

TL studies (paired *t*-test, $P=0.05$, Table 1 by Eisenberg¹⁰). If lower paternal survival is associated with increased incidence of critically short TLs, the paternal TL distribution will be truncated leading to lower father–offspring correlations in older populations. Tentatively suggestive that this explanation is worthy of further consideration, across the limited set of available populations ($n=8$), there is a nonsignificant trend toward greater father–offspring correlations (relative to mother–offspring correlations, difference) in studies with increasing numbers of father–offspring pairs (relative to mother–offspring pairs, ratio; Pearson correlation, $r=0.37$, $P=0.37$).

As suggested above, biological phenomena may provide particularly important explanations for the inheritance discrepancy before fertilization, whereas methodology-associated biases may arise more after birth. A large-scale TL inheritance study of neonatal offspring along with both parents might identify whether biology or methodology lies at the origin of the observed discrepancy. Methodology-induced biases will most likely be related to measurement method, parental age at conception, parental and offspring age at measurement, and parental survival. Statistical adjustment for the latter variables, or selection of appropriate population subsets might allow discernment of the effect(s) at play. The use of nonparametric statistics or better-validated TL measurements might eliminate the possibility that the discrepancies are related to nonlinearity in the TL measurement scale. In summary, there are several possible explanations for the parental TL inheritance discrepancy, and the net effect most likely depends on the specific study design and characteristics of the included populations. A novel (meta-)analysis of the different data sets adjusting for the above-suggested putative confounding variables might enable the identification of the underlying mechanism, or at least might eliminate several methodological possibilities.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Parental expression is overvalued in the interpretation of rare inherited variants

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Rare copy number variations (CNVs) contribute to genetic risk for many developmental and neuropsychiatric conditions. The growing uptake of chromosomal microarray analysis in clinical practice^{1,2} is predicated on the assumption that there exist valid protocols to guide the interpretation of very rare or private CNVs. Many protocols

currently in use can be strongly influenced by the results of parental studies.^{1,3,4} We used a simple Bayesian model to demonstrate that in the case of inherited variants, the observed parental phenotype will be heavily and predictably influenced by the decreased reproductive fitness associated with disease expression. This means that transmitting parents with high penetrance variants will nonetheless often be 'unaffected'.

BIASES INHERENT IN PARENTAL STUDIES

In any individual most genetic variants, including rare variants, are inherited.⁵ Pathogenicity of very rare or private inherited CNVs is often inferred in part from the parental phenotype. Disease concordance between parent and offspring may be used to conclude that a shared CNV likely had a causal role, despite the fact that the *a priori* probability of both the parent and a child sharing that CNV (given that the parent had the CNV) was 50% in most cases. Disease discordance in the form of an 'unaffected' or 'healthy' parent, on the other hand, may be used to argue that a shared variant is likely benign. However, by definition, transmitting parents have been able to find a partner and to reproduce (and were available and willing to

