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REVIEW

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Challenges in providing residual risks in carrier testing

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Abstract

The probability an individual is a carrier for a recessive disorder despite a negative carrier test, referred to as residual risk, has been part of carrier screening for over 2 decades. Residual risks are calculated by subtracting the frequency of carriers of pathogenic variants detected by the test from the carrier frequency in a population, estimated from the incidence of the disease. Estimates of the incidence (and therefore carrier frequency) of many recessive disorders differ among different population groups and are inaccurate or unavailable for many genes on large carrier screening panels for most of the world's populations. The pathogenic variants detected by the test and their frequencies also vary across groups and over time as variants are newly discovered or reclassified, which requires today's residual carrier risks to be continually updated. Even when a residual carrier risk is derived using accurate data obtained in a particular group, it may not apply to many individuals in that group because of misattributed ancestry or unsuspected admixture. Missing or inaccurate data, the challenge of determining meaningful ancestry-specific risks and applying them appropriately, and a lack of evidence they impact management, suggest that patients be counseled that although carrier screening may miss a small fraction of carriers, residual risks with contemporary carrier screening are well below the risk posed by invasive prenatal diagnosis, even if one member of the couple is a carrier, and that efforts to provide precise residual carrier risks are unnecessary.

Key Points

What's already known about this topic? What does this study add?

- There has been no published discussion of the methods and uncertainties involved in the calculation of residual risk that are discussed here
- There has been much discussion of using ancestry in genetic testing but this review highlights the serious problems that arise in calculating and assigning ancestry-specific residual carrier risks at specific disease loci
- The review questions what has not been questioned before: Is there clinical utility to providing what are mostly imprecise residual carrier risks

1 | INTRODUCTION

Residual carrier risk (RCR) after carrier testing is the chance an individual with a negative carrier test could still be a carrier of the disorder. This review will discuss how residual risks after negative carrier screening are determined, the problems with providing them to clinicians and patients, and their limited clinical utility. Examples will be drawn from cystic fibrosis (CF) carrier screening, where the practice of providing RCRs began, but are relevant to all recessive disorders.

2 | THE ORIGIN OF RCRs

Cystic fibrosis carrier screening began over 2 decades ago with a test that screened the cystic fibrosis transmembrane conductance regulator gene (*CFTR*) for 25 pathogenic variants (PVs) chosen because they were present in greater than 1 in 500 people in the U.S. general population and in certain subpopulations, such as Black or Ashkenazi Jewish individuals.[1,2] While this strategy identified a majority of carriers in many populations, a RCR remained after a negative carrier screen because the rest of the >900 rarer PVs in *CFTR* were omitted in order to reduce the complexity and cost of the test and improve its positive rate. Guidelines, therefore, recommended that the providers "… define as accurately as possible, based on current knowledge, the residual risk that the person tested could be a carrier of an untested or unknown mutation."[1] This recommendation was made out of concern that practitioners and patients alike were unfamiliar with the concept of clinical false negatives in genetic testing and needed to be alerted that false negatives will occur. Because an even greater fraction of the PVs responsible for CF outside the United States and Northern Europe were omitted, an even greater RCR existed in non-Europeans testing negative for this panel.[2] Therefore, an additional recommendation was made that different RCRs be provided that are tailored to an individual's "racial/ethnic" background without addressing pitfalls in calculating accurate ancestry-specific RCRs or in determining an individual patient's ancestry.

3 | CALCULATING RCRs

RCR after negative carrier screening is the difference between the frequency of carriers of *every* PV responsible for a recessive disease in a population, and the frequency of carriers of just those PVs that can be *detected by the screening test* (See Appendix A). Next-generation sequencing makes sequencing of the entire *CFTR* gene (and many others) cost-effective and reduces false negatives by allowing detection of essentially all previously described PVs as well as many novel variants classifiable as PVs (because of the type of DNA change), a yield far exceeding the ~25 PVs detected using old technology.[3] Other variants also detected but not reported in carrier screening include variants of uncertain significance (VUSs) that lack definitive classification (although subject to future reinterpretation as pathogenic or benign) and benign variants of no medical significance.[4]

The frequency of carriers of every PV responsible for a rare disease in a population is difficult to measure directly and so is usually approximated as twice the square root of the observed incidence of the disease (Appendix A) using the Hardy-Weinberg law[5] (Appendix B). The frequency of the carriers of the PVs that can be detected by the test must also be determined, either by directly sequencing large numbers of people in the population and counting the number of carriers or, more commonly, by sequencing a large number of unrelated *affected* individuals and using the proportion of PVs found to estimate their allele frequencies, and therefore carrier frequencies (Appendix A). The latter approach is well suited to rare disorders because just affected individuals are sequenced rather than a large number of unaffected individuals looking for rare carriers. Similar calculations for X-linked inheritance are in Appendix A.

