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Cascade genetic testing for mismatch repair gene mutations

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Abstract Mismatch repair gene mutation carriers have a high risk of developing colorectal cancer, and can benefit from appropriate surveillance. A combined population based ascertainment cascade genetic testing approach provides a systematic and potentially effective strategy for identifying such carriers. We have developed a Markov Chain computer model system which simulates various factors influencing cascade genetic testing; including demographics, uptake, genetic epidemiology and family size. This was used to evaluate cascade genetic testing for mismatch repair gene mutations in theory and practice. Simulations focussed on the population of Scotland by way of illustration, and were based on a 20-year programme in which index cases were ascertained from colorectal cancer cases aged <55 years at onset. Results indicated that without practical barriers to cascade genetic testing, 545 (95% CI = 522, 568) carriers could be identified; 42% of the population total. This comprised approximately 140 index cases, 302 asymptomatic relatives and 104 previously affected relatives. However, when realistic ascertainment and acceptance rates were used to inform simulations, only 257 (95% CI = 246, 268) carriers, about 20% of the carrier population, were identifiable. Of these approximately 112 were index cases, 108 were asymptomatic relatives, and 37 were previously affected relatives. This contrast emphasises the importance of ascertainment and acceptance rates. Likewise the low number of index cases shows that case identification is a limiting factor. In the absence of robust data from epidemiological studies, these findings can inform decisions about the use of cascade genetic testing for mismatch repair gene mutations.

Keywords Colorectal cancer · Genetic screening · Mismatch repair · Genetic services · Genetic epidemiology

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Abbreviations

MMR	Mismatch repair
CRC	Colorectal cancer
CGTM	Cascade Genetic Testing Model
GPM	Genetic Population Model

Introduction

A small but significant sub-set of colorectal cancer (CRC) cases are caused by pathogenic mutations in mismatch repair (MMR) genes [1–6], which confer an approximate lifetime risk of 80% in males and 40% in females [7]. Colonoscopic screening of carriers can have significant health benefits in terms of both reduced incidence and life-years saved [8]. Consequently, there is a compelling

rationale for identifying MMR gene mutation carriers before they develop disease.

This presents a major challenge, however. Population prevalence is uncertain but is certainly low: the carrier frequency in Scotland has been estimated to be approximately 1:3,139 (95% CI = 1:1,247, 1:7,626) [9]. By extrapolation, this implies that there are about 1,200 carriers aged 15–74 in the Scottish population of around 5 million. Population genetic testing for MMR gene mutations is not currently viable, and further information regarding the underlying genetic epidemiology and the effectiveness of intervention strategies is needed before it can even be considered [10–14]. Alternative approaches are thus required.

One current strategy for finding asymptomatic mutation carriers is to offer genetic testing to close relatives of a known carrier. The specific mutation present can thus be traced through an expanded pedigree in a process known as cascade genetic testing. In practice, index cases are often selected on the basis of family history. Tests can be offered to first-degree relatives only, or to a wider group of relatives; a concept which can be referred to as "depth" of testing.

Cascade and population screening represent opposite ends of a continuum. A further option for identifying asymptomatic MMR gene mutation carriers, which is potentially both feasible and effective, could, therefore, be to combine features of both methodologies. A systematic, population-based approach to carrier ascertainment would thus be targeted to individuals at a relatively high risk of carrying a mutation, for example those with early onset CRC. Cascade genetic testing would then be employed, using these individuals as starting points. By only testing individuals at very high risk of harbouring a pathogenic mutation, such an approach would limit the number of non-carriers tested whilst maximizing the number of carriers identified.

The hypothesis that a combined population based ascertainment cascade approach may be both effective and efficient is supported by theoretical work [15]. Cascade genetic testing has also been systematically applied to other complex diseases with adult onset, notably familial hypercholesterolaemia [16, 17], for which it has been shown to be cost effective [18, 19]. However, the outcomes of cascade genetic testing are highly context specific, and this strategy remains to be thoroughly evaluated at the population level for MMR gene mutations. The overarching aim of this paper is to address this gap in current knowledge.

Methods

Overview

The complex process of cascade genetic testing was evaluated using a computer model system, implemented using Java and the database software MySQL. Design was function driven, but in applying probabilities to events in a stochastic manner the model system is essentially an example of a Markov Chain.

