GENETIC ASPECTS OF ISCHEMIC STROKE

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Summary: Stroke is one of the most common causes of death and long term disability throughout the world. It is not only induced by classic vascular risk factors like hypertension, cigarette smoking and diabetes mellitus, but also by genetic factors. The genetic etiology of ischemic stroke is polygenic. The candidate «stroke risk» genes may be conveniently divided into five groups, affecting (i) lipid metabolism, (ii) the renin-angiotensin system, (iii) haemostasis, (iv) nitric oxide production, and (v) homocysteine metabolism. Since stroke is a complex disease comprising a heterogeneous group of disorders with multiple risk factors, research into genetics of stroke presents some unique challenges. Numerous studies have investigated the role of genetics in the pathogenesis of stroke, with varied and often contradictory results. Additional knowledge of the role of genes in ischemic stroke may improve our understanding of the cause of stroke, provide new insights into prevention and the factors that influence the outcome of stroke, and new therapeutic targets when preventive strategies have failed.

Key words: ischemic stroke, genetics, candidate genes

Introduction

As one of the leading causes of death within both the developed and the developing world, stroke is a worldwide problem. It primarily affects elderly people, but about 20% of strokes occur before the age of 65. Stroke describes a syndrome of different pathophysiological processes all resulting in the common end point of focal cerebral ischemia. Eighty five per cent have ischemic aetiology and 15% hemorrhagic (intracerebral and subarachnoid). Different pathophysiological processes are responsible for ischemic stroke, including cardioembolism, large vessel atherosclerosis with thromboembolism, and small vessel disease. Pathophysiological mechanisms and underlying genetic influences may differ for the different subtypes.

Besides well-documented conventional risk factors like hypertension, cigarette smoking and diabetes mellitus, genetic factors influence the risk of stroke. Twin and family-based studies, together with observations in animal models, have provided evidence that genetic factors are very important in the pathogenesis of stroke. The genetic etiology of ischemic stroke is polygenic and reflects the influence of many different loci modulating different pathophysiological processes. Genetic factors may act either by predisposing to conventional risk factors (hyperlipidemia, diabetes, hypertension), by modulating the effects of such conventional risk factors on the end organs, or by direct independent effect on stroke risk.

Genetic factors in stroke risk

Human single-gene disorders associated with stroke

A large number of single-gene disorders can cause stroke by several pathophysiological mechanisms. These include cardioembolism, large artery disease, hematological disorders, small vessel disease, mitochondrial disorders, ion channel disorders, and connective tissue disorders leading to arterial dissection. One of the best examples is the NOTCH3 gene, mutations of which cause cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), a condition that leads to lacunar infarcts and vascular dementia.
Mutations of the APP, CST3, and BRI genes can cause autosomal dominant amyloid angiopathies, which lead to cerebral hemorrhage, vascular dementia, or both. KRIT1 has been identified as one of the genes causing cavernous angiomas. Identification of these genes by reverse genetics has provided new and fascinating insights into the pathophysiology of stroke. This work is also the basis for clinically useful molecular diagnostic tests. Despite their low prevalence, monogenic conditions should always be considered in a young patient who presents with stroke or in a patient of any age with no evident vascular risk factor, especially when there is a family history. Indeed, the risk of stroke in persons carrying the mutated gene and in their relatives is very high. For example, in the case of an autosomal dominant disorder with complete penetrance, all persons who carry the mutated gene will have a stroke, as will half of their first-degree relatives.

Evidence for the genetic background of stroke

Both epidemiological and animal-based studies provide strong evidence that genetic factors are important in the pathogenesis of stroke. Epidemiological studies have used twin, affected sibling pair and family-based approaches. Twin studies provide the most robust evidence for genetic influences on stroke. The principle is the comparison of concordance rates between monozygotic and dizygotic twins for a disorder. It is assumed that, apart from genetic factors, monozygotic and dizygotic twins will be similar in other respects, such as environmental exposures. From the degree of concordance it is then possible to determine the heritability of a disorder, defined as the proportion of the phenotype that can be attributed to genetic factors. Twin and sibling studies have also shown that the intermediate phenotypes for stroke are under strong genetic control. Family-based studies have examined the relationship between a family history of stroke among first-degree relatives and risk of stroke in proband. However, most studies suggest that a family history of stroke is an independent risk factor for stroke, and this is consistent with a genetic component operating outside the usual risk factors. Recent observations in animal models have provided strong evidence for the existence of stroke susceptibility genes. A well-established experimental tool in the study of hypertension has been the spontaneously hypertensive rat (SHR).

Identifying genetic factors in ischemic stroke

Quantitative trait locus mapping in stroke-prone animals and candidate gene studies in man are the most frequently used methods in the identification of genetic factors in ischemic stroke. In polygenic stro-

ke, the situation is difficult because of numerous factors: (i) late onset: the late onset of stroke makes genetic comparisons between living relatives difficult; (ii) phenotypic heterogeneity: the variety of stroke subtypes or phenotypes is likely to reflect different aetiologies; (iii) genetic heterogeneity: mutations in any one of several genes might result in an identical phenotype; (iv) phenoype: some individuals who do not inherit a predisposing allele will still manifest stroke because of random or environmental causes; (v) variable penetrance: some individuals who inherit a predisposing allele may not manifest the disease; causes of variable penetrance include gene dose, gene–environment interaction and epistatic phenomena; (vi) confounders: the presence of coexistent risk factors, such as hypertension and diabetes, may make the effects of a single gene difficult to assess in affected individuals.

Human studies: the candidate gene approach

Candidate gene studies in stroke can be considered as belonging to two broad categories: (i) those investigating the role of genes which may influence stroke risk, and (ii) those investigating genes which determine infarct size after vessel occlusion by influencing vascular reactivity and collateral supply, and neuronal responses to injury. The candidate «stroke risk» genes may be conveniently divided in a few groups, affecting (i) haemostasis (genetic variants in components of the coagulation cascade: factor V, VII, XIII, prothrombin, fibrinogen; PAI-1, platelet glycoprotein receptor polymorphism), (ii) nitric oxide production (polymorphisms of genes encoding both the neuronal and the inducible form of nitric oxide synthase) (iii) lipid metabolism (polymorphic variants of genes encoding the apolipoproteins, lipoprotein receptors and the key enzyme of plasma lipoprotein metabolism), and (iv) homocysteine metabolism (genetic polymorphism of MTHFR, cystathionine beta-synthase), (v) the renin-angiotensin system (ACE gene insertion/deletion polymorphism). From the data a lot of association studies in ischemic stroke: hemostatic system, endothelial nitric oxide, lipid metabolism, homocysteine, and renin angiotensin pathway, are presented in Table 1 (1–125).

