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Estimating the Efficacy and Efficiency of Cascade Genetic Screening

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Screening for genetic variants that predispose individuals or their offspring to disease may be performed at the general population level or may instead be targeted at the relatives of previously identified carriers. The latter strategy has come to be known as "cascade genetic screening." Since the carrier risk of close relatives of known carriers is generally higher than the population risk, cascade screening is more efficient than population screening, in the sense that fewer individuals have to be genotyped per detected carrier. The efficacy of cascade screening, as measured by the overall proportion of carriers detected in a given population, is, however, lower than that of population-wide screening, and the respective inclusion rates vary according to the population frequency and mode of inheritance of the predisposing variants. For dominant mutations, we have developed equations that allow the inclusion rates of cascade screening to be calculated in an iterative fashion, depending upon screening depth and penetrance. For recessive mutations, we derived only equations for the screening of siblings and the children of patients. Owing to their mathematical complexity, it was necessary to study more extended screening strategies by simulation. Cascade screening turned out to result in low inclusion rates (<1%) when aimed at the identification of heterozygous carriers of rare recessive variants. Considerably higher rates are achievable, however, when screening is performed to detect covert homozygotes for frequent recessive mutations with reduced penetrance. This situation is exemplified by hereditary hemochromatosis, for which up to 40% of at-risk individuals may be identifiable through screening of first- to third-degree relatives of overt carriers (i.e., patients); the efficiency of this screening strategy was found to be ~50 times higher than that of population-wide screening. For dominant mutations, inclusion rates of cascade screening were estimated to be higher than for recessive variants. Thus, some 80% of all carriers of the factor V Leiden mutation would be detected if screening were to be targeted specifically at first- to third-degree relatives of patients with venous thrombosis. The relative cost efficiency of cascade as compared with population-wide screening (i.e., the overall savings in the extra managerial cost of the condition) is also likely to be higher for dominant than for recessive mutations. This notwithstanding, once screening has become cost-effective at the population level, it can be expected that cascade screening would only transiently represent an economically viable option.

Introduction

Genetic screening, defined by the European Society of Human Genetics (ESHG) as "any kind of test performed for the systematic early detection or exclusion of a genetic disease, the predisposition or resistance to such a disease, or to determine whether a person carries a gene variant which may produce disease in offspring," is a central issue in community genetics (ESHG Web site). The recent determination of the human genome sequence (International Human Genome Sequencing Consortium 2001; Venter et al. 2001) should promote research into the molecular basis of human genetic disease, and it appears likely that the first practical spin-off from such endeavors will lie in the sphere of gene diagnostics. Even in the absence of an understanding of the actual pathophysiological processes involved, the abundance of new polymorphisms available for analysis and their association with specific human traits should pave the way for the development of predictive tests for conditions not hitherto amenable to genetic diagnosis. Two major goals of genetic screening can be distinguished: First, to facilitate the early provision of therapeutic or prophylactic measures, it is important to identify (as yet) asymptomatic heterozygotes or homozygotes for mutations that cause predisposition to late-onset disease. Second, for many recessive conditions, the goal is to recognize (asymptomatic) heterozygous carriers to aid the process of reproductive decision making.

The success of medical screening programs is often assessed in terms of their acceptance rates—that is, by the proportion of target individuals who, after appro-

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priate counselling, eventually opt for the test (Bowling 1989, Arveux et al. 1992, Weller et al. 1995). However, with genetic screening, the potential for emotional, ethical, and moral conflict is exceptionally high, and any efficiency assessment based exclusively on the level of client appreciation would be overly narrow. For example, programs aimed at the identification of carriers predisposed to late-onset diseases do not normally provide ideal preventive options. Only some of those tested would actually benefit personally from the screening test result and, even if preventive measures were available, these measures could have detrimental side effects. On the other hand, screening programs aimed at carriers of disease alleles that only manifest in their children often present the target individuals with a personal dilemma. In view of this, it appears justified to assess the success of the educational and testing components of a genetic screening program separately. Whereas the educational component can be evaluated simply by determination of the proportion of target individuals who received sufficient information to decide whether or not to have the test, the success of the testing component should be assessed by its *efficacy* and *efficiency*—that is, by the proportion of carriers detected, both in absolute terms and in relation to the screening effort.

Two distinct types of genetic-screening strategy have been proposed. One offers testing to the population at large; the other targets relatives of carriers previously identified through the testing of their phenotypes or genotypes. The latter strategy, which has been most intensively assessed for cystic fibrosis (Holloway and Brock 1994; Marteau 1994; Super et al. 1994*a*, 1994*b*), is usually referred to as "cascade screening." The major difference between population and cascade screening lies in the design of the educational component. The first strategy targets individuals who have equal prior risks and are largely ignorant about the disease in question. By contrast, cascade screening is offered to individuals with an increased risk who usually already have some knowledge of the disease. Although cascade-like strategies are undoubtedly more efficient, they are less efficacious and have lower inclusion rates than population-wide screening—that is, the overall proportion of carriers detected is smaller.