The model system has the flexibility to be applied to any population. Results presented here used the Scottish population for illustrative purposes. The model system comprises two integrated models, which operate sequentially:

1. Genetic Population Model (GPM)—simulates demographics, family structure and genetic epidemiology in a dynamic population.
2. Cascade Genetic Testing Model (CGTM)—superimposes cascade genetic testing on populations simulated by the GPM.

Genetic Population Model (GPM)

The GPM constitutes a flexible and powerful research tool for studying the inheritance of pathogenic mutations at the population level. A dynamic population is simulated over time, with demographics and mutation prevalence at the starting point defined by the model user. A set of variables is assigned for each individual: these include a unique identifier, year of birth, sex, mutation status, disease status and date of death. In the course of the simulation these variables are updated annually, using a series of algorithms which apply a probability for the event in question. In this manner, the occurrence of events such as births, death and disease onset over time is simulated. Obtaining input probability values from real data enables actual demographic and epidemiological trends to be simulated. Importantly, the probability of disease is determined by mutation status as well as age, sex and cohort-specific population incidence. Hence information about the prevalence and penetrance of MMR gene mutations was used to simulate their role in CRC.

Another crucial feature is that partnerships are established within the population, and 'birth' algorithms are applied to females with a current partner, with age-specific probability of birth adjusted to account for existing parity. For each birth event, mother and father identity is known and mutation status can be assigned according to Mendelian inheritance. Hence realistic family relationships and inheritance patterns evolve in the simulated population.

To provide the necessary framework for simulating cascade genetic testing beginning in the present day, the GPM was used to produce a dynamic simulation of the Scottish population from the time of the 1901 census to the year 2049.

In view of the uncertainty surrounding the prevalence and penetrance of MMR gene mutations (see "Discussion"), these input values were considered as assumptions

rather than robust estimates. A single estimate of 0.016%, extrapolated from the study by Dunlop et al. [9], was used to represent the collective allele frequency for pathogenic mutations in hMLH1 and hMSH2. Penetrance was estimated to be 80% in males and 40% in females [3, 7].

A summary of key inputs to the GPM is shown in Appendix 1.

Cascade Genetic Testing Model (CGTM)

The CGTM simulates the progress of a cascade genetic testing programme over time, using weeks as time units. Initially, the population database is interrogated to identify CRC cases occurring in the current week. Ascertainment and acceptance of genetic testing is determined using probability algorithms, with test results reflecting true mutation status. Another algorithm is run to determine if mutation carriers will allow their relatives to be contacted. If the outcome is affirmative, the CGTM then retrieves relatives of the newly identified mutation carrier from the population database, beginning a new cycle of ascertainment and testing.

All simulations were based on applying cascade genetic testing to a 'depth' of all first- and second-degree relatives. To reflect the long-term nature of the strategy, the CTGM simulated cascade genetic testing over a 20-year period. Restricting genetic testing to early-onset cases is a commonly used approach to enrich for mutation carriers. For the purposes of the study, it was assumed that all CRC cases presenting under the age of 55 would be offered MMR gene mutation analysis as part of routine clinical practice. Realistic delays were also built into the model system.

Uptake of cascade genetic testing, determined by both ascertainment and acceptance rates, is likely to have a significant impact on the above outcomes. To study this, two sets of input parameters were defined:

(i) Optimum: assumes 100% ascertainment and acceptance and thus provides an indication of the theoretical utility of cascade genetic testing.
(ii) Realistic: current 'best estimates' of ascertainment and acceptance rates, based on published data [1, 20, 21] and the practical experience of our research group. These represent conservative assumptions about what may be achievable in practice.

Table 1 provides a summary of parameters used to inform the CGTM.

Strategy for evaluating cascade genetic testing using integrated model system

The two models were considered as one integrated system. Each set of results was generated by running the GPM first, and then using the CGTM to model cascade genetic testing in the simulated population database. Variation is therefore the net result of variability in the entire model system. The number of repeat simulations was minimised to generate robust estimates whilst reducing computing time. Results presented here are based on mean estimates from multiple simulations (n = 25) run with identical parameters. Standard deviation and confidence intervals were calculated by standard methods.

