On certain genetic and metabolic risk factors for carotid stenosis and stroke

Pär W. Wanby

Linköpings universitet
FACULTY OF HEALTH SCIENCES

Department of Medicine and Care, Division of Internal Medicine,
Faculty of Health Sciences, Linköping University,
SE-581 85 Linköping, Sweden.

Department of Internal Medicine, County Hospital of Kalmar,
SE-391 85 Kalmar, Sweden.

Linköping and Kalmar 2006

*"Knowledge is like a sphere, the greater its volume,
 the larger its contact with the unknown"*

Blaise Pascal

To Anna-Stina, Ingrid, Märta and Nils

ABSTRACT

The present study evaluated genetic and metabolic factors influencing the risk of acute cerebrovascular disease (CVD) and internal carotid artery stenosis (ICA stenosis) in a Swedish community. The threonine (T) containing protein of the FABP2 A54T gene polymorphism has a greater affinity for long chain fatty acids (FFAs) than the alanine (A) containing protein. This altered affinity for FFAs has been shown to affect the intestinal absorption of fatty acids and consequently the fatty acid composition of serum lipids, in particularly postprandially. Endothelium derived NO is a potent vasodilator and antiatherogenic agent. Asymmetric dimethyl arginine (ADMA) is an endogenous competitive inhibitor of endothelial nitric oxide synthase (eNOS). ADMA has been shown to be involved in the pathogenesis of atherosclerotic disease, and ADMA inhibits eNOS by displacement of L-arginine from the enzyme, which in turn is believed to affect the amount of NO available within the endothelium.

The FABP2 A54T gene polymorphism was analyzed in 407 patients with acute CVD and also in a subset of these patients whose carotids had been evaluated with ultrasound. Both the FABP2 polymorphism and a common polymorphism of the eNOS gene, Glu298Asp, were analyzed in a different population consisting of 54 matched pairs of patients with ICA stenosis and controls. ADMA levels were measured in both study populations.

We found that the T54 allele was more frequent in patients with transient ischaemic attacks (TIA), and that the TT genotype was more prevalent in young, non-smoking patients with CVD than in controls.

Increased concentrations of ADMA were observed in cardio-embolic infarction and TIA, but not significantly in non-cardio-embolic infarction nor in haemorrhagic stroke. In multivariate logistic regression models, CVD increased across quartiles of ADMA in all subgroups, but this association was only significant in the TIA group. A decreased arginine/ADMA ratio, a measure of NO availability was associated with CVD in the entire study population. Patients with severe carotid stenosis had significantly higher ADMA levels than the controls. Allele and genotype frequencies of the FABP2 and eNOS polymorphisms did not differ between patients with ICA stenosis and controls.

Our results indicate that ADMA is a strong marker for TIA and severe ICA stenosis, and that relative defiency of arginine, measured as L-arginine/ADMA, is present in acute CVD. We also conclude that a common polymorphism of the FABP2 gene increases susceptibility to ischaemic stroke and TIA.

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals:

I. Wanby P, Palmqvist P, Rydén I, Brattström L, Carlsson M. The FABP2 gene polymorphism in cerebrovascular disease. Acta Neurol Scand 2004; 110: 355-360.

II. Pär Wanby, MD; Petter Palmqvist, MD; Lars Brudin, MD, PhD; Martin Carlsson, MD, PhD. Genetic variation of the intestinal fatty acid-binding protein 2 gene in carotid atherosclerosis. Vascular Medicine 2005; 10: 103-108.

III. P. Wanby, T. Teerlink, L. Brudin, L. Brattström, I. Nilsson, P. Palmqvist, M. Carlsson. Asymmetric dimethylarginine (ADMA) in a Swedish population with acute cerebrovascular disease. Atherosclerosis 2006; 185: 271-277.

IV. Pär Wanby, Ingela Nilsson, Lars Brudin, Ida Nyhammar, Martin Carlsson. Asymmetric dimethylarginine and polymorphisms of fatty acid-binding protein 2 and endothelial nitric oxide synthase genes in carotid atherosclerosis. Manuscript.

Contents

Papers I-IV

Abbreviations

ADMA	asymmetrical dimethylarginine
Ala	alanine
Arg	arginine
BMI	body mass index
cAMP	cyclic AMP (adenosine monophospate)
cGMP	cyclic GMP (guanosine monophosphate)
CRP	C-reactive protein
CVD	cerebrovascular disease
DDAH	dimethylarginine dimethylamino-hydrolase
eNOS	endothelial nitric oxide synthase
FABP2	fatty acid-binding protein 2
FFA	free fatty acid
HDL	high density lipoproteins
HPLC	high performance liquid chromatography
ICA	internal carotid artery
IMT	intima media thickness
LDL	low density lipoproteins
NO	nitric oxide
NOS	nitric oxide synthase
OR	odds ratio
PCR	polymerase chain reaction
PDE4D	phosphodiesterase 4D
RFLP	restriction fragment length polymorphism
RR	relative risk
PRMT	protein arginine methyltransferase
PUFA	polyunsaturated fatty acid
SDMA	symmetrical dimethylarginine
SNP	single nucleotide polymorphism
TG	triglyceride
Thr	threonine
TIA	transient ischaemic attack
TNF-α	tumour necrosis factor-alpha
TRL	triglyceride-rich lipoproteins
SAH	subarachnoidal haemorrhage
VLCFA	very long-chain fatty acid
VLDL	very low density lipoprotein

Definitions

Codon	A group of three mRNA bases, each of which specifies an amino acid when translated
Hardy-Weinberg law	Specifies an equilibrium relationship between gene frequencies and genotype frequencies
Linkage disequilibrium	A higher frequency of combined genetic markers than expected from the normal frequency of recombination
Fatty acids; short-chain	Fatty acid which contains 2-6 carbon atoms
medium-chain	Fatty acid which contains 8-12 carbon atoms
long-chain	Fatty acid which contains more than 12 carbon atoms
very long-chain (VLCFA)	Fatty acid which contains 18 or more carbon atoms
omega 3	A family of polyenoic acids with three or more cis-unsaturated centers separated from each other by one methylene group and having the first unsaturated centre three carbons from the methyl end
omega 6	A family of polyenoic acids with two or more cis-unsaturated centers separated from each other by one methylene group and having the first unsaturated centre on the sixth carbon from the methyl end
Odds ratio	The ratio of odds of having the target disorder in the disease group relative to the odds in favour of having the target disease in the control group
Polygenic disease	Disease which is caused by the combined effects of multiple genes
Polymorphism	A locus in which two or more alleles have gene frequencies greater than 1 % in a population
Population attributable risk	The influence of an exposure on the risk of disease throughout the entire population
Relative risk	The ratio of disease or death among the exposed to the disease or death among the non-exposed
Risk factor	A factor that indicates that an individual has increased risk of developing a disease, but which does not necessarily cause the disease

1. INTRODUCTION

Stroke is a devastating condition, which affects 30,000 Swedes every year, of which 20,000 are affected for the first time (National Stroke Register, 2005). In the western world the annual incidence of stroke in elderly populations approaches 2% and stroke is the third leading cause of death (Bonita and Beaglehole, 1993). The mean age of stroke patients in Sweden is 75 years (Asplund, 2003). Cerebrovascular disease and stroke also cause vascular dementia, the second most common form of dementia after Alzheimer´s disease. Stroke is a clinical syndrome defined as "rapidly developing clinical signs of focal (or global) disturbance of cerebral function, with symptoms lasting 24 hours or longer, or leading to death, with no apparent cause other than of vascular origin" (Aho et al., 1980). In transient ischaemic attacks (TIAs) focal disturbances disappear within 24 hours. Depending on the localisation in the brain and the severity of the brain damage the neurological deficit in stroke presents with a wide range of symptoms, such as hemiparesis, hemianopsia, dysarthria, diplopia, dysphagia, vertigo and aphasia.

Stroke is a complex multi-factorial disease. Prevention of stroke is dependent on identification of factors associated with risk and prevention of stroke is currently the best way of eliminating its consequences. Different subtypes of stroke have different aetiological mechanisms and therefore different risk factor profiles. Among several classifications, the Trial of Org 10172 in acute stroke treatment (TOAST) classification (Adams et al., 1993) is often used (Table 1). Stroke is, on pathophysiological grounds divided into two broad categories: ischaemic stroke and cerebral haemorrhage. Cerebral haemorrhage may further be subdived into intracerebral haemorrhage and subarachnoidal haemorrhage (SAH). Approximately 10 % of all strokes in Sweden are caused by intracerebral haemorrhage, 5 % are caused by SAH and the remaining 85 % by ischaemic stroke (National Stroke Register, 2005). Subdural and epidural haematoma and TIA are not included in the stroke concept. The term acute cerebrovascular disease includes both stroke and TIA (Fig 1).

TIAs are caused by large artery atherosclerosis, small vessel disease, or cardio-embolic disease. In a study on TIAs by Purroy and co-workers (2004), large artery disease was detected in 45 % of the patients. TIA is a severe risk factor of stroke. After a first TIA, more than 10 % of patients suffer a stroke within the next 90 days (Johnston et al., 2000).

Table 1. TOAST classification of ischaemic stroke_____

Ischaemic stroke
1. large artery disease
2. cardioembolic
3. lacunar (small vessel disease)
4. other determined aetiology
5. undetermined aetiology and multiple possible aetiologies

_____
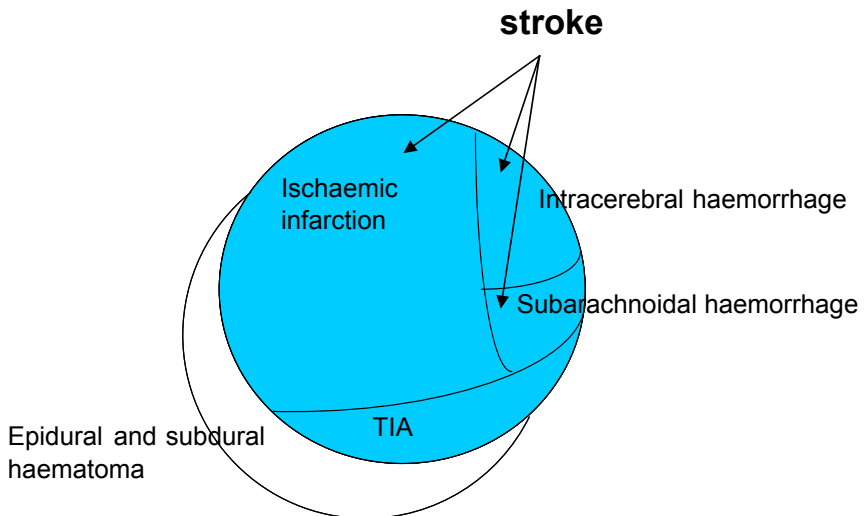
= acute cerebrovascular disease

Fig 1. Acute cerebrovascular disease and its subtypes. Epidural and subdural haematoma
are not included in the concept of acute cerebrovascular disease.

With the exception of SAH, the underlying process in stroke is, in most cases, atherosclerosis.
The pathogenesis of small vessel disease (lacunar infarction) is incompletely understood, but
it is generally thought of as to result of both microatheroma and lipohyalinosis (Lammie et al.,
2002). The conventional risk factors such as hypertension, cigarette smoking, diabetes and
hyperlipidaemia are estimated to account for about half the risk for stroke (Sacco et al., 1989).

Increasing evidence suggests that, particularly in younger individuals, the remaining risk is at least partly due to genetic factors, that is, a genetic component appears to operate outside the usual risk factors (Hassan and Markus, 2000). In addition, the well-documented conventional risk factors are themselves believed to be partly under genetic control (Brass and Alberts, 1995).

In recent years it has become clear that the endothelium, which forms a monolayer of cells in all blood vessels, along with inflammatory cells, plays a pivotal role in the development of atherosclerosis and vascular disease. Impaired endothelial function may promote the development of atherosclerosis through its effects on vasoregulation, platelet and monocyte adhesion, vascular smooth muscle cell growth, and coagulation (Böger 2003). Dysfunction of the endothelium is present in cardiovascular risk factors such as hypertension (Panza et al., 1990) and hypercholesterolaemia (Böger et al., 1998). Triglyceride-rich lipoproteins (TRL) and free fatty acids (FFAs) may also impair endothelial function (Hennig et al., 1985). Endothelium-derived nitric oxide (NO) is a potent endogenous vasodilator with antiatherogenic properties (Cooke and Dzau, 1997). Asymmetric dimethyl arginine (ADMA) is an endogenous, competitive inhibitor of NO synthase (Vallance et al., 1992), and ADMA has been shown to be elevated in large artery disease (Miyazaki et al., 1999).

The present study was undertaken to examine the role of two genetic factors, a polymorphism of the fatty acid-binding 2 gene, a polymorphism of the eNOS gene, and a metabolic factor, ADMA, in cerebrovascular disease.

2. BACKGROUND

2.1 Risk factors in stroke

Risk factors for stroke can be classified according to potential for modification (nonmodifiable, modifiable or potentially modifiable) (Goldstein et al., 2001). Nonmodifiable risk factors are *age, gender, ethnicity and family history*.

Age is the strongest risk factor for stroke. The incidence of stroke doubles in each successive decade after 55 years of age (Brown et al., 1996).

Stroke is more prevalent in men than in women (Wolfe et al., 2000) but due to their greater life expectancy, more women will suffer a stroke during their lifetime (Elkind and Sacco 1998).

The incidence of SAH and intracerebral haemorrhage is increased in African Americans compared to Caucasians (Broderick et al., 1992), and African Americans may also be at higher risk for lacunar infarction and intracranial large vessel disease (Gorelick, 1998). Stroke incidence also appears to be higher in Chinese than in Caucasians (Thorvaldsen et al., 1995). Family history of stroke and genetics in stroke are discussed below.

Hypertension, smoking, diabetes, carotid stenosis, atrial fibrillation, and *hyperlipidaemia* are modifiable risk factors.

Hypertension is a major risk factor for stroke (Collins and MacMahon, 1994). The incidence of stroke increases in proportion to both systolic and diastolic blood pressure, and control of high blood pressure strongly contributes to prevention of stroke. In the Nurses´ Health Study the relative risk was about 2.7 for intracranial haemorrhage, about 2.3 for embolic infarction, about 3.2 for large-artery infarction and about 2 for lacunar infarction (Iso et al., 2000).

Smoking affects both the vasculature and blood rheology. Cigarette *smoking* is a risk factor of stroke with a relative risk of about 2 and the population attributable risk associated with all forms of exposure to tobacco smoke is substantial (Whisnant, 1997).

Both type 1 and type 2 d*iabetes* are associated with an increased risk of stroke. *Diabetes* type 2 is associated with a relative risk of stroke of 4.1 in men and 5.8 in women (Stegmayr and Asplund, 1995). In lacunar infarction a relative risk of about 4 has been reported, of about 1.1 in subarachnoid and intracranial haemorrhage, and of 3.1 in embolic infarction (the Nurses´ Health Study [Iso et al., 2000]).

A carotid stenosis (unilateral and asymptomatic) of $\geq$ 50% was detected in the general

population (Framingham cohort) in 7% of the women and in 9% of the men aged 66 to 93 years (Fine-Edelstein et al., 1994). Other studies suggest that the rate of unheralded stroke ipsilateral to a hemodynamically significant extracranial carotid artery stenosis is approximately 1 to 3 % annually (Bogousslavsky et al., 1986; Inzitari et al., 2000). A symptomatic severe stenosis is associated with a much higher risk of stroke, approaching 30% over the next two years (European Carotid Surgery Trialist´Collaborative Study, 1998). While it is likely that some strokes associated with carotid artery disease result from hypoperfusion (Ringelstein et al., 1988), the majority of such strokes appear to result from embolization from an atherosclerotic plaque or acute occlusion of the carotid and propagation of a thrombus distally (Golledge et al., 2000).

Atrial fibrillation is a cardioembolic source of stroke. The annual overall risk of stroke in patients with *atrial fibrillation* is 3-5% (Wolf et al., 1978). Younger patients free of cardiac disease, diabetes, or hypertension have a low rate of stroke despite atrial fibrillation, 1.3% over 15 years (Kopecky et al., 1987). Other sources of cardio-embolic stroke include myxoma and cardio-valvular disease (Ferro, 2003).

Historically, the etiologic link between *hyperlipidaemia* and stroke has been less clear than between lipids and coronary heart disease. The conflicting results may have several causes. Many studies, particulary in the era before computed tomography, grouped all stroke subtypes together. A meta-analysis (Qizilbash et al., 1991) demonstrated a pooled total stroke risk ratio in hypercholesterolaemia (5.7 mmol/L) of a modest 1.3. Another meta-analysis found no such association (Prospective Studies Collaboration, 1995 [13,000 strokes from 45 cohorts]). Since some studies have shown an inverse relationship between plasma cholesterol concentration and cerebral haemorrhage (Iso et al, 1989; Benfante et al., 1994) the inclusion of cerebral haemorrhage is likely to have concealed or lessened a positive association with ischaemic stroke. Another possible reason for the lack of association in the literature between hypercholesterolaemia and stroke is that the impact of cholesterol may be different in different subtypes of ischemic stroke. In addition, stroke occurs at later age, so that studies of lipids in middle aged people, where heart valve disease and carotid/vertebral artery dissection are common causes of stroke, may not be sensitive to the occurrence of stroke in older subjects. Furthermore, most studies assessing the role of lipids in stroke have not accounted for a possible hypolipaemic effect of acute stroke or TIA (Mendez et al., 1987; Hollanders et al., 1975) thereby possibly missing hyperlipaemia by testing too early.

Several stroke subtypes have been associated with different lipids or lipoproteins (Table 2), although conflicting results also have been reported. In a study on the metabolic syndrome and the risk of stroke (Ninomiya et al., 2004), hypertriglyceridemia was the strongest predictor for stroke.

Table 2. Results from studies on different lipids in stroke subtypes and carotid stenosis.

Lipid subclass	Stroke subtype	Findings	Reference
High total cholesterol	Ischemic stroke	RR=1.4 (lowest vs highest quartile)	Benfante et al., 1994
		OR=1.7 (>6.0 mmol/L)	Qizilbash et al., 1991
		OR=1.4 (upper vs lower tertile)	Koren-Morag et al., 2002
	Large vessel disease (carotid stenosis)	OR=2.4/1.0 mmol/L	Fine-Edelstein et al., 1994
	Cerebral haemorrhage	OR=3 (<4.1 mmol/L)	Iso et al., 1989
	Ischaemic stroke	*n.s.*	Tilvis et al., 1987; Iso et al., 2002
	Large vessel disease (carotid stenosis)	*n.s.*	Ingall et al., 1991
	Small vessel disease	*n.s.*	Iso et al., 2002
High LDL-cholesterol	Ischaemic stroke	6.0 vs 5.4 mmol/L (cases vs controls)	Hachinski et al., 1996
	Large vessel disease	OR=2.0 (4.1 vs < 2.6 mmol/l)	Heiss et al., 1991
	Large vessel disease	*n.s.*	Iso et al., 2002
High HDL-cholesterol	Ischemic stroke	OR=0.29 (protective)	Hachinski et al., 1996
	Large vessel disease	OR=0.53 (protective)	Sacco et al., 2001
Low HDL-cholesterol	Small vessel disease	0.92 vs 1.3 mmol/L (cases vs controls)	Lindgren et al., 1992
High triglycerides	Ischaemic stroke	OR=1.7	Ninomiya et al., 2004
		OR=1.1/1.0mmol/L	Lindenström et al., 1994
	Ischaemic stroke	OR=1.5	Hachinski et al., 1996
	Ischaemic stroke/TIA	OR=1.3	Tanne et al., 2001
	Large vessel disease (carotid plaques)	OR=1.8/1mmol/L	Palomaki et al., 1993
	Small vessel disease	2.3 vs 1.5 mmol/L (cases vs controls)	Laloux et al., 2004
	Cardioembolic stroke	1.7 vs 1.1mmol/l (cases vs controls)	Lindgren et al., 1992
	Ischaemic stroke	*n.s.*	Wannamethe et al., 2000; Sacco et al., 2001
	Large vessel disease	*n.s.*	Ford et al., 1985 (carotid stenosis); Iso et al., 2002

Studies on lipids and extracranial carotid intima thickness give indirect support to *hyperlipidaemia* as a riskfactor in stroke (O´Leary et al., 1992). Several studies have also reported a significant association between hypertriglyceridaemia and extracranial arterial atherosclerosis (Palomaki et al., 1993; Ryu et al., 1992). Other studies have reported a positive association with lacunar infarction and cardioembolic stroke.

The apolipoprotein B/apoprotein A-1 ratio has been shown to be a strong predictor of myocardial infarction (Walldius and Jungner, 2005) and the ratio has also shown similar results in stroke (personal communication Walldius).

