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Common Genetic Mutations as Possible Aetiological Factors in Stroke

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Well-known mutations, such as the Leiden V, prothrombin G20210A, F.V. R306T Cambridge and F.V. R306G Hong Kong mutations, can cause thrombotic processes, mainly in the venous part of the circulatory system. The roles of the Leiden V [1–3], R306T Cambridge [4], R306G Hong Kong [5] and methylene tetrahydrofolate reductase (MTHFR) mutations [6–9] and of angiotensin-converting enzyme polymorphism (ACE polymorphism) [10, 11] in the pathogenesis of stroke are still debated. The aim of this case report is to demonstrate the value of molecular genetic examinations in facilitating an understanding of the actiology and pathogenesis of stroke.

Case Description

A 16-year-old male patient was admitted with acutely developing weakness in the right limbs. He had had no history of venous or arterial thrombotic events. He did not smoke tobacco or drink alcohol. Drug abuse could be excluded as a possibility. Two weeks before admission, he had suffered a common viral infection. Neurological examination revealed central facial paresis, hemiparesis, pyramidal signs and hemihypaesthesia on the right side. A detailed physical examination did not reveal any somatic alterations.

The weakness of the limbs gradually worsened during 2 days. Computer tomography (CT) and magnetic resonance imaging (MRI) examinations were carried out 2 days after the beginning of the symptoms. Both the MRI examination and the CT scan demonstrated a large subcortical lesion on the left side (fig. 1).

Search for Actiological Factors. Carotid, vertebral duplex and cardiac transesophageal ultrasonography furnished negative results. A right-to-left shunt through the heart could be excluded by means of an injection of agitated saline during the transesophageal echocardiogram. Ultrasound examination screening of the lower extremities for deep vein thrombosis was negative. 24-hour arterial blood pressure monitoring did not register abnormal values. Magnetic resonance angiography and transcranial Doppler examination did not reveal any arterial vascular anomalies. The results of bilateral carotid and vertebral angiography were normal. No alteration was found either in the venous phase of the angiograms. A 24-hour ECG examination demonstrated no rhythmic alterations. Myeloproliferative disorders, malignancy and complications of chemotherapy could be excluded. Both the abdominal ultrasound examination and the chest X-ray findings were negative. Routine blood testing and urine analysis furnished normal data. The routine cerebrospinal fluid analysis showed no alteration. Oligoclonal band were negative. Routine fungal and mycobacterial cultures of cerebrospinal fluid were negative. Anticariolipin, antileucocyte, antimitochondrial, anti-DNA antibody and lupus anticoagulant were not detected. Protein C, protein S and antithrombin deficiencies were not found. The antithrombin III level was in the normal range. An activated protein C resistance was discovered. An elevated total plasma homocysteine level (14.6 μmol/l) was measured 4 days after the beginning of the symptoms.

By means of the PCR technique, heterozygous Leiden V and MTHFR C677T mutations were detected. The F.V. R306T Cambridge, F.V. R306G Hong Kong and prothrombin G20210A mutations were not observed. The I/D genotype for ACE polymorphism was detected. The APO E 3/3 genotype was found.