Since both types of genetic screening possess advantages and disadvantages, their relative merits and demerits depend upon many factors, each of which is variably relevant to different diseases and different target populations. One important issue in this context is the disease (and carrier) frequency in the population of interest. The disease frequency influences the level of public awareness prior to a screening program and, importantly, determines both the efficacy and the efficiency of the program. Perhaps surprisingly, no comprehensive formal mathematical treatment of the relative advantages of population versus cascade genetic screening programs has so far been presented. The present study represents an attempt to fill that gap. To this end, we have employed a Poisson-type model of reproduction in a large outbred population, in order to calculate (*i*) the inclusion rates of different cascade screening strategies and (*ii*) the efficiency of cascade screening as compared to population-wide screening. The theoretical findings will be applied to different genetic models underlying disease causation or predisposition, and the practical implications will be discussed for three specific conditions: cystic fibrosis, hemochromatosis, and factor V Leiden as a risk factor for venous thrombosis. Although cascade and population screening will be dealt with as separate entities, in reality the two scenarios are likely to represent opposite ends of a continuum. Thus, case-finding in order to identify the entry points for cascade screening procedures may, in practice, exhibit some characteristics of a population-based approach, whereas family-based identification of at-risk individuals is likely to precede—and, therefore, to serve to initiate—any population-wide searches.

Methods and Results

In the following, we shall refer to a screening strategy that targets all relatives of a given degree of an affected individual as "comprehensive cascade screening." If only first-degree relatives of carriers are tested that have themselves been identified in a previous screening cycle, this will be referred to as "cascade screening sensu stricto."

Autosomal Dominant: Heterozygous Carriers of Mutations with Reduced Penetrance

Let X_n denote the property that a heterozygous carrier of an autosomal dominant mutation is clinically asymptomatic and has no affected offspring in the immediately following n generations. Let Y_n denote the property that all siblings of a heterozygous carrier have property X_n. We shall henceforth assume that X_n and Y_n are statistically independent; that is, that the penetrance, ψ, of the mutation is the same for all carriers and therefore does not depend upon the number of affected family members. Then, for the probability f_0 of X_0,

$$f_0 = P(X_0) = 1 - \psi \ . \quad (1)$$

We shall further assume that, in the population of interest, the number of children per individual follows a Poisson distribution with parameter λ. A carrier has property X_n if—and only if—he/she is clinically asymptomatic and if all his/her carrier children have property X_{n-1}. Assuming that the population frequency, p, of the

Table 1

Model Parameters of Cascade Screening for Carriers of Autosomal Dominant Mutations, with $\lambda = 2$

ψ	f_0	g_0	f_1	g_1	f_2	g_2	f_3	g_3
.05	.950	.968	.904	.939	.863	.914	.828	.894
.10	.900	.937	.814	.886	.748	.849	.699	.823
.25	.750	.850	.584	.766	.495	.724	.453	.706
.50	.500	.727	.303	.644	.249	.623	.236	.618
.75	.250	.624	.118	.576	.104	.572	.102	.571
.90	.100	.570	.041	.551	.038	.550	.038	.550

disease allele is sufficiently small (i.e., $p < .05$), this implies that

$$f_n = P(X_n) = f_0 \cdot \sum_{i=0}^{\infty} \frac{(\frac{\lambda}{2})^i}{i!} \cdot e^{-\frac{\lambda}{2}} \cdot f_{n-1}^i = f_0 \cdot e^{-\frac{\lambda}{2}(1-f_{n-1})} , \quad (2)$$

since the number of heterozygous children of a heterozygote follows a Poisson distribution with parameter $\lambda/2$. Similarly,

$$g_n = P(Y_n) = \sum_{i=0}^{\infty} q_i \cdot z_{i,n} ,$$

where

$$q_i = \frac{e^{-\lambda}}{1-e^{-\lambda}} \cdot \frac{\lambda^{i+1}}{(i+1)!}$$

is the probability that the carrier's parents have i additional children ($i \geqslant 0$), and

$$z_{i,n} = \sum_{j=0}^{i} \binom{i}{j} \cdot \left(\frac{1}{2}\right)^i \cdot f_n^j = \left(\frac{1+f_n}{2}\right)^i$$

is the probability that, among these additional children, all carriers have property X_n. This implies that

$$g_n = \frac{2}{1+f_n} \cdot \frac{e^{-(\lambda/2)(1-f_n)} - e^{-\lambda}}{1-e^{-\lambda}} . \quad (3)$$

Equations (1), (2), and (3) define a recursive relationship by which f_n and g_n can be calculated from λ and ψ. By adopting $\lambda = 2$ throughout the following, we shall assume that the population of interest is of constant size. The resulting f_n and g_n values (table 1) facilitate the assessment of the inclusion rate R for almost any cascade-screening strategy. This is exemplified below by three distinct strategies aimed at detecting carrier relatives of an affected individual in either the same or succeeding generations.