Table 1 Key parameters for Cascade Genetic Testing Model

Parameter type	Parameter estimate applied
Depth of cascade genetic testing	First- and second-degree relatives
Length of programme	20 years
Age limit for index cases	55 years
Age limit for relatives	75 years
Minimum age for genetic testing	18 years[a]
Sensitivity of genetic test	100%
Specificity of genetic test	100%
Realistic ascertainment rate for colorectal cancer cases	90%
Realistic acceptance rate for genetic testing by colorectal cancer cases	85%
Realistic proportion of index cases permitting/facilitating ascertainment of relatives	98%
Realistic acceptance rate for genetic testing by relatives	50%
Time required for genetic test in colorectal cancer case	52 weeks
Time required for specific genetic test in relative of carrier	1 week
Optimal time for ascertainment of relatives	4–12 weeks
Realistic time for ascertainment of relatives	1–2 years[b]

[a] Eligible individuals aged less than 18 years were not processed until they reached this minimum age

[b] Practical experience suggests that ascertainment of relatives can be a prolonged process, and accordingly the 'realistic' set of simulations modelled presentation of relatives over a period of up to 2 years

Results

Cascade genetic testing identifies mutations in affected individuals, or in relatives of these index cases. Inevitably, some relatives will have developed disease previously, whereas others will be asymptomatic. This latter group constitutes the key outcome against which the effectiveness of cascade genetic testing can be judged. Figures 1 and 2 illustrate the cumulative total number of carriers identified in each category, in simulations using, respectively, 'optimal' and 'realistic' assumptions about ascertainment and acceptance. Estimates are based on the mean value from multiple simulations, with 95% confidence intervals.

Under optimum ascertainment and acceptance rates, simulations suggested that an average of 140 (95% CI = 135, 145) index cases could be identified over 20 years, at a rate of approximately seven per year. The corresponding average number of asymptomatic mutation carriers identifiable through cascade genetic testing was 302 (95% CI = 287, 317). A further 104 (95% CI = 98, 110) relatives who had previously developed CRC were predicted to be identifiable.

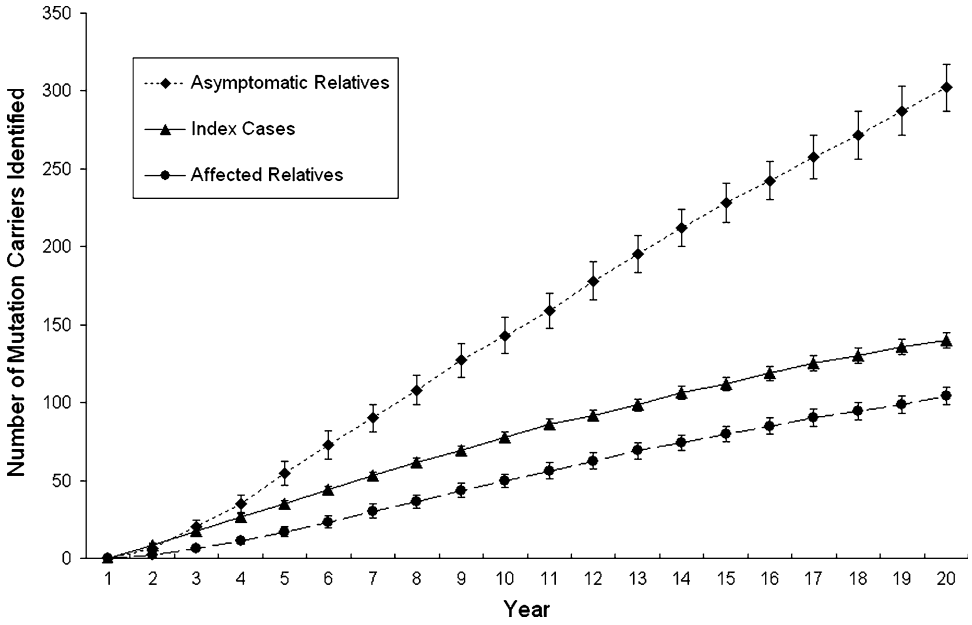

Fig. 1 Average number of mutation carriers identified in simulations assuming 'optimal' ascertainment and acceptance rates

