

# Age of onset in genetic prion disease and the design of preventive clinical trials

## Supplementary materials

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## Supplementary Methods

### **Data collection details.**

Data sources included nine study centers listed in Table S2, with data collection methods as previously described<sup>1-7</sup>.

### **Life tables and hazard curves.**

We tabulated, for each *PRNP* mutation and for each age from 1-100, the number of individuals alive at the beginning of the interval (lives; *l*), becoming sick or dying within the interval (deaths; *d*), or being censored – alive and well at last followup or dead of a different cause – within the interval (withdrawals; *w*). The raw hazard (*q*) was computed as onsets divided by the mean number of people observed over the interval:  $q = d / (l - w/2)$ , and a smoothed hazard (*q\_smooth*) was computed by passing a Gaussian filter (*sd*=3 years, maximum width=15 years) over the raw hazard. The proportion surviving for each interval (*p*) was 100% for the first year and was computed as  $(1-q)$  times the proportion surviving in the previous interval for every year thereafter. To compute the 95% confidence intervals on the smoothed hazard, we sampled each mutation's data, with replacement, 1000 times, generated life tables for iteration, and then chose the 2.5<sup>th</sup> and 97.5<sup>th</sup> percentile of the hazards in the bootstrapped distributions at each age.

### **Assumptions.**

To determine a reasonable assumption for withdrawal rate, we performed Google Scholar searches for preventive trials in neurology (*N*=2) or cardiology (*N*=6). The annual withdrawal rate was computed as  $w = 1 - \exp(\log(A)/t)$ , where *A* is the proportion of patients completing the trial at time *t*. Results are summarized in Table S3.

### **Power calculation.**

The number of events (disease onsets, *d*) required was computed per Schoenfeld et al<sup>8</sup> (equation 1). The number of patients required in order to observe that number of disease onsets was computed using an exponential model per Kohn et al<sup>9</sup>. Hazard in the placebo group was the baseline hazard specified in the text (4.6% for Table 2), and hazard for the drug group was the baseline hazard times the hazard ratio. The cumulative event rate in each group was computed as  $C = (h/(h+w)) * (1 - \exp(-(h+w)*t))$ , where *h* = hazard, *w* = withdrawal rate, and *t* = years of followup. The overall cumulative event rate *C<sub>tot</sub>* was the average of the cumulative event rates for the two groups, weighted by proportion treated (in this case, 50/50). The number of randomized individuals required for *d* events to be observed was calculated as  $d / C_{tot}$ . To account for ignoring the first *g* years of data, we reasoned that the cumulative rate of events usable in the final dataset would be  $C_{usable} = (h/(h+w)) * (1 - \exp(-(h+w)*t)) - (h/(h+w)) * (1 - \exp(-(h+w)*g))$ , which simplifies to  $C_{usable} = (h/(h+w)) * (\exp(-(h+w)*g) - \exp(-(h+w)*t))$ .

## Supplementary Discussion

### *Estimation of number of individuals available for trials*

We considered worldwide trials but we found we did not have adequate data to estimate the number of genetically tested presymptomatic individuals worldwide, analogous to our approach for the U.S. The NHS National Prion Clinic in the U.K. has seen 72 presymptomatic individuals with *PRNP* mutations since 1990, and the French surveillance center in Paris has delivered 18 positive *PRNP* predictive test results since 2004, but the other centers involved in this report did not have comprehensive data on predictive testing in their respective countries. A large number of E200K mutation carriers are suspected to exist in Slovakia and Israel due to founder mutations, although fewer than 100 carriers appear to have been identified in each country to date<sup>10,11</sup>.

A less conservative estimate of the true number of high penetrance *PRNP* mutation carriers could be derived from lifetime risk. Prion disease is responsible for ~1 in 5,000 deaths<sup>12</sup>. 1,176 prion disease cases in a recent case series harbored a *PRNP* variant classified here as highly penetrant, out of 10,460 sequenced cases or 16,025 total cases<sup>12</sup>. Thus, 7 - 11% of prion disease cases have a high penetrance *PRNP* variant, suggesting that ~1 in 45,000 to 71,000 deaths are due to a high penetrance *PRNP* variant. The carrier rate among the living population will be somewhat lower because these variants reduce life expectancy, so perhaps ~1 in 100,000 people harbors a high penetrance *PRNP* variant. Data from population controls are consistent, with 1 high penetrance allele (E200K) out of 141,456 individuals in the gnomAD database as of October 2018 (<http://gnomad.broadinstitute.org/>)<sup>13</sup>, and a similar frequency in the 23andMe cohort<sup>12</sup>. If the true carrier rate is 1 in 100,000, then there may exist 3,000 people in the United States with high penetrance *PRNP* variants. However, this figure greatly overestimates the number of people available for trials, as most of these individuals have not undergone predictive testing. Indeed, many are likely not even aware that they are at risk, perhaps because a family history is absent or a family member was not diagnosed correctly.

All of the above estimates only consider genetic status and age. Of course, willingness, geography, and various exclusion criteria will dramatically lower the number of individuals actually enrolled in any trial. On the other hand, it is likely that approval of a first prion disease drug would increase the number of patients available for future trials. A drug could improve diagnosis rates, as prion disease is not currently prioritized in the differential diagnosis of rapidly progressive dementia due to its being untreatable<sup>14</sup>. The U.S. observes an incidence of ~1 prion disease case per million population per year, but up to twice that incidence has been observed in countries with more intense surveillance systems<sup>15</sup>. Because many prion disease patients die undiagnosed, their relatives may never learn that they are at risk for a *PRNP* mutation. A drug might also increase the uptake of predictive genetic testing among those who do learn that they are at risk. The 23% uptake observed for prion disease<sup>16</sup> is consistent with other currently “medically inactionable” indications such as Huntington’s disease<sup>17</sup>, while as noted in the main text, “actionable” indications such as *BRCA1/2* mutations appear to have much higher uptake<sup>18</sup>. Finally, the existence of a drug may promote general awareness of the disease and improve the infrastructure for surveillance, registries, and patient ascertainment.