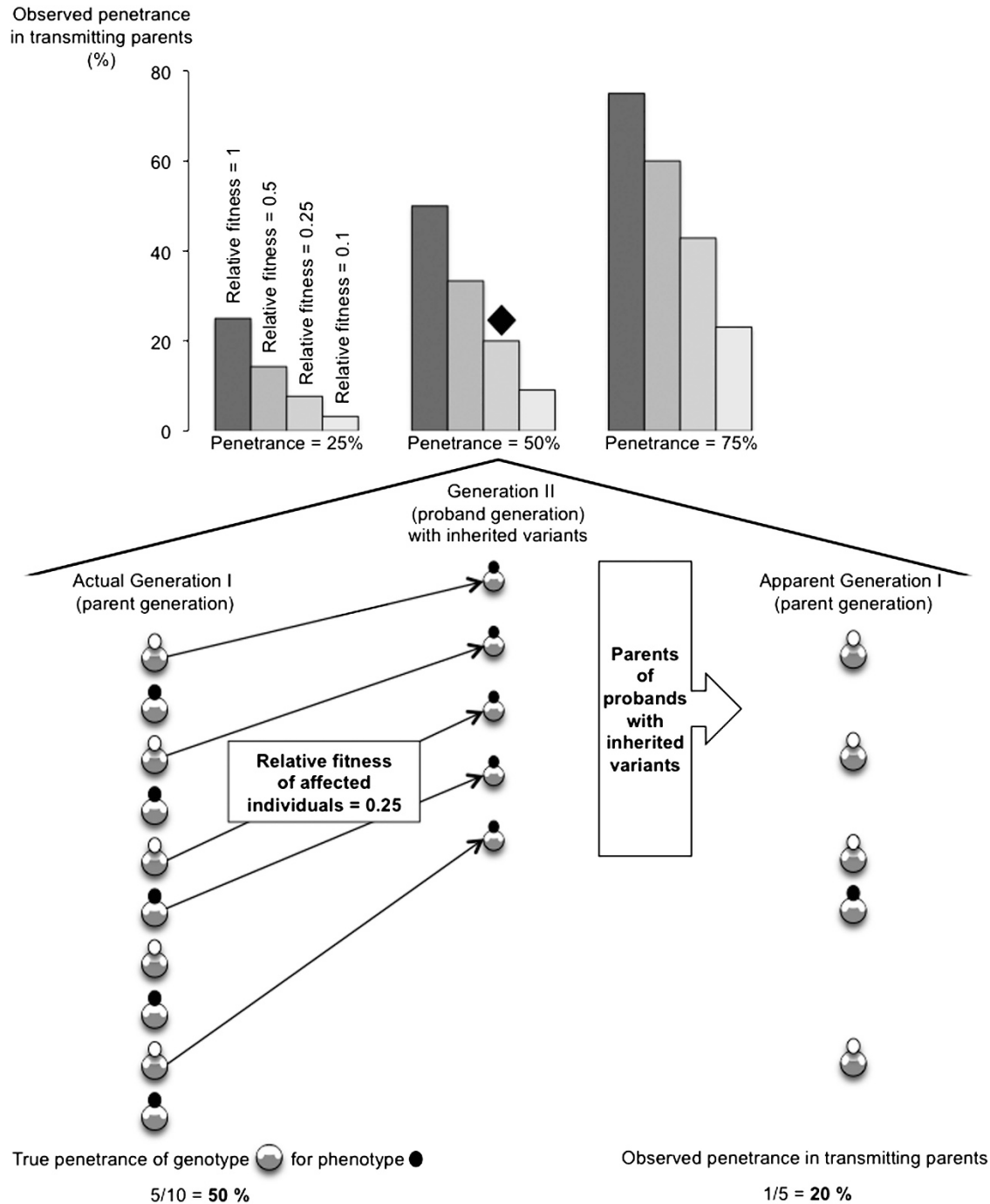


Figure 1 Observed penetrance in transmitting parents for major phenotypes associated with reduced reproductive fitness. See text, Equation (1), and Supplementary Tables S1 and S2 for details.

have genetic testing). This will bias downward the observed prevalence in transmitting parents of major phenotypes associated with reduced reproductive fitness (Figure 1), such as major developmental and neuropsychiatric conditions.⁶

A SIMPLE BAYESIAN MODEL

One can quantify the potential impact of reproductive fitness differences associated with expression of a disease (X) on the observed prevalence of X in transmitting parents with a rare autosomal

dominant-acting CNV (g). Let P be the penetrance of variant g for disease X and f be the relative reproductive fitness of individuals with g and with X (compared with individuals with g and without X), where $0 < P, f < 1$. Using standard Bayesian analysis methods (Supplementary Table S1), the probability of disease X in a randomly selected transmitting parent is:

$$\text{Probability}[\text{affected parent}] = \frac{P \cdot f}{P \cdot f + (1 - P)} \quad (1)$$

In those cases where $P < 1/(1+f)$ (eg, where a variant has penetrance $P = 0.5$ for disease X but where those affected with X have on average only half of the number of offspring of those without X), only a minority of transmitting parents would be expected to be affected with X (Figure 1). Thus, while the observation in clinical practice that a particular very rare or novel CNV has been inherited from an unaffected parent may demonstrate that penetrance is incomplete, it is less informative with respect to quantifying penetrance.