A considerable body of evidence suggests that circulating hemostatic factors are risk factors for stroke. In the past, studies have proposed excess coagulation factors, increased levels of fibrinolytic inhibitors, or both. Circulating levels are subject to considerable biological influence, and the acute-phase response that accompanies an acute stroke event may hinder the interpretation of levels in a case-control study. Prospective studies are not subject to the confounding influence of acute phase reaction, but large numbers would be required.

<p>| Table 1 |</p>
<table>
<thead>
<tr>
<th>Gene</th>
<th>Reference</th>
<th>Polymorphism</th>
<th>Methodology</th>
<th>Phenotype</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor V</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>C677T</td>
<td>Q2056 Leiden</td>
<td>Case-control</td>
<td>348 cases, 247 controls</td>
<td>Ischemic stroke</td>
<td>Negative</td>
</tr>
<tr>
<td>Q2056 Leiden</td>
<td>Case-control: 45 patients, population controls</td>
<td>Ischemic stroke &lt;45 years</td>
<td>Negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kostulas et al. (1999)</td>
<td>Q2056 Leiden</td>
<td>Case-control: 236 cases, 137 controls</td>
<td>Ischemic stroke</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Nakata et al. (1998)</td>
<td>Q2056 Leiden</td>
<td>Nested case-control: 208 cases, 209 controls</td>
<td>NIH or minor ischemic stroke</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Albuchner et al. (1996)</td>
<td>Q2056 Leiden</td>
<td>Case-control: 30 cases, 75 controls</td>
<td>Ischemic stroke</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>Chmiolew et al. (1996)</td>
<td>Q2056 Leiden</td>
<td>Case-control: 53 cases, 397 controls</td>
<td>Ischemic stroke 18-50 years</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>Fisher et al. (1996)</td>
<td>Q2056 Leiden</td>
<td>Case-control: 63 cases, 31 controls</td>
<td>Ischemic stroke</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Press et al. (1996)</td>
<td>Q2056 Leiden</td>
<td>Case-control: 116 cases, 54/161/287 controls</td>
<td>Ischemic stroke in elderly/elderly controls with and without risk factors/young controls</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>van der Born et al. (1996)</td>
<td>Q2056 Leiden</td>
<td>Case-control: 112 cases, 222 controls</td>
<td>Ischemic stroke</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Markus et al. (1996)</td>
<td>Q2056 Leiden</td>
<td>Case-control: 181 cases, 80 controls</td>
<td>Ischemic stroke</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Martinelli et al. (1997)</td>
<td>Q2056 Leiden</td>
<td>Case-control: 155 cases, 155 controls</td>
<td>Ischemic stroke</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Sanchez et al. (1997)</td>
<td>Q2056 Leiden</td>
<td>Case-control: 66 cases, 66 controls</td>
<td>Ischemic stroke</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Halbmaier et al. (1997)</td>
<td>Q2056 Leiden</td>
<td>Case-control: 229 cases, 71 controls</td>
<td>Ischemic stroke</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>De Luca et al. (1997)</td>
<td>Q2056 Leiden</td>
<td>Case-control: 14 cases, 25 controls</td>
<td>Young ischemic strokes from 3 families</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Longstreth et al. (1998)</td>
<td>Q2056 Leiden</td>
<td>Case-control: 106 cases, 391 controls</td>
<td>Ischemic stroke young women aged 18-44 years</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Martinelli et al. (1999)</td>
<td>Q2056 Leiden</td>
<td>Nested case-control: 259 cases, 1744 controls</td>
<td>Ischemic stroke</td>
<td>Positive trend</td>
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<tr>
<td>Nishiuma et al. (1999)</td>
<td>Q2056 Leiden</td>
<td>Case-control: 81 cases, 81 controls</td>
<td>TIA or minor ischemic stroke</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>Heywood et al. (1997)</td>
<td>Q2056 Leiden</td>
<td>Case-control: 286 cases, 197 controls</td>
<td>Ischemic stroke</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Ressler et al. (1997)</td>
<td>Q2056 Leiden</td>
<td>Case-control: 277 cases, 225 controls</td>
<td>Ischemic stroke</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Carr et al. (1997)</td>
<td>J448(1/2)</td>
<td>Case-control: 305 cases, 197 controls</td>
<td>Ischemic stroke</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>Nishiuma et al. (1998)</td>
<td>Q2056 Leiden</td>
<td>Case-control: 85 cases, 85/84 controls</td>
<td>Hypertensive stroke</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>Schmidt et al. (1998a)</td>
<td>F148(1/2)</td>
<td>Cross-sectional: 399 cases</td>
<td>Carotid atherosclerosis</td>
<td>Positive</td>
<td></td>
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<tr>
<td>PAI 1</td>
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<tr>
<td>Catto et al. (1997)</td>
<td>Q435Q</td>
<td>Case-control: 421 cases, 172 controls</td>
<td>Ischemic stroke</td>
<td>Negative</td>
<td></td>
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<tr>
<td>Factor XII</td>
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<tr>
<td>Catto et al. (1998)</td>
<td>Va43Leu</td>
<td>Case-control: 529 cases, 437 controls</td>
<td>Ischemic stroke</td>
<td>Negative</td>
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<tr>
<td>GpIIb/IIIa</td>
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<tr>
<td>Roller et al. (1998)</td>
<td>1PA2</td>
<td>Nested case-control: 208 cases, 209 controls</td>
<td>Ischemic stroke</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Cariss et al. (1997)</td>
<td>1PA1/1PA3</td>
<td>Case-control: 218 cases, 165,321 controls</td>