4 | FACTORS AFFECTING THE ACCURACY OF RCRs

The accuracy of RCRs depends on how well disease incidence is known. Although estimates of the incidence of CF in the United States and many European Union (EU) countries are accurate because of CF newborn screening and the widespread practice of managing all CF patients in specialized CF clinics, measurements of CF incidence are challenging for most areas of the world lacking these programs.[6] In Latin America,[7] only four countries, Brazil, Uruguay, Argentina, and Chile, had national newborn CF screening programs as of 2020, yet even with newborn screening, estimates of CF incidence in Brazil range 9-fold, from 1/1,600 to 1/14,000, due to varying degrees of genetic admixture of different socioeconomic groups within Brazil. In countries without CF newborn screening, ascertainment is incomplete and estimates of incidence are probably artifactually low. For example, the published incidence figures for CF In India vary 2.5-fold (1/40,000 and 1/100,000) and 3.5-fold in Japan (1/100,000 and 1/350,000).[7] If disease ascertainment is incomplete even for a well-studied disease like CF, it is even less complete for most other rarer disorders and nationalities.

Compounding the difficulties in measuring disease incidence is the problem of using the Hardy-Weinberg equation[5] to estimate the frequency of all PVs from disease incidence in a population. The Hardy-Weinberg equation requires simplifying assumptions including that a population consists of randomly mating individuals (Appendix B). In practice, disease incidence, no matter how accurate, is not measured and reported from an idealized population of randomly mating individuals. Instead, incidence is generally determined in groups defined by a common language, ethnicity, geographic ancestry, or national boundary within which there is still genetic stratification due to non-random mating patterns.

Stratification and consanguinity inflate the incidence of autosomal recessive disorders beyond what would be expected from the actual carrier frequency under the Hardy-Weinberg equation and consequently cause overestimation of carrier frequency and, therefore, RCR derived from incidence.[5,8]

As an example of stratification, the population of the United Arab Emirates (U.A.E.) consists of subgroups of different nationalities and/or ethnicities, with 25% from India, 12% from Pakistan, 9% indigenous Emirati, 7% Bengali, 5% Filipino, 6% immigrants from Europe, Australia, North America, and Latin America, and Britain, with the remaining ~30% from other Arab states.[9] Intermarriage between members of many of these groups is limited for reasons of religion or country-of-origin, and consanguinity is present.[10,11] The overall incidence of cystic fibrosis in the U.A.E. is 1/15,876 but differs among these different ancestral subgroups.[7] An average CF carrier frequency calculated from the observed average incidence is $2 \times \sqrt{(1/15{,}876)} = 1/63$ in the U.A.E but is likely not applicable to many individuals in any of these subgroups.

Accurate measurements of the carrier frequency of PVs that can be detected by sequencing are also required for accurate RCRs. For example, a CF screening program in Yucatan, Mexico[12] screened 96,071 consecutive newborns by sequencing their *CFTR* genes and ascertained 7 patients with a firm diagnosis of CF, for an incidence of 1/13,724, and carrier frequency = 1/59 (0.017) (Appendix C). Upon sequencing of the 14 *CFTR* genes in these 7 patients, 9 of the 14 (64%) PVs were seen, giving a carrier frequency of detectable PVs = $0.64 \times 1/59 = 1/92$ (0.011). The RCR for a patient from Yucatan testing negative for these nine alleles = 1/164 (95% confidence limit 1/96–1/625). Two missense VUS's in three patients were also seen but could not be classified as PVs and two putative PVs were not found.

