Network: Obesity and Related Disorders

Project: Polymorphism and Frequency of Obesity Candidate Genes in Stroke Patients

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Introduction

Background: Stroke is the third leading cause of death in adults in the Western world. The two principal types of stroke are ischemic and hemorrhagic stroke. Both are strongly associated with chronic arterial hypertension, diabetes mellitus, hyperlipidemia and a multitude of other vascular risk factors (see Fig. 1). However, the relationship between obesity and stroke is less clear. A recent guideline of the American Heart Association lists obesity is a potentially modifiable risk factor for stroke (Goldstein et al., 2001). In the United States an estimated 64% of all adults are either overweight or obese (National Center For Health Statistics, 1999). Obesity predisposes to cardiovascular disease in general and to stroke in particular (Mokdad et al., 2003; Hubert et al., 1983). The Honolulu Heart Study found a positive correlation between BMI and stroke risk in non-smoking men (Abott et al., 1994). Several other large-scale studies suggest abdominal obesity, rather than BMI or general obesity, as more closely related to stroke risk (Walker et al., 1996). The age-adjusted relative risk of stroke in a recently published study on abdominal obesity was 3.0 in a comparison of the extreme quintiles of waist-to-hip ratios in an ethnically and sexually diverse population in Northern Manhattan (Suk et al., 2003) The Nurses' Health Study found that in women, obesity was associated with an increased risk of ischemic stroke with increasing levels of BMI. The relative risk ranged from 1.75 for a BMI of 27 to 28.9 kg/m^2 to 2.37 for BMI of ≥32 kg/m^2. Weight gain after the age of 18 years was also related to ischemic stroke, with increasing weight associated with increasing stroke risk (Rexrode et al., 1997). Thus, recent evidence supports abdominal obesity in men and women and obesity and weight gain in women as independent risk factors for stroke.

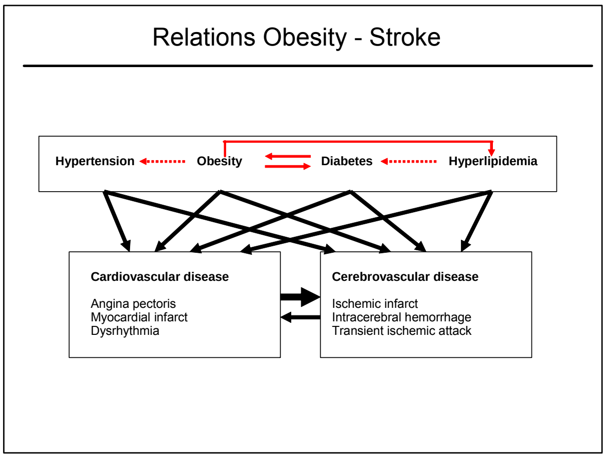

Fig 1: *The pathophysiology of cardio- und cerebrovascular disease is characterized by differential risk profiles and a variety of interdependencies.*

Genetics and stroke: Mendelian disorders associated with stroke have long been known and include metabolic disorders of inborn origin, hemoglobinopathies, dyslipo-proteinemias and cardioembolic disorders. Examples of such are sickle cell disease, cerebral autosomal dominant arteriopathy with subcortical infarct and leukoencephalopathy (CADASIL), mitochondrial disorders such as mitochondrial encephalopathy with lactic acidosis and stroke-like episodes (MELAS), familial atrial myxoma and Fabry's disease. However, all these well recognized and phenotypically distinct causes for stroke include only a small minority of stroke cases. Defining the genetic basis of common stroke, without distinct phenotypical features, has been more challenging. Family history (Kiely et al., 1993) and twins studies support the existence of genetic susceptibility to stroke (Liao et al., 1997). The risk of ischemic stroke is increased fivefold in monozygotic versus dizygotic twins with stroke (Brass et al., 1992) (see Fig. 2). Hemorrhagic stroke has a familial predilection, particularly intracranial aneurysms and subarachnoid hemorrhages (SAH). Family members of an individual with SAH are more likely to suffer from SAH than control groups (Ronkainen et al., 1997).

Candidate genes for stroke: Stroke is a heterogeneous disease of different etiologies which make it highly unlikely to find a single gene defect accounting for the disease in general. In smaller groups of patients genes involved in coagulation, such as mutations in the factor V-Leiden gene, are known to cause stroke (Ridker et al., 1995). Notch3 mutations cause another rare form of stroke, namely CADASIL (Joutel et al., 1996). The Ischemic Stroke Genetics Study is currently underway, testing the correlation between polymorphisms of β-fibrinogen, platelet glycoprotein Ia, Iba, and IIb/IIIa and ischemic stroke. This study will separate ischemic stroke types following the TOAST criteria (Meschia et al., 2003). Until recently, studies have failed to show mutations in these candidate genes that would increase stroke risk in the general stroke population (Hassan and Markus, 2000).

