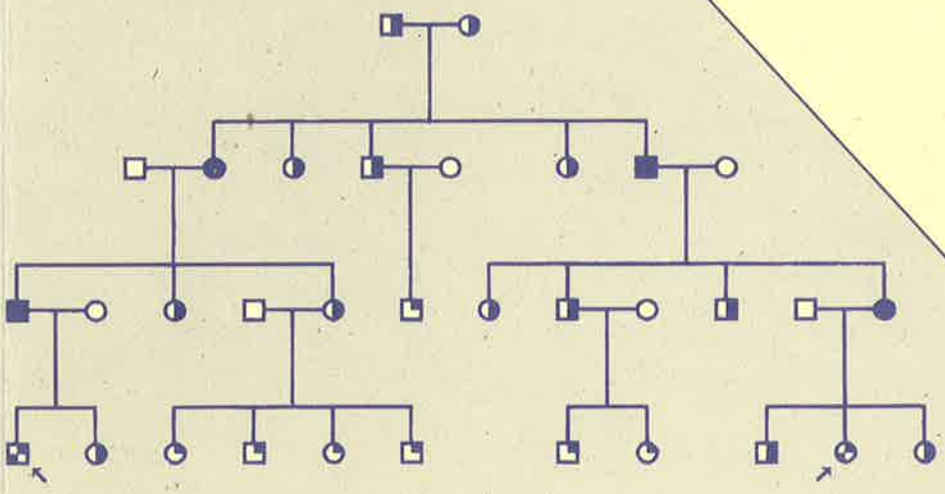
The 'Family Tree Mortality Ratio':

A Study of the Natural History of Hereditary Disorders in Past and Present



Elysée T.M. Hille

The 'Family Tree Mortality Ratio':

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Proefschrift

ter verkrijging van de graad van Doctor
aan de Rijksuniversiteit te Leiden,
op gezag van de Rector Magnificus Dr. W. A. Wagenaar,
hoogleraar in de faculteit der Sociale Wetenschappen,
volgens besluit van het College voor Promoties
te verdedigen op dinsdag 16 juni 1998
te klokke 16.15 uur

door

Elisabeth Theodora Maria Hille
geboren te Eindhoven in 1969

Promotiecommissie

Promotoren:	Prof dr J.P. Vandenbroucke
	Prof dr F.R. Rosendaal
Referent:	Prof dr M.H. Breuning
Overige leden:	Prof dr E. Briët, Universiteit van Amsterdam
	Prof dr L.P. ten Kate, Vrije Universiteit Amsterdam

The studies described in this thesis were performed at the Department of Clinical Epidemiology, Leiden University Medical Center, The Netherlands, and were financially supported by a grant from the Netherlands Organization for Scientific Research (NWO grant no 904-61-063).

Stellingen

1. Doordat de sterftecijfers in de algemene bevolking over de afgelopen eeuw gedaald zijn, is sterfte als gevolg van genetische defecten belangrijker geworden.
   Dit proefschrift

2. Dragers van het gen voor de ziekte van Huntington hebben, wat betreft levensverwachting, niet geprofiteerd van vooruitgang in de geneeskunde.
   Dit proefschrift

3. Het feit dat een genetisch defect niet altijd leidt tot een verminderde levensverwachting, onderstreept de invloed van additionele risicofactoren.
   Dit proefschrift

4. Toegenomen aandacht voor familieonderzoek kan selectiebias introduceren. Door naar generaties te stratificeren kan een overschatting van het effect op sterfte in families met een erfelijke aandoening zichtbaar gemaakt worden.
   Dit proefschrift

5. Een verhoogde plasma cholesterol concentratie beschermt tegen infecties en kanker en bevordert zo de vergrijzing.

6. De betrouwbaarheid van sterfteonderzoek in families is recht evenredig met de compleetheid der data.

7. Het idee dat de 'informed consent' van de patiënt zelf voldoende zou zijn om eventuele misstanden bij een wetenschappelijk onderzoek te voorkomen is onjuist.

Stellingen behorende bij het proefschrift
The 'Family Tree Mortality Ratio':
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8. Een "puur genetische ziekte" is een ziekte waar de omgevingsfactor nog niet van bekend is.

9. Het uitzoeken van een genealogie op familienaam is als een onderzoek van het Y-chromosoom.

10. Het vervallen van het automatisme dat een kind de familienaam van de vader krijgt, versterkt de genetische onzekerheid over het vaderschap.

11. Het abonnement op een wekelijks biologisch geteeld groentepakket verandert de hamvraag "Wat zullen we vanavond eten" in "Wat is dit en hoe maak ik het klaar".

12. Zingen bevordert de levensverwachting.
Uit: BMJ, 1996; 313: 1577-80

Leiden, 16 juni 1998
Elysée T.M. Hille

Living and Dying

As life departs, death arrives.
Three people in ten are followers of life.
Three people in ten are followers of death.
Three people in ten cling to life and follow death as a result.
Why is this so?
Because they strive excessively for life.
One who knows how to live can walk in the hills without
fear of rhinoceros or tiger.
He can go into battle without being touched by weapons.
The rhinoceros can find no place to thrust his horn.
The tiger can find no place to clasp his claws.
The weapon can find no place to penetrate.
Why is this so?
Because he has no room for death.

(Tao Te Ching: Backward down the path / Jerry O. Dalton)

Contents

General Introduction

General Introduction

The Family Tree Mortality Ratio (FTMR) method, which literally means that it measures mortality ratios in family trees, can be used to estimate changes in life expectancy in pedigrees over the past centuries. Developed at the Department of Clinical Epidemiology in Leiden to study the natural history of hereditary disorders, it allows us to determine the survival of carriers of a genetic disease even in times when the disease was unknown. With this knowledge of the "natural" life expectancy in the past, the challenge will be to estimate how much mortality of a hereditary disorder might be prevented by disease prevention strategies in the future.

Research Objectives

Our research contained two areas of investigation; firstly, the FTMR method was used to determine the impact of several (autosomal dominant) hereditary disorders on mortality, and secondly, methodological aspects of the FTMR method itself were explored. By quantifying the impact of a genetic defect on mortality, it will become clear how much excess mortality has been or can maximally be prevented with therapeutic and screening activities. This is of specific interest for diseases with an evident genetic origin, an onset in middle age, and a progressive clinical course.

Outline of This Thesis

Chapter I describes the background that led to the development of the FTMR method. The FTMR technique and its opportunities are explained, as well as the interpretation of the results obtained and possible uncertainties. We also examined the advantages and disadvantages of various control populations. The general Dutch population turned out to be the ultimate comparison group, and was used in the investigation of the first research objective. In the remaining chapters, five different autosomal dominant hereditary disorders on mortality are described.

Chapter II is about mortality in families with Huntington's disease, and acts as a "positive control" for the FTMR method, because in this monogenetic disorder with almost complete penetrance there was a known deleterious effect of the genetic defect on mortality. Chapter III extends the study of the FTMR method by including analysis of the causes of death in parents of patients with resistance to activated protein C (the factor V Leiden mutation). These patients were unselected consecutive cases with a first episode of venous thrombosis, who carried this recently discovered, widespread genetic cause of thrombosis. In chapter IV we investigated mortality in one extended apolipoprotein E3-Leiden pedigree associated with familial dysbetalipoproteinemia. Because this underlying genetic defect is due to a remarkable insertion of seven amino acids in the APOE gene, the pedigree could be traced back to 1718. Chapters V and VI deal with hereditary cancer syndromes. Causes of death in families with the familial atypical multiple mole-melanoma syndrome, all showing the same 19 base pair deletion in the CDKN2 gene, are discussed in chapter V. In the last chapter, in contrast, pedigrees with different underlying genetic defects leading to autosomal dominant expression of breast or ovarian cancer are studied, even though breast cancer is a common disease among Caucasian women. This suggests that the FTMR method proved feasible to estimate the impact of an underlying hereditary risk based on life expectancy in the past for various autosomal dominant hereditary disorders.

Chapter I

A Method to Assess the Impact of Genetic Influences on Mortality in Families

Elysée T.M. Hille, Jan P. Vandenbroucke, Frits R. Rosendaal
Submitted for publication

Measuring Prognosis in Families

Why

Over the next decade, medicine will face an explosion of "gene-disease" associations. Especially as a consequence of the Human Genome Project, the construction of a genetic and physical map of the human genome and the sequencing of human genes will expand enormously [1-4]. The genome will become sprinkled with easy to identify markers at short distances from one other. Linkage analysis of these markers with a variety of human diseases will be greatly facilitated. The next problem will be to translate this genetic technology and the forthcoming knowledge of biological processes into disease prevention strategies [5-8]. Assessing how much disease is due to the carriage of a particular gene will help determine whether preventive or therapeutic action is warranted.

One candidate method to meet this challenge from an epidemiologic point of view is the application of the technique of calculating observed and expected disease experience in family trees. This method of indirect standardization has been used in the past to investigate whether a disease is familial. Examples of this kind of studies can be found throughout the literature, e.g. studies of families of patients with breast cancer [9-12] and Hodgkin's disease [13] . Here, we have used the natural history of family members to estimate for any hereditary disease the excess mortality, due to genetic components, by restricting the analysis to certain and highly probable gene carriers. The results show how much mortality might maximally be prevented by screening and intervention, if screening and intervention were completely effective.

The First Application: Therapeutic Dilemma of Inherited Thrombophilia

Some dramatic family histories with several members developing lethal thrombosis at young ages led some clinicians to propose that all relatives of affected persons ought to be screened so that all people who shared the genetic defect might be treated with anticoagulation for life, whether or not they had

previously had a thrombotic episode [14-16]. Others resisted this idea and pointed to the small but definite risk of the preventive therapy itself [17]. Long-term anticoagulation has a risk of severe bleeding of about 3 per 100 person-years and a risk of death of about 2 to 6 per 1000 person-years [18-21]. If such a therapy is to be instituted, we should first be certain that the risk of gene carriership is higher than the risk of complications from anticoagulation. A standard method to resolve problems of therapeutic uncertainty, the randomized controlled trial, is not feasible: the number of identified families is very small and, although markedly increased, the thrombotic episodes remain infrequent.

The maximal achievable benefit of prevention by anticoagulant treatment of asymptomatic carriers might be estimated as the surplus mortality associated with the carriage of the gene in untreated persons, if anticoagulation was to prevent all episodes of thrombosis and has no side effects. Next, we realized that the presence of an elevated mortality might be quantified retrospectively by comparing the mortality in the families of the affected persons, in particular among the family members who were likely carriers of the gene, with the expected mortality of their contemporaries in the general population. Hence, since this approach is in effect a standardized mortality ratio for families, we have called it the Family Tree Mortality Ratio (FTMR).

Family Tree Mortality Ratio

FTMR technique

With the FTMR method, pedigrees are constructed to extend the number of carriers into the previous generations. The mode of reasoning can be followed in figure 1. The upper part denotes a hypothetical pedigree of a family in which an autosomal dominant defect is diagnosed in two index cases A and B. They are proven carriers, who appeared to be two second cousins. Supposing this is a rare genetic defect, we can be certain that A's father and paternal grandmother have

been affected, as well as B's mother and maternal grandfather, since they transmitted the same allele from the common great-grandparents. All persons who must have transmitted the allele to the next generation can be defined as obligate carriers. Furthermore, all siblings and offspring of proven and obligate carriers have a 50 percent probability of gene carriership, which also holds for the pair of common ancestors at the top of the family tree; we call all these individuals potential carriers. In this way, Mendelian probabilities can be assigned to all individuals in the pedigree: all second degree relatives of proven and obligate carriers have a 25 percent probability of gene carriership. The lower part of figure 1 shows the hypothetical pedigree with the probabilities of gene carriership for all family members. From this stylized pedigree, it is clear that a few index cases are the source of a much larger number of obligate or potential carriers extending to many generations in the past.

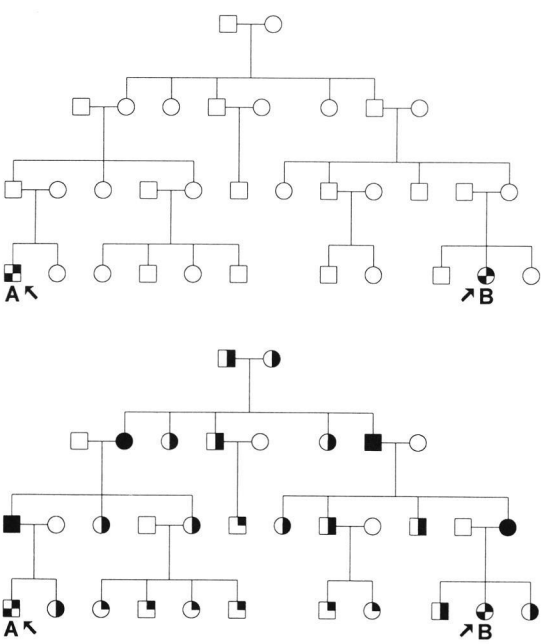


Figure 1. ***Hypothetical pedigree.*** *The upper part represents the family after identification of the two second cousins, A and B, by DNA-testing. No other family members were tested. The lower part represents the family after allocation of the probabilities of gene carriership. The closed individuals are obligate gene carriers, because they transmitted the gene from the common great-grandparents to A and B. One of these great-grandparents also carried the gene, so each of them has a probability of 50% of being affected (potential carriers), as well as all siblings and children of affected individuals. All children of potential carriers have a probability of 25% of being affected.*

Data Collection

The prerequisite of the method of data collection is the ability to complete pedigrees with at least two affected persons branching out to all relatives with some predefined probability for gene carriership. Of all these persons the date of birth and date of death, or survival up to a common date, has to become known. This is greatly facilitated by the existence of accessible genealogical and population registries. In The Netherlands all births, marriages and deaths must be reported by law since 1811 in municipal population records and State archives. For the period before 1811 parish registers can be used. In most cases verification is necessary, because pedigrees drawn for clinical or molecular research purposes may be incomplete; especially individuals who have died may be missing. Comparative mortality rates from the general population of the same age and sex over the centuries can be obtained from official vital statistics publications.

In general, the follow-up extends from the date of birth to the date of death or a common end-of-study date. The follow-up is slightly different, however, for probands and the obligate carriers. The members of the pedigree who have secured the transmission of the gene to further generations must have survived until reproductive age. Therefore, follow-up of the obligate carriers before the birth of the affected child should not be taken into account. Furthermore, the pedigrees could be constructed only because the probands had been recognized. Consequently, follow-up of probands before diagnosis should be ignored for the analysis. A simplified solution to avoid this "reproduction bias" is to limit all comparisons to mortality from age 20 onwards. An additional advantage of both types of "left truncation" is that early childhood mortality is likely to be the most unreliable figure in the vital statistics of the past.

Statistical Analysis

When the follow-up of all relatives with at least a predefined probability of gene carriership has been completed, the observed mortality in the study population can be compared with the expected mortality from the

contemporary general population of the same age and sex. This classical epidemiologic technique consists of calculating the mortality that would have occurred in the study population if the mortality rates of the general population had prevailed. The mortality exposure of proven and obligate carriers gives a direct effect estimation, while that of potential carriers will yield an effect which is diluted by a known amount of misclassification, i.e. on average 50 percent. The method allows us to estimate the mortality of the cohort for different ages, different sexes and different calendar times, as well as for family subgroups.

Continuation of Inherited Thrombophilia Example: Antithrombin

The FTMR method was first applied to antithrombin deficiency [22], which was suitable for the FTMR method for several reasons. First, the disease is certainly not so lethal as to preclude procreation. Second, it is highly plausible that the allele was already present in the previous generations of the pedigree. Third, all individuals who inherit the genetic defect show the biochemical abnormality, with a clear-cut difference between normal individuals and carriers of the abnormality, although it is uncertain how many affected individuals will eventually develop thrombosis. In 10 families with at least 2 antithrombin deficient individuals the FTMR method yielded information on the survival of 171 proven, obligate, and potential carriers from 1830 onwards (i.e. a time when the disease was not known and no treatment for thrombosis was available) and for a total of more than 8,000 person-years. The mortality was compared with the expected in the general population. A total of 56 deaths was observed, whereas 58.8 deaths were expected (SMR 1.0; 95% CI 0.7-1.2). Whatever subdivision we used in our analysis of the antithrombin families, the relative risk remained around unity [22]. This indicates that a policy of extensive screening of family members has no priority, since life-long treatment with anticoagulants in symptom-free antithrombin deficient individuals is unlikely to improve survival over and beyond that of the general population, and may well have adverse effects due to bleeding [22,23].

Possibilities of the Family Tree Mortality Ratio

Overall Rate Ratios

One way to present the observed and expected number of deaths is as a standardized mortality ratio (SMR). This SMR is a relative risk, i.e. the ratio of two incidence rates [24]. When we analyze family data with the FTMR method, we normally start calculating SMRs for the various sexes, age groups, calendar periods, and families. A SMR of 1 means that the mortality in the index population and the standard population are equal. If the SMR is greater than 1 there is excess mortality in the index population, and if the SMR is less than 1 the index population lives longer than the standard population. Confidence limits for the SMR are based on a Poisson distribution for the observed number of deaths [24].

Secular Trend of Absolute Risks

To determine the evolution of mortality over the study period, we calculated the absolute observed and expected mortality rates per 1,000 person-years, adjusted for differences in age distribution during the calendar time. We used the age distribution of the index population over the whole study period as an external standard for age standardization. In this way a secular trend of mortality during the century can be followed, e.g. in the age group with the greatest impact of the involved gene. Changes occurring over time, such as the identification of the disease or availability of treatment or screening, can be evaluated. The risk difference (absolute difference of the two incidence rates) between the index population and the standard population over the calendar periods is important, because it gives insight in what happened over calendar time. If, for example, the mortality rates associated with a genetic disease are constant between 1900 and 1990, and the population mortality rates go down over time, this means that, both the relative risk and the risk difference will increase: by and large, the disease has a larger impact. In the "interpretation of results" examples of different scenarios will be presented.

Causes of Death

In addition to observed and expected overall mortality, deaths from specific causes can be studied. Information about the cause of death can be obtained from the patient record, the family or from the death certificate. However, since our main interest is the comparison between causes of death in the general population *versus* the families, only the use of the information on the death certificates for both groups guarantees a valid comparison as the classification was the same for the study cohort and the general population. There may be some misclassification, which will be similar, however, in both groups. The availability of death certificates differs among countries. In the Netherlands a copy of each death certificate is kept at the Central Bureau of Statistics. The causes of death are routinely coded by this Bureau using the coding rules from the International Classification of Diseases [25-29].

Statistical Extensions

More sophisticated modeling, such as Poisson or Cox regression models may be used to make comparisons between families, probabilities of gene carriership, or line of inheritance, and to adjust for other risk factors than sex, age and calendar time. If the general population is the reference group, as will normally be the case with the standardized mortality ratio, observed and expected mortality can best be estimated with the Poisson model. All age groups, calendar periods, confounders, and risk factors have to be included in the regression model. However, if a reference group has been defined within the study population, a Cox regression model will give a more accurate estimate of the risk parameters. Instead of different age groups and calendar periods, the individuals birth date and age or date at entry are the factors to adjust for (both as continuous variables). In theory, both models should give the same risk estimates. The difference is that in a Poisson model the basis is a particular combination of age group and calendar period, whereas in a Cox model the basis is an individual, which in general is easier to understand. Furthermore, as in the Poisson model age groups and calendar periods are fitted, the model will sooner become unstable because of the many categorical variables.

Interpretation of Results

Examples: Choice of Different Patterns

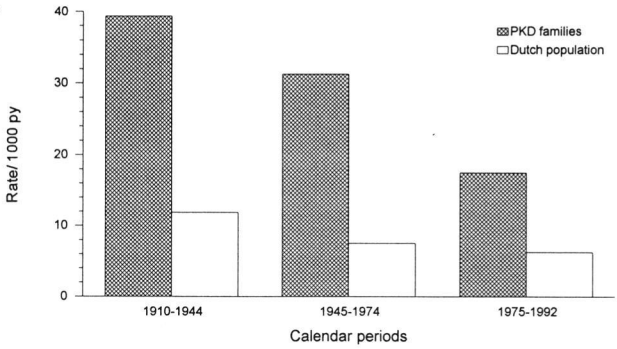
Until now, we have used the FTMR method for a range of genetic diseases. These disorders differ in mean age at onset and severity of the disease. They have an autosomal dominant inheritance pattern in common, and do not lead to excessive mortality in childhood. We studied polycystic kidney disease [32], inherited thrombophilia (antithrombin deficiency [22,23], protein C deficiency [30], and activated protein C resistance [31]), familial dysbetalipoproteinemia [34], familial hypercholesterolaemia [35], familial atypical multiple mole-melanoma [33], hereditary breast-ovarian cancer [36], and Huntington's disease [37]. Comparing the overall mortality patterns in these various diseases makes clear how secular trends vary when preventive and therapeutic strategies are different. In figures 2a-d and figures 3a-d the extremes are shown. For each disease separately, the age groups with the greatest impact of the gene were analyzed for different calendar periods. Next, in order to compare the mortality rates of the calendar periods, both families and general population were directly standardized to the age distribution of the families over the whole calendar time as the external standard population.