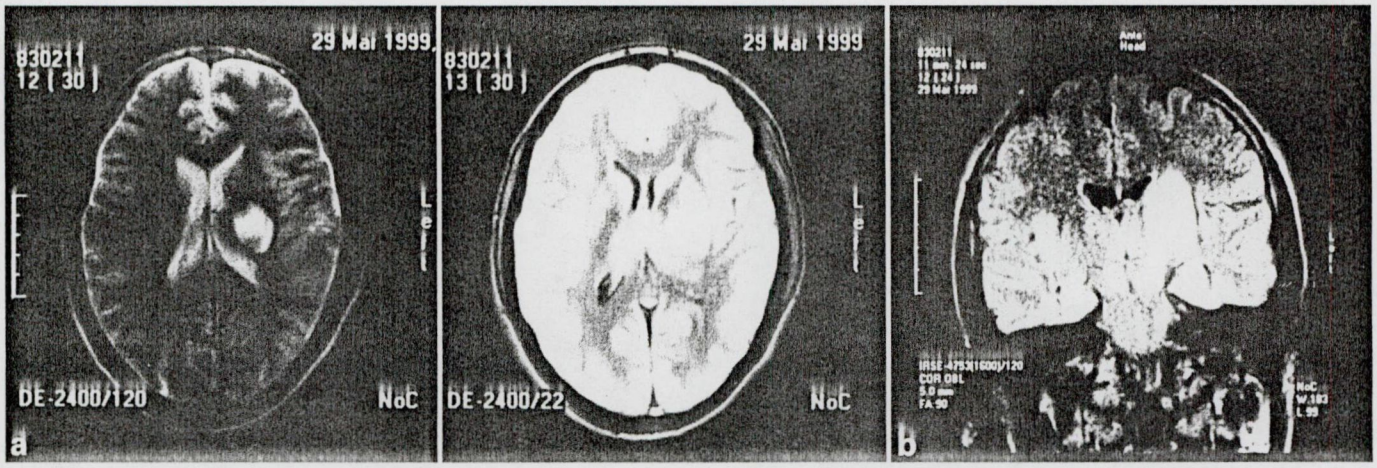

Fig. 1. Axial T_2 WI, proton density (**a**), and coronal FLAIR (**b**) images revealed a hyperintense (2 × 3 × 5 cm) lesion in the area of the basal ganglia, the capsular interna and the upper mesencephalon region on the left side. The lesion caused a mild mass effect, but did not exhibit a contrast enhancement.

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Discussion

The clinical picture, the MRI, the CT scan and the molecular genetic examinations led us to attribute the alteration to ischaemic stroke. No common risk factors for stroke were found. None of the clinical tests performed yielded any evidence of an embolic event. They did not demonstrate thrombosis in either the main arterial or the venous streams. The clinical picture did not allow a decision as to the venous versus arterial origin of the stroke. A thrombotic process in the arteriae perforantes of the middle cerebral artery could not be excluded, however. The roles of the Leiden V, MTHFR C677T and ACE I/D mutations in the pathological chain of events are open to question. Although these mutations occur very frequently in the normal population and along have not been proved to be significant risk factors [11, 12], they could have been additive pathological factors. Some evidence has been published that the combined occurrence of the MTHFR C677T and Leiden V mutations might unfavourably affect the clinical course of ischaemic stroke [8]. In the published clinical studies, the allele frequencies of the heterozygous Leiden V [12, 13] and MTHFR C677T [14] mutations and the ACE I/D genotype [15, 16] in different European areas have been estimated to be 3–7, 30–40 and 40–50%, respectively. Accordingly, the probability of the three mutations occurring together in one person is calculated to be 0.36–1.4%. The prevalence of the heterozygous Leiden V [2] and ACE I/D genotype [16] in the young ischaemic stroke population has not been proved to be significantly higher than that in healthy controls. In a subgroup of patients with cryptogenic cerebral ischaemia, however, a significantly higher frequency of the Leiden V mutation was found [2]. A significant decline in the frequency of the ACE D allele with increasing age has also been described in ischaemic stroke patients [16]. No such data are available for the heterozygous MTHFR C677T mutation. With regard to the estimated occurrences of the mutations and from a consideration of the direct pathophysiological role of the Leiden V mutation in thrombophilia, the Leiden V mutation could have been the main actiological factor. The MTHFR C677T mutation causing an elevated serum homocysteine level might have had an unfavourable effect on the vasoregulation. Although a heterozygous MTHFR C677T mutation alone has not been observed to cause an elevated serum homocysteine level [17], a second common mutation in the MTHFR gene (A1298C) has been desribed which, in combination with a heterozygous MTHFR C677T mutation, can have a similar pathobiochemical effect to that of a homozygous MTHFR C677T mutation [18]. The presence of this second mutation, which was not examined in this case, may be speculated as an explanation for the elevated serum homocysteine level. Another possible explanation for the elevated serum homocysteine level is that the previous viral infection and the state of fever might have created a demand for a greater folate intake. During the infection, the fever could also have decreased the function of the MTHFR with the termolabile C677T mutation. ACE I/D polymorphism is an important genetic factor in the renin-angiotensin system (RAS). RAS activation seems to have both short- and long-term regulatory roles in the cardiovascular system [15]. The ACE I/D genotype causing the higher ACE level has been considered to have an unfavourable effect on the vasoregulation system too [15]. These three genetic factors might have been actiological factors clustered together in the clinically risk-free stroke patient. Certain mutations which alone do not prove to be significant actiological factors for stroke might together cause a thombotic process in a young person not exhibiting any other common actiological factors. This case suggests that the roles of combinations of all potential genetic risk factors should be studied under standardized circumstances, in special subgroups of stroke (arterial versus venous infarction) at different ages.