Strategy 1: Screening of all children and all siblings of an affected individual.—An asymptomatic heterozygous carrier has no affected parent and no affected sibs if he/she has property Y_0 and if their carrier parent has property X_0 (i.e., the mutation is not penetrant). Since these two properties are statistically independent, the probability of their combined occurrence is $f_0 \cdot g_0$. Therefore,

$$R_1 = 1 - f_0 \cdot g_0$$

Strategy 2: Additional screening of all grandchildren, nieces, nephews and first cousins.—An asymptomatic carrier has no clinically affected grandparent, no affected uncles or aunts, and no affected first cousins, if the carrier grandparent has property X_0 and the carrier parent has property Y_1. Owing to statistical independence,

$$R_2 = 1 - (1 - R_1) \cdot f_0 \cdot g_1 = 1 - f_0^2 \cdot g_0 \cdot g_1 .$$

Strategy 3: Additional screening of all great-grandchildren, grandnieces, grandnephews, and second-degree nieces, nephews, and cousins.—On the basis of arguments similar to those employed in strategies 1 and 2,

$$R_3 = 1 - (1 - R_2) \cdot f_0 \cdot g_2 = 1 - f_0^3 \cdot g_0 \cdot g_1 \cdot g_2$$

and, generally, for screening depth n,

$$R_n = 1 - (1 - R_{n-1}) \cdot f_0 \cdot g_{n-1} = 1 - f_0^n \cdot \prod_{i=0}^{n-1} g_i .$$

Inclusion rates for some representative values of ψ, and assuming $\lambda = 2$, are summarized in table 2. Inclusion rates for generations other than the succeeding ones can be derived similarly from f_n and g_n values. For example, screening of the parents of clinically affected individuals yields an inclusion rate of $1 - f_1$; extension of screening to the offspring, siblings, uncles, and aunts of patients results in an inclusion rate of $1 - f_1 \cdot f_0 \cdot g_1$, etc. It should be noted that the inclusion rate is independent of

Table 2

Inclusion Rate and Efficiency of Cascade Screening for Autosomal Dominant Mutations, with $\lambda = 2$

							E_{pop} at p =		
ψ	R_1	E_1	R_2	E_2	R_3	E_3	.001	.01	.05
.05	.081	2.1	.180	3.2	.288	5.1	526.3	52.6	10.5
.10	.157	2.1	.327	3.3	.486	5.1	555.6	55.6	11.1
.25	.362	2.3	.634	3.5	.801	4.9	666.7	66.7	13.3
.50	.637	3.0	.883	4.1	.964	5.0	1000.0	100.0	20.0
.75	.844	5.0	.978	6.1	.997	6.5	2000.0	200.0	40.0
.90	.943	11.0	.997	12.1	>.999	12.2	5000.0	500.0	100.0

whether the cascade screening is performed comprehensively or sensu stricto.

The efficiency of a genetic-screening strategy can be assessed by the number, E, of unaffected individuals who have to be screened for one heterozygous carrier to be detected. E equals the inverse of the respective carrier risk and, with strategies $i = 1, \ldots, 3$ as outlined above, amounts to $(2^i - \psi)/(1 - \psi)$ for those carriers who are detectable by strategy i but not by strategy $i - 1$. Therefore, with comprehensive screening of relatives,

$$E_n = \frac{1}{(1-\psi)\cdot R_n}\sum_{i=1}^{n}(R_i - R_{i-1})\cdot(2^i - \psi) ,$$

with $R_0 = 0$. If screening is performed strictly in cascade fashion, then target individuals have a carrier risk equal to that given in strategy 1. Therefore, the efficiency of cascade screening sensu stricto equals E_1 for all screening depths. The efficiency of screening the whole population equals

$$E_{\text{pop}} = \frac{1}{2p\cdot(1-\psi)} ,$$

provided that p is sufficiently small.

