Stroke Research in GenomEUtwin

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Stroke is one of the leading causes of severe disability and death in the world. In the present article we outline possibilities and limitations for future stroke research within the GenomEUtwin. The combined sample of twins born before 1958 from Denmark, Finland, and Sweden, and available for follow-up into the second millennium for non-fatal and fatal stroke events through national inpatient and death registers exceeds 70,000 twin pairs. This sample size will enable the study of genetic influences on stroke and major stroke subtypes. Large samples of twins in GenomEUtwin have been followed up repeatedly through interviews and questionnaires concerning a variety of exposures and potential risk factors for stroke. We briefly outline how this information can be combined with the health register information for epidemiologic and genetic epidemiologic studies of stroke. We also present the number of twin pairs concordant and discordant for stroke in Denmark, Finland and Sweden, and time lags between events for twins concordant for stroke. This information illustrates that the number of affected sib pairs for linkage studies is relatively limited, but the sample sizes are promising for association studies.

Stroke is one of the leading causes of severe disability and death in the world. A large number of studies have focused on non-genetic risk factors of stroke, such as sex, age, uncontrolled hypertension, diabetes, cigarette smoking, and excess alcohol consumption. A number of rare conditions inherited in a Mendelian pattern are known to be associated with an increased risk of primarily young-onset stroke, and in some disorders the responsible genes have been identified. Mutations in the NOTCH3 gene, for example, cause cerebral autosomal dominant arteriopathy (CADASIL), which leads to lacunar infarcts and vascular dementia (Joutel, 1996). It is important to realise that these disorders are only present in a minority of stroke patients. Although classical patterns of inheritance cannot be demonstrated for common cases of stroke, genetic influences of a more complex nature have been indicated by epidemiologic studies including family studies (Bromberg, 1995; Gaist, 2000; Kiely, 1993; Jousilahti, 1997; Liao, 1997; Wannamethee, 1996) and a small number of twin studies (Bak, 2002; Brass, 1992; de Faire, 1975). These findings have spurred an increasing interest in the genetics of stroke, an area of research that was recently coined "a field of needs" (Boerwinkle, 1999).

Scandinavian and other European twin registers have repeatedly collected data on twins through questionnaires and face-to-face interviews. Furthermore, Scandinavian registers have the potential to monitor their twin cohorts for fatal and non-fatal stroke events through national population-based registers. The aim of the present paper is to present ways in which the twin registers in GenomEUtwin can contribute to stroke research using the tools of genetic epidemiology, epidemiology, and molecular genetics. The components and scope of the project are described in an accompanying paper by Peltonen et al. (2003, this issue).

Family and Twin Studies of Stroke

Family studies have estimated that the relative risk of stroke in a first-degree relative of a patient who has had a stroke is between 1.5 and 2.5 (Kiely, 1993; Jousilahti, 1997; Liao, 1997; Tournier-Laserve, 2002; Wannamethee, 1996), although a minority of reported family studies were negative (Boysen, 1988; Herman, 1983). As pointed out by Hassan and Markus, ascertainment of the exact stroke subtype was a major problem in most of these inter-generational studies (Hassan, 2000).

Twin studies are considered more informative than traditional family studies, since they can disentangle the effects of genetic factors and common environment. Only three twin studies of stroke have been performed so far. In a study of stroke death based on death certificates carried out in a cohort of 10,900 Swedish twin pairs, no difference between concordance rates for stroke death was found in monozygotic compared with dizygotic pairs (de Faire, 1975). Only 19 concordant pairs were identified and follow-up was only 12 years. In a questionnaire study of 2722 pairs of United States (US) male army veterans, Brass et al. (1992) found probandwise concordance rates for stroke of 0.18 for monozygotic and 0.04 for dizygotic twins. This corresponded with a four-fold increase in the relative risk of stroke in monozygotic compared with dizygotic twin pairs.

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However, only 8 pairs were concordant for stroke in this study, which by its design was restricted to surviving self-reported cases of stroke, and hence studied concordance for stroke and survival after stroke. The aforementioned results were based on questionnaire results from 1984. In an extended follow-up of the cohort, where the subjects were 67 to 77 years of age, a total of 35 MZ pairs and 29 DZ twin pairs concordant for stroke were identified, which corresponded to a relative risk of 1.63 (95% CI 1.0–2.63) (Brass, 1996).