<td>Ischemic stroke</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Carr et al. (1998)</td>
<td>1PA2</td>
<td>Case-control: 505 cases, 462 controls</td>
<td>Ischemic stroke</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Wagner et al. (1998)</td>
<td>1PA2</td>
<td>Case-control: 63 cases, 122 controls</td>
<td>Ischemic stroke young women aged 15-44 years</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>eNOS</td>
<td></td>
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<tr>
<td>Yoo et al. (1998)</td>
<td>eNOS 4a/b</td>
<td>Case-control: 144 cases, 91 controls</td>
<td>Atherosclerosis</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Markus et al. (1999)</td>
<td>Glu208App</td>
<td>Case-control: 361 cases, 236 controls</td>
<td>Ischemic stroke</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Macioci et al. (1999)</td>
<td>Glu208App</td>
<td>Case-control: 265 cases, 253 controls</td>
<td>Ischemic stroke</td>
<td>Negative</td>
<td></td>
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<tr>
<td>MTHFR</td>
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<tr>
<td>Markus et al. (1997)</td>
<td>C677T</td>
<td>Case-control: 345 cases, 161 controls</td>
<td>Ischemic stroke</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Neumann et al. (1998)</td>
<td>C677T</td>
<td>Case-control: 37 cases, 166 controls</td>
<td>Ischemic stroke</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>De Stefano et al. (1998)</td>
<td>C677T</td>
<td>Case-control: 71 cases, 198 controls</td>
<td>Ischemic stroke &lt;50 years</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Neuner et al. (1998)</td>
<td>C677T</td>
<td>Case-control: 31 cases, 182 controls</td>
<td>Ischemic stroke</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Kosultas et al. (1998)</td>
<td>C677T</td>
<td>Case-control: 126 cases, 126 controls</td>
<td>Ischemic stroke</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Motta et al. (1998)</td>
<td>C677T</td>
<td>Case-control: 296 cases, 325 controls</td>
<td>Ischemic stroke</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>Saloga et al. (1998)</td>
<td>C677T</td>
<td>Case-control: 422 cases, 173 controls</td>
<td>Ischemic stroke</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Hermon et al. (1998)</td>
<td>C677T</td>
<td>Case-control: 174 cases, 183 controls</td>
<td>Ischemic stroke C1 proven &gt;60 years</td>
<td>Inconclusive</td>
<td></td>
</tr>
<tr>
<td>Krätser et al. (1999)</td>
<td>C677T</td>
<td>Case-control: 80 cases, 41 controls</td>
<td>Ischemic stroke</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Nakato et al. (1999)</td>
<td>C677T</td>
<td>Case-control: 81 cases, 81 controls</td>
<td>TIA or minor ischemic stroke</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Magugajou et al. (1999)</td>
<td>C677T</td>
<td>Case-control: 208 cases, 1038 controls</td>
<td>Ischemic stroke</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>Notou et al. (1999)</td>
<td>C677T</td>
<td>Case-control: 147 (74 cases, 214/209 controls)</td>
<td>Ischemic stroke</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>Press et al. (1999)</td>
<td>C677T</td>
<td>Case-control: 136 cases, 52 controls</td>
<td>Ischemic stroke</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Ehrbroch et al. (2000)</td>
<td>C677T</td>
<td>Case-control: 219 cases, 205 controls</td>
<td>Ischemic stroke</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Voelkel et al. (2000)</td>
<td>C677T</td>
<td>Case-control: 153 cases, 225 controls</td>
<td>Ischemic stroke</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Yoo et al. (2000)</td>
<td>C677T</td>
<td>Case-control: 122 cases, 217 controls</td>
<td>Ischemic stroke</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Zheng et al. (2000)</td>
<td>C677T</td>
<td>Case-control: 115 cases, 122 controls</td>
<td>Ischemic stroke</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Gopala et al. (2001)</td>
<td>C677T</td>
<td>Case-control: 100 cases, 238 controls</td>
<td>Ischemic stroke</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Topic et al. (2001)</td>
<td>C677T</td>
<td>Case-control: 16 cases, 124 controls</td>
<td>Ischemic stroke</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>Wu et al. (2001)</td>
<td>C677T</td>
<td>Case-control: 77 cases, 229 controls</td>
<td>Ischemic stroke</td>
<td>Positive (women)</td>
<td></td>
</tr>
<tr>
<td>Zheng et al. (2001)</td>
<td>C677T</td>
<td>Case-control: 102 cases, 200 controls</td>
<td>Ischemic stroke</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Gopala et al. (2001)</td>
<td>C677T</td>
<td>Case-control: 91 cases, 184 controls</td>
<td>Ischemic stroke</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Mcilroy et al. (2002)</td>
<td>C677T</td>
<td>Case-control: 64 cases, 71 controls</td>
<td>Ischemic stroke</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Pozzi et al. (2002)</td>
<td>C677T</td>
<td>Case-control: 31 cases, 35 controls</td>
<td>Ischemic stroke</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Yindong et al. (2002)</td>
<td>C677T</td>
<td>Case-control: 43 cases, 42 controls</td>
<td>Ischemic stroke</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Choi et al. (2003)</td>
<td>C677T</td>
<td>Case-control: 195 cases, 196 controls</td>
<td>Ischemic stroke</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>Pozzi et al. (2003)</td>
<td>C677T</td>
<td>Case-control: 125 cases, 149 controls</td>
<td>Ischemic stroke</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Soiroki et al. (2003)</td>
<td>C677T</td>
<td>Case-control: 867 cases, 743 controls</td>
<td>Ischemic stroke</td>
<td>Negative</td>
<td></td>
</tr>
</tbody>
</table>
Ischemic stroke

