Survival in families with hereditary protein C deficiency, 1820 to 1993

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Abstract

Objectives—To establish the survival of individuals heterozygous for hereditary protein C deficiency, who have an increased risk of venous thrombotic events, and to compare it with the survival of the general population.

Design—Retrospective study in pedigrees of 23 families with hereditary protein C deficiency for period 1820 and 1993.

Setting—23 completed family trees of 24 probands from various parts of the Netherlands with symptoms of protein C deficiency.

Subjects—All 736 members of the 23 families with a 50% or 100% probability of being (or having been) heterozygous for the genetic defect on the basis of DNA analysis or their place in the pedigrees, following mendelian rules.

Main outcome measures—Observed mortality compared with the mortality of the general Dutch population; the standardised mortality ratio was calculated by dividing the observed mortality by the expected mortality.

Results—No excess mortality was found in the 206 proved heterozygous individuals and "obligatory transmitters" (those who have definitely passed on the deficiency) (standardised mortality ratio 0.95 (95% confidence interval 0.5 to 1.2)) or in the 530 family members with a 50% genetic probability of heterozygosity (1.10 (0.9 to 1.3)).

Conclusion—Heterozygous individuals with hereditary protein C deficiency type I have normal survival compared with the general population. Prophylactic anticoagulant treatment may prevent thrombotic events in heterozygous individuals but may not be expected to improve their survival.

Introduction

Protein C is a natural clotting inhibitor that in its activated form can render inactive factors Va and VIIIa.^{1,2} In families with symptoms of hereditary protein C deficiency heterozygosity for the defect is associated with an increased risk of venous thrombotic events: superficial thrombophlebitis and deep vein thrombosis, which are seldom life threatening, and pulmonary embolism.³⁻⁶ Prophylactic anticoagulant treatment may prevent thrombotic events. It is becoming standard practice to give anticoagulant drugs to heterozygous people for life after recurrent episodes of thrombosis. Moreover, if people with protein C deficiency have a higher mortality than the general population then prophylactic anticoagulant treatment may improve not only reduced thrombotic events in people with heterozygosity for the deficiency but also their overall survival. Anticoagulant treatment, however, is not without risks.⁷ To investigate the effect of prophylactic anticoagulation on the survival of heterozygous people in a placebo controlled prospective study would require a long follow up and would pose ethical problems. We therefore performed a retrospective study of 1820 to 1993 in which we compared the mortality in 233 families with protein C deficiency with that in the Dutch general population. We applied the same method of investigation that we developed for

an earlier study in 10 families with hereditary antithrombin III deficiency.⁸

Subjects and methods

STUDY POPULATION

At the start of our study 80 probands with protein C deficiency type I and a history of venous thrombotic disease were known in our centre. We took a random sample of 25 probands. In 24 of these we found a mutation in one of the protein C genes. In a previous study we investigated in each family all family members who had a 50% genetic probability of being heterozygous (parents, children, and siblings of the proband; and siblings of the deficient parent).⁹ We used DNA analysis to identify heterozygous and normal individuals. We completed the family pedigrees and found common ancestry in two families. We also found that two other families already known in our centre were related to two of the 24 families. This resulted in 23 separate pedigrees, each complete with all family members who had a 50% or 100% genetic probability of being heterozygous for the genetic defect, on the basis of their place in the pedigree and following mendelian rules. Figure 1 shows the largest pedigree, derived from the families of three probands.

The dates of birth, death, and emigration were given by the family members, and when necessary we checked and completed this information using municipal population records and state archives (all births, deaths, and emigrations have by law had to be reported in the Netherlands since 1809). For the period before 1809 we also used local church registries. For 12 individuals who were born before 1820 we could find no reliable data, and we excluded their whole generation as well as earlier generations from the analysis. Six heterozygous individuals had participated anonymously in earlier studies, and we could not identify their offspring.

Many family members had been tested for the deficiency. Including them in the study implies selection of those still alive; inclusion on the basis of test results would therefore result in bias. For this reason we used as an inclusion criterion only genetic probability, regardless of later test results, except for the probands. Of all the family members with a 50% genetic probability of being heterozygous, 380 who had never been tested and 150 who had later had a negative result for the deficiency entered the analysis for the year after their year of birth, as did 53 who were later identified as deficient and who had no children. To avoid false low standardised mortality ratios caused by selection of individuals who had lived long enough to procreate, we ignored the years lived before the (possible) date of transmission of the defect for 126 individuals who had children and who were heterozygous on the basis of subsequent DNA tests or their position in the pedigree. We counted their survival from the date of birth of the first heterozygous child, or if the diagnosis in the children was unknown, the date of birth of the middle child or a date in between the births of the two middle children. We included all 27 probands of the 23 families in the study, but to avoid false low standardised mortality ratios we ignored the

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years lived by the probands before the deficiency was diagnosed. As we knew that this analysis included 150 individuals who later had normal results and 380 individuals with a prior probability of 50% who were never tested, we performed a subsequent analysis on the 206 certain heterozygous individuals (on the basis of laboratory results or on their place in the pedigree).