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A clustering of unfavourable common genetic mutations in stroke cases

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Objectives – The aetiological role of common genetic mutations was analysed in a subgroup of stroke patients. *Material and methods* – A total of 406 patients were examined because of ischaemic stroke. After a detailed clinical scrutiny, 5 were found who did not exhibit any of the classical clinical risk factors. In this clinically homogeneous subgroup of stroke patients, the prothrombin A20210G, Hong Kong, Cambridge and methylenetetrahydrofolate reductase C677T (MTHFR C677T) mutations, angiotensin-converting enzyme polymorphism (ACE polymorphism) and apolipoprotein E (APO E) genotype were examined. *Results* – In all 5 patients, the same type of clustering of three mutations was manifested. A heterozygous Leiden V mutation was observed in all 5 subjects, while a heterozygous MTHFR C677T mutation and an I/D genotype for ACE polymorphism were detected in 4 of them, and a homozygous D/D genotype and a homozygous MTHFR C677T mutation in 1. This type of clustering of the mutations was not observed in the remaining 401 stroke patients. *Conclusion* – These results suggest that the Leiden mutation might possibly be an aetiological factor for stroke in a rare subgroup of patients who do not display any of the classical risk factors. The roles of ACE D polymorphism and the MTHFR C677T mutation in stroke, should also be taken into consideration in this subgroup of stroke patients. These unfavourable genetic factors might be aetiological factors if they are clustered together in a stroke patient not presenting any of the standard clinical risk factors.

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Well-known mutations such as the Leiden V, F.V.R 306 T Cambridge and F.V.R 306 G Hong Kong mutations can cause thrombotic processes, mainly in the venous part of the circulatory system. It has been reported that the prothrombin G 20210 A mutation can be detected more frequently in young patients who have suffered a stroke than in healthy persons (1–4). The roles of the Leiden (5–17), Cambridge (18), Hong Kong (19) and methylenetetrahydrofolate reductase (MTHFR) mutations (20–28) and angiotensin-converting enzyme polymorphism (ACE polymorphism) (29–31) in the pathogenesis of stroke are still debated. The Leiden and prothrombin G 20210 A mutations might give rise to stroke if they are associated with hypertension, diabetes mellitus or some other clinical risk factor (14). There is no evidence that these pathological mutations alone can cause stroke. Our aim was to examine the roles of these genetic factors in stroke cases in which they are not associated with other well-known clinical risk factors.

Material and method

A total of 406 Hungarian patients were examined and treated because of acutely developing ischaemic stroke. After a detailed clinical scrutiny, 5 were found who did not exhibit any classical clinical risk factors. No clinical explanation for the aetiology of stroke could be established in these subjects. The criteria of exclusion included any of the following

conditions: diabetes mellitus, hypertension, ischaemic heart disease, cardiac valvular disease, cardiac dysrhythmia, cardiac failure, stenosis of the carotid internal artery or vertebral artery, arterial dissection, atherosclerotic signs in the carotid internal artery and vertebral artery, aneurysm diseases, microangiopathic signs in the retina, age above 60 years, hypertriglyceridaemia, hypercholesterinaemia, the presence of anticardiolipin antibody or lupus anticoagulant, heavy smoking or drinking. The aim of the exclusion was to create a clinically homogenous subgroup in which the role of the previously mentioned genetic factors in the aetiology of stroke could be analysed. A battery of genetic examinations was carried out on all 406 subjects. The genetic data were evaluated with regard to whether the genetic factors could have given rise to the pathological process in the 5 classical clinical risk factor-free patients and whether any special type of clustering of the genetic mutations could be observed in these subjects in comparison with the remaining 401 stroke subjects. Because of the importance of the clinical homogeneity of the subgroup, the clinical findings on the 5 patients are briefly summarized.

Case descriptions

All 5 subjects presented with acutely developing focal neurological signs. The main positive clinical findings are listed in Table 1. The patients had no history of venous or arterial thrombotic events. None of them suffered from hypertension or diabetes mellitus. They did not smoke or drink alcohol. Drug abuse could be excluded as a possibility. There were no meningeal signs. The body temperatures were normal. A detailed physical examination did not reveal any somatic alterations.