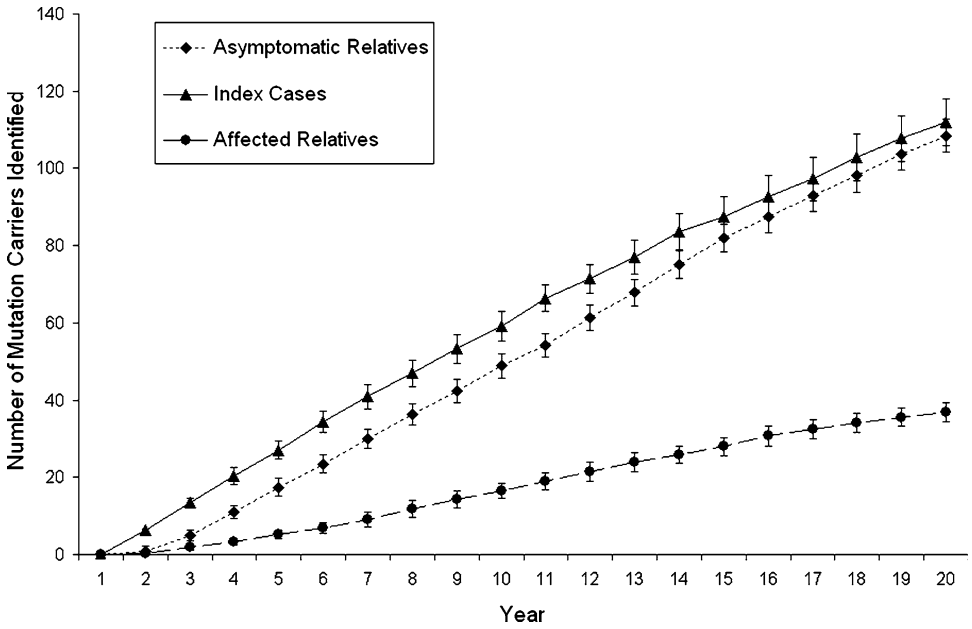

Fig. 2 Average number of mutation carriers identified in simulations assuming 'realistic' ascertainment and acceptance rates

In simulated populations generated by the GPM, the average number of mutation carriers aged 15–74 years was 1,290. This value is consistent with the published estimate used to inform the model system [9], illustrating the validity of the model in this respect. The above results suggest that under optimal (i.e. 100%) ascertainment and acceptance rates, an average of 545 (95% CI = 522, 568) mutation carriers would be identified through cascade genetic testing over a 20-year period. Therefore, this implies that roughly 42% of the total number of carriers in this age group aged in Scotland could theoretically be identified through a systematic cascade genetic testing programme spanning 20 years.

Results of simulations in which the CGTM was informed by more realistic estimates of ascertainment and acceptance demonstrate the major impact that these factors are likely to have on the outcomes of cascade genetic testing. These results suggest that approximately 112 (95% CI = 108, 116) index cases, 108 (95% CI = 102, 115) asymptomatic relatives, and 37 (95% CI = 34, 39) previously affected relatives would be identified when realistic ascertainment and acceptance rates applied. The total number of mutation carriers that would be identified by cascade genetic testing according to these simulated results was 257 (95% CI = 246, 268), or approximately 20% of all carriers in the population. Notably, the ratio of asymptomatic carriers identified to index cases ascertained is much lower when 'realistic' ascertainment and acceptance rates for relatives are applied.

The ratio of asymptomatic carriers identified to genetic tests performed is a key indicator of the efficiency of cascade genetic testing. The relatively complex process of searching for an unspecified MMR gene mutation in a CRC case is likely to be the most resource-intensive aspect of a cascade genetic testing programme. Simulations using the CGTM suggest that under optimal ascertainment and acceptance parameters, approximately 8,400 potential index cases need to undergo genetic testing in order to identify 302 asymptomatic relatives, a ratio of about 28:1. In the more realistic simulations, an average of 6,500 potential index cases underwent genetic testing and around 112 asymptomatic relatives were identified, an overall ratio of about 58:1 that suggests that limitations in ascertainment and acceptance rates make the process far less efficient.

Extending the time period of cascade genetic testing beyond 20 years in these simulations showed that this strategy remains useful over an even longer period, although return diminishes slightly as the pool of untested mutation carriers dwindles. Simulations under 'optimal' assumptions suggested that about 72% of carriers could be identified after 40 years. This observation implies cascade genetic testing could in theory identify the majority of carriers in the population, but is unlikely to do so within a reasonable timeframe.

