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Quantifying penetrance in a dominant disease gene using large population control cohorts

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Abstract

More than 100,000 genetic variants are reported to cause Mendelian disease in humans, but the penetrance - the probability that a carrier of the purported disease-causing genotype will indeed develop the disease - is generally unknown. Here we assess the impact of variants in the prion protein gene (*PRNP*) on the risk of prion disease by analyzing 16,025 prion disease cases, 60,706 population control exomes, and 531,575 individuals genotyped by 23andMe, Inc. We show that missense variants in *PRNP* previously reported to be pathogenic are at least 30× more common in the population than expected based on genetic prion disease prevalence. While some of this excess can be attributed to benign variants falsely assigned as pathogenic, other variants have genuine effects on disease susceptibility but confer lifetime risks ranging from <0.1% to ~100%. We also show that truncating variants in *PRNP* have position-dependent effects, with true loss-of-function alleles found in healthy older individuals, supporting the safety of therapeutic suppression of prion protein expression.

INTRODUCTION

The study of pedigrees with Mendelian disease has been tremendously successful in identifying variants that contribute to severe inherited disorders (1–3). Causal variant discovery is enabled by selective ascertainment of affected individuals, and especially of multiplex families. Although efficient from a gene discovery perspective, the resulting ascertainment bias confounds efforts to accurately estimate the penetrance of disease-causing variants, with profound implications for genetic counseling (4–7). The development of large-scale genotyping and sequencing methods has recently made it tractable to perform unbiased assessments of penetrance in population controls. In several instances, such studies

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³⁴A list of consortium members may be found at <http://exac.broadinstitute.org/about>

Source code availability

Data processing, analysis, and figure generation utilized custom scripts written in Python 2.7.6 and R 3.1.2. These scripts, along with vector graphics of all figures and tab-delimited text versions of all supplementary tables, are available online at https://github.com/ericminikel/prnp_penetrance

Author contributions

E.V.M., S.M.V. and D.G.M. conceived and designed the study. E.V.M. analyzed data, generated figures, and wrote the manuscript. S.M.V. and E.V.M. reviewed literature and IGV screenshots. K.E.S. performed constraint analyses. M. Lek, K.E., K.E.S., K.J.K., A.H.O.-L., M.J.D., and D.G.M. consulted on data analysis and interpretation. J.F.S., C.Y.M., J.Y.C., and L.P.C.Y. prepared and consulted on analysis of 23andMe data. P.G., J.B., S.Z., Y.C., W.C., M.Y., T.H., N.S., H.M., Y.N., T.K., S.J.C., A.B., R.G.W., R. Knight, C.P., I.Z., T.F.J.K., S.E., A.G., M.C., J.d.P.C., S.H., J.-L.L., E.B.-A., J.-P.B., S.C., P.P., A.L., A.P., R. Kraaij., J.G.J.v.R., S.J.v.d.L., R.M., and C.v.D. prepared and consulted on analysis of prion surveillance data. E.V.M., J.L.M., M.B., M. Laakso, K.M., A.K., K.C., S.A.M., P.S., C.M.H., S.M.P., P.S., C.v.D., F.R.R., A.H., A.I., S.J.v.d.L., J.M.V.-D., and A.G.U. prepared and consulted on analysis of data regarding protein-truncating variants. ExAC provided exome sequence data.

have suggested that previously reported Mendelian variants, as a class, are substantially less penetrant than had been believed (8–11). To date, however, all of these studies have been limited to relatively prevalent ($>0.1\%$) diseases, and point estimates of the penetrance of individual variants have been limited to large copy number variations (8, 11).

Here we demonstrate the use of large-scale population data to infer the penetrance of variants in rare, dominant, monogenic disease, using the example of prion diseases. These invariably fatal neurodegenerative disorders are caused by misfolding of the prion protein (PrP, the product of *PRNP*) (12) and have an annual incidence of 1 to 2 cases per 1 million population (13). A small, albeit infamous, minority of cases ($<1\%$ in recent years (14, 15)) are acquired through dietary or iatrogenic routes. The majority ($\sim 85\%$) of cases are defined as sporadic, occurring in individuals with two wild-type *PRNP* alleles and no known environmental exposures. Finally, $\sim 15\%$ of cases occur in individuals with rare, typically heterozygous, coding variants in *PRNP*, including missense variants, truncating variants, and octapeptide repeat insertions or deletions (Table S1). Centralized ascertainment of cases by national surveillance centers (Materials and Methods) makes prion disease a good test case for using reference datasets to assess the penetrance of these variants.

PRNP was conclusively established as a dominant disease gene due to clear Mendelian segregation of a few variants with disease (16–18). Yet ascertainment bias (19), low rates of predictive genetic testing (20), and frequent lack of family history (21, 22) confound attempts to estimate penetrance by survival analysis (19, 23–26). Meanwhile, the existence of non-genetic etiologies leaves doubt as to whether novel variants are causal or coincidental.