Computer tomography (CT) and magnetic resonance imaging (MRI) examinations were carried out 2 days after the first observation of the symptoms. The MRI was performed with a 0.23 T resistive magnet (Picker, Outlook) with the use of a head coil.

Search for aetiological factors

Carotid, vertebral duplex and cardiac transoesophageal ultrasonography furnished negative results. A right to left shunt through the heart could be excluded by means of an injection of agitated saline during the transoesophageal echocardiogram. Ultrasound examinations screening the lower extremities for deep vein thrombosis were negative. Arterial blood pressure monitoring every 24 h did not register abnormal values. Magnetic resonance angiography did not reveal any vascular anomalies. The transcranial Doppler did not indicate any embolic events. ECG examinations demonstrated no rhythmic alterations. Myeloproliferative disorders, malignancy and complications of chemotherapy could be excluded. Both abdominal ultrasonographic and chest X-ray findings were negative. No common risk factors were found.

Routine blood tests and urine analyses proved normal. Routine cerebrospinal fluid analyses showed no alteration. Oligoclonal bands were negative. Routine fungal and mycobacterial cultures of cerebrospinal fluid were negative. The serum immunoglobulin A, G and M levels were normal. Anticardiolipin, antileukocyte, antimitochondrial, anti-DNA antibody and lupus anticoagulant were not detected. Protein C, protein S and antithrombin deficiencies were not found. The antithrombin III levels were in the normal range. An activated protein C resistance was discovered in all 5 patients.

By means of the PCR technique, the Leiden, Hong Kong, Cambridge, prothrombin G 20210 A, and MTHFR C677T mutations, and ACE and apolipoprotein E (APO E) polymorphism were examined. DNA isolation (33) and the PCR technique for the Leiden V (34), MTHFR C677T (35) and prothrombin G20210 A mutations (36)

Table 1. Clinical data on the 5 classical clinical risk-free patients

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Age	21	57	50	17	52
Sex	Female	Female	Female	Male	Female
Site of lesion	Deep, subcortical lacunar infarcts in white matter in left fronto-temporo-parietal region	Solitary, deep, subcortical infarct with diameter of 2 cm in left capsula interna region	Cortical and subcortical infarct with diameter of 1–2 cm in left fronto-temporal regions	Solitary, infarct in region of medial basal ganglia, capsula interna and upper mesencephalon on left	Solitary, deep, subcortical infarct with diameter of 2 cm in right frontal region
Clinical manifestation	Contralateral hemiparesis, hemisensory loss	Contralateral hemiparesis, hemisensory loss, motor aphasia	Contralateral hemiparesis, motor aphasia, central facial paresis	Contralateral hemisensory loss, hemiparesis	Contralateral hemiparesis

and APO E polymorphism (37) were carried out in accordance with the original descriptions. ACE polymorphism was examined by a modification of the original (38) description for the increased detection of heterozygotes (39).

Results

Heterozygous Leiden V mutations were detected in all 5 patients. Heterozygous MTHFR C677T mutations were detected in patients 1–4, and the homozygous MTHFR C677T mutation in patient 5. The F.V.R 306 T Cambridge, F.V.R 306 G Hong Kong and prothrombin G 20210 A mutations were not observed in any of the 5 subjects. I/D genotypes were detected for ACE polymorphism in patients 1–4, and D/D genotype in patient 5. APO E 3/3 genotypes were found in all 5 subjects. The results of the genetic examinations in the 5 patients are reported in Table 2.

Discussion

The clinical picture and the results of the MRI, the CT scan and the molecular genetic examinations led us to attribute all the alterations in these 5 patients to ischaemic stroke. The results of the routine blood tests were normal and no common risk factors for stroke were found. The clinical data excluded embolic events. The findings in the genetic and clinical examinations led to the supposition of thrombotic processes in different brain regions. In this clinically homogeneous subgroup of stroke patients, the Leiden and the MTHFR C677T mutations and the D allele for ACE polymorphism were present together in the same subjects.

In the published clinical studies, the allele frequency of the Leiden mutation in different European areas has been estimated as 3.56–7% (40, 41). As no such data are available for our region, the alleles of all 406 stroke patients were examined. The Screening of 812 alleles revealed a Leiden frequency of 2.46%. In the given 5 stroke patients, however, the corresponding allele frequency was calculated as 50%. The carrier rate was 100%.