### *Simulation of power for randomized preventive trials with a clinical endpoint*

Individuals were assigned one of the three *PRNP* mutations and a starting age distributed between 40 and 80, weighted by mutation prevalence and by the proportion of individuals surviving at each age. As above, we assigned half of individuals to drug and half to placebo, and assumed a  $w=15.2\%$  annual withdrawal rate, a  $P=0.05$  statistical threshold, and a 5-year trial duration with a 1-year "run-in" period. For each year of the trial, each individual withdraws with probability  $w$ , becomes sick with a probability corresponding to the hazard function for their particular *PRNP* mutation and age at the time, multiplied by the simulated hazard ratio if drug treated, or else continues on in the trial. At the end of each simulated trial, we analyzed the censored trial data to determine a  $P$  value. For non-stratified simulations, drug/placebo status was assigned without regard to mutation, and survival status was regressed on drug/placebo status alone using a log-rank test, with the overall  $P$  value as the readout. For stratified trials, drug/placebo status was assigned 50/50 within each mutation, and mutation was included as a covariate in a Cox proportional hazards regression, with the  $P$  value for the "drug" parameter as the readout.

We then compared this model to the power calculation results by taking the calculated required numbers of individuals for 80% power (Table 2) and then running the simulation (500 iterations) to determine the power for this number of individuals. The results (Table S6) show overall good agreement between the power calculation and the simulation — for most scenarios tested, the power is indeed close to 80%, with or without stratification. Stratification actually reduces statistical power for the conditions with low  $N$  and low hazard ratios. Under such conditions, it is a common occurrence that there may be zero disease onsets either in one randomized group (usually the drug-treated group) or in one mutation, resulting in an infinite regression coefficient or beta in the Cox model. Thus, the regression never converges, and the simulated trial results turn out statistically non-significant.

### **Codon 129 effects on age of onset and disease duration**

To determine whether codon 129 affects age of onset for the three most prevalent mutations considered here, we used a log-rank model based on codon 129 diplotype (phased genotype) where available (Table S7). In this model, only D178N showed clear evidence for genetic modification of age of onset and disease duration, with  $P$  values significant after multiple testing correction. To determine the nature of this genetic modification, we plotted survival curves by codon 129 diplotype and, because phase was unknown for many codon 129 heterozygous individuals, we also considered phaseless genotypes. In pairwise tests for D178N, M/M was not significantly different from M/V (nominal  $P = 0.14$ ) nor from V/M (nominal  $P = 0.69$ ), and in the phaseless survival curve, MV was overall similar to MM (Figure S4D). These results suggest that the significant codon 129 effect on D178N age of onset is most likely driven primarily by a younger age of onset in V/V individuals compared to other diplotypes. Despite the strong statistical significance of this difference, the small number of D178N-129VV individuals means that codon 129 does not add any explanatory power for age of onset in the dataset as a whole. As noted in the main text, mutation alone explains limited variance in age of onset (adjusted  $R^2 = 0.15$ ,  $P = 1.3e-33$ ). Adding *cis* and *trans* codon 129 to this model decreases the variance explained (adjusted  $R^2 = 0.14$ ,  $P = 3.6e-18$ ).

We also investigated in further detail previously reported associations. For disease duration, D178N M/M and V/V were significantly more rapid than either heterozygous diplotype, consistent with previous reports. Although codon 129 diplotype did not have a significant effect on E200K disease duration overall (nominal  $P = 0.10$ ), a phaseless genotypic model was suggestive (nominal  $P = 0.031$ ), with MV heterozygotes appearing to have a slightly longer disease duration than MM homozygotes, a direction of effect consistent with previous reports<sup>4,19</sup>.

Whereas P102L age of onset was reported to be higher for M/V than M/M individuals<sup>20</sup>, here we find no evidence for this and, ignoring phase, the non-significant trend is towards younger onset in MV than MM individuals (nominal  $P = 0.056$ ).

### ***Potential age of onset confounders***

Because our data were gathered from a variety of study centers using a variety of methodologies, we asked whether any confounders might affect age of onset (Table S7). There was no difference in age of onset between directly and indirectly ascertained individuals ( $P = 0.78$ ). Age of onset was correlated with year of birth after controlling for mutation ( $P < 1e-48$ ), which is a previously reported artifact caused by our relatively limited ability to ascertain individuals whose onset has not yet arrived (though we ascertain some of them through predictive testing) or whose onset occurred before genetic diagnosis of prion disease was possible (though we ascertain some of them through family histories)<sup>4</sup>. This correlation does not affect estimation of overall age of onset distributions. Age of onset appeared to differ slightly among the nine contributing study centers after controlling for mutation, although it was not significant after multiple testing correction (nominal  $P = 0.012$ , Bonferroni  $P = 0.26$ , two-way ANOVA), and it only marginally increased variance explained (adjusted  $R^2 = 0.16$ ) compared to mutation alone (adjusted  $R^2 = 0.15$ , see above). Year of onset showed evidence of positive correlation with age of onset after controlling for study center and mutation (nominal  $P = 0.00032$ , Bonferroni  $P = 0.008$ , linear regression), although the effect size was small (+0.12 years of age per calendar year, or in other words, cases in 2010 have on average an age of onset 1.2 years older than cases in 2000) and, again, the effect on variance explained was minimal (adjusted  $R^2 = 0.18$ ). This slight positive correlation might be due to improved ascertainment of older-onset cases as prion surveillance strengthens over time.