Polycystic Kidney Disease

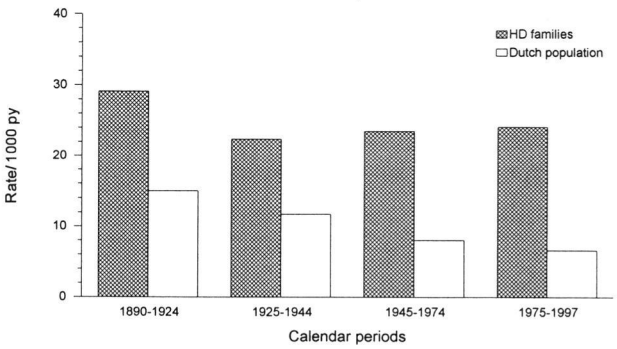
Figures 2a and 3a have been adaped from the study of Florijn *et al* [32]. He investigated families with autosomal dominant polycystic kidney disease (PKD) to estimate how much the life expectancy has improved due to the introduction of antibiotics, antihypertensive therapy, and renal transplant therapy. Compared to the general Dutch population, there was a much more pronounced decline in mortality rates among the PKD patients, especially after the 1970s (Figure 2a), although total mortality remained more than twofold higher than the general population figures (Figure 3a) [32].

Figure 2. ***Absolute evolution of mortality in affected families compared with the general Dutch population.***

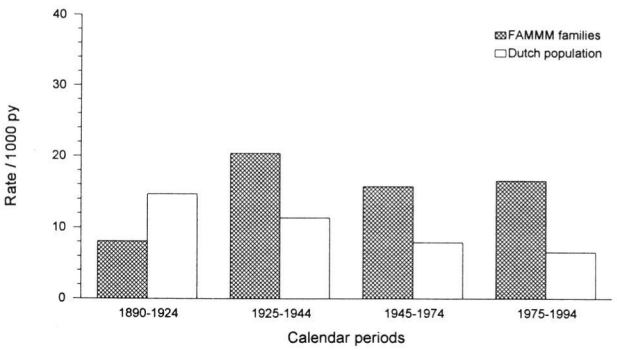
2a) Absolute mortality rates per 1000 person-years in families with polycystic kidney disease (PKD) and in the Dutch population between 50 and 60 years of age[32].



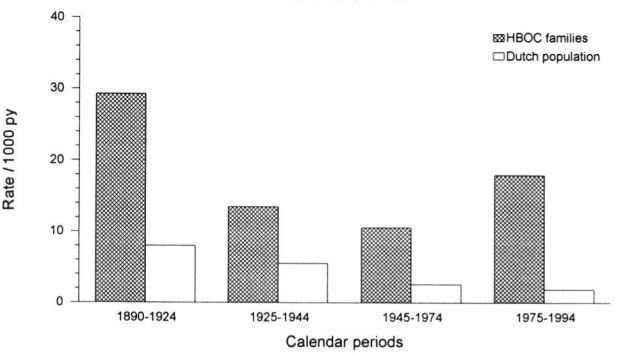
2b) Absolute mortality rates per 1000 person-years in families with Huntington's disease (HD) and in the Dutch population between 40 and 70 years of age[37].



2c) Absolute mortality rates per 1000 person-years in families with the familial atypical multiple mole-melanoma (FAMMM) syndrome and in the Dutch population between 40 and 70 years of age[33].



2d) Absolute mortality rates per 1000 person-years in families with hereditary breast-ovarian cancer (HBOC) and in the Dutch population between 30 and 60 years of age[36].



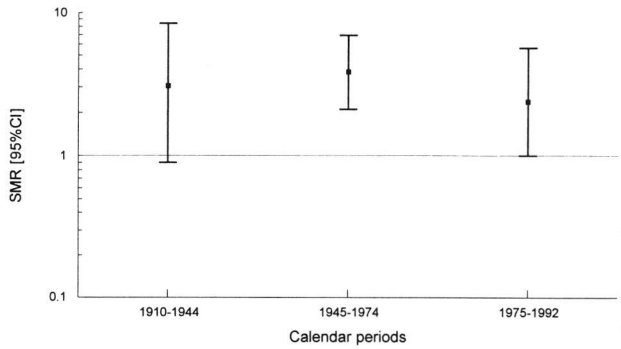
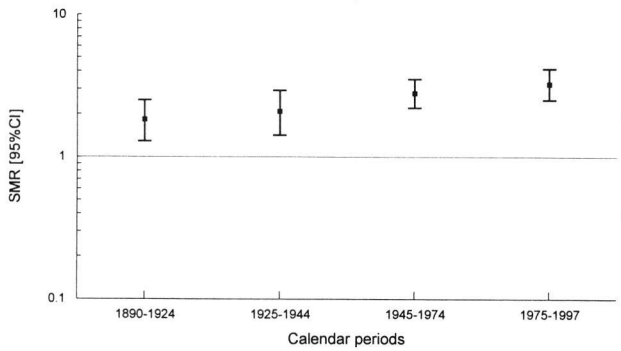
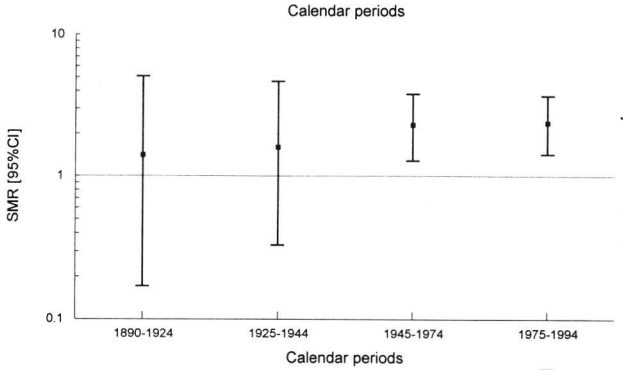


Figure 3. ***Relative evolution of mortality in affected families compared with the general Dutch population.***

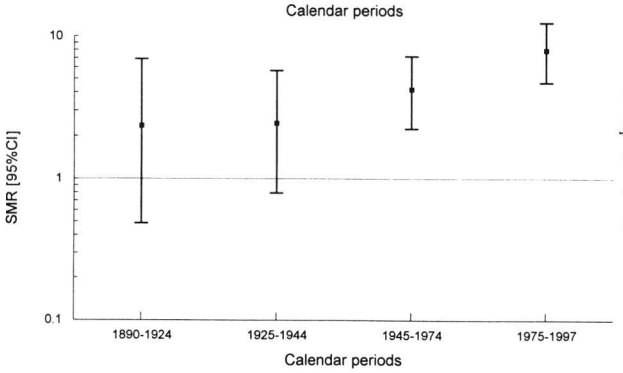
3a) SMRs with 95% CI in families with polycystic kidney disease compared with the Dutch population between 50 and 60 years of age [32].



3b) SMRs with 95% CI in families with Huntington's disease compared with the Dutch population between 40 and 70 years of age [37].



3c) SMRs with 95% CI in families with the familial atypical multiple mole-melanoma syndrome compared with the Dutch population between 40 and 70 years of age [33].



3d) SMRs with 95% CI in families with hereditary breast-ovarian cancer compared with the Dutch population between 30 and 60 years of age [36].

Huntington's Disease

Figure 2b is an example of the evolution of overall mortality in patients with Huntington's disease (HD), who did not seem to benefit from any advances in medical care. Total mortality rates of the general Dutch population have continuously declined over the years, but among the members of families with Huntington's disease this decline was absent. This phenomenon implies that the impact of the HD gene is rising, which is shown by the increasing SMR (Figure 3b) [37]. It is still too early to expect to see an effect of DNA-testing in these families, which is available since the 1980s.

Familial Atypical Multiple Mole-Melanoma

The third example of a secular trend is shown in figure 2c. Individuals with a deletion in the tumor suppressor gene p16 did not seem to have excess mortality compared to the general Dutch population in the previous century. Since 1925 however, the mortality rates have been higher than those in the population and have not declined, and because of this, the SMR has doubled (Figure 3c). The appearance of excess mortality is likely to be the result of new exposure to an exogenous risk factor, e.g. sunbathing, to which individuals with the p16 deletion are more susceptible than others in the general population. As a result of extensive follow-up programmes for deletion carriers since the 1980s, we expect that the excess mortality in these families with the familial atypical multiple mole-melanoma syndrome will decrease during the next decade [33].

Hereditary Breast-Ovarian Cancer

In figures 2d and 3d two effects seem to be going on. Around 1900 the mortality rates of women in families with hereditary breast-ovarian cancer were very high. During the twentieth century these rates rapidly declined, although relative mortality compared to women in the general Dutch population remained high. In the last twenty years the rates have increased again, which may suggest that women in these families were more susceptible to changing exogenous risk factors for breast cancer than other women in the population; one may think of the number of children or contraception use. For several

reasons detailed in Chapter VI, the recent increase may, at least in part, be the result of selection bias (ascertainment bias) in the most recent generations. This means that the mortality peak in the last calendar period is likely to be an overestimation of the true effect, due to selection in the most recent generations [36].

So, the FTMR method is capable of showing whether excess mortality is confined to certain age groups, and whether the impact of a hereditary defect has changed over the calendar time. The evolution of mortality over calendar time depends on preventive and therapeutic possibilities and changes in exogenous risk factors. In understanding the changing mortality patterns and their impact on the population, both relative risks and the differences of absolute risks need to be taken into account.

Options for the Comparison Group

General Population

The basic idea of the FTMR was the SMR analysis, i.e. indirect standardization of the age specific mortality rates of the standard population applied to the corresponding age distribution of the index population. As our goal is to determine how much the life span in the families under investigation differ from that in the rest of the population, the best possible comparison group is that same general population. Other advantages of using the general population as the comparison group are the availability of mortality rates from official vital statistics publications [38] and the size of the comparison population which leads to a negligible standard error for the expected mortality rates. On the other hand, it is plausible that being a member of a still existing family implies a smaller intrinsic risk of dying, because the population figures also include individuals from extinct families. If such is the case, the families will be, on average, a healthier cohort than the general population. If in comparison with

the general population, the mortality in the families would turn out to be equal, an exsisting excess mortality would never be noticed.

Spouse Controls

A comparison group that seems to overcome part of the problem of the healthy cohort effect are spouse controls. Spouse controls are spouses of family members taken from each generation throughout the pedigrees. Since they are as much part of the pedigree as the index cases, the comparison is fair. Another advantage of using spouse controls is that they come from the same region and are likely to have, on average, the same socioeconomic status. Furthermore, when completing data from family members, the information about their spouses can easily be obtained. A possible bias, however, is that married individuals have a higher life expectancy than single persons. Spouses are, by definition married, but the pedigrees also contain unmarried family members. In its purest form, therefore, the analysis should be restricted to index-spouse control pairs and exclude individuals without spouses.

Families of Spouses

In theory, using families of spouses as the comparison group seems the best of both worlds: they often have the same social background, live in the same region and consist of complete families, including unmarried individuals. By family of spouse we refer to the family of the spouse of the proband. One disadvantage, however, is that this is the most time consuming method, as now not only do we need complete families with the hereditary disease of interest, but also as many complete families of the spouses. For both methods of spouse controls, the standard error for the mortality rates of this comparison group are no longer negligible. When environment and socioeconomic status appear to be important confounders, it may be worthwhile to collect data on families of spouses.

Control Families

A fourth possibility is to assemble a pool of control families, that can be used as

a comparison group for families, irrespective of the genetic disease of interest. We will present data of control families and their spouse controls in this review.

Family Members with Small Probability of Carriership

More sophisticated modeling might be used to make comparisons within pedigrees between persons with different gene carrier probabilities, extending to 25 or 12.5 percent. The latter might yield an internal control group, since these "controls" will share fewer environmental characteristics the more genetically remote they are. Although in theory this approach might give the most optimal control population, it would be very time consuming to complete families for all second and third degree relatives. One has to decide in advance what will be the benefit from comparing with internal controls rather than with the general population.

Comparing Comparison Groups

We have explained that there are various possiblities for the comparison group, which is needed to estimate the expected mortality in the hereditary diseases of interest. Since our hypothesis is that the impact of a gene on mortality can be quantified by the reduction in life expectancy in the families under investigation against the life expectancy of their contemporaries, the comparison with the general population is preferred. The healthy family effect may, however, bias the estimates of the SMR. Estimations from control families can provide the evaluation of a correction factor assessing this healthy cohort effect.

Subjects and Methods

Study population

We compiled three types of control groups: two types of families and spouse controls. For the first type of control families, we collected six published

genealogies from the Central Bureau of Genealogy in the Netherlands, constructed by amateur genealogists, and in which both male and female descendants had been traced. In these complete genealogies, we started to look at the most recent adult generation. Next we searched for the great-great-grandparents of the most informative kindred (four generations backwards). This couple became the top of the control family (in all six pedigrees these ancestors were born in the first quarter of the nineteenth century). Starting with this common ancestor pair, we registered data on birth, death, and gender for all family members and spouses descending from this ancestor pair. We accepted the information collected by the genealogists as correct and complete.

A second type of control families was constructed. In September 1993 we randomly selected ten individuals from the Dutch phone book. For these persons we tried to trace the great-grandparents in the male line of inheritance through municipal registers. In four cases the tracing was broken off, because either the ancestors had never lived in the Netherlands, the parents could not be traced, or the local authorities could not cooperate. The other individuals yielded six common great-grandparent pairs, all born in the first quarter of the nineteenth century. Using parish records, municipal registers and national archives, we traced the dates of birth and death for all offspring, including spouses, of these common great-grandparents and stored the information into a database.

The third type of control group were the spouses of all twelve control families. The spouses of the genealogist families we called as 'spouse G' and the spouses of the phone book families we called as 'spouse P'.

Statistical analysis

The data were analyzed with the Family Tree Mortality Ratio method [22,31]: the overall mortality of the study population (observed) was compared with that of the Dutch general population (expected). The expected mortality was calculated by multiplying the total number of years lived by the study population with the

sex, age, and calendar period specific population mortality rates from the annual reports of the Netherlands Central Bureau of Statistics, using the computer program 'Person-Years' [39]. Confidence limits for the SMR are based on a Poisson distribution for the observed number of deaths [24]. The calendar periods were divided into a fifty-year interval from 1800 through 1849, twenty-year intervals from 1850 through 1889, a fifteen-year interval from 1890 through 1904, and into ten-year intervals from 1905 through 1997. To each of these periods we applied the population mortality rates of the mid-interval year, subdivided by sex and into five-year age groups.

Follow-up for all individuals extended from twenty years after the date of birth to the date of death or to December 31, 1997. While the main issue is the choice of an appropriate comparison group for families with autosomal dominant disorders, we directly compared descendants of phone book families and descendants of families from genealogists with the spouse controls in a Cox regression model adjusted for gender and year of birth.

Results on Different Control Groups

Of the six genealogists families, 599 relatives were analyzed over 13,637 person-years and 252 spouses over 8,050 person-years, from 20 years of age onward. In addition, of the six phone book families, 639 relatives were analyzed over 17,976 person-years and 351 spouses over 12,857 person-years, from 20 years of age onward. In table 1 the observed and expected number of deaths are shown for all control families and spouse groups separately. Fam 1 through 6 are the genealogists families with their spouse control group (spouse G), and fam 7 through 12 are the phone book families with their spouse control group (spouse P). The overall SMR for the control families did not differ from unity, although in half of the families from genealogists and in one phone book family there was excess mortality. In both groups of spouse controls the number of deaths was as expected. There was no difference between men and women in either control families or spouse controls.

Table 1. Characteristics and observed and expected number of deaths for all control families separately and spouse groups, 20 years of age onward

	Number in analysis	Observed deaths	Expected deaths	SMR [95% CI]	Person-years
Genealogists families	599	120	86.74	1.38 [1.15-1.65]	13,637
Fam 1	123	20	16.84	1.19 [0.73-1.83]	2,687
Fam 2	90	15	16.31	0.92 [0.52-1.52]	2,099
Fam 3	105	27	16.77	1.61 [1.06-2.34]	2,741
Fam 4	32	15	8.32	1.80 [1.01-2.97]	893
Fam 5	37	25	10.26	2.44 [1.58-3.60]	1,268
Fam 6	212	18	18.23	0.99 [0.59-1.56]	3,949
Spouses G	252	56	47.62	1.18 [0.89-1.53]	8,050
Phone book families	639	154	159.17	0.97 [0.82-1.13]	17,976
Fam 7	195	52	67.11	0.78 [0.58-1.02]	5,875
Fam 8	29	9	7.58	1.19 [0.54-2.25]	801
Fam 9	54	7	8.68	0.81 [0.32-1.66]	1,114
Fam 10	159	43	19.95	2.16 [1.56-2.90]	4,484
Fam 11	119	33	44.31	0.75 [0.51-1.05]	4,129
Fam 12	96	10	11.53	0.87 [0.42-1.59]	1,574
Spouses P	351	96	112.33	0.86 [0.69-1.04]	12,857
Control families	1238	274	245.91	1.11 [0.99-1.25]	31,613
Spouses	603	152	159.95	0.95 [0.81-1.11]	20,907

SMR: standardized mortality ratio; 95% CI: 95 percent confidence interval

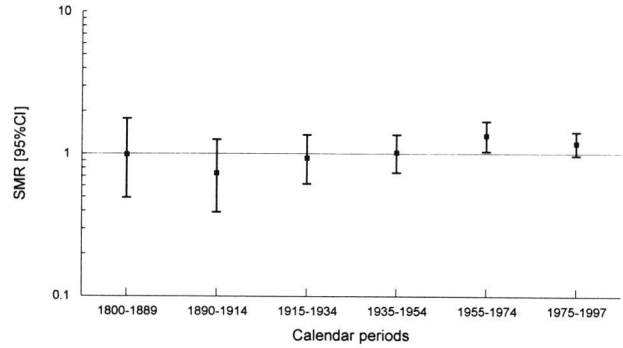
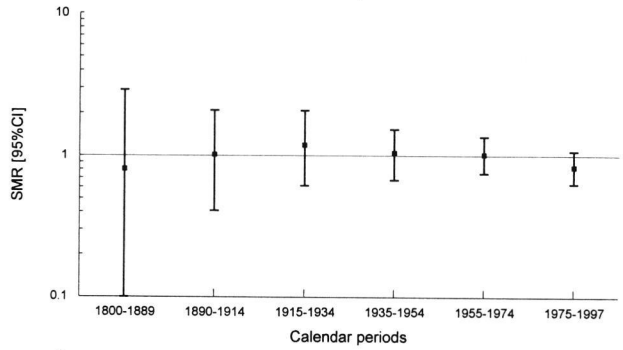


Figure 4. ***Relative evolution of mortality in different control groups compared with the general Dutch population.***

4a) SMRs with 95% CI in control families compared with the Dutch population according to calendar periods.



4b) SMRs with 95% CI in spouse controls compared with the Dutch population according to calendar periods.

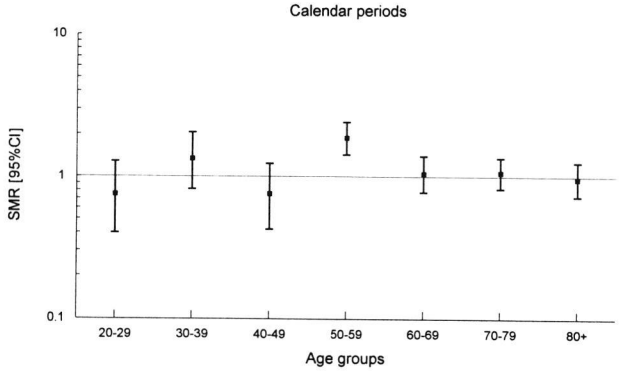
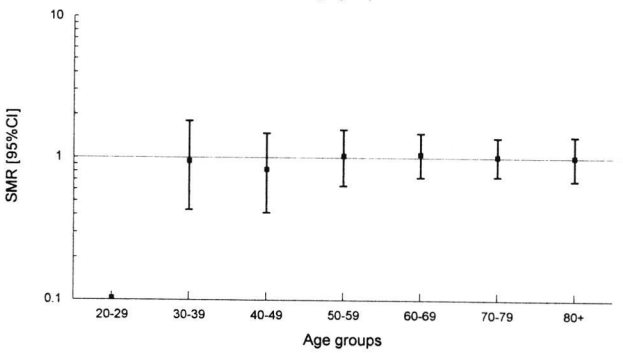


Figure 5. ***Relative mortality in different control groups compared with the general Dutch population over age groups.***

5a) SMRs with 95% CI in control families compared with the Dutch population according to age groups.



5b) SMRs with 95% CI in spouse controls compared with the Dutch population according to age groups.

Figures 4a and 4b show the SMRs over calendar time for control families and spouses respectively. Only in the calendar period 1955-1974 there was a slight increase of the SMR in the control families. In addition, figures 5a and 5b show the SMRs over the different age groups for control families and spouses respectively. Excess mortality was shown for the age group 50-59 years of age in the control families, due to an increase in the families from genealogists. In the spouses there was only one death observed in the age group 20-29 years of age, whereas almost 10 deaths were expected.

The mean life expectancy of all spouse controls (spouse G + spouse P) was 75.6 years (95% CI 74.0-77.3), that of the genealogists family members was 70.2 years (95% CI 68.0-72.5) and the mean life span of the phone book family members was 74.7 years (95% CI 72.9-76.5) (log rank 15.9, p-value 0.0004). In a Cox regression model adjusted for sex and year of birth, it was confirmed that mortality in relatives of the genealogists families was slightly increased compared to the spouse controls and phone book families: compared to the spouse controls the relative risk of the genealogists families was 1.4 (95% CI 1.1-1.8), whereas the relative risk of the phone book families did not differ from unity (95% CI 0.8-1.2) compared to the spouse controls.

