the control group (C/C, 14.3%; C/T, 47.9%; T/T, 37.8%).

Polymorphism analysis
As shown in table 2, the allele frequencies were compared by Student’s t test. The allele frequencies were also compared using Student’s t test. Multiple logistic regression methods were used to control for possible confounding factors. All statistical analyses were performed using Statview software (ver 5.0 for windows, SAS Institute, CA, USA).

DISCUSSION
The present study is the first to examine the relation between CVD and the Notch3 polymorphism. The Japanese population has a higher C allele frequency in T6746C polymorphism than European populations. Our study shows that the Notch3 polymorphism is not associated with CVD, even in low risk subjects.

Mutations in the Notch3 gene are missense mutations characteristically leading to the loss or gain of a cysteine residue in one of the EGF-like domains of the protein. The abnormal Notch3 allele may encode for a protein product with a normal conformation due to disruption of the disulphide bonding of cysteine residues. Five genetic polymorphisms, which lead to amino acid substitutions, have been reported in the coding sequence of Notch 3. Among them, the most common polymorphism, T6746C, results in an amino acid dimorphism (Val/Ala) at residue 2223, which is located in the intracellular domain. Because the intracellular domain of Notch3 is thought to be involved in signal transduction, this polymorphism has been suggested to be directly associated with Notch3 function. However, our data yielded no evidence of an association between T6746C polymorphism and CVD. In our preliminary study, we analyzed other polymorphisms in the Notch3 coding region, although they seem to be rare in the Japanese population. Therefore, it is unlikely that they are associated with common diseases, such as CVD.

Finally, several points should be kept in mind when interpreting the results of the present study. Our study refers to the association between this polymorphism and CVD only in the Japanese population, where the prevalence of stroke is especially higher than that in most western European countries. The relevance of this polymorphism should be investigated in other populations as well as in prospective studies.

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