Autosomal Recessive: Homozygotes for Frequent Mutations with Reduced Penetrance

Homozygotes for an incompletely penetrant recessive mutation have two carrier parents, and, if the frequency of the mutation is high, some parents may even be homozygous themselves. This results in multiple branches of possible inheritance for the mutation, thereby complicating the efficiency assessment of cascade screening strategies. In the following, we shall first assume that the reproductive fitness of overt homozygotes is the same as that of covert homozygotes or as that of any other genotype (which is typically the case for late-onset diseases, or for diseases for which therapeutic intervention is possible). Under these assumptions, and under Hardy-Weinberg conditions, the probability of the combined parental genotype is

$$a_i = \binom{2}{i} p^{2-i}(1-p)^i , \tag{4}$$

where $i = 0$, 1, or 2 is the number of heterozygous (as opposed to homozygous) parents. The probability, f_0, that neither parent is clinically affected themselves depends upon i, in that $f_0 = f_{0,i} = (1\text{-}\psi)^{2\text{-}i}$. The probability, g_0, that a homozygote has no clinically affected siblings is also a function of i and, by arguments similar to those intrinsic to equation (3), it follows that

$$g_{0,i} = \frac{2^i}{2^i - \psi}\cdot\frac{e^{-\lambda\psi 2^{-i}} - e^{-\lambda}}{1 - e^{-\lambda}} . \tag{5}$$

The inclusion rate, R_1, of screening all siblings and all children of an affected individual equals 1 minus the weighted sum of the $f_{0,i}$ and $g_{0,i}$ values; that is,

$$R_1 = 1 - \sum_{i=0}^{2} a_i \cdot f_{0,i}\cdot g_{0,i}$$

Results obtained for some representative values of p and ψ, under the assumption that $\lambda = 2$, are summarized in table 3.

The efficiency of screening strategy 1 equals the inverse of the homozygosity risk of its target individuals, which, in turn, depends upon parental genotypes. If the parental genotypes comprise i heterozygotes and $2 - i$ homozygotes for the mutation in question, then the homozygosity risk of an unaffected child equals $(1 - \psi)/(2^i - \psi)$. If one parent lacks a mutation, the risk is 0. Probabilities of combined parental genotypes, analogous to equation (4) but conditional upon the presence of an unaffected individual and at least one affected parent or sibling, can be calculated using Bayes' formula (not shown). The overall homozygosity risk of target individuals then equals the average of the parental genotype-specific risks, weighted by the paternal genotype probabilities. Screening efficiency E_1 equals the inverse of this average risk (table 4, *top*). Note that, for cascade screening sensu stricto, the efficiency equals E_1 for any screening depth. The efficiency of screening the whole population (table 4, *bottom*) is calculated as

$$E_{\text{pop}} = \frac{1 - \psi p^2}{(1-\psi)p^2} .$$

Owing to the variable number and structure of mutant lineages leading to a homozygote, the inclusion rate of extended cascade screening is difficult to assess analytically and was therefore evaluated by simulation. To this

Table 3

Inclusion Rate (R_1) of Screening for Homozygotes for Autosomal Recessive Mutations among First-Degree Relatives of Patients, with $\lambda = 2$

	R_1 AT $p =$					
ψ	.05	.10	.20	.30	.40	.50
.05	.023	.029	.042	.056	.069	.083
.10	.045	.057	.082	.108	.133	.159
.25	.107	.135	.190	.244	.297	.348
.50	.198	.244	.334	.419	.498	.572
.75	.276	.335	.446	.547	.638	.719
.90	.318	.383	.502	.608	.702	.783

Table 4

Efficiency (E_1 and E_{pop}) of Screening for Homozygotes for Autosomal Recessive Mutations, with λ = 2

	E_1 AT p =					
ψ	.05	.10	.20	.30	.40	.50
.05	7.6	5.9	4.0	3.0	2.4	2.0
.10	8.0	6.2	4.2	3.2	2.5	2.1
.25	9.7	7.5	5.1	3.8	3.0	2.4
.50	14.8	11.5	7.8	5.7	4.3	3.4
.75	30.2	23.5	15.7	11.3	8.4	6.3
.90	76.2	59.5	39.5	28.1	20.5	15.2
	E_{pop} AT p =					
	.05	.10	.20	.30	.40	.50
.05	421.0	105.2	26.3	11.6	6.5	4.2
.10	444.3	111.0	27.7	12.2	6.8	4.3
.25	533.0	133.0	33.0	14.5	8.0	5.0
.50	799.0	199.0	49.0	21.2	11.5	7.0
.75	1597.0	397.0	97.0	41.4	22.0	13.0
.90	3991.0	991.0	241.0	102.1	53.5	31.0

end, an ancestral genealogy was constructed in each run, conditional upon the presence of a pivotal affected offspring. Further offspring were added to each generation, according to a Poisson model (λ = 2), allowing, however, for the presence of at least one child for each ancestor of the pivotal homozygote. The inclusion rates of screening strategies 2 and 3 were then evaluated by counting the number of successes in 1,000,000 simulation runs per parameter combination (table 5). It should again be noted that the inclusion rates are the same for comprehensive cascade screening and for cascade screening sensu stricto.