Conclusions about Choice of Control Group

The mortality in both control families and spouse controls did not differ from that in the general Dutch population. From these results we conclude that the comparison of family data with the general population is valid for the study of genetic influences on mortality. A group of control families is a fair representation of the general population, although in such a group an individual family may have an increased or a decreased mortality ("chance variation"). Only if the families under investigation come from a specific geographic region, where total mortality differs from that in the general population, or if the families represent a specific socioeconomic group, it may be advisable to compare with spouse controls or family members with a small probability of carrying the gene.

Some observations in subgroups of these control families and spouse controls may deserve comment. In the spouses there was only one death in the age group 20-29 years of age. This probably represents the fact that marriage, which usually occurs in this age group, implies a selection on good health ("healthy cohort effect"). We also found a slight excess mortality in the control families in the age group 50-59 years of age. This could be attributed to a few families with increased mortality (families 3, 4, 5 and 10), and is therefore an example of the above mentioned "chance variation" among individual families. Although the overall SMR for the control families did not differ from unity, there was excess mortality in the families from the genealogists. It is possible that the genealogists became interested in their own pedigree because of a genetic disease; we did not verify the reason for the hobby. It is more likely that the pedigrees were not as complete as they first appeared to be. They probably contained some bias towards individuals in poor health, who are less mobile and therefore easier to trace. Originally, uncertainty about the completeness of the genealogist pedigrees was the most important reason for us to start collecting the second group of control families (the phone book families).

Swift *et al* [40,41] studied deaths and incidence of cancer in families of patients with ataxia-telangiectasia. They compared blood relatives and spouse controls to the general population and came to the conclusion that, since among both relatives and spouses the observed was consistently lower than expected, spouse controls may be a better comparison group than population rates. In other studies by the same research group [42-44], they confirmed that families included in genetic studies may not be a random sample of the national population. The same finding has been reported in families of patients with Hodgkin's disease [13] and systemic lupus erythematosus [45]. We, however, found that the observed number of deaths in both control families and spouse controls did not differ from the expected number of deaths based on the general Dutch population. From these results we therefore conclude that, although families may not always be a random sample of the general population, the comparison with the national population rates is preferred.

General Discussion: Problems and Opportunities

Left Truncation

Left-truncation refers to the moment in time a family member enters the follow-up period, in case that moment is not birth. In many of our analyses, for instance, individuals entered the follow-up period at age 20, i.e. left-truncation occurred at age 20. When counting the number of person-years contributing to the life-times of the family members, there are two reasons for left-truncation. One was already discussed above: persons who transmitted the gene could not have died before the age of procreation, otherwise their branch of the family tree would not exist. In some studies [33,36,37], we chose the rather crude solution of omitting the first 20 years of life in all calculations, which suited us because of the uncertainty of both family information and vital statistics on early deaths in the previous century. In the first antithrombin study [22] the first 20 years of life were discarded from parents and grandparents of probandi, and all others were counted from day of birth. In later studies on mortality in families with inherited thrombophilia [23,30,31] obligate carriers entered the study at the date of birth of their first affected child. The same procedure was used in the studies investigating mortality in families with polycystic kidney disease [32] and with familial dysbetalipoproteinemia [34]. Analyzing the data from 20 years onwards, both options made little difference (unpublished data). An estimate of excess mortality during childhood and puberty can only be given if precise left-trunction is applied.

The second reason for left truncation is more complicated, and confined to the present-day contemporary siblings of the index cases. In, for instance, the antithrombin example [22] several of the brothers and sisters had been screened for the presence of the deficiency while others had not, either because they had declined screening or because they had died before the screening became available. Those who had been screened, had a known certainty of either 100 percent or zero percent probability of carriership. The others remained at their prior 50 percent probability level (potential carriers). The screenees were often

adults, however, and by the very fact of being screened they had survived up to the time of screening in the late 1980s. Excluding siblings who appeared to be non-carriers after the DNA-test, would introduce selection bias towards deceased siblings. The exact treatment of the problem consists of counting those whose carrier status is known by laboratory investigation, from the date of this investigation onward. So, screenees are potential carriers until the date of the test. After the test individuals who appeared to be non-carriers leave the study population and individuals who appeared to be gene carriers move to the 100 percent cohort. Another option is to disregard the screening information altogether and assign all, who are not obligate carriers by Mendelian reasoning, to the 50 percent cohort. In that case, test results are only used to enlarge the pedigrees. In our applications [22,23,30-34] we chose the last option. However, with advancing years the exact treatment of DNA-testing becomes more important, because sibships can be screened independent of survival.

Inferential Uncertainties

Another general problem is that probands become probands because they have several characteristics: they developed the disease and possess an allele which is believed to facilitate the disease development. In addition, however, they might very well possess other alleles or environmental characteristics, which concentrate in their persons and which are necessary in combination to produce the disease. The further the distance from a proband in a pedigree, either laterally or into the past, the lesser will be the amount of sharing of these other alleles or environmental characteristics. Thus, there is the possibility that the effect of a certain genotype will always become diluted. One of the reasons for starting a genetic analysis from a complete geographical survey of index cases is to counter a type of ascertainment bias in which only patients come under the attention of the geneticist. It is no complete guarantee, however, in instances when the index disease is the same as the disease which is studied. An analysis which stratifies for "closeness to probands", might give valuable additional information: a certain genotype might have a different effect if an individual is closely related to the proband than if faraway.

The proposed analysis regroups members from several different pedigrees into cohorts, as if independent observations had been made. In theory, statistical independence is uncertain and this should probably be accounted for in the calculation of the variance of the estimates. We chose to forgo this problem in our applications, as do others in the literature, since there were in every studied gene defect between six and a dozen families, which made extensive sharing of genetic or environmental risk factors unlikely. Still, when challenged about the existence of some families with increased risk, we looked at the mortality ratio of individual families to verify that the overall results would not be biased by some particular pedigrees.

"Background" Mortality

The mortality of the contemporary general population which is the minimal "background" mortality to which the pedigree members have been exposed, was much higher in the past than in more recent times, at all ages and for both sexes. In consequence, when the same surplus risk - in terms of risk difference - is superimposed on a higher "background", the mortality ratio will be closer to unity in the past and increase towards the present. Another way of presenting the data under such conditions may be to calculate the risk difference after direct standardization (secular increase) [32]. The interpretation might present further difficulties, if it is imagined that the mortality in the past was so high that the extra genetic risk could never be seen, as most people had already died of other diseases before the gene could express itself. In addition, it is possible that certain genes only express themselves under present-day conditions of general nutritional affluence or in well-to-do families of the past, and in the presence of modified risk factors.

Future Perspectives

The method may be extended to diseases with other inheritance patterns, i.e. recessive or X-linked hereditary diseases. Our applications in autosomal dominant diseases with expression relatively late in life, are the easiest and yield the largest family trees. The crude classification of the categories of gene

carriership might be refined by maximum likelihood methods, which calculate the probability of gene carriership from the complete information of the surrounding pedigree. For example, a person with an a priori probability of 50 percent who is also the parent of a number of unaffected offspring, thereby sees his or her probability of gene carriership much reduced. However, for large pedigrees the necessary computations can become unwieldy.

Conclusion

The family tree mortality ratio (FTMR) permits the study of part of the natural history of a disease linked to a certain genotype, even in times when the disease was not yet known, its genetic basis not suspected, and therapy was not available. Using the FTMR method it is possible to assess the burden of disease that might be prevented by screening and treatment. On average, 10 percent increase of the overall SMR equals one year reduction of life expectancy due to the genetic factor [37]. The applications of the FTMR method show how every hereditary disease has its own methodological complications and needs its own interpretation of the results. Knowledge of various aspects of the diseases is, therefore, necessary to draw conclusions from the FTMR analyses. It shows whether excess mortality is confined to certain age groups, and whether the impact of a hereditary defect has changed over the calendar time. The evolution of mortality over calendar time depends on preventive and therapeutic possibilities and changes in exogenous risk factors. In understanding the changing mortality patterns and its impact on the population, both relative risks and the difference of absolute risks, need to be taken into account. In our opinion, the FTMR method is of specific interest for monogenetic diseases with an age at onset after reproduction, and indicates how much mortality might maximally be prevented, if screening and intervention in affected families were completely effective.

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Chapter II

Two Centuries of Mortality in Ten Large Families with Huntington's disease: A Rising Impact of Gene Carriership

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Abstract

To estimate the impact of the Huntington gene on mortality, we studied ten large families with Huntington's disease, whose records started before 1800. We investigated mortality from 1800-1997 in 257 proven and obligate carriers of the Huntington gene and 474 potential carriers. Follow-up extended from 20 years after the date of birth to the date of death or end-of-study date. The observed number of deaths was compared with the expected number, based on the general population, adjusted for sex, age and calendar time. To study the influence of the family and parental transmission, we used Cox regression, calculating hazard ratios adjusted for sex, probability of carrying the gene, and year of birth.

On 25,013 person-years 420 deaths occurred, where 278 deaths were expected (SMR 1.5; 95% CI 1.4-1.7). Excess mortality was confined to the ages 40-70 and was strongest in the age group 45-60 years (SMR 2.7; 95% CI 2.3-3.2). To study the evolution of mortality over time in this age group, where the Huntington gene expresses its major impact, we calculated absolute mortality rates per calendar period. From 1800 onward mortality rates in the general population continuously declined, but among the families with Huntington's disease this decline was absent. There were only small differences in risk between families, and the risk for paternal over maternal transmission was 1.2 (95% CI 0.9-1.5). The main finding of our study is that persons who carry the Huntington gene and reach middle age, have not benefitted from any advances in medical care and overall increase in life expectancy.

Introduction

Huntington's disease (HD) is a slowly progressive autosomal dominant neuro-degenerative disease with a variable age at onset [1]. Clinical manifestation

consists of gradually evolving involuntary movements (chorea), progressive dementia, and psychiatric disturbances, especially mood disorders and personality changes. HD affects between 3 and 7 per 100,000 individuals in populations of Caucasian descent, but it has been described in populations of many different ancestries [1]. In the majority of patients the age at onset is round 40 years of age, although extremes of 2 and 80 years have been reported [1-4]. The mean duration of the disease is 16 years and is independent of the age at onset [1,3,5,6]. There is no treatment to prevent the onset or to delay the fatal course of the disease. Approximately 80% of juvenile patients inherit the HD gene from their father [2,4,5,7-10], while a preponderance of maternal transmission was noted in late-onset disease [11-13]. However, in the Dutch late-onset patients the sex of the affected parent was equally distributed [4,8]. In addition it has been reported that, although the mean age at onset between affected mothers and their offspring did not differ greatly, affected children of fathers had a lower mean age at onset than their fathers [14]. This anticipation phenomenon was already reported in the 1970s as a difference in age at death between offspring of men and their fathers [15,16].

In 1993, the HD gene has been identified as an expansion and instability of a specific CAG trinucleotide repeat on chromosome 4 p16.3 [17]. In HD patients this highly polymorphic CAG repeat is expanded to a range of 36 to 121 copies. Age at onset of the disorder is inversely correlated with the number of CAG repeats [18-20], but there was a wide range of age at onset for any specific repeat number [21]. The repeat length accounts for approximately 50% of the variation in the age at onset [20]. During meioses the HD repeat is unstable showing both increases and decreases in size with the largest expansions in alleles of paternal origin. This could be an explanation for the anticipation phenomenon [18-20].

Because anticipation may be observed as a consequence of ascertainment bias, the unbiased impact of the HD gene and its variation between families and line of inheritance, can best be studied during long-term follow-up. We developed a method to study the survival of family members retrospectively by extending

the follow-up to the past [22]. The aim of the present study was to compare mortality between 1800 and 1997 in members of HD families with that in the general Dutch population using the Family Tree Mortality Ratio method. The amount of excess mortality in the HD families provides an estimate of the impact of gene carriership on life expectancy. In addition, in these ten families the influence of transmission of the HD gene from either maternal or paternal origin on mortality ratios was determined.

Methods

Study population

For more than 30 years clinical and genealogical data of the great majority of Huntington patients and their families in the Netherlands have been compiled in the Leiden Roster [4,6,8,23]. From these files ten large pedigrees, between seven and nine successive generations, have been selected. All proven carriers of the HD gene, obligate and potential carriers were included in this study. In the present generations the diagnosis of HD was confirmed by DNA-analysis. In the previous generations we used pathological records and Mendelian reasoning to identify persons with a 50 or 100 percent probability of carrying the HD gene. Obligate carriers were family members who had passed on the HD gene from common ancestors to their affected offspring. Potential carriers were defined as all first degree relatives of carriers (i.e. children and siblings). Thus, Mendelian probabilities can be assigned to all individuals in the pedigree. Using parish records, municipal registers and national archives, we verified and completed the dates of birth and death for all proven, obligate and potential HD carriers. Follow-up for all individuals extended from twenty years after the date of birth to the date of death or to June 30, 1997. The reason for ignoring the first 20 years of life was that the obligate carriers, who have passed on the HD gene to their affected offspring had to be alive at the start of the reproductive period.

Statistical analysis

Mortality data were analyzed with the Family Tree Mortality Ratio method[22,24]: the overall mortality of the study population (observed) was compared with that of the Dutch general population (expected) adjusted for age, sex and calendar period. The ratio of observed to expected number of deaths is the standardized mortality ratio (SMR), which is a relative risk. The expected mortality was calculated by multiplying the total number of years lived by the study population with the sex, age and calendar period specific population mortality rates from the annual reports of the Netherlands Central Bureau of Statistics, using the computer program 'Person-Years' [25]. Confidence limits for the SMR are based on a Poisson distribution for the observed number of deaths [26]. The calendar periods were divided into a fifty-year interval from 1800 to 1849, twenty-year intervals from 1850 to 1889, a fifteen-year interval from 1890 to 1904, and into ten-year intervals from 1905 to 1997. To each of these periods we applied the population mortality rates of the mid-interval year, subdivided by sex and into five-year age groups.

Because of distinctions in structure of person-years over the calendar and age groups, one SMR cannot be compared with another SMR. Therefore, to study the influence of the family or of line of inheritance, we performed Cox regression analysis. In the two oldest generations it was, per definition, not known which parent transmitted the HD gene. In the third till ninth generation individuals the HD gene was transmitted through either the father or the mother. The multivariate analysis calculated hazard ratios for the ten families and for parental transmission adjusted for sex, probability of carrying the HD gene, and year of birth.

Table 1. Characteristics and relative mortality (SMR) in the ten Huntington's disease (HD) families, from 20 years of age onward

HD family	Number	(Obligate) carriers	Potential carriers	Mean age at death [SD]	Mean life expectancy [SE]	Observed	Expected	SMR [95% CI]	Person-years
Fam 1	91	36	55	60 [16]	65 [2.2]	48	32.21	1.49 [1.10-1.98]	2,924
Fam 2	63	28	35	61 [16]	65 [2.3]	45	27.88	1.61 [1.18-2.16]	2,442
Fam 3	167	44	123	61 [16]	66 [1.5]	91	64.53	1.41 [1.14-1.73]	5,807
Fam 4	12	5	7	49 [8]	49 [3.1]	7	1.23	5.69 [2.29-11.7]	281
Fam 5	30	8	22	58 [14]	60 [3.2]	15	6.99	2.15 [1.20-3.54]	812
Fam 6	46	18	28	63 [17]	66 [2.5]	34	26.71	1.27 [0.88-1.78]	1,848
Fam 7	188	70	118	62 [18]	68 [1.6]	106	81.05	1.31 [1.07-1.58]	6,805
Fam 8	56	23	33	55 [15]	59 [2.3]	31	12.72	2.44 [1.66-3.46]	1,643
Fam 9	49	19	30	55 [17]	58 [2.9]	28	13.88	2.02 [1.34-2.92]	1,351
Fam 10	29	6	23	66 [15]	70 [3.0]	15	10.72	1.40 [0.78-2.31]	1,100
	731	257	474	60 [16]#	65 [0.7]$	420	277.91	1.51 [1.37-1.66]	25,013

HD: Huntington's disease; SD: standard deviation; SE: standard error; SMR: standardized mortality ratio; 95% CI: 95% confidence interval

#: F-test 1.52, p-value 0.14; $: log rank test 33.20, p-value 0.0001

Results

After removal of spouses and family members with less than 50 percent probability of carrying the HD gene, 849 persons out of ten HD families had at least 50 percent probability of carrying the HD gene. 731 individuals were 20 years of age and older and contributed person-years to the analysis. From these ten families 143 affected men and 114 affected women were identified with certainty, with respectively 123 and 88 persons dying during the study period; 241 men and 233 women were potential HD gene carriers, with respectively 119 and 90 dying in the study period. The mean life expectancy in men was 63 years (95% confidence interval (95% CI) 61-65 years) and the mean life expectancy in women was 68 years (95% CI 65-70 years). The mean life expectancy in proven and obligate HD carriers was 59 years (95% CI 57-61 years) and the mean life expectancy in the potential HD carriers was 73 years (95% CI 71-75 years).

On a total of 25,013 person-years 420 deaths were counted (242 in men and 178 in women) (Table 1). The expected number of deaths 278 (154 in men and 124 in women), leading to an overall SMR of 1.5 (95% CI 1.4-1.7) in both men and women. Table 1 shows the characteristics and relative mortality for the ten HD families separately. In all families there was excess mortality, although there were differences in the magnitude of the SMR. The smallest family (Fam 4) had the lowest mean life expectancy and highest SMR, possibly due to chance.

During this century there was a secular increase of mortality (Figure 1). Before 1935 the SMR did not differ from unity; from 1935 onward the SMR fluctuated between 1.4 and 2.2. The excess mortality was limited to the age group 40-70 years (Figure 2) and was strongest in the age group 45-60 years (SMR 137/51=2.7; 95% CI 2.3-3.2). To determine the evolution of mortality in this particular age group, where the HD gene expresses its major impact, we calculated the absolute mortality rates per 1000 person-years in the HD

families and in the general Dutch population per calendar period, adjusted for the age structure of the HD families over the whole time period (Figure 3). During the past two centuries the absolute mortality rates of the general Dutch population continuously declined, but among the HD family members this decline was absent. Because of this phenomenon the relative risk increased from 1.3 in the nineteenth century to 3.7 in the period 1975-1997 for the age group 40-70 years.

The differences in risk between the HD families were small (Table 2), given that the smallest family (Fam 4) had the highest hazard ratio. Furthermore, the year of birth did not influence the mortality rate (as was already shown in figure 3 by the fact that the absolute mortality rate remained stable over the century in the HD family members). The risk of dying was 1.4 times greater for men than for women (as was also evident from the difference in mean life expectancy), and proven and obligate carriers had a higher mortality rate than potential carriers (as was already expressed in the difference in mean life expectancy) (Table 2). The SMR for both paternal and maternal inheritance was 1.7 (95% CI 1.5-1.9), but the SMR for unknown transmission (meaning the first and second generation) did not differ from unity. In a Cox regression model adjusted for family, sex, probability of carrying the HD gene and year of birth, individuals who inherited the HD gene from their father had a 20 percent higher risk of dying than family members who inherited the HD gene from their mother (Table 2). This effect was apparent in four families, but in three families the risk of dying might be lower for paternal than for maternal transmission, though not significant (Table 3). In most families both paternal and maternal inheritance were present, although in families 4 and 5 most individuals inherited the HD gene from their mother and in families 2, 6 and 10 there were more individuals who inherited the HD gene from their father (Table 3).