This Yucatan example demonstrates many of the problems associated with RCR calculations. First, the frequency of *all* pathogenic variants is calculated from the incidence by assuming random mating and applying the Hardy-Weinberg law, which may not be the case here as population genetic studies of indigenous individuals across Mexico suggest significant stratification with excess homozygosity.[13,14] Second, because genetic testing of affected individuals is limited, especially in understudied demographic groups,[15] sample sizes are small, which means that estimates of allele frequencies will be imprecise, with wide confidence limits, and rarer PVs will be missed until more sequencing of affected individuals identifies more PVs and VUSs, requiring an amended RCR calculation. There may be a delay before sufficient data accumulate to allow the two missense VUSs to be classified as either pathogenic or benign; if they are classified as PVs, RCR falls to 1/409. Finally, additional sequence analysis may ultimately identify the two undetected variants, which may also at first be classified as VUSs but later reclassified to PVs, which would further decrease the RCR. Known variants (benign or VUS) may be reclassified to or from being PVs, thereby altering the set of PVs detected by the test whose carrier frequencies are used to calculate the RCR.

5 | ANCESTRY AFFECTS RCR

Groups differ in disease incidence and PVs responsible for disease.[16,17] For example the incidence of CF is 1/1353 in Ireland, 1/8500 in Mexico, 1 in 25,000 in Finland and between 1/150,000 and 1/300,000 in Japan.[7,18] Similarly, the particular PVs responsible for CF and their allele frequencies differ among groups, with some PVs either enriched in, or even exclusive to, one group.[7] For example, the most common *CFTR* PV in Europe, p.Phe508del, is found in ~72% of CF patients of non-Hispanic European ancestry but only 39% of CF patients of Asian ancestry and an even smaller percentage in people of African ancestry.[2] Such differences among groups in disease incidence and in the frequency of PVs detectable by the screening test result in different RCRs. Unfortunately, the two values needed for an RCR, accurate disease incidence data and frequency of PVs detected by the test, are lacking or incomplete for most groups and more epidemiological and molecular data are needed to fill these gaps if RCRs are to be generally reliable.[15]

6 | PITFALLS IN APPLYING ANCESTRY-SPECIFIC RCRs TO AN INDIVIDUAL PATIENT

Even assuming an RCR can be accurately determined for a particular ancestral, national or ethnic group, providing the relevant RCR figure to an individual following a negative carrier screen requires the screened individual be correctly assigned to a group for which the RCR was determined. Ancestral group assignment has most often relied on self-reported ancestry (SRA) or on ethnicity based on a shared language (e.g., Spanish), or history/religion (Ashkenazi Jewish). However, for individuals with mixed genetic ancestry, especially when the mixture includes ancestries from different continents where PV frequencies may differ dramatically, the SRA is not an accurate reflection of either the individual's overall genetic ancestry, but even more importantly, will lack precision regarding the genetic ancestry of the alleles within the gene involved in the disease in question.[7,19,20] There is no clear consensus among geneticists and genetic testing laboratories on how SRA should be defined or used[21] and it is unknown how often inaccurate RCRs based on an incorrect SRA are provided to patients. For example, using SRA (e.g., "Brazilian") to decide which RCR applies to a Brazilian of mixed ancestry will miss the mark because we do not know the ethnic origin of her *CFTR* alleles (e.g., African, Native American, or European)[7] and can only offer a weighted average across these ancestral possibilities.

If SRA alone, especially for an admixed individual, does not provide the specificity needed to calculate an RCR used in counseling that individual,[20,21] genetic ancestry derived from single nucleotide polymorphisms (SNPs) is an alternative. SNPs are genetic markers whose allele frequencies differ among people whose ancestors historically have lived in distinct, separated geographic locations throughout the world.[20,22–24] SNP alleles and their frequencies, determined in cohorts of individuals whose ancestors are known to have originated from particular geographical regions, are used to determine the likely ancestral origin of every location in the genome, depending on the number and informativeness of SNPs around that location, how extensive the reference datasets for their allele frequencies are, and when admixture occurred in that individual. These

chromosomal ancestry estimates are summed to obtain genetic ancestry averaged across an individual's entire genome.