Negative
Positive
Case-control: 69 cases, 68 controls
Lacunar stroke
Cross-sectional: 234 cases
Case-control: 689 cases, 652 controls
I/D
Case-control: 322 cases, 1126 controls
Ischemic stroke/ Carotid atherosclerosis
I/D
CT 2/3
Case-control: cases 79, controls 126
Case-control: 388 cases
Ischaemic stroke
Cerebral infarction
IMT in patients with and without CHD
Cerebral atherosclerosis
Ischaemic stroke and mortality
I/D
Aolto-Setala et al. (1998)
Ischemic stroke
I/D
Positive
Case-control: 193 cases, 147 controls
Ischemic stroke
Negative
Positive
Case-control: 104 cases, 94 controls
Carotid atherosclerosis in patients with ischemic stroke
I/D
Negative
Negative
Cross-sectional: 280 cases
I/D
Carotid atherosclerosis
Ischemic stroke
Case-control: cases 689, controls 652
Aolto-Setala et al. (1998)
Ischemic stroke
Case-control: 100 cases, 100 controls
Positive
Case-control: 55 cases, 61 controls
Case-control: 100 cases, 137 controls
Case-control: 125/56 cases, 95 controls
Negative
Positive
Cross-sectional: 234 cases
I/D
Ischaemic stroke
Ischemic stroke
Case-control: cases 491, controls 400
Cohort study: 1077 subjects
Ischemic stroke
Ischemic stroke or TIA
Positive
Positive
Positive
I/D
Case-control: 767 cases, 735 controls
Ischemic stroke
Case-control: 228 cases, 90/104 controls
Negative
I/D
Ischaemic stroke
Ischemic stroke
I/D
Negative
Negative
Ischaemic stroke and OCSP subtype
Case-control: cases 189
Positive
Cerebral infarction
I/D
Ischemic stroke
Ischaemic stroke
I/D
Positive
Cerebral atherosclerosis
Ischemic stroke
Case-control: 65 cases, 61 controls
Ischemic stroke
Positive
Ischemic stroke
Negative
Case-control: 306 cases, 300 controls
Negative
Ischemic stroke
Positive
Ischemic stroke
SstI
Positive
Cao et al. (1998)
glu122asp
Cross-sectional: 197 cases
IMT in patients with ischemic stroke > 60 years
SstI
Positive
Cases 5401
Carotid atherosclerosis
Negative
I/D
Aso-Setala et al. (1998) SstI Cross-sectional: 234 cases
Carotid atherosclerosis in patients with ischemic stroke > 60 years
Negative
Patch et al. (1994) XmnI Cross-sectional: 268 cases
IMT in groups with different lipid profiles
Positive
apo A1/CII
Aso-Setala et al. (1998) XbaI Cross-sectional: 234 cases
Carotid atherosclerosis in patients with ischemic stroke > 60 years
Negative
Lipoprotein lipase
Huanga et al. (1997) A201G Case-control: 125/56 cases, 95 controls
Ischemic stroke / carotid atherosclerosis
Negative
ACE
Sharma et al. (1994) ID
Case-control: 100 cases, 73 controls
Ischaemic stroke
Negative
Markus et al. (1995) ID
Case-control: 100 cases, 137 controls
TONST subtype
Positive
Linda et al. (1995) ID
Case-control: 488 cases, 188 controls
Ischaemic stroke and OCSP subtype
Case-control: 100 cases
Ischaemic stroke
Positive
Yang et al. (2002) ID
Case-control: 488 cases, 188 controls
Ischaemic stroke and OCSP subtype
Positive
Lutfia et al. (2002) ID
Case-control: 488 cases, 188 controls
Ischaemic stroke
Positive
Sakurada et al. (2002) ID
Case-control: 488 cases, 188 controls
Ischaemic stroke
Positive
Kouyoumdjian et al. (2001) ID
Case-control: 338 cases, 99 controls
Ischaemic stroke / carotid stenosis
Positive
MacLeod et al. (2001) ID
Case-control: 491 cases, 491 controls
Ischaemic stroke
Positive
Sinistere et al. (2001) ID
Case-control: 79, controls 126
Ischemic stroke
Positive
Sakura et al. (2001) ID
Case-control: 388 cases, 115 controls
Ischaemic stroke
Positive
Ischaemic stroke
I/D
Positive
Negative
Cross-sectional: 234 cases
IMT in NIDDM
IMT NIDDM patients
Case-control: 60 cases, published controls
Meta-analysis: 1918 cases, 722 controls
I/D
Cases 5401
Ischemic stroke (atherothrombotic)
Cerebral infarction
I/D
Carotid atherosclerosis
Ischaemic stroke
I/D
Non hypertensive patients with ischemic stroke aged <60 years
Symptomatic stroke or MRI silent lacunae in hypertensives, Hypertensives/nonhypertensive controls
Positive
Hosoi et al. (1996) ID
Case-control: 228 cases, 90/104 controls
Symptomatic stroke or MRI silent lacunae in hypertensives, Hypertensives/nonhypertensive controls
Positive
Sepe et al. (1996) ID
Case-control: 50 cases, 25 controls
Cerebral atherosclerosis
Positive
Nakata et al. (1997) ID
Case-control: 55 cases, 61 controls
Ischaemic stroke
Positive
Doi et al. (1997) ID
Case-control: 181 cases, 271 controls
Ischaemic stroke (atheroembolic/lacunar)
Positive
Watanabe et al. (1997) ID
Case-control: 228 cases, 90/104 controls
Symptomatic stroke or MRI silent lacunae in hypertensives, Hypertensives/nonhypertensive controls
Positive
Hosoi et al. (1996) ID
Cross-sectional: 288 cases
IMT in NIDDM patients
Positive
Sepe et al. (1996) ID
Cross-sectional: 288 cases
IMT in NIDDM patients
Positive
Nakata et al. (1997) ID
Cross-sectional: 197 cases
Positive
Miyazaki et al. (1998) ID
Case-control: 52 cases, 80 controls
Non hypertensive patients with ischemic stroke
Positive
Pichl et al. (1998) ID
Case-control: 388 cases
Ischaemic stroke / carotid artery stenosis
Positive
Setino et al. (1998) ID
Case-control: 26 cases, 28 controls
Cerebral infarction
Positive
Lee et al. (1999) ID
Neurologic case-control: 348 cases, 348 controls
Ischaemic stroke/PRCH
Positive
Koistinen et al. (1999) ID
Case-control: 100 cases, 100 controls
Ischaemic stroke / carotid stenosis
Positive
Li et al. (2000) ID
Case-control: 306 cases, 306 controls
Stroke
Negative
Locci et al. (2000) ID
Case-control: 406 cases
Stroke
Positive
Gin et al. (2001) ID
Case-control: 106 cases, 498 controls
Cerebral infarction
Positive
Sairiaux et al. (2000) ID
Case-control: 689 cases, 689 controls
Cerebral infarction
Positive
Gin et al. (2003) ID
Case-control: 306 cases, 319 controls
Cerebral infarction
Positive
Karagianis et al. (2004) ID
Cross-sectional: 264 cases
Ischaemic stroke
Positive
Barley et al. (1995) R2251
Cross-sectional: 100 cases, 45 spouse controls
Ischaemic stroke / carotid atherosclerosis
Negative
Aalto-Setala et al. (1998) R2251
Cross-sectional: 55 cases, 61 controls
Ischaemic stroke
Negative
because stroke event rates are comparatively low (in comparison with MI), making this a resource-intensive task. In addition, circulating levels obtained from peripheral blood samples may bear no relationship to local intracerebral levels of hemostatic proteins. One way to overcome this problem is to study the genetic regulation of the circulating levels of the hemostatic proteins, and the remainder of this article considers a number of hemostatic proteins (excluding platelet glycoproteins) and their genetic variants in relation to CVD.