A fully penetrant disease genotype should be no more common in the population than the disease that it causes. This observation allows us to leverage two large population control datasets to re-evaluate the penetrance of reported disease variants in *PRNP*. The recently reported Exome Aggregation Consortium (ExAC) dataset (27) contains variant calls on 60,706 people ascertained for various common diseases, without any ascertainment on neurodegenerative disease. 23andMe's database contains genotypes on 531,575 customers of its direct-to-consumer genotyping service who have opted in to participate in research, pruned to remove related individuals (first cousins or closer; Materials and Methods), preventing enrichment due to large families with prion disease.

RESULTS

We began by asking whether reportedly pathogenic variants are as rare as expected in these population control datasets. The proportion of people alive in the population today who harbor completely penetrant variants causal for prion disease can be approximated by the product of three numbers: the annual incidence of prion disease, the proportion of cases with such a genetic variant, and the life expectancy of individuals harboring these variants. Based on upper bounds of these numbers (Figure 1A), and assuming ascertainment is neutral with respect to neurodegenerative disease, we would expect no more than ~ 1.7 such individuals in the 60,706 exomes in the ExAC dataset (27), and ~ 15 such individuals among the $\sim 530,000$ genotyped 23andMe customers who opted to participate in research.

Through reviews (28–30) and PubMed searches, we identified 63 rare genetic variants reported to cause prion disease (Table S2). We reviewed ExAC read-level evidence for every rare (<0.1% allele frequency) variant call in *PRNP* (Materials and Methods; Table S3–4) and found that 52 individuals in ExAC harbor reportedly pathogenic missense variants (Figure 1B), at least a 30-fold excess over expectation if all such variants were fully penetrant. Similarly, in the 23andMe database we observed a total of 141 alleles of 16 reportedly pathogenic variants genotyped on their platform (Table S5).

Individuals with reportedly pathogenic *PRNP* variants did not cluster within any one cohort within ExAC (Table S6), arguing against enrichment due to comorbidity with a common disease ascertained for exome sequencing. ExAC does include populations, such as South Asians, in which prion disease is not closely surveilled and we cannot rule out a higher incidence than that reported in developed countries, yet the individuals with reportedly pathogenic variants in either ExAC or 23andMe were of diverse inferred ancestry (Table S7–9). These individuals' ages were consistent with the overall ExAC age distribution (Figure S1), rather than being enriched below some age of disease onset. ExAC genotypes at the prion disease modifier polymorphism M129V (34) were consistent with population allele frequencies (Table S7), rather than enriched for the lower-risk heterozygous genotype. Certain *PRNP* variants are associated with highly atypical phenotypes (35, 36), which are mistakable for other dementias and may not be well ascertained by current surveillance efforts. Most of the variants found in our population control cohorts, however, have been reported in individuals with a classic, sporadic Creutzfeldt-Jakob disease phenotype (22, 28, 30, 37–39), arguing that the discrepancy between observed and expected allele counts does not result primarily from an underappreciated prevalence of atypical prion disease.

Having observed a large excess of reportedly pathogenic variants over expectation in two datasets, and having excluded the most obvious confounders, we hypothesized that the unexpectedly high frequency of these variants in controls might arise from benign and/or low-risk variants.

We investigated which variants were responsible for the observed excess (Figure 2). Variants with the strongest prior evidence of pathogenicity are absent from ExAC and cumulatively account for 5 alleles in 23andMe, consistent with the known rarity of genetic prion disease. Much of the excess allele frequency in population controls is due, instead, to variants with very weak prior evidence of pathogenicity (Figure 2 and Supplementary Discussion). For four variants observed in controls (V180I, R208H, V210I, and M232R), pathogenicity is controversial (40, 41) or reduced penetrance has been suggested (42, 43), but quantitative estimates of penetrance have never been produced, and the variants remain categorized as causes of genetic Creutzfeldt-Jakob disease (21, 22). Although we cannot prove that any one of the variants we observe in population controls is completely neutral, the list of reported pathogenic variants likely includes false positives. Indeed, the observation that 0.4% (236 / 60,706) of ExAC individuals harbor a rare (<0.1%) missense variant (Table S4) suggests that ~4 of every 1000 sporadic prion disease cases will, by chance, harbor such a variant, which in many cases will be interpreted and reported as causal given the long-standing classification of *PRNP* as a Mendelian disease gene.

At least three variants, however (V180I, V210I, and M232R) fail to cluster with either the likely benign or likely Mendelian variants (Figure 2). Because each of these three appears primarily in one population in both cases and controls (Tables S1, S5, S7), we compared allele frequencies in matched population groups. Each has an allele frequency in controls that is too high for a fully penetrant, dominant prion disease-causing variant, and yet far lower than the corresponding allele frequency in cases (Figure 3).