Discussion

We have developed a robust computer model system that facilitates the study of cascade genetic testing, focussing on its theoretical and practical utility in a specific clinical context.

Precise outcomes of cascade genetic testing will be population-specific due to variations in demographics and genetic epidemiology. Penetrance may vary as the specific mutations present and the proportion of hMLH1 compared to hMSH2 mutations will differ between populations. In addition, the behaviour of individuals in a cascade genetic testing programme may be influenced by social context and external factors such as the availability of free healthcare. Nonetheless, Scotland serves as a typical example of a developed country with a high incidence of CRC and low prevalence of MMR gene mutations. Hence, the methods, results and conclusions of this work have much wider relevance.

Model outcomes are critically dependent on input data, so the quality of these data merits further consideration. Robust national statistics were used to simulate the demographics of the Scottish population and to simulate CRC epidemiology, although early historical data and projected estimates had less precision. In contrast, data on family size and structure was limited and relied heavily on one publication [22]. Input data relating to the genetic epidemiology of MMR gene mutations were regarded as assumptions. Published penetrance estimates vary widely [2, 3, 6], and ascertainment bias in previous studies may have led to overestimation [21]. This study considered all mutations in hMLH1 or hMSH2, although these are highly heterogenous and mutations in other MMR genes may also be clinically relevant. More detailed information could not be incorporated into the simulations because of the lack of robust input data.

Due to the stochastic nature of the model and the limitations outlined above, both random variation and some minor systematic differences were evident between simulated data and the 'actual' parameters of the Scottish population. Nonetheless, the system reliably produced simulated populations that were representative of the actual Scottish population in terms of demographics, genetic epidemiology, and family size and structure.

The depth to which cascade genetic testing is applied is a crucial consideration. It is desirable to test only close relatives with a very high probability of having a mutation, but this can be rather restrictive, especially in small families. Conversely, extending cascade genetic testing to

distant relatives is more resource-intensive and may lead to reduced uptake. In reality, factors such as family history and test results in other relatives may also guide the cascade process.

In this, as in any, cascade genetic testing programme, the identification of index cases is extremely important and potentially a major limiting factor [23]. Applying genetic tests to CRC cases is the simplest approach. To improve efficiency, genetic testing can be targeted to a sub-group of cases with early onset. Family history provides an alternative means of targeting testing, although use of the stringent Amsterdam criteria [24] would miss a large proportion of carriers, and high risk families may not be representative [21]. Incorporating information on tumour pathology into the genetic testing strategy could also be an effective means of targeting testing [25]. Specific approaches, such as the two-stage model described by Barnetson et al. [26] which incorporated clinical variables in stage 1 and microsatellite instability and immunohistochemistry in stage 2, may prove useful. However, data on the use of pathology features in this context is limited, and the required pathology data are not always routinely collected. Because of this, and the inherent limitations and complexity of using family history criteria, the age alone model was chosen for the current study. Although their inclusion would theoretically permit identification of additional index cases, other cancers associated with MMR gene mutations were not simulated due to lack of quality evidence to support estimates of penetrance and test uptake.

Under optimum conditions, simulations suggested that approximately 140 index cases could be identified over 20 years. In reality though, ascertainment and acceptance will be sub-optimal [20, 27–29]. Results based on more realistic estimates, which suggest that 112 index cases could be identified, are thus more appropriate.

Simulations suggested that cascade genetic testing could theoretically identify about 40% of MMR gene mutation carriers in Scotland, most of which were predicted to be identified at an asymptomatic stage. This is consistent with estimates of theoretical effectiveness presented by Krawczak et al. [15], although results are not directly comparable since this previous study had no chronological component, and did not relate to a specific disease or population.

Published data concerning the achievable uptake for a cascade genetic testing programme is limited, and relates to large hereditary non-polyposis colorectal cancer (HNPCC) families. Figures quoted are highly variable, ranging from 33.6 [29] to 75% [27]. Low education and depression act as barriers to uptake [20], and other factors, including age, sex, and relationship to mutation carrier may also be involved [20, 27, 29]. The manner in which relatives are approached is another vital factor. Current 'best practice' in clinical genetics, designed to comply with ethical guidelines, dictates that communication with relatives must be initiated through the index case or another family member. The onus is then on the relatives to contact a genetics clinic. For the current study, this difficulty was addressed by using an estimate of 50% to reflect the overall proportion of eligible relatives who will undergo a genetic test in 'realistic' simulations. Based on the practical experience of our research group, we consider this to be a conservative estimate.