Coagulation and fibrinolytic system in stroke

Factor V Leiden (G1691A). A misense mutation in codon 506 (Arg206.Gln) of the factor V Leiden gene (G1691A) is directly associated with resistance to activated protein C (APC) through prolongation of the action of factor Va. Two to seven percent of the general population carry this mutation, and the mutation is now considered to be the most common inherited form of venous thrombosis, occurring in 10–40% of cases with venous thromboembolism. Most larger case-control studies have failed to find an association between prothrombotic states, such as activated protein C resistance or the underlying Leiden factor V mutation, and ischemic stroke in older individuals. This gene defect may be responsible for stroke in some younger individuals, but these prothrombotic states are unlikely to be important causes of multifactorial stroke in middle-aged and elderly patients. Evidence suggests that factor V Leiden and another mutation in the 3’-UTR of the prothrombin gene (G20210A) are candidate genes for stroke resulting from cerebral venous thrombosis.

Fibrinogen. A number of prospective studies have shown a strong association between elevated fibrinogen levels and risk for MI and cerebral infarction. Interestingly, levels of fibrinogen are associated with peripheral vascular disease, which itself is associated with a poorer outcome of ischemic stroke (although whether this is mediated through fibrinogen is not known). In the Northwick Park Heart Study, it was noted that in middle-aged men an increase of only 0.6 g/dl in fibrinogen levels (equivalent to 1 SD) was associated with an 84% increased risk of MI over 5 years. Circulating fibrinogen levels are determined by a number of factors including smoking, age, and gender. In contrast to other hemostatic factors, there is evidence for a relatively strong genetic component to fibrinogen levels. As much as 57% of the variation in levels can be attributed to genetic factors. There are fewer studies of fibrinogen and genotype with risk for CVD compared to ischemic heart disease. However, the Austrian Stroke Prevention Study studied 399 subjects for an association between the [beta]-chain–148C/T fibrinogen polymorphism, fibrinogen levels, and carotid atherosclerosis. The 1/T genotype had a greater degree of atherosclerosis than C allele carriers ($p = 0.003$). The T/T genotype was a significant (OR 6.17) predictor of disease in a multivariate model, although there were relatively small numbers of subjects with this genotype. However, no association was found between genotype and fibrinogen levels. A separate group has reported an association between b-chain 448 fibrinogen variant and CVD in 149 female patients compared with controls free from CVD. This is one of the few examples of a possible gender-specific association with fibrinogen levels and suggests a protective role for the lysine allele in females. There is also a relationship between the [beta]-promoter polymorphism G/A −455 with stroke in Japanese subjects and also the C/T 148 with carotid atheroma. Raised fibrinogen levels may predispose to stroke both by accelerated atherosclerosis and prothrombotic mechanisms.

Factor XIII. Relatively little is known about the molecular structure and function of the fibrin clot in vascular disease, and nothing is known about fibrin structure and function in ischemic stroke or PICH. A common G-T point mutation (Val34Leu) in exon 2 of the [alpha]-subunit of the factor XIII gene in relation to vascular disorders was investigated, demonstrating that possession of the Leu allele is protective against atherothrombotic disease and venous thrombosis, but appears to be involved in the pathogenesis of ICH. Data indicate that among 642 subjects with CVD (the pathologic type of which was defined by cranial CT scan and the Oxfordshire Community Stroke Project classification) and 750 healthy controls, in the 62 subjects with PICH there was a higher prevalence of the Leu allele. This supports the hypothesis that factor XIIIVal34Leu may play a role in the stability of the fibrin clot, and extends previous observations by indicating that possession of the Leu allele is protective against thrombotic disease but increases the risk of hemorrhage. The GENIC investigations found a protective association.

The Leu allele appears to alter fibrin structure/function, and the protective effect is mediated by increased rates of fibrinolysis compared to the Val allele in the presence of platelets and to an interaction between Val34Leu and elevated fibrinogen that alters the rate of activation of factor XIII. The clinical and laboratory findings implicate factor XIIIVal34Leu in both thrombotic and hemorrhagic disease and provide clear therapeutic targets for intervention. Decreased fibrin porosity, turbidity and fiber mass-length ratios for factor XIII Leu allele were also found. The few clinical investigations into ex-vivo fibrin clot structure have been in CAD, but they have produced contrasting findings: decreased permeability of fibrin clots from patients with MI due to tight and rigid fibrin with reduced fiber mass-length ratio, increased fiber mass-length ratio. Fibrin structures with reduced fiber mass-length ratio and reduced pore size are associated with slower rates of lysis by plasmin, while cross-linking of fibrin by factor XIII increases resis-
tance of the clot to lysis. Increased cross-linking by factor XIII has been found in acute MI, but whether the same property operates in ischemic stroke or PICH has not been reported. However, it is probable that genetic variants in the hemostatic system also play a role in the pathogenesis of intracranial hemorrhage. Polymorphism in the coding region of the α-chain (Thr312Ala) was also investigated. This is of interest because it lies close to the factor XIII cross-linking site at position 328. It is also in a region involved in factor XIII A- and B-subunit dissociation and factor XIII activation. We studied aThr312Ala in post-stroke mortality, and possession of the A allele was associated with a dose-related significant reduction in survival compared to those subjects homozygous for the T allele. These findings support the hypothesis that possession of the A allele influences clot stability, although further clinical and in vitro studies are now under way to clarify this issue.