### ***Justification for trial duration assumptions***

In the main text, we argued that a longer trial duration could be considered for a post-marketing study because it would run concurrently with, rather than reducing, the drug's effective market exclusivity period (the period before generic equivalents can be approved). In the U.S., new drugs may be protected by patent exclusivity granted by the Patent and Trademark Office and/or by market exclusivity measures granted by FDA; these exclusivity periods are not additive. Patents last 20 years beginning from their filing, which is usually during the preclinical development phase. The 1984 Hatch-Waxman Act allows sponsors to recover up to 5 years of additional exclusivity, not to exceed a total of 14 years of market exclusivity, to make up for time the drug spends in FDA review<sup>21</sup>. FDA can offer varying periods of market exclusivity depending upon the indication and treatment modality, including 12 years for new biologics<sup>22</sup> and 7 years for rare disease drugs granted Orphan Drug designation<sup>23</sup>. In practice, new drugs receive on average about 12 years of effective market exclusivity<sup>24,25</sup>. The vast majority of pivotal trials supporting new drug approvals last less than one year<sup>26</sup>. While there are rare examples of 5-year trials<sup>27</sup>, a 10- or 15-year prevention trial would exhaust most or all of a drug's effective market exclusivity period. In contrast, as noted in the Discussion, there do exist precedents for very long-term surveillance of patients receiving a drug after approval.

### ***Historical control trial simulation***

As for the simulation of randomized trials, individuals were assigned one of the three *PRNP* mutations and a starting age distributed between 40 and 80, weighted by mutation prevalence and by the proportion of individuals surviving at each age. Again, we assumed a  $w=15.2\%$  annual withdrawal rate (Table S5), a  $P=0.05$  statistical threshold, and a 1-year "run-in" period where disease events are ignored. Distinct from the randomized trial simulation, here all simulated individuals are treated with the drug. For each year of the trial, each individual withdraws with probability  $w$ , becomes sick with a probability corresponding to the hazard function for their particular *PRNP* mutation and age at the time, multiplied by the simulated hazard ratio, or else continues on in the trial. At the end of each simulated trial, the censored trial data on treated individuals are compared to our original dataset as historical controls (Supplementary Life Tables). To determine a  $P$  value we used a Cox proportional hazards counting model accounting for different left-truncation times<sup>28</sup>: for untreated individuals in the original dataset, we assumed age 0 as a start time, while for treated individuals, we assumed left truncation at the age at trial enrollment, plus one year to account for the "run-in" year.

While we cannot currently rule out the possibility that our dataset is biased relative to the *true* hazards facing mutation carriers in real life (see main text Discussion), we sought to confirm that our simulation method is not itself biased. We reasoned that if our simulation was unbiased, then for a drug with hazard ratio equal to 1 (a completely ineffective drug), even long trials with large numbers of individuals should have power equal to  $\alpha$ , by the definition that  $\alpha$  is the false positive rate when the null hypothesis (no efficacy) is true. We therefore ran 1000 iterations of a simulation with a hazard ratio of 1 and 1000 individuals followed for 20 years. We observed a significant result at  $P < 0.05$  in only 5.5% of iterations, consistent with the expected 5%.

In contrast to the result for randomized trials (see discussion above and Table S6), we found that stratification by mutation in the analysis of historical control trial simulation did just slightly increase statistical power. For example, with  $N=156$  individuals followed for 15 years with a hazard ratio of 0.5, power was 90.6% (906/1000 iterations) without stratification and 94.1% (941/1000 iterations) with stratification. This difference from the randomized trial simulation may be a property of the Cox counting model, combined with the fact that our historical comparison dataset has  $N=1,000$  individuals, and we considered follow-up periods of up to 15 years, meaning that the dataset was large enough for the small explanatory power of different *PRNP* mutations to matter. Nevertheless, for consistency with the methods used for the randomized trial simulations, we chose not to stratify in the simulations used for Table 3 and Figure S6.

We performed power calculations for post-marketing studies using historical controls under a range of assumptions in addition to those explored in Table 3 in the main text. In one set of experiments, we considered the effects of varying the length of the follow-up period. For a hazard ratio of 0.5, 80% power could be achieved within 9 years for  $N=156$  participants, but is never achieved for  $N=60$  participants (Figure S6A). This is because statistical power eventually plateaus for lack of participants: our assumption of a 15.2% withdrawal rate compounded annually means that after 10 years, only 19% of the original participants remain in the trial. If the set of drug recipients followed in a post-marketing study were fixed shortly after approval, then this is a realistic concern. If, on the other hand, study design allows new individuals who are prescribed the drug to be added to the monitored cohort continually, the number of individuals in the trial could stay constant or even grow. To simulate this possibility, we also considered a zero withdrawal rate scenario. Under this assumption, even with  $N=60$  individuals, 80% power is achieved in 10 years (Figure S6A).

In another set of experiments, we compared the power for post-marketing studies with historical controls, with or without modeling withdrawal, in comparison to pre-approval randomized trials, for a range of hazard ratios (Figure S6B). For the same hazard ratio and level of statistical power, post-marketing trials generally required only about one fifth as many individuals, and if withdrawal is set to zero, simulating continuous enrollment, only one twentieth as many, as pre-approval randomized trials.

Certainly, a post-marketing study is not a panacea, and under certain assumptions even this trial design is not well-powered: for instance, for a drug of marginal efficacy (hazard ratio 0.9, delaying onset by ~1 year) even a 15-year trial with no withdrawal could not achieve 80% power with 1,000 participants. But, under a range of moderate assumptions, a post-marketing study is more feasible than randomized pre-approval trials with a clinical endpoint.