If the reproductive fitness of an overt homozygote is lower than that of other genotypes, the inclusion rate of cascade screening is bound to decrease. If patients have no children at all (i.e., if the disease state is "biologically lethal"), then equation (4) has to be replaced by

$$a_i^* = \frac{1}{(1-\psi p)^2}\binom{2}{i}p^{2-i}(1-p)^i \cdot (1-\psi)^{2-i} ,$$

and $f_0 = f_{0,i} = 1$, for all i. This implies that

$$R_1^* = 1 - \sum_{i=0}^{2} a_i^* \cdot g_{0,i} = \frac{R_1 - \psi p}{1 - \psi p} \leqslant R_1 ,$$

where the last relationship is strict ("<") if $R_1 < 1$ and $\psi p > 0$. Assuming λ = 2, inclusion rates R_1^* (table 6) and R_1 (table 3) are very similar for a wide range of values of p and ψ, suggesting that the reproductive fitness of patients has no substantial influence upon the inclusion rate of cascade screening strategies.

Autosomal Recessive: Heterozygous Carriers of Rare, Fully Penetrant Mutations

For rare autosomal recessive diseases, virtually all heterozygous carriers represent offspring of matings between a (clinically unaffected) heterozygote and either a heterozygote or homozygote for the wild-type allele. Under Hardy-Weinberg conditions, the probabilities of these two mating types are p and $1 - p$, respectively. Only pairs of two heterozygotes will have affected children, so that heterozygous children of a single heterozygous parent always lack an affected sibling. The probability that a heterozygous child of two heterozygous parents has no affected sibling equals $g_{0,2}$, as defined in formula (5), assuming $\psi = 1$. Thus, the proportion R_1 of heterozygotes who have an affected sibling is calculated as $R_1 = 1 - (p \cdot g_{0,2} + 1 - p) = p \cdot (1 - g_{0,2})$. This implies that the proportion of unaffected carriers who are detectable via the screening of siblings of patients decreases linearly with the disease-allele (and, therefore, carrier) frequency. If λ = 2, for example, $R_1 = .273 \cdot p$.

Under a Poisson-type model of sibship size, the average number of siblings of a given individual equals

$$s(\lambda) = \frac{\lambda}{1 - e^{-\lambda}} - 1 .$$

Whereas the average number of heterozygous siblings of patients is thus $s(\lambda) \cdot 1/2$, heterozygous first cousins of patients number $2 \cdot s(\lambda) \cdot \lambda \cdot 1/4$. Here, $2 \cdot s(\lambda) \cdot \lambda$ is the average number of first cousins, and 1/4 is their individual carrier risk. The ratio, r_1, of the number of heterozygous first cousins versus heterozygous siblings therefore equals λ. By similar arguments, it follows that $r_2 = \lambda^2/2$ for sec-

Table 5

Inclusion Rates (R_2 and R_3) of Screening for Homozygotes for Autosomal Recessive Mutations, with λ = 2

	R_2 AT p =					
ψ	.05	.10	.20	.30	.40	.50
.05	.035	.056	.102	.155	.211	.271
.10	.068	.106	.190	.279	.369	.457
.25	.159	.241	.397	.540	.660	.758
.50	.285	.409	.613	.763	.863	.926
.75	.387	.532	.744	.871	.941	.976
.90	.439	.590	.798	.909	.964	.987
	R_3 AT p =					
	.05	.10	.20	.30	.40	.50
.05	.055	.106	.234	.381	.525	.653
.10	.106	.198	.404	.599	.752	.857
.25	.238	.411	.696	.869	.951	.983
.50	.406	.628	.883	.971	.994	.999
.75	.530	.754	.949	.992	.999	>.999
.90	.588	.805	.967	.996	>.999	>.999

Table 6

Inclusion Rate (R_1^*) of Screening for Homozygotes for Autosomal Recessive Mutations, Assuming Biological Lethality of the Overt Homozygous Genotype, with $\lambda = 2$

	R_1^* AT p =					
ψ	.05	.10	.20	.30	.40	.50
.05	.020	.024	.033	.041	.050	.059
.10	.040	.048	.064	.080	.097	.114
.25	.095	.112	.147	.182	.218	.255
.50	.177	.205	.260	.316	.372	.429
.75	.248	.281	.349	.416	.483	.551
.90	.286	.321	.393	.464	.535	.606

ond cousins and, generally, $r_n = \lambda^n/2^{n-1}$. If $\lambda = 2$, this implies that $r_n = 2$ for all n; that is, the number of clinically unaffected carriers who are first, second, etc. cousins of patients is always twice that of carrier siblings of patients.