Trials on the risk of stroke in regard to the use of the lipid-lowering drugs, statins, support a role for *lipids* in stroke (Scandinavian Simvastatin Survival Study [4S], 1994; Cholesterol and Recurrent Events [CARE], 1996; Long-term Intervention with Pravastatin in Ischemic Disease [LIPID], 1998; Treating to New Targets [TNT], 2005) and subsequent meta-analysis (Herbert et al., 1997; Crouse et al., 1997, Baigent et al., 2005), estimate the risk reduction to 12-48 %. These studies are conducted in patients with coronary heart disease, and therefore are not necessarily representative of the overall stroke population. How the lipid lowering agents provide stroke protection is uncertain. Although some of the stroke reduction may be due to lipoprotein alteration, statins have additional therapeutic effects that could reduce stroke incidence, including upregulation of NO, plaque stabilization and anti-inflammatory properties (Rosenson and Tangney, 1998). Due to its link with HDL-cholesterol level, it has been difficult to interpret the importance of hypertriglyceridemia in stroke. In the Veteran Affairs High-Density Lipoprotein Cholesterol Intervention Trial (VA-HIT: Rubins et al., 1999) a fibrate (gemfibrozil) was associated with a 24 % risk reduction of stroke. In this study triglyceride levels were reduced by 31 % and HDL-cholesterol was increased by 6%. In the Bezafibrate Infarction Prevention (BIP, 2000) trial, patients with low HDL-cholesterol were treated either with bezafibrate or placebo. A 21 % decrease of triglycerides was observed, but it was not accompanied by a reduction of ischemic stroke.

In the Lyon Diet Heart Study (de Lorgeril et al., 1999), a low fat diet was compared with a Mediterranean diet rich in n-3 fatty acids. Cholesterol values were unaltered, but fatal myocardial infarction lessened with 50-60 %. Similar results were seen in in the GISSI prevention trial (1999) and Indo-Mediterranean Diet Study (Singh et al., 2002). In the first two of these studies stroke incidence was also evaluated and was found to be lowerer in patients with coronary heart disease and a diet rich in n-3 fatty acids. The n-3 fatty acids have a wide range of biological effects leading to improvements in blood pressure and cardiac function, arterial compliance, endothelial function (see below), lipid and lipoprotein

metabolism, reduced neutrophil and monocyte cytokine formation, and potent anti-platelet and anti-inflammatory effects (Constant, 2004).

The role of different fatty acids as a risk factor in stroke and stroke subtypes is largely unknown. In 197 Japanese stroke patients, low levels of linoleic acid (C18:2) and higher proportions of saturated and monosaturated fatty acids, compared to controls, were found, but levels of n-3 polyunsaturated fatty acids were similar in both groups (Iso et al., 2002). In another study by Simon and coworkers (1995), an increase of 13 % in n-3 alpha-linolenic acid (C18:3) was associated with a 37 % decrease in the risk of stroke having adjusted for other risk factors. This finding is supported by another Japanese study (Hino et al., 2004) in which eating patterns were evaluated and carotid intimal-media thickness (IMT) was measured in 1902 subjects. Intake of the very long-chain n-3 fatty acids (n-3 VLCFA) [C20:5, eicosa-pentaenoic acid (EPA) and C22:6, docosahexaenoic acid (DHA)] was in this study significantly and inversely related to carotid IMT. A decreased intake of saturated fatty acids has been associated with a reduced progression of carotid and femoral IMT (Bemelmans et al., 2002).

In addition there are numerous less well-documented or potentially modifiable risk factors to stroke such as *hyperhomocysteinemia, inflammatory processes, obesity*, *physical inactivity, alcohol and drug abuse, hypercoagulability, hormone therapy, migraine,* and *socio-economic factors* (Goldstein et al., 2001).

It has become evident that an *inflammatory* component is of importance in the development of atherosclerosis. Both recent and chronic *inflammation* may contribute to stroke risk (Grau et al., 1995, Feigin et al., 2002). Markers of inflammation, such as activated T-cells and macrophages, are present in carotid endarterectomy specimens of recently symptomatic patients and C-reactive protein (CRP) levels are elevated in the metabolic syndrome and stroke (Ridker et al., 1997). An association between inflammatory markers and the metabolic syndrome has been shown, and it has been proposed that cytokines are the link between dysregulated metabolism and inflammation. Elevations of not only CRP, but also IL-6 (Yudkin et al., 1999) and tumour necrosis factor-alpha (TNF-α) have been demonstrated in the metabolic syndrome (Saghizadeh et al., 1996).

2.2 Genetics in stroke

There are several examples of mutations in specific genes that cause rare forms of stroke. However, in the vast majority of cases, stroke is not a single-gene disease, but a multifactorial disease that is caused by the simultaneous operation of multiple genetic and environmental factors, each of which has a relatively small effect. This complexity complicates the study of stroke.

2.2.1 Evidence for the role of genetic factors in stroke

Twin studies provide an opportunity to assess the relative importance of genetic factors in disease. So far, three different twin studies assessing genetic risk in stroke have been performed. de Faire et al (1975) studied cerebrovascular mortality in the Swedish Twin Register and did not, in a small sample of twins find a significant difference in concordance rates in monozygotic and dizygotic twins. Brass and co-workers (1992 and 1996) found a relative risk of stroke of 4.3 in monozygotic twins among US veterans. The Danish Twin Register provides the most recent twin study (Bak et al, 2002). In this study, 10% of monozygotic twins were concordant for stroke death compared to 5% dizygotic twins (relative risk of 2.1).

Family based studies have provided further evidence for a genetic component in stroke. In a Finnish population a doubled increase in stroke among patients with a parental history of stroke was seen (Jousilahti et al., 1997) and similar results have been found in several other studies (Kiely et al., 1993; Wannamethee et al., 1996). Despite methodological concerns and some studies with contradictory results, twin studies as well as family history studies generally suggest that a genetic predisposition is of importance in addition to the usual risk factors.

2.2.2 Polygenic disorders

For the vast majority of stroke cases a classical, Mendelian, pattern of inheritance cannot be demonstrated. An exception may be the phosphodiesterase 4D (PDE4D) gene, identified by the deCode group on Iceland (Gretarsdottir et al., 2003). This gene was demonstrated, in the Icelandic population, to be a strong and common risk factor for ischemic stroke, in particular to cardiogenic and large artery stroke, but the results could not be repeated in two other European populations (Bevan et al., 2005). The activity of PDE4D influences the second messenger camp which, possibly at lower levels, is involved in the atherosclerotic process. Rather, like in other complex traits, stroke phenotypes are more likely to result from the interactions of environmental factors with multiple polymorphisms in several genes (Fig 2). Some of these polymorphisms may encode key proteins involved in the pathophysiology of different stroke subtypes. The spectrum of disease alleles is probably wide with polygenic inheritance, each gene contributing with a small relative risk, but the population attributable risk for a particular gene may be substantial. On an individual level several genes may increase the risk of disease in an additive manner or by synergistic co-effects, and in addition a gene may interact with another risk factor and modulate its effect (Alberts, 2003). At a younger age environmental and behavioural factors have not had the time to substantially modify the phenotype, and genetic factors may therefore have an age-dependent effect on stroke risk, with a more prominent influence in early-onset disease (Pezzini et al., 2005; Hassan et al., 2000).
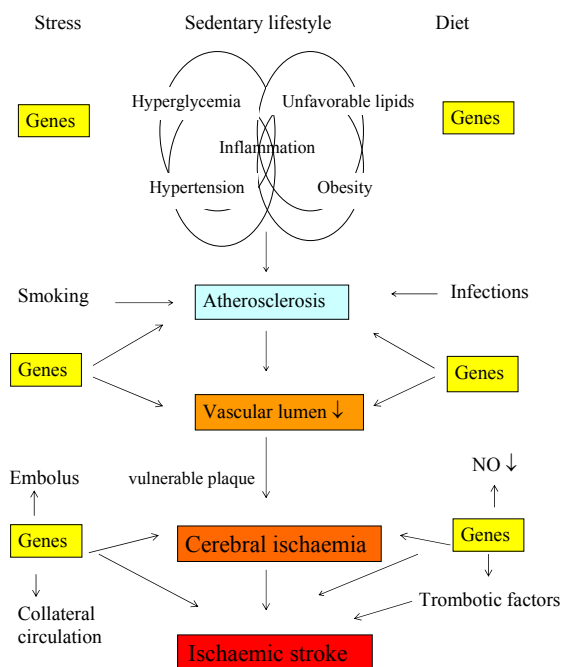
Fig 2. Stroke is a polygenic disease where multiple genes interact with environmental factors in a complex manner. This figure only outlines ischemic stroke; somewhat different genetic and environmental factors are likely to be involved in intracerebral haemorrhage.

2.2.3 Methods of identifying gene variants in stroke

The majority of stroke genetic studies have employed *association studies,* which examine the frequency of DNA variants of interest in candidate genes, mainly through case-control studies. These studies have the advantage of having larger statistical power than linkage analysis and they do not require family based collections (Rosand and Altshuler, 2003). There is however a risk of "linkage disequilibrium" if the studied polymorphism has a close localisation to the pathogenic polymorphism.

To date, many association studies of candidate genes, mainly single-nucleotide polymorphisms (SNPs), have yielded non-reproducible results. In lipid metabolism apolipoprotein E (APOE) has been of particular interest, since a polymorphism of the APOE gene is associated with high levels of triglycerides, LDL- and total-cholesterol, low HDL-cholesterol, and also with the risk of premature atherosclerosis. Nevertheless, studies on APOE and the risk of stroke have only reported a weak association with stroke. Another rather extensively studied gene is the angiotensin converting enzyme (ACE) gene. Selections of gene polymorphisms that recently have been reported to be associated with

ischaemic stroke are shown in Table 3.

Table 3. Reported significant associations with polymorphisms and ischaemic stroke. The polymorphisms are described by their most commonly used designations.

Gene	Polymorphism	Number of patients/controls	Findings	Reference
Apoprotein E; APOE	APOε2, ε3, ε4	926/890	APOε4 associated with stroke (OR=1.7)	McCarron et al., 1999
Paraoxonase-1; PON1	Gln(Q)192Arg(R)	118/118	RR genotype associated with stroke (OR=4.1)	Voetsch et al., 2002
Low-density lipoprotein receptor; LDLR	A370T	465/8432	TT genotype associated with stroke (OR=3.6)	Frikke-Schmidt et al., 2004
Phosphodiesterase 4D; PDE4D	Haplotypes AX,GO, GX	864/908	GO (one copy) associated with stroke (cardiogenic or carotid, OR=1.8)	Gretarsdottir et al., 2003
Angiotensin converting enzyme 1; ACE-1	Insertion (I)/ Deletion (D)	1196/722	ACE D allele associated with stroke (OR=1.3)	Sharma et al., 1998
Metylentetrahydrofolate-reductase; MTHFR	C677T	1823/1832	TT genotype associated with stroke (OR=1.3)	Li et al., 2003
Interleukin 6; IL6 and intracellular adhesion molecule-1; ICAM1	C174G ICAM-1 E/K	119/133	GG genotype associated with stroke (OR=8.6) EE genotype associate with stroke (OR=4.0, both GG and EE: OR=10.1)	Pola et al., 2003
Glycoprotein IIIa; GpIIIa	A1/A2	92/184	A2: OR=2.5 in large vessel disease	Slowik et al., 2004
Nitric oxide synthase; eNOS (NOS3)	Intron 4ab	300/600	Intron 4a variant protective in small vessel disease (OR=0.4)	Hassan et al., 2004
Atrial Natriuretic Peptide; ANP	T2238C	206/236	CC genotype associated with stroke (OR=3.8)	Rubattu et al., 2004
Cyclooxygenase 2; COX-2	G765C	864/864	CC: OR=5.8	Cipollone et al., 2004

2.2.4 Intermediate phenotypes

Since numerous genes probably influence common stroke, conventional case control studies may not be sufficiently powerful to detect the contribution of an individual disease allele. Instead *intermediate phenotypes,* which represent a characteristic component of the disease process may be studied and may offer a short-cut to the discovery of important disease genes. For ischaemic stroke, carotid intimal media thickness (IMT) is often used as an intermediate phenotype for large vessel disease. Carotid IMT is a surrogate measure of sub-clinical atherosclerosis (Geroulakos et al., 1994) and a strong predictor of future stroke (O´Leary et al., 1999). Carotid IMT correlates with established risk factors for atherosclerotic disease (Heiss et al; 1991).

Stenosis of the exteracranial carotid is a sign of advanced atherosclerosis, and internal carotid (ICA) stenosis is recognised as a risk factor of stroke. Conversely, carotid endarterectomy is effective in the prevention of stroke secondary to severe ICA stenosis (Rothwell et al., 2003). ICA stenosis also represents an intermediate phenotype. Known risk factors for ICA stenosis include age, male gender, hypertension, diabetes, and cigarette smoking (Khaw, 1996). ICA stenosis has also been associated with elevated triglycerides, low HDL and hyper-cholesterolemia (Ritto et al., 2001).

Some polymorphisms of genes that have shown association with high carotid IMT and/or ICA stenosis are given in Table 4.

Matrix metalloproteinases (MMP:s) and their endogenous inhibitors regulate the accumulation of extracellular matrix during tissue injury. Disruption of this balance has been implicated in atherosclerosis and plaque rupture (Woessner, 1991, Ghilardi et al., 2002). A common polymorphism in the promotor sequence of the MMP3 gene has been identified (Ye et al., 1996) in which one allele has six adenosine (6A) nucleotides and wheras the other has only five (5A). The 6A-variant has been associated with carotid stenosis (Ghilardi et al., 2002).

Table 4. Significant associations between gene polymorphisms and carotid IMT/carotid stenosis.

Gene	Polymorphism	Findings	Reference
Matrix metallo-proteinase 3; Matrix 3; MMP3	5A/6A	> carotid IMT in 6A ICA stenosis more frequent in 6A allele	Rundek T et al., 2002 Ghilardi et al., 2002
apolipoprotein E; APOE	APOε2, ε3, ε4	> carotid IMT in	Cattin et al., 1997
Angiotensin converting enzyme 1; ACE-1	Insertion (I) or Deletion (D)	> carotid IMT in D allele Extracranial artery stenosis more frequent in DD genotype	Hosoi et al., 1996 Pfohl et al., 1998
Metylentetrahydrofolate-reductase; MTHFR	C677T	> carotid IMT in T genotype ICA stenosis more frequent in TT genotype	Lim et al., 2001 Inamoto et al., 2003
Nitric oxide synthase; eNOS (NOS3)	Asp298Glu	> carotid IMT in D	Paradossi et al., 2004
Fractalkine receptor 1; CX3CR1 (inflammation)	T280M	ICA stenosis more frequent in M genotype	Ghilardi et al., 2004

2.3 THE FATTY ACID-BINDING PROTEIN 2

2.3.1 Intestinal fatty acid uptake

After a fat meal, long-chain free fatty acids (FFAs), a major hydrolysis product of dietary triglycerides, are absorbed from the intestinal lumen into enterocytes of the small intestine. Persons with healthy gastrointestinal tracts have > 93% fat absorption (Alcock, 1999). Short- and medium-chain fatty acids are albumin-bound upon absorption, and transported directly to the liver. Following absorption, long-chain FFAs are reincorporated into triglycerides, the majority of which are secreted as chylomicrons (Karpe et al., 1998) and transported from the enterocytes via the intestinal lymphatic system to the thoracic duct and into the plasma compartment (fig 3).
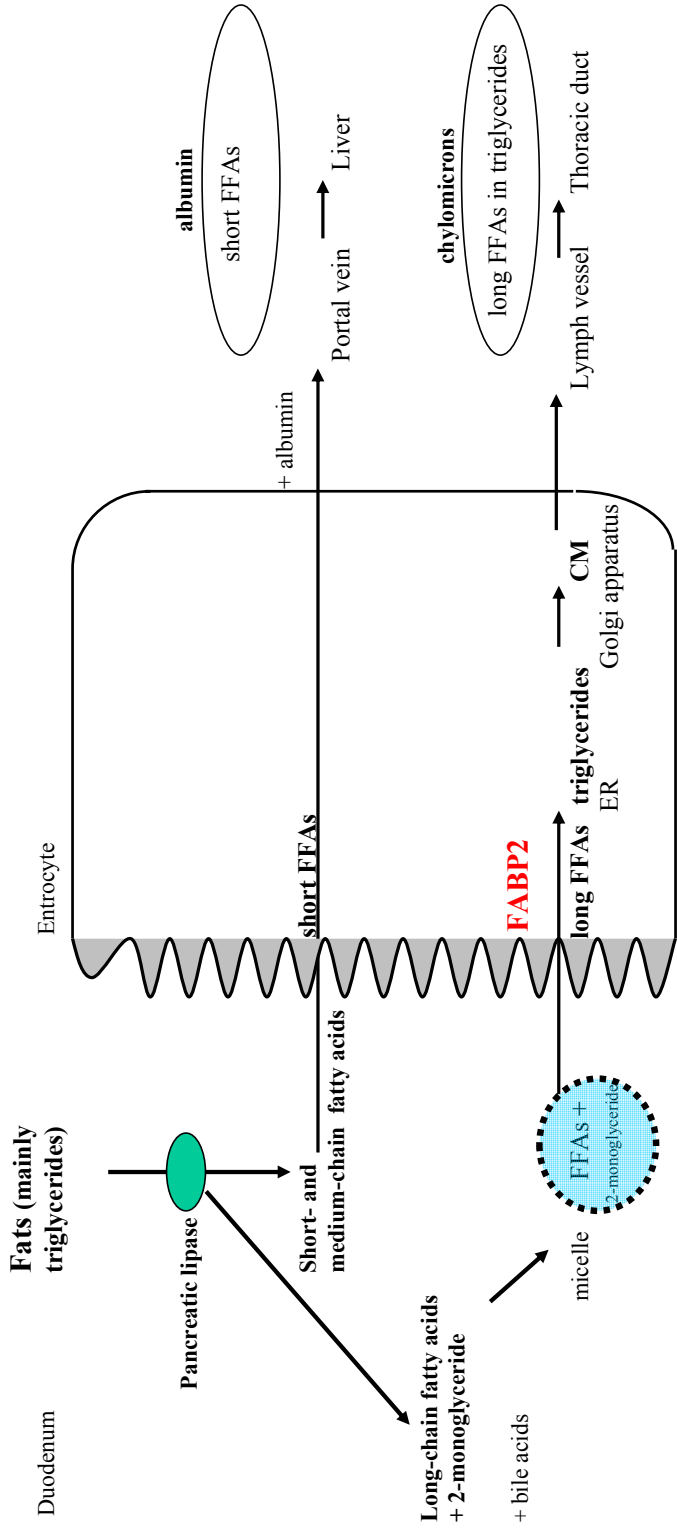
Duodenum

Fats (mainly triglycerides)

Pancreatic lipase

Short- and medium-chain fatty acids

Long-chain fatty acids + 2-monoglyceride

+ bile acids

Enterocyte

short FFAs

FABP2

long FFAs   **triglycerides**   **CM**

micelle

FFAs + 2-monoglyceride

ER   Golgi apparatus

+ albumin

albumin

short FFAs

Portal vein → Liver

chylomicrons

long FFAs in triglycerides

Lymph vessel   Thoracic duct

Fig 3. Digestion and absorption of fat. After the chyme has passed from the stomach into the duodenum, fat, mainly in the form of triglycerides, is broken down by pancreatic lipase, and free fatty acids and 2-monoglycerides are formed. The medium- and short-chain fatty acids are more water soluble and diffuse directly from the intestinal lumen into the enterocytes into the blood. The long-chain fatty acids must form micelles in order to reach the enterocyte membrane. At the inside of the enterocyte membrane the fatty acids are taken up by the transport protein, FABP2, for transportation through the aqueous cytosol to the endoplasmic reticulum (ER). In the endoplasmic reticulum the fatty acids are reesterified into triglycerides. In the Golgi apparatus the triglycerides are packaged into chylomicrons (CM), which are released from the enterocytes into the lymph vessels and reach the circulation by way of the thoracic duct.

2.3.2 Fatty acid binding proteins

Intestinal fatty acid binding protein 2 (FABP2) [fig 4] belongs to a large family of lipid binding proteins, some of which capture fatty acids at the plasma membrane and transport them through to the aqueous cytosol to cytosolic compartments for esterification or oxidation. These proteins are needed especially in cells that have either a high flux of FFAs or high demand for FFAs as substrate for energy, and the structure of these proteins seem to be highly conserved between species. Since the first description of the first FABP (Ockner et al, 1972) almost twenty members of fatty acid binding protein have been described (Schroeder et al., 1998). Some examples of FABPs isolated from different tissues include, FABP1 from liver, FABP3 from striated muscle and heart muscle, and FABP4 from adipocytes. Suggested functions of the FABPs other than in intracellular fatty acid transport are modulation of enzyme activity (e.g., lipoprotein lipase and hepatic lipase) and protection of the cytosol from the cytotoxic effects of FFAs (Besnard, 1996; Van Nieuwenhoven et al., 1996).
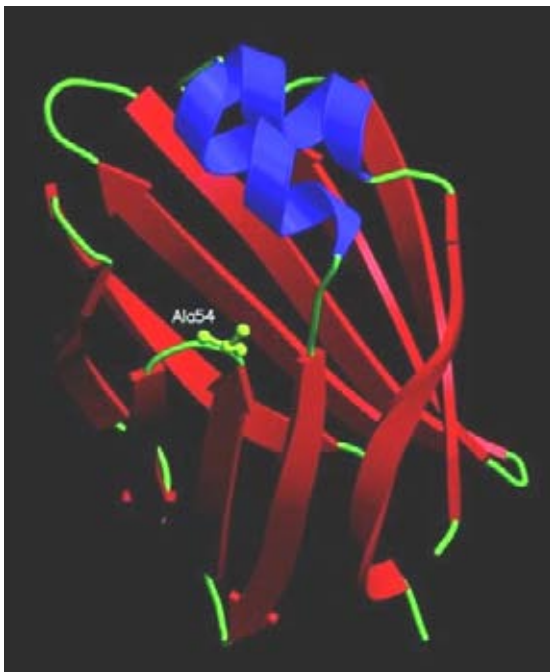

Fig 4. The apo-structure (without bound fatty acid) of the fatty acid binding protein 2 (FABP2). The straight red ribbons represents β-strands, which collectively form two β-sheets arranged as a β-clam (type of structure). The coiled blue ribbons represent α–helices that cap one end of the β-clam (Zhang et al., 1997). The protein contains a single ligand-binding site that binds long-chain fatty acids. With kind permission from the American Physiological Society.