However, basing an RCR calculation for CF, for example, on average ancestry derived from genome-wide SNPs will be inaccurate for individuals who differ in the ancestry of the alleles in the region of *CFTR*, regardless of how similar their average genome-wide ancestry might be. For example, the degree of admixture of African, Native American, and European ancestry for 96 self-identified Puerto Rican individuals is shown in Figure 1.[25] All subjects carried a contribution from all three ancestral populations with an average of 66% European, 16% African, and 18% Native American ancestries. However, the proportion of ancestral alleles from each of these three continental ancestries varied among individuals, with African ancestry between ~10% and ~55%, European ancestry between ~30% and ~80%, and Native American ancestry between ~5% and ~20%. Individuals 95 and 96 have nearly identical contributions of alleles of the three continental origins. Yet, if the alleles at the *CFTR* locus were among the ~20% of alleles of European origin in individual 95 and among the ~50% of alleles of African ancestry in individual 96, their RCRs following a negative CF screening test would be different. In contrast, individual 1 far exceeds individual 96 in the relative contribution of European versus African ancestral alleles. Yet, if the alleles at the *CFTR* locus were among the ~80% of alleles of European ancestry in individual 1 and among the ~20% of alleles of European ancestry in individual 96, their RCR following a negative CF screening test would be similar, regardless of how these two individuals self-identify and regardless of the total proportions of other European and non-European alleles they carried across their genomes.

The admixture problem does not only affect communities with a clear historical record of admixture of individuals originating on different continents. Individuals of mixed ancestry are present even within supposedly homogeneous groups such as Europeans whose allele frequencies vary across Europe and the Middle East, and even for Northern Europeans such as the Irish or Finns.[6]

7 | CLINICAL UTILITY OF RCRs

Given that RCRs are imprecise and difficult to apply in an ancestry-specific manner, does knowing the approximate values of even imprecise RCRs impact management? The utility in knowing an RCR depends on whether it leads to a residual risk for a disorder in a pregnancy high enough to change clinical management. In the preconception setting, it is difficult to predict how any particular value of risk to a prospective offspring would affect the reproductive plans different couples make (e.g., pregnancy with invasive prenatal diagnosis [IPD], sperm or egg donation, in vitro fertilization with preimplantation diagnosis, and adoption). In the prenatal setting, however, we can benchmark offering IPD as the actionability threshold, using the past ACOG recommendation that IPD be discussed with all pregnant women regardless of personal or family history or advanced maternal age based on the chance of chromosomal aneuploidy (≥~1/500) outweighing the procedural risk (1/1,000–1/333).[26,27] Applying a similar argument, when one member of a couple is known to be a carrier and the other has tested negative, the chance of there being an affected fetus is one-fourth × RCR; RCR would need to exceed 1/125 to make the risk of the disorder in the pregnancy exceed the 1/500 actionability threshold. Given modern sequencing methods, even imprecise estimates of RCRs place nearly all of them well below levels that would trigger actionability. When both members of the couple are carrier screen negative, an RCR would need to exceed ~1/11 to make the risk of an affected pregnancy (one-fourth × $[RCR]^2$) exceed a 1/500 threshold, an RCR that is never seen after negative carrier screening when the entire coding region of a gene is examined (Table 1). Determining precisely how far an RCR is below the level that would trigger a management change makes no additional contribution to clinical decision making.

8 | CONCLUSION AND RECOMMENDATIONS

Counseling patients about their carrier risk and offering carrier screening to identify couples at risk so they can receive counseling and learn about reproductive options are essential components of preconception and prenatal care. Couples in which one or both members have negative carrier screens benefit from learning their risk is reduced. However, for all the reasons discussed in this review, the current practice of trying to precisely quantify that reduction in carrier risk, with or without ancestry specificity, is both flawed and, furthermore, of little utility. There are a number of options for dealing with the current unsatisfactory situation.

One is to only continue to provide RCRs when there are ancestry-specific incidence and PV data of sufficient quality and quantity to justify their use, including confidence limits or ranges on the estimate, and the ancestry of the alleles for the disorder in the individual screened, and not overall ancestral proportions, is well known from SNP-based ancestry studies. In this case, patients of diverse ancestries would receive different ancestry-specific RCRs for differing sets of genes, but this approach is difficult for clinicians and testing laboratories to execute and is problematic as social policy.