Discussion

Population-wide genetic screening for disorders for which a successful therapy exists has been practiced for many years. For example, newborns in Europe, North America, Australia, New Zealand, Japan, and many other countries worldwide are routinely screened for the presence of phenylketonuria (PKU), an autosomal recessive disorder for which a carefully monitored diet from an early age provides significant amelioration of the clinical phenotype (Scriver and Kaufman 2001). Genetic screening for curable conditions is clearly beneficial and therefore widely accepted, since test results help to confirm the diagnosis, improve the prognosis, and may serve to identify individualized treatments. Even at a birth prevalence as low as that of PKU (1 case per 10,000 live births [Scriver and Kaufman 2001]), the benefits of population-wide screening accruing to patients outweigh most ethical and economic concerns, thereby rendering efficiency and efficacy considerations irrelevant.

Heterozygote Screening for Rare Autosomal Recessive Diseases: Cystic Fibrosis

For severe diseases lacking a viable therapeutic option, newborn screening would still be helpful if the early detection of the condition in question were to have some benefits that served to improve the quality of life of both the patients and their families. Autosomal recessive diseases falling into this category include cystic fibrosis (CF), the most frequent severe single-gene disorder in North America and northern Europe (Scotet et al. 2000). For a recessive disease such as CF, it may also be possible to target genetic screening at heterozygous carriers prior to reproduction, to either identify at-risk pregnancies or facilitate alternative reproductive choices prior to conception (Wildhagen et al. 1998). However, since selective abortion represents one possible option in the case of a positive prenatal diagnosis, carrier screening for severe recessive diseases is less widely regarded as being ethically acceptable (Schmidtke 1998). Nevertheless, even with CF, for which the disease-allele frequency p is ~1/50 in most European populations, the inclusion rate of carrier screening among siblings of patients would only be 0.5%. Extension of screening to first and second cousins would still leave more than 97.5% of clinically unaffected heterozygous carriers in the population undetected.

This result is broadly consistent with previous findings of Holloway and Brock (1994), who calculated an inclusion rate R of 4%–13% for CF. Their estimate of R is somewhat higher than ours, since it was based upon a constant sibship size of 2 or 3, an assumption which would have put insufficient weight upon those heterozygotes who do not reproduce at all. Second, the 1977 data on sibship size in Scotland, as employed by Holloway and Brock (1994), broadly fit a Poisson model, but with $\lambda = 2.5$ instead of $\lambda = 2.0$. Furthermore, the census only referred to married individuals and excluded singles, who can be assumed to have had fewer children. Finally, Holloway and Brock (1994) took heterozygotes from generations preceding the index patient into account; these we chose to ignore, since most of the target individuals would have completed reproduction by the time of screening. Despite some minor discrepancies, the conclusions drawn by Holloway and Brock are nevertheless in accord with our own in that, even if cascade screening were to be offered to relatives of patients suffering from CF (or any other rare recessive disease), "most carrier couples will not be informed of their risk status before they have an affected child" (Holloway and Brock 1994, p. 164). Additionally, the sensitivity of genetic testing for CF carriership varies greatly according to the mutations screened for and the population offered the screening; screening of the 70 most common mutations would thus detect ~90% of *CFTR* gene lesions in whites but a much smaller proportion in other ethnic groups (Girodon-Boulandet et al. 2000).

Screening for Homozygous At-Risk Individuals: Hemochromatosis

Hereditary hemochromatosis (HH) is one of the most common autosomal recessive disorders in people of northern European descent, with $p = .05$ or higher in many populations (Beutler et al. 2001*a*). In HH, the body absorbs an excessive amount of iron from the diet, which is then deposited in various organs. Most homozygotes have some degree of pathological iron over-

load, but it is as yet unclear how many of them become overtly affected. Whereas some studies suggest that as many as 50% of homozygotes may display at least one clinical manifestation (Bradley et al. 1996*a,* 1996*b;* Olynyk et al. 1999), others imply much lower penetrance values (Beutler et al. 2001*b*). Feder et al. (1996) identified a genetic change in the *HFE* gene on chromosome 6 which appears to underlie most cases of HH, and a genetic test for *HFE* mutations identifies >90% of at-risk individuals in the United Kingdom (The U.K. Haemochromatosis Consortium 1997). Regular measurement of transferrin saturation and serum ferritin concentration can be used to detect acute iron overload in patients, which can then be treated by phlebotomy. The possibility of clinical intervention to prevent morbidity and mortality, together with the fact that genetic testing is only required once in a lifetime, provide good justification for screening for covert homozygotes or compound heterozygotes who might develop disease at a later stage (Allen and Williamson 1999). Inspection of table 5 ($p = .05$) reveals that, at a penetrance of $\psi = .50$, some 40% of unaffected homozygotes or compound heterozygotes for *HFE* mutations would be detectable by screening first to third degree relatives of patients. At a penetrance of $\psi = .25$, the inclusion rate would equal some 24%. Since, in both cases, the number of people that have to be tested to identify a single at-risk individual is 50 times smaller than with population-wide screening, cascade screening thus appears to represent an efficient alternative.