Because we lack genome-wide SNP data on cases we are unable to directly correct for population stratification, which thus may contribute to the observed differences in allele frequencies. Geographic clusters of genetic prion disease have been recognized for decades (26, 31, 58). For example, nearly half of Italian prion disease cases with the V210I variant are concentrated within two regions of Italy (59), so any non-uniform geographic sampling in cases versus controls would add some uncertainty to our penetrance estimates.

Nonetheless, the magnitude of the enrichment of certain variants in cases over controls in our datasets makes substructure an implausible explanation for the entire difference. In order for V210I to be neutral and yet appear with an allele frequency of 8.1% in Italian cases despite an apparent allele frequency of 0.02% in Italian controls, it would need to be fixed in a subpopulation comprising 8% of Italy's populace. Under this scenario, this subpopulation would need to be virtually unsampled in any of our control cohorts, and V210I cases would contain many homozygotes. In reality, no cases have been reported homozygous for this variant. Conversely, if V210I were fully penetrant, family history would be positive in most cases, and the variant's appearance on 13 alleles in 23andMe (Table S5) would indicate that this variant alone accounts for three times the known prevalence of genetic prion disease (Figure 1A). Finally, if the low family history rate were due to many *de novo* mutations, then V210I cases would be more uniformly distributed across populations (Table S1). Similar arguments rule out V180I being either benign or Mendelian. M232R, though clearly not Mendelian, could still be benign as it exhibits only 4- to 6-fold enrichment in cases, an amount that might conceivably be explained by Japanese population substructure alone. However, because even common variants in *PRNP* affect prion disease risk with odds ratios of 3 or greater (60–62), it is not implausible that M232R has a similar effect size, and our data suggest this a more likely scenario than it being neutral.

Satisfied that these three variants are likely neither benign nor Mendelian, we estimated lifetime risk in heterozygotes (Materials and Methods). The ~2 in 1 million annual incidence of prion disease translates into a baseline lifetime risk of ~1 in 5,000 in the general population (Materials and Methods). Because prion diseases are so rare, even the massive enrichment of heterozygotes in cases (Figure 3), implying odds ratios on the order of 10 to 1,000, corresponds to only low penetrance, with lifetime risk for M232R, V180I and V210I estimated near 0.1%, 1%, and 10%, respectively. Although our estimates are imperfect due to population stratification, they accord well with family history rates (Figure 3) and explain the unique space that these variants occupy in the plot of case versus control allele count (Figure 2). These data indicate that *PRNP* missense variants occupy a risk continuum rather than a dichotomy of causal versus benign.

We asked whether the same was true of protein-truncating variants. *PRNP* possesses only one protein-coding exon, so premature stop codons are expected to result in truncated polypeptides rather than in nonsense-mediated decay. Prion diseases are known to arise from a gain of function, as neurodegeneration is not seen in mice, cows, or goats lacking PrP (63–66), and the rate of prion disease progression is tightly correlated with PrP expression level (67). Yet heterozygous C-terminal (residue 145) truncating variants are known to cause prion disease, sometimes with peripheral amyloidosis (35). Some of these patients also experience sensorimotor neuropathy phenotypically similar to that present in homozygous, but not heterozygous, PrP knockout mice (68), but attributed to amyloid infiltration of peripheral nerves, rather than loss of PrP function (35).

We identified, for the first time, heterozygous N-terminal (residue 131) truncating variants in four ExAC individuals and were able to obtain Sanger validation (Figure S1) and limited phenotype data (Table S11) for three. These individuals are free of overt neurological disease at ages 79, 73, and 52, and report no personal or family history of neurodegeneration nor of peripheral neuropathy. Therefore, the pathogenicity of protein-truncating variants appears to be dictated by position within PrP's amino acid sequence (Figure 4). Observing three *PRNP* nonsense variants in ExAC is consistent with the expected number (~3.9) once we adjust our model (69) to exclude codons 145, where truncations cause a dominant gain-of-function disease. Thus, we see no evidence that *PRNP* is constrained against truncation in its N terminus. This, combined with the lack of any obvious phenotype in individuals with N-terminal truncating variants, suggests that heterozygous loss of PrP function is tolerated.

DISCUSSION

Over 100,000 genetic variants have been reported to cause Mendelian disease in humans (73, 74). Many such reports do not meet current standards for assertions of pathogenicity (75, 76), and if all such reports were believed, the cumulative frequency of these variants in the population would imply that most people have a genetic disease (27). It is generally unclear how much of the excess burden of purported disease variants in the population is due to benign variants falsely associated, and how much is due to variants with genuine association but incomplete penetrance.

Here we leverage newly available large genomic reference datasets to re-evaluate reported disease associations in a dominant disease gene, *PRNP*. We identify some missense variants as likely benign while showing that others span a spectrum from <0.1% to ~100% penetrance. Our analyses provide quantitative estimates of lifetime risk for hundreds of asymptomatic individuals who have inherited incompletely penetrant *PRNP* variants.