In a more recent study, a large cohort of twin pairs identified through the population-based Danish Twin Register was followed-up for hospital discharge diagnoses or death diagnoses of stroke through linkage with national morbidity (Inpatient Register) and mortality (Cause-of-Death) registers (Bak, 2002). Cases with subarachnoid hemorrhage were excluded. Survival analysis revealed that after adjustment for sex and age, co-twins of monozygotic twins had a double risk of stroke death and a 1.5 times increased risk of hospitalisation or death from stroke compared with co-twins of dizygotic twins. Structural equation modelling showed modest heritability effects for stroke death (0.32 (95% CI, 0.04–0.47)) and stroke hospitalisation or death (0.17 (95% CI 0.00–0.44)). None of these studies took into account twin similarity for risk factors for stroke, hence leaving open the question of whether familial aggregation in stroke is independent of familial aggregation of risk factors for stroke.

Thus, overall, twin studies seem to indicate modest genetic effects on the risk of stroke. None of the published twin studies looked at stroke subtypes, where stronger genetic effects may be at work. The risk of subarachnoid hemorrhage in first degree relatives of patients with this disorder, for instance, has been found to be four-fold to six-fold increased compared with the background population (Bromberg, 1995; Gaist, 2000). According to Woo et al. (2002), first degree relatives with intracranial haemorrhage (ICH) were reported 12 times more often in cases of lobar ICH and 6.3 times more often in cases of non-lobar ICH compared with matched controls. The results of a recent case-control study indicate that genetic influences may also vary within different types of ischaemic stroke (Jerrard-Dunne, 2003). In this study, self-reported family history of stroke before the age of 65 years was a risk factor for large-vessel disease (odds ratio 1.67 (1.08–2.66)) and for small-vessel disease (1.49 (0.94–2.37), but not for cardioembolic stroke (0.60 (0.26–1.39)) or stroke of undetermined etiology (1.11 (0.70–1.77)).

Phenotype Considerations

Phenotype considerations are central in stroke genetic research. Stroke is a syndromic diagnosis and comprises of two basic types: ischaemic stroke and the less common haemorrhagic stroke. Both of these basic types can be further subdivided into categories that are clinically meaningful since they differ with regard to treatment and prognosis. Several lines of evidence underscore that the various subtypes of stroke differ in their pathophysiology. Genetic effects may therefore show considerable variation by stroke subtype, a point that may be overlooked in studies using a broad definition of the phenotype. Several researchers have therefore argued that reducing phenotypic heterogeneity by studying specific subtypes of stroke may facilitate the discovery of genes that influence the risk of stroke (Hassan, 2000; Meschia, 2002a,b; Tournie-Laserve, 2002). This seems exemplified by a recent Japanese genome-wide linkage study that mapped intracranial aneurysm to chromosome 7q11 (Onda et al., 2001). Choice of a narrow phenotype with known underlying pathophysiological mechanisms may forge studies that enable the testing of hypotheses regarding genetic influences. This was elegantly illustrated by O'Donnell et al. (2000) who showed that apolipoprotein E genotype predicted the risk of recurrent lobar intracerebral hemorrhage, a disorder associated with cerebral amyloid angiopathy.

Attempts to address the heterogeneity of stroke by focusing on subtypes are not without limitations. Subtyping of ischaemic stroke through the TOAST classification system has frequently been used in stroke genetic research (Meschia, 2002a). This classification system requires extensive work-up of the patient and as many as 47% of subjects may not be classifiable (Figure derived from Odense Stroke Database, Bak, 2003). Furthermore, although intuitively attractive from a neurologist's point of view, the narrow phenotype approach has been called to question by the findings of a recent linkage study in Iceland. The researchers defined stroke as ischaemic stroke including transitory ischaemic attacks, and intracerebral haemorrhage, and only excluded cases of subarachnoid hemorrhage or the Icelandic type of hereditary cerebral hemorrhage with amyloidosis. A significant linkage to region of 5q12 was reported suggesting the presence of a stroke-susceptibility gene within this region (Gredasdottir et al., 2002). Replication of this finding is of course desirable before any firm conclusions can be drawn.