ANALYSIS

With the method that we used in our study in families with antithrombin III deficiency,⁸ we compared the observed mortality in the study population with the mortality in the Dutch general population, adjusted for age, sex and calendar period. The study subjects were grouped by age and sex. In each calendar period we counted the number of years lived by the subjects and the number of deaths that occurred (observed mortality). The expected mortality was calculated by multiplying the total number of years lived by the subjects in a certain calendar period by the mortality of the general population (age and sex specific) in the same calendar period.

Mortality data were obtained from the Central Bureau of Statistics. The calendar periods in our study were divided into 20 year intervals from 1820 to 1945 and into 10 year intervals from 1945 to 1993. For the calculations for each of these periods we used the mortality of the mid-interval year given by the Central Bureau of Statistics, subdivided by sex and into five year age groups. As there was uncertainty about the way the mortality data for children <1 year old were obtained and as we surmised that the reporting of infant deaths in the early years of the period under investigation may have been incomplete, we did not include the first 12 months of life. As no reliable mortality data exist for the period before 1820 we used the earliest rates available to compare the mortality of family members who lived before 1820.

We calculated the standardised mortality ratio as the observed mortality divided by the expected mortality based on the population rates. A standardised mortality ratio of <1.0 shows that the subjects on average have lived longer than the general population; a ratio of >1.0 shows excess death in the subjects. The 95% confidence interval for the standardised mortality ratio is based on a Poisson distribution for the observed number of deaths.⁹

Results

We analysed 23 families in which protein C deficiency was confirmed in the current generation by DNA analysis. Autosomally dominant inherited single mutations in the protein C genes cosegregated in each family with low protein C concentrations.⁶

The number of family members included in the general analysis varied from 9 to 151 per family; the number of generations that could be analysed varied from three to five. Years of birth ranged from 1821 to 1992, and years of death from 1822 to 1992. Eight individuals had emigrated and were excluded from analysis after the emigration date.

In all, 736 probands and family members (368 men and 368 women) with a 50% or 100% genetic probability of being heterozygous were included in the study. They lived a total number of 26 063 years after their date of entry in the analysis.

Over the whole period of 1820 to 1993 the standardised mortality ratio was 0.95 (observed mortality 145, expected mortality 152.4; 95% confidence interval 0.8 to 1.1) for all age groups combined; 0.95 (60, 63.0; 0.7 to 1.2) for women of all age groups; and 0.95 (85, 89.4; 0.8 to 1.2) for men of all age groups (fig 2).

No significant difference in mortality was apparent in the 23 families (table) or in the various age groups

FIG 1—Pedigree of family 7, the largest family, derived from families of three probands. Squares represent men, circles women. Dots indicate later testing for heterozygosity. Black symbols indicate original three probands; black and white symbols indicate certain heterozygous individuals (proved by testing or "obligatory" because of their place in pedigree); white symbols indicate possible heterozygous individuals (50% genetic probability, including individuals who later had normal test result). The first three generations were excluded from analysis because of incomplete dates of death in nine individuals

FIG 2—Mortality for 368 men and 368 women with 50% or 100% genetic probability of being heterozygous for hereditary protein C deficiency type I and for 206 individuals with 100% probability of being heterozygous, on basis of DNA analysis or their place in pedigree

(fig 3). The mortality in the youngest age group (1 to 9 years) was relatively low. This may be due to selection bias, as in genealogical studies records of individuals who lived to procreate are more likely to be found than those of children who died very young, especially in the early decades when mortality in children was relatively high. No difference in mortality was found in the calendar periods in our study (fig 4).

We found no excess mortality in 206 proved heterozygous individuals and "obligatory transmitters" (those who have definitely passed on the deficiency, proved by testing other family members): the standardised mortality ratio was 0.83 (26, 31.4; 0.5 to 1.2). In the 380 individuals with an unknown diagnosis and a 50% genetic probability of being heterozygous the standardised mortality ratio was 1.10 (118, 107.2; 0.9 to 1.3). These figures seem to confirm that limiting the analysis to tested individuals leads to a slight bias of underestimation.