Available datasets are only now approaching the size and quality required for such analyses, resulting in limitations for our study. The confidence intervals on our lifetime risk estimates span more than an order of magnitude, and our inability to perfectly control for population stratification injects additional uncertainty. We have been unable to reclassify those *PRNP* variants that are very rare both in cases and in controls (Supplementary Discussion). We have avoided analysis of large insertions that are poorly called with short sequencing reads, though we note that existing literature on these insertions is consistent with a spectrum of

penetrance similar to that which we observe for missense variants (28, 77). Penetrance estimation in Mendelian disease will be improved by the collection of larger case series, particularly with genome-wide SNP data to allow more accurate population matching. This, coupled with continued large-scale population control sequencing and genotyping efforts, should reveal whether the dramatic variation in penetrance that we observe here is a more general feature of dominant disease genes.

Because PrP is required for prion pathogenesis and reduction in gene dosage slows disease progression (67, 78–80), several groups have sought to therapeutically reduce PrP expression using RNA interference (81–83), antisense oligonucleotides (84), or small molecules (85, 86). Our discovery of heterozygous loss-of-function variants in three healthy older humans provides the first human genetic data regarding the effects of a 50% reduction in gene dosage for *PRNP*. Both the number of individuals and the depth of available phenotype data are limited, and lifelong heterozygous inactivation of a gene is an imperfect model of the effects of pharmacological depletion of the gene product. With those limitations, our data provide preliminary evidence that a reduction in *PRNP* dosage, if achievable in patients, is likely to be tolerated. Increasingly large control sequencing datasets will soon enable testing whether the same is true of other genes currently being targeted in substrate reduction therapeutic approaches for other protein-folding disorders.

Together, our findings highlight the value of large reference datasets of human genetic variation for informing both genetic counseling and therapeutic strategy.

MATERIALS AND METHODS

Prion disease case series

Prion disease is considered a notifiable diagnosis in most developed countries, with mandatory reporting of all suspect cases to a centralized surveillance center. Surveillance was carried out broadly according to established guidelines (87, 88), with specifics as described previously for Australia (89), France (90), Germany (91–93), Italy (94), Japan (22), and the Netherlands (95). Sanger sequencing of the *PRNP* open reading frame was performed as described (96). We included only prion disease cases classified as definite (autopsy-confirmed) or probable according to published guidelines (88). Criteria for genetic testing vary between countries and over the years of data collection, with testing offered only on indication of family history in some times and places, and testing of all suspect cases with tissue available in other instances. Summary statistics on the total number and proportion of cases sequenced are presented in Table S1.

Exome sequencing and analysis

The ascertainment, sequencing, and joint calling of the ExAC dataset have been described previously (97). We extracted all rare (<0.1%) coding variant calls in *PRNP* with genotype quality (GQ) ≥ 10, alternate allele depth (AD) ≥ 3 and alternate allele balance (AB) ≥ 20%. Read-level evidence was visualized using Integrative Genomics Viewer (IGV) (98) for manual review. Because most ExAC exomes were sequenced with 76bp reads and the *PRNP* octapeptide repeat region (codons 50–90 inclusive) is 123bp long, it was impossible to

determine whether genotype calls in this region were correct, and they were not considered further. After review of IGV screenshots, 87% of genotype calls were judged to be correct and were included in Table S3. Of the genotype calls judged to be correct, 99% had genotype quality (GQ) ≥ 95, 99% had allelic balance (AB) between 30% and 70%, and 97% had ≥ 10 reads supporting the alternate allele. All participants provided informed consent for exome sequencing and analysis. The Exome Aggregation Consortium's aggregation and release of exome data have been approved by the Partners Healthcare Institutional Research Board (2013P001339). ExAC data have been publicly released at <http://exac.broadinstitute.org/> and IGV screenshots of the rare *PRNP* variants deemed to be genuine and included in this study are available at https://github.com/ericminikel/prnp_penetrance/tree/master/supplement/igv