2.3.3 The FABP2 A54T polymorphism

FABP2 is an abundant cytosolic protein expressed exclusively in the simple columnar epithelial cells of the proximal small intestine. FABP2 is believed to be involved in fatty acid absorption and intracellular transport (Zhang et al., 1997; Cohn et al., 1992) of dietary long chain (C16-C20) FFAs (Lowe et al., 1987). FABP2 consists of 131 amino acid residues and has a molecular mass of 15 kDa (Bernlohr et al., 1997). The FABP2 gene is located on the long arm of chromosome 4q. The gene has four exons containing 700 base pairs and three introns containing 2,650 base pairs. An A to G single base polymorphism at codon 54 results in replacement of alanine (A) with threonine (T) affecting the structure of the clam-shaped protein. *In vitro*, the T containing protein thus has a 2-fold greater affinity for long-chain FFAs (Baier et al., 1995; Baier et al., 1996), which on a transformed human carcinoma cell line (Caco-2) resulted in increased triglyceride transport across intestinal cells (Baier et al., 1996).

A variable but high frequency of the polymorphism has been found in different populations (Table 5). The T allele frequency is much lower among Tongan and aboriginal Canadians compared to other populations; only 12-14%.

Table 5. Frequency of T-allele of the FABP2 A54T polymorphism in different populations.

Population	T allele frequency	Reference
Pima Indians	0.29	Baier et al., 1995
Canadian men	0.31	Baier et al., 1995
Canadian aboriginals (Inuits)	0.14	Hegele et al., 1996
Japanese	0.27	Yamada et al., 1997
Europeans	0.27	Tahvanainen et al., 2000
Chilean women	0.32	Albala et al., 2004
Guadeloupe (Indian desendents)	0.30	Boullu-Sanchis et al., 1999
Tonga	0.12	Duarte et al., 2003

The T54 allele has been associated with lipid abnormalities, including higher fasting triglyceride concentrations, higher fasting plasma HDL and LDL-cholesterol and increased postprandial lipemic response (Table 6). In several studies assessing the lipemic response to an oral fat load, FFAs peaks earlier and significantly higher postprandially in subjects with the T54 allele, although all case-control studies could not replicate these data (Table 6).

Table 6. Studies on fasting lipid values, postprandial lipid values and fatty acid profile in subjects with the T54 allele of the FABP2 A54T polymorphism.

Studied effect	Effect (+/-) FABP2 T54 allele	Reference
Fasting lipids	+ (f-cholesterol, f-TGs)	Carlsson et al., 2000
Fasting lipids	+ (f-TGs)	Hegele et al., 1996
Fasting lipids	-	Vidgren et al., 1996
Fasting lipids	- (f-cholesterol, f-TGs, f -FFA: p=0.09)	Sipiläinen et al., 1997
Postprandial hyperlipidemia	+ (HDL-TGs)	Berthier et al., 2001
Postprandial hyperlipidemia	+ (FFA:s), - (TGs)	Prately et al., 2000
Postprandial hyperlipidemia	+ (TGs)	Ågren et al., 1999
Postprandial hyperlipidemia	- (cholesterol, TGs)	Tahvanainen et al., 2000 Marin et al., 2005
Fatty acid profile	- (in serum TGs, cholesterol esters or phospholipids)	Vidgren et al., 1997
Fatty acid profile	- (in membrane phospholipids of skeletal muscle and adipose tissue)	Prately et al., 2000
Fatty acid profile	- (in serum cholesteryl esters)	Erkkilä et al., 2002

In a study (Carlsson et al., 2000) using genotype-discordant sibling-pairs, siblings with more T54 alleles had higher triglycerides and cholesterol concentrations compared with their siblings with less A54 alleles. This study also suggested that the T54 allele in the FABP2 gene might increase susceptibility to stroke, as a higher parental prevalence of stroke was found in TT and TA genotype carriers compared with AA genotype carriers.

The T54 allele of the FABP2 A54T polymorphism has also been associated with obesity (Hegele et al., 1996), but these findings have not been confirmed in all studies (Sipiläinen et al., 1997; Vidgren et al., 1997; Tahvanainen et al., 2000). In a recent study by Cannai and coworkers (2005) on renal disease in type 2 diabetes, the TT genotype was over-represented. Numerous studies have assessed the FABP2 gene as a possible candidate gene for diabetes mellitus, but although some studies have demonstrated an association with insulin resistance (Baier et al., 1995, Hegele et al., 1996, Hayakawa et al., 1999), the T54 allele has only been associated with diabetes in the Indian population of Guadeloupe (Boullu-Sanchis et al., 1999). Many studies have not assessed the type of fat ingested. Albala and coworkers (2004)

suggested that the A54T polymorphism affects the differential absorption of n-3 and n-6 polyunsaturated fatty acids (PUFAs), and that this, in turn, may have an impact on the production of inflammatory cytokines. This research group demonstrated elevated levels of TNFα in obese subjects with the T54 genotype. Elevated levels of TNFα have in other studies been shown to increase the risk of insulin resistance (Hotamisligil et al., 1993). The absorption of different types of fatty acids in relation to the A54T polymorphism has however only been partly studied. In a study by Marín et al. (2005) patients heterozygous for the T54 allele had higher levels of fatty acids and decreased insulin sensitivity when on a diet rich in saturated fatty acids as compared to diets rich in either monusaturated fatty acids or carbohydrates.

Carriers of the T allele have reduced excretion of feaecal bile acids compared with A allele carriers and dietary fibre intake has been shown to affect cholesterol concentrations differently in T and A allele carriers (Hegele et al., 1997).

2.3.4 Postprandial hyperlipidemia and atherosclerosis

A number of reports have pointed out an association between impaired metabolism of postprandial triglyceride-rich lipoproteins and the presence or development of coronary artery disease (Hyson et al, 2003). Peak postprandial triglyceridemia (Ryu et al., 1992), early postprandial triglyceride levels (Boquist et al., 1999) and late postprandial triglyceride levels (Karpe et al., 1998), have all been found to be associated with carotid IMT.

After fat feeding, in parallel with chylomicron secretion from the intestine, very low density lipoproteins (VLDL) particles are secreted by the liver. Chylomicrons and VLDL are both rich in triglycerides (TRL) and compete for the same sites of lipolysis by lipoprotein-lipase (LPL) explaining some of the accumulation of triglycerides postprandially. Further delipidation and cholesterol enrichment of these particles generates small and potentially atherogenic remnant lipoproteins.

In vitro and *in vivo* studies suggest that TRL and products of TRL hydrolysis, including FFA, may impair endothelial function (Hennig et al., 1985; Lundman et al., 1997), which can be partly abolished by L-arginine administration (Marchesi et al., 2001; Bae et al., 2001).

2.4 The endothelium

The endothelium is the largest organ in the body and consists of a monolayer of cells located between the vascular lumen and the smooth muscle cells of the vessel wall (Endemann and Schiffrin, 2004). Dysfunction of the endothelium may be interpreted as the ruin of the homeostasis of the constricting and relaxing factors and is often measured as flow-mediated vasodilation (Rubanyi et al., 1993). A broader understanding of endothelium dysfunction includes not only reduced vasodilatation but also a proinflammatory and protrombotic state. Endothelium dysfunction is considered an early characteristic of atherosclerosis (Celermajer et al., 1992). Endothelial dysfunction is typically present in patients with cardiovascular disease and in subjects with risk factors for such disease (Engler et al., 2003; Rizzoni et al., 2001; Park and Schifferin, 2001; Oida et al., 2003). Although the degree of endothelial dysfunction appears to correlate with the burden of traditional risk factors, there is considerable heterogeneity in the magnitude of dysfunction observed in individuals with similar risk factor profiles (Halcox et al., 2002).

2.4.1 Nitric oxide (NO)

One of the major endothelium derived vasoactive mediators is nitric oxide (NO), which is synthesized from L-arginine by the enzyme endothelial NO synthase (eNOS) [fig 6, page 26]. The discovery of an endothelium-derived relaxing factor (EDRF) resulted from the observation that functionally intact endothelium was necessary for acetylcholine (Ach)-induced vasodilation and that EDRF mediated this endothelium-dependent vasodilation. EDRF was later shown to be NO (Furchgott and Zawadzski, 1980). Among other vasodilatory substances produced by the endothelium are prostacyclin, C-type natriuretic peptide, histamine, serotonin (5-hydroxy-tryptamine, [5-HT]), and Ach. Vasoconstrictors include endothelin-1 (ET-1), angiotensin II, tromboxane A_2, and reactive oxygen species (ROS). The development of cardiovascular risk factors, such as type 2 diabetes, results in increased oxidative stress. Oxidative stress in turn result in endothelial dysfunction by altering the balance between vasoconstrictors and vasodilators. Oxidative stress also promotes inflammation and a protrombotic environment (Quinones, et al., 2005).

NO is continuously produced by the endothelium leading to a constant state of dilation of blood vessels in the resting state (Vallance et al., 1989). NO production (which can be further increased by shear stress or circulating factors such as Ach, bradykinin and 5-HT) activates soluble guanylate cyclase, increasing cyclic guanosine monophosphate (cGMP) concentration

(Moncada and Higgs, 1993). cGMP as the second messenger mediates many of the biological effects of NO, including relaxation of smooth muscle cells resulting in vasodilation. In addition to vasodilation, NO inhibits platelet adhesion and aggregation (Stamler et al., 1989), attenuates monocyte adhesion and infiltration (Kubes, 1991), suppresses myointimal hyperplasia (Garg and Hassid, 1989; Böger et al., 1998), and reduces the vascular production of superoxide radicals (Böger et al., 1995).

2.4.2 Asymmetric dimethylarginine (ADMA)

Asymmetric dimethylarginine, ADMA) is a naturally occurring amino acid that circulates in plasma and is metabolised in the liver or excreted in the urine (Fig 5). ADMA is synthesized when arginine residues in proteins are methylated by the action of protein arginine methyltransferases (PRMTs; type 1 in the endothelium). Protein methylation is a ubiquitously present mechanism of post-translational modification of proteins. Post-translational methylation of arginine residues results in a modification of the tertiary structure and function of proteins. Proteolysis is subsequently necessary to release free methylarginines such as ADMA. ADMA is a competitive inhibitor of NO synthase (Vallanceet al., 1992) (fig 5). In the presence of suboptimal concentrations of arginine, high LDL-cholesterol or ADMA concentrations, the catalytic mechanism of the enzyme is "uncoupled" which results in the formation of superoxide (O_2^-) instead of NO and citrulline (Vasquez-Vivar et al., 1998). Superoxide rapidly inactivates already existing NO by the formation of highly oxidative peroxynitrite. Superoxide is also formed through other metabolic pathways and is prevalent in conditions with increased oxidative stress. Under such conditions NO half-life is thus reduced by inactivation by superoxide. Oxidant excess will also result in reduction of tetrahydro-biopterin (BH_4), a cofactor of eNOS, leading to the uncoupling of eNOS dicussed above. Symmetric dimethylarginine (SDMA) is also found in human plasma but does not inhibit NO synthesis. SDMA is excreted in the urine, while ADMA is metabolised mainly by the enzyme dimethylarginine dimethylamino-hydrolase (DDAH). The activity of DDAH seems to be critical in regulating ADMA levels (McAllister et al., 1994). DDAH activity may be inhibited by elevated concentrations of glucose (Lin et al., 2002), cholesterol (Ito, 1999) and homocysteine (Stűhlinger et al, 2003). Other possible mechanisms by which homocysteine may increase levels of ADMA (and subsequently impair endothelial function) include increased expression or activity of the enzyme PRMT type 1 and enhanced proteolysis (fig 6). It has also been shown that oxidative stress induced by oxidized LDL or TNFα decrease DDAH activity *in vitro* (Ito et al., 1999).
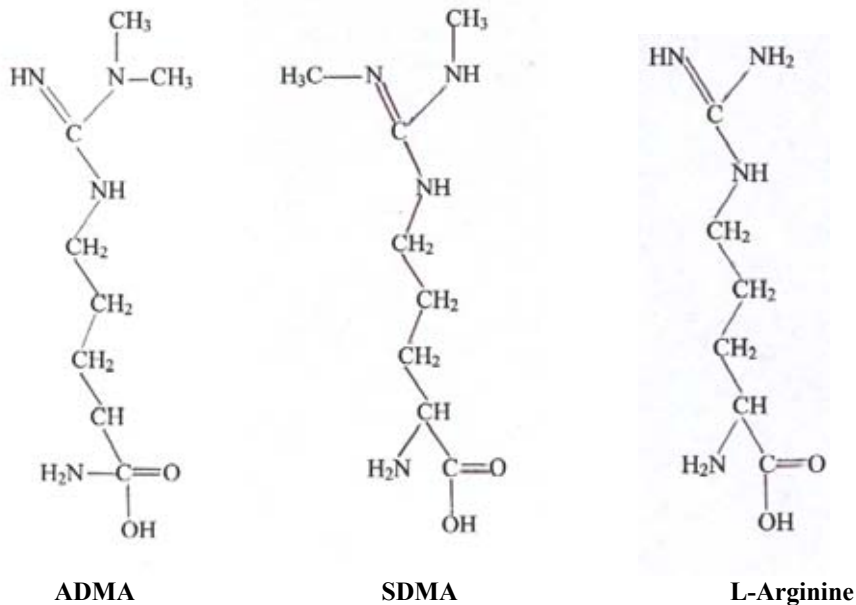
Fig 5. Chemical structure of N^G,N^G-dimethyl-L-arginine (asymmetric dimethylarginine, ADMA), $N^G,N^{G'}$-dimethyl-L-arginine (symmetric dimethylarginine, SDMA) and L-Arginine (Böger, 2003). L-Arginine does not contain the two methyl-groups which in ADMA are placed asymmetrical and in SDMA symmetrical.

2.4.3 ADMA in metabolic and atherosclerotic disease

Data from experimental studies suggest that ADMA inhibits vascular NO production at concentrations found in patophysiological conditions and ADMA administered intravenously in humans, resulting in elevated blood pressure, vascular resistance and heart rate (Kielstein et al., 2004).

ADMA has been shown to be increased in patients with conditions accompanied by endothelial dysfunction such as hypercholesterolemia (Böger et al., 1998), hyperhomocysteinemia (Stűhlinger et al., 2003), hypertension (Higashi et al., 1997), diabetes mellitus (Abbasi et al., 2001), insulin resistance (Stűhlinger, 2002), chronic renal failure (Kielstein et al., 1999), and in patients with atherosclerotic disease (Miyazaki et al, 1999). In patients with renal disease it was reported that the progression of carotid IMT was best predicted by ADMA and CRP levels (Zoccali et al., 2002).

Increased levels of ADMA have also been correlated with coronary heart disease (Valkonen et al., 2001). Furthermore, in a study from South Korea on 52 patients with ischemic stroke plasma levels of ADMA were elevated (Yoo and Lee, 2001).

Elevated ADMA levels cause a relative L-arginine defiency even in the presence of normal plasma L-arginine levels. Studies on dietary supplementation with L-Arginine show that the inhibitory action of ADMA on eNOS can be reversed (Chan et al., 2000). This has also been shown to improve clinical symptoms of cardiovascular disease in some studies (Rector et al., 1996; Maxwell et al., 2000), but not all (Cross et al., 2001; Walker et al., 2001).

2.4.5 Polymorphisms of the eNOS gene

Because of the multiple antiatherogenic actions of NO, the eNOS gene must be considered a candidate gene for atherosclerosis. Knocking out the eNOS gene in mice results in significant hypertension, and aortic rings from these animals studied *ex vivo* display no relaxation in response to acetylcholine (Huang et al., 1995) and furthermore, augmenting vascular NO production by local delivery of NOS in animal models improves endothelial function and increases regression of atherosclerotic lesions (Channon et al., 2000). The eNOS gene (NOS3) is located on chromosome 7q (Mardsen et al., 1993) and is composed of 26 exons that spans 21 kb. Multiple polymorphisms of the eNOS gene have been reported. A common variant of the eNOS gene, located in exon 7 (G894→T) results in the substitution of glutamic acid (Glu) with aspartic acid (Asp) at amino acid position 298, and the Asp298 variant has been associated with carotid IMT in young non-smoking subjects (Paradossi et al., 2004), with atherosclerotic plaques in the common carotid of the general population, and in a low-risk group also with carotid IMT (Wollf et al., 2005). Furthermore, the Asp allele has been associated with the presence of carotid plaques (Lembo et al., 2001), coronary spasm (Yoshimura et al., 2000), acute myocardial infarction (Hibi et al., 1998), preeclampsia (Serrano et al., 2004), diabetic nephropathy (Shin Shin et al., 2004) and with hypertension (Shoji, 2000) in some, but not all studies (MacLeod et al., 1999; Markus et al., 1998). However, in one study (Elbaz et al., 2000), the Glu allele and not the Asp allele was associated with stroke in general and lacunar stroke in particular. In yet another study on stroke, no association with this polymorphism and cerebrovascular disease or its subtypes was found (Marcus et al., 1998). In contrast, in a recent study the eNOS Glu/Asp or Asp/Asp genotypes in combination with the methylentetrahydrofolate-reductase (MTHFR) 677TT or ACE DD genotype was associated with the risk of ischemic stroke (Szolnoki et al., 2005). The Glu298Asp eNOS polymorphism is the only polymorphism of the eNOS gene known to result in amino acid substitution in the eNOS protein (Wolff et al., 2005). The eNOS gene

with this polymorphism generates protein products with differing susceptibility to cleavage and subsequent inactivation (Tesauro et al., 2000). Furthermore, in a study in healthy young subjects (Paradossi et al., 2004), the Asp/Asp genotype was an independent predictor of endothelial- dependent flow-mediated brachial artery dilation. Other studies, however, have not been able to demonstrate a direct functional effect on vascular NO bioactivity (Guzik et al., 2001), suggesting that the aspartate mutation may act merely as a marker for a functional mutation in either eNOS or a nearby gene (Cai et al., 1999).

Other polymorphisms of the eNOS gene, studied in the context of vascular disease, include a 27 bp repeat polymorphism in intron 4 (Wang et al., 1997), cytosine adenosine repeats in intron 13 (Bonnardeux et al., 1995) and a polymorphism in the 5' flanking region of the eNOS gene (T786→C) (Nakayama et al., 1999). These polymorphisms have, however, not been associated with an altered amino acid sequence.
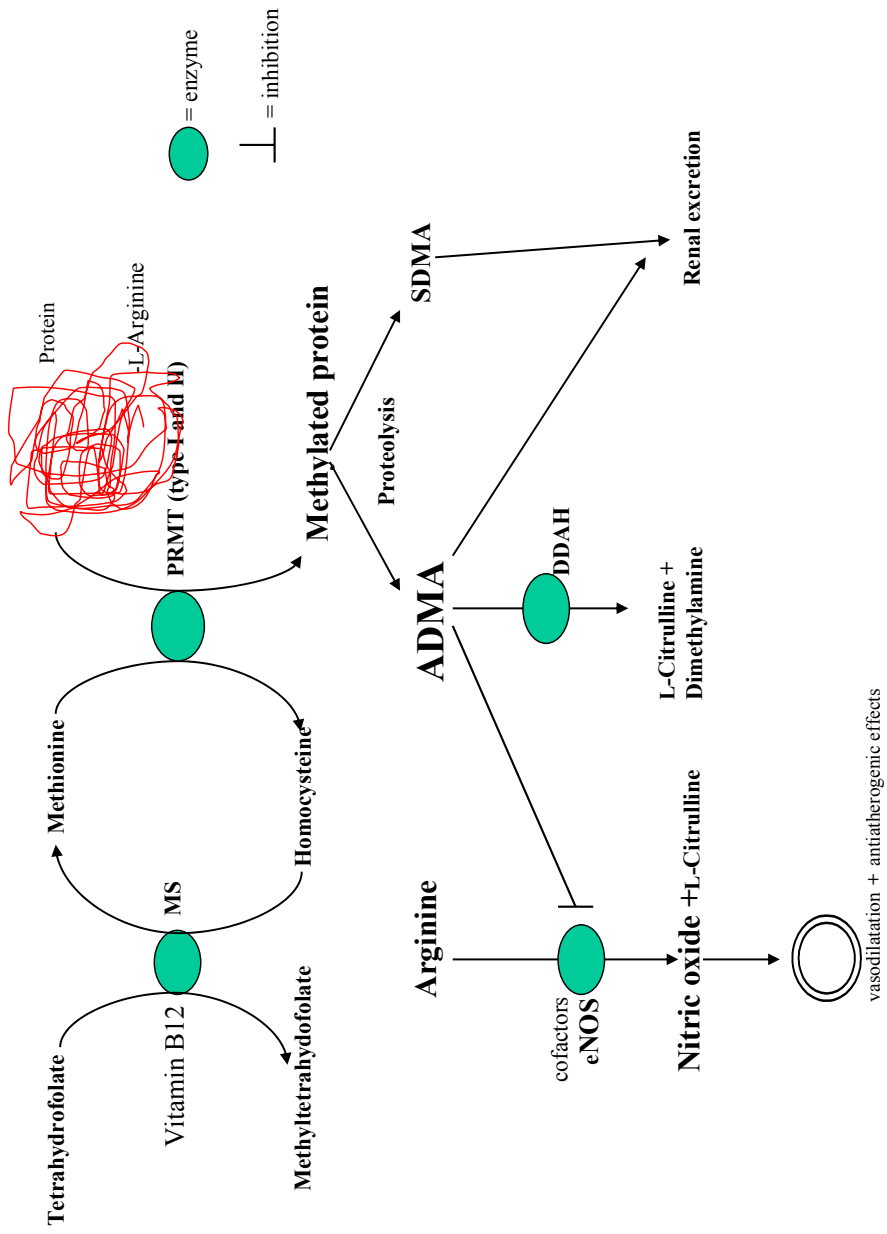
Fig 6. Asymmetric dimethylarginine (ADMA) is formed in the same methionine-dependent (MS = methionine synthase) reaction as homocysteine by methylation of arginine residues of proteins. Free ADMA is released in the turnover of proteins and modulates eNOS by competitive inhibition. Possible mechanisms by which homocysteine may increase levels of ADMA include increased expression or activity of Protein-arginine methyltransferase (PRMT) type 1, enhanced proteolysis or decreased Dimethylarginine dimethylamino hydroxylase (DDAH) activity.