FIG 3—Mortality in individuals with hereditary protein C deficiency type I, by age

FIG 4—Mortality in individuals with hereditary protein C deficiency type I, by calendar period

Discussion

We studied the overall survival in families with hereditary protein C deficiency type I. In these 23 families heterozygosity has previously been shown to be associated with a history of venous thrombotic disease.⁶ Most individuals in the study lived before anticoagulant treatment became available. A comparison of their survival with that of the general population shows whether such treatment may have a beneficial effect on overall survival.

We studied all individuals with a 50% or 100% probability of being heterozygous, on the basis of the pedigrees, regardless of later laboratory results proving them to be heterozygous or normal. This allowed us to extend the size of the study population and of the study period. Also, it reduced the bias of selecting only the heterozygous individuals who had procreated or who had survived until the time of laboratory investigation. The method implies that some of our outcomes are diluted with results of normal family members. We did not find, however, any important difference in mortality, compared with the whole study population, in the proved heterozygous individuals and obligatory transmitters or in the undiagnosed individuals with a 50% probability of being heterozygous for hereditary protein C deficiency.

We found no excess mortality, compared with the general population, in the heterozygous individuals and those with a 50% probability of being heterozygous.

The mortality in men and women was the same. No difference in mortality was apparent between any of the 23 families in the study, although they had 10 different mutations as underlying cause of the protein C deficiency (table). Only in family 17 did we find a mortality significantly higher than that of the general population. We ascribe this to chance: three brothers in this family of 19 individuals had died at a young age in road accidents.

Families with hereditary protein C deficiency where heterozygous members remain asymptomatic have been described. In such families, infants who are homozygous or doubly heterozygous for the deficiency often have life threatening thrombotic complications.¹⁰⁻¹² No such infants or families have been reported in the Netherlands to date. As the sources and the reliability of reported infant death in our families are uncertain we excluded the first year of life from our analysis. Owing to natural selection of families whose members lived to procreate and grow large family trees, we may have missed the families in which infant deaths after the first year of life, including deaths possibly caused by homozygosity or double heterozygosity, occurred more often.

If we introduced bias by selecting the families with the most severe thrombotic events in heterozygous adults, this would most likely lead to overestimation of mortality rather than to underestimation. As no excess mortality was found, this bias is unlikely. The increased risk for venous thrombotic events associated with protein C deficiency does not seem to influence overall survival. This is also true for the period before anticoagulant treatment became available. An earlier study in families with antithrombin III deficiency showed the same results.⁸

CONCLUSION

We conclude that in families with symptoms of heterozygous protein C deficiency type I, where heterozygosity for the genetic defect is associated with an increased risk of venous thrombotic events, survival of the heterozygous individuals is not significantly different from that of the general population. This implies that prophylactic anticoagulation should not in general be expected to improve survival. On an individual basis, however, prophylactic anticoagulation can be of value in trying to prevent thrombotic events such as deep vein thrombosis and pulmonary embolism, in all heterozygous individuals—with or without symptoms.

Gene mutations and standardised mortality ratios in 23 families with symptoms of hereditary protein C deficiency type I

Family No (members in pedigree)	Mutation	Standardised mortality ratio (95% confidence interval)
Family 1 (9)	¹³² Gln→stop	0.8 (0.1 to 2.9)
Family 2 (13)	¹³² Gln→stop	0.9 (0.2 to 2.6)
Family 3 (18)	¹³² Gln→stop	1.4 (0.6 to 2.5)
Family 4 (28)	¹³² Gln→stop	1.4 (0.5 to 2.8)
Family 5 (29)	¹³² Gln→stop	1.3 (0.6 to 2.6)
Family 6 (37)	¹³² Gln→stop	0.6 (0.2 to 1.5)
Family 7 (151)	³⁰⁸ Arg→stop	1.1 (0.7 to 1.5)
Family 8 (11)	²³⁰ Arg→Cys	0.9 (0.1 to 3.4)
Family 9 (16)	²³⁰ Arg→Cys	3.2 (0.1 to 17.8)
Family 10 (25)	²³⁰ Arg→Cys	0.7 (0.2 to 1.7)
Family 11 (27)	²³⁰ Arg→Cys	0.7 (0.1 to 2.0)
Family 12* (85)	²³⁰ Arg→Cys	1.5 (0.9 to 2.3)
Family 13* (131)	²³⁰ Arg→Cys	0.7 (0.4 to 1.4)
Family 14 (10)	¹⁰² Cys→Tyr	1.1 (0.03 to 6.0)
Family 15 (19)	¹⁰² Cys→Tyr	0.6 (0.2 to 1.3)
Family 16 (14)	⁷⁴ Phe→Leu	0.7 (0.1 to 2.4)
Family 17 (19)	⁷⁴ Phe→Leu	3.3 (1.2 to 7.3)
Family 18 (19)	⁷⁴ Phe→Leu	3.4 (0.4 to 12.2)
Family 19 (11)	²⁹² Gly→Ser	0.4 (0.04 to 1.5)
Family 20 (14)	¹⁷² Arg→Trp	0.7 (0.02 to 2.2)
Family 21 (16)	²⁵² Asn→Asp	1.1 (0.1 to 3.8)
Family 22 (22)	⁴⁰³ Ile→Met	0.9 (0.3 to 2.4)
Family 23 (12)	3222 G→T†	0.2 (0.0001 to 1.3)