## Supplementary Tables

**Table S1. Literature review to identify probable high penetrance variants**

"Mendelian segregation" indicates the presence of at least one family with at least three affected individuals in a pattern consistent with Mendelian segregation. "De novo" indicates a case with a confirmed de novo mutation. — indicates neither of these criteria was present.

variant	evidence for high penetrance	comments
P39L	— <sup>29</sup>	
2-OPRD	— <sup>30,31</sup>	
1-OPRI	— <sup>32,33</sup>	
2-OPRI	— <sup>34</sup>	
3-OPRI	— <sup>35</sup>	
4-OPRI	— <sup>36</sup>	most cases have a negative family history
5-OPRI	Mendelian segregation <sup>37</sup>	
6-OPRI	Mendelian segregation <sup>38</sup>	
7-OPRI	Mendelian segregation <sup>39</sup>	
8-OPRI	Mendelian segregation <sup>39,40</sup>	
9-OPRI	Mendelian segregation <sup>41</sup> , <i>de novo</i> <sup>42</sup>	
12-OPRI	Mendelian segregation <sup>43</sup>	
P84S	— <sup>44</sup>	
S97N	— <sup>45</sup>	
P102L	Mendelian segregation <sup>20</sup>	
P105L	Mendelian segregation <sup>46</sup>	2 sibs affected & genotyped, 1 ungenotyped parent likely affected
P105S	— <sup>47</sup>	
P105T	Mendelian segregation <sup>48</sup>	
G114V	Mendelian segregation <sup>49,50</sup>	pedigree suggests penetrance high though not 100%
A117V	Mendelian segregation <sup>51</sup>	
129insLGGLGGYV	<i>de novo</i> <sup>52</sup>	
G131V	— <sup>53,54</sup>	positive family history in one case
S132I	Mendelian segregation <sup>55</sup>	extensive family history, only proband genotyped
A133V	— <sup>56</sup>	
Y145X	— <sup>57</sup>	
R148H	— <sup>31</sup>	
R156C	— <sup>58</sup>	
Q160X	Mendelian segregation <sup>59</sup>	
Y162X	Mendelian segregation <sup>60</sup>	
Y163X	Mendelian segregation <sup>61,62</sup>	
D167G	— <sup>63</sup>	
D167N	— <sup>3</sup>	
Y169X	Mendelian segregation <sup>62</sup>	
V176G	— <sup>64</sup>	
D178Efs25X	Mendelian segregation <sup>65</sup>	only proband genotyped
D178N	Mendelian segregation <sup>66</sup> , <i>de novo</i> <sup>67</sup>	



V180I	— 68	
T183A	Mendelian segregation <sup>69</sup>	
H187R	Mendelian segregation <sup>70</sup>	
T188A	— 71	
T188K	— 72	some patients have a positive family history <sup>72-74</sup>
T188R	— 72,75	
V189I	— 76	
T193I	— 77	
K194E	— 5	
E196A	— 78	
E196K	Mendelian segregation <sup>79</sup>	only proband genotyped
F198S	Mendelian segregation <sup>80,81</sup>	
F198V	— 45	
E200G	— 82	
E200K	Mendelian segregation <sup>83</sup>	
T201S	— 84	
D202G	Mendelian segregation <sup>85</sup>	only proband genotyped
D202N	— 86	
V203I	— 87	
R208C	— 45	
R208H	— 88	
V210I	— 89,90	
E211D	Mendelian segregation <sup>91</sup>	supplement describes 1 family with 3 affected
E211Q	— 79	2 sibs affected
Q212P	— 3	
I215V	— 92	
Q217R	— 81	2 affected
Y218N	Mendelian segregation <sup>93</sup>	
A224V	— 94	
Y226X	— 95	
Q227X	— 95	
M232R	— 68	
M232T	— 96	
P238S	— 2	

**Table S2. Descriptive statistics regarding sources of age of onset data**

<b>study center</b>	<b>N</b>
Japanese national prion surveillance network (Shimotsuke & Kanazawa, Japan)	215
MRC Prion Unit (London, U.K.)	211
French national reference center for CJD (Paris, France)	168
UCSF Memory and Aging Center (San Francisco, U.S.)	147
Spanish National Center for Epidemiology (Madrid, Spain)	114
German Reference Center for TSEs (Göttingen, Germany)	101
DOXIFF study at the Mario Negri Institute (Milan, Italy)	65
Reference Center for CJD at University of Bologna (Bologna, Italy)	49
Australian National CJD Registry (Melbourne, Australia)	24
<i>total</i>	<i>1094</i>
<b>method of ascertainment</b>	<b>N</b>
direct (clinical visit, autopsy, or surveillance report)	843
indirect (family history)	251
<i>total</i>	<i>1094</i>
<b>vital status</b>	<b>N</b>
censored — died due to intercurrent illness without developing prion disease	4
censored — alive and well at last follow-up	101
symptomatic with prion disease at last follow-up	81
died of prion disease	908
<i>total</i>	<i>1094</i>

**Table S3. Age of onset statistics on supplementary variants**

<b>mutation</b>	<b>without censored data</b>		<b>survival curve including censored data</b>		
	<b>mean ± sd</b>	<b>N</b>	<b>median (IQR)</b>	<b>range</b>	<b>N</b>
5-OPRI	46.8 ± 6.0	14	49 (44 - 53)	34 - 56	18
6-OPRI	35.1 ± 5.8	31	35 (32 - 39)	23 - 47	34
P105L	46.5 ± 8.5	13	47 (40 - 51)	31 - 61	13
A117V	41.2 ± 7.8	26	41 (37 - 45)	25 - 58	28

**Table S4. Withdrawal rates in preventive clinical trials**

*w*, annual withdrawal rate. CHD, coronary heart disease. NSAID, non-steroidal anti-inflammatory drug. See Methods for details.

category	trial	description	w
cardiology	WOSCOPS <sup>97</sup>	pravastatin for CHD	6.9%
cardiology	AFCAPS/TexCAPS <sup>98</sup>	lovastatin for CHD	7.1%
cardiology	OSLER <sup>99</sup>	evolocumab for CHD	9.0%
cardiology	JUPITER <sup>100</sup>	rosuvastatin for CHD	14.1%
neurology	ADAPT <sup>101</sup>	NSAIDs for Alzheimer's	16.3%
cardiology	ODYSSEY LONG TERM <sup>102</sup>	alirocumab for CHD	19.0%
cardiology	NCT00607373 <sup>103</sup>	mipomersen for homozygous <i>LDLR</i> hypercholesterolemia	22.1%
neurology	PRECREST <sup>104</sup>	creatine for Huntington's disease	54.9%