26

3. THE PRESENT INVESTIGATION

3.1 Aims of the present study

- To study if the FABP2 A54T genotype is associated with an increased risk of acute CVD or with any subgroups of acute CVD (Study I).

- To study if the FABP2 A54T genotype is associated with ICA stenosis in CVD patients (Studies II and IV).

- To study if an increased level of ADMA is associated with acute CVD or with any subgroups of acute CVD (Study III).

- To study whether ADMA and the eNOS Asp 298Glu and FABP2 A54T polymorphisms, are associated with ICA stenosis (Study IV).

3.2 Materials and methods

3.2.1 Study populations

Studies I-III

Between June 2000 and December 2003 a total of 598 patients, initially diagnosed with stroke or TIA, were recruited upon their admission to the stroke unit of the County Hospital of Kalmar, Sweden. The outline of the thesis is shown in figure 7. Patients with subarachnoid haemorrhage were not included in the study. The first 497 (407 remained after exclusion) of the 598 patients were studied in Study I. A subset of the patients in Study I was studied in Study II. In Study III 442 patients wey studied (386 remained after exclusion). For Study I we also recruited 158 blood donors as controls, and for Study III we recruited 48 patients without cardiovascular disease, either undergoing elective prostethic surgery or recruited from an influenza vaccination unit, as controls. From the blood doners it was only possible to obtain DNA samples. From the controls in Study III we obtained several blood samples, and these controls were also better phenotyped.

The stroke patients were classified either with ischemic stroke or intracerebral haemorrhage, and the patients with ischemic stroke were further classified as non-cardioembolic infarction or as cardioembolic infarction, but no distinction was made between large artery disease and lacunar infarction. If the patient on admission had an ongoing atrial fibrillation, a history of atrial fibrillation or cardio-valvular disease, the stroke was considered to be due to a cardiac embolism unless the CT scan showed haemorrhage. Patients with identifiable possible causes of stroke and patients with a possible lipid altering treatment were excluded from the present investigation. These causes or treatments consisted of patients with a presence of systemic malignancy, SLE or a history of migraine, and patients with a kidney transplantation. Patients with oestrogen, thyroxine and lipid-lowering treatment were also excluded from the Studies I-III.

Study IV

In paper IV, 108 non-smoking patients under the age of 75 years were included from a database data file containing consecutive information on patients who had undergone investigation with ultrasound of their internal carotids (ICA) at the County Hospital of Kalmar between December 1997 and February 2005. Patients with a severe ICA stenosis (>70%) or a normal ultrasonographic outcome were contacted and invited to participate in the

study. Patients previously included in Studies I-III were not included in Study IV. Patients and controls were non-smokers and matched pair-wise for age and gender.
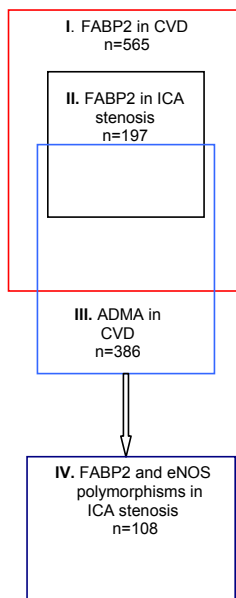

Fig 7. Studies I - IV

3.2.2 Ethical aspects

Before the subjects were allowed to participate in the study, the purpose, nature and potential risks were explained to them. If the patients because of their disease were not receptive to the information, permission to include the patient in the study was asked from the patients´ relatives. All participants, or their relatives, gave informed consent to participate in the study, which received the approval of the ethics committee of Linköping University.

3.2.3 Questionnaire

Information about the medical history of all patients concerning previous stroke/TIA, diabetes, hypertension and smoking habits was obtained from the patient or a relative following a written nurse-administrated standardised questionnaire. A history of hypertension was defined as the use of anti-hypertensive drugs or blood pressure more than 140/90 and diabetes mellitus as the use of anti-diabetic drugs or diet treatment only or a fasting plasma glucose value ≥ 7.0 mmol/L.

In study IV the patients were also questioned about their personal medication.

When possible the information from the questionnaire was supplemented with an examination of relevant medical journals.

3.2.4 Antropomethric measurements

Antropomethric measurements included measurements of height and weight. Weight and height were, if possible, measured with the subject in light clothing without shoes, otherwise estimated, and the body mass index (BMI) was calculated as kilograms per m^2. Blood pressure was measured with the subject in the supine position.

3.2.5 Analytic assays

At approximately 7 a.m. during the first days after admission to hospital fasting venous blood, samples were drawn for DNA and for measurements of glucose, HbAIc, creatinine, triglycerides, total-cholesterol, LDL- and HDL-cholesterol, free fatty acids (FFAs) (Studies I-III), and, ADMA, symmetric dimethylarginine (SDMA), and arginine (Study III). FFAs were not sampled if the subject was on medication with heparin or low-molecular weight heparins. In Study IV venous blood samples were obtained following a light breakfast, at approximately 9 a.m.

3.2.6 Genotyping

DNA was extracted from peripheral blood leucocytes by standard methods.

The following polymorphisms were genotyped:

- In Studies I, II and IV, the G to A nucleotide substitution in codon 54 of the FABP2 gene (A54T).

- In Study IV, the G to T nucleotide substitution in codon 298 of the eNOS gene

  (Asp298Glu)

The polymorhisms were genotyped by polymerase chain reaction (PCR), followed by allele specific enzymatic cleavage by restriction enzymes (RFLP) or pyrosequencing. The digested PCR products were separated on agarose gels containing ethidium bromide, which enabled visualisation of the DNA fragments under ultraviolet light. Detailed descriptions of the particular PCR-RFLP methods used are given in the respective original papers (I, II and IV). Pyrosequencing: In pyrosequencing, single-stranded DNA from the PCR, placed on a DNA template is used. Nucleotides are dispensed in a cyclic manner. Each incorporation event generates a release of photons proportional to the number of nucleotides incorporated, displayed as a peak in a pyrogram. In the absence of incorporation of a nucleotide, no photon is released. Detailed descriptions of the pyrosquencing technique are given in paper IV.

3.2.7 Ultrasonography

Patients in Studies II, III and IV were investigated with Duplex sonography combining B-mode imaging, colour flow and pulsed Doppler spectrum analysis (Sonos 5000 HP™ or Acuson Sequoia™ 512). A consultant clinical physiologist (MD) performed examinations using an 8 MHz probe with the subject in supine position. The Doppler angle was chosen as close to 60° as possible. Doppler measurements were made with the sampling volume in the common carotid artery (CCA) proximal to the bifurcation, in the internal carotid artery (ICA; where the highest peak velocity was chosen) and in the external carotid artery (ECA). A maximum systolic peak ICA velocities above the normal limit 1.05 m/s were used to classify a stenosis of the ICA (ranging from 50% to 100%; degrees of stenosis less than 50% are not possible to detect with this method) and above 2.4 m/s as severe stenosis of the ICA ($\geq$70%) [Bluth, et al., 1988]. Both the right and left carotid in each subject were investigated, and the highest ICA-stenosis value of the two was used when the subjects were classified as either without stenosis (<50% stenosis irrespective of the characteristics of a possible plaque) or with stenosis ($\geq$50 % in Studies II and III; $\geq$70% in Study IV).

Both the right and left carotid in each subject were investigated and the highest ICA-stenosis value of the two was used. In paper IV patients with a severe stenosis and an ipsilateral TIA/stroke with symptoms compatible with the carotic territory were further classified as having symptomatic ICA stenosis.

3.2.8 Statistical methods

All statistical analyses were performed with a commercially available statistical data programme, STATISTICA (version 6.0, StatSoft®, Tulsa, USA). Variables not normally distributed are given as median (range). The results for continuous normally distributed variables are given as mean ± SD and for categorical variables as percentages. For variables not normally distributed (HbA1c, creatinine, triglycerides and FFAs) group differences were analysed using, the Kruskal-Wallis non-parametric test, followed by Mann-Whitney's test in case of significance. In Study II logistic regression analysis was used to investigate associations between the dependent variable, ICA stenosis and the independent variables, FABP2 genotype together with possible risk factors for atherosclerosis, of which some were fixed in the model (see below). Both univariate and multivariate (stepwise, backwards) analysis were used (blood donors excluded).

In Study III Pearson's correlation coefficient was calculated to examine a possible correlation between ADMA on the one hand and other continuous variables on the other hand (using log values for log-normally distributed variables). The relationships between ADMA and other possible risk factors and CVD and its subgroups were analysed with logistic regression.

In Study IV genotype and allele frequencies of the FABP2 and eNOS polymorphisms were compared using an exact test corresponding to McNemar´s paired Chi-2 modification first proposed by Lidell (1983) [Armitage, 2002]. Associations between FABP2 and eNOS on the one hand and the various risk factors on the other were tested with multiple logistic regression.

P values ≤ 0.05 were considered significant.

3.3 The FABP2 A54T gene polymorphism in cerebrovascular disease (I)

In Study I we determined whether the FABP2 A54T genotype is associated with an increased risk of acute CVD or any of its subgroups.

3.3.1 Subjects

497 patients initially diagnosed with stroke or TIA (acute cerebrovascular disease) were recruited for the study. After exclusion, using the above mentioned criteria, a total of 497 patients (age 73.0 ±10.8), 252 with an ischemic infarction, 91 with a cardioembolic infarction, 23 with a haemorrhagic stroke and 41 with TIA were to be further evaluated. Clinical characteristics of these patients are given in Table 7.

As controls 158 volunteering healthy blood donors (age=55.4 ± 3.9, 83 males, 75 females) were invited to participate in the study. The control patients were free from drugs and without a history of diabetes or coronary, peripheral or cerebrovascular disease.

3.3.2 Methods

Allele and genotype frequencies of the FABP2 A54T polymorphism were assessed in the patients with acute CVD and in the control subjects.

3.3.3 Results

Allele and genotype frequencies did not differ between subjects with acute CVD (TT, 9.6%; TA, 41.0%; AA, 49.4%) compared with controls (TT, 7.6%; TA, 41.4%; AA, 51.3%). In subgroup analysis the combined TT and TA genotype frequency in TIA (n=41) was more frequent than in controls (65.9 % vs. 48.7%, p=0.05). This association was not seen in ischemic infarction, embolic infarction or intracerebral haemorrhage (Table 8). As genetic factors are likely to be of more importance at a younger age of onset and since smoking is a known environmental independent risk factor for stroke we further analysed the data in a subgroup of non-smoking patients under the age of 70 years. In this group of patients with acute CVD (n=106) a tendency towards a more frequent TT genotype was seen (TT, 17.0 %; TA: 36.8 %; AA: 46.2 %) compared with controls (TT, 7.6%; TA, 41.1%; AA, 51.3 %) (p=0.06). In subgroup analysis, the TT genotype was significantly more frequent in ischemic infarction under the age of 70 (n=77, age 57.6 ± 6.3; TT 18.2%; TA 28.6%; AA 53.2 %) than in controls (TT, 7.6%; TA, 41.1%; AA, 51.3 %, p=0.023).

3.3.4 Discussion

The results of study I do not support that the A54T polymorphism of the FABP2 gene is associated with stroke or acute cerebrovascular disease in general. However, the subgroup analysis suggested an involvement of the T allele in the pathogenesis of CVD. Firstly, among patients with TIA we found an increased frequency of the combined TT and TA genotype compared with controls. Secondly, an increased TT genotype frequency among younger and non-smoking patients with ischemic infarction compared with age-matched controls was seen, which may indicate that homozygous subjects with the FABP2 A54T polymorphism have an increased risk for ischemic stroke.

Table 7. Clinical characteristics of 407 patients with acute cerebrovascular disease

	Ischemic infarction	Cardio-embolic infarction	Haemorrhagic stroke	TIA
No. (male/female)	251 (147/104)	92 (47/45)	23 (15/8)	41 (27/14)
Age (years)	71.3±11.2	78.2±7.7 [*]	72.6±13.0	71.3±9.7
Current smokers (%)	17.5	7.6	13.0	19.5
Earlier hypertension (%)	39.4	42.4	43.5	46.3
Earlier stroke (%)	20.5	22.8	26.1	19.5
Diabetes (%)	28.0	25.0	21.7	14.6
Systolic BP (mmHg)	160±27	156±25	168±18	154±26
Diastolic BP (mmHg)	85±12	84±12	89±10	86±12
BMI (kg/m^2)	26.1±4.7	25.2±3.3	24.9±4.1	25.8±3.2
f-Glucose (mmol/L)	6.5±3.1	6.3±2.2	6.6±2.0	5.8±1.3
HbA1c (%)	4.8 (3.1-11.4)	4.8 (3.7-13.1)	4.5 (3.7-8.5)	4.7 (3.2-7.3)
f-Triglycerides (mmol/L)	1.4 (0.4-4.6)	1.1 (0.5-4.8)	1.2 (0.7-3.1)	1.4 (0.6-2.7)
f-Cholesterol (mmol/L)	5.9±1.3	5.5±1.4	5.4±1.2	5.9±1.0
f-LDL-cholesterol (mmol/L)	3.8±1.2	3.5±1.2	3.2±1.0	3.7±0.9
f-HDL-cholesterol (mmol/L)	1.4±0.4	1.4±0.4	1.6±0.6	1.5±0.4
f-Free fatty acids (mmol/L)	0.59 (0.06-1.86)	0.62 (0.24-1.72)	0.66 (0.34-0.90)	0.56 (0.19-1.18)

Data are mean±SD or median and range
[*] P<0.001 vs. other subgroups

Table 8. Patients with subgroups of acute cerebrovascular disease, control subjects and FABP2 genotypes.

FABP2 genotypes	Ischemic infarction	Cardioembolic infarction	Haemorrhagic stroke	TIA	Controls
TT n, (%)	24 (9.5)	12 (13.1)	1 (4.4)	2 (4.9)[*]	12 (7.6)
TA n, (%)	95 (37.5)	36 (39.1)	11 (47.8)	25 (61.0)	65 (41.1)
AA n, (%)	132 (53.0)	44 (47.8)	11 (47.8)	14 (34.1)	81 (51.3)
FABP2 alleles					
T n, (%)	143 (28.5)	60 (32.6)	13 (28.3)	29 (35.4)	89 (28.1)
A n, (%)	359 (71.5)	124 (67.4)	33 (71.7)	53 (64.6)	227 (71.8)

*TT/TA in TIA vs controls: p=0.05.

3.4 Genetic variation of the intestinal fatty-acid binding protein 2 gene in carotid atherosclerosis (II)

In Study II we investigated wether the FABP2 A54T genotype is associated with ICA stenosis.

3.4.1 Subjects

Those of the patients in Study I who had been investigated in connection with their stroke or TIA using ultrasound of the carotids (n=196) were identified and included in this study. Depending on the result of the ultrasound assessment, they were either classified as cases (patients with ICA stenosis [≥50%], age 74.0 ±7.2) or as controls (patients without ICA stenosis [<50%], age 66.7 ±10.1). Of the study group (n=196), one hundred and thirty three subjects had received a final diagnosis of ischemic cerebral infarction, 30 had cardio-embolic infarction, 29 had TIA, and 4 patients had been found not to have suffered a stroke or TIA. It is not known whether the patients with stenosis suffered a stroke/TIA due to their stenosis or atherosclerosis elsewhere, i.e. if the stenosis were ipsilateral to the brain injury and can be considered symptomatic.

As a reference of FABP2 genotype frequencies in a healthy population we used 158 genotyped blood donors from the same hospital (age=55.4 ± 3.9 years, 83 males, 75 females) whose internal carotid arteries had not been assessed with ultrasound.

3.4.2 Methods

Subjects were genotyped for the FABP2 A54T polymorphism. For details see paper II.

3.4.3 Results

The group of patients with ICA stenosis were older, suffered from more diabetes and had lower concentrations of cholesterol and LDL-cholesterol than patients without ICA stenosis. There were no group differences in the proportions that smoked or had a history of earlier hypertension, nor were there any differences in systolic or diastolic blood pressure or in BMI, fasting glucose, HbA1c, or in the use of lipid-lowering therapy (statins), oral anti-diabetics or insulin. No association between different genotypes of the FABP2 gene polymorphism and blood pressure, BMI, fasting glucose, HbAIc and lipid concentrations were seen.

Due to few patients with the TT genotype (only two patients with ICA-stenosis), the TT and TA genotypes were combined. Allele and genotype frequencies in the group of ICA stenosis (TT + TA, 68.0 %; AA, 32.0 %) did not differ significantly from the group of patients without ICA stenosis (TT + TA, 49.7 %; AA, 50.3 %; p=0.09), although a trend towards higher T frequency in ICA stenosis was seen. There were univariate statistically significant correlations between ICA stenosis and age, and with a history of diabetes.

We also compared the genotype frequencies of the group of patients with cerebrovascular disesase with the genotype frequencies in apparently healthy blood donors. The genotype frequencies of blood donors did not differ significantly from the group of patients with ICA stenosis (TT + TA, 48.7 %; AA, 51.3 %; p=0.07) [Table 9].

Nevertheless, there was a statistically significant multivariate relationship between ICA stenosis on the one hand and TT+TA genotype, known diabetes mellitus, and systolic blood pressure on the other (p= 0.04) [Table 10].

3.4.4 Discussion

In study II, which can be considered a pilot study on the A54T FABP2 polymorphism in carotid atherosclerosis, we observed that the T 54 allele was associated with ICA stenosis in CVD subjects. A limitation of this study was the relatively small number of patients with carotid stenosis.

Table 9. Genotype and allele frequencies in patients without ICA stenosis, with ICA stenosis and in healthy blood donors.

FABP2 genotype	Patiens without ICA-stenosis	Patients with ICA-stenosis	Blood donors
N	171	25	158
TT (N, %)	17 (9.9)	2 (8.0)	12 (7.6)
TA (N, %)	68 (39.8)	15 (60.0)	65 (41.1)
AA (N, %)	86 (50.3)	8 (32.0)[a]	81 (51.3)[b]
FABP2 alleles			
T (N, %)	102 (29.8)	19 (38.0)	89 (28.1)
A (N, %)	240 (70.2)	31 (62.0)[c]	227 (71.8)

[a]p=0.09, TT+TA vs. AA in patients with and without stenosis
[b]p= 0.07, TT+TA vs. AA in patients with stenosis and blood donors
[c]p= 0.25, T allele vs. A allele in patients with and without stenosis

Table 10. Multivariate logistic regression analysis for risk factors in ICA stenosis.

	Odds ratio	95 % Confidence interval	P-value
Diabetes mellitus no	1.00		
yes	4.93	1.76-13.8	**<0.01**
FABP2 genotype AA	1.00		
TT+TA	2.88	1.08-7.70	**0.04**
Systolic blood pressure < 160 mmHg	1.00		
≥ 160 mmHg	2.51	0.91-6.95	0.08
Age < 67 years	1.00		
67-74 years	1.57	0.98-2.52	
75-81 years	2.47	0.96-6.36	
> 81 years	3.89	0.94-16.0	0.06
Current smoker no	1.00		
yes	2.27	0.74-7.0	0.15

3.5 Asymmetric dimethylarginine (ADMA) in a Swedish population with acute cerebrovascular disease (III)

In Study III we investigated wether an increased level of ADMA is associated with acute CVD or with any of its subgroups.

3.5.1 Subjects

363 consecutive patients who were clinically suspected of having a stroke were included in the study; 71 patients with cardio-embolic infarction, 239 with non-cardio-embolic infarction, 31 with transitory ischaemic attack (TIA), and 31 with intracerebral haemorrhage. The stroke study was well under way when we decided to measure plasma levels of ADMA in the patients, whereby ADMA measurements for the first 156 patients in study I is missing.