A genetic linkage analysis on the extensive genealogical data bank in Iceland (deCODE, an associated partner of this application) has identified a linkage between stroke and a region on the long arm of chromosome 5 (5q12) (Gretarsdottir et al., 2002). This locus was recently mapped and encodes phosphodiesterase 4D (PDE4D) that seems to be involved in the pathogenesis of carotid and cardiogenic stroke (Gretarsdottir et al., 2003) (Fig. 2). However, this finding has not been reproduced in central Europe patient populations (Bevan et al., 2005; Lohmussaar et al., 2005).

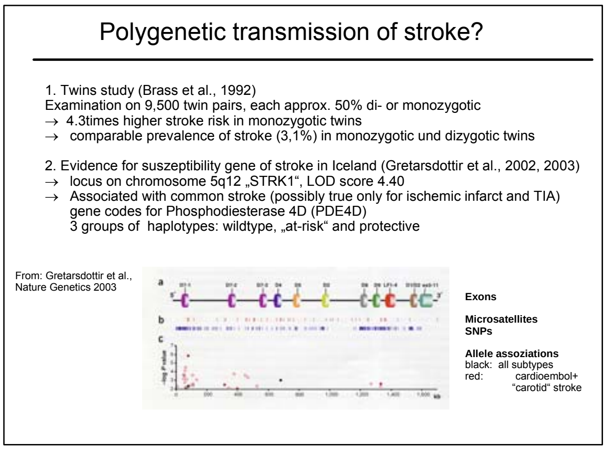

Fig 2: *Arguments for a polygenetic transmission of common stroke.*

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Current study: We investigate the role of well-defined candidate genes for obesity/leanness on stroke risk. In this respect, we rely on the work to be done in workblocks 1 and 2 of the present network. We plan to obtain blood samples of 800 to 1000 consecutive patients with ischemic stroke, intracerebral hemorrhage (ICH) or transient ischemic attacks (TIAs) who present to our Department of Neurology. Stroke and TIA is defined according to the WHO (WHO MONICA Project Principal Investigators, 1988) and classified according to the TOAST criteria (Adams et al., 1993). The obesity phenotype is obtained by measuring the body mass index (BMI) and the waist-to-hip ratio (WHR). Candidate genes will also be tested without adjustment to BMI in a large sample of stroke patients and regionally matched controls in cooperation with Prof. Berger, German Competence Network for Stroke.

In cooperation with Prof. Neumaier, Institute for Clinical Chemistry, Mannheim, blood samples will be processed (DNA extraction, storage). In close cooperation with Prof. Hebebrand/Dr. Hinney (Essen) testing for candidate genes will be performed (a) with adjustment for BMI and waist-to-hip ratio in our own patient/control cohorts and (b) without adjustment for BMI in a large cohort of stroke patients and matched controls as mentioned above. Controls will be matched for age, sex and BMI by using the KORA cohorts in cooperation with Dr. Gieger (Neuherberg). The statistical analysis will be supported by Prof. Schäfer (Marburg) by an established cooperation (see Fig. 3)

The following data will be recorded on case report forms: patient history, physical examination, CT and/or MRI of the head, complete blood cell count, casual or fasting blood glucose, prothrombin time, activated partial thromboplastin time, vital signs (height, weight, blood pressure, temperature), international normalized ratio and lipid profile. The body mass index will be calculated following the internationally standardized formular (National Institute of Health, 2003). Due to the importance of abdominal obesity as risk factor for stroke the waist-to-hip ratio will also be measured as described previously (Suk et al., 2003). Before blood sampling informed consent will be obtained. The approval by the Ethics Committee of the Medical Faculty of the Klinikum Mannheim has been successfully obtained.

Study design

Genetic association study to explore the frequency of obesity candidate genes in cerebrovascular patients

1. Characterization of the phenotype:

- Stroke subtype: application of TOAST criteria
- Measurement of BMI and WHR (abdominal obesity)
  BMI = 25 kg/m^2 = overweight, BMI = 30 kg/m^2 = obesity
- Informations on NIHSS, mRS, risk profile and life style, comorbidity, neuroimaging results, Doppler ultrasound, laboratory findings

2. Characterization of the genotype :

Testing of candidate genes of obesity/leanness and stroke

- Melanocortine-4 receptor gene polymorphism V103I (Geller et al., 2004)
- GAD2 gene (codes for glutamine acid decarboxylase) (Boutin et al., 2003)
- SLC6A14 gene (codes for Na-/Cl-dependent amino acid transporter B^{0+}) (Suviolahti et al., 2003)
- Inclusion of candidate genes of stroke, e.g., ALOX5AP gene (Lohmusaar et al. 2005)
- Further candidate genes of workblocks 1 and 2