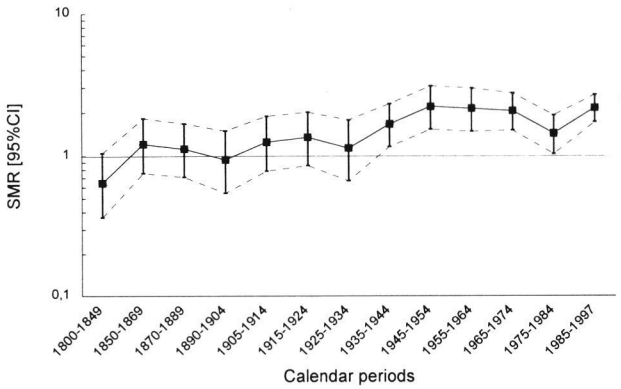

Figure 1. All-cause mortality, SMR with 95% CI, in HD families compared with the general Dutch population according to calendar periods, ≥20 years of age

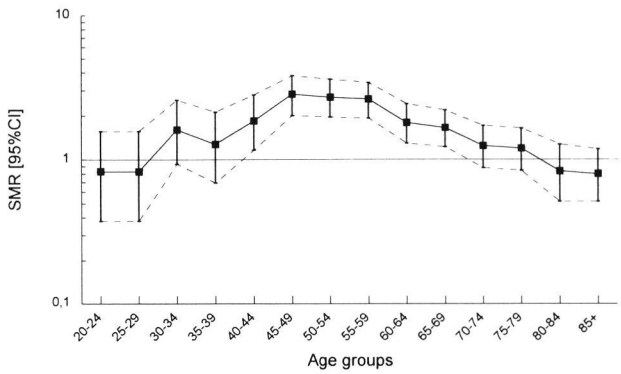

Figure 2. All-cause mortality, SMR with 95% CI, in HD families compared with the general Dutch population according to age groups from 1800 till 1997

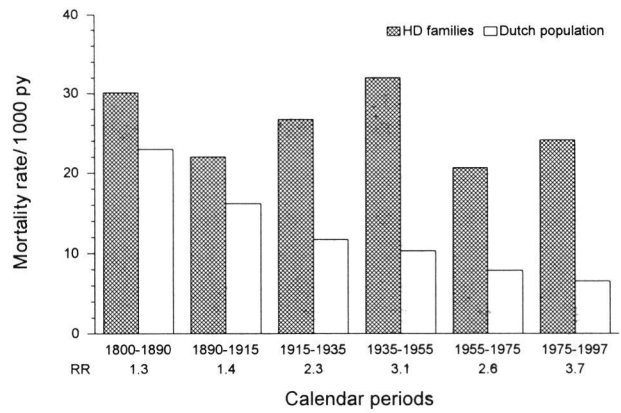

Figure 3. Absolute mortality rate per 1000 person-years (py) in the HD families and in the Dutch population according to calendar periods in the age group 40-70 years of age, adjusted for the age structure of the HD families over the whole time period. The RR is the adjusted mortality rate of the HD families to that of the Dutch population per calendar period

Table 2. Adjusted hazard ratio (HR) and 95% confidence interval (95% CI) for mortality from 20 years of age onward

Variable	Number	HR	95% CI
Line of inheritance			
Paternal transmission	403	1.18	0.94 - 1.49
Maternal transmission	267	1	
Unknown transmission	61	0.96	0.81 - 1.13
Family			
Fam 1	91	1	
Fam 2	63	0.85	0.56 - 1.30
Fam 3	167	0.96	0.68 - 1.38
Fam 4	12	3.69	1.64 - 8.30
Fam 5	30	1.55	0.86 - 2.81
Fam 6	46	0.72	0.45 - 1.13
Fam 7	188	0.74	0.53 - 1.05
Fam 8	56	1.25	0.79 - 1.98
Fam 9	49	1.67	1.03 - 2.70
Fam 10	29	1.00	0.55 - 1.83
Probability of carrying HD gene			
(Obligate) carrier	257	2.55	2.06 - 3.15
Potential carrier	474	1	
Gender			
Men	384	1.43	1.17 - 1.75
Women	347	1	
Year of birth	731	1.00	1.00 - 1.00

Discussion

We studied the mortality patterns from 1800 through 1997 in ten large pedigrees with Huntington's disease. The overall lifetime SMR for proven, obligate and potenial carriers of the HD gene was 1.5 (95% CI 1.4-1.7), with no significant differences between men and women. Excess mortality was

Table 3. Hazard ratio (HR) and 95% confidence interval (95% CI) of paternal versus maternal inheritance for mortality from 20 years of age onward and adjusted for sex, probability of carrying the HD gene and year of birth

Family	Number offspring paternal / maternal	HR (paternal vs maternal)	95% CI
Fam 1	44 / 38	1.28	0.64 - 2.53
Fam 2	51 / 10	0.84	0.32 - 2.17
Fam 3	90 / 68	1.34	0.83 - 2.17
Fam 4	2 / 6	0	0 - ∞
Fam 5	5 / 21	0	0 - ∞
Fam 6	35 / 4	1.48	0.30 - 7.27
Fam 7	107 / 75	1.42	0.92 - 2.13
Fam 8	27 / 22	0.63	0.18 - 2.26
Fam 9	21 / 20	0.92	0.30 - 2.84
Fam 10	18 / 3	∞	0 - ∞

confined to the age group 40-70 years. We found a secular increase of relative mortality in the HD families, mainly because of decreasing mortality rates in the general Dutch population over the past two centuries. This means that the impact of the HD gene is rising: in comparison with the general Dutch population it becomes more disadvantageous to carry this gene. The fact that the mortality rates in the HD family members remained stable over calendar time implies that the duration of the disease has not changed (around 16 years), and it reflects the absence of medical therapy to delay the disease progression [1].

According to the literature, in some families, HD follows a milder course with longer survival [1]. In our study all HD families showed excess mortality. The SMR varied between 1.3 and 5.7 and the mean life expectancy between 70 and 49 years of age. The most important factor associated with differences in mortality rate was the number of individuals contributed to the analysis by a specific family: the smallest family (Fam 4) had the highest risk. Taken the

family size into account, these HD families might have a homogeneous genetic background (Table 2).

In the literature the mean age at death lies around 60 years, but differs between the studies based on the interval of observation [1,15,16,27,28]. In our study the mean age at death for affected family members and their first degree relatives was 60 years of age, but corrected for surviving individuals the observed mean was 5 years older (mean life expectancy 65 years of age). Comparing the mean life expectancy of men and women (difference 5 years) with the adjusted hazard ratio for men versus women (43% increase), and the mean life expectancy of certain (proven and obligate) carriers and potential carriers (difference 14 years) with the risk for certain versus potential carriers (155% increase), it becomes clear that an increase of approximately 10% in risk might be equated with a decrease of one year in mean life expectancy. Thus, an adjusted hazard ratio of 1.2 for paternal over maternal transmission might imply that all offspring of affected fathers will, on average, live two years shorter than offspring of affected mothers. This was in comparison with earlier results from the literature [15,16], although the purpose of these studies was to determine anticipation between generations. In the literature there has been discussion why and how paternal transmission can lead to earlier disease onset in their offspring [5,7,9,10,13,14,29,30]. After the identification of the HD gene [17], the mechanism of expansion and instability of the CAG trinucleotide repeat length has been added to the discussion [18-21,31,32].

The mortality rate for an individual was slightly higher if the HD gene was transmitted through the father, but this was not true for all families. In at least three of the studied HD families (Fam 2, Fam 8 and Fam 9) the line of inheritance had no influence on the mortality rate or might even have a reverse effect. In addition, if paternal transmission leads to earlier age at onset and therefore to higher mortality, one would expect a higher mortality rate in families with mainly paternal transmission as compared to families with mainly maternal transmission. However, in the families under investigation this was

not the case: the two families with preponderantly maternal inheritance had slightly higher mortality rates than the reference family, whereas the families with primarily paternal inheritance all had slightly lower mortality rates than the reference family. Since this phenomenon was most prominent in smaller families, this should be a chance finding. Further studies are needed to investigate possible risk factors within and between HD families that might influence the impact of parental transmission on age at onset and mortality. The main finding of our study is that individuals who carry the HD gene and reach middle age, have not benefitted from any advances in medical care and overall expansion in life expectancy. This means that HD carriership becomes progressively more disadvantageous.

Acknowledgments

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Chapter III

Mortality and Causes of Death in Families with the Factor V Leiden Mutation (Resistance to Activated Protein C)

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Abstract

To investigate whether resistance to activated protein C (APC resistance) because of a mutation in the factor V gene (factor V Leiden) leads to a decrease in life expectancy, we analyzed overall and cause-specific mortality in 171 parents whose offspring carried this mutation. Compared with the Dutch general population, and after adjustment for age, sex and calendar period, we found no excess deaths in the parents (standardized mortality ratio [SMR], 1.0, 95% confidence interval [CI], 0.8 to 1.2). The cause-specific SMR for malignant neoplasms (1.0; 95% CI, 0.6 to 1.4), diseases of the circulatory system (1.0; 95% CI, 0.7 to 1.4) and cerebrovascular disease (1.0; 95% CI, 0.4 to 1.9) also did not differ from unity. The SMRs for diseases of the respiratory system (1.4; 95% CI, 0.6 to 2.6) and for ischemic heart diseases (1.1; 95% CI, 0.7 to 1.7) were slightly increased. Under the age of 45 years, there was a ninefold increase of dying from ischemic heart disease. Thromboembolic complications were mentioned only once (venous embolism or thrombosis) as an underlying ("primary") cause of death (SMR, 2.3; 95% CI, 0.1 to 13.0) and three times (pulmonary embolisms) as a contributing ("secondary") cause of death (SMR, 1.5; 95% CI, 0.3 to 4.4). We conclude that there is no major effect of APC resistance on life expectancy. Therefore, long-term anticoagulation in carriers of factor V Leiden, on the basis of the carrier state alone, is not indicated.

Introduction

A poor anticoagulant response to activated protein C (APC resistance) is a common autosomal inherited abnormality, which is associated with a tendency to venous thrombosis [1-3]. This APC resistance is associated with a mutation at the APC cleavage site in the factor V gene (factor V Leiden) [4,5]. The risk of deep-vein thrombosis in unselected carriers of the mutation is increased

eightfold [2,6]. Concern for premature death has been raised about the effect of APC resistance by a case report of fatal pulmonary embolism in a 32-year-old man with a profound, and possibly homozygous APC resistance [7].

Life expectancy in individuals with the factor V Leiden mutation can be studied during long-term follow-up periods. However, by genetic reasoning, we know that, because of its autosomal dominant mode, at least one parent of each heterozygous index case carried the abnormality and passed it on to his/her offspring and that in homozygous carriers both parents were affected. Therefore, a deleterious effect of the mutation on mortality could also be studied in the parents of a proband with the factor V Leiden mutation. We questioned whether an excess mortality in parents of individuals with APC resistance exsists and, especially, whether the risk of dying from thromboembolic complications has been increased. The method used in this study has been extensively described in former reports on mortality in hereditary antithrombin deficiency [8] and protein C deficiency [9].

Methods

Study population

Our investigation was based on the Leiden Thrombophilia Study (LETS). The design of this population-based case-control study on hereditary venous thrombosis has been specified previously [2]. A total of 474 consecutive patients younger than 70 years of age, with an objective diagnosis of a first episode of deep venous thrombosis between 1988 and 1993 and without an underlying malignant disorder, were selected from the files of three anticoagulation clinics in the Netherlands. In 92 of the patients, APC resistance was subsequently confirmed by DNA-analysis [5]. Using municipal registers and state archives (where, since 1811, all births, marriages, and deaths must be reported by Dutch law), we retrieved the dates of birth and death for the 184 parents of all

carriers. Because the parents had to live until the birth of the index case to pass on the mutation, the years lived before this birth were ignored. Therefore, the follow-up period extended from the date of birth of their affected offspring to the date of death or to December 31, 1994.

Causes of death

We were able to survey all causes of death of the parents who were deceased in the Netherlands between 1941 and 1994. These were made available by the Netherlands Central Bureau of Statistics (Voorburg, The Netherlands), where one copy of each death certificate is kept. The causes of death are routinely coded by this Bureau using the coding rules from the International Classification of Diseases, fifth through ninth revisions (ICD-5 through ICD-9) [10-14]. For each death, both the underlying (primary) cause of death and any other contributing (secondary) causes of death, if listed, were made available. For the analysis, the older ICD codes were recoded conform the ninth revision. We classified the deceased persons into six groups of underlying causes of death: malignant neoplasms (ICD-9 codes 1400-2099), diseases of the circulatory system (ICD-9 codes 3900-4599), cerebrovascular diseases (ICD-9 codes 4300-4389), ischemic heart diseases (ICD-9 codes 4100-4149), diseases of the respiratory system (ICD-9 codes 4600-5199), and thromboembolic complications. We defined thromboembolic complications as pulmonary embolism (ICD-9 code 4151), (thrombo)phlebitis (ICD-9 codes 4510-4519), venous embolism or thrombosis (ICD-9 codes 4530-4539), venous complications in pregnancy and the puerperium (ICD-9 codes 6710-6719) and obstetrical pulmonary embolism (ICD-9 codes 6730-6739).

On the death certificate, a maximum of three secondary causes of death is recorded. These conditions may be contributing causes, conditions that are part of the causal pathway, or any other conditions mentioned on the certificate [15,16]. As venous thromboembolic complications are frequently coded as a contributing or fatal cause of death, we also studied the occurrence of these complications within the group of secondary causes of death. Mackenbach *et*

al [16] reported that, between categories of primary causes of death, there are major differences in the prevalence at death of other secondary causes. The number of reports on all secondary causes of death should give us an indication as to whether, in our study group, the frequency of secondary causes of death might be compared with that of the general population.

Statistical analysis

The overall and cause-specific mortality of the parents (observed) was compared with that of the Dutch general population adjusted for age, sex, and calendar period (expected). The ratio of observed to expected number of deaths is the standardized mortality ratio (SMR), which can be viewed as a relative risk. The expected mortality was calculated by multiplying the total number of years lived by the study population per sex, age, and calendar period with the cause-specific population mortality rates from the annual reports of the Netherlands Central Bureau of Statistics, using the computer program "Person-Years" (Prof J. Peto, Institute of Cancer Research, London, UK) [17]. Confidence limits for the SMR are based on a Poisson distribution for the observed number of deaths [18].

The calendar periods were assigned to 5-year intervals from 1941 to 1994 and subdivided by sex and into 5-year age classes. To each of these periods, we applied the overall and cause-specific population mortality rates of the mid-interval year, starting from the mid-interval year 1943 (period, 1941 through 1945), 1948 (period, 1946 through 1950) until 1993 (period, 1991 through 1994). Only since the early 1950s has the number of secondary causes of death been mentioned in the annual reports. Besides, since 1993, the secondary causes of death have no longer been integrated in these reports. These facts imply that the calculation of expected mortality for the secondary causes of death started with the mid-interval year 1953 for the period from 1951 through 1955 until 1992 for the last calendar period. Furthermore, only since 1971 has the total of all secondary causes of death been given in the annual reports; therefore, the expected overall secondary causes of death could only be calculated for the period from 1971 through 1994.

Results

Characteristics of the study population

For 171 parents (86 mothers and 85 fathers), the dates of birth and the dates of death or the end-of-study dates were analyzed. The parents of 4 probands had never lived in the Netherlands, and 5 parents could not be traced up to the end-of-study date. Year of birth ranged from 1873 to 1948, and year of death from 1943 to 1994. The mean age at the end-of-study date was 66 years (range, 46 to 87 years). The mean age of the parents at which their affected offspring was born was 30 years (range, 18 to 46 years) for the mothers and 32 years (range, 19 to 49 years) for the fathers. From 1941 until 1994, the 171 parents were followed up over 5,810 person-years, in which 86 deaths took place. A total of 50 men died in 2,769 person-years, and 36 women died in 3,041 person-years. The mean age of death was 70 years (range, 40 to 96 years) in men and 71 years (range, 33 to 96 years) in women. Two men died in foreign countries and 2 death certificates (from 1 man and 1 woman) could not be linked; therefore, for the analysis of the causes of death, 82 death certificates were reviewed. To prevent the introduction of selection bias, the person-years of these 4 parents were excluded from the calculation of the cause-specific expected number of deaths.

All-cause mortality

The overall mortality in the study group and the general population was equal (SMR, 1.0; 95% confidence interval (CI), 0.8 to 1.2). In men the SMR was 0.9 (95% CI, 0.7 to 1.2), and in women the SMR was 1.0 (95% CI, 0.7 to 1.4). Figure 1 shows the relative mortality risks for different calendar periods from 1941 to the present. In none of these periods did the SMR significantly differ from unity; although, for the period from 1941 through 1955, 10 deaths were observed, whereas 7.3 deaths had been expected (SMR, 1.4; 95% CI, 0.7 to 2.5). Moreover, in none of the age groups was there a significant difference between the observed and expected mortality (Figure 2). However, 5 parents (2 men and 3 women) died in the youngest age category, including a man who

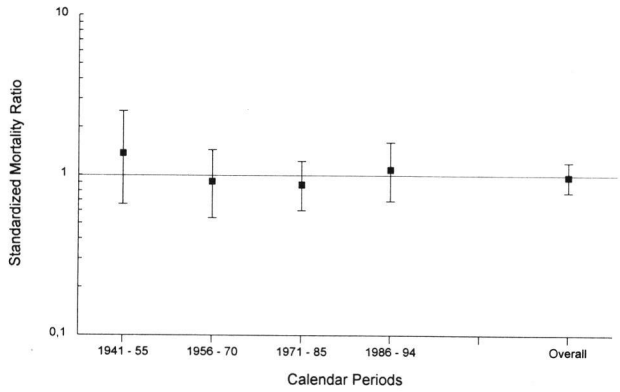

Figure 1. All-cause mortality in 171 parents of affected offspring by calendar periods from date of birth of their affected offspring to end-of-study date

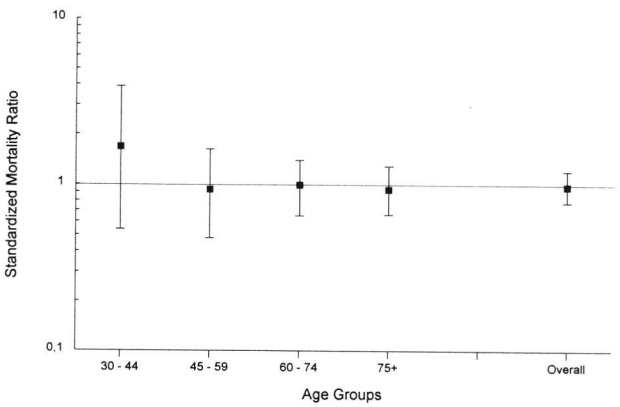

Figure 2. All-cause mortality in 171 parents of affected offspring by age groups between 1941 and 1994

died in World War II and a woman with an unknown cause of death (SMR, 1.7; 95% CI, 0.5 to 3.9).

Causes of death

The observed number of deaths from malignant neoplasms, circulatory diseases, and cerebrovascular diseases matched the expected number of deaths. The SMRs for diseases of the respiratory system and for ischemic heart diseases were slightly increased (Table 1). There were 2 deaths from ischemic heart disease before the age of 45 years, whereas only 0.2 deaths were expected before this age (SMR, 9.2; 95% CI, 1.1 to 32.8). Thromboembolic complications were mentioned only once (venous embolism or thrombosis) as a primary cause of death. However, because this cause of death is even more rare in the general

Table 1. Observed and expected number of deaths for the different groups of primary causes of death between 1941 and 1994

		Total				Men			Women		
condition	ICD-9	O	E	SMR	95% CI	O	SMR	95% CI	O	SMR	95% CI
All causes of death		86	88.3	1.0	0.8-1.2	50	0.9	0.7-1.2	36	1.0	0.7-1.4
Malignant neoplasms	1400-2099	23	24.1	1.0	0.6-1.4	14	1.0	0.5-1.6	9	0.9	0.4-1.8
Circulatory diseases[1]	3900-4599	39	37.5	1.0	0.7-1.4	23	1.0	0.7-1.6	16	1.0	0.6-1.7
Cerebrovascular diseases	*4300-4389*	*9*	*9.1*	*1.0*	*0.4-1.9*	*4*	*0.9*	*0.2-2.2*	*5*	*1.1*	*0.4-2.6*
Ischemic heart diseases	*4100-4149*	*21*	*18.4*	*1.1*	*0.7-1.7*	*12*	*1.0*	*0.5-1.7*	*9*	*1.4*	*0.7-2.7*
Other disorders[2]		*9*	*10.0*	*0.9*	*0.4-1.7*	*7*	*1.3*	*0.5-2.7*	*2*	*0.4*	*0.1-1.6*
Respiratory diseases	4600-5199	9	6.4	1.4	0.6-2.6	6	1.4	0.5-2.9	3	1.5	0.3-4.4
Thromboembolic complications		1	0.43	2.3	0.1-13.0	-	-	-	-	-	-
Pulmonary embolism	*4151*	*0*	*0.24*	*0*	-	-	-	-	-	-	-
Venous embolism and thrombosis or (thrombo)phlebitis	*4530-4539 4510-4519*	*1*	*0.18*	*5.5*	-	-	-	-	-	-	-
of the puerperium	*6710-6719 6730-6739*	*0*	*0.01*	*0*	-	-	-	-	-	-	-

O: observed number of deaths; E: expected number of deaths; SMR: standardized mortality ratio; 95% CI: 95 percent confidence interval.

1: excluding thromboembolic complications; 2: ICD-9 codes 3900-4059, 4160-4299, 4400-4489, 452, and 4540-4599.

population, this number was much higher than expected, although with a very broad confidence interval (SMR, 2.3; 95% CI, 0.1 to 13.0; see Table 1). The number of reports on all secondary causes of death was about equal to that of the general population, indicating that the cause-specific secondary mortality rates were informative. Among these contributing causes of death, 3 were classified as pulmonary embolism (Table 2), including the one caused by the venous thromboembolism; 2 of them were men in the age group of 45 through 59 years, and 1 was a woman in the age group of 60 through 74 years.

Discussion

We studied the overall mortality in parents whose children were carriers of the factor V Leiden mutation. No excess mortality was found, and confidence limits were narrow. Obviously, only half of the parents of heterozygous and both parents of homozygous patients will have carried the mutation at the APC cleavage site in the factor V gene. Among the 171 individuals, 14 were the parent of 7 homozygous patients. Therefore, at least 92 of 171 individuals studied were themselves carriers of the defect. The inclusion of a substantial number of "normal" parents might have diluted any effect on mortality but cannot explain the absence of any increased mortality risk. This is so because the diluted SMR is composed of half of the SMR of the APC resistant individuals plus half of the SMR of normal persons. Because our observed SMR was 1 and, by definition, the SMR of normal persons is 1, the SMR of the APC resistant individuals can also only be 1. However, had we found an SMR of 3, this diluted observed SMR would then point to a true SMR of 5 for the APC resistant individuals.