Accordingly, in all 5 patients, the Leiden V mutation could have been the main aetiological factor in the thrombotic process.

Among the 406 stroke patients, the frequencies of the homozygous and heterozygous MTHFR C667T mutations were 0.7 and 0.26 respectively; and the distribution of the DD, II, and ID genotypes was 32%, 23% and 45% respectively. The roles of the MTHFR C677T and ACE D mutations in the pathological chain of events are open to question. Although they occur very frequently in the normal population and alone have not been proved to be significant risk factors (32, 42, 43), the clustering of the same 3 mutations in all 5 patients suggests that they might possibly be additive pathological factors. We did not find the same clustering of the 3 mutations in any other of the remaining 401 stroke subjects. Because of the small number of subjects in the apparently clinically risk-free group, we did not compare the allele frequencies of the MTHFR C6677T and the ACE D mutations with the respective allele frequencies in the remaining 401 stroke patients.

The PCR technique and genetic examinations could provide more exact data relating to the pathogenesis of stroke. On the basis of these cases, physicians should consider the possibility of a Leiden mutation in patients with ischaemic stroke if no other aetiology is evident. The contributions of the MTHFR C677T mutation and ACE I/D polymorphism to a thrombotic event (not as major risk factors, but as additive ones) should also be taken into consideration under such special circumstances. Some mutations which alone do not prove to be significant aetiological factors for stroke might together cause a thrombotic process in patients not exhibiting any other common aetiological factor. The present cases suggest that it might be important to carry out large prospective studies in which the roles of all the potential genetic risk factors could be examined together under standardized circumstances, in special subgroups

Table 2. Results of the genetic examinations in the 5 classical clinical risk-free patients

	Patient Number 1	Patient Number 2	Patient Number 3	Patient Number 4	Patient Number 5
Leiden V	Heterozygous	Heterozygous	Heterozygous	Heterozygous	Heterozygous
Prothrombin G20210A	No mutation	No mutation	No mutation	No mutation	No mutation
ACE polymorphism	I/D	I/D	I/D	I/D	D/D
MTHFR C677T	Heterozygous	Heterozygous	Heterozygous	Heterozygous	Homozygous
Hong Kong	No mutation	No mutation	No mutation	No mutation	No mutation
Cambridge	No mutation	No mutation	No mutation	No mutation	No mutation
APO E	3/3	3/3	3/3	3/3	3/3

of patients of different ages. In the future, it might become possible to predict the relative risk of a stroke event in special subgroups of patients by using the results of genetic examinations.

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Real-Time PCR Assay with Fluorescent Hybridization Probes for Exact and Rapid Genotyping of the Angiotensin-converting Enzyme Gene Insertion/Deletion Polymorphism, *Ferenc Somogyvári,*[1*] *Zoltán Szolnoki,*[2] *János Márki-Zay,*[1] *and Lajos Fodor*[1] ([1] Central Laboratory and [2] Department of Neurology and Neurophysiology, Pándy Kálmán County Hospital, H-5700 Gyula, Semmelweis 1, Hungary; * address correspondence to this author at: Central Laboratory, Pándy Kálmán County Hospital, H-5700 Gyula, Budapest krt. 58, Hungary; fax 36-66-463474, e-mail fsoma99@hotmail.com)

The serum angiotensin-converting enzyme (ACE) concentration depends on the *ACE* gene insertion/deletion (I/D) polymorphism. This insertion is a 287-bp-long *alu* repetitive sequence localized in intron 16 of the gene *(1)*. The D/D genotype is associated with a higher serum ACE concentration, and it has been demonstrated that it may be associated with ischemic heart diseases *(2–4)*. We are interested in the role of the *ACE* D allele in ischemic stroke *(5, 6)*.

The PCR method reported by Rigat et al. *(1)* has been modified several times because of preferential amplification of the D allele. In the past, I/D heterozygotes had been mistyped as D/D homozygotes; the extent of this misclassification has been estimated at ~5% *(2, 7, 8)*.

Some of the modified techniques are listed below.

The confirmatory PCR method proposed by Shangmugam et al. *(9)* uses a third PCR primer inside the I allele, just like multiplexed PCR. Other authors consider that multiplexed PCR is not acceptable in this method *(10)*.

The step-down PCR described by Chiang et al. *(11)* is a modified touchdown PCR involving some initial cycles with an annealing temperature higher than the melting point of the primers, followed by annealing temperatures reduced stepwise to the melting point. Unfortunately, this method is time-consuming, and some thermocyclers are not programmable for this kind of method.