Using 'realistic' ascertainment and acceptance rates as input data for the CGTM produced results that are strikingly different to theoretical outcomes. These simulations suggest that a 20-year cascade genetic testing programme would identify a much smaller number of mutation carriers; approximately 260, or 20% of the population total. Less than half of these carriers are likely to be identified while asymptomatic. This may be regarded as a relatively poor return from a long-term, resource-intensive testing programme, although identifying a group of mutation carriers does offer the potential to trace mutations in future generations.

The identification of asymptomatic MMR gene mutation carriers is merely the first step towards disease prevention in this population sub-group. Carriers are recommended to undergo intensive surveillance, ideally comprising biennial colonoscopy [30]. Such screening has been shown to reduce CRC incidence and mortality in MMR gene mutation carriers [8]. In practice though, incomplete compliance with surveillance is likely to constrain the potential health benefits [8, 29].

Our findings support the hypothesis that cascade genetic testing has the theoretical potential to be both effective and efficient as a means of identifying MMR gene mutation carriers from within a population. However, we have illustrated that incomplete ascertainment and acceptance has a significant impact. The practical barriers to pursuing cascade genetic testing effectively must therefore be given due consideration when deciding on the appropriateness of implementing this strategy.

In summary, we provide data on the potential effectiveness and efficiency of cascade genetic testing both in theory and in practice. These data and the associated observations and conclusions that have been prompted can be used to inform decisions about the use of cascade genetic testing as part of a strategy to address the health needs of MMR gene mutation carriers.

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Appendix 1

Key input data to Genetic Population Model

Input data	Point estimate (if applicable)	Source/ reference	Comments on data accuracy/quality	Generalisability
Population size	5,094,800 (2005)	GROS	Robust estimate based on census data	Scotland is a relatively small country but data may be extrapolated to populations with similar demographics
Demographic structure		GROS	Robust estimate	Scotland has a demographic structure that is typical of an aging population in a developed country
Deaths (historical data)		GROS	Robust national data	Scotland is broadly representative of developed countries with respect to mortality rate
Deaths (projections)		GAD	Projections only	The projected continuation of decreasing mortality rates for Scotland is typical of developed countries
Births		GROS	Robust national data	Fertility in Scotland, as with many developed countries, has decreased during the latter part of the 20th century
Births (projections)		GAD	Projections only	The projected continuation of decreasing fertility rates for Scotland is typical of developed countries
Disease incidence		ISD	Disease incidence has been recorded with a high degree of accuracy since the 1960s; earlier data is of poor quality	Scotland has a relatively high incidence of colorectal cancer, but incidence is comparable with other countries in which the disease is a public health issue
Disease incidence (projections)		ISD	Projections, based on current trends and demographic projections	The increasing incidence projected for Scotland is reflected in many other populations
Penetrance of MMR gene mutations	To age 70: males: 80% females: 40%	Scientific literature	Limited sources of data, various methods, wide confidence intervals	There is currently no evidence of population variations since differences in published estimates are likely to be due to methods used and lack of statistical power. However, penetrance may vary according to the precise mutation involved and numerous other factors, and may therefore vary by population
Prevalence of MMR gene mutations	1:3,139 (95% CI 1:1,247, 1:7,626)	Scientific literature	One source of data only, wide confidence intervals	Prevalence data are scarce, and the single published estimate relates to Scotland. Prevalence is likely to vary by country, particularly when common founder mutations are present in the population (e.g. Finland), but there is no evidence to suggest that Scotland is not representative in this respect

Appendix 1 continued

Input data	Point estimate (if applicable)	Source/reference	Comments on data accuracy/quality	Generalisability
Disease survival		ISD	Robust national data	In terms of survival, Scotland is representative of countries with modern health care systems
Family structure		ONS	Data on frequency distribution of children as well as completed family size is limited to one reliable source	Family structure may vary across populations according to cultural, social and demographic trends. However, Scotland has a stable population which is useful for comparisons, and the observed trend of decreasing family size leading to stabilizing or decreasing population is common

GROS = General Register Office Scotland, GAD = Government Actuary's Department, ISD = Information Services Division Scotland, ONS = Office of National Statistics, CI = Confidence interval

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