It has been argued that population-wide screening for hemochromatosis could be cost-effective (Schöffski et al. 2000, Beutler et al. 2001*a*). Generally, a screening strategy, s, is cost-effective if the overall diagnostic costs, $N \cdot p^2 \cdot R_s \cdot E_s \cdot c_d$, are lower than the extra costs arising from managing—rather than preventing or ameliorating—the disease, calculated as $N \cdot p^2 \cdot R_s \cdot c_m$. Here N denotes the population size, and c_d and c_m are the diagnostic and extra managerial costs per person, respectively. Note that, under certain conditions, $c_m < 0$. This could occur when a large number of individuals with a positive screening result obtain expensive treatment that would otherwise only be required by a small number of them. Depending upon the context in which cost considerations are being made, c_d and c_m could include either economical or emotional costs, or both. In any case, cost-effectiveness is equivalent to $E_s \cdot c_d < c_m$. Given that a particular screening strategy is cost-effective, then any strategy that is more efficient (i.e., one with a smaller E value) is also going to be cost-effective. It thus follows that, if population-wide screening for hemochromatosis is cost-effective, so is cascade screening. However, for a strategy, s, to be superior to another strategy, t, the total savings have to be greater with strategy s than with strategy t, which is the case if $R_s \cdot (c_m - E_s \cdot c_d) > R_t \cdot (c_m - E_t \cdot c_d)$. Apart from a proportionality factor on both sides of the inequality, $R_{pop} = 1$ for hemochromatosis, so that the criterion of higher savings becomes

$$c_d > \frac{1 - R_s}{E_{pop} - R_s \cdot E_s} \cdot c_m$$

Since $R_s \cdot E_s$ is small compared to E_{pop}, we may conclude that, if approximately $c_m > E_{pop} \cdot c_d > 0.6c_m$ (or $c_m > E_{pop} \cdot c_d > 0.76c_m$, if $\psi = .25$ and thus $R_s = .24$), population-wide screening for hemochromatosis is cost-effective, but cascade screening is predicted to be even more cost-effective. However, the current trend in molecular medicine is such that, for most diseases, c_m is bound to increase, whereas high-throughput genotyping facilities will serve to reduce c_d dramatically. It is therefore likely that population-wide genetic screening for many diseases will become cost-effective in the near future, and that diagnostic costs will eventually become so low that cascade screening will become increasingly unattractive. With hemochromatosis, for example, for which $c_m > E_{pop} \cdot c_d$ can currently be assumed, any reduction in the diagnostic costs per head by >40% (or 24% if $\psi = .25$) would render cascade screening less cost-effective than population-wide screening. Furthermore, R_s might be substantially reduced for diseases for which not all patients are correctly diagnosed. This is especially true for HH, which appears to be a massively underdiagnosed condition. Reduction of R_s, however, renders cascade screening less cost-effective than population-wide screening at even higher diagnostic costs c_d. Finally, cascade screening requires the availability of an index case, the finding of which can either be through active search or through the "opportunistic" retrospective approach of known patients. In any case, an emotional burden is likely to be involved, which would not arise from population screening. Taking the extra emotional costs pertaining to the index case and their relatives into account could therefore detract further from the appeal of a cascade-screening strategy.

Autosomal Dominant Mutations Underlying Complex Disease: Factor V Leiden

Most prominent fatal genetic disorders, such as cancer and heart disease, are multifactorial in nature. Mutations in many genes and in different combinations are required to cause one of these complex disorders. The clinical severity of the phenotype is also likely to depend upon how the different genetic factors interact with environmental influences. The efficiency of genetic screening for a multifactorial disease will thus be critically dependent upon (*i*) its etiological complexity and (*ii*) the net effect that individual genetic variants have upon the

disease phenotype. Some disorders will be caused by just one of many possible disruptions in a particular metabolic or developmental pathway, with each case or family in effect representing a separate, high-penetrance Mendelian disorder. These disorders may appear as if they are complex, but they are, in fact, caused by private mutations with strong effects. Population screening is unlikely to be very efficacious in these cases. The same applies to disorders at the other end of the complexity spectrum, which involve many genes of small effect operating in concert in any given case.