A second would be to accept that RCR estimates are uncertain and assigning patient ancestry is challenging, so, using the best data available, provide all patients with a range of "ancestry-agnostic" RCRs, with confidence limits, for only the most common disorders with the greatest RCRs and not deliver RCRs based on difficult-to-assign "'racial/ethnic' background of the tested individual."[1] Although not taking ancestry into account means using average RCRs that could be discrepant from their true values for a given individual, errors in assigning ancestry are likely to result in just as many erroneous RCR assignments. There is precedent for not taking ancestry into account when providing genetic screening. The American College of Obstetricians and Gynecologists (ACOG) initially considered it appropriate to only screen individuals for CF if there were a high carrier frequency of CF in the ancestral group to which the individuals belong.[28] However, increasing recognition of the difficulty of assigning an individual accurately to a single ancestry led

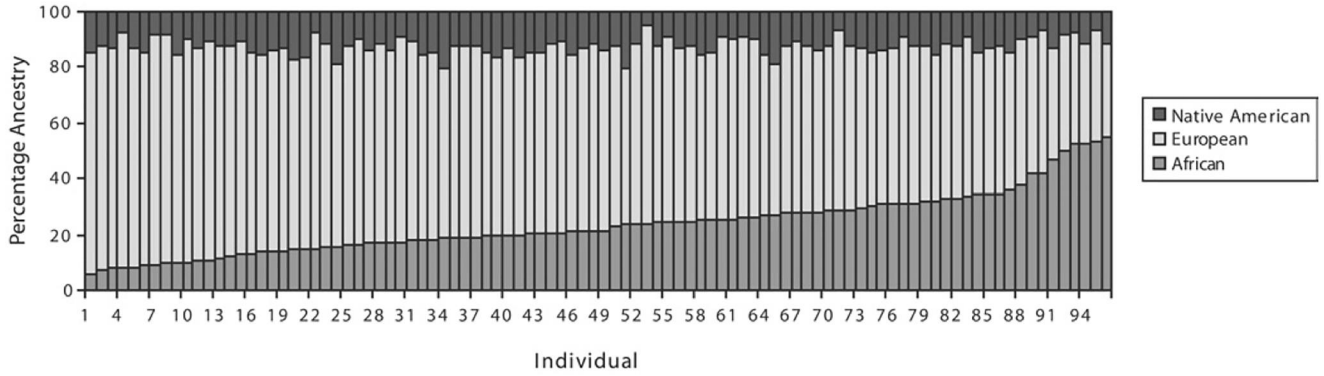

FIGURE 1 Graph showing the proportions of alleles of three different continental ancestries, African, Native American, and European, respectively, assessed at >116,000 SNP loci from across the genome in 96 self-identified Puerto Rican individuals. Each vertical bar represents a single individual, beginning with individual 1 on the left to 96 on the far right, arranged by increasing percentage of alleles of African continental origin carried (Figure from Burchard et al.).[25]

TABLE 1 Residual Carrier Risks in a couple that exceed a threshold for clinical actionability

		RCR in individuals with negative carrier test that would cause risk to offspring to exceed Actionability Threshold[a]	
		Carrier screen results in members of couple	
	Actionability threshold	Both test negative	One is a carrier Other tests negative
Prenatal setting	1/500	RCR > 1/11	RCR > 1/125

Abbreviation: RCR, residual carrier risk.

[a]When one member of the couple is of unknown carrier status, his/her chance of being a carrier is between the RCR of a person with a negative test (if he/she were to be tested and tests negative) and 1 (if he/she were to be tested and tests positive). The RCR needed to exceed threshold for actionability would be between the RCRs for when both test negative and the RCR for when one tests positive, the other negative.

ACOG to retract the previous guideline and recommend that practitioners should "offer CF carrier screening to all patients."[29,30]

Finally, RCRs estimates could be eliminated from most carrier testing. Despite inaccuracies, the vast majority of RCRs do fall below an actionability threshold for all but the most common disorders even in the most challenging situation, when the other member of the couple is known to be carrier; attempts at precision or ancestry-matching will not increase their actionability. Patients should therefore be counseled honestly that no genetic test is perfectly sensitive, carrier screening will miss a small fraction of carriers, but contemporary carrier testing reduces RCRs well below the risk posed by invasive prenatal diagnosis, even if one member of the couple is a carrier.

RCRs were a well-intentioned but now vestigial instructional tool from 20 years ago when individuals were screened for only a limited number of variants. More complete sequencing, inaccurate or unobtainable population data, a more sophisticated appreciation of the genetic complexity of ancestry, and the recognition of their limited utility together render them unnecessary. Professional guidelines should be revised with respect to the recommendation to offer RCRs as part of clinical care.

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No original data were generated for this manuscript.