The patients in study III were compared with 48 controls, who were clinically free from cardiovascular disease.

3.5.2 Methods

Plasma concentrations of ADMA, symmetric dimethylarginine (SDMA) and L-arginine (μmol/L) were compared between patients and controls. ADMA, SDMA and L-arginine were analyzed with HPLC.

3.5.3 Results

ADMA concentrations in the overall group of patients with acute CVD were increased compared with controls ($p<0.01$). In the subgroup analysis, ADMA was increased in cardio-embolic infarction ($p<0.001$) and TIA ($p<0.001$) compared with controls, but not in non-cardio-embolic infarction ($p=0.56$) or in haemorrhagic stroke ($p=0.77$). In the whole group of CVD patients, ADMA was not associated with previous stroke/TIA ($p=0.08$), but when patients with haemorrhagic stroke were excluded from the CVD patients such an association was seen ($p=0.01$).

In the patients with CVD, plasma concentrations of ADMA were positively associated with age, renal function (creatinine), SDMA and L-arginine, but not with traditional CVD risk factors such as blood pressure, BMI, glucose or lipids.

In multivariate logistic regression models, CVD increased across quartiles of ADMA in all subgroups, but this association was only significant in the TIA group (odds ratio for highest vs lowest quartile 13.1; 95% CI: 2.9-58.6; p=0.001). A decreased L-arginine/ADMA ratio was significantly associated with acute CVD (p<0.01), cardio-embolic infarction (p<0.01), haemorrhagic stroke (p=0.01) and TIA (p=0.03), but not with non-cardio-embolic infarction (p=0.12).

3.5.4 Discussion

Study III is the first study comparing plasma levels of ADMA in different subtypes of acute CVD. A previous study (Yoo et al., 2001), conducted in an Asian population, demonstrated elevated ADMA levels in patients with ischemic stroke. In the present study we observed slightly increased ADMA levels in patients with earlier ischaemic stroke or TIA. In multiple logistic regression analysis, increased ADMA concentration was strongly and independently associated with TIA. A tendency towards increased risk associated with elevated concentrations of ADMA was seen also in cardio-embolic infarction and in the group of CVD as a whole but did not reach statistical significance. The results indicate that ADMA is a weak independent marker for acute stroke and a strong marker for TIA.

A reduced plasma L-arginine concentration has previously been reported in patients with hypercholesterolemia (Jeserich et al., 1992) and was in Study III observed in patients with acute CVD, cardio-embolic and haemorrhagic stroke, and a reduced L-arginine/ADMA ratio was seen in these groups as well as patients with TIA. A reduced level of L-arginine or a decreased L-arginine/ADMA ratio is compatible with ADMA acting as a competitive inhibitor of NO synthase with L-arginine as its substrate, resulting in a reduced NO synthesis. L-arginine/ADMA ratio is thus a relevant biological measure of relative arginine deficiency.

In contrast to what would have been expected from previous reports, we did not find any associations between high ADMA levels and diabetes (Abbasi et al., 2001), high blood pressure (Surdacki et al., 1999) or dyslipidemia (Lundman et al., 2001; Böger et al., 1998). The results of Study III, therefore, raise some doubts to the applicability of ADMA as a marker of the metabolic syndrome, which has been suggested (Böger 2003).

3.6 Asymmetric dimethylarginine and polymorphisms of fatty acid-binding protein 2 and endothelial nitric oxide synthase genes in carotid atherosclerosis (IV)

In Study IV we investigated the relationship between ADMA levels and the eNOS gene Glu298Asp polymorphism in patients with carotid stenosis and control subjects. We also investigated the FABP2 A54T polymorphism in these groups. A limitation of Study II was the relatively small number of patients with carotid stenosis and in addition, the FABP2 genotype frequencies only reached statistical significance in the multivariate regression analysis, not in the univariate analysis. The aim of Study IV was therefore, also to evaluate the FABP2 Ala54Thr genotype in a larger population.

3.6.1 Subjects
54 patients who had severe carotid stenosis ($\geq$70%) and 54 control patients without significant carotid stenosis (< 50%), were matched for age and sex. All 108 patients were under the age of 75 years.

3.6.2 Methods
DNA was amplified in a PCR reaction and the eNOS gene Glu298ASp polymorphism was determined by pyrosequencing and the FABP2 A54T polymorphism with RFLP technique. In contrast to study III, ADMA was analysed with an ELISA method (for details see paper IV).

3.6.3 Results
Clinical characteristics of the study population are given in Table 11. Of the 108 patients included, 54 with and 54 without ICA stenosis, 72 (67 %) had suffered a previous stroke or TIA. There were no differences between the two groups regarding systolic and diastolic blood pressure, BMI or creatinine and total- or LDL-cholesterol. Patients with ICA stenosis had more often suffered from stroke or TIA (p=0.03), had higher prevalence of hypertension (p<0.001), claudication (p<0.001) and angina pectoris (p=0.001), and used more lipid-

lowering agents (p<0.001) than subjects without ICA stenosis. The patients with ICA stenosis also had higher triglycerides (p<0.001) than patients without ICA stenosis.

In the whole group of patients, plasma ADMA concentrations were strongly associated with creatinine and we, therefore, also calculated ADMA concentration corrected for creatinine (ADMAcorr). In the group of patients without ICA stenosis both ADMA (0.70 ± 0.14) and ADMAcorr (0.70 ± 0.13) concentrations were lower than in the patients with ICA stenosis (0.76 ± 0.16 and 0.75 ± 0.15 μmol/L, respectively; p<0.01 for both).

Table 11. Clinical characteristics in patients without and with ICA stenosis

	Controls	ICA stenosis	p-value
n	54	54	
Gender (females/males)	20/34	20/34	*
Age (years)	67.1 ± 5.8	67.1 ± 5.9	*
History of stroke/TIA (%)	31 (57)	41 (76)	0.03
History of hypertension (%)	22 (41)	39 (72)	<0.001
History of claudication (%)	2 (4)	17 (31)	<0.001
History of angina pectoris (%)	10 (19)	28 (52)	<0.001
Prevalence of diabetes (%)	7 (13)	9 (17)	0.54
Prevalence of lipid-lowering therapy (%)	12 (22)	36 (67)	<0.001
SBP (mmHg)	148 ± 21	153 ± 20	0.25
DBP (mmHg)	78 ± 10	78 ± 12	0.72
BMI (kg/m^2)	25.8 ± 3.0	26.3 ± 3.2	0.43
Glucose (mmol/L)	6.2 ± 1.6	6.6 ± 2.3	0.19
HbA1c (%)	4.7 (3. 9 - 7.5)	4.9 (2.6 - 8.9)	0.13**
Creatinine (μmol/L)	79 ± 23	82 ± 22	0.61
Total Cholesterol (mmol/L)	5.3 ± 1.2	5.3 ± 1.6	0.71
LDL cholesterol (mmol/L)	3.2 ± 0.9	2.9 ± 1.1	0.61
HDL cholesterol (mmol/L)	1.5 ± 0.6	1.3 ± 0.4	0.03
Triglycerides (mmol/L)	1.5 (0.6 - 5.1)	2.3 (0.6 -7.8)	<0.001**
ADMA (μmol/L)	0.70 ± 0.14	0.76 ± 0.16	<0.01

Data presented as mean ± SD, or as median and range or fraction (%). Group differences analysed by Student's paired t-test for continuous variables and Fisher's exact test for categorical variables

*The two groups were matched according to gender and age
**Student's t-test using logarithmic values

Allele and genotype frequencies of the eNOS gene and the FABP2 polymorphisms did not differ between patients and controls and ADMA levels did not differ in subjects with different eNOS genotypes.

Because of few patients with the TT genotype (n=6) of the FABP2 A54T polymorphism, the TT and TA genotypes were combined. Allele and genotype frequencies in the group without ICA stenosis (TT + TA, 59.3 %; AA, 40.7 %) did not differ significantly from the group of patients with ICA stenosis (TT + TA, 51.9 %; AA, 48.1 %; p=0.09).
The A allele of FABP2 A54T polymorphism was associated with previous stroke/TIA (p=0.04), but neither the A nor the T allele was associated with plasma ADMA levels or with any traditional risk factor for atherosclerosis. In multivariate logistic regression, correcting for traditional risk factors for ICA stenosis, as well as ADMA levels, previous stroke/TIA remained the only independently factor associated with the A-allele of FABP2 A54T polymorphism (OR 2.40 (1.02 – 5.66)) (p=0.04).

Because of few patients with the Asp/Asp genotype of the eNOS (Glu→Asp) polymorphism, the Asp/Asp and Asp/Glu genotypes were combined. Allele and genotype frequencies in the group without ICA stenosis were not significantly different from that in the stenosis group and frequencies of Asp/Asp and Asp/Glu vs Glu/Glu were identical in the two groups.
The polymorphism was not associated with plasma ADMA levels (t-test, p=0.58; in multivariate logistic regression, p=0.11) or with any traditional risk factor for atherosclerosis. Neither was the polymorphism associated with the FABP2 A54T polymorphism (p=0.06).

3.6.4 Discussion
In agreement with the finding of an increased plasma ADMA level in TIA (Study III) we also found an increased level of ADMA in the group of patients with a ICA stenosis compared to the group of patients without ICA patients (Study IV). In Study IV we also observed that triglycerides, in this study measured postprandially, were increased in subjects with carotid stenosis. Postprandial hypertriglyceridemia is an early sign of insulin-resistance (Axelsen et al., 1999) and has, as endothelial dysfunction, been associated with early atherosclerosis (Boquist S et al., 1999). ADMA, a marker of endothelial dysfunction, is increased in clinical disease related to atherosclerosis (Usui et al., 1998; Pettersson et al., 1998; Paradossi et al.,

2004; Shin Shin et al., 2004) and ADMA has earlier also been associated with increased triglyceride levels (Fard et al., 2000; Lundman et al., 2001). The mechanism behind this increase is not yet fully understood, but reduced degradation of ADMA is one mechanism proposed to be involved in its accumulation (Fard et al., 2000).

It is thus conceivable that the more advanced atherosclerosis in ICA stenosis, for which ADMA is a marker and possibly also a causal factor, may partly be explained by the hyper-triglyceridemia observed among patients with ICA stenosis.

Study IV was higher powered and better matched than Study II. In Study IV a comparison was made between patients with severe ICA stenosis (> 70%) and patients without ICA stenosis (< 50%) which is also an advantage compared with Study II in which patients with ICA stenosis (> 50%) and without stenosis (< 50%) were compared. These differences speak in favour of the result in Study IV. On the other hand both studies made comparisons between two groups of patients where all (Study II) or many (Study IV) of the patients suffered from vascular disease. A better design might have been to compare patients with ICA stenosis with, control patients having less atherosclerotic disease. It can also be argued that in Study IV we investigated a population years after they have survived a stroke, whereas in Study II, we do not know if the patients were alive some years after their stroke. The population of patients with ICA stenosis in Study II may thus represent a group with more unstable carotid plaques and the studies may not be entirely comparable. We, however, conclude that it is not likely that the A54T polymorphism of the FABP2 gene is associated with ICA stenosis.

We also conclude that the Glu298Asp polymorphism of the eNOS gene not is associated with severe ICA stenosis.

4. GENERAL DISCUSSION

We have studied two different polymorphisms in relation to stroke and carotid atherosclerosis, the FABP2 A54T and the eNOS Glu298Asp, from two different genes that can be considered candidate genes for atherosclerosis. The FABP2 protein is involved in lipid metabolism and eNOS affects the amount of NO available within the vascular endothelium. ADMA, an endogenous inhibitor of NO syntethase, has also been studied in the present investigation.

4.1 Methodological considerations

One problem when studying the etiology of stroke is that it is a heterogenous disease. Not only is stroke itself made up of many different subtypes which may have different genetic bases, but also different types of stroke have different stages in their patogenesis each of which may have different genetic influences. One approach to simplify the situation is to use intermediate phenotypes such as carotid atherosclerosis. In Studies II and IV we used ICA stenosis as an intermediate phenotype. Such an intermediate phenotype has the advantage of overcoming the problem of incomplete penetrance in clinical disease. A control patient in a stroke study may have subclinical disease and develop a stroke a few years after having been recruited as a control.

Historically, stroke phenotyping in stroke studies has often been incomplete and all types of strokes have been lumped together (Alberts, 2003). We have, therefore, tried to subclassify stroke patients (although a distinction between large and small vessel was not possible). Another problem in stroke studies is that the distribution of polymorphisms between different ethnic groups may vary widely. This may lead to so-called population stratification, which occurs when cases and controls are unintentionally included at different ratios from two or more subgroups that have different ethnic or genetic background (Rosand and Alshuler, 2003). The present investigation, however, has been carried out in a relatively ethnically homogenous population.

Many stroke studies analyze polymorphisms without a functional affect on the expression of the gene or function of the encoded protein (Alberts, 2003). The A54T FABP2 polymorphism has been shown to have a clear effect on the structure of the protein.

4.2 The A54T FABP2 polymorphism in atherosclerotic disease

In contrast to myocardial infarction, hypercholesterolemia seems to be a relatively weak risk factor in stroke, but increased levels of postprandial triglyceride-rich lipoproteins has on the other hand been associated with endothelial dysfunction and carotid intima media thickness (IMT), which is a strong predictor of stroke.

The threonine containing protein of the FABP2 A54T polymorphism has a greater affinity for long chain FFAs than the alanine containing protein. This altered affinity for FFAs in the intestine has been shown to affect the absorption and, consequently, the fatty acid composition of serum lipids. Several studies have demonstrated that the FABP2 T54 allele is associated with postprandial lipemia, and carriers of this polymorphism have therefore a presumptive increased risk for atherosclerosis and stroke.

Interestingly, saturated fatty acids have been associated with an increased endothelial dysfunction (Sarabi et al., 2001; Steer et al., 2003) and polyunsaturated fatty acids (PUFAs) with a favourable endothelial function (Mozaffarian, 2005). Measuring the fatty acid composition of serum phospholipids reflects weeks to months´ qualitative dietary intake of fatty acids (Zock et al., 1997). When studying 58 stroke patients and 30 healthy controls we found that CVD patients had higher proportion of saturated fatty acids and lower proportions of total monounsaturated and total diunsaturated fatty acids vs controls in serum phospholipids (Carlsson et al., 2005). The CVD patients also had lower proportions of the polyunsaturated n-3 fatty acid C:20:5, eicosapentaenoic acid (EPA) and the n-6 fatty acid C:18:2, linoleic acid, than the controls. Stroke patients with TT or TA genotypes of the FABP2 AT54 polymorphism had higher saturated fatty acids compared with patients with AA genotype. Furthermore, stroke patients with TT or TA genotype had the highest relative amount of saturated fatty acids and the lowest relative amount of linoleic acid and EPA. Inversely, the controls with AA genotype had the lowest relative amount of saturated fat (fig 8) and the highest amount of linolenic acid and EPA. This study, although with a small number of patients, thus underscores a role for FABP2 in stroke and confirms previous findings of a role of fatty acids in stroke (Iso et al, 2002).
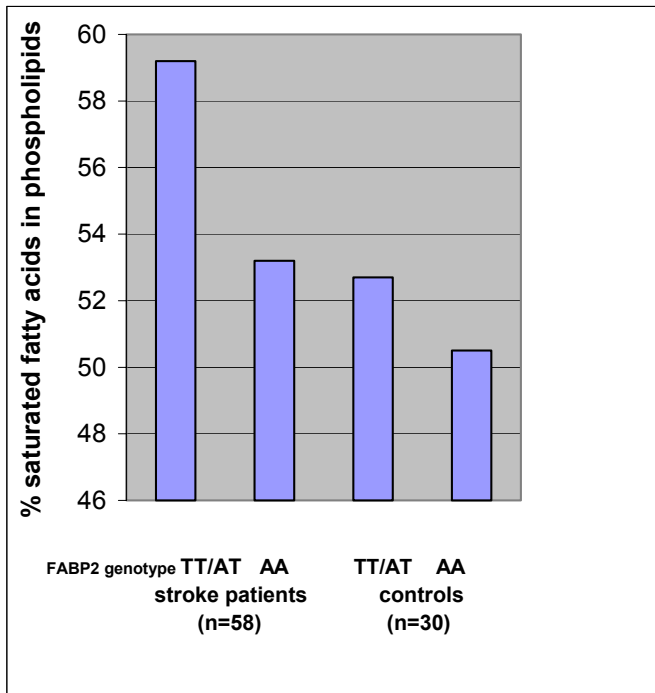
Fig 8. Gene-environment interaction in 58 stroke patients and 30 controls. Serum phospholipids composition of saturated fatty acids in relation to the FABP2 A54T gene polymorphism (p<0.001).

An analysis of the total level of FFAs in patients with acute CVD gives further support to the notion that the T genotype influences the uptake of FFAs. It is well known that FFAs are elevated in insulin deficiency. If only FFAs in CVD patients (from Study I) with normal glucose values are analyzed in regard to FABP2 genotype, FFAs are increased in the TT genotype (fig 9).

How can the divergent results (Studies II and IV) on the FABP2 A54T gene polymorphism then be explained? TIA is in many instances an attribute of large vessel disease, and if the association for the T allele with TIA holds true, we would have expected to find a similar association with carotid artery stenosis, but much to our surprise, in contrast to Study II, in Study IV a trend towards an overrepresentation of the A allele in patients with severe carotid stenosis was found. If we compare the two study populations in Studies II an IV, some differences in the populations used may in part account for the discrepant results in the two studies. In Study IV the study group consisted of a much larger number of patients with ICA

stenosis and also a well-characterized and matched control group compared with our previous study. The conclusion must be that the T allele of the A54T FABP2 polymorphism is not likely associated with carotid stenosis.

If in Study I we exclude the patients with carotid stenosis, TT/TA genotypes were near significant (p=0.07) in TIA (n= 35) vs controls, and the same was true for the TT genotype in ischaemic stroke among younger (< 70 years) and non-smoking patients (n=90; p=0.06).

As suggested by our study on fatty acids in stroke (Carlsson et al., 2005) the T allele of the FABP2 A54T gene polymorphism influences uptake of saturated fatty acids and in combination with dietary fat intake influences the atherosclerotic process and the risk of stroke. The study by Marín et al. (2005) likewise suggested a pathogenic role of the T allele only if patients were fed a diet rich in saturated fatty acids. An explanation to the positive findings of the T allele of the FABP2 A54T gene polymorphism in Studies I and II, and negative findings in Study IV could be that the T allele is associated with intracranial large or small vessel disease, but not with extracranial carotid atherosclerosis. Galluzi (2001), in a large study, did not find an association with the FABP2 A54T gene polymorphism and coronary artery disease. Coronary artery disease has in turn been associated with carotid atherosclerosis (Crouse JR 3rd., 1991). We therefore speculate that the intracranial endothelium is more susceptible to saturated fatty acids in postprandial hyperlipidemia than the endothelium of the carotid and coronary arteries.
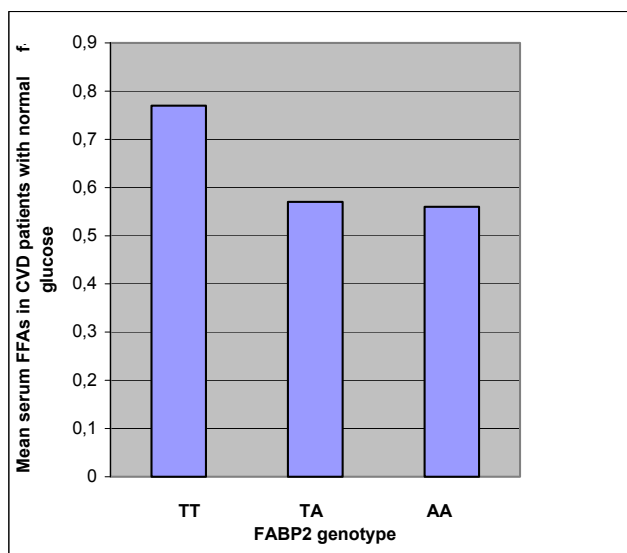
Fig 9. Mean fasting serum FFA concentrations (mmol/L) in different FABP2 genotypes in normoglycemic CVD patients (<6.1 mmol/L).

4.3 ADMA

Dysfunction of the endothelium is a common mechanism by which several cardiovascular risk factors mediate certain deleterious effects on the vascular wall. Although the degree of endothelial dysfunction appears to correlate with the burden of traditional risk factors, there is considerable heterogeneity in the magnitude of dysfunction observed in individuals with similar profiles of traditional risk factors (Halcox et al., 2002). The endothelial function is crucially influenced by the availability of NO, since NO is involved in a variety of mechanisms maintaining vascular homeostasis. ADMA appears to be a strong marker of endothelial dysfunction, and accumulating evidence indicates that ADMA is a novel cardiovascular risk factor which might contribute to the identification of patients at risk of developing such disease beyond traditional risk factors.

In Study III we demonstrated an increased concentration of ADMA in patients with TIA and a decreased L-arginine/ADMA ratio in CVD and in most of its subgroups. In Study IV we demonstrated an increase of ADMA in patients with severe carotid artery stenosis. These results thus support ADMA as an indicator of atherosclerotic disease.