**Table S5. Power calculations under alternative assumptions.**

Each block of this table is equivalent to Table 3 but with different assumptions as indicated (except where stated, other assumptions are identical to those in Table 2). A) Best case scenario: overall average hazard is 4.8% (the higher figure including the less common mutations shown in Table S2 and Figure S1), the withdrawal rate is 6.9% per year (the lowest rate in any of the trials we reviewed, see Table S4), and there is no run-in period — the drug is effective immediately and so disease onsets within the 1st year of the trial are included. B) Worst case scenario: overall average hazard is only 3.5% — one quarter lower than calculated in this manuscript, because our data are biased due to under-inclusion of asymptomatic individuals, and/or because predominantly younger people enroll in a trial — and the withdrawal rate is 54.9% per year (the highest rate in any trial we reviewed, see Table S4). C) Targeted trial scenario: only the mutations with higher hazards — 5-OPRI, 6-OPRI, P105L, and A117V — are targeted for recruitment, resulting in a higher baseline hazard of 5.2%. Although the enrollment requirements for this scenario are lower than in Table 2, these mutations are also approximately one order of magnitude rarer<sup>12</sup>, making achievement of these enrollment numbers yet more unlikely. D) Long follow-up scenario: trial duration is 15 years. This reduces the required numbers somewhat, but this benefit is limited by the withdrawal rate, which means that few individuals are still enrolled after 15 years. E) Zero withdrawal scenario: withdrawal rate is set to zero.

alternate scenario	hazard ratio	years of life added	onsets required	participants required
A (best case)	0.1	undefined*	6	59
	0.2	21	12	110
	0.3	13	22	182
	0.4	9	37	291
	0.5	7	65	475
	0.6	5	120	821
	0.7	3	247	1,589
	0.8	2	631	3,850
	0.9	1	2,828	16,434
B (worst case)	0.1	undefined*	6	357
	0.2	21	12	666
	0.3	14	22	1,097
	0.4	10	37	1,757
	0.5	7	65	2,866
	0.6	5	120	4,955
	0.7	4	247	9,586
	0.8	2	631	23,196
	0.9	1	2,828	98,897
C (targeted trial)	0.1	undefined*	6	92
	0.2	undefined*	12	171
	0.3	10	22	280
	0.4	7	37	449
	0.5	5	65	732
	0.6	4	120	1,267

	0.7	3	247	2,457
	0.8	2	631	5,958
	0.9	1	2,828	25,471
D (long follow-up)	0.1	undefined*	6	59
	0.2	21	12	108
	0.3	14	22	178
	0.4	10	37	285
	0.5	7	65	465
	0.6	5	120	806
	0.7	4	247	1,568
	0.8	2	631	3,816
	0.9	1	2,828	16,384
E (zero withdrawal)	0.1	undefined*	6	66
	0.2	21	12	123
	0.3	14	22	202
	0.4	10	37	323
	0.5	7	65	527
	0.6	5	120	912
	0.7	4	247	1,767
	0.8	2	631	4,285
	0.9	1	2,828	18,314

**Table S6. Comparison of power calculation and simulation results.**

The first four columns are reproduced from Table 2 for ease of comparison. The number of participants required was calculated to yield 80% power; the final two columns show the power for this number of participants, at  $P=0.05$ , indicated by simulation. See Supplementary Discussion above for details of the method.

hazard ratio	years of life added	onsets required	participants required	calculated power	simulated power without stratification	simulated power with stratification
0.1	undefined*	6	101	80.0%	62.2%	35.0%
0.2	21	12	189	80.0%	71.8%	69.2%
0.3	14	22	311	80.0%	78.6%	76.6%
0.4	10	37	498	80.0%	80.0%	81.2%
0.5	7	65	813	80.0%	80.0%	81.8%
0.6	5	120	1406	80.0%	83.2%	79.8%
0.7	4	247	2,724	80.0%	80.4%	80.8%
0.8	2	631	6,602	80.0%	81.4%	77.6%
0.9	1	2,828	28,204	80.0%	80.6%	78.0%

**Table S7. Tests for modifiers and confounders of age of onset**

All *p*-values are two-tailed. As explained in Supplementary Discussion, diplotypes (phased genotypes) are indicated with a slash (*cis/trans* to the mutation) while unphased genotypes have no slash. We were unable to obtain phase data for many 129MV individuals, so the genotypic tests represent not only a different grouping of data but also include more data points than the corresponding diplotypic tests. Thus, we considered them as independent tests for the purposes of multiple testing correction. *p* (raw) indicates the raw *p* value; *p* (bc) is Bonferroni-corrected for 22 tests. For parent-child comparisons *n* is the number of pairs. For linear regressions, child year of birth was included in the model as a covariate. The prior column represents the prior expectation of whether there would be a significant difference in each test based on previous reports in the literature.