From the three major categories of specific causes of death (malignant neoplasms and diseases of the circulatory and of the respiratory system), only the number of deaths from respiratory diseases was slightly increased. We can

Table 2. Observed and expected number of deaths for all diseases and thromboembolic complications as secondary causes of death

condition	ICD-9	O	E	SMR	95% CI
All secondary causes of death[1]		41	37.8	1.1	0.8-1.5
Thromboembolic complications[2]		3	2.01	1.5	0.3-4.4
Pulmonary embolism	*4151*	*3*	*1.60*	*1.9*	-
Venous embolism and thrombosis or (thrombo)phlebitis	*4530-4539 4510-4519*	*0*	*0.41*	*0*	-
of the puerperium	*6710-6719 6730-6739*	*0*	*0.00*	*0*	-

1: calendar period 1971-1994; 2: calendar period 1951-1994; O: observed number of deaths; E: expected number of deaths; SMR: standardized mortality ratio; 95% CI: 95 % confidence interval

roughly split these diseases into chronic obstructive pulmonary diseases and pneumonia. It is possible that reports of mortality by pneumonia could be caused by missed pulmonary embolism. However, among these 9 observed deaths caused by respiratory diseases, only 1 was an "unspecified pneumonia".

Ischemic heart disease gave excess mortality under 45 years of age. Although based on only 2 deaths, it might point to the factor V Leiden mutation as a risk factor for arterial thrombosis in relatively young individuals. This finding is supported by the findings of some other investigators but possibly is only pronounced in homozygous individuals [19-21]. However, there are just as many studies that do not find an association [22-25].

If small, an effect on the risk of death of thromboembolic complications may not be apparent from the overall figures. Among 86 deaths, only 1 was classified as being caused by thromboembolism. Even though this 1 event exceeded the expected number (because of the rareness of fatal thromboembolisms in the general population), this does not point to a high rate of deaths by thrombotic causes. Similarly, the 3 deaths in which pulmonary embolism was listed as a secondary cause of death are not sufficient enough to cause alarm.

Given our sample size, if we had found 3 or more primary deaths from thromboembolic complications, it would have given us an SMR significantly greater than unity. Such a rate would have meant a clear deleterious effect of the mutation on life expectancy. At present, an increase in mortality, if present at all, can only be very small. To quantify more precisely such a small increase in mortality from thromboembolic complications caused by the factor V Leiden mutation, a study about 10 times larger would have been necessary.

In theory, these fatal thromboembolisms might have been prevented by long-term prophylactic anticoagulation treatment. However, it is not likely that this would have improved overall survival rates. On the contrary, previous studies estimated the incidence of major bleeding episodes during oral anticoagulant therapy to be 2% to 3% per year [26,27]. Furthermore, the risk of fatal hemorrhage brought about by anticoagulant therapy was reported to be 6 per 1,000 treatment-years [27]. Because our total follow-up time was nearly 6,000 person-years, long-term prophylactic anticoagulation treatment would have induced a considerable number of fatal hemorrhages in our cohort, leading to excess mortality. Even for the use of prophylaxis restricted to high-risk situations, such as pregnancy or the puerperium, it is not obvious whether the induced complications outweigh the thrombotic risk in individuals who have never experienced a thrombosis [28]. Because we have no information about the parents' medical histories, it can not be eliminated that some of them were treated with anticoagulants for shorter or longer periods for recurrent thrombosis. The time frame of the follow-up study renders it unlikely that this included a substantial number of individuals. The absence of excess mortality by hemorrhage in our study also does not point to widespread use of anticoagulants.

Our findings are in accordance with our previous reports on thrombophilia caused by hereditary antithrombin [8] and protein C deficiency [9], in which we also did not find any indication of excess deaths related to the abnormality. However, in contrast to thrombophilia associated with the deficiency of protein C, protein S, or antithrombin, APC resistance is common. The carrier rate in

Europe is estimated at 2% to 7% [2,3,5,29] and may be the result of evolutionary advantages for the heterozygous state at the time that thrombophilia may have been less damaging because of the absence of some lifestyle risk factors. A study by Mari *et al* [30] among healthy centenarians supported our findings; they found similar allele frequencies in centenarians and noncentenarians, suggesting that the factor V Leiden mutation is compatible with extreme longevity.

Because of the high prevalence of factor V Leiden, it may be feasible to confirm our results in a prospective follow-up study and to quantify the association between the factor V Leiden mutation and arterial and venous thromboembolism with and without fatal outcome. Our results apply only to heterozygous factor V Leiden carriership, and it remains to be elucidated whether the homozygous state confers an additional risk, as it does for nonfatal venous thrombosis (80-fold) [31]. It may well be worthwhile to test the families of carriers of the factor V Leiden mutation for the defect for clinical management of thrombosis, but there is no need for major concern about a deleterious effect on life expectancy.

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Chapter IV

Mortality and Causes of Death from 1800 to 1994 in One Extended Apolipoprotein E3-Leiden Pedigree with Familial Dysbetalipoproteinemia

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Abstract

Background - The apolipoprotein E3-Leiden (APOE*3-Leiden) variant is one of the causes of autosomal dominant familial dysbetalipoproteinemia (FD). In general, FD leads to premature coronary and peripheral atherosclerosis in one third to more than half of these individuals.

Purpose - We investigated all-cause mortality from 1800-1994 and causes of death from 1941-1994 in 266 proven, obligate and potential carriers of the APOE*3-Leiden allele in one extended pedigree with FD (ancestors born in 1718), to estimate the impact of this hereditary defect on mortality.

Methods - The overall and cause-specific mortality of the family members (observed) was compared with that of the general Dutch population (expected) adjusted for sex, age and calendar period. The ratio of observed to expected is the standardized mortality ratio (SMR), a relative risk measure.

Results - Between 1800-1994 over 9,067 person-years, 110 deaths occurred and 107 deaths were expected (SMR 1.0; 95% CI 0.8-1.2). In none of the calendar periods the SMR differed from unity. Among the age groups there was some variation in the SMR, especially at younger ages. Between 1941-1994 there also was no excess overall mortality (SMR 0.7; 95% CI 0.4-1.1). Most individuals died of circulatory diseases, but the observed number of circulatory deaths equalled the expected number (SMR 1.1; 95% CI 0.6-2.0). Only two individuals died of cancer (SMR 0.3; 95% CI 0.03-1.0).

Conclusions - There was no excess mortality in this extended pedigree with APOE*3-Leiden associated FD. Therefore, screening of family members of carriers of the APOE*3-Leiden variant for the mutation is unlikely to offer survival benefits.

Introduction

Familial dysbetalipoproteinemia (FD), also called type III hyperlipoproteinemia is a genetic disorder of the lipoprotein metabolism, characterized by elevated plasma cholesterol and triglyceride levels due to the accumulation in the plasma of cholesterol-rich β-VLDL [1-3]. The primary metabolic defect of FD is the presence of mutant forms of apolipoprotein E (apoE). ApoE plays a central role in the lipoprotein metabolism by serving as a ligand for the binding of lipoproteins to lipoprotein receptors. Mutant forms of apoE lead to an impaired clearance of chylomicron and very low density lipoprotein remnants (collectively referred to as β-VLDL) by the liver [2]. In the APOE gene, located on chromosome 19 as part of an apolipoprotein gene cluster [4], three major alleles (APOE*2, APOE*3 and APOE*4) [5-7] can be distinguished. Their resultant protein products (apoE2, apoE3 and apoE4) have a significant impact on normal variation of plasma lipid and lipoprotein parameters [8].

In more than 90% of FD patients, the underlying defect is homozygosity for the isoform apoE2 [1,9]. About 1% of the general population is homozygous for apoE2, and although all these subjects have detectable β-VLDL, only about 1-10% of them will develop type III hyperlipoproteinemia [1]. In a minority of cases, heterozygosity for certain rare mutants in or near the LDL receptor-binding domain of the apoE protein has been shown to be associated with FD [1,2,10]. In these instances where dominant transmission has been ascertained, there is almost complete penetrance for FD [1]. All these rare mutants, except the apoE3-Leiden variant, involve substitutions of neutral or acidic amino acid residues for basic one in residues 136 to 150, the region of apoE that interacts directly with the LDL receptor [1,2]. ApoE3-Leiden has a seven amino acid tandem duplication of residues 121 to 127 [11,12].

The prevalence of FD is about 1 to 10 per 10000 individuals [2]. Clinical manifestations among apoE2/E2 associated FD are more prevalent in men than in women, and women usually do not express the disorder until after

menopause [13]. Premature coronary and peripheral atherosclerosis occur in one third to more than half of individuals with FD [2]; in 1975, Morganroth[13] described the occurrence of atherosclerotic vascular disease in about 43% of 49 FD patients and in 1983, Stuyt [14] reported a percentage of 56% in 39 patients. Recently, Dobmeyer [15] found atherosclerosis in 41% of 78 FD patients. In all studies, this was equally distributed over coronary and peripheral arteries [13-15]. Because of the familial nature of FD and the predisposition of FD for atherosclerotic vascular disease, it is common practice to screen family members of affected patients.

In kindreds with FD due to heterozygosity for certain rare mutants, such as the APOE*3-Leiden variant, the penetrance is about 100%, the age at onset of clinical symptoms appears to be much younger, and many of the affected family members are premenopausal women [1,16]. However, little is known about the development of atherosclerotic vascular disease in these autosomal dominant FD patients. Therefore, we investigated overall mortality from 1800 to the present and causes of death from 1941 through 1994 in proven, obligate and potential carriers of the APOE*3-Leiden allele in one extended pedigree with FD (ancestors born 1718), to obtain an estimate of the impact of the hereditary defect of this allele on mortality and causes of death.

Methods

Study population

By screening of FD patients, five probands heterozygous for the APOE*3-Leiden allele were identified in lipid clinics in Nijmegen and Leiden. Genealogical studies revealed that these five probands share common ancestors in 1718 through four main branches. This pedigree has been described in detail elsewhere [16] (updated pedigree in appendix). All proven carriers of the APOE*3-Leiden allele, obligate and potential carriers were included in this analysis.

Proven carriers had been confirmed by DNA analysis. Obligate carriers were family members who had passed on the allele from their ancestors to their offspring. Potential carriers were defined as all first degree relatives of carriers (i.e., parents, children, siblings). Some of the potential carriers were also screened for the APOE*3-Leiden allele. However, the outcome of the DNA test was only used to extend the pedigree, meaning that all potential carriers were part of the study population regardless of their DNA test. Since only living family members could undergo the test, excluding potential carriers *after* the test would introduce bias.

Using parish records, municipal registers and national archives, we retrieved the dates of birth and death for all individuals. Follow-up extended from the date of birth until the date of death or until December 31, 1994. Because the obligate carriers had lived until the birth of the proven carrier to pass on the mutation, the person-years lived before the birth of this child were ignored in these obligate carriers. For the five probands, person-years were counted from the date of testing onwards.

Causes of death

In addition to overall mortality, we were able to obtain information on all causes of death of persons who died in the Netherlands between 1941 and the end of 1994. These were made available by the Netherlands Central Bureau of Statistics, where one copy of each death certificate is kept. The causes of death are routinely coded by this Bureau using the coding rules from the *International Classification of Diseases*, Fifth through Ninth Revision (ICD-5 through ICD-9 [17-21]). For the analysis, the older ICD codes were recoded to the Ninth Revision. We categorized the causes of death into malignant neoplasms (ICD-9 codes 140.0-209.9), diseases of the circulatory system (ICD-9 codes 390.0-459.9), and remaining causes of death.

Statistical analysis

Mortality data were analyzed with the family tree mortality ratio method [22,23]:

the overall and cause-specific mortality of the family members (observed) was compared with that of the general Dutch population (expected) adjusted for sex, age and calendar period. The ratio of observed to expected number of deaths is the standardized mortality ratio (SMR), which can be viewed as a relative risk associated with carriership. The expected mortality is derived by multiplying the total number of years lived by the study population per category of sex, age and calendar period with the corresponding cause-specific population mortality rates obtained from the annual reports of the Netherlands Central Bureau of Statistics, using the computer program 'Person-Years' [24]. The 95 percent confidence interval (95% CI) for the SMR is based on a Poisson distribution for the observed number of deaths [25].

The calendar periods were divided into a fifty-year interval from 1800 to 1850, twenty-year intervals from 1850 to 1890, a fifteen-year interval from 1890 to 1905, and into ten-year intervals from 1905 through 1994, based on the availability of population rates. To each of these periods we applied the population mortality rates of the mid-interval year, subdivided by sex and into five-year age groups. For the causes of death the calendar periods were assigned to five-year intervals from 1941 through 1994, subdivided by sex and into five-year age classes. To each of these periods we applied the cause-specific population mortality rates of the mid-interval year, starting from the mid-interval year 1943 (period 1941-1945), 1948 (period 1946-1950) until 1993 (period 1991-1994).

Results

Characteristics of the study population

Table 1 gives an overview of the characteristics of the different parts of the pedigree. This pedigree consisted of nine generations (Appendix). The top of the pedigree were the six earliest generations and the four branches contained

Table 1. Characteristics and observed and expected number of deaths for the different parts of the pedigree between 1800 and 1994

Branches#	Total number	(Obligate) carriers	Potential carriers	Mean life span [SE]	Obs	Exp	SMR [95% CI]	Person-years
top	117	20	97	46 [3.1]	82	71.2	1.2 [0.9-1.4]	3,315
10 (CV)	47	8	39	75 [4.3]	11	16.4	0.7 [0.3-1.2]	2,043
11 (GW)	12	6	6	80 [4.6]	2	2.6	0.8 [0.1-2.8]	466
12 (JE)	31	11	20	80 [2.3]	3	7.2	0.4 [0.1-1.2]	1,189
13 (AB-JB)	59	23	36	69 [3.5]	12	9.4	1.3 [0.7-2.2]	2,054
	266	68	198	60 [2.8]	110	106.8	1.0 [0.8-1.2]	9,067

 # initials of the five probands between parentheses (see appendix for overview of the pedigree)
The top of the pedigree are the six earliest generations with the common ancestors.
Branches 10, 11, 12, 13 are the four branches of the pedigree, each consisting of the four latest generations.
SE: standard error; SMR: standardized mortality ratio; 95% CI: 95% confidence interval

the four most recent generations. From 1800 through 1994, 266 family members were followed over 9,067 person-years (145 men over 4,622 person-years and 121 women over 4,445 person-years). Of the 68 APOE*3-Leiden carriers, 43 were certain carriers, proven by testing, and the other 25 were obligate carriers, proven by transmission. A total of 149 potential carriers were a first degree relative of a carrier and had not been tested themselves, while the other 49 potential carriers appeared to be non-carriers after testing. Nevertheless to avoid bias, all 198 potential carriers were included in the analysis. The mean life expectancy of the whole study population, both in men and women, was 60 years (standard error (SE) 2.8 years). The top of the pedigree had a lower mean life expectancy (46 years, SE 3.1 years) than the four recent branches of the pedigree (Table 1).

All-cause mortality

We studied the mortality patterns among proven, obligate and potential carriers in this pedigree with apoE3-Leiden associated FD. From 1800 through 1994, 110 deaths (59 in men and 51 in women) occurred and 107 deaths were

expected (SMR 1.0; 95% CI 0.8-1.2). Excess mortality was absent in both men and women. Although the SMR varied among the top of the pedigree and the four recent branches, there was no excess or deficit mortality in any part of the pedigree (Table 1). Furthermore, as shown in figure 1, in none of the calendar periods the SMR differed from unity. There was more variation among the different age groups, especially at younger ages (Figure 2); in childhood (1-9 years) the SMR was halved (95% CI 0.2-1.0), whereas in puberty (SMR 2.1; 95% CI 0.9-4.1) and late twenties (SMR 2.5; 95% CI 1.2-4.4) the SMR was more than doubled (Figure 2).

Causes of death

Between 1941 and 1994, 139 individuals from the four recent branches of the pedigree could be followed over 4,676 person-years (84 men over 2,532 person-years and 55 women over 2,144 person-years). From 1941 onward, 19 family members died, whereas 27 deaths were expected (SMR 0.7; 95% CI 0.4-1.1).

For the analysis of the causes of death, all 19 death certificates (13 men and only 6 women) could be reviewed. The observed and expected number of deaths for the different causes of death are given in table 2. Most individuals died of diseases from the circulatory system (12 out of 19: 63%), and the observed number of deaths from circulatory diseases did not differ from the expected number (SMR 1.1; 95% CI 0.6-2.0). Half of the deaths from the circulatory system were due to ischemic heart diseases (SMR 1.1; 95% CI 0.4-2.4), and there were two deaths from cerebrovascular diseases (SMR 0.8; 95% CI 0.1-2.8). Four family members died of other circulatory disorders, such as peripheral atherosclerosis and hypertension (SMR 1.5; 95% CI 0.4-3.8). It was striking that there were only two cancer deaths, whereas 7.2 individuals were expected to die from malignant neoplasms (SMR 0.3; 95% CI 0.03-1.0). Only two of the 19 verified deaths occurred before the age of 60, both in the category of "remaining" causes of death.

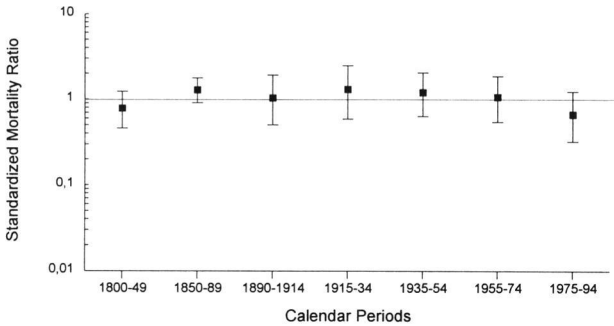

Figure 1. Standardized mortality ratios and 95% CI for the different calendar periods between 1800 and 1994 over all age groups

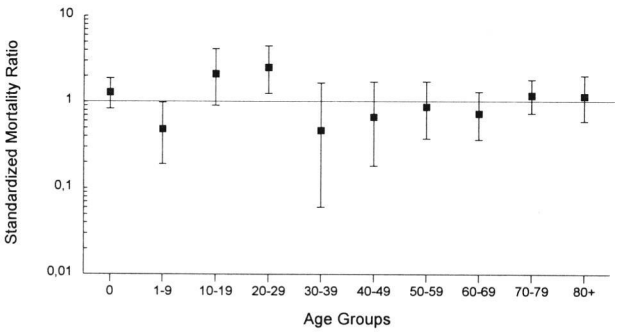

Figure 2. Standardized mortality ratios and 95% CI for the different age groups between 1800 and 1994

Table 2. Observed and expected number of deaths for the different causes of death between 1941 and 1994

Condition	Gender	Observed	Expected	SMR [95% CI]
All-cause mortality	All	19	27.0	0.7 [0.4 - 1.1]
	Men	13	16.5	0.8 [0.4 - 1.4]
	Women	6	10.5	0.6 [0.2 - 1.2]
Malignant neoplasms	All	2	7.2	0.3 [0.03 - 1.0]
	Men	1	4.3	0.2 [0.01 - 1.3]
	Women	1	2.9	0.4 [0.01- 1.9]
Circulatory diseases	All	12	10.8	1.1 [0.6 - 2.0]
	Men	8	6.7	1.2 [0.5 - 2.4]
	Women	4	4.1	1.0 [0.3 - 2.5]
Remaining causes of death	All	5	9.0	0.6 [0.2 - 1.3]
	Men	4	5.5	0.7 [0.2 - 1.9]
	Women	1	3.5	0.3 [0.01 - 1.6]

Discussion

We studied the mortality patterns in one extended pedigree with APOE*3-Leiden associated FD. Between 1800 and 1994 the overall mortality did not differ from unity (SMR 1.0; 95% CI 0.8-1.2) and there were no major distinctions among the different parts of the pedigree. In addition, there was no excess mortality in any calendar period. The absence of excess mortality in the family members compared to the general population leads to the conclusion that carriership alone is not likely to result in premature death. This is a surprising result, because in a previous study all subjects exhibiting heterozygosity for the APOE*3-Leiden allele expressed clinical symptoms of FD [16].

Among the age groups there was some variation in the SMR, especially at younger ages; in childhood (1-9 years) the SMR was 0.5 (95% CI 0.2-1.0), whereas the SMR was doubled in puberty (SMR 2.1; 95% CI 0.9-4.1) and late twenties (SMR 2.5; 95% CI 1.2-4.4). Above thirty years of age the SMR did not differ from unity. It is possible that the decreased SMR in childhood may be associated with a reduced mortality risk from infectious disease due to high cholesterol concentrations in APOE*3-Leiden carriers. In the literature, there are several indications for an inverse relation between total cholesterol levels and mortality risk from cancer, respiratory and infectious disease [26,27]. In general, in individuals with dominant FD, such as the APOE*3-Leiden variant, the age at onset of clinical symptoms is much younger than in recessive FD, and include teenagers [1,16]. Probably, the family members who died between 10 and 30 years of age, were APOE*3-Leiden carriers who were, also due to the presence of other risk factors, highly susceptible for the development of atherosclerotic vascular disease. As the calendar time from the mortality of this particular age group was before 1940, we could not verify this hypothesis with death certificates.