Factor VII. Data on the association of FVII levels with IHD are contradictory: the prospective Northwick Park Study related elevated levels to fatal (but not non-fatal) MI, but the Edinburgh Artery Study found no association. The gene coding for factor VII is polymorphic, and probably accounts for approximately 30% of variance in levels of factor VII. There is no clear relationship between the polymorphic variants and vascular disease. A factor VII gene polymorphism (R353Q) has been associated with higher levels of factor VII:C, but in a later study no association was found between levels of factor VII:C or between the R353Q variant and ischemic cerebrovascular disease. To our knowledge, there is only one study of factor VII levels and genotype in ischemic stroke. The results support prior findings of a relationship between genotype and levels of factor VII, but neither were associated with ischemic CVD. This is consistent with the results of a separate large study of arterial thrombotic events. Another study examining factor VII polymorphisms in hypertensive small vessel disease also gave negative results.

Plasminogen activator inhibitor-1. Plasminogen activator inhibitor-1 (PAI-1) is the fast-acting inhibitor of tissue plasminogen activator (t-PA). Evidence to support the role of the fibrinolytic system comes from studies demonstrating suppressed fibrinolysis or high PAI-1 levels to CAD presenting as either angina or MI. In prospective studies, diminished fibrinolysis predicts MI and high PAI-1 activity predicts recurrence of MI in young men but not in older patients. The evidence for a role of PAI-1 levels in stroke is less clear. In some studies, elevated levels of PAI-1 are seen in acute stroke, whereas in others, suppression or no difference in PAI-1 levels is seen. The conflicting results probably reflect differing analytical techniques for PAI-1 and the population of stroke subjects studied. The gene coding for PAI-1 has several polymorphic loci, and the 4G/5G polymorphism exhibits differential transcriptional responses to interleukin-1 in HepG2 cells, with higher rates of PAI-1 synthesis in cells with the homozygous 4G/4G genotype. In one large case-control study involving over 600 subjects with CVD, there was no difference in genotype frequency compared to a control group and no relationship between the 4G/5G genotype and PAI-1 levels, although Margaglione did suggest an association of the 5G/5G genotype with stroke. It is likely that the overall influence of the 4G/5G genotype on the pathogenesis of ischemic stroke is small. However, it has recently been suggested that the 4G allele is associated with a reduced risk for cerebrovascular mortality in females. The authors suggested that PAI-1 might be acting via pathways other than fibrinolysis. They hypothesize that PAI-1 might protect against destabilization of the atherosclerotic plaque, or inhibit the neurotoxic action of tissue plasminogen activator in the brain.

Platelet glycoprotein receptor. The role of platelet glycoprotein receptor polymorphisms has also been studied extensively in patients with ischemic stroke. These molecules are members of the integrin family and, when activated, bind fibrinogen, von Willebrand factor or collagen, and therefore promote platelet aggregation and thrombosis. The P1A2 variant of the platelet fibrinogen receptor Gp IIa/IIIb has been reported a risk factor for acute coronary syndromes specifically in young patients. Subgroup analysis in a case–control study has suggested that the P1A2 allele may also be an important risk factor in stroke patients aged less than 50 years. The other study failed to find an overall association between this polymorphism and cerebral infarction in young women. Conflicting genotype–phenotype correlations have also been found with the HPA2 (human platelet antigen 2) and VNTR (variable number of tandem repeats) variants of the platelet von Willebrand factor receptor, Gp Ia/IIa. It has been reported recently that a silent point mutation (Gpla C807T), correlating with increased expression of the collagen receptor in vitro, is an independent risk factor for stroke in young patients. However, association studies of different polymorphisms in this gene have revealed a lack of association.

Homocystein. In recent years, attention has focused on the role of plasma homocyst(e)ine (Hcy) as one such candidate risk factor. Following early observations of premature atherothrombotic complications in children with severe hyperhomocyst(e)emia (hyper-Hcy; Hcy level, > 100 µmol/L) due to inborn errors of metabolism, subsequent studies have supported a possible relationship between an elevated Hcy level and atherosclerotic vascular disease. Several studies have addressed the question of a potential association between hyper-Hcy and CVD defined as clinical stroke, carotid atherosclerosis, or intima–media thickening. Some of these have reported no evidence of an association, whereas others have reported a strong link between hyper-Hcy and
CVD. Interpretation of the results of these studies as a group is complicated by the variability in study design, sample size, entry criteria, and outcome measures employed. The prevalence of hyperhomocysteinemia for the cystathionine $\beta$-synthase gene is estimated at 0.5–0.15% of the population, heterozygous individuals possessing $\sim$30% of the normal enzyme activity. Several studies determined whether allele heterozygosity is itself a significant risk factor for polygenic ischemic stroke. Two studies found an increased frequency of heterozygotes in patients with occlusive cerebrovascular disease compared with controls, and this has also been shown in patients with asymptomatic carotid artery atherosclerosis. In contrast, no association was found between heterozygosity for the cystathionine $\beta$-synthase gene and either carotid intima media thickness or asymptomatic carotid atherosclerosis. These inconsistencies may reflect the different ages of the patients examined, mechanisms other than wall disease through which homocysteine acts, or the influence of multiple risk factors on homocysteine levels and carotid artery damage in carriers. Very rarely, patients with homocysteinuria have complete deficiency of methylene tetrahydrofolate reductase, a folate-dependent enzyme catalysing the rate-limiting step in the methylation of homocysteine to methionine. In 1988, a common thermolabile variant of methylene tetrahydrofolate reductase associated with decreased enzyme activity and mildly elevated plasma homocysteine levels was identified. A single base pair (677C$\rightarrow$T) substitution in the human MTHFR gene predicts phenotypic expression of a heat-sensitive variant with reduced enzymatic activity. This variant has been considered an ideal candidate genetic polymorphism for predisposition to ischemic stroke, as it is common in many populations studied to date and the genotype correlates highly with the plasma Hcy level in a dose-dependent manner. However, studies that have examined the risk for atherosclerotic vascular disease and stroke associated with the MTHFR 677C$\rightarrow$T polymorphism have reported conflicting results, which has prevented a definitive conclusion to date. A small influence (pooled relative risk, 1.23) of the MTHFR 677TT genotype on stroke risk, which tended toward, but did not reach, the threshold for statistical significance was found. Despite these considerations, evidence from several sources supports the concept that moderate elevations in the plasma Hcy concentration may contribute to the pathogenesis of atherosclerosis and ischemic stroke. First, children with homocystinuria due to mutations in homocysteine-pathway enzymes develop complications of premature atherosclerosis and thrombosis, in the absence of other vascular risk factors. Second, prospective cohort studies have demonstrated a dose-dependent relationship between plasma Hcy concentration and other measures of vascular disease, such as carotid atherosclerosis and intima–media thickening. Third, several studies have described in vivo reversible impairment of endothelial-dependent vasodilatation associated with an elevated Hcy level, thus supporting the concept of Hcy-mediated endothelial injury. Finally, experimental and clinical data suggest that in vivo auto-oxidation of homocysteine thiol groups results in the formation of reactive oxygen species, promoting peroxidation of lipids bound to low-density lipoproteins. Both endothelial injury and low-density lipoprotein–lipid peroxidation are thought to be important early pathophysiologic processes in the development of atherosclerotic lesions. Most studies have demonstrated that the TT genotype is consistently associated with greater plasma Hcy concentrations, compared with those in normal CC or CT heterozygous subjects. Despite this observation, studies that have investigated the potential role of the 677C$\rightarrow$T polymorphism in determining susceptibility to ischemic stroke have obtained widely differing results, some reporting no association, whereas others have reported a greater than threefold increased risk associated with the homozygous state. The explanation for these findings is unclear at this time. A meta-analysis found that the C677T variant was associated with mild homocystinemia but not increased vascular risk. There is an interaction with folate, and it is still possible that the methylene tetrahydrofolate reductase polymorphism may be a risk factor in younger individuals with low folate intake, but further studies are required in these populations.