*Combination of two families with common ancestry.

†Donor splice site 3' side of exon 5.

Key messages

- Individuals heterozygous for hereditary protein C deficiency have an increased risk of venous thrombotic events, including pulmonary embolism
- Anticoagulant prophylaxis may prevent such events, reducing morbidity
- Whether anticoagulant prophylaxis will also reduce mortality is not known
- This study shows that mortality is no higher in heterozygous individuals than in the general population
- Anticoagulant prophylaxis should not therefore be expected to improve the survival of heterozygous individuals

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Conflict of interest: None.

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Concordance rates of insulin dependent diabetes mellitus: a population based study of young Danish twins

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Abstract

Objective—To study the genetic contribution to the aetiology of insulin dependent diabetes mellitus.

Design—Historical cohort study of twins, with information on diabetes being gathered by questionnaire, verification of the diagnosis by the subject's diabetologist or general practitioner, and clinical examination in available twins.

Setting—Danish twin register and diabetic clinics and general practices throughout Denmark.

Subjects—20 888 twin pairs born during 1953-82, included in a population based nationwide register.

Main outcome measures—Crude and cumulative concordance rates and heritability in monozygotic and dizygotic twins.

Results—The crude probandwise concordance rate was 0.53 (95% confidence interval 0.33 to 0.73) for monozygotic twin pairs and 0.11 (0.05 to 0.21) for dizygotic twin pairs. When adjusted for age at onset of diabetes and age at last observation among unaffected twin partners the cumulative probandwise risk from birth to age 35 was estimated as 0.70 (0.45 to 0.95) for monozygotic twins and 0.13 (0.05 to 0.20) for dizygotic twins. The correlations of liability for monozygotic and dizygotic twin pairs were estimated as 0.96 (SE 0.09) and 0.58 (0.07), with a heritability estimate of 0.72 (0.21).

Conclusions—The risk of insulin dependent diabetes in monozygotic twins is higher than previously thought and for dizygotic twins is higher than in ordinary first degree relatives. Based on the findings of this study the genetic component to the disease seems more important than hitherto believed.

Introduction

Insulin dependent diabetes develops on the basis of genetic susceptibility interacting with environmental

factors. Research in twins represents unique opportunities for investigating the importance of genetic and non-genetic factors in the causation of insulin dependent diabetes mellitus. There are, however, difficulties with twin studies. Proband should be ascertained at random—that is, diabetic twins should be ascertained independently of the cotwin's disease status. Many twin studies have been based on ascertainment by advertising or sampling from diabetic clinics¹⁻⁶ with the inherent possibility of bias due to disproportionate sampling of concordant or monozygotic pairs, or both. Population based studies are preferred but have until now been carried out in the old twin registries in Scandinavia,^{7,8} which include many deceased twins as a consequence of study designs. The results of earlier twin studies of insulin dependent diabetes mellitus are inconsistent and in the earliest studies difficult to interpret because of the changing criteria for diagnosis and classification as well as lack of detailed clinical data.^{3,4,7,9}

In order to provide a framework for twin studies into disease aetiology we have established a new population based register of young Danish twins. This paper reports the concordance rates, recurrence risks for monozygotic and dizygotic twins, and an evaluation of the genetic contribution to insulin dependent diabetes as ascertained from the register.

Subjects and methods

TOTAL TWIN COHORT

We established a new Danish twin register comprising 20 888 twin pairs (41 776 subjects) born during 1953-82.¹⁰ The register includes 74.4% of twins born during 1953-67 and 97.4% of twins born during 1968-82. All twins who were alive and resident in Denmark in 1991 were sent a single page questionnaire asking about the presence of diabetes mellitus, their

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