4.4 The eNOS Glu298Asp gene polymorphism in carotid atherosclerosis

The Glu298-to-Asp variant of the NOS3 gene has in several studies been associated with manifestations of atherosclerotic disease (Paradossi et al., 2004; McDonald et al., 2004). We did not find an increased frequency of the T allele in patients with ICA stenosis compared to patients without ICA stenosis, nor did the variant influence plasma ADMA concentrations. Our findings suggest that the eNOS Glu298Asp is not of major importance for development of internal carotid stenosis.

4.5 A possible connection between FABP2 and ADMA
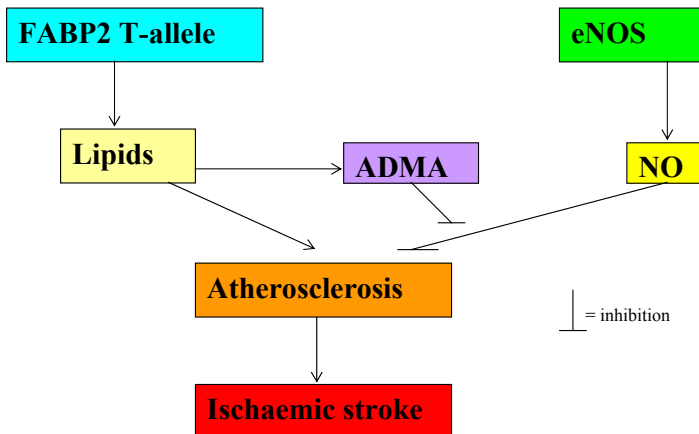
Fig 10. A possible link between the T-allele of the FABP2 A54T polymorphism, ADMA and stroke. The T-allele has been associated with an increased affinity of fatty acids to the FABP2 protein and an increased uptake of saturated fatty acids (Carlsson et al., 2005) as with postprandial hypertriglyceridemia, which in turn has been associated with endothelial dysfunction (Lundman et al., 1997). High intake of saturated fatty acids (Sarabi et al., 2001; Steer et al., 2003) and low PUFAs (Mozaffarian, 2005) have also been associated with endothelial dysfunction. Endothelial dysfunction due to hypertriglyceridemia may be explained by an inhibition of DDAH, with a concomitant increase of ADMA and a decrease of NO bioavailability (Fard et al., 2000). A connection between n-3 fatty acids and ADMA is supported by a study done on spontaneously hypertensive rats, in which supplementation with n-3 fatty acids decreased ADMA levels (Raimondi et al., 2005).

5. SUMMARY

1. Carriers of the T54 variant of the FABP2 gene have an atherogenic blood lipid profile, and the T allele of the A54T polymorphism of the FABP2 gene appears to be associated with ischaemic stroke and TIA.

2. The T allele of the A54T polymorphism of the FABP2 gene is probably not associated with ICA stenosis. The Asp allele of the Glu298Asp eNOS gene is not associated with ICA stenosis.

3. ADMA is an independent marker for CVD. Relative defiency of arginine, measured as L-arginine/ADMA ratio, is present in acute CVD.

4. ADMA levels are elevated in patients with carotid stenosis, and the eNOS Glu298Asp polymorphism does not influence ADMA levels.

6. CONCLUSIONS

Levels of ADMA are elevated in both TIA and carotid stenosis, and the common polymorphism A54T of the FABP2 gene increases susceptibility to ischaemic stroke and TIA.

7. ACKNOWLEDGEMENTS

8. POPULÄRVETENSKAPLIG SAMMANFATTNING

Syftet med de studier som ligger till grund för denna avhandling är att studera genetiska och metabola riskfaktorer för slaganfall (stroke) och åderförkalkning.

Strokesjukdomen utgör ett enormt hälsoproblem, både i västvärlden, men numera också i tredje världen. I Sverige är stroke den tredje största dödsorsaken efter hjärtinfarkt och cancer och den vanligaste orsaken till handikapp. Ungefär 30 000 människor insjuknar per år i Sverige. Därutöver insjuknar cirka 8000 personer per år i sk transitorisk ischemisk attack (TIA), ett tillstånd där de neurologiska bortfallssymptomen försvinner inom 24 timmar.

Trots framsteg inom den genetiska forskningen är det först under de senaste åren som forskningen kring genetik vid stroke på allvar tagit fart. Allt fler studier, bland annat de som jämför insjuknandefrekvensen i stroke hos enäggs- och tvåäggstvillingpar, talar för att genetiska komponenter är betydelsefulla. Kända riskfaktor, såsom högt blodtryck, diabetes, rökning, kan förklara ungefär hälften av alla uppkomna stroke, medan resterande del troligen kan förklaras utifrån genetiska faktorer.

Stroke är i huvudsak en komplex polygen sjukdom, vilket innebär att många gener samverkar med omgivningsfaktorer vid uppkomsten av sjukdomen. Att många gener och omgivningsfaktorer är inblandade i sjukdomsprocessen gör strokesjukdomen svårstuderad. Sannolikt bidrar olika gener i olika faser av sjukdomsförloppet och dessutom har troligen olika undergrupper av stroke olika genetisk bakgrund.

För att minska antalet inblandade gener studeras ibland istället sk intermediära fenotyper (en del av sjukdomsprocessen). En ofta studerad intermediär fenotyp inom strokegenetiken är karotisstenos (förträngning av halspulsådern), som är ett tecken på avancerad åderförkalkning och som medför en kraftigt ökad risk för stroke.

Fettsyrebindande protein 2 (FABP2) är ett protein (äggviteämne) som är involverat i fettupptaget i tunntarmen. En ärftlig variant av genen som kodar för detta protein har visats öka upptaget av långa fettsyror till blodet och därmed även påverka blodfetterna, ffa de fria fettsyrorna, efter måltid. Stegring av blodfetterna efter måltid har i sin tur visats kunna leda

till kärlsjukdom. FABP2 kan därför betraktas som en kandidatgen för åderförkalknings-sjukdom.

Endotelial dysfunktion, dvs ett tillstånd med rubbad balans i blodkärlsväggen, har vuxit fram som ett centralt begrepp vid åderförkalkning av blodkärlen. En nyckelsubstans i detta sammanhang är kväveoxid, NO, som bildas kontinuerligt i endotelet (cellerna närmast blodströmmen) från aminosyran arginin med hjälp av enzymet endotelialt kväveoxidsyntas, eNOS. NO har egenskaper som motverkar åderförkalkning, bland annat påverkar det kärlets förmåga att vidga sig och blodplättarnas tendens att klumpa ihop sig. NO påverkar också graden av inflammation. En minskad bildning av NO anses därför viktig vid uppkomsten av åderförkalkning. Då eNOS påverkar tillgången på NO har även eNOS genen betraktats som en kandidatgen för åderförkalkning. En ärftlig variant av eNOS genen har visats vara av speciellt intresse.

Asymmetriskt dimetylarginin, ADMA, bildas från argininändar av proteiner. Fritt ADMA hämmar eNOS. En hög ADMA koncentration kan därför leda till sänkt NO produktion. ADMA har visats vara förhöjt vid endotelial dysfunktion och åderförkalningssjukdom.

De olika studierna:

I studie I studerade vi den ärftliga varianten (A54T) av FABP2 genen hos 407 patienter med stroke eller TIA och jämförde med 158 friska blodgivare.

I studie II studerade vi A54T varianterna av FABP2 genen hos de patienter i den första studien som i samband med strokeinsjuknandet blivit undersökta med ultraljud för före-komst av karotisstenos.

I studie III studerade vi ADMA koncentrationerna hos 386 patienter med stroke och TIA och 48 friska kontroller.

I studie IV studerade vi ADMA koncentrationerna hos 54 patienter med tät förträngning av halskärlen och 54 parvis matchade personer utan förträngning av halskärlen. I denna studie studerade vi också de ärftliga varianterna av FABP2 genen (A54T) och eNOS genen (Glu298Asp).

Detta avhandlingsarbete har visat:

1. Bärare av T54-varianten av FABP2 genen har en blodfettsprofil som gynnar uppkomsten av åderförkalkning och T54 varianten förefaller vara associerad till uppkomsten av stroke och TIA (studie I).

2. T54-varianten av FABP2 genen och Asp298-varianten av eNOS genen är inte associerade till uppkomsten av karotisstenos (studie II och IV).

3. ADMA är en oberoende markör för akut cerebrovaskulärsjukdom. Relativ arginin-brist, mätt som arginin-ADMA-kvot, förekommer vid akut cerebrovaskulärsjukdom (studie III).

4. ADMA nivåer är förhöjda vid karotisstenos (studie IV) men påverkas inte av eNOS Glu298Asp varianten.

Sammanfattningsvis kan vi konstatera att ADMA nivåer är förhöjda både vid TIA och karotisstenos och att en vanligt förekommande variant i FABP2 genen ökar benägenheten för ischemiskt stroke och TIA.

9. References

Abbasi F, Asagmi T, Cooke JP, Lamendola C, McLaughlin T, Reaven GM, et al. Plasma concentrations of asymmetric dimethylarginine are increased in patients with type 2 diabetes mellitus. Am J Cardiol 2001; 88: 1201-1203.

Adams HP Jr, Bendixen BH, Kappelle LJ, Biller J, Love BB, Gordon DL, Marsh EE 3rd. Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of Org 10172 in acute stroke treatment. Stroke 1993; 24: 35-41.

Albala C, Santos JL, Cifuentes M, Villarroel AC, Lera L, Liberman C, et al. Intestinal FABP2 A54T polymorphism: association with insulin resistance and obesity in women. Obes Res 2004; 12: 340-345.

Alberts MJ. Stroke genetics update. Stroke 2003; 34: 342-344.

Alcock NW. Laboratory tests for assessing nutritional status. In: Shils ME, Olsson JSA, Shike M, Ross AC (eds), Modern nutrition in health and disease. 9th ed. Williams & Wilkins Baltimore, MD, US 1999: 927-928.

Aho K, Harmsen P, Hatano S, Marquardsen J, Smirnov VE, Strasser T. Cerebrovascular disease in the community: results of a WHO collaboration study. Bull World Health Organ 1980; 58: 113-130.

Amarenco P. Hypercholesterolemia, lipid-lowering agents, and the risk for brain infarction. Neurology 2001; 57: 35-44.

Armitage P, Berry G, Matthews JNS: Statistical Methods in Medical Research, Fourth edition, London, Blackwell Scientific Publications, 2002, p 677.

Asplund K. Riks-Stroke – A Swedish National Quality Register for Stroke Care. Cerebrovascular Dis 2003; 15: 5-7.

Axelsen M, Smith U, Eriksson JW, Taskinen MR, Jansson PA. Postprandial hypertriglyceridemia and insulin resistance in normoglycemic first-degree relatives of patients with type 2 diabetes. Ann Intern Med 1999; 131: 27-31.

Bae JH, Bassenge E, Kim KB, Kim YN, Kim KS, Lee HJ, et al. Postprandial hypertriglyceridemia impairs endothelial function by enhanced oxidant stress. Atherosclerosis 2001; 155: 517-523.

Baier LJ, Sacchettini JC, Knowler WC, Eads J, Paolisso G, Tataranni PA, et al. An amino acid substitution in the human intestinal fatty acid binding protein is associated with increased fatty acid binding, increased fat oxidation, and insulin resistance. J Clin Invest 1995; 95: 1281-1287.

Baier LJ, Bogardus C, Sacchettini JC. A polymorphism in the human intestinal fatty acid-binding protein alters fatty acid transport across Caco-2 cells. J Biol Chem 1996; 271: 10892-10896.

Baigent C, Keech A, Kearney PM, Blackwell L, Buck G, Pollicino C, et al.; Cholesterol Treatment Trialists' (CTT) Collaborators. Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90,056 participants in 14 randomised trials

of statins. Lancet 2005; 366: 1267-1278.

Bak S, Gaist D, Sindrup SH, Skytthe A, Christensen K. Genetic liability in stroke: a long-term follow-up study of Danish twins. Stroke 2002; 33: 769-774.

Bemelmans WJ, Lefrandt JD, Feskens EJ, Broer J, Tervaert JW, May JF, Smit AJ. Change in saturated fat intake is associated with progression of carotid and femoral intima-media thickness, and with levels of soluble intercellular adhesion molecule-1. Atherosclerosis 2002; 163: 113-120.

Benfante R, Yano K, Hwang LJ, Curb JD, Kagan A, Ross W. Elevated serum cholesterol is a risk factor for both coronary heart disease and thromboembolic stroke in Hawaiian Japanese men. Implications of shared risk. Stroke 1994; 25: 814-820.

Bernlohr DA, Simpson MA, Hertzel AV, Banaszak LJ. Intracellular lipid-binding proteins and their genes. Annu Rev Nutr 1997; 17: 277-303.

Berthier MT, Couillard C, Prud'homme D, Nadeau A, Bergeron J, Tremblay A, et al. Effects of the FABP2 A54T mutation on triglyceride metabolism of viscerally obese men. Obes Res 2001; 9: 668-675.

Besnard P, Niot I, Bernard A, Carlier H. Cellular and molecular aspects of fat metabolism in the small intestine. Proc Nutr Soc 1996; 55: 19-37.

Bevan S, Porteous L, Sitzer M, Markus HS. Phosphodiesterase 4D gene, ischemic stroke, and asymptomatic carotid atherosclerosis. Stroke 2005; 36: 949-53.

BIP: Secondary prevention by raising HDL cholesterol and reducing triglycerides in patients with coronary artery disease: the Bezafibrate Infarction Prevention (BIP) study. Circulation 2000; 102: 2-4.

Bluth EI, Wetzner SM, Stavros AT, Aufrichtig D, Marish KW, and Baker JD. Carotid duplex sonography: A multicenter recommendation for standardized imaging and Doppler criteria. RadioGraphics 1988; 8: 487-506, Figure 22.

Bogousslavsky J, Despland PA, Regli F. Asymptomatic tight stenosis of the internal carotid artery: long-term prognosis. Neurology 1986; 36: 861-3.

Bonita R, Beaglhole. Explaining stroke mortality trends. Lancet 1993; 341: 1510-1511.

Bonnardeaux A, Nadaud S, Charru A, Jeunemaitre X, Corvol P, Soubrier F. Lack of evidence for linkage of the endothelial cell nitric oxide synthase gene to essential hypertension. Circulation 1995; 91: 96-102.

Boquist S, Ruotolo G, Tang R, Bjorkegren J, Bond MG, de Faire U, et al. Alimentary lipemia, postprandial triglyceride-rich lipoproteins, and common carotid intima media thickness in healthy, middle-aged men. Circulation 1999; 17: 723-728.

Boullu-Sanchis S, Lepretre F, Hedelin G, Donnet JP, Schaffer P, Froguel P, et al. Type 2 diabetes mellitus: association study of five candidate genes in an Indian population of Guadeloupe, genetic contribution of FABP2 polymorphism. Diabetes Metab 1999; 25: 150-156.

Brass LM, Isaacsohn JL, Merikangas KR, Robinette CD. A study of twins and stroke. Stroke 1992; 23: 1361-1366.

Brass LM, Alberts MJ. The genetics of cerebrovascular disease. In: Balliére´s Clinical Neurology Vol 4, No 2, Aug 1995.

Brass LM, Carrano D, Hartigan PM, Hartigan PM, Concato J, and Page WF. Genetic risk for stroke: a follow-up study of the National Academy of Sciences/Veteranes Administration Twin Registry (abstract). Neurology 1996; 46: A212.

Broderick JP, Brott T, Tomsick T, Huster G, Miller R. The risk of subarachnoid and intracerebral hemorrhage in blacks as compared with whites. N Engl J Med 1992; 326: 733-736.

Brown RD, Whisnant JP, Sicks JD, O'Fallon WM, Wiebers DO. Stroke incidence, prevalence, and survival: secular trends in Rochester, Minnesota, through 1989. Stroke 1996; 27: 373-380.

Böger RH, Bode-Böger SM, Mugge A, Kienke S, Brandes R, Dwenger A, Frolich JC. Supplementation of hypercholesterolaemic rabbits with L-arginine reduces the vascular release of superoxide anions and restores NO production. Atherosclerosis 1995; 117: 273-284.

Boger RH, Bode-Boger SM, Kienke S, Stan AC, Nafe R, Frolich JC. Dietary L-arginine decreases myointimal cell proliferation and vascular monocyte accumulation in cholesterol-fed rabbits. Atherosclerosis 1998; 136: 67-77.

Boger RH, Bode-Boger SM, Szuba A, Tsao PS, Chan JR, Tangphao O, et al. Asymmetric Dimethylarginine (ADMA): A Novel Risk Factor for Endothelial Dysfunction Its Role in Hypercholesterolemia. Circulation 1998; 98: 1842-1847.

Böger RH. The emerging role of asymmetric dimethylarginine as a novel cardiovascular risk factor. Cardiovascular Research 2003; 59: 824-833.

Cai H, Wilcken DE, Wang XL. The Glu-298-->Asp (894G-->T) mutation at exon 7 of the endothelial nitric oxide synthase gene and coronary artery disease. J Mol Med 1999; 77: 511-514.

Canani LH, Capp C, Ng DP, Choo SG, Maia AL, Nabinger GB et al. The Fatty Acid-Binding Protein-2 A54T Polymorphism Is Associated With Renal Disease in Patients With Type 2 Diabetes. Diabetes 2005; 54: 3326-3330.

CARE: Sacks FM, Pfeffer MA, Moye LA, Rouleau JL, Rutherford JD, Cole TG, et al. for the Cholesterol and Recurrent Events Trial Investigators. The effect of pravastatin on coronary events after myocardial infarction in patients with average cholesterol levels. N Engl J Med 1996; 336: 1001-1009.

Carlsson M, Orho-Melander M, Hedenbro J, Almgren P, Groop LC. The T 54 allele of the fatty acid-binding protein 2 is associated with a parental history of stroke. J Clin Endocrinol Metab 2000; 85: 2801-2804.

Carlsson M, Nilsson I, Wanby P, Gustafsson I, Palmqvist P, Brudin L. The FABP2 gene polymorphism, serum fatty acids and the risk of stroke. Cerebrovascular diseases 2005; 19 (suppl 2) p 141, poster nr 14 (abstracts 14th European Stroke Conference Bologna, Italy, May 25-28, 2005).

Cattin L, Fisicaro M, Tonizzo M, Valenti M, Danek GM, Fonda M, et al. Polymorphisms of the apolipoprotein E gene and early carotid atherosclerosis defined by ultrasonography in asymptomatic adults. Arterioscler Thromb Vasc Biol 1997; 17: 91-94.

Celermajer DS, Sorensen KE, Gooch VM, Spiegelhalter DJ, Miller OI, Sullivan ID, et al. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. Lancet 1992; 340: 1111-1115.

Chambers JC, McGregor A, Jean-Marie J, Obeid OA, Krooner JS. Demonstration of rapid onset vascular endothelial dysfunction after hyperhomocysteinemia: an effect reversible with vitamin C therapy. Circulation 1999; 99: 1156-1160.

Chan JR, Boger RH, Bode-Boger SM, Tangphao O, Tsao PS, Blaschke TF, Cooke JP. Asymmetric dimethylarginine increases mononuclear cell adhesiveness in hypercholesterolemic humans. Arterioscler Thromb Vasc Biol 2000; 20: 1040-1046.

Channon KM, Qian H, George SE. Nitric oxide synthase in atherosclerosis and vascular injury: insights from experimental gene therapy. Arterioscler Thromb Vasc Biol 2000; 20: 1873-1881.

Cipollone F, Toniato E, Martinotti S, Fazia M, Iezzi A, Cuccurullo C, et al.; Identification of New Elements of Plaque Stability (INES) Study Group. A polymorphism in the cyclooxygenase 2 gene as an inherited protective factor against myocardial infarction and stroke. JAMA 2004; 291: 2221-2228.

Cohn SM, Simon TC, Roth KA, Birkenmeier EH, Gordon JI. Use of transgenic mice to map cis-acting elements in the intestinal fatty acid binding protein gene (Fabpi) that control its cell lineage-specific and regional patterns of expression along the duodenal-colonic and crypt-villus axis of the gut epithelium. J Cell Biol 1992: 119: 27-44.

Collins R, MacMahon S. Blood pressure, antihypertensive drug treatment and the risks of stroke and of coronary heart disease. Br Med Bull 1994; 50: 272-298.

Constant J. The role of eggs, margarines and fish oils in the nutritional management of coronary artery disease and strokes. Keio J Med 2004; 53:131-136.

Cooke JP, Dzau VJ. Nitric oxide synthase: role in the genesis of vascular disease. Annu Rev Med 1997; 48: 489-509.

Cross JM, Donald AE, Kharbanda R, Deanfield JE, Woolfson RG, MacAllister RJ. Acute administration of L-arginine does not improve arterial endothelial function in chronic renal failure. Kidney Int 2001; 60: 2318-2323.

Crouse JR 3rd. Carotid and coronary atherosclerosis. What are the connections? Postgrad Med 1991; 90: 175-179.