23andMe research participants and genotyping

Participants were drawn from the customer base of 23andMe, Inc., a personal genetics company (accessed February 6, 2015). All participants provided informed consent under a protocol approved by an external AAHRPP-accredited IRB, Ethical & Independent Review Services (E&I Review). DNA extraction and genotyping were performed on saliva samples by National Genetics Institute (NGI), a CLIA-licensed clinical laboratory and a subsidiary of Laboratory Corporation of America. Samples were genotyped on one of four Illumina platforms (V1-V4) as described previously (99). Of the *PRNP* SNPs considered, two (P105L and E200K) were genotyped on all four platforms while the other 14 were genotyped only on V3 and V4, resulting in differing numbers of total samples genotyped (Table S5). Genotypes were called with Illumina GenomeStudio. A 98.5% call rate were required for all samples. As with all 23andMe research participants, individuals whose genotyping analyses failed to reach the desired call rate repeatedly were recontacted to provide additional samples. A maximal set of unrelated individuals was chosen based on segmental identity-by-descent (IBD) estimation(100). Individuals were defined as related if they shared more than 700 cM IBD (approximately the minimal expected sharing between first cousins). Allele counts between 1 and 5 were rounded up to 5 to protect individual privacy (Table S5). Rounding down to 1 instead would raise our estimates of penetrance for V180I to 7.7% (95%CI, 1.2% – 50%) and for P102L, A117V, D178N and E200K collectively to 100% (95%CI, 100% – 100%), but the confidence intervals would still overlap those based on ExAC allele frequencies, and the overall conclusions of our study would remain unchanged.

23andMe ancestry composition

Ancestral origins of chromosomal segments were assigned on a continental level (European, Latino, African, and East Asian) and a country level (Japanese) as described by Durand et al (101). Briefly, after phasing genotypes using an out-of-sample implementation of the Beagle algorithm (102), a string kernel support vector machine classifier assigns tentative ancestry labels to local genomic regions. Then an autoregressive pair hidden Markov model was used to simultaneously correct phasing errors and produce reconciled local ancestry estimates and confidence scores based on the initial assignment. Finally, isotonic regression models were used to recalibrate the confidence estimates.

Europeans and East Asians were defined as individuals with more than 97% of chromosomal segments predicted as being from the respective ancestries. Because African Americans and Latinos are highly admixed, no single threshold of genome-wide ancestry is sufficient to distinguish them. However, segment length distributions of European, African, and Native American ancestries are different between African Americans and Latinos, due to distinct admixture timing in the two ethnic groups. Thus, a logistic classifier based on segment length of European, African, and Native American ancestries was used to distinguish between African Americans and Latinos.

At the country level, individuals were classified as Japanese based on the fraction of the respective local ancestry using a threshold of 90% for classifying Japanese ancestry. This threshold is based on the average fraction of local ancestry in the reference population (23andMe research participants with all four grandparents from the reference country): 94% (5% SD, $N=533$) for Japanese. Using the same approach, we were unable to obtain a confident set of Italian individuals for analysis of V210I due to extensive admixture. 23andMe research participants with all four grandparents from Italy only have 66% (18% SD, $N=2090$) Italian ancestry, and only ~60 participants have >90% Italian ancestry.

ExAC ancestry inference

We computed ten principal components based on ~5,800 common SNPs as described (27, 103). A centroid in eigenvalue-weighted principal component space was generated for each HapMap population based on 1000 Genomes individuals in ExAC. The remaining individuals in ExAC were assigned to the HapMap population with the nearest centroid according to eigenvalue-weighted Euclidean distance. Ancestries of all individuals, including those with reportedly pathogenic variants, are summarized in (Tables S7, S8).

Prion disease incidence and baseline risk

The reported incidence of prion disease varies between countries and between years, with much of the variability explained by the intensity of surveillance, as measured by the number of cases referred to national surveillance centers (13). Rates of ~1 case per million population per year have been reported, for instance in the U.S. (104) and in Japan (22), however, the countries with the most intense surveillance (greatest number of referrals per capita), such as France and Austria, observe incidence figures as high as 2 cases per million population per year (13). Only in small countries where the statistics are dominated by a particular genetic prion disease founder mutation, such as Israel and Slovakia (23, 26), has an incidence higher than 2 per million been consistently observed (105). We therefore accepted 2 cases per million as an upper bound for the true incidence of prion disease. Assuming an all-causes death rate of ~10 per 1,000 annually (106), this incidence corresponds to prion disease accounting for ~0.02% of all deaths, which we accepted as the baseline disease risk in the general population.

Lifetime risk estimation

By Bayes' theorem, the probability of disease given a genotype (penetrance or lifetime risk, $P(D|G)$) is equal to the proportion of individuals with the disease who have the genotype (genotype frequency in cases, $P(G|D)$) times the prevalence of the disease (baseline lifetime

risk in the general population, $P(D)$), divided by the frequency of the genotype in the general population (here, population control allele frequency, $P(G)$). The use of this formula to estimate disease risk dates back at least to Cornfield's estimation of the probability of lung cancer in smokers (107), with later contributions by Woolf (108) and a synthesis by C.C. Li with application to genetics (109).