variable	mutation	comparison	n	test	<i>P</i> (raw)	<i>P</i> (bc)	prior evidence
onset	P102L	M/M vs. M/V vs. V/V	125 vs. 13 vs. 1	log-rank	0.18	1	mixed <sup>20,38,105</sup>
onset	P102L	MM vs. MV vs. VV	125 vs. 32 vs. 1	log-rank	0.057	1	
onset	D178N	M/M vs. M/V vs. V/M vs. V/V	133 vs. 18 vs. 9 vs. 13	log-rank	0.000062	0.0014	none <sup>105–108</sup>
onset	D178N	MM vs. MV vs. VV	133 vs. 58 vs. 13	log-rank	0.000018	0.00040	
onset	E200K	M/M vs. M/V vs. V/M vs. V/V	286 vs. 33 vs. 10 vs. 5	log-rank	0.13	1	none for <i>trans</i> allele <sup>4,105,108,109</sup> , suggestive for <i>cis</i> allele <sup>4</sup>
onset	E200K	MM vs. MV vs. VV	288 vs. 92 vs. 5	log-rank	0.30	1	
duration	P102L	M/M vs. M/V	89 vs. 8	log-rank	0.55	1	none <sup>20</sup>
duration	P102L	MM vs. MV	89 vs. 21	log-rank	0.93	1	
duration	D178N	M/M vs. M/V vs. V/M vs. V/V	62 vs. 13 vs. 8 vs. 10	log-rank	0.000081	0.0018	yes <sup>107</sup>
duration	D178N	MM vs. MV vs. VV	62 vs. 33 vs. 10	log-rank	0.00000010	0.0000022	
duration	E200K	M/M vs. M/V vs. V/M vs. V/V	208 vs. 21 vs. 6 vs. 5	log-rank	0.10	1	yes <sup>4,10,19</sup>
duration	E200K	MM vs. MV vs. VV	210 vs. 50 vs. 5	log-rank	0.031	0.68	
onset	P102L	parent vs. child	32	linear regression	0.44	1	suggestive <sup>110</sup>
onset	D178N	parent vs. child	15	linear regression	0.12	1	none
onset	E200K	parent vs. child	40	linear regression	0.68	1	none <sup>4</sup>
onset	top three	men vs. women	446 vs. 492	Cox	0.22	1	none
duration	top three	men vs. women	264 vs. 281	linear regression	0.02	0.44	yes <sup>19</sup>
onset	top three	direct vs. indirect ascertainment	843 vs. 251	Cox	0.78	1	none
duration	top three	direct vs. indirect ascertainment	544 vs. 90	linear regression	0.64	1	none
onset	top three	year of birth	697	linear regression	3.6E-49	7.9E-48	yes <sup>4</sup>
onset	top three	study centers	973	two-way ANOVA	0.012	0.26	none
onset	top three	year of onset	697	linear regression	0.00032	0.0070	none

***Supplementary Life Tables***

Uploaded as a separate Excel file. These tables are also made available as .tsv and .xls files in the code and data repository for this manuscript: [https://github.com/ericminikel/prnp\\_onset](https://github.com/ericminikel/prnp_onset)

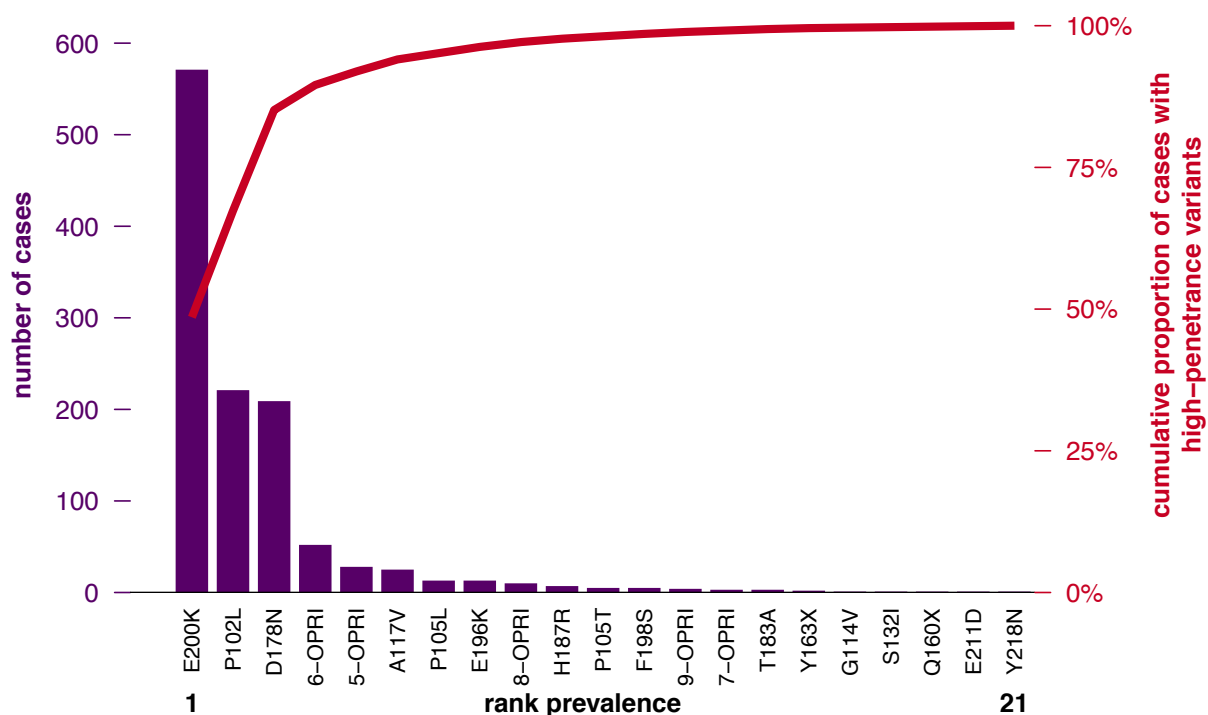
***Supplementary Duration Tables***

Uploaded as a separate Excel file. These tables are made available as .tsv and .xls files in the code and data repository for this manuscript: [https://github.com/ericminikel/prnp\\_onset](https://github.com/ericminikel/prnp_onset)