This notwithstanding, it can be expected that genetic variants will represent useful risk predictors for a substantial proportion of complex diseases and that these variants will formally segregate in the population as dominant mutations with age-dependent—and therefore cross-sectionally reduced—penetrance. A typical example of this category of mutation is factor V Leiden, a G→A transition in the gene (*F5*) encoding human coagulation factor V, leading to an Arg→Gln replacement at codon 506. Factor V Leiden has a frequency of ~4% in white populations and represents the single-most-frequent genetic risk factor for venous thrombosis (Cooper and Krawczak 1997). The odds ratio equals 6, leading to a percentage attributable risk of 16.7% in the general population. Although in-depth studies of the economic implications are lacking, such a high impact upon population health suggests that screening for the factor V Leiden mutation could be cost-effective.

A comparison between table 2 and tables 3, 4, and 5 reveals that the inclusion rates of cascade screening for at-risk individuals are higher for dominant than for recessive mutations. With a penetrance of $\psi = .25$—the approximate lifetime risk for venous thrombosis of a factor V Leiden carrier (Cooper and Krawczak 1997)—screening of first- to third-degree relatives of affected individuals allows the detection of almost 80% of covert carriers in the population (table 2). The cost-efficiency is also higher than for recessive conditions, since only four individuals would have to be screened to detect one carrier. Furthermore, once population-wide screening has become cost-effective, diagnostic costs would have to drop by 80% for population screening to become more cost-effective than cascade screening. At present, the cost-effectiveness of population-wide screening for factor V Leiden remains to be determined. It has been shown, however, that screening of all U.S. women using oral contraceptives for factor V Leiden carriership would involve costs of >$100 million per preventable death by venous thrombosis, attributable to the interaction of the two risk factors (Creinin et al. 1999). Taking a family history of venous thromboembolic events was thus advocated as a better and more cost-effective option. These findings lend further support to the notion that cascade screening is an economically more sensible option for dominant—as opposed to recessive—conditions, and that cascade screening may also represent a viable option for complex disease, if and when high-risk predisposing mutations have been characterized.

Further Considerations

Throughout the derivation of inclusion rates *R* and efficiency values *E*, we have assumed that reproduction in the population of interest results from random mating. If this assumption is violated, some of our conclusions may be inaccurate. For example, some 55% of marriages in the Pakistani proportion of the U.K. population occur between first cousins (Darr and Modell 1988). With inbreeding, the carrier risk of close relatives of carriers for a disease-predisposing mutation would be markedly increased over that applying in a panmictic population. This implies that both the efficacy and efficiency of cascade screening are higher under inbreeding than under random mating, and that cascade screening, even for heterozygosity for recessive mutations, may be cost-efficient in some populations or subpopulations.

Similarly, if a population is substantially structured and comprises genetically distinct strata, or if the effect of the mutation in question is influenced by other environmental and/or genetic factors, then both the frequency and the penetrance of a disease-predisposing lesion may also vary significantly between different subpopulations. In addition, since the managerial and preventive costs could be chosen so as to include emotional (in addition to pecuniary) costs, "economic" parameters could vary according to social and cultural affiliation. Under such circumstances, it would appear sensible to assess the efficiency and efficacy of a screening strategy separately for each subpopulation. Thus, for example, screening for *BRCA1* and *BRCA2* mutations predisposing to breast cancer has been shown to be cost-effective in Ashkenazi Jewish women, among whom the respective variants have a particularly high prevalence (Grann et al. 1999).

In the present study, we have confined our analysis to autosomal gene mutations affecting males and females at equal rates. A substantial proportion of genetic variants for which screening might be worth considering would, however, be located on a sex chromosome or in the mitochondrial genome. Other lesions, such as mutations in the *BRCA1* and *BRCA2* genes, may specifically predispose only one gender. Although the basic concepts developed herein should, in principle, be transferable to sex-specific penetrance values and/or modes of inheritance, the analytical treatment of such cases will be much more complicated than that of autosomal gene mutations affecting both genders at equal rates. For example, the recursive definition of parameters f_n and g_n (i.e., the probabilities of patients or carriers absent from

the progeny of individuals and their siblings), by analogy with equations (2), (3), and (5), would have to allow for gender and would have to take the exact sex distribution among an individual's children into account. As in the case of autosomal recessive mutations, the efficacy and efficiency of cascade screening under sex-specific genetic models is therefore more easily assessed by simulation. A simulation study specifically addressing the efficacy of cascade screening for fragile-X syndrome has recently revealed that testing first- to third-degree relatives of patients would allow the detection of 12% of at-risk couples who would otherwise go unnoticed (Wildhagen et al. 1999). This inclusion rate, which is intermediate to those of heterozygosity for CF and of covert homozygosity for hemochromatosis, was deemed to indicate that cascade screening for fragile X is not very effective. Since fragile-X syndrome is inherited in a semidominant fashion, the findings of Wildhagen et al. (1999) will be relatively independent of the frequency of the causative mutation and should therefore be applicable to a wide range of similar conditions.

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Electronic-Database Information

The URL for data in this article is as follows:

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