Crouse JR III, Byington RP, Hoen HM, Furberg CD. Reductase inhibitor mono therapy and stroke prevention. Arch Intern Med 1997; 157: 1305-1310.

de Faire U, Friberg L. and Lundman T. Concordance for mortality with special reference to ischemic heart disease and cerebrovascular disease: a study on the Swedish Twin Registry. Preventive Medicine 1975; 4: 509-517.

de Lorgeril M, Salen P, Martin JL, Monjaud I, Delaye J, Mamelle N. Mediterranean diet, traditional risk factors, and the rate of cardiovascular complications after myocardial infarction: final report of the Lyon Diet Heart Study. Circulation 1999; 99: 779-785.

Duarte, Colagiuri, Palu, Wang XL, Wilcken DE. Obesity, Type II diabetes and the Ala54Thr polymorphism of fatty acid binding protein 2 in the Tongan population. Mol Genet

Metab 2003; 79: 183-188.

Elbaz A, Poirier O, Moulin T, Chedru F, Cambien F, Amarenco P. Association between the Glu298Asp polymorphism in the endothelial constitutive nitric oxide synthase gene and brain infarction. The GENIC Investigators. Stroke 2000; 31:1634-1639.

Elkind MS, Sacco RL. Stroke risk factors and stroke prevention. Seminars in neurology 1998; 18; 429-440.

Endemann DH, Schiffrin EL. Endothelial dysfunction. J Am Soc Nephrol 2004; 15: 1983-1992.

Engler MM, Engler MB, Malloy MJ, Chiu EY, Schloetter MC, Paul SM, et al. Antioxidant vitamins C and E improve endothelial function in children with hyperlipidemia: Endothelial Assessment of Risk from Lipids in Youth (EARLY) Trial. Circulation 2003; 108: 1059-1063.

Erkkila AT, Lindi V, Lehto S, Pyorala K, Laakso M, Uusitupa MI. Variation in the fatty acid binding protein 2 gene is not associated with markers of metabolic syndrome in patients with coronary heart disease. Nutr Metab Cardiovasc Dis 2002; 12: 53-59.

European Carotid Surgery Trialist´Collaborative Group. Randomised trial of endarectomy for recently symptomatic carotid stenosis: final results of the MRC European Carotid Surgery Trial (ECST). Lancet 1998; 351: 1379-1387.

Fard A, Tuck CH, Donis JA, Sciacca R, Di Tullio MR, Wu HD, et al. Acute elevations of plasma asymmetric dimethylarginine and impaired endothelial function in response to a high-fat meal in patients with type 2 diabetes. Arterioscler Thromb Vasc Biol 2000; 20: 2039-2044.

Feigin VL, Andersson CS, and Mhurchu CN. Systemic inflammation, endothelial dysfunction, dietary fatty acids and micronutrients as risk factors for stroke; a selective review. Cerebrovascular Disease 2002; 13: 219-224.

Ferro JM. Cardioembolic stroke: an update. Lancet Neurol 2003; 2: 77-188.

Fine-Edelstein JS, Wolf PA, O'Leary DH, Poehlman H, Belanger AJ, Kase CS, D'Agostino RB. Precursors of extracranial carotid atherosclerosis in the Framingham Study. Neurology 1994; 44: 1046-1050.

Fisher CM. The arterial lesions underlying lacunes. Acta Neuropathol (Berl) 1969; 12: 1-15.

Ford CS, Crouse JR, Howard G, Toole JF, Ball MR, Frye J. The role of plasma lipids in carotid atherosclerosis. Ann Neurol 1985; 17: 301-303.

Forte P, Copland M, Smith LM, Milne E, Sutherland J, Benjamin N. Basal nitric oxide synthesis in essential hypertension. Lancet 1997; 349: 837-842.

Frikke-Schmidt R, Nordestgaard BG, Schnohr P, Tybjaerg-Hansen A. Single nucleotide polymorphism in the low-density lipoprotein receptor is associated with a threefold risk of stroke. A case-control and prospective study. Eur Heart J 2004; 25: 943-951.

Furchgott R, Zawadzski D. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 1980; 288: 373-376.

Galluzi JR, Cupples LA, Otvos JD, Wilson PWF, Schaefer EJ, Ordovas JM. Association

of the A /T54 polymorphism in the intestinal fatty acid binding protein with variations in plasma lipids in the Framingham offspring study. Atherosclerosis 2001; 159: 417-424.

Garg UC, Hassid A. Nitric oxide-generating vasodilators and proliferation of cultured rat vasculature smooth muscle cells. J Clin Invest 1989; 1774-1777.

Geroulakos G, O'Gorman DJ, Kalodiki E, Sheridan DJ, Nicolaides AN. The carotid intima-media thickness as a marker of the presence of severe symptomatic coronary artery disease. Eur Heart J 1994; 15: 781-785.

Ghilardi G, Biondi ML, DeMonti M, Turri O, Guagnellini E, Scorza R. Matrix metalloproteinase-1 and matrix metalloproteinase-3 gene promoter polymorphisms are associated with carotid artery stenosis. Stroke 2002; 33: 2408-2412.

Ghilardi G, Biondi ML, Turri O, Guagnellini E, Scorza R. Internal carotid artery occlusive disease and polymorphisms of fractalkine receptor CX3CR1: a genetic risk factor. Stroke 2004; 35: 1276-1279.

GISSI-Prevention trial: Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial. Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico. Lancet 1999; 354: 447-455.

Glatz JFC, van der Vusse GJ. Fatty acid-binding protein and the early detection of acute myocardial infarction. Prog Lipid Res 1996; 35: 243-282.

Goldstein LB, Adams R, Becker K, Furberg CD, Gorelick PB, Hademenos G, et al. Primary prevention of ischemic stroke: A statement for healthcare professionals from the Stroke Council of the American Heart Association. Circulation 2001; 103: 163-182.

Golledge J, Greenhalgh RM, Davies AH. The symptomatic carotid plaque. Stroke 2000; 31: 774-781.

Gorelick PB. Cerebrovascular disease in African Americans. Stroke 1998; 29: 2656-2664.

Grau AJ, Buggle F, Steichen-Wiehn C, Heindl S, Banerjee T, Seitz R, et al. Clinical and biochemical analysis in infection-associated stroke. Stroke 1995; 32: 1520-6

Gretarsdottir S, Thorleifsson G, Reynisdottir ST, Manolescu A, Jonsdottir S, Jonsdottir T, et al. The gene encoding phosphodiesterase 4D confers risk of ischemic stroke. Nat Genet 2003; 35: 131-138.

Griendling KK, FitzGerald GA. Oxidative stress and cardiovascular injury: Part I: basic mechanisms and in vivo monitoring of ROS. Circulation 2003; 108: 1912-1916.

Guzik TJ, Black E, West NE, McDonald D, Ratnatunga C, Pillai R, Channon KM. Relationship between the G894T polymorphism (Glu298Asp variant) in endothelial nitric oxide synthase and nitric oxide-mediated endothelial function in human atherosclerosis. Am J Med Genet 2001; 100: 130-137.

Hachinski V, Graffagnino C, Beaudry M, Bernier G, Buck C, Donner A, et al. Lipids and stroke; a paradox resolved. Arch Neurol 1996; 53: 303-308.

Halcox JP, Schenke WH, Zalos G, Mincemoyer R, Prasad A, Waclawiw MA, Nour KR, et al. Prognostic value of coronary vascular endothelial dysfunction. Circulation 2002; 106: 653-658.

Hassan A and Markus HS. Genetics and ischaemic stroke. Brain 2000; 123: 1784-1812.

Hassan A, Gormley K, O'Sullivan M, Knight J, Sham P, Vallance P, et al. Endothelial nitric oxide gene haplotypes and risk of cerebral small-vessel disease. Stroke 2004; 35: 654-659.

Hayakawa T, Nagai Y, Nohara E, Yamashita H, Takamura T, Abe T, et al. Variation of the fatty acid binding protein 2 gene is not associated with obesity and insulin resistance in Japanese subjects. Metabolism 1999; 48: 655-657.

Hegele RA, Harris, Hanley AJG, Sadikian S, Connelly PW, Zinman B. Genetic variation of intestinal fatty acid-binding protein associated with variation in body mass in aboriginal Canadians. J Clin Endocrinol Metab 1996; 81: 4334-4337.

Hegele RA, Wolever TM, Story JA, Connelly PW, Jenkins DJ. Intestinal fatty acid-binding protein variation associated with variation in the response of plasma lipoproteins to dietary fibre. Eur J Clin Invest 1997; 27: 857-862.

Heiss G, Sharrett AR, Barnes R, Chambless LE, Szklo M, Alzola C. Carotid atherosclerosis measured by B-mode ultrasound in populations: associations with cardiovascular risk factors in the ARIC study. American Journal of Epidemiology 1991; 134: 250-256.

Hennig B, Shasby DM, Spector AA. Exposure to fatty acid increases human low density lipoprotein transfer across cultured endothelial monolayers. Circ Res 1985; 57: 776-80

Herbert PR, Gaziano JM, Chan KS, Hennekens CH. Cholesterol-lowering with statin drugs, risk of stroke, and total mortality. An overview of randomized trials. JAMA 1997; 278: 313-321.

Hibi K, Ishigami T, Tamura K, Mizushima S, Nyui N, Fujita T, Ochiai H, Kosuge M, Watanabe Y, Yoshii Y, Kihara M, Kimura K, Ishii M, Umemura S. Endothelial nitric oxide synthase gene polymorphism and acute myocardial infarction. Hypertension 1998; 32: 521-526.

Higashi Y, Oshima T, Sasaki N, Ishioka N, Nakano Y, Ozono R, et al. Relationship between insulin resistance and endothelium dependent vascular relaxation in patients with essential hypertension. Hypertension 1997; 29: 280-285.

Hino A, Adachi H, Toyomasu K, Yoshida N, Enomoto M, Hiratsuka A, et al. Very long chain N-3 fatty acids intake and carotid atherosclerosis: an epidemiological study evaluated by ultrasonography. Atherosclerosis 2004; 176: 145-149.

Hollanders FD, Shafar J, Burton P. Serum lipids changes following the completed stroke syndrome. Postgrad Med J 1975; 51: 386-389.

Hosoi M, Nishizawa Y, Kogawa K, Kawagishi T, Konishi T, Maekawa K, et al. Angiotensin-converting enzyme gene polymorphism is associtaed with carotid arterial wall thickness in non-insulin-dependent diabetic patients. Circulation 1996; 94: 704-707.

Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science 1993; 259: 87-91.

Hsu KT, Storch J. Fatty acid transfer from liver and intestinal fatty acid-binding proteins to membranes occurs by different mechanisms. J Biol Chem 1996; 271: 13317-13323.

Huang PL, Huang Z, Mashimo H, Bloch KD, Moskowitz MA, Bevan JA, Fishman MC. Hypertension in mice lacking the gene for endothelial nitric oxide synthase. Nature 1995; 21: 239-242.

Hyson D, Rutledge JC, Berglund L. Postprandial lipemia and cardiovascular disease. Curr Atheroscler Rep 2003; 5: 437-444.

Inamoto N, Katsuya T, Kokubo Y, Mannami T, Asai T, Baba S, et al. Association of methylenetetrahydrofolate reductase gene polymorphism with carotid atherosclerosis depending on smoking status in a Japanese general population. Stroke 2003; 34: 1628-1633.

Ingall TJ, Homer D, Baker HL Jr, Kottke BA, O'Fallon WM, Whisnant JP. Predictors of intracranial carotid artery atherosclerosis. Duration of cigarette smoking and hypertension are more powerful than serum lipid levels. Arch Neurol 1991; 48: 687-691.

Inzitari D, Eliasziw M, Gates P, Sharpe BL, Chan RK, Meldrum HE, Barnett HJ. The causes and risk of stroke in patients with asymptomatic internal-carotid-artery stenosis. North American Symptomatic Carotid Endarterectomy Trial Collaborators. N Engl J Med 2000; 342: 1693-1700.

Iso H, Jacobs DR, Wentworth D, Neaton J, Cohen JD, for the MRFIT Research Group. Serum cholesterol levels and 6-year mortality from stroke in 350,977 men screened for the Multiple Risk Factor Intervention Trial. N Engl J Med 1989; 320: 904-910.

Iso H, **Rexrode K**, **Hennekens CH**, **Manson JE.** Application of computer tomography-oriented criteria for subtype classification in a prospective study. Ann Epidemiol 2000; 10: 81-87.

Iso H, Sato S, Umemura U, Kudo M, Koike K, Kitamura A, et al. Linoleic acid, other fatty acids, and the risk of stroke. Stroke 2002; 33: 2086-2093.

Ito A, Tsao PS, Adimoolam S, Kimoto M, Ogawa T, Cooke JP. Novel mechanism for endothelial dysfunction: dysregulation of dimethylarginine dimethylaminohydrolase. Circulation 1999; 99: 3092-3095.

Jeserich M, Műnzel T, Just H, Drexler H. Reduced plasma L-arginine in hypercholesterolemia. Lancet 1992; 339: 561.

Johnston SC, Gress DR, Browner WS, Sidney S. Short-term prognosis after emergency department diagnosis of TIA. JAMA 2000; 284: 2901-2906.

Jousilahti P, Rastenyte D, Tuomilehto J, Sarti C, Vartiainen E. Parental history of cardiovascular disease and risk of stroke. A prospective follow-up of 14 371 middle-aged men and women in Finland. Stroke 1997; 28: 1361-1366.

Karpe F, de Faire U, Mercuri M, Hellenius M-L, Hamsten A. Magnitude of alimentary lipemia is related to intima-media thickness of the common carotid artery in middle-aged men. Atherosclerosis 1998; 141: 307-314.

Kielstein JT, Boger RH, Bode-Boger SM, Schaffer J, Barbey M, Koch KM, Frolich JC. Asymmetric dimethylarginine plasma concentrations differ in patients with end-stage renal disease: relationship to treatment method and atherosclerotic disease. J Am Soc Nephrol 1999; 10: 594-600.

Kielstein JT, Impraim B, Simmel S, Bode-Boger SM, Tsikas D, Frolich JC, et al. Cardiovascular effects of systemic nitric oxide synthase inhibition with asymmetrical dimethylarginine in humans. Circulation 2004; 109: 172-177.

Kiely DK, Wolf PA, Cupples LA, Beiser AS, Myers RH. Familial aggregation of stroke: the Framingham Study. Stroke 1993; 24: 1366-1371.

Khaw KT. Epidemiology of stroke. J Neurol Neurosurg Phsychiatry 1996; 61: 333-38.

Kopecky SL, Gersh BJ, McGoon MD, Whisnant JP, Holmes DR Jr, Ilstrup DM, Frye RL.The natural history of lone atrial fibrillation. A population-based study over three decades. N Engl J Med 1987; 317: 669-674.

Koren-Morag N, Tanne D, Graff E, Goldbourt U. Low- and high-density lipoprotein cholesterol and ischemic cerebrovascular disease: the bezafibrate infarction prevention registry. Arch Intern Med 2002; 162: 993-999.

Kubes P, Suzuki M, Granger DN. Nitric oxide: an endogenous modulator of leukocyte adhesion. Proc Natl Acad Sci USA 1991; 88: 4651-4655.

Laloux P, Galanti L and Jamart J. Lipids in alchemic stroke subtypes. Acta Neurol belg 2004; 104: 13-19.

Lammie GA. Pathology of lacunar infarction. In: G. Dfonnan, B. Norrving, J. Bamford, and J. Bogousslavsky (eds), Subcortical stroke. 2nd edn. Oxford University Press, Oxford, UK 2002; 37-46.

Lembo G, De Luca N, Battagli C, Iovino G, Aretini A, Musicco M, et al. A common variant of endothelial nitric oxide synthase (Glu298Asp) is an independent risk factor for carotid atherosclerosis. Stroke 2001; 32: 735-740.

Li Z, Sun L, Zhang H, Liao Y, Wang D, Zhao B, et al. Elevated plasma homocysteine was associated with hemorrhagic and ischemic stroke, but methylenetetrahydrofolate reductase gene C677T polymorphism was a risk factor for thrombotic stroke: a Multicenter Case-Control Study in China. Stroke 2003; 34: 2085-2090.

Lim PS, Hung WR, Wei YH. Polymorphism in methylenetetrahydrofolate reductase gene: its impact on plasma homocysteine levels and carotid atherosclerosis in ESRD patients receiving hemodialysis. Nephron 2001; 87: 249-256.

Lin KY, Ito A, Asagami T, Tsao PS, Adimoolam S, Kimoto M, et al. Impaired nitric oxide synthase pathway in diabetes mellitus: role of asymmetric dimethylarginine and dimethylarginine dimethylaminohydrolase. Circulation 2002; 106: 987-992.

Lindenström E, Boysen G, Nyboe J. Influence of total cholesterol, high density lipoprotein cholesterol, and triglycerides on risk of cerebrovascular disease: the Copenhagen City Heart Study. BMJ 1994; 309: 11-15.

Lindgren A, Nilsson-Ehle P, Norrving B, Johansson BB. Plasma lipids and lipoproteins in subtypes of stroke. Acta Neurol Scand 1992; 86: 572-578.

LIPID: The Long-Term Intervention with Pravastatin in Ischemic Disease (LIPID) Study Group. Prevention of cardiovascular events and death with pravastatin in patients with coronary heart disease and a broad range of initial cholesterol levels. N Engl J Med 1998; 339:

1349-1357.

Lowe JB, Sacchettini JC, Laposata M, McQuillan JJ, Gordon JI. Expression of rat intestinal fatty acid-binding protein in Escherichia coli. Purification and comparison of ligand binding characteristics with that of Escherichia coli-derived rat liver fatty acid-binding protein. J Biol Chem 1987; 262: 5931-5937.

Lu TM, Ding YA, Lin SJ, Lee WS, Tai HC. Plasma levels of asymmetrical dimethylarginine and adverse cardiovascular events after percutaneous coronary intervention. Eur Heart J 2003; 24: 1912-1919.

Lundman P, Eriksson M, Schenck-Gustafsson K, Karpe F, Tornvall P. Transient triglyceridemia decreases vascular reactivity in young, healthy men without risk factors for coronary heart disease. Circulation 1997; 96: 3266-3268.

Lundman P, Eriksson MJ, Stűhlinger M, Cooke JP, Hamsten A, Tornvall P. Mild-to-moderate hypertriglyceridemia in young men is associated with endothelial dysfunction and increased plasma concentrations of asymmetric dimethylarginine. J Am Coll Cardiol 2001; 38: 111-116.

MacLeod MJ, Dahiyat MT, Cumming A, Meiklejohn D, Shaw D, St Clair D. No association between Glu/Asp polymorphism of NOS3 gene and ischemic stroke. Neurology 1999; 22; 418-420.

Maggi FM, Raselli S, Grigore L, Redaelli L, Fantappie S, Catapano AL. Lipoprotein remnants and endothelial dysfunction in the postprandial phase. J Clin Endocrinol Metab 2004; 89: 2946-50.

Marchesi S, Lupattelli G, Siepi D, Roscini AR, Vaudo G, Sinzinger H, Mannarino E. Oral L-arginine administration attenuates postprandial endothelial dysfunction in young healthy males. Clin Pharm Ther 2001; 26: 343-349.

Marsden PA, Heng HH, Scherer SW, Stewart RJ, Hall AV, Shi XM, et al. Structure and chromosomal localization of the human constitutive endothelial nitric oxide synthase gene. J Biol Chem 1993; 15: 17478-17488.

Marin C, Perez-Jimenez F, Gomez P, Delgado J, Paniagua JA, Lozano A, et al. The Ala54Thr polymorphism of the fatty acid-binding protein 2 gene is associated with a change in insulin sensitivity after a change in the type of dietary fat. Am J Clin Nutr 2005; 82: 196-200.

Markus HS, Ruigrok Y, Ali N, Powell JF. Endothelial nitric oxide synthase exon 7 polymorphism, ischemic cerebrovascular disease, and carotid atheroma. Stroke 1998; 29: 1908-1911.

Maxwell AJ, Anderson BE, Cooke JP. Nutritional therapy for peripheral arterial disease: a double-blind, placebo-controlled, randomized trial of HeartBar. Vasc Med 2000; 5: 11-19.

McAllister RJ, Fickling SA, Whitley GSJ, Vallance P. Metabolism of methylarginines by human vasculature: implications for the regulation of nitric oxide synthesis. Br J Pharmacology 1994; 112: 43-48.

McCarron MO, Delong D, Alberts MJ. APOE genotype as a risk factor for ischemic cerebrovascular disease: a meta-analysis. Neurology 1999; 53: 1308-1311.

McDonald DM, Alp NJ, Channon KM. Functional comparison of the endothelial nitric oxide synthase Glu298Asp polymorphic variants in human endothelial cells. Pharmacogenetics 2004; 14: 831-883.

Mendez I, Hachinski VC, Wolfe BM. Serum lipids after stroke. Can J Neurol sci 1987; 37: 507-511.

Miyazaki H, Matsuoka H, Cooke JP. Endogenous nitric oxide synthase inhibitor. A novel marker of atherosclerosis. Circulation 1999; 99: 1141-1146.

Moncada S, Higgs A. The L-arginine-nitric oxide pathway. N Engl J Med 1993; 329: 2002-2012.

Mori TA, Beilin LJ. Omega-3 fatty acids and inflammation. Curr Atheroscler Rep 2004; 6: 461- 477.

Mozaffarian D. Does alpha-linolenic acid intake reduce the risk of coronary heart disease? A review of the evidence. Altern Ther Health Med 2005; 11: 24-30; quiz 31, 79.