We used an allelic rather than genotypic model, such that lifetime risk in an individual with one allele is equal to case allele frequency (based on the number of prion disease cases that underwent *PRNP* sequencing) times baseline risk divided by population control allele frequency, $P(D|A) = P(A|D) \times P(D) / P(A)$. Note that we assume that our population control datasets include individuals who will later die of prion disease, thus enabling direct use of the ExAC and 23andMe allele frequencies as the denominator $P(A)$. Following Kirov (11), we compute Wilson 95% confidence intervals on the binomial proportions $P(A|D)$ and $P(A)$, and calculate the upper bound of the 95% confidence interval for penetrance using the upper bound on case allele frequency and the lower bound on population control allele frequency, and vice versa for the lower bound on penetrance.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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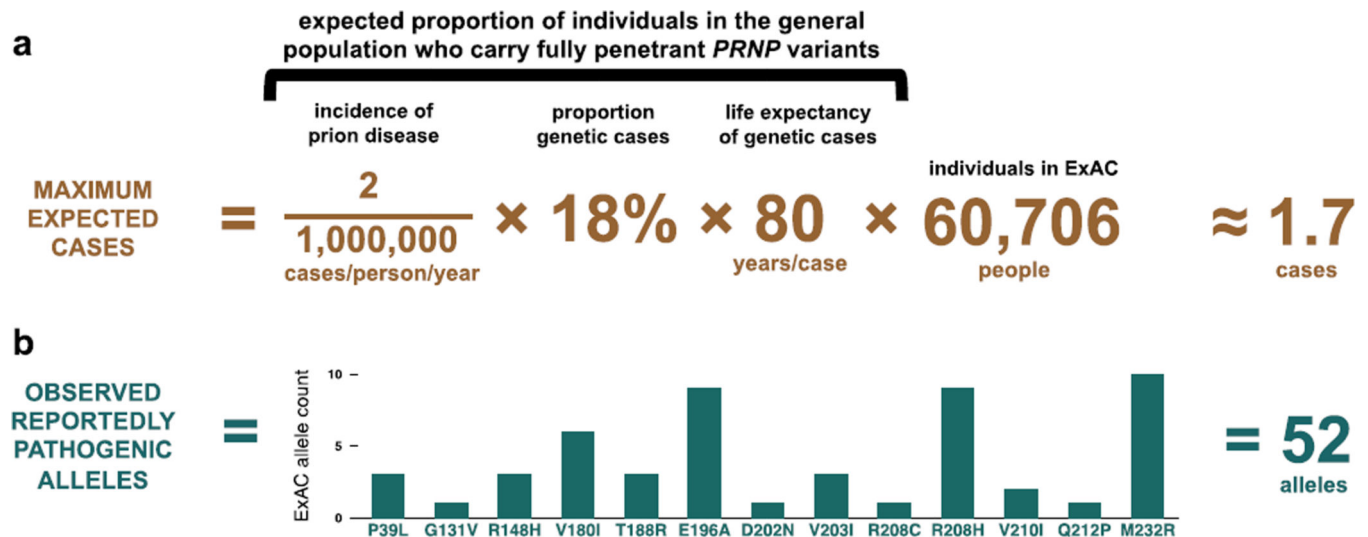


Figure 1. Reportedly pathogenic *PRNP* variants are >30 times more common in controls than expected based on disease incidence

Reported prion disease incidence varies with the intensity of surveillance efforts (13), with an apparent upper bound of ~2 cases per million population per year (Materials and Methods). In our surveillance cohorts, 65% of cases underwent *PRNP* open reading frame sequencing, with 12% of all cases, or 18% of sequenced cases, possessing a rare variant (Table S1), consistent with an oft-cited estimate that 15% of cases of Creutzfeldt-Jakob disease are familial (31). Genetic prion diseases typically strike in midlife, with mean age of onset for different variants ranging from 28 to 77 (22, 32) (Table S10); we accepted 80, a typical human life expectancy, as an upper bound for mean age of onset, and to be additionally conservative, we assumed that all individuals in ExAC and 23andMe were below any age of onset, even though both contain elderly individuals (33) (Figure S1). Thus, no more than ~29 people per million in the general population should harbor high-penetrance prion disease-causing variants. Therefore at most ~1.7 people in ExAC (A) and ~15 people in 23andMe would be expected to harbor such variants. In fact, reportedly pathogenic variants are seen in 52 ExAC individuals (B) and on 141 alleles in the 23andMe database.