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Appendix A

Suppose q_{PATH} = sum of the allele frequencies of *each and every* pathogenic variant (PVs), both identified and not yet identified, responsible for the disorder in a particular ancestral group or nationality. This set of all PVs is made up of three classes of variants defined as follows:

i. All known PVs detectable by sequencing that are responsible for the disease in a group, with frequency q_{KWN}.
ii. All VUSs that are detectable by sequencing but are not currently known to be pathogenic, although they eventually will be shown to be pathogenic ("proto-PVs" or PPVs), with frequency q_{PPV}

iii. All other PVs that are not detectable by sequencing because they are outside the reportable range of the test, with frequency q_{UND}.

$$q_{PATH} = q_{KWN} + q_{PPV} + q_{UND} \quad (A1)$$

Let p = Sum of the frequency of all benign alleles, including variants currently known to be benign as well as known VUS's and still undiscovered variants that ultimately will all be found to be benign. Then, p is what is left after subtracting the frequency of all PVs from 1. Then, Residual carrier risk (RCR) due to proto-PVs and undetected variants,

$$RCR = 2p(q_{PPV} + q_{UND}) \quad (A2)$$

Substituting (q_{PATH} – q_{KWN}) for (q_{PPV} + q_{UND}) from Equation A1 and letting $p = 1 - q_{PATH} \cong 1$, since q_{PATH} is a small number (PVs are rare in the population),

$$RCR = \cong 2(q_{PATH} - q_{KWN}) \quad (A3)$$

To calculate RCR, from Equation A3, Appendix A, we need values for q_{PATH} and q_{KWN}. Measuring q_{PATH} depends on the incidence of the disease and the inheritance pattern of the disorder. For an autosomal recessive disorder q_{PATH} can be estimated from the disease incidence I and the Hardy-Weinberg equation by $(q_{PATH})^2 = I$, so $q_{PATH} = \sqrt{I}$. If the assumptions of Hardy-Weinberg (Appendix B) do not apply and there is stratification or consanguinity, the incidence of autosomal recessive disorders is inflated which, in turn, leads to an overestimation of the frequency of pathogenic variants (alleles) and therefore RCR.[5,11]

For an *X*-linked recessive disorder q_{PATH} can be estimated directly from the disease incidence in males, I_m, as $q_{PATH} = I_m$. The incidence of *X*-linked disorders and carrier frequency in the population are not inflated by stratification and consanguinity but are influenced by the degree to which reproductive fitness is reduced in affected males and how much male and female mutation rates differ for the gene involved.[31] Decreased reproductive fitness of males with the disease reduces the chance a female can become a carrier by inheriting a PV from an affected father. In addition, new, as opposed to inherited, PVs are frequent contributors to the incidence. These two phenomena associated with *X*-linked disease lead to overestimation of carrier frequency from disease incidence and an RCR based on it.[31]

To measure q_{KWN}, there are three approaches:

i. Estimate q_{KWN} from a sample of genotypes from affected individuals as follows:
   - Identify N affected individuals in a group and sequence them to determine the genotypes at the appropriate gene.
   - Suppose that k different distinct PVs are seen in the $2N$ alleles in this sample of N affecteds, with x_1 instances of PV1, x_2 instances of PV2, x_k instances of PVk, etc. These PVs are either known to be PVs or can be inferred to be PVs by the nature of the change in the gene.
   - Let M = the number of times a known/inferred PV is seen in the $2N$ alleles $M = \sum_{i=1}^{k} x_i$.
   - The fraction of known PVs in this sample of affected = $f_{KWN} = M/2N$, and therefore, q_{KWN} can be estimated as $f_{KWN} \times q_{PATH}$. Then, RCR $\cong 2(q_{PATH} - q_{KWN}) = 2(\sqrt{I} - f_{KWN}\sqrt{I}) = 2(\sqrt{I})(1 - f_{KWN})$.
   - Standard deviation for $M = \sqrt{[2N(M/2N)(1 - (M/2N))]} = \sqrt{[M(1 - (M/2N))]}$.