Nakayama M, Yasue H, Yoshimura M, Shimasaki Y, Kugiyama K, Ogawa H, et al. T-786-->C mutation in the 5'-flanking region of the endothelial nitric oxide synthase gene is associated with coronary spasm. Circulation 1999; 99: 2864-2870.

National Stroke Register, Sweden 2005; http://www.riks-stroke.org/files/contents.html

Ninomiya JK, L´Italien G, Criqui MH, Whyte JL, Gamst A, Chen R. Association of the metabolic syndrome with history of myocardial infarction and stroke in the Third National Health and Nutrion Examination Survey. Circulation 2004; 109: 42-46.

Ockner RK, Manning JA, Poppenhausen RB, Ho WK. A binding protein for fatty acids in cytosol of intestinal mucosa, liver, myocardium, and other tissues. Science 1972; 177: 56-58.

Oida K, Ebata K, Kanehara H, Suzuki J, Miyamori I. Effect of cilostazol on impaired vasodilatory response of the brachial artery to ischemia in smokers. J Atheroscler Thromb 2003; 10: 93-98.

O'Leary DH, Polak JF, Kronmal RA, Kittner SJ, Bond MG, Wolfson SK Jr, et al. Distribution and correlates of sonographically detected carotid artery disease in the Cardiovascular Health Study. The CHS Collaborative Research Group. Stroke 1992; 23: 1752-1760.

O'Leary DH, Polak JF, Kronmal RA, Manolio TA, Burke GL, Wolfson SK Jr. Carotid artery intima media thickness as a risk factor for myocardial infarction and stroke in older adults. New Engl J Med 1999; 349: 14-22.

Palomaki H, Kaste M, Raininko R, Salonen O, Juvela S, Sarna S. Risk factors for cervical atherosclerosis in patients with transient ischemic attack or minor ischemic stroke. Stroke 1993; 24: 970-975.

Panza JA, Quyyumi AA, Brush JE Jr, Epstein SE. Abnormal endothelium-dependent vascular relaxation in patients with essential hypertension. N Engl J Med 1990; 323: 22-7.

Paradossi U, Ciofini E, Clerico A, Botto N, Biagini A, Colombo MG. Endothelial function and carotid intima-media thickness in young healthy subjects among endothelial nitric oxide synthase Glu298-->Asp and T-786-->C polymorphisms. Stroke 2004; 35: 1305-1309.

Park JB, Schiffrin EL. Small artery remodeling is the most prevalent (earliest?) form of target organ damage in mild essential hypertension. J Hypertens 2001; 19: 921-930.

Pettersson A, Hedner T, Milsom I. Increased circulating concentrations of asymmetric dimethyl arginine (.ADMA), an endogenous inhibitor of nitric oxide synthesis, in preeclampsia. Acta Obstet Gynecol Scand 1998; 77: 808-13.

Pezzini A, Grassi M, Del Zotto E, Archetti S, Spezi R, Vergani V, et al. Cumulative effect of predisposing genotypes and their interaction with modifiable factors on the risk of ischemic stroke in young adults. Stroke 2005; 36: 533-539.

Pfohl M, Fetter M, Koch M, Barth CM, Rudiger W, Haring HU. Association between angiotensin I-converting enzyme genotypes, extracranial artery stenosis, and stroke. Atherosclerosis 1998; 140: 161-166.

Pola R, Flex A, Gaetani E, Flore R, Serricchio M, Pola P. Synergistic effect of -174 G/C polymorphism of the interleukin-6 gene promoter and 469 E/K polymorphism of the intercellular adhesion molecule-1 gene in Italian patients with history of ischemic stroke. Stroke 2003; 34: 881-885

Pratley RE, Baier L, Pan DA, Salbe AD, Storlien L, Ravussin E, Bogardus C. Effects of an Ala54Thr polymorphism in the intestinal fatty acid-binding protein on responses to dietary fat in humans. J Lipid Res 2000; 41: 2002-2008.

Prospective Studies Collaboration. Cholesterol, diastolic blood pressure, and stroke: 13,000 strokes in 450,000 people in 45 prospective. Lancet 1995; 346: 1647-1653.

Purroy F, Montaner J, Rovira A, Delgado P, Quintana M, Alvarez-Sabin J. Higher risk of further vascular events among transient ischemic attack patients with diffusion-weighted imaging acute ischemic lesions. Stroke 2004; 35: 2313-2319.

Qizilbash N, Jones L, Warlow C, Mann J. Fibrinogen and lipid concentrations as risk factors for transient ischaemic attacks and minor ischaemic strokes. BMJ 1991; 303: 605-609.

Qizilbash, N, Duffy SW, Warlow C, Mann J. Lipids are risk factors for ischaemic stroke: overview and review. Cerebrovasc Dis 1992; 2: 127-136.

Quinones MJ, Nicholas SB, Lyon CJ. Insulin resistance and the endothelium. Curr Diab Rep 2005; 5: 246-253.

Raimondi L, Lodovici M, Visioli F, Sartiani L, Cioni L, Alfarano C, et al. n-3 polyunsaturated fatty acids supplementation decreases asymmetric dimethyl arginine and arachidonate accumulation in aging spontaneously hypertensive rats. Eur J Nutr 2005; 44: 327-333.

Rector TS, Bank AJ, Mullen KA, Tschumperlin LK, Sih R, Pillai K, Kubo SH. Randomized, double-blind, placebo-controlled study of supplemental oral L-arginine in patients with heart failure. Circulation 1996; 93: 2135-2141.

Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. N Engl J Med 1997; 336: 973-979.

Ringelstein EB, Sievers C, Ecker S, Schneider PA, Otis SM. Noninvasive assessment of CO_2-

induced cerebral vasomotor response in normal individuals and patients with internal carotid artery occlusions. Stroke 1988; 19: 963-969.

Rittoo D, Cramb R, Odogwu S, Khaira H, Duddy M, Smith S, Vohra R. Worsening lipid profile is associated with progression of carotid artery stenosis. Int Angiol 2001; 20: 47-50.

Rizzoni D, Porteri E, Guelfi D, Muiesan ML, Piccoli A, Valentini U, et al. Endothelial dysfunction in small resistance arteries of patients with non-insulin-dependent diabetes mellitus. J Hypertens 2001; 19: 913-919.

Rosand J, Altshuler D. Human genome sequence variation and the search for genes influencing stroke. Stroke 2003; 34: 2512-2516.

Rosenson RS, Tangney CC. Antiatherothrombotic properties of statins: implications for cardiovascular event reduction. JAMA 1998; 279: 1643-1650.

Rothwell PM, Eliasziw M, Gutnikov SA, Fox AJ, Taylor DW, Mayberg MR, et al.; Carotid Endarterectomy Trialists' Collaboration. Analysis of pooled data from the randomised controlled trials of endarterectomy for symptomatic carotid stenosis. Lancet 2003; 361: 107-116.

Rubanyi GM. The role of the endothelium in cardiovascular homeostasis and disease. J Cardiovasc Pharmacol 1993; 22 (suppl): S1-S4.

Rubattu S, Stanzione R, Di Angelantonio E, Zanda B, Evangelista A, Tarasi D, et al. Atrial natriuretic peptide gene polymorphisms and risk of ischemic stroke in humans. Stroke 2004; 35: 814-818.

Rubins HB, Robins SJ, Collins D, Fye CL, Anderson JW, Elam MB, et al. Gemfibrozil for the secondary prevention of coronary heart disease in men with low levels of high-density lipoprotein cholesterol. Veterans Affairs High-Density Lipoprotein Cholesterol Intervention Trial Study Group. N Engl J Med 1999; 341: 410-418.

Rundek T, Elkind MS, Pittman J, Boden-Albala B, Martin S, Humphries SE. Carotid intima-media thickness is associated with allelic variants of stromelysin-1, interleukin 6, and hepatic lipase genes: the Nothern Manhattan Prospective Cohort Study. Stroke 2002; 33: 1420-23.

Ryu JE, Howard G, Craven TE, Bond G, Hagaman AP, Crouse III JR. Postprandial triglyceridemia and carotid atherosclerosis in middle-aged subjects. Stroke 1992; 23: 823- 828.

Sacco RL, Ellenberg JH, Mohr JP, Tatemichi TK, Hier DB, Price TR, Wolf PA. Infarcts of undetermined cause: the NINCDS Stroke Data Bank. Ann Neurol 1989; 25: 382-390.

Sacco RL, Benson RT, Kargman DE, Boden-Albala B, Tuck C, Lin IF, et al. High-density lipoprotein cholesterol and ischemic stroke in the elderly: the Northern Manhattan Stroke Study. JAMA 2001; 285: 2729-2735.

Saghizadeh M, Ong JM, Garvey WT, Henry RR, Kern PA. The expression of TNF alpha by human muscle. Relationship to insulin resistance. J Clin Invest 1996; 9: 1111-1116.

Sarabi M, Vessby B, Millgard J, Lind L. Endothelium-dependent vasodilation is related to the fatty acid composition of serum lipids in healthy subjects. Atherosclerosis 2001; 156: 349-55.

Scandinavian Simvastatin Survival Study Group. Randomised trial of cholesterol lowering in

4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S). Lancet 1994; 344: 1383-1389.

Schroeder F, Jolly CA, Cho TH, Frolov A. Fatty acid binding protein isoforms: structure and function. Chem Phys Lipids 1998; 92: 1-25.

Serrano NC, Casas JP, Diaz LA, Paez C, Mesa CM, Cifuentes R, et al. Endothelial NO synthase genotype and risk of preeclampsia: a multicenter case-control study. Hypertension 2004; 44: 702-707.

Simon J, Fong J, Bernert J, Browner W. Serum fatty acids and the risk of stroke. Stroke 1995; 26: 778-782.

Sharett AR, Chambless LE, Heiss G, Paton CC, Patsch W. Association of postprandial triglyceride and retinyl palmitate responses with asymptomatic carotid artery atherosclerosis in middle-aged men and women. Arterioscler Tromb Vasc Biol 1995; 15: 2122-2129.

Sharma P. Meta-analysis of the ACE gene in ischaemic stroke. J Neurol Neurosurg Psychiatry 1998; 64: 227-230.

Shin Shin Y, Baek SH, Chang KY, Park CW, Yang CW, Jin DC, et al. Relations between eNOS Glu298Asp polymorphism and progression of diabetic nephropathy. Diabetes Res Clin Pract 2004; 65: 257-65.

Shoji M, Tsutaya S, Saito R, Takamatu H, Yasujima M. Positive association of endothelial nitric oxide synthase gene polymorphism with hypertension in northern Japan. Life Sci 2000; 66: 2557-62.

Singh RB, Dubnov G, Niaz MA, Ghosh S, Singh R, Rastogi SS, et al. Effect of an Indo-Mediterranean diet on progression of coronary artery disease in high risk patients (Indo-Mediterranean Diet Heart Study): a randomised single-blind trial. Lancet 2002; 360: 1455-1461.

Sipilainen R, Uusitupa M, Heikkinen S, Rissanen A, Laakso M. Variants in the human intestinal fatty acid binding protein 2 gene in obese subjects. J Clin Endocrinol Metab 1997; 82: 2629-2632.

Slowik A, Dziedzic T, Turaj W, Pera J, Glodzik-Sobanska L, Szermer P, et al. A2 alelle of GpIIIa gene is a risk factor for stroke caused by large-vessel disease in males. Stroke 2004; 35: 1589-1593.

Stamler J, Mendelsohn ME, Amarante P, Smick D, Andon N, Davies PF, et al. N-Acetylcysteine potentiates platelet inhibition by endothelium-derived relaxing factor. Circ Res 1989; 65: 789-795.

Stamler JS, Silvka A. Biological chemistry of thiols in the vasculature and in vascular-related disease. Nutr Rev 1996; 54: 1-30.

Steer P, **Vessby B**, **Lind L.** Endothelial vasodilatory function is related to the proportions of saturated fatty acids and alpha-linolenic acid in young men, but not in women. Eur J Clin Invest 2003; 33: 390-6.

Stegmayr B, Asplund K. Diabetes as a risk factor for stroke. A population perspective. Diabetologia 1995; 38: 1061-1068.

Stuhlinger MC, Abbasi F, Chu JW, Lamendola C, McLaughlin TL, Cooke JP, et al.

Relationship between insulin resistance and an endogenous nitric oxide synthase inhibitor. JAMA 2002; 287: 1420-1426.

Stuhlinger MC, Oka RK, Graf EE, Schmolzer I, Upson BM, Kapoor O, et al. Endothelial dysfunction induced by hyperhomocyst(e)inemia: role of asymmetric dimethylarginine. Circulation 2003; 108: 933-938.

Surdacki A, Nowicki M, Sandmann J. Reduced urinary excretion of nitric oxide metabolites and increased plasma levels of asymmetrical dimethylarginine in men with essential hypertension. J Cardiovasc Pharmacol 1999; 33: 652-658.

Szolnoki Z, Havasi V, Bene J, Komlosi K, Szoke D, Somogyvari F, et al. Endothelial nitric oxide synthase gene interactions and the risk of ischaemic stroke. Acta Neurol Scand 2005; 111: 29-33.

Tahvanainen E, Molin M, Vainio S, Tiret L, Nicaud V, Farinaro E, et al. Intestinal fatty acid binding protein polymorphism at codon 54 is not associated with postprandial responses to fat and glucose tolerance tests in healthy young Europeans. Results from EARS II participants. Atherosclerosis 2000; 152: 317-325.

Tanne D, Koren-Morag N, Graff E, Goldbourt U. Blood lipids and first-ever ischemic stroke/transient ischemic attack in the Bezafibrate Infarction Prevention (BIP) Registry: high triglycerides constitute an independent risk factor. Circulation 2001; 104: 2892-2897.

Tesauro M, Thompson WC, Rogliani P, Qi L, Chaudhary PP, Moss J. Intracellular processing of endothelial nitric oxide synthase isoforms associated with differences in severity of cardiopulmonary diseases: cleavage of proteins with aspartate vs. glutamate at position 298. Proc Natl Acad Sci U S A 2000; 97: 2832-2835.

Tilvis RS, Erkinjuntti T, Sulkava R, Farkkila M, Miettinen TA. Serum lipids and fatty acids in ischemic strokes. Am Heart J 1987; 113: 615-619.

Thorvaldsen P, Asplund K, Kuulasmaa K, Rajakangas AM, Schroll M. Stroke incidence, case fatality, and mortality in the WHO MONICA project. World Health Organization Monitoring Trends and Determinants in Cardiovascular Disease. Stroke 1995; 26: 361-367.

TNT: LaRosa JC, Grundy SM, Waters DD, Shear C, Barter P, Fruchart JC, et al.; Treating to New Targets (TNT) Investigators. Intensive lipid lowering with atorvastatin in patients with stable coronary disease. N Engl J Med 2005; 352: 1425-1435.

Usui M, Matsuoka H, Miyazaki H, Ueda S, Okuda S, Imaizumi T. Increased endogenous nitric oxide synthase inhibitor in patients with congestive heart failure. Life Sci 1998; 62: 2425-2430.

Valkonen VP, Päivä H, Salonen JT, Lakka TA, Lehtimaki T, Laakso J, Laaksonen R. Risk of acute coronary events and serum concentration of asymmetrical dimethylarginine. Lancet 2001; 358: 2127-2128.

Vallance P, Collier J, Moncada S. Effects of endothelium-derived nitric oxide on peripheral arteriolar tone in man. Lancet 1989; 2: 997-1000.

Vallance P, Leone A, Calver A, Collier J, Moncada S. Endogenous dimethylarginine as an inhibitor of nitric oxide synthesis. J Cardiovasc Pharmacol 1992; 20: S60-S62.

Van Nieuwenhoven FA, Van der Vusse GJ, Glatz JF. Membrane-associated and cytoplasmic

fatty acid-binding proteins. Lipids 1996; 31 Suppl: S223-227.

Vasquez-Vivar J, Kalyanaraman B, Martasek P, Hogg N, Masters BS, Karoui H, et al. Superoxide generation by endothelial nitric oxide synthase: the influence of cofactors. Proc Natl Acad Sci U S A 1998; 95: 9220-9225.

Veerkamp JH, Maatmaan RGHJ. Structural and functional studies on different human FABP2 types. Pro Lipid Res 1995; 34: 17-52.

Vidgren HM, Sipiläinen RH, Heikkinen S, Laakso M, Uusitupa MI. Threonine allele in codon 54 of the fatty acid binding protein 2 gene does not modify the fatty acid composition of serum lipids in obese subjects. Eur J Clin Invest 1997; 27: 405-408.

Voetsch B, Benke KS, Damasceno BP, Siqueira LH, Loscalzo J. Paraoxonase 192 Gln -->Arg polymorphism: an independent risk factor for nonfatal arterial ischemic stroke among young adults. Stroke 2002; 33: 1459-1464.

Walldius G, Jungner I. Rationale for using apolipoprotein B and apolipoprotein A-I as indicators of cardiac risk and as targets for lipid-lowering therapy. Eur Heart J 2005; 26: 271-278.

Walker HA, McGing E, Fisher I, Boger RH, Bode-Boger SM, Jackson G, et al. Endothelium-dependent vasodilation is independent of the plasma L-arginine/ADMA ratio in men with stable angina: lack of effect of oral L-arginine on endothelial function, oxidative stress and exercise performance. J Am Coll Cardiol. 2001; 38: 499-505.

Wang XL, Mahaney MC, Sim AS, Wang J, Wang J, Blangero J, et al. Genetic contribution of the endothelial constitutive nitric oxide synthase gene to plasma nitric oxide levels. Arterioscler Thromb Vasc Biol 1997; 17: 3147-3153.

Wannamethee SG, Shaper GA, Ebrahim S. History of parental death from stroke or heart trouble and the risk of stroke in middle-aged men. Stroke 1996; 27: 1492-1498.

Wannamethee SG, Shaper AG, Ebrahim S. HDL-Cholesterol, total cholesterol, and the risk of stroke in middle-aged British men. Stroke 2000; 31: 1882-1888.

Whisnant JP. Modeling of risk factors for stroke: the Willis Lecture. Stroke 1997; 28: 1840-1844.

Woessner JF Jr. Matrix metalloproteinases and their inhibitors in connective tissue remodeling. FASEB J 1991; 5: 2145-2154.

Wolf PA, TR Dawber, HE Thomas, WB Kannel. Epidemological assessment of chronic atrial fibrillation and risk of stroke: The Framingham Study. Neurology 1978; 28: 973-977.

Wolfe CD. The impact of stroke. Br Med Bull 2000; 56: 275-286.

Wolff B, Braun C, Schluter C, Grabe HJ, Popowski K, Volzke H, et al. Endothelial nitric oxide synthase Glu(298)-->Asp polymorphism, carotid atherosclerosis and intima-media thickness in a general population sample. Clin Sci (Lond) 2005; 109: 475-481.

Yamada K, Yuan X, Ishiyama S, Koyama K, Ichikawa F, Koyanagi A, et al. Association between Ala54Thr substitution of the fatty acid-binding protein 2 gene with insulin resistance and intra-abdominal fat thickness in Japanese men. Diabetologia 1997; 40: 706-710.

Ye S, Eriksson P, Hamsten A, Kurkinen M, Humphries SE, Henney AM. Progression of coronary atherosclerosis is associated with a common genetic variant of the human stromelysin-1 promoter which results in reduced gene expression. J Biol Chem 1996; 271: 13055-13060.

Yoo J-H, Lee S-C. Elevated levels of plasma homocysteine ans asymmetric dimethylarginine in elderly patients with stroke. Atherosclerosis 2001; 158: 425-430.

Yoshimura M, Yasue H, Nakayama M, Shimasaki Y, Ogawa H, Kugiyama K, et al. Genetic risk factors for coronary artery spasm: significance of endothelial nitric oxide synthase gene T-786-->C and missense Glu298Asp variants. J Invest Med 2000; 48: 367-374.

Yudkin JS, Stehouwer CD, Emeis JJ, Coppack SW. C-reactive protein in healthy subjects: associations with obesity, insulin resistance, and endothelial dysfunction: a potential role for cytokines originating from adipose tissue? Arterioscler Thromb Vasc Biol 1999; 19: 972-978.

Zhang F, Lücke C, Baier LJ, Sacchettini JC, Hamilton JA. Solution structure of human intestinal fatty acid binding protein: Implications for ligand entry and exit. J Biomol NMR 1997; 9: 213-228.

Zoccali C, Benedetto FA, Maas R, Mallamaci F, Tripepi G, Malatino LS, Boger R. Asymmetric dimethylarginine (ADMA), C-reactive protein and carotid intima-media thickness in end–stage renal disease. J Am Soc Nephrol 2002; 13: 490-496.

Zock PL, Mensink RP, Harryvan J, de Vries JH, Katan MB. Fatty acids in serum cholesteryl esters as quantitative biomarkers of dietary intake in humans. Am J Epidemiol 1997; 145: 1114-1122.

Ågren JJ, Valve R, Vidgren H, Laakso M, Uusitupa M. Postprandial lipemic response is modified by the polymorphism at codon 54 of the fatty acid-binding protein 2 gene. Arterioscler Thromb Vasc Biol 1998; 18: 1606-1610.

"A man is as old as his arteries"

P.J.G. Cabanis, French philosopher and physiologist, ca. 1800