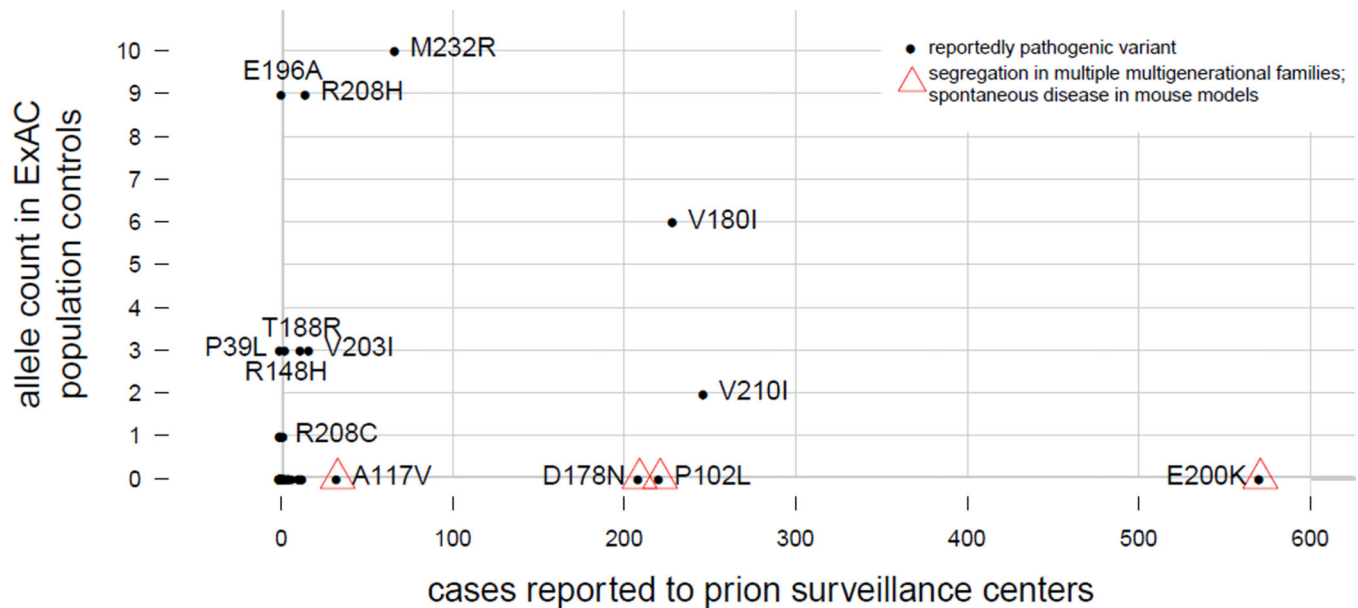


Figure 2. Reportedly pathogenic *PRNP* variants include Mendelian, benign, and intermediate variants

Prior evidence of pathogenicity is extremely strong for four missense variants - P102L, A117V, D178N and E200K - each of which has been observed to segregate with disease in multiple multigenerational families (16–18, 44–48) and to cause spontaneous disease in mouse models (49–54). These account for >50% of genetic prion disease cases (Table S1), yet are absent from ExAC (Table S3), and collectively appear on 5 alleles in 23andMe's cohort (Table S5), indicating allele frequencies sufficiently low to be consistent with the prevalence of genetic prion disease (Figure 1). Conversely, the variants most common in controls and rare in cases had categorically weak prior evidence for pathogenicity. R208C (8 alleles in 23andMe) and P39L were observed in patients presenting clinically with other dementias, with prion disease suggested as an alternative diagnosis solely on the basis of finding a novel *PRNP* variant (55, 56). E196A was originally reported in a single patient, with a sporadic Creutzfeldt-Jakob disease phenotype and no family history (37), and appeared in only 2 of 790 Chinese prion disease patients in a recent case series (57), consistent with the ~0.1% allele frequency among Chinese individuals in ExAC (Tables S5 and S8). At least three variants (M232R, V180I, and V210I) occupy a space inconsistent with either neutrality or with complete penetrance (see main text and Figure 3). R148H, T188R, V203I, R208H and additional variants are discussed in Supplementary Discussion.