Most family members died of diseases from the circulatory system, which was

as expected, but the observed number of circulatory deaths was equal to the expected number (SMR 1.1; 95% CI 0.6-2.0). From these 12 deaths from the circulatory system six were due to ischemic heart diseases, two to cerebrovascular diseases, and four to other circulatory disorders, such as peripheral atherosclerosis and hypertension. In the literature [13-15], coronary and peripheral atherosclerosis were equally present in patients homozygous for APOE*2 with type III hyperlipoproteinemia (in contrast with the distribution in the population and in patients with hypercholesterolemia) and cerebrovascular diseases were rare. This seems in accordance with our findings although only one death from other circulatory disorders was due to peripheral atherosclerosis. It has been shown that FD patients homozygous for APOE*2, had a slightly higher incidence of ischemic heart disease [2,28]. In the present study we found no indication of excess mortality, neither for all causes nor for ischemic heart disease. Borrie [28] also found no increased death from ischemic heart disease in first degree relatives of patients with type III hyperlipoproteinemia. Slack and Nevin [29], investigating first degree relatives of patients with hypercholesterolemic xanthomatosis with hypertriglyceridemia, made a similar finding. Unfortunately, these two studies did not mention whether overall mortality in the family members was different from that in the general population.

From 1941 through 1994 the overall mortality appeared slightly decreased (SMR 0.7; 95% CI 0.4-1.1). The main reason for this decreased all-cause SMR between 1941 and 1994 was that only two family members died of cancer (SMR 0.3; 95% CI 0.03-1.0). Obviously, this might have been a chance finding. Another explanation might be that these families are truly at a lower risk for cancer. In the literature, several mechanisms have been proposed for the finding that low cholesterol concentrations may be associated with an increased mortality risk from cancer and respiratory disease [26]. Additionally, a recent study in people older than 85 years, showed a reduced mortality risk from cancer and infection among individuals in the highest cholesterol category, explaining longevity in this category [27]. As patients with FD are charaterized by

elevated plasma cholesterol and triglyceride levels, the APOE*3-Leiden variant potentially may lead to human longevity, providing that atherosclerotic vascular diseases could be prevented. This would be in line with recent findings of longevity associated with the apoE2 variant [30]. However, the number of observed deaths in our investigation from 1941 onwards, are too small for definite conclusions on cause-specific mortality.

The specific DNA mutation involved in APOE*3-Leiden is probably limited to this single family. Nevertheless, our findings might be generalizable, because there are no clear indications in the literature that the effect of the APOE*3-Leiden allele may be distinct from the common recessive type of FD. The generalizability is further enhanced, because this is a very large pedigree, going back several centuries: there is so much admixture with other genes from the surrounding Dutch population, that the only genetic similarity between all persons of this pedigree might be the relatively short stretch of DNA, where the mutation can be found. Clinical symptoms of FD occur in less than 10% of patients associated with homozygosity for the isoform apoE2, whereas almost all patients with dominant transmission of apoE mutants show clinical symptoms and at a younger age [1,2,16]. For a long time it has been suggested that, also because of the low penetrance of FD in homozygous APOE*2, other genetic or environmental factors are required to develop clinical manifestations [1,2,5,31]. As seen in our previous study about the same APOE*3-Leiden pedigree [16], the effect of FD is enhanced by increasing age and by APOE*2 as the second allele. This might also indicate that other genetic or environmental factors are necessary to lead to clinical expression. From the present study we conclude that, there was no overall excess mortality in this extended pedigree with APOE*3-Leiden associated FD, although all carriers develop clinical symptoms of FD. Therefore, screening of family members of APOE*3-Leiden carriers for the mutation is unlikely to offer survival benefits.

Acknowledgments

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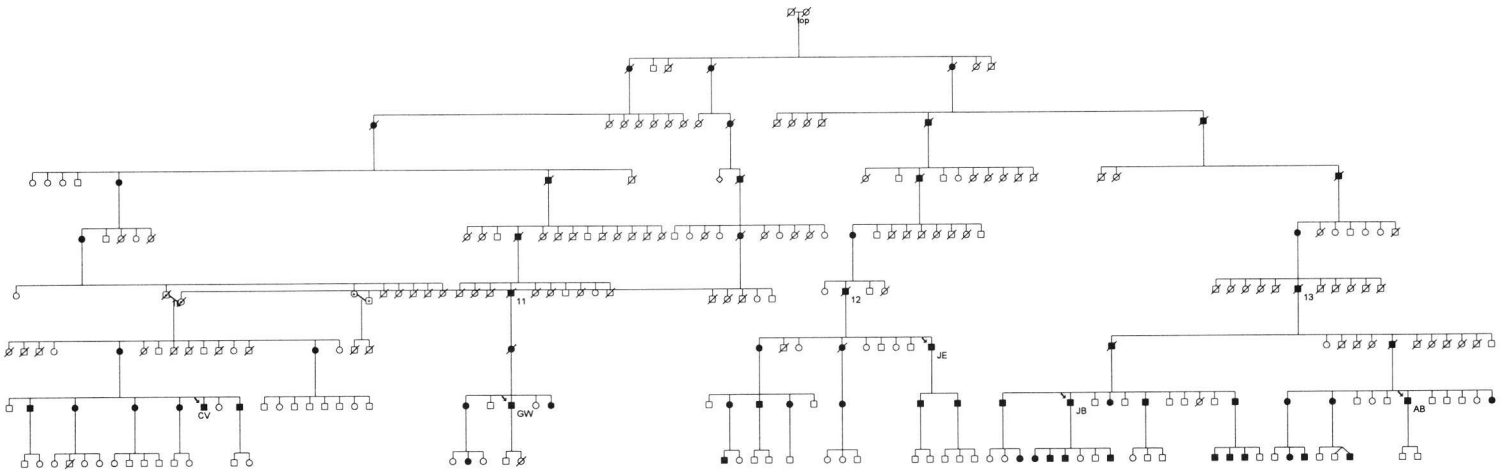

*Appendix. The extended apolipoprotein E3-Leiden pedigree with all 266 proven, obligate and potential carriers without spouses. The pedigree consists of nine successive generations. The probands are indicated with an arrow and with their initials. Each of the four recent branches is indicated with a family number (10, 11, 12 or 13). In family 13 there are two probands. ●/■: proven or obligate APOE*3-Leiden carriers; ○/□: potential APOE*3-Leiden carriers; ⊙/◙: subjects with 50%-100% probability of carrying APOE*3-Leiden, in table 1 classified as being potential carriers; □/■: men; ○/●: women; ◇: gender unknown; crossed off subjects are individuals with a known date of death.*

Chapter V

Excess Cancer Mortality in Six Dutch Pedigrees with the Familial Atypical Multiple Mole-Melanoma Syndrome from 1830 to 1994

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Abstract

In some reports of familial atypical multiple mole-melanoma kindreds an increased incidence of systemic cancers has been described. If the gene defect underlying the familial atypical multiple mole-melanoma syndrome is not only important for the development of melanoma of the skin, the impact of the defect on life expectancy may be much higher than previously thought. We investigated all-cause mortality from 1830 to the present and causes of death from 1941 to 1994 in proven, obligate and potential CDKN2 mutation carriers, to obtain an estimate of the impact of a hereditary defect of the CDKN2 gene on mortality. From 1830 to 1994, 65 deaths took place, whereas only 42 deaths were expected (SMR 1.6, 95% CI 1.2-2.0) and the SMR doubled with calendar time. Excess mortality was shown in most of the families, but was confined to the ages 35 to 70 y (SMR 2.1, 95% CI 1.5-2.9). Excess mortality could be fully attributed to cancer mortality, especially to pancreatic carcinoma and melanoma of the skin. Among the families there appeared to be some heterogeneity, especially due to the specific cancer pattern within a family. The impact of the defect of the CDKN2 gene is rising over calendar time, mainly because the mortality in the general population has been falling. Excess mortality was not only due to melanoma, but also to pancreatic carcinoma. Therefore, follow-up programs of affected family members should not be confined to regular check of the atypical nevi.

Introduction

Since 1951 there has been mounting evidence that up to 12% of cutaneous melanomas cluster in families [1-5]. In many of these families with a susceptibility to melanoma, the individuals have an underlying syndrome of multiple atypical nevi [3,4,6]. This condition, known as the familial atypical multiple mole-melanoma (FAMMM) syndrome [7], is also known as the B-K mole syndrome [8],

the dysplastic nevus syndrome [9], or the atypical mole syndrome [10]. The underlying gene defect has an autosomal dominant inheritance pattern with variable expression and incomplete penetrance [6], but it seems plausible that more genes are involved [4]. The three known melanoma susceptibility loci are located on 1p36 [11], 12q13 [12], and 9p21 [13]. The latter region contains a tumor suppressor gene, the CDKN2 gene also known as $p16^{INK4}$. The protein p16 is a cell cycle regulator and inhibits the kinase activity of CDK4/cyclin D [14].

In some reports of FAMMM kindreds an increased incidence of systemic cancers has been described [15-17]; according to others this can be attributed to the method of ascertainment of the families [18]. Neoplasms most often found in those families, besides melanoma of the skin, are non-melanoma skin cancer, and cancers of the lung, breast, stomach, pancreas, large bowel, and endometrium [3]. If the gene defect underlying the FAMMM syndrome is not only important for the development of melanoma of the skin, but also for a whole range of other life threatening systemic neoplasms, the impact of the defect on life expectancy may be much higher than previously thought. Knowledge of the natural history of the effects of mutations in the CDKN2 gene may have consequences for follow-up programs of affected family members.

Life expectancy in individuals with the FAMMM syndrome can only be studied during a long-term follow-up period. We developed a method to study the survival of family members retrospectively by extending the follow-up period to the past [19]. In this study we selected six FAMMM pedigrees and investigated all-cause mortality from 1830 to the present and causes of death from 1941 to 1994 in proven, obligate and potential carriers, to obtain an estimate of the impact of a hereditary defect of the CDKN2 gene on mortality and causes of death.

Methods

Study population

In February 1982 the Pigmented Lesions Clinic of the Department of Dermatology was established, facilitating studies on familial melanoma. From this database, 10 families were found with more than two melanoma patients in at least two consecutive generations and in which several family members showed atypical nevi. These FAMMM families have been described in detail elsewhere [6,16,20,21]. Six families, all showing an autosomal dominant pattern of inheritance for a 19 bp deletion in exon 2 of the CDKN2 gene, were included in our current investigation [22]. Two of the four remaining families had the deletion but were not suitable for the mortality analysis because of an unclear inheritance pattern of the CDKN2 gene and two families originated from a different part of the Netherlands and did not show the deletion.

We restricted the study population to proven carriers of the deletion in CDKN2, obligate and potential gene carriers. Proven CDKN2 deletion carriers had been confirmed by DNA analysis to carry the mutation [22]. Obligate carriers were family members who had passed on the gene from common ancestors to gene carriers amongst their offspring. Potential carriers were defined as all first degree relatives of gene carriers. Some of the potential carriers could be tested for the deletion; however, the outcome on the DNA test was only used to enlarge the pedigrees, meaning that all potential carriers were part of the study population regardless of their DNA test. (Because only living family members could undergo the test, excluding potential carriers after the test would introduce bias towards deceased people.)

Using church and municipal registers of births, marriages, and deaths, we retrieved the dates of birth and death for all individuals. Because the obligate carriers had to live until the birth of the proven carrier to pass on the mutation, the person-years lived before 20 y of age were ignored in these carriers. Besides, because only a few melanoma occur before 20 y of age, the best estimate of the

gene impact on life expectancy can be made from this age on. Therefore, follow-up extended from 20 y after the date of birth to the date of death or to December 31, 1994.

Causes of death

We were able to survey all causes of death of persons who were deceased in the Netherlands between 1941 and 1994. These were made available by the Netherlands Central Bureau of Statistics, where one copy of each death certificate is kept. The causes of death are routinely coded by this Bureau using the coding rules from the International Classification of Diseases, fifth through ninth revision (ICD-5 through ICD-9 [23-27]). For the analysis, the older ICD codes were recoded conform the ninth revision. We classified the deceased persons into malignant neoplasms (ICD-9 codes 140.0-209.9), diseases of the circulatory system (ICD-9 codes 390.0-459.9), and remaining causes of death. Malignant neoplasms were further divided into cancers of the digestive organs other than pancreas (ICD-9 codes 150.0-159.9, excl. 157), pancreatic carcinoma (ICD-9 codes 157.0-157.9), melanoma of the skin (ICD-9 codes 172.0-172.9), and remaining cancers. The comparison of causes of death was based on death certificate classifications to guarantee a valid comparison as the classification was the same for both study and control populations.

Statistical analysis

Mortality data were analyzed with the Family Tree Mortality Ratio method[19,28]: the overall and cause-specific mortality of the FAMMM-families (observed) was compared with that of the general Dutch population adjusted for sex, age, and calendar period (expected). The ratio of observed to expected number of deaths is the standardized mortality ratio (SMR), which can be viewed as a relative risk. The expected mortality was derived by multiplying the total number of y lived by the study population per category of sex, age and calendar period with the corresponding cause-specific population mortality rates obtained from the annual reports of the Netherlands Central Bureau of Statistics, using the computer program 'Person-Years' [29]. The 95% confidence interval (95% CI) for

the SMR is based on a Poisson distribution for the observed number of deaths [30].

The calendar periods were divided into 20 y intervals from 1830 to 1889, a 15 y interval from 1890 to 1904, and into 10 y intervals from 1905 to 1994. To each of these periods we applied the population mortality rates of the mid-interval year, subdivided by sex and into 5 y age groups. For the causes of death the calendar periods were assigned to 5 y intervals from 1941 to 1994, subdivided by sex and into 5 y age classes. To each of these periods we applied the cause-specific population mortality rates of the mid-interval year, starting from the mid-interval year 1943 (period 1941-1945), and running until 1993 (period 1991-1994).

Finally, to study differences among the families, we extended the SMR analysis with Poisson regression analysis, using 'EGRET' [31]. This multivariate analysis calculated rate ratios for the different families adjusted for sex, age, and calendar periods. We determined the differences between the families for all-cause mortality and for mortality from malignant neoplasms from 1941 to 1994. The pedigree that contributed most of the individuals and person-years to the analysis was taken as the reference category.

Table 1. Characteristics of the study population, ≥20 y of age

FAMMM family	Number	(Obligate) carriers [a]	Potential carriers	Mean age at death (SD) [b]	Number of deaths	Person-years
1	25	12	13	61.7 (14.8)	9	646
3	40	15	25	69.5 (17.0)	18	1418
4	52	35	17	58.4 (17.5)	16	1316
5	20	9	11	50.1 (13.6)	4	437
10	56	24	32	58.9 (17.4)	12	1439
19	31	15	16	58.6 (12.1)	6	766
	224	110	114	61.5 (16.7)	65	6022

[a] *proven and obligate carriers;* [b] *SD: standard deviation*

Results

Characteristics of the study population

Table 1 gives an overview of the characteristics of the study population for the six families. From 1830 through 1994, 224 family members, who were at least 20 y of age, were followed over 6022 person-years. There were 87 proven, 23 obligate, and 114 potential CDKN2 gene carriers. Birth year ranged from 1829 to 1974 and year of death from 1874 to 1994. The mean age at the end-of-study date was 40.9 y (SD 15.4). For the entire study population the mean age at death was 61.5 y (SD 16.7). In the 126 men the mean age at death was 59.1 y (SD 17.0), whereas the 98 women had a mean age at death of 65.3 y (SD 15.8).

All-cause mortality

Over the whole period 65 deaths occurred, whereas only 42 deaths were expected (SMR 1.6, 95% CI 1.2-2.0). In men, 40 individuals died in 3,497 person-years, leading to a SMR of 1.5 (95% CI 1.1-2.0), and 25 women died in 2,525 person-years (SMR 1.6, 95% CI 1.1-2.4). Excess mortality was shown in most of the families, although there was some heterogeneity between the families (Figure 1). The SMR doubled with calendar time, from 1870 to the present (Figure 2). From the calendar period 1925-1944 onward, the SMR differed from unity, but the increase was only significant from 1975 onwards. Furthermore, figure 3 shows that excess mortality was confined to the ages 35-70 y (SMR 42/19.7=2.1, 95% CI 1.5-2.9), with the mortality peak between 40 and 50 (SMR 15/3.8=4.0, 95% CI 2.2-6.6).

Causes of death

For the analysis of the causes of death between 1941 and 1994, 59 death certificates were reviewed. The all-cause SMR over this period was slightly higher than for the whole period, as a result of a higher SMR in women (Table 2). Excess mortality can fully be attributed to malignancies, especially to melanoma of the skin and pancreatic carcinoma, although half of the malignant

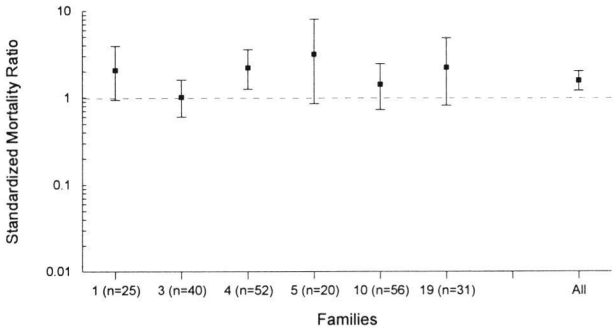

Figure 1. ***Excess mortality is present in most families.*** *SMR with 95% CI, per FAMMM-family from 1850 till 1994, ≥20 y of age*

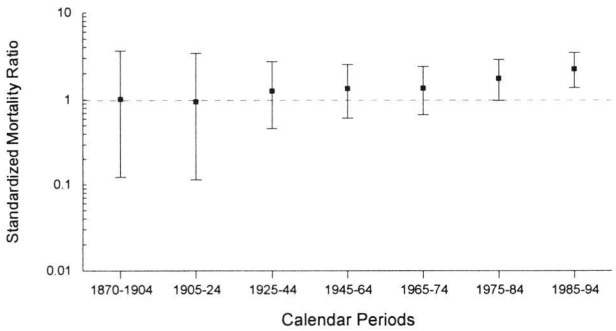

Figure 2. ***The SMR is rising over calendar time.*** *SMR with 95% CI, in FAMMM-families according to calendar periods, ≥20 y of age*

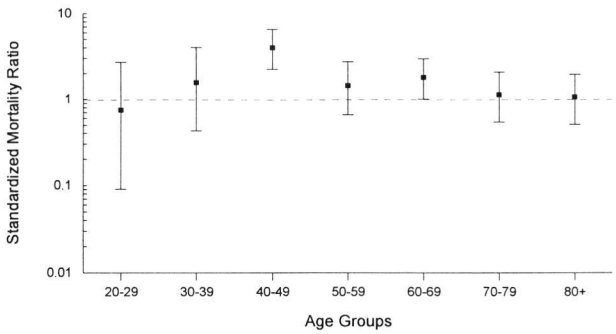

Figure 3. ***Excess mortality is confined to age groups 40-70 y.*** *SMR with 95% CI, in FAMMM-families according to age groups from 1850 till 1994*

neoplasms resulted from several tumors, such as lung, breast and stomach cancers (Table 2). Separating the study population into proven and obligate CDKN2 gene carriers *versus* potential carriers led to very similar patterns in both groups, with, as expected, the most pronounced risks for those who were carriers with certainty (Table 3). Because these families have been ascertained on the basis of a history of melanoma, there may be some bias in the melanoma mortality. The unbiased result would be mortality other than melanoma of the

Table 2. Observed and expected number of deaths for the different causes of death between 1941 and 1994, ≥20 y of age

Condition	Gender	O [a]	E [b]	SMR [c]	95% CI [d]
All-cause mortality	All	59	34.6	1.7	1.3-2.2
	Men	35	22.6	1.6	1.1-2.2
	Women	24	12.0	2.0	1.3-3.0
Malignant neoplasms	All	44	9.4	4.7	3.4-6.3
	Men	26	6.1	4.3	2.8-6.2
	Women	18	3.3	5.5	3.2-8.6
Digestive cancer [e],	All	7	2.7	2.6	1.1-5.4
excl. pancreas	Men	3	1.6	1.8	0.38-5.3
	Women	4	1.0	3.9	1.1-9.9
Pancreatic carcinoma	All	12	0.38	31.4	16-55
	Men	7	0.25	28.0	11-58
	Women	5	0.13	37.6	12-90
Melanoma of skin	All	10	0.09	110	53-204
	Men	5	0.06	87	27-194
	Women	5	0.03	148	54-389
Remaining cancers [f]	All	15	6.2	2.4	1.4-4.0
	Men	11	4.1	2.7	1.3-4.8
	Women	4	2.1	1.9	0.52-4.9
Circulatory diseases	All	8	13.9	0.57	0.25-1.1
	Men	5	9.2	0.54	0.18-1.3
	Women	3	4.8	0.63	0.13-1.8
Remaining causes of death	All	7	11.3	0.62	0.25-1.3
	Men	4	7.3	0.55	0.15-1.4
	Women	3	3.9	0.76	0.16-2.2

[a] observed number of deaths; [b] expected number of deaths; standardized mortality ratio; 95% confidence interval; [e] 4 stomach, 2 biliary ducts, 1 colon; [f] 5 lung, 3 breast, 2 brain, 1 salivary gland, 1 nasopharynx, 1 prostate, 1 abdomen, 1 disseminated

skin. In these families there were 49 deaths for reasons other than melanoma, leading to a SMR of 1.4 (95% CI 1.1-1.9) and there were 34 deaths from cancers other than melanoma, with a SMR of 3.6 (95% CI 2.5-5.1).