A PRACTICAL ILLUSTRATION: 22q11.2 DELETION SYNDROME AND SCHIZOPHRENIA

As a practical illustration of the general principle highlighted by Equation (1), recent data^{6,7} can be used to explain historical observations regarding the prevalence of schizophrenia in transmitting parents with 22q11.2 deletion syndrome (22q11.2DS; OMIM #188400/#192430). Take genetic variant g to be a typical 22q11.2 deletion and disease X to be schizophrenia. The penetrance P for schizophrenia in 22q11.2DS is ~ 0.25 (25%).⁷ Individuals with 22q11.2DS and schizophrenia have approximately one tenth as many children as do individuals with 22q11.2DS and without schizophrenia, and thus the relative fitness f associated with schizophrenia in 22q11.2DS is ~ 0.10 (10%).⁶ The observed penetrance of schizophrenia in transmitting parents with 22q11.2DS would therefore be on the order of 0.03 (3%) (Figure 1; Supplementary Table S2). Early in the study of 22q11.2DS, most adults reported in the literature and being seen in clinical practice were transmitting parents.⁸ As expected per the above calculation, only a small minority was reported to have a serious psychotic illness.^{8,9} Thus, this ascertainment bias effectively masked the true extent of the association of schizophrenia with 22q11.2DS for some time. Most adults with other genomic disorders thus far reported in the literature are transmitting parents;^{10,11} this will similarly confound accurate genotype–phenotype correlations across the life span.

OTHER IMPLICATIONS FOR CLINICAL AND RESEARCH PRACTICE

The above observations may seem self-evident, and yet there is some evidence to suggest that this ‘transmitting parent’ bias has affected decision-making in clinical practice. For example, in a recent large-scale study of prenatal microarray testing,¹² a maternally inherited 10q11.2 duplication (chr10:46,482,304–51,558,563; hg18) was deemed of uncertain significance and ultimately not reported to the family, while a comparable *de novo* 10q11.2 duplication (chr10:49,033,015–51,005,023 and 51,407,449–52,135,488) was reported. A corollary to this caveat of parental studies is that imposing a major distinction between *de novo* variants and those that might have appeared in the immediate preceding generation may sometimes be unnecessarily artificial.

There are also implications for designing family-based gene hunting studies in complex diseases. A disease-concordant sibpair design has had a major perceived disadvantage with respect to the discovery of associated autosomal dominant mutations: any variant shared by both affected siblings would typically also have to be present in an unaffected parent. The above discussion and calculations suggest that such a study design may nonetheless have the ability to discover high penetrance variants. Expression in siblings may be a better way of gauging penetrance than expression in parents, albeit with the potential for different biases like stoppage rules.¹³

CONCLUSIONS

In summary, simple probabilistic reasoning challenges a convenient narrative: that *de novo* variants and variants inherited from a similarly affected parent are likely associated with the proband’s phenotype, whereas those inherited from an unaffected parent are likely benign. We do not advocate abandoning existing protocols and conventions; rather, we simply urge caution in mistaking reasonable (and generally useful) heuristics for absolute tenets. Just as *de novo* variants are not necessarily causal,¹⁴ very rare or private variants inherited from apparently unaffected parents are not necessarily benign. The interpretation of rare variants in complex disease is not yet driven by an understanding of the underlying etiopathogenesis. In the future, current approaches to rare CNV interpretation may be superseded by population-based estimates of disease-specific penetrance (for recurrent variants) and additional functional approaches to variant classification.

Studying contemporary reproductive fitness in genomic disorders will have positive repercussions for genetic counseling and understanding the evolutionary biology of these structural rearrangements.⁶ As for 22q11.2DS,⁶ reproductive fitness and transmission patterns of pathogenic CNVs will change over time. Regardless, the purpose of Equation (1) is to model a general principle that is immediately relevant to clinical practice, rather than to perform specific calculations in the (rare) cases where the necessary data would be available. Finally, although we have focused on clinical adjudication of rare CNVs, many of the observations and results are generalizable to rare sequence variants and to discovery-based science. Use of whole exome and whole-genome sequencing in clinical practice provides further impetus to optimize methods of interpreting rare variants.

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