A better solution, which uses a second, independent PCR amplification with a primer pair that recognizes an insertion-specific sequence, has been described by Lindpainter et al. *(2)*. In this method, the I/D genotypes that were mistyped as D/D in the first PCR reaction can be detected. Unfortunately, this method is also time-consuming.

To reduce the time requirements, we suggest a rapid PCR with fluorescently labeled oligonucleotide hybridization probes on the LightCycler™ instrument (Roche Diagnostics) with subsequent fluorescent probe melting point analysis. The proposed assay has been confirmed by acrylamide gel electrophoresis.

The fluorescence hybridization probe was constructed to fit into the insertion-specific sequence, which can also be amplified by the confirmatory primer pair mentioned above *(2)*. Thus, validation of the D/D genotype with the independent PCR reaction is feasible with the same fluorescent probes.

Previously, hybridization probes have been constructed to detect point mutations or short insertions. In the case of

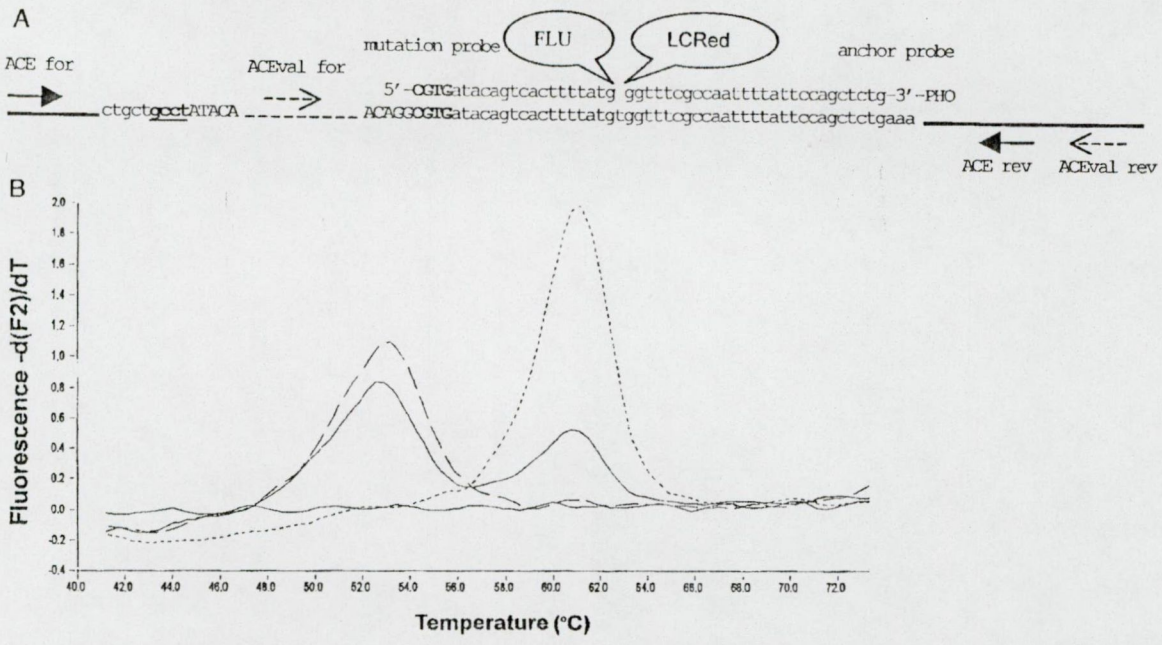

Fig. 1. Relative placements of the primers and the hybridization probes detecting the *ACE* gene I/D polymorphism (*A*) and derivative melting curves of *ACE* D/D, I/D, and I/I genotypes (*B*).

(*A*), the insertion is indicated by *upper case letters* and the *dashed line*. *ACE for* and *ACE rev*, *ACE* primers; amplicon length, 490 bp with and 190 bp without insertion. *ACEval for* and *ACEval rev*, *ACE* insertion-specific primer pair; amplicon length, 335 bp only in the presence of an insertion (the site of the mismatch in the case of the D allele is *underlined*). *FLU*, fluorescein; *PHO*, phosphorylated end. (*B*), (— — —), D/D; (———), I/D; (- - - -), I/I.

short insertions, the hybridization probe bridges the whole mutation site (*12, 13*). For the detection of a long insertion, a general solution could involve designing the probe to overlap 1–5 bases on the insertion. The scheme of our solution is shown in Fig. 1A.