Fig 3: *Basic characteristics of our study design.*

Project Status

We have started to recruit patients with ischemic stroke, TIA or intracerebral hemorrhage in January 2005. Until August 2005, about 150 patients have been enrolled for the study. In order to increase the patient number we are currently in the process of gaining regional partners: the neurological Department of the Municipal Hospital in Ludwigshafen as well as neurological centers in Heidelberg are possibly contributing to our project in the future. A databank has been created to document patient data for further statistical analysis. In order to reach the desired enrollment numbers, patient data and samples have to be collected over more than 24 months depending also on the contribution of the centers participating in the project. Controls are amply available both from research groups in Essen (Hebebrand/ Hinney) and in Munich/Neuherberg (KORA samples).

Outlook

We have started to collect at the same time plasma and serum probes in order to extend studies in direction of proteomics and further analysis of lipid metabolism. A contract with KORAgen (Dr Gieger, Neuherberg) for providing controls is in process. Two further neurological centers of the region (Hospital Speyrer Hof, Heidelberg, and the Municipal Hospital Ludwigshafen) have confirmed to become partners of the project. Possibly, the Dept. of Neurology of Heidelberg University will join, too.

Contribution to the goal of the Network

This study will allow a first analysis as to whether or not alleles/genotypes relevant in obesity/leanness also predispose to cerebrovascular disorders and may distinguish stroke subtypes and relevant BMI ranges.

Lit.: ***1.*** *Abott RD et al. Body mass index and thromboembolic stroke in non-smoking men in older middle age: the Honolulu Heart Program. Stroke 1994; 25:2370–2376.* ***2.*** *Adams HP Jr et al.. 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.* ***3.*** *Brass LM et al. A study of twins and stroke. Stroke 1992;23:221-223.* ***4.*** *Goldstein LB et al. AHA Scientific Statement: primary prevention stroke: a statement for healthcare professionals from the Stroke Council of the American Heart Association. Stroke. 2001;32: 280–299.* ***5.*** *Gretarsdottir S et al. Localization of a susceptibility gene for common forms of stroke to 5q12. Am J Hum Genet 2002;70:593-603.* ***6.*** *Gretarsdottir S et al. The gene encoding phosphodiesterase 4D confers risk of ischemic stroke. Nat Genet 2003;35:131-138.* ***7.*** *Hassan A, Markus HS. Genetics and ischaemic stroke. Brain 2000;123:1784-1812.* ***8.*** *Hubert HB et al.. Obesity as an independent risk factor for cardiovascular disease: a 26-year follow-up of participants in the Framingham Heart Study. Circulation 1983; 67:968-977* ***9.*** *Joutel A et al. Notch3 mutations in CADASIL, a hereditary adult-onset condition causing stroke and dementia. Nature 1996;383:707-710.* ***10.*** *Kiely DK et al.. Familial aggregation of stroke. The Framingham Study. Stroke 1993;24:1366-1371.* ***11.*** *Liao D et al. Familial history of stroke and stroke risk. The Family Heart Study. Stroke 1997;28:1908-1912.* ***12.*** *Lohmussaar E et al. ALOX5AP gene and the PDE4D gene in a central eurpoean population of stroke patients. Stroke 2005; 36:731-736.* ***13.*** *Meschia JF et al. The Ischemic Stroke Genetics Study (ISGS) Protocol. BMC Neurol 2003;3:4.* ***14.*** *Mokdad AH et al.. Prevalence of Obesity, Diabetes, and Obesity Related health Risk Factors, 2001. JAMA 2003:289;76–79.* ***15.*** *Rexrode KM et al. A prospective study of body mass index, weight change, and risk of stroke in women. JAMA. 1997; 277: 1539–1545.* ***16.*** *Ridker PM et al.. Mutation in the gene coding for coagulation factor V and the risk of myocardial infarction, stroke, and venous thrombosis in apparently healthy men. N Engl J Med. 1995; 332: 912–917.* ***17.*** *Suk SH et al.. Abdominal Obesity and Risk of Ischemic Stroke. The Northern Manhattan Stroke Study. Stroke 2003;34:1586-1592.* ***18.*** *Walker SP et al. Body size and fat distribution as predictors of stroke among US men. Am J Epidemiol. 1996; 144: 1143–1150.* ***19.*** *WHO MONICA Project Principal Investigators: The World Health Organization MONICA Project (monitoring trends in cardiovascular disease): a major international collaboration. J Clin Epidemiol 1988;41:105-11.*