## Supplementary Figures

### **Figure S1. Variant prevalence among prion disease cases with a high penetrance variant.**

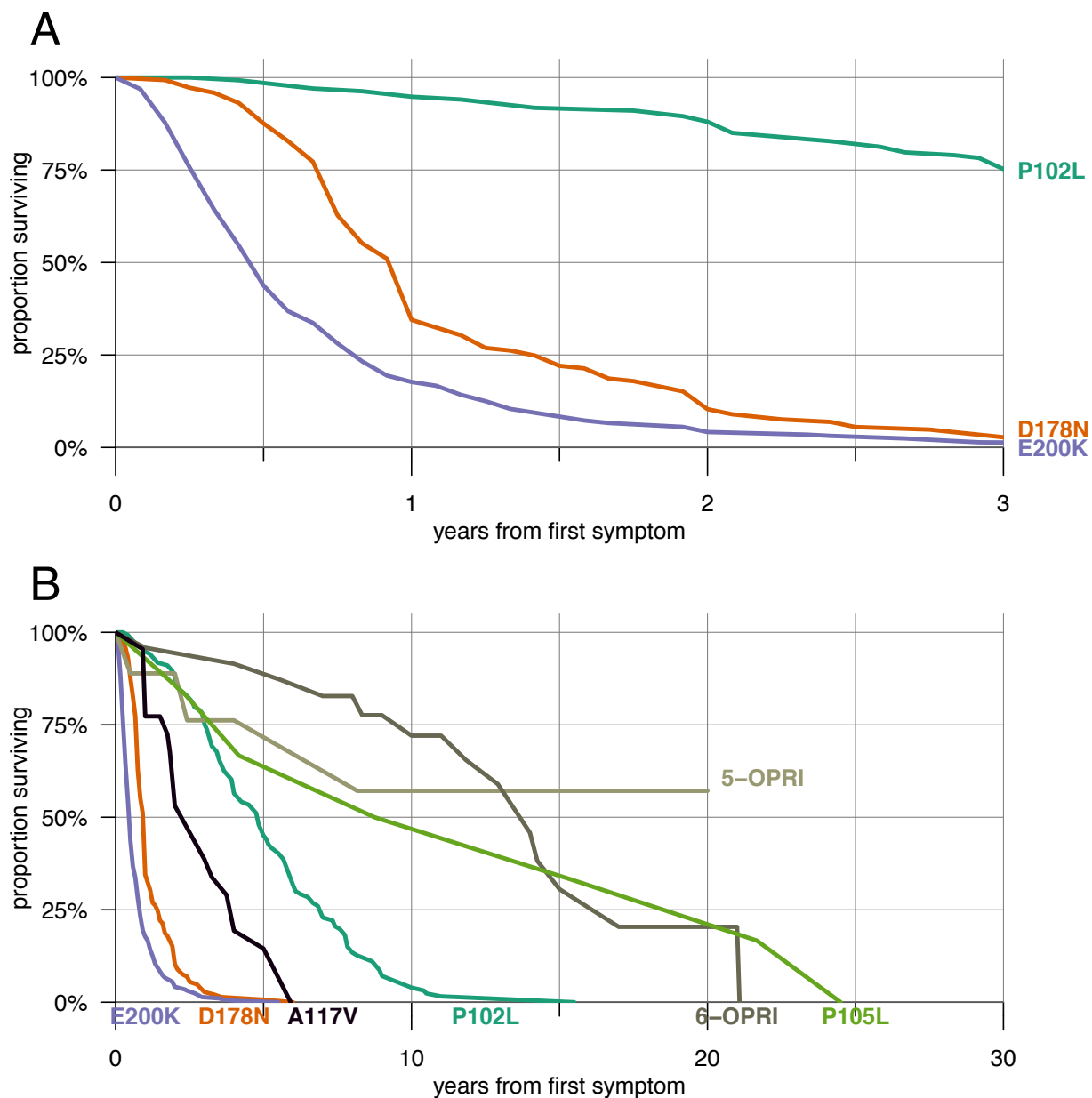
Genetic variants deemed highly penetrant based on the literature review in Table S1 are plotted by the rank (x axis) versus number (left axis) and cumulative proportion (right axis) of high penetrance cases they explain in a recent case series<sup>12</sup>.





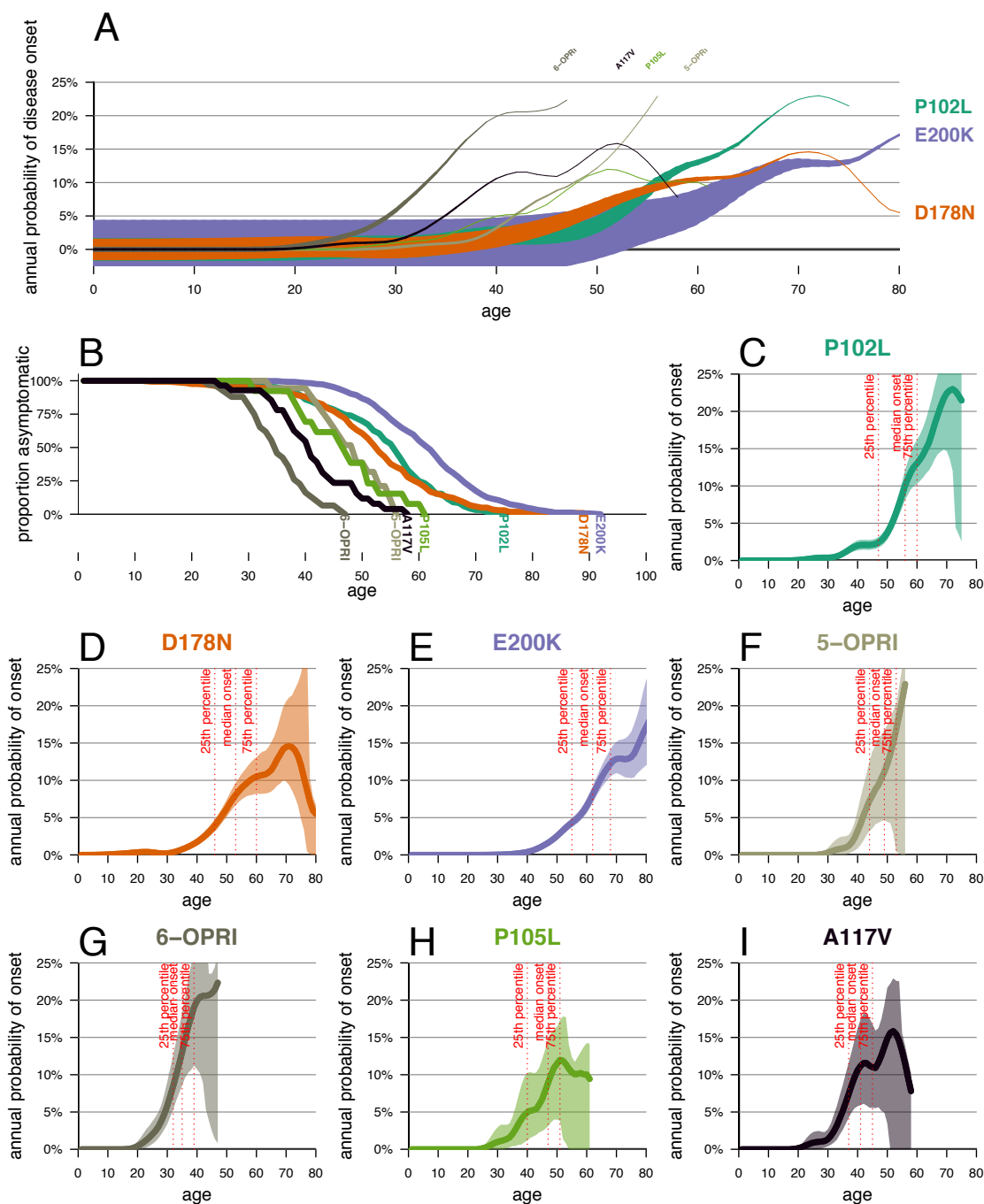
**Figure S2. Disease duration by mutation**