Genomic DNA was extracted from 200 μL of peripheral blood anticoagulated with EDTA with the QIAamp blood reagent set (Qiagen) according to the manufacturer's instruction. All blood samples were kept at −20 °C until DNA isolation.

PCR was performed in disposable capillaries (Roche Diagnostics). The reaction volume was 10 μL, containing 1 μL of DNA (40–80 ng), 0.2 μM each of the primers reported by Rigat et al. (*1*), 1 μL of reaction buffer (LightCycler DNA master hybridization probes 10× buffer; Roche Diagnostics), 0.4 μL of 25 mM $MgCl_2$ stock solution, 0.5 μL of dimethyl sulfoxide, and 0.1 μM each of the probes. The detection probe specific for the 3′ end of the insertion (underlined four bases in the 5′ end of the probe) was labeled at the 3′ end with fluorescein (5′-CGT GAT ACA GTC ACT TTT ATG-3′). The anchor probe (5′-GGT TTC GCC AAT TTT ATT CCA GCT CTG-3′) was labeled with LightCycler Red 640 at the 5′ end and was modified at the 3′ end by phosphorylation to block extension.

The PCR conditions were as follows: initial denaturation at 95 °C for 60 s, followed by 40 cycles of denaturation (95 °C for 0 s, 20 °C/s), annealing (61 °C for 10 s, 20 °C/s), and extension (72 °C for 15 s, 20 °C/s). The melting curve analysis consisted of 1 cycle at 95 °C for 10 s and 40 °C for 10 s, followed by an increase of the temperature to 65 °C at 0.2 °C/s. The fluorescence signal (F) was monitored continuously during the temperature ramp and then plotted against the temperature (*T*). These curves were transformed to derivative melting curves [$(-dF/dT)$ vs T]. The derivative melting curves for the three genotypes (I/I, I/D and D/D) are depicted in Fig. 1B.

The PCR conditions were the same when the confirmatory primer pair were used, except that the annealing temperature was 67 °C. In this case, the derivative melting curve exhibited only the peak characteristic of the insertion allele.

Of 103 patient samples tested, 23.3% were I/I, 44.7% were I/D, and 33.0% were D/D.

The proposed technique and the electrophoresis yielded identical results. No *ACE* D/D misclassification was found even when the confirmatory, independent primer pair was used.

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Detection of Anti-Survivin Antibody in Gastrointestinal Cancer Patients, *Atsuhito Yagihashi,[1] Koichi Asanuma,[1] Masashi Nakamura,[1] Jan Araya,[2] Yoshinori Mano,[3] Torigoe Torigoe,[3] Daisuke Kobayashi,[1] and Naoki Watanabe[1]** (Departments of [1] Clinical Laboratory Medicine, [2] Surgery, and [3] Pathology, Sapporo Medical University School of Medicine, Sapporo 060-8543, Japan; * address correspondence to this author at: Department of Clinical Laboratory Medicine, Sapporo Medical University, South-1, West-16, Chuo-ku, Sapporo 060-8543, Japan; fax 81-11-622-7502, e-mail watanabn@sapmed.ac.jp)

Survivin, a recently described member of the inhibitor of apoptosis protein family, contains a single baculovirus inhibitor of apoptosis protein repeat and lacks a C-terminal RING finger (*1*). Like other proteins in the inhibitor of apoptosis protein family, survivin binds specifically to terminal effector cell death proteases, specifically caspase-3 and -7. The consequences are substantially reduced caspase activity and reduced cell death in response to diverse apoptotic stimuli (*2*). Expression of survivin is detected during fetal development in humans, but is not detected in healthy adult tissues except for the thymus and placenta (*1*). Survivin is expressed by most cancers, including carcinoma of the lung, stomach, colon, pancreas, breast, and prostate, as well as by neuroblastomas, melanomas, and lymphomas (*1, 3–10*).

Overexpression of survivin by cancer cells may lead to anti-survivin antibody responses and cytotoxic T-lymphocyte responses against the cancer (*11–12*). In the present study, we examined the occurrence of antibody response against survivin in patients with various gastrointestinal cancers.

Blood samples were collected from 33 healthy blood donors and 63 gastrointestinal cancer patients after histo-