Table 3. Similar mortality pattern for potential carriers, though diluted, as for proven and obligate carriers

	(Obligate) carriers		Potential carriers	
Condition	Observed	SMR	Observed	SMR
All-cause mortality	29	2.9	30	1.2
Malignant neoplasms	25	8.7	19	2.9
Digestive cancer, excl. pancreas	4	5.0	3	1.6
Pancreatic carcinoma	7	63.6	5	17.8
Melanoma of skin	6	150	4	66.7
Remaining cancers	8	4.2	7	1.6
Circulatory diseases	2	0.6	6	0.6
Remaining causes of death	2	0.6	5	0.6

Table 4 shows all-cause mortality and deaths from malignant neoplasms for the different families compared with the general Dutch population (SMR) and compared with pedigree 10 (poisson rate ratio; the largest family). None of the differences between the families were significant. Furthermore, it was striking that in two kindreds (FAMMM 1 and 5) no one died from pancreatic cancer, and that in pedigree number 5 even no deaths from melanoma of the skin were mentioned on the death certificates (Table 4).

Discussion

We studied the mortality patterns in six FAMMM pedigrees. The all-cause lifetime SMR for proven, obligate and potential carriers with a deletion in the CDKN2 gene was 1.6, with no significant differences between men and women. Excess mortality started after the age of 30 y and could be fully attributed to cancer mortality. We found a secular increase of mortality in the FAMMM

families, mainly because of decreasing mortality rates in the Dutch population over the past century. From the calendar period 1925-44 onward, the SMR differed from unity, but the increase was only significant from 1975 onwards. This means that the impact of the 19 bp deletion in exon 2 of the CDKN2 gene is rising; in comparison with the general population it becomes more disadvantageous to carry this gene.

All the FAMMM families were detected from the Pigmented Lesions Clinic as six separate families; however, when we found out that these families all had the same 19 bp deletion in exon 2 of the CDKN2 gene, we started looking for common ancestors, especially because all pedigrees came from the region of Leiden. From the finding of shared haplotype among the melanoma patients, it had already been suggested that there was a common mutation in the pedigrees [22,32]. Genealogical investigation could confirm this assumption: families 1 and 4 have common ancestors in 1775, and together with kindred 3, they have a common ancestor in 1707. Pedigrees 10 and 19 have common ancestors in 1763. We expect all these FAMMM families to have one founder (far) before 1700. This means that the differences between the families can be seen as differences between branches of one pedigree. As a consequence of new incoming genes through spouses, differences between the different branches develop.

There appears to be some heterogeneity among the families, especially when taking into account the specific cancer pattern within a family. In FAMMM families 1 and 5, there were no deaths from pancreatic carcinomas and in kindred 5 no deaths from melanoma of the skin were mentioned. This can be due to small numbers. Compared with family 10, all other kindreds had higher mortality rates, though not significant. Families 1 and 4 were expected to be more equal than the other families, because of nearness of their common ancestor. For the all-cause mortality and total cancer mortality estimates this seemed to hold, although the specific cancer patterns in these families differed.

Table 4. All-cause mortality and deaths from malignant neoplasms for the different families compared to the general Dutch population (SMR) and compared to each other (PRR) between 1941 and 1994, ≥20 y of age

	All-cause mortality			Malignant neoplasms						
FAMMM family	O [a]	SMR [b]	PRR [c]	O [a]	SMR [b]	PRR [c]	Dig [d]	Pan [e]	Mel [f]	Rem [g]
1	8	2.2 [0.9-4.3]	2.1 [0.7-5.9]	6	5.4 [2.0-12]	2.8 [0.8-9.5]	2	0	2	2
3	18	1.2 [0.7-1.9]	1.3 [0.5-3.1]	14	3.9 [2.1-6.5]	2.0 [0.7-5.8]	3	4	2	5
4	16	2.4 [1.4-3.9]	1.9 [0.8-4.6]	11	6.0 [3.0-11]	2.2 [0.8-6.5]	0	4	2	5
5	3	2.5 [0.5-7.3]	1.6 [0.4-5.9]	2	4.9 [0.6-18]	1.5 [0.3-7.8]	0	0	0	2
10	8	1.5 [0.6-2.9]	1	5	3.2 [1.0-7.5]	1	1	2	2	0
19	6	2.2 [0.8-4.8]	1.5 [0.5-4.4]	6	6.7 [2.5-15]	2.3 [0.7-7.6]	1	2	2	1

[a] observed number of deaths; [b] standardized mortality ratio with 95% CI in brackets; Poisson rate ratio with the largest family (family 10) as the reference group and 95% CI in brackets; [d] observed number of death from digestive cancer, excl. pancreas; observed number of death from pancreatic carcinoma; observed number of death from melanoma of skin; [g] observed number of death from remaining cancers

Half of the cancer deaths were due to melanoma (10 deaths) and pancreatic neoplasms (12 deaths). In addition, we also found an increased mortality from digestive carcinomas other than pancreatic (four deaths from stomach cancer), and from remaining cancers such as lung (five deaths) and breast (three deaths) cancers. All these types of neoplasms had already been described in previous reports of FAMMM kindreds, and relative risk estimates for melanoma and pancreatic carcinoma were similar [3,15-17]. These results confirm the recent findings from Whelan [33] that CDKN2 plays an important role in both melanoma and pancreatic tumorgenesis. In the same issue, Goldstein [34] concluded that for the occurrence of pancreatic cancer in FAMMM families, a mutation in the CDKN2 gene is required and that differences in cancer patterns between families might be explained by the kind of mutation; however, from the Dutch families it has already been suggested that the differences in cancer patterns cannot exclusively be explained by the kind of CDKN2 mutation, but have to be influenced by other environmental or genetic factors[35]. The impression that it is really the CDKN2 gene that is causing the pancreatic carcinoma, is reinforced as the mortality risk was stronger in proven and obligate gene carriers than in potential gene carriers. In both groups excess mortality could be fully attributed to cancer mortality, especially to pancreatic carcinoma and melanoma of the skin. Further evidence was given by the study of Gruis [22], where loss of heterozygosity of CDKN2 was shown in pancreatic carcinoma tissue of one of the family members.

These FAMMM pedigrees have been ascertained on having more than two melanoma patients in at least two consecutive generations from the Pigmented Lesions Clinic. Therefore, the results could be biased for deaths from melanoma. When omitting the melanoma mortality, the excess mortality remained, meaning that deaths from other cancers were as important as the melanoma mortality. This is slightly different from the morbidity figures, where melanoma incidence seems the most pronounced. Follow-up programs for the CDKN2 mutation carriers in these FAMMM kindreds, at this moment, consist of yearly check of the atypical nevi; however, on the basis of our results the

question is whether these gene carriers should not be screened for cancers other than melanoma, especially for pancreatic carcinoma. The problem is that currently no appropriate intervention programs are available. From that perspective, it might be worthwhile to study, whether screening on serum tumor markers, like CA19-9 or CA494 [36-40] in combination with regular endoscopic ultrasonography or magnetic resonance imaging, for pancreatic carcinoma in CDKN2 mutation carriers, might prevent more early mortality in FAMMM families.

Acknowledgments

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Chapter VI

Mortality from 1840 to 1994 in Six Large Families With Hereditary Breast-Ovarian Cancer

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Abstract

To estimate the impact of a hereditary defect on mortality in women from breast-ovarian cancer families, we selected six families with hereditary breast-ovarian cancer, four of which carried a mutation in the BRCA1 gene. We investigated overall mortality from 1840-1994 in 57 affected women, 10 obligate and 108 potential carriers. Follow-up extended from 20 years after birth to death or end-of-study date. The observed number of deaths was compared with the expected number, based on the Dutch population, adjusted for age and calendar time. The ratio of observed to expected is the standardized mortality ratio (SMR).

On 5162 woman-years 69 deaths occurred, where 35 were expected (SMR 2.0; 95% CI 1.5-2.5). Excess mortality varied between families and was confined to the ages 30-60 (SMR 4.4; 95% CI 3.1-6.0). When dividing the follow-up time for these women in four calendar periods, there was a stepwise increase of the SMR, related to excessive mortality in the youngest generations. The SMR in nulliparous women and women with 1-2 children was two times greater than in women with 3+ children. The information for the oldest generations, which was least likely to be affected by ascertainment bias, showed a decreased absolute risk over calendar time.

Introduction

In the past few years it has become clear that a fraction of all breast or ovarian cancer cases is associated with a hereditary defect. Most of these defects are rare in the general population. About five percent of all breast cancer is related to highly penetrant genes such as BRCA1 [1,2] or BRCA2 [3,4] , while another ten percent show familial clustering without a clear mode of inheritance [5-7]. Because of these understandings women in families with multiple and early-onset breast

or ovarian cancer cases qualify for genetic and medical counseling. Despite the molecular genetic discoveries the clinical message is not yet clear. For the purpose of counseling, tables with risk estimations have been formulated for women with a positive family history, to determine to what extent these women should be screened [8,9]. In this light, knowledge of the natural history of women with the genetic predisposition can be used to estimate the mortality that might be prevented.

The impact of the genetic susceptibility on life expectancy can only be investigated during long-term follow-up. Therefore, we developed a method to study the survival of family members retrospectively [10,11]. This method is useful in families with hereditary breast-ovarian cancer, although the contribution of one specific gene mutation is unlikely. In the present study we selected six pedigrees with multiple cases of early-onset breast or ovarian cancer, irrespective of a known defect at a particular locus. We investigated all-cause mortality from 1840 to the present in affected family members, obligate and potential carriers to obtain an estimate of the impact of a hereditary defect on mortality.

Methods

Families

All families, with a history of multiple and early-onset breast or ovarian cancer cases, who were known at and had at least one case under care of the Daniël den Hoed Cancer Center and the Department of Clinical Genetics in Rotterdam, were eligible for study. The pattern of cancer occurrence within each family had to be consistent with Mendelian inheritance of an autosomal dominant susceptibility gene. Six pedigrees had been selected at random, four of which had (evidence for) a mutation in the BRCA1 gene (Table 1). In three families mutations showed up in exon 11 of the BRCA1 locus using the protein

truncation test [12]. In pedigrees 92008 and 93024 a frame shift mutation at codon 431 (1409insT) has been found [12], while the exact mutation in family 91011 has not yet unequivocally been characterized. In family 92011 the lodscore for linkage at the BRCA1 locus was over 3, whereas in the families 93010 and 94068 there was insufficient evidence for linkage (Table 1).

We restricted the study population to affected women, obligate and potential carriers of the disease in the six families. Obligate carriers were women who had passed on the breast or ovarian cancer predisposition from common ancestors to affected individuals (transmitters). Potential carriers were defined as women who were first degree relatives of patients or transmitters. Table 1 gives an overview of the number of successive generations, the number of obligate and potential carriers, and breast or ovarian cancer patients in each of the six families. Overall 57 women had breast or ovarian cancer, 10 women were transmitters of the disease and 108 women had at least one affected first degree relative. We retrieved all dates of birth and death for the study population, using genealogical data provided by the families, supplemented with information from hospital records, municipal registers and state archives. Follow-up extended from 20 years after birth to the date of death or to December 31, 1994. There were two reason for ignoring the first 20 years of life: first, the women who transmitted the predisposition to their offspring had to be alive at the start of the reproductive period, to be able to pass on a mutation, and second, we did not expect a major impact of the predisposition before the procreation period, because otherwise the genetic susceptibility would have died out.

Statistical Analysis

Mortality data were analyzed with the family tree mortality ratio method [10,11]: the all-cause mortality of the study population (observed) was compared with that of the general Dutch female population adjusted for age and calendar period (expected). The expected number of deaths was derived by multiplying the total number of woman-years lived by the study population per category of

age and calendar period with the corresponding mortality rates of the general population obtained from the annual reports of the Netherlands Central Bureau of Statistics, using the computer program 'Person-Years' [13]. The ratio of observed to expected number of deaths is the standardized mortality ratio (SMR). The 95% confidence interval (95% CI) for the SMR is based on a Poisson distribution for the observed number of deaths [14]. The calendar periods were divided into twenty-year intervals from 1830 through 1889, a fifteen-year interval from 1890 through 1904, and into ten-year intervals from 1905 through 1994. To each of these periods we applied the population mortality rates of the mid-interval year subdivided into five-year age groups.

Besides the relative mortality estimates we analyzed life expectancy with the method of Kaplan and Meier [15] to study the influence of birth cohort and parity. Birth cohort was classified into women born before 1900, between 1900 and 1949 and born after 1950. For parity we made a distinction between women who had no children, women with one or two children and those with three or more children.

In order to assess the effect of ascertainment bias, we divided the women in each family into youngest, middle and oldest generations. For the family with three generations in the analysis, the third generation belongs to the youngest generations, and the first and second generation were categorized into the middle generations. In the families with four or five generations in the analysis, the fourth and fifth generation were classed into the youngest generations, the third generation is the middle generation, and the first and second generation were categorized into the oldest generations. For the family with six generations in the analysis, the sixth and fifth generation belong to the youngest generations, the fourth generation is the middle generation, and the first, second and third generation were classed into the oldest generations. Ascertainment bias may play a role in the younger generations; the older generations are unbiased and give therefore the true impact of the genetic susceptibility on mortality.

Table 1. Characteristics of the women in the six families

Family number	Gener-ations	Affected members	*Breast cancer*	*Ovarian cancer*	Obligate carriers	Potential carriers	Mutation BRCA1
91011	V	9	*4*	*5*	3	20	exon 11
92008	V	6	*6*	*2*	2	12	exon 11
92011	VI	10	*10*	*0*	3	24	lod 3.1
93010	IV	14	*6*	*11*	0	22	lod -0.52
93024	III	6	*8*	*0*	0	5	exon 11
94068	IV	12	*12*	*0*	2	25	lod 0.07
		57	*46*	*18*	10	108	

Table 2. Mortality in the separate families, ≥20 years of age

Family number	Total in analysis	Mean age at death*	Number of deaths	Expected	SMR [95% CI]	Woman-years
91011	28	61 [30 - 91]	16	9.4	1.7 [1.0 - 2.8]	1,069
92008	18	51 [32 - 74]	9	1.7	5.4 [2.5 - 10]	502
92011	26	45 [24 - 80]	16	2.7	6.0 [3.4 - 9.8]	685
93010	35	54 [31 - 82]	16	5.8	2.8 [1.6 - 4.5]	1,194
93024	10	62 [46 - 80]	3	1.7	1.7 [0.4 - 5.0]	355
94068	36	69 [25 - 95]	9	14	0.6 [0.3 - 1.2]	1,357
	153	56	69	35.3	2.0 [1.5 - 2.5]	5,162

** range between brackets; SMR: standardized mortality ratio; 95% CI: 95% confidence interval*

Table 3. Mean life expectancy in different birth cohort by parity, ≥20 years of age

Parity	Born <1900		Born 1900-50		Born ≥1950*		All women
	N	Age [SE]	*N*	Age [SE]	*N*	Age [SE]	Age [SE]
0	*2*	56 [18]	*31*	70 [4]	*24*	41 [1]	67 [4]
1-2	*3*	50 [23]	*25*	66 [4]	*11*	40 [2]	65 [5]
≥3	*19*	66 [4]	*34*	72 [3]	*4*	45 [0]	70 [3]
		64 [4]		70 [2]		42 [1]	68 [2]

** limited up to 45 years of age; SE: standard error*

Results

We analyzed the dates of birth and death or the end-of-study date for 153 women, who were at least 20 years of age. Twenty-two women were younger than 20 years at the end of 1994. Dates of birth and death ranged from 1841 to 1968 and from 1894 to 1994 respectively. The mean age of women still alive at the end-of-study date was 51 years (range 26 to 86). On a total of 5162 person-years 69 deaths occurred with a mean age at death of 56 years (range 24 to 95), whereas 35 deaths would have been expected. This leads to a life-time SMR of 2.0 (95% CI 1.5 to 2.5) for women adjusted for age groups and calendar periods. Mortality in men was not increased (data not shown). At least two thirds of these women died of cancer (46 out of 69 deaths), whereas in the Dutch population less than 25% of the women die of malignant neoplasia. All-cause mortality varied strongly between the six separate families (Table 2), and excess mortality was confined to the ages 30 to 60 years (SMR 39/8.8=4.4; 95% CI 3.1-6.0) (Figure 1).

Figure 2 shows the absolute mortality rates over the calendar time for the women between 30 and 60 years, adjusted for the age structure of the families over the whole time period. The corresponding SMR, for all generations together, in the calendar period 1890-1924 was 2.4 (95% CI 0.5 to 6.9), in the calendar period 1925-1944 it was 2.4 (95% CI 0.8 to 5.7), during 1945-1974 it was 4.2 (95% CI 2.2-7.2) and in the most recent period the SMR was 8.0 (95% CI 4.7 to 12.6). The mortality peak in the last twenty years had occurred mainly in the women in the youngest generations (SMR 24; 95% CI 11 to 49). For the women in the oldest generations, which are least likely to be affected by ascertainment bias, mortality rates decreased with calendar time, although its corresponding relative mortality increased from 2.5 (95% CI 0.5 to 7.3) in the period 1890-1924, to 2.7 (95% CI 0.9 to 6.3) in the period 1925-1944, to 5.2 (95% CI 2.3 to 10) in the period 1945-1974.

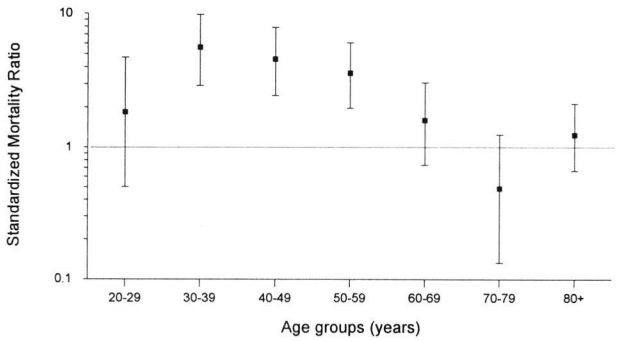

Figure 1. Standardized mortality ratios with 95% CI in 153 affected and potentially affected women according to age

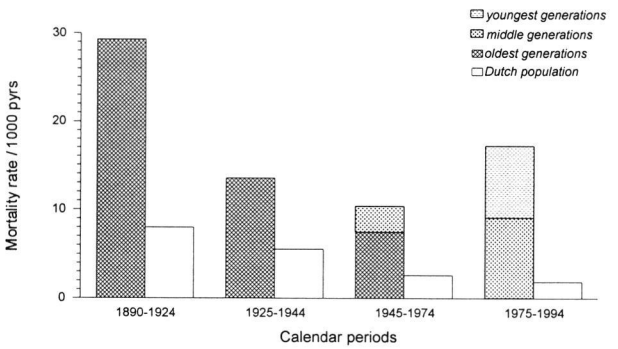

Figure 2. Absolute mortality rates in 153 women in age group 30-60 years, divided into generations, compared with the absolute mortality rates in the Dutch population over calendar periods, adjusted for the age structure of the families over the whole time period

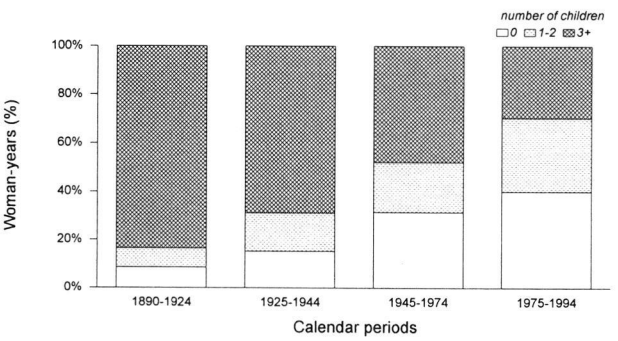

Figure 3. Percent of person-years in 153 affected and potentially affected women ≥20 years of age according to calendar periods by parity

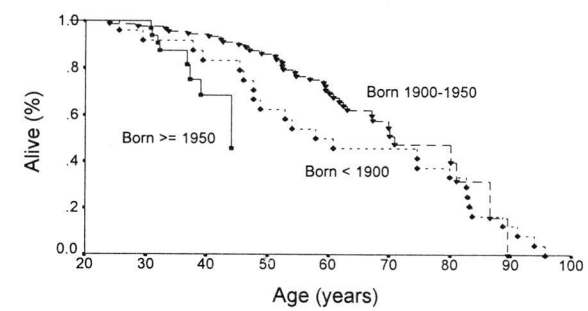

Figure 4. Mean life expectancy in 153 affected and potentially affected women ≥20 years of age divided into birth cohorts

The influence of birth cohort and parity on the secular increase of mortality was studied. The SMR for nulliparous women was 3.2 (95% CI 1.8 to 5.1), for women with one or two children it was 2.9 (95% CI 1.7 to 4.6) and for women with three or more children it was 1.5 (95% CI 1.0 to 2.0). However, there was no difference in life expectancy between these groups (Table 3). From 1890 to the present the number of nulliparous women has increased together with a decrease in the number of women who had three or more children (Figure 3); 80% of the women born before 1900 had three or more children, whereas 62% of the women born after 1950 was still nulliparous (Table 3). In addition, women born between 1900 and 1950 had the highest life expectancy (mean life expectancy 70 years) compared with women born before 1900 (mean life expectancy 64 years) or women born after 1950 (Figure 4, log-rank test 11.28, p=0.0036).

Discussion

Our study on the impact of the genetic susceptibility on mortality in six families with multiple and early-onset breast or ovarian cancer cases showed that women in these families had a twofold increased mortality, confined to ages 30 to 60. There was considerable variation between the families. The families with the highest excess mortality (numbers 92008 and 92011) both had (evidence for) a mutation at the BRCA1 gene. Two other families, however, with a mutation at the BRCA1 gene (numbers 91011 and 93024) had less increased mortality. In pedigree 94068, a family without evidence for linkage in BRCA1, mortality was similar to that of the general Dutch population, although this family had the largest number of early-onset breast cancer patients.