A) Disease duration (time from first symptom to death) in genetic prion disease. D178N and E200K are classified as rapidly progressive mutations, with >50% of individuals dying within one year of first symptom. B) Zoomed out to 30 years (note y axis) and including supplementary mutations. Disease duration data are provided in the Supplementary Duration Tables.



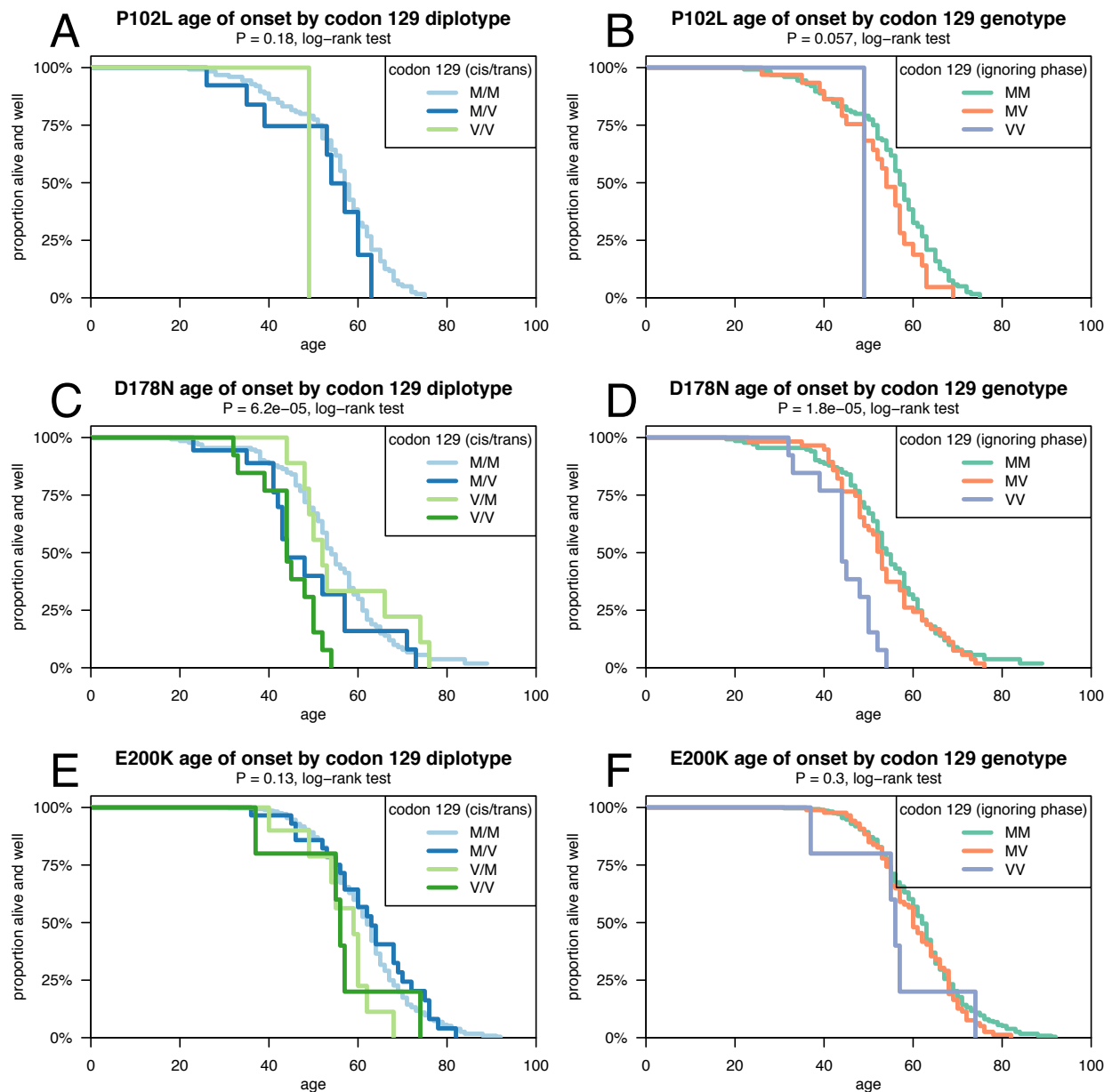
**Figure S3. Survival and hazard curves.**

A) Hazard vs. time with line thickness representing survival, as Figure 1 but including the top 7 mutations. B) Survival curves for the 7 mutations. C-I) Hazard vs. age with 95% confidence intervals displayed in 50% transparency.