Renin–angiotensin system. The ACE gene is probably the most extensively investigated candidate gene in ischemic stroke, after an initial study by Cambien and co-workers which suggested that an intron 16 insertion/deletion polymorphism was associated with myocardial infarction. A number of studies have reported an association with stroke, with a relative risk usually of the order of 1.5–2.5, but other studies have failed to find a significant association. A meta-analysis has evaluated the risk of stroke in 1918 subjects versus 722 controls from seven studies. It was concluded that the ACE genotype conferred a small but modest effect, with an odds ratio of 1.31 (95% confidence interval 1.06–1.62), according to a dominant model of inheritance. A weaker association was seen under a recessive model. A nested case–control study was performed on the US Physicians Health cohort and a negative result was reported. Although reducing the risk of false-positive results due to selection bias, such prospective cohort studies tend to suffer from poor stroke phenotyping; in this study the cases included both ischemic and haemorrhagic stroke, and no subtyping of ischemic stroke subtypes was performed. Such analyses will fail to detect a selective association with a particular stroke phenotype, and this may be of particular importance with the ACE gene. A number of studies have reported an association that was strongest or exclusively with lacunar stroke, and these findings are consistent with reported associations between the deletion allele and MRI-detected silent small vessel disease in hyper-
tensives. A weak association was found between the deletion polymorphism and all ischemic stroke cases in Han Chinese in Taiwan. When a further study was performed with more detailed investigations allowing recruitment of only lacunar stroke patients, a much stronger positive association was found. ACE deletion/insertion polymorphism is not a major risk factor in an unselected group of patients with ischemic stroke, but it may be a risk factor for small vessel disease. A variant of the angiotensinogen gene (M235T) has also been implicated in vascular disease, but its evaluation in stroke so far suggests that it does not behave as an important risk factor. However, it has recently been proposed that an epistatic interaction with the ACE gene may exist.

**Lipid metabolism.** Individuals with higher levels of plasma cholesterol, increased HDL (high-density lipoprotein) and decreased LDL (low-density lipoprotein) have a higher risk of premature atherosclerosis. The phenotype may arise not only from single gene disorders, as discussed above, but also from a number of genetic and environmental factors, including polymorphic variants of genes encoding the apolipoproteins, lipoprotein receptors and the key enzymes of plasma lipoprotein metabolism. The role of apolipoproteins and enzymes in relation to stroke and carotid artery disease is considered in Table 1. The studies to date have produced conflicting results as to the importance of apolipoproteins in predisposition to ischemic stroke. In small case–control or cross-sectional studies, both the 2/3 genotype and the E4 allele have been over-represented in patients with ischemic stroke. Other groups have examined the role of the apolipoprotein E genotype in modulating the outcome of cerebral infarction as this lipoprotein appears to be an important regulator of lipid turnover within the brain and of neuronal membrane maintenance and repair. Studies in patients with head injury and intracerebral hemorrhage have indicated that the E4 allele is a predictor of poor outcome in terms of death and disability, and this is consistent with studies of cognitive decline in 4 carriers with cerebrovascular disease. McCaron and colleagues found a favorable effect of E4 on stroke outcome. Stanković and colleagues (126) gave detailed description of the conflicting results as to the importance of apolipoprotein E alleles in predisposition to ischemic stroke.

**Endothelial nitric oxide synthase.** The activity of the L-arginine/nitric oxide synthase system is an important mediator of endothelial function. Nitric oxide (NO) plays a key role in the regulation of vascular tone, and reduces vascular smooth muscle cell proliferation and adhesion of platelets and leucocytes. A few studies on coronary disease suggest that NO deficiency may have a genetic basis, while others did not find any association of the Glu298Asp polymorphism and the 27-basepair repeat in intron 4 of the endothelial constitutive NO synthase gene with ischemic stroke. However, preliminary results of the GÉNIC study are in favour of an association of the GG genotype of the Glu298Asp polymorphism with ischemic stroke, and more particularly with lacunes. A higher frequency of the nn (GG) genotype was found in lacunes compared with other subtypes. Strong evidence from animal and human studies indicates that the activity of this system is under genetic control. Work in the stroke-prone SHR rat has suggested that impaired endothelial dysfunction is an important predisposing factor leading to stroke. In addition, knockout mice deficient in endothelial nitric oxide synthase are highly sensitive to focal cerebral ischaemia and have marked vessel wall abnormalities. An earlier study had demonstrated that a functional variant of nitric oxide synthase (ecNOS 4a) was associated with increased risk of significant coronary artery disease and myocardial infarction in smokers. However, neither this nor another variant with unknown functional significance (Glu298Asp) has been shown to be an important risk factor for ischemic cerebrovascular disease. The genes encoding both the neuronal and the inducible form of nitric oxide synthase are potential candidate genes for stroke. In animal models, their inhibition reduces infarct size, which is also smaller in knockout mice. Both genes have been cloned and common polymorphisms described.