Another approach to reducing the heterogeneity of stroke has been to study intermediate phenotypes that represent specific components of the disease process. Ultrasonography determination of the thickness of common carotid artery intimal media thickness as a measure of early carotid atherosclerosis and carotid plaque size as a measure of advanced disease have been used as intermediate phenotypes for large artery stroke (Fox et al., 2003). Jartti et al. (2002) estimated a heritability of 36% for carotid artery intimal media thickness in male Finnish twin pairs, compared to an estimate of 24% for flow-mediated brachial arterial dilation, an early marker of atherosclerosis. Silent white matter hyperintensities (WMH) seen on T2-weighted magnetic resonance imaging may represent an intermediate phenotype for small vessel disease stroke. A single twin study investigating this potential intermediate phenotype reported a heritability estimate for WMH of 71% (Carmelli et al., 1998). Reducing heterogeneity through use of the intermediate phenotype approach decreases the number of necessary subjects, bypasses problems of survival-bias, and frequently involves measures that can be treated as quantitative traits, as illustrated by the above mentioned examples. The relationship between the intermediate phenotype and the ultimate stroke phenotype is frequently complex (Hassan & Markus,

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2000), which along with the fact that intermediate phenotypes at best only explain a minority of strokes, are disadvantages, which make this approach less attractive.

Possibilities for Future Twin Stroke Research

Genetic Epidemiological and Epidemiological Studies

Classic Twin Studies

A broad definition of the phenotype has been used in twin studies of stroke so far. Further subtype classification was probably impeded by two main obstacles: sample size issues, and logistical difficulties in ascertaining the diagnosis at a subtype level for cases occurring over large time-periods.

Issues of sample size can be overcome through international collaborations. The population-based twin registers in Sweden (Lichtenstein, 2002; Pedersen, 2002), Finland (Kaprio, 2002) and Denmark (Skytthe, 2002) are particularly attractive for this purpose because they include cohorts in age groups where sporadic stroke is a relatively frequent event. Also, in all three countries all residents have access to tax-supported medical care, which is organized in a highly comparable manner. National inpatient registers and cause-of-death registers that code diagnoses according to the International Classification of Diseases have been operational for decades in Scandinavia. The unique and permanent personal registration number, used in all Scandinavian countries, is recorded in the aforementioned registers and enables simple and correct linkage across the data sources. Thus, large cohorts of twins can be followed up for cases of fatal and non-fatal stroke by means of a fairly homogeneous detection instrument (i.e., National Inpatient and Death Registers). The feasibility of this method in stroke genetic research has already been demonstrated in the Danish Twin Registry (Bak, 2002) and the logistics of epidemiologic collaboration across the Nordic twin registries have been previously demonstrated for other phenotypes (Lichtenstein, 2000).

The number of twin pairs available for follow up in Denmark, Finland and Sweden is shown in Table 1 (with the number of twin pairs born before 1930 stated in parentheses). We limited the tables to pairs where both twins were alive at the time the health registers became functional at a national level in the respective countries (for details see footnote to Table 1). Twins born before 1930 are particularly interesting, since we expect the rate of stroke, due to age effects, to be relatively high in these cohorts in the 1990s and onwards. The results of neuroimaging, which became widely accessible during the same period, will probably enable subclassification of cases of interest.

Combining the three twin registers will increase the sample size considerably compared with the previously published results from the Danish register and will enable the further study of the influence of age and gender on stroke genetic effects. The combined sample will enable the study of genetic effects on stroke subtypes. Twin studies may thus be able to test which phenotype definition, narrow versus broad, would be the most rewarding, at least for the major subtypes of stroke, ie. ischaemic stroke and intracerebral haemorrhage. The crude MZ pairwize concordances for stroke mortality were very similar in the three countries as computable from the numbers of concordant and discordant pairs in Table 2: in Denmark 0.10, in Finland 0.10 and Sweden 0.07 despite differences in age structure and follow-up periods of the cohorts. Thus increasing follow-up is unlikely to change these estimates substantially.