   For *X*-linked disorders, $M = \sum_{i=1}^{k} x_i$ with k different distinct PVs in the N alleles in this sample of N affected males and $f_{KWN} = M/N$. Then RCR $\cong 2(q_{PATH} - q_{KWN}) = 2(I - f_{KWN}I) = 2(I)(1 - f_{KWN})$.

ii. Screen a large random sample of a population to determine the frequency of carriers of known PVs. Since only a small fraction are carriers of an autosomal recessive condition, this approach requires very large sample sizes to prevent underestimating the carrier frequency because the rarer PVs escape detection. Measure q_{KWN} directly by determining,
   a. X, the number of individuals with one known PV, and
   b. Y, the number of homozygotes or compound heterozygotes with two known PVs, and calculate q_{KWN} as $q_{KWN} = (X + 2Y)/2N$. If the random sample of N individuals is chosen so as to avoid including any affected individuals, then $Y = 0$ and $q_{KWN} = (X)/2N$.

iii. Use large public databases of alleles and their frequencies to determine the frequency of carriers of PVs. For example, the Genome Aggregation Database (gNOMAD)[32] contains the identity and frequency of variants from 141,456 unrelated individuals, often with different frequencies listed for different ancestral or national groups. The pathogenicity, or lack thereof, of some variants listed in gNOMAD can be determined by their population frequency to be benign, or by the nature of the DNA change (e.g., premature termination with loss of function) to be pathogenic, but assessing the pathogenicity of most variants requires cross-referencing to variant annotation databases, such as ClinVar[33] or gene-specific databases, like CFTR2 for *CFTR* variants.[34] Such an approach, however, may also underestimate carrier frequencies for groups underrepresented in public databases since the variants will either be missing if those groups are absent, or, if present, are likely to remain VUSs due to scant clinical data.

Appendix B

The Hardy-Weinberg law[5] applies when the following conditions are met: the population under study is large, and mating is random; allele frequencies remain constant over time; there is no appreciable rate of new mutation; individuals with all genotypes are equally capable of mating and passing on their genes (there is no selection against any particular genotype); and there has been no significant immigration of individuals from a population with allele frequencies very different from the endogenous population.

If all disease-causing variants for an autosomal recessive disorder have a combined frequency in a population of q, the Hardy-Weinberg law states the frequency of affected homozygous or compound heterozygous individuals = q^2 and will remain as q^2 in the next generation. Since the incidence (I) of the autosomal recessive disorder (assuming full penetrance) is the frequency of affected homozygous or compound heterozygous individuals, $q^2 = I$, and therefore, $q = \sqrt{(I)}$.

If the remaining (non-disease causing) variants have a combined frequency in a population of p, then $p = 1 - q$ and heterozygous carriers will have a frequency in the population of $2pq$ and will remain as $2pq$ in the next generation as long as the conditions apply. Since q is generally small when it is the frequency of pathogenic variants for a rare, recessive disorder, $p = 1 - q \approx 1$, and the carrier frequency becomes $2q = 2\sqrt{(I)}$.

Although one assumption underlying the Hardy-Weinberg Law, that there be no selection against any particular genotype, does not hold true when we are dealing with a serious medical condition in homozygotes or compound heterozygotes, the impact for autosomal recessive conditions is small and the Hardy-Weinberg formula can still be used as an approximation.

Appendix C

Calculating residual risks for being a carrier of CF in an individual from Yucatan.[12]

To determine carrier frequency from incidence,

- Incidence of CF = 7/96,071 = 1/13,724 = 0.000073
- Frequency of PVs responsible for CF = $\sqrt{0.000073}$ = 0.0085 by Hardy-Weinberg equation (Appendix B)
- Carrier frequency $\cong 2 \times 0.0085 = 0.017$

Determining the frequency of PVs detectable by screening affected individuals[12] (Appendix A).

- Sequence 7 affected unrelated individuals and analyze their 14 variants.
  - Seven of 14 alleles are known PVs.
  - Two of 14 are previously unknown premature termination variants (Thr1299HisFs*2, Asp1270Metfs*8) that are classified as PVs according to clinical laboratory guidelines for variant interpretation.[35]
  - Two missense variants (Lys536Glu and Ala559Pro) accounting for three of the 14 alleles in *CFTR* affected individuals lack evidence for pathogenicity and are classified as VUSs.[35]
  - Two *CFTR* variants are not detected.
- 9/14 = 64% of PVs were detectable in affected patients

The frequency of detectable PVs in the population = $0.64 \times (0.0085) = 0.0055$, carrier frequency of detectable PVs $= 2 \times 0.0055 = 0.011$ and an RCR for a patient from Yucatan testing negative for these 9 PVs $\cong 0.017 - 0.011 = 0.006 = 1/167$ (95% confidence limit 1/96–1/625) (Method described in Appendix A).