Our family tree mortality ratio method is a historical cohort analysis of all-cause mortality in all affected family members, obligate and potential carriers in

the six families. The selection of the cohort is based on the assumption of Mendelian inheritance. In the case of breast and ovarian cancer, it is likely that in the comparison between families several genetic and environmental predispositions are involved. Moreover, even in a family with a proven mutation we cannot exclude the existence of "phenocopies", as breast cancer occurs in 1 up to 10 women in the Dutch population.

To explore the apparent secular increase of mortality from 1890 through 1994, the women were first classified into birth cohorts, which supported the increasing SMRs with time. Some studies found indications for an increasing penetrance over time in families with BRCA1 gene mutation carriers [16,17] resulting in shorter life expectancy for the youngest birth cohort. In earlier studies mean age at onset of breast cancer among mothers (or aunts) of patients had been compared with the mean age at onset among breast cancer patients (or sisters). They showed an intrapair difference between 4 and 13 years in favor with the older generation [18-22]. This cohort effect appeared to be the result of a genetic susceptibility. However, the problem with most of these studies is that daughters and sisters tend to be younger than their mothers and aunts, because of incomplete follow-up of the younger generation [23,24]. The calculation of death rates adjusted for age and calendar period (Figure 2) or of a survival curve (Figure 4) can only partially overcome this problem.

Another explanation for the apparent secular increase of mortality in these families was the relative increase of the number of nulliparous women from 1890 to the present. The relative mortality in these women and women with one or two children was two times greater than in women with three or more children. This finding seemed inconsistent with a recent observation from the Nurses' Health Study, where no protection was found from multiple births among women with a positive family history [25]. However, according to table 3, within each birth cohort the influence of parity was limited, and in each stratum of parity the cohort effect still existed, indicating that the number of children in itself might not influence the impact of the genetic predisposition

on mortality.

At the same time, in the Dutch population there has been a steady increase in breast cancer incidence in all birth cohorts between 1880-89 and 1940-49, but in women born after 1950 the risk may have declined [26]. However, breast cancer mortality rates have been relatively constant over time [27]. As the overall standardized mortality for all causes of death has diminished markedly, the contribution of breast cancer mortality to overall mortality rose from 3 to 6%, whereas in women in the age category 30 to 60 up to 20% of all deaths are caused by breast cancer.

The influence of birth cohort and parity on the apparent secular increase of mortality in these families together with the stable breast cancer mortality in the Dutch population may suggest that hereditary breast-ovarian cancer families are more susceptible to exogenous risk factors for breast cancer than the general Dutch population. On the other hand, we also tried to disentangle the increasing impact from the way these families have been ascertained, by distinguishing the oldest and middle generations from the youngest generations. All pedigrees were completed with all obligate and potential carriers backwards to the common ancestors (3 to 6 generations). Because of this completeness there was no selection towards the most severe branches of the pedigrees, meaning that the oldest generations are unbiased. Still, ascertainment bias could have played a role in the youngest generations and might have played a role in the middle generations. This means that the mortality peak in the last calendar period (1975-1994) is, most likely, an overestimation of the effect. Therefore, we feel that the real impact of the genetic predisposition on mortality is given by the mortality rates of the oldest (and middle) generations, which showed a decreased absolute risk over calendar time.

The excess all-cause mortality in the study population is confined to early ages, which is the kind of (genetically determined) mortality we would like to prevent. We are fairly certain that the described excess death is the result of the

genetic susceptibility, since the majority of the women died of cancer and no increased mortality in men was found. A model like ours gives a first estimate of how much early mortality is related to a genetic predisposition in these families. Because of the possibility of ascertainment bias, our estimation of overall mortality due to the genetic susceptibility is perhaps a maximal one. Further elucidation of the impact of genetic factors on all-cause and cause-specific mortality will need a study of very large and unselected pedigrees with a single mutation, investigating differences between branches of the families. Such a study might teach us whether it is worthwhile to screen women in hereditary breast-ovarian cancer families and how much early mortality might be prevented. Modifying factors, like reproductive behaviour and life-style, that influence the penetrance of the genetic influences might best be investigated in case-control studies within the pedigrees.

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Summary

Summary

Despite advances in molecular genetics and the availability of DNA-testing for more and more genetic diseases, insight in clinical outcome of hereditary disorders with an onset in middle age is lacking. The Family Tree Mortality Ratio (FTMR) method permits the study of the natural history of a disease linked to a certain genotype, even in times when the disease was not yet known, its genetic basis not suspected or no therapy was available. In the FTMR method we select from pedigrees with (autosomal dominant) hereditary disorders, all family members with at least 50% probability of gene carriership. Next, with indirect standardization the observed number of deaths is compared with the expected number in a comparison group adjusted for sex, age and calendar time. The ratio of observed to expected mortality is the standardized mortality ratio (SMR). The general Dutch population appeared to be a valid control group in most situations (Chapter I).

In this study, the FTMR method was applied to five autosomal dominant hereditary disorders with a different age-at-onset and severity of the disease, resulting in distinct impact on mortality within the families (Chapter II - VI).

In chapter II we compared the mortality in ten large families with Huntington's disease with that of the general Dutch population. Huntington's disease is a slowly progressive autosomal dominant neurodegenerative disease caused by an expansion and instability of a specific CAG repeat on chromosome four. The clinical manifestations (chorea, dementia, psychiatric disturbances, and emaciation) start round 40 years of age in the majority of patients. Between 1800 and 1997, 257 carriers of the Huntington gene and 474 first-degree relatives have been followed up from 20 years onwards. Over the whole period, 420 deaths occurred, whereas 278 deaths were expected (SMR 1.5; 95% CI 1.4-1.7). Excess mortality was strongest in the age group 45-60 years (SMR 2.7; 95% CI 2.3-3.2). In this age group, where the Huntington gene expresses its

major impact, mortality rates in the general Dutch population continuously declined over the past century, but among the families with Huntington's disease this decline was absent. The main finding, therefore, is that persons who carry the Huntington gene and reach middle age, have not benefitted advances in medical care and overall increase in life expectancy as have individuals of the general Dutch population.

Chapter III specifies causes of death in families with the factor V Leiden mutation. Resistance to activated protein C (APC resistance) because of this factor V Leiden mutation is the most important genetic cause for venous thrombosis. Therefore, gene carriership was hypothesized to result in more deaths from thromboembolic complications. By genetic reasoning we know that, because of its autosomal dominant mode, at least one parent of each patient carried the abnormality. A deleterious effect of the mutation on life expectancy should therefore be visible among parents of a patient. After the detection of the factor V Leiden mutation in 1993, all dates of birth and death of the parents of all 92 index cases from the Leiden Thrombophilia Study were traced. Observed mortality in the parents was equal to the expected mortality in the general Dutch population (SMR 86/88=1.0; 95% CI 0.8-1.2). The cause-specific SMR for cancer, cardiovascular diseases, and respiratory diseases also did not differ from unity. Thromboembolic complications were mentioned only once as an underlying cause of death (SMR 2.3; 95% CI 0.1-13.0) and three times as a contributing cause of death (SMR 1.5; 95% CI 0.3-4.4). We conclude that there is no major effect of APC resistance on life expectancy. Therefore, long-term anticoagulation in carriers of factor V Leiden, on the basis of the carrier state alone, is not indicated to reduce excess mortality.

The next hereditary disorder with possibly excess mortality at middle age was familial dysbetalipoproteinemia (Chapter IV), characterized by elevated levels of cholesterol and triglyceride due to accumulation in the plasma of β-VLDL. More than 90% of the patients is homozygous for apolipoprotein E2, and about 40% of them suffers from cardiovascular diseases. In a minority of cases,

familial dysbetalipoproteinemia is associated with dominant transmission of mutants, like the apolipoprotein E3-Leiden variant. In the Netherlands, 5 probands were known with this gene defect, who had common ancestors in 1718, leading to one extended pedigree. We investigated all-cause mortality from 1800 to 1994 and causes of death from 1941 to 1994 in 68 apolipoprotein E3-Leiden carriers and 198 first-degree relatives. The life expectancy did not differ from that in the general Dutch population (SMR 110/107=1.0; 95% CI 0.8-1.2). Most individuals died of cardiovascular diseases, but the observed number of cardiovascular deaths equalled the expected number (SMR 1.1; 95% CI 0.6-2.0). It was striking that only two family members died of cancer (SMR 0.3; 95% CI 0.03-1.0). So, there was no excess mortality in this extended pedigree. Therefore, screening of family members of apolipoprotein E3-Leiden carriers for the mutation is unlikely to offer survival benefits.

In chapter V the familial atypical multiple mole-melanoma syndrome was discussed. There are indications that this syndrome is not only associated with the development of melanoma of the skin, but also with an increased incidence of systemic cancers. In that case the impact on life expectancy is much higher than previously thought. Out of six families, 110 gene carriers and 114 first-degree relatives have been followed up between 1830 and 1994. Overall, there was 60% excess mortality (SMR 65/42=1.6; 95% CI 1.2-2.0), and the SMR has doubled since the beginning of this century. The impact of the familial atypical multiple mole-melanoma syndrome is rising over calendar time, mainly because the mortality in the general Dutch population has been falling. Excess mortality was confined to the ages 35 to 70 years (SMR 2.1; 95% CI 1.5-2.9), and could be fully attributed to cancer mortality (SMR 4.7; 95% CI 3.4-6.3). Half of the cancer deaths was caused by pancreatic carcinoma (SMR 31; 95% CI 16-55) and melanoma of the skin (SMR 110; 95% CI 53-204). Therefore, follow-up programmes of affected family members should not be confined to regular check of the atypical nevi.

Finally, we described the effect on mortality in six families with autosomal dominant transmission of breast-ovarian cancer (Chapter VI). These families had different underlying gene defects; four families carried a mutation in BRCA1, but in the two other families the genetic cause was not yet clarified. Between 1840 and 1994, 57 affected women, 10 women who transmitted the genetic predisposition, and 108 sisters and daughters have been followed up from 20 years onwards. Mortality was doubled (SMR 69/35=2.0; 95% CI 1.5-2.5). Two-third of the women died of cancer and mortality was confined to the ages 30 to 60 years (SMR 4.4; 95% CI 3.1-6.0). In this age group, the SMR was 2.5 until 1945; after 1945 there was a stepwise increase of the SMR to 8.0 in the calendar period 1975-1994, related to excessive mortality in the youngest generations. Even the oldest generations, which were least likely to be affected by ascertainment bias, showed a rising impact of the genetic predisposition, despite decreasing absolute mortality over the calendar time.

In conclusion, the FTMR method gives a realistic idea of the impact of a hereditary disorder on mortality in families. It shows whether excess mortality is confined to certain age groups, and whether the impact of a hereditary defect has changed over the calendar time in absolute as well as relative terms. Causes of death contain additional information, but will be difficult to trace for the period before 1940. Especially in time periods where no therapy was available, the FTMR method indicates how much mortality might maximally be prevented, if screening and intervention in affected families are completely effective.

Samenvatting

Samenvatting

Ondanks een zeer snelle ontwikkeling in het diagnostiseren van erfelijke aandoeningen, ontbreekt vooral bij aandoeningen die op middelbare leeftijd tot expressie komen, het inzicht in de klinische gevolgen. De 'Family Tree Mortality Ratio' (FTMR) methode geeft dit inzicht door het "effect" van een bepaald gen defect na te gaan, ook in een tijdvak dat noch de ziekte, noch het gen bekend waren. In de FTMR methode selecteren we uit stambomen waarin zich een (autosomaal dominante) erfelijke aandoening voordoet, alle familieleden die tenminste 50% kans op dragerschap hebben. Vervolgens wordt dan met behulp van indirecte standaardisatie hierin het aantal overledenen (waargenomen sterfte) vergeleken met het verwachte aantal in een controlegroep, waarin het gen zelden voorkomt, gecorrigeerd voor geslacht, leeftijd en kalenderperiode. De ratio van waargenomen en verwachte sterfte is de 'standardized mortality ratio' (SMR). De Nederlandse bevolking bleek in bijna alle situaties een betrouwbare controlegroep te zijn (Hoofdstuk I).

In dit onderzoek is de FTMR methode toegepast op een vijftal autosomaal dominant overervende afwijkingen met verschillende age-at-onset en ernst van de ziekte en daardoor een verschillende impact op de sterfte binnen de families (Hoofdstuk II t/m VI).

In hoofdstuk II hebben we de sterfte in tien grote families met de ziekte van Huntington vergeleken met die in de Nederlandse bevolking. De ziekte van Huntington is het gevolg van een CAG repeat expansie in het Huntington gen. De eerste symptomen (chorea, dementie, persoonlijkheidsveranderingen en vermagering) beginnen in de meeste gevallen rond het 40e jaar, waarna de ziekteduur tot het overlijden gemideld 15 jaar is. Tussen 1800 en 1997 zijn 731 familieleden met de ziekte van Huntington en al hun eerstegraadsfamilieleden vanaf 20 jarige leeftijd gevolgd. In deze periode overleden er 420 personen, terwijl er 278 verwacht werden (SMR 1,5; 95% BI 1,4-1,7). De oversterfte was

het hoogst tussen de 45 en 60 jaar (SMR 2,7; 95% BI 2,3-3,2). In de Nederlandse bevolking is binnen deze leeftijdscategorie vanaf 1800 een continue afname van de absolute sterfte zichtbaar, maar binnen de aangedane families is deze daling afwezig. De belangrijkste bevinding is dan ook dat dragers van het Huntington gen, die de middelbare leeftijd bereiken, niet hebben kunnen profiteren van vooruitgang in de geneeskunde en geen verbeterde levensverwachting hebben gekregen.

Hoofdstuk III gaat over de doodsoorzaken in families met de factor V Leiden mutatie. Resistentie tegen geactiveerd proteïne C ten gevolge van de factor V Leiden mutatie is de belangrijkste genetische oorzaak van veneuze trombose. Het is mogelijk dat gendragers hierdoor ook vaker aan een trombo-embolische complicatie zullen overlijden. Vanwege de overerving weten we dat tenminste één van de ouders van elke patiënt de mutatie ook gehad moet hebben. Een schadelijk effect van de mutatie op het sterfte-patroon zou daarom ook zichtbaar moeten zijn in de ouders van een proband. Nadat eind 1993 de factor V Leiden mutatie was ontdekt, zijn de geboorte- en overlijdensdata van de ouders van alle 92 patiënten met deze mutatie uit de Leiden Erfelijke Trombose Studie achterhaald. De waargenomen sterfte in de ouders was gelijk aan de verwachte sterfte (SMR 86/88=1,0; 95% BI 0,8-1,2). Ook overleden er relatief evenveel ouders aan kanker, hart- en vaatziekten, en luchtwegaandoeningen als in de Nederlandse bevolking. Er waren vier trombo-embolische complicaties, eenmaal als primaire doodsoorzaak (SMR 2,3; 95% BI 0,1-13) en driemaal als bijkomende doodsoorzaak (SMR 1,5; 95% BI 0,3-4,4). Onze conclusie is dat de levensverwachting niet door dragerschap van factor V Leiden alleen verlaagd wordt.

Een volgende erfelijke aandoening met een mogelijke verhoogde sterfte op middelbare leeftijd was familiaire dysbetalipoproteïnemie (Hoofdstuk IV), dat gekarakteriseerd wordt door verhoogde cholesterol en triglyceride plasma concentraties als gevolg van β-VLDL stapeling. Meer dan 90% van de patiënten is homozygoot voor apolipoproteïne E2 en ongeveer 40% van hen lijdt aan hart-

en vaatziekten. Soms wordt familiaire dysbetalipoproteïnemie veroorzaakt door autosomaal dominante overerving van mutanten, zoals bij de apolipoproteïne E3-Leiden variant. In Nederland waren vijf probanden bekend met deze variant, die gemeenschappelijke voorouders hadden in 1718. Wij hebben de sterfte vanaf 1800 en de doodsoorzaken vanaf 1941 voor alle 68 gendragers en 198 eerstegraadsfamilieleden in kaart gebracht. De levensverwachting verschilde niet van die in de Nederlandse bevolking (SMR 110/107=1,0; 95% BI 0,8-1,2). Hart- en vaatziekten kwamen het meeste voor, maar de waargenomen sterfte hieraan was gelijk aan de verwachte (SMR 1,1; 95% BI 0,6-2,0). Opvallend was dat er na 1940 slechts twee familieleden aan kanker waren overleden (SMR 0,3; 95% BI 0,03-1,0). Apolipoproteïne E3-Leiden leidt dus niet tot oversterfte. Daardoor zal screening van familieleden van gendragers geen voordelen bieden voor de levensverwachting.

Het familiaire atypische multipele moedervlek-melanomen syndroom wordt behandeld in hoofdstuk V. Dit syndroom leidt mogelijk niet alleen tot melanomen, maar ook tot een verhoogde incidentie van andere kankers. In dat geval is de impact op de levensverwachting mogelijk groter dan tot op heden werd aangenomen. Uit zes families zijn 110 gendragers en 114 eerstegraads-familieleden van 1830 tot 1994 gevolgd. In het totaal was er 60% oversterfte (SMR 65/42=1,6; 95% BI 1,2-2,0), die sinds het begin van deze eeuw geleidelijk is toegenomen tot een SMR van 2,2 in de periode 1985-1994 (95% BI 1,4-3,4). De impact van het familiaire atypische multipele moedervlek-melanomen syndroom is dus groter geworden in de 20e eeuw, vooral door dalende sterftecijfers in de algemene bevolking. De oversterfte beperkte zich tot de leeftijdscategorie 35 tot 70 jaar (SMR 2,1; 95% BI 1,5-2,9) en kon volledig toegeschreven worden aan kankersterfte (SMR 4,7; 95% BI 3,4-6,3). De helft van de kankersterfte werd veroorzaakt door pancreascarcinomen (SMR 31; 95% BI 16-55) en melanomen (SMR 110; 95% BI 53-204). Hierdoor rijst de vraag of aangedane familieleden, naast de jaarlijkse check van atypische moedervlekken, niet ook regelmatig onderzocht zouden moeten worden op de aanwezigheid van andere kankersoorten.

Tenslotte hebben we het effect op de sterfte bestudeerd in zes families waarin borst-ovarium kanker dominant overerft (Hoofdstuk VI). Deze families hadden niet allemaal dezelfde mutatie; in vier families was een mutatie gevonden in het BRCA1 gen, maar in de twee andere families was nog geen onderliggende genetische oorzaak bekend. Over de periode 1840-1994 werden 57 vrouwen met borst- en/of ovariumkanker, 10 vrouwen die de genetische predispositie gehad moeten hebben, en 108 eerstegraadsfamilieleden gevolgd vanaf 20 jarige leeftijd. De sterfte was verdubbeld (SMR 69/35=2,0; 95% BI 1,5-2,5). Tenminste tweederde van de vrouwen was overleden aan kanker en de mortaliteit was het hoogste tussen de 30 en 60 jaar (SMR 4,4; 95% BI 3,1-6,0). In deze leeftijdsgroep lag de SMR tot 1945 rond de 2,5, daarna steeg de SMR van 4,2 in de periode 1945-1975 tot 8,0 in de periode 1975-1994, onder invloed van excessieve stijging van het aantal overledenen in de meest recente generatie. Waarschijnlijk speelt selectiebias een rol bij de sterftecijfers in de meest recente kalenderperiode. Maar ook in de oudere generaties was er een toegenomen impact van de genetische predispositie voor borst- en ovariumkanker, ook al daalde de absolute sterfte.

Geconcludeerd kan worden dat de FTMR methode een reëel beeld geeft wat de impact van een genetische afwijking is op de sterfte in families. Er kan onder andere zichtbaar gemaakt worden, of de oversterfte zich beperkt tot een bepaalde leeftijdscategorie, en of de impact van een gen defect veranderd is in de loop van de kalendertijd zowel in absoluut als relatief opzicht. De doodsoorzaken voegen veel informatie toe, maar hebben het nadeel dat ze voor 1940 veel moeilijker te achterhalen zijn. Met name in perioden dat er nog geen therapie beschikbaar was, is goed vast te stellen wat het maximale effect van preventie of therapie kan zijn in de aangedane families.

Nawoord

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Curriculum Vitae

Elisabeth Theodora Maria Hille werd op 11 september 1969 in Eindhoven geboren. Na de lagere school en het gymnasium β op het Lorentz Lyceum in Eindhoven ging zij in 1987 in Leiden Gezondheidswetenschappen studeren. Na een uitstapje naar Rotterdam waar zij in 1991 haar propaedeuse 'Beleid en Management in de Gezondheidszorg' behaalde, slaagde ze in 1993 voor het doctoraal examen Biomedische Wetenschappen. Van juli 1993 tot juni 1998 was ze onderzoeker op de afdeling Klinische Epidemiologie van het Academisch Ziekenhuis in Leiden. In het kader van de opleiding tot Epidemioloog B volgde zij onder andere de '14th Annual New England Epidemiology Summer Program' in Boston en de '9th European Educational Programme in Epidemiology' in Florence. Vanaf juli 1998 zal zij werkzaam zijn bij TNO Preventie en Gezondheid, divisie Jeugd, in Leiden.