Epidemiologic Studies

Large samples of twins within relevant age-groups have been followed up repeatedly through interviews or questionnaires concerning a wide range of exposures, sociodemographic information, health status, cognitive skills and so forth (Christensen, 2000; Finkel, 2003; Gaist, 2000; Haapanen, 1989; Kaprio, 2000; McClearn, 1997). Several of these surveys included information on potential risk factors for stroke. These cohorts can be utilized as individuals in traditional epidemiologic designs (e.g., case-control and cohort studies of risk factors of stroke).

Table 1

The Number of Twin Pairs Eligible for Follow-up for Stroke Through Population-based Inpatient and Cause of Death Registries in Denmark, Finland, and Sweden

	No. of eligible* twin pairs (no. pairs born before 1931)			
	Denmark†	Finland†	Sweden†	All
Death Register#				
MZ-pairs	5305 (2949)	4184 (1096)	19,770 (8500)	29,259 (12,545)
DZ-pairs§	10,727 (5542)	9257 (2400)	33,227 (15,753)	53,211 (23,695)
All pairs	16,032 (8491)	13,441 (3496)	52,997 (24,253)	82,470 (36,240)
Inpatient Register#				
MZ-pairs	4009 (1731)	4,184 (1096)	16,939 (6092)	25,132 (8919)
DZ-pairs§	8204 (3240)	9,257 (2400)	27,995 (11,263)	45,456 (16,903)
All pairs	12,213 (4971)	13,441 (3496)	44,934 (17,355)	70,588 (25,822)

Note: *Twins were considered eligible if both twins in a pair were alive at the time the register in question became operational (i.e., had complete national coverage).
†Cohorts from which twin-pairs were identified:
Denmark: 1870–1952; Finland: 1875–1957; Sweden: 1886–1958
#Complete computerised national coverage offered since the year:
Inpatient Register: Denmark: 1977; Finland: 1972; Sweden: 1987.
Death Register: Denmark: 1943; Finland: 1970; Sweden: 1959.
§Only DZ-same sex twins included.

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An informative way to study simultaneously the effect of genes and other known risk factors on stroke is to limit the analyses to pairs of twins where one twin has suffered a stroke and the co-twin is alive and free from stroke (Bak, 2002). The risk of subsequent stroke in the co-twin can then be estimated through multivariate models including information on suspected risk factors and, as a measure of genetic influences, zygosity.

The potential of these cohorts for co-twin control analyses of risk is illustrated in Tables 2–3. The numbers of pairs discordant for stroke suggest that we should have substantial power for not only co-twin analyses of all zygosities, but also of the more powerful discordant MZ pairs.

GenomEUtwin also comprises cohorts from Southern Europe (Stazi, 2002). Stroke diagnoses are only available for a minority of Italian twins at present. Once the diagnosis is ascertained in a larger proportion of the cohorts, the Italian twins will add value, enabling the comparison of data in countries within the GenomEUtwin with different distributions of disease and risk factors.

Molecular Studies

As described in the accompanying paper by van Houwelingen et al. (2003, this issue), a powerful method for identifying chromosomal regions of potential interest for phenotypes measured on a categorical scale is the affected sib-pair method. Affected sibs are identified and genome-wide scans are performed on DNA through micro-satellites in order to identify chromosomal regions linked to the phenotype. The main advantage of the method is that no candidate genes have to be identified a priori. An example of such a study is the ongoing Siblings With Ischaemic Stroke Study (SWISS), where sib-pairs with stroke are identified through the collected efforts of 50 hospital-centres (Meschia et al., 2002).

As is apparent through recently published estimates (Hassan, 2002), the sample size necessary to confidently identify stroke risk genes through affected sib-pair linkage studies depends on the predicted stroke loci and can be daunting. For instance, in order to detect significant linkage through genome wide screen (LOD score = 3.6), based on a sibling relative risk of 3.08 and a predicted total of 5 stroke loci, the number of affected sib-pairs required would be 2446. (Hassan, 2002).

Dizygotic twin pairs concordant for stroke could be used for an affected sib-pair linkage study. The practical limitations of such an approach are highlighted by the findings from the Scandinavian twin registers presented in Tables 2 and 3. For instance, over a period of 22 years of prospective monitoring of a large cohort of Danish twins through the national Inpatient Register only 45 pairs of dizygotic twins concordant for stroke were identified (Table 3). Despite our ability to identify 237 Swedish dizygotic pairs concordant for stroke from the Inpatient registry, both members of only 13 pairs were alive in 2003 and available for blood sample collection. The small sample size along with the considerable time-lag between stroke events in the concordant pairs indicate that this approach would not be attractive within a twin-register setting, even in the context of a study across the Scandinavian countries.