**Atrial natriuretic peptide.** Evidence for the existence of genes directly contributing to stroke occurrence was first obtained in the animal model of stroke-prone spontaneously hypertensive rat through a linkage analysis approach in F2 segregating hybrid populations. Several quantitative trait loci were detected in different chromosomes of the rat. Candidate genes were identified (ANP, BNP, adrenomedullin) and subsequently analyzed to obtain information on the fine disease mechanisms possibly dependent on specific sequence mutations. Gene encoding ANP appeared to play a role in the disease. Characterization of both BNP and adrenomedullin failed to identify differences between the stroke-prone animal model and its related control. Furthermore, we were unable to demonstrate an involvement of these genes in the human disease, and no positive findings have so far been reported by other groups. In contrast, our experience with the gene encoding ANP has been so far quite promising. Among other genes, the atrial natriuretic peptide (ANP) gene has been involved in the pathogenesis of stroke. In fact, structural abnormalities of ANP are significantly associated with stroke in both an animal model, the stroke-prone spontaneously hypertensive rat, and the North American white population of male physicians recruited in the Physicians Health Study (PHS) and an Italian population from Sardinia. These two human studies identified a twofold risk of stroke independent of hypertension, obesity, and diabetes in subjects-carriers of an exon 1 mutation of the ANP gene carrying the exon 1 mutation (mutation responsible for a Val/Met trans-
position within the 1-30 proANP (long-acting natriuretic peptide) and 3.8-fold increased risk of ischemic stroke in carriers of a stop codon mutation (responsible for the synthesis of a 30 rather than 28 aa mature ANP peptide) (127).

Phosphodiesterase 4D. Linkage based approaches applied to Icelandic stroke patients identified a locus for a gene for common stroke on chromosome 5q12. Initial analyses suggested that it was a risk factor for ischemic stroke but not for other types of vascular disease such as myocardial infarction or peripheral arterial disease. By further analyses, the responsible gene-phosphodiesterase 4D (PDE4D) was identified and characterized. The PDE4D gene encodes a cyclic nucleotide phosphodiesterase which is involved in the selective degradation of second messenger cAMP, which has a central role in signal transduction and regulation of physiological responses. In vascular smooth muscle cells, low cAMP levels lead to cell proliferation and migration that is mediated in part by PDE4D, and increase immune functions which lead to the development and progression of atherosclerosis. The highest risk haplotype (present in 9% of controls) conferred a twofold relative risk. A protective haplotype (present in 21% of controls) was identified with a relative risk of 0.7. However, none of the associated variants were present in protein coding or gene splicing regions, suggesting that the identified or associated variants affect gene regulation (for example expression level) rather than having a direct functional effect on the protein. The association between PDE4D and stroke now needs replication in independent populations. This may represent a completely new pathophysiological process causing stroke and could open the way for new therapeutic opportunities for disease prevention (128).

5-lipoxygenase activating protein. The identification and characterisation of ALOX5AP, a gene coding for 5-lipoxygenase activating protein, in which certain common haplotypes double the risk of both stroke and myocardial infarction, was reported. The initial finding was a suggestive linkage to a region of chromosome 13 in a series of 296 Icelandic families with multiple affected members. A case-control association study was carried out using a high density of markers across the implicated region (containing 40 known genes) which led to the identification of the ALOX5AP susceptibility gene. This was confirmed in a UK population, although the associated haplotype was different. The individual or combination of variants associated with disease risk remain to be identified. ALOX5AP and 5-lipoxygenase together convert unesterified arachidonic acid to the leukotriene LTA4, which is further converted to LTB4 or LTC4. These are important proinflammatory mediators which are active in macrophages and leukocytes invading atherosclerotic lesions (129).

Inflammatory molecules. In recent years, there has been increasing appreciation of the fact that inflammatory molecules, as well as single nucleotide polymorphisms of genes encoding inflammatory mediators, may contribute to the development and progression of a large number of pathological conditions (130). Single nucleotide polymorphism of proinflammatory and anti-inflammatory genes may strongly influence the plasma levels and biological activity of the corresponding proteins with potentially important clinical implications. It has been suggested that proinflammatory gene variations may act synergistically and determine genetic profiles associated with increased risk for diseases. The polymorphisms of C-reactive protein (1059G/C), interleukin-4 (582C/T), interleukin-6 (174G/C), macrophage migration inhibitory factor (173G/C), monocyte chemoattractant protein-1(2518A/G), intracellular adhesion molecule-1 (469E/K), E-selectin (Ser128Arg), P-selectin (Val640Leu), matrix metalloproteinase-3 genes (11715A/6A), were associated with ischemic stroke in different studies. Also, the relationship between single nucleotide polymorphism (SNP) in the gene that encodes the amiloride-sensitive epithelial sodium channels (ENaCs) and ischemic cerebrovascular event was identified in the newer studies.

Conclusion

The identification of novel and important genes that appear to be responsible for some cases of ischemic stroke will open new avenues of investigation for those interested in genetics and ischemic stroke. Additional knowledge of the role of genes in ischemic stroke may improve our understanding of the cause of stroke, provide new insights into prevention and the factors that influence the outcome of stroke, and new therapeutic targets when preventive strategies have failed. Stroke therapy will undergo a great revolution in the present decade. The knowledge of the human genome, gene interactions and proteomics will permit a new concept of drug development for stroke. Gene therapy by modification of gene expression will be useful in treating atherosclerosis and hypertensive microangiopathy, or in the acute phase, when we will manipulate the acute gene expression induced by ischemia or the apoptotic gene program. However, a single abnormal gene, as in monogenic diseases, is easier to replace than several genes in complex multifogenic disorders. Gene therapy, stem cell therapy and neurological grafts for stroke are still in the experimental phase, and many hurdles will have to be jumped before the introduction of these therapies into human clinical stroke trials. A more immediate clinical application of genetics to stroke therapy is the development of pharmacogenetics that analyzes the influence of genetic variability of individuals on drug response. A new era of personalized therapy is dawning where specific DNA biochips will help stroke clinicians decide on the better use of thrombolytics, neuroprotectants, anti thrombotics, statins or antihypertensives.
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