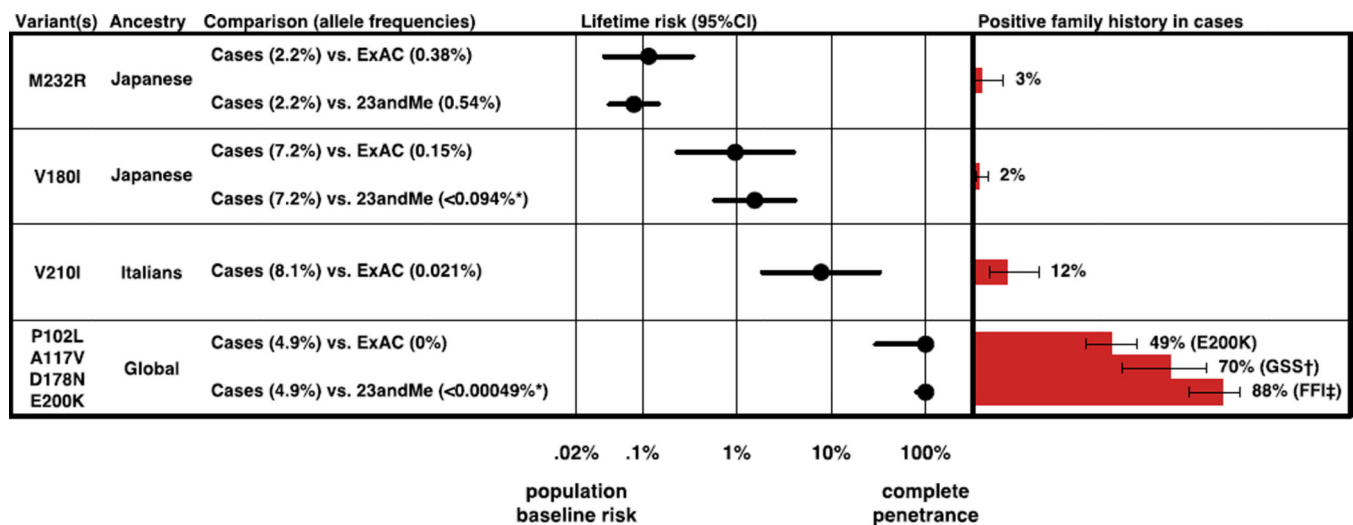


Figure 3. Certain variants confer intermediate amounts of lifetime risk

M232R, V180I, and V210I show varying degrees of enrichment in cases over controls, indicating a weak to moderate increase in risk. Best estimates of lifetime risk in heterozygotes (Materials and Methods) range from ~0.08% for M232R to ~7.8% for V210I, and correlate with the proportion of patients with a positive family history. Allele frequencies for P102L, A117V, D178N and E200K are consistent with up to 100% penetrance, with confidence intervals including all reported estimates of E200K penetrance based on survival analysis, which range from ~60% to ~90% (19, 23–26). Rates of family history of neurodegenerative disease in Japanese cases are from (Table S10) and in European populations are from Kovacs et al (21), with Wilson binomial 95% confidence intervals shown. *Based on allele counts rounded for privacy (Materials and Methods). †GSS, Gerstmann Straussler Scheinker disease associated with variants P102L, A117V and G131V. ‡FFI: fatal familial insomnia associated with a D178N *cis* 129M haplotype.

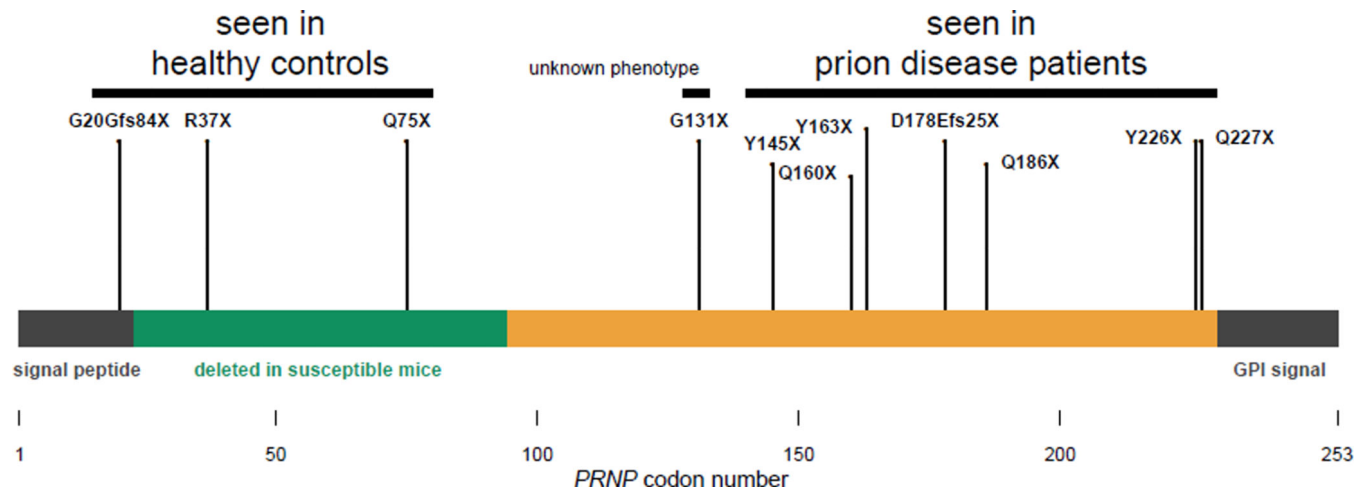


Figure 4. Effects of truncating variants in the human prion protein are position-dependent

Truncating variants reported in prion disease cases in the literature (Table S2) and in our cohorts (Table S1) cluster exclusively in the C-terminal region (residue 145), while truncating variants in ExAC are more N-terminal (residue 131). The ortholog of each residue from 23–94 is deleted in at least one prion-susceptible transgenic mouse line (70). C-terminal truncations abolish PrP's glycosylphosphatidylinositol anchor but leave most of the protein intact, a combination that mediates gain of function through mislocalization, causing this normally cell-surface-anchored protein to be secreted. Consistent with this model of pathogenicity, mice expressing full-length secreted PrP develop fatal and transmissible prion disease (71, 72). By contrast, the N-terminal truncating variants that we observe retain only residues dispensable for prion propagation, and are likely to cause a total loss of protein function.