We believe that designs other than the affected sib-pair would be more fruitful in twin studies of stroke. Biological material has been collected from several subsamples of twins in registers participating in the GenomEUtwin study. Such samples can be used to test candidate genes (e.g., in association and follow up studies).

The main strength of twin registers compared with other currently available resources for molecular studies is the plethora of information on environmental factors. This information is available for very large population-based cohorts, often with repeated measurements and high participation rates. The incidence of stroke rises steeply with age, from 0.1–0.3 per 1000 person-years in those aged less than 45 years to 12.0–20.0 per 1000 person-years

Table 2

Number of Danish, Finnish and Swedish Twin Pairs Concordant and Discordant for Stroke Death (Subarachnoid Haemorrhage Excluded) and Time Lag Between Events in Concordant Pairs

	No. of pairs			
	Both alive or dead from other causes	Discordant for stroke	Concordant for stroke	Time-lag between stroke events in concordant twins Median (IQR)**, years
Denmark*, 1943–1993				
Monozygotic	3501	316	35	3.8 (1.7–7.5)
Dizygotic	7073	605	34	5.9 (2.8–10.2)
Finland†, 1974–2001				
Monozygotic	4037	132	15	6.4 (3.2–10.2)
Dizygotic	8968	270	19	4.1 (1.7–6.7)
Sweden‡, 1961–2000				
Monozygotic	9470	497	38	NA#
Dizygotic	18,774	988	40	NA#

Note: *To be included, both twins in a pair had to be born before 1943 and be alive in on January 1, 1943.
†To be included, both twins in a pair had to be born before 1958 and be alive on January 1, 1974.
‡To be included, both twins in a pair had to be born 1958 and be alive on January 1, 1961.
**IQR: Interquartile range.
#NA: not available at present.
Data obtained through linkage of twin registries with national cause of death registries.

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Table 3

Number of Danish and Swedish Twin Pairs Concordant and Discordant for Stroke Hospitalisation (Subarachnoid Haemorrhage Excluded) and Time Lag Between Events in Concordant Pairs

	No. of pairs			
	Both unaffected by stroke	Discordant for stroke	Concordant for stroke	Time-lag between stroke events in concordant twins Median (IQR‡), years
Denmark*, 1977–1993				
Monozygotic	3321	355	36	4.1 (1.9-6.1)
Dizygotic	7156	685	45	4.9 (1.8-9.2)
Sweden†, 1977-2000				
Monozygotic	8820	1,040	145	NA**
Dizygotic	14,431	2083	237	NA**

Note: *To be included, both twins in a pair had to be born before 1953 and be alive on January 1, 1977.
†To be included, both twins in a pair had to be born before 1958 and be alive on January 1, 1987.
‡IQR: Interquartile range.
**NA: not available at present.
Data obtained through linkage of twin registries with national inpatient registries.

in those aged 75–84 years (Feigin, 2003). The lifetime risk of stroke is high: one in five persons aged 85 will have suffered from a stroke (Bonita, 1992; Thorvaldsen et al., 1999). The age distribution of the twins included in the registers in Denmark, Finland and Sweden, indicates that large numbers of cases of stroke will be available. In the not so distant future, when the era of gene-identification will be replaced by a need to understand the interplay between genes and environment, the detailed information on environmental factors available in twin registers will be invaluable. At present, however, biological material is only available from a minority of twins. We therefore believe that efforts and resources should be directed not at sampling biological material from single affected individuals, but rather from whole cohorts of twins that can be subsequently monitored for events.

Conclusion

Data from Scandinavian twin registers hold excellent promise for epidemiologic research targeted at estimating the genetic liability to stroke through register-linkage studies, which may extend to stroke subtypes. Epidemiologic studies of potential non-genetic stroke risk factors can be addressed in case co-twin control studies. While probably less suitable for affected sib-pair linkage studies of stroke, twin registers may offer opportunities for association studies and gene-environment interaction studies, particularly if efforts are directed at extending the collection of biological material from large cohorts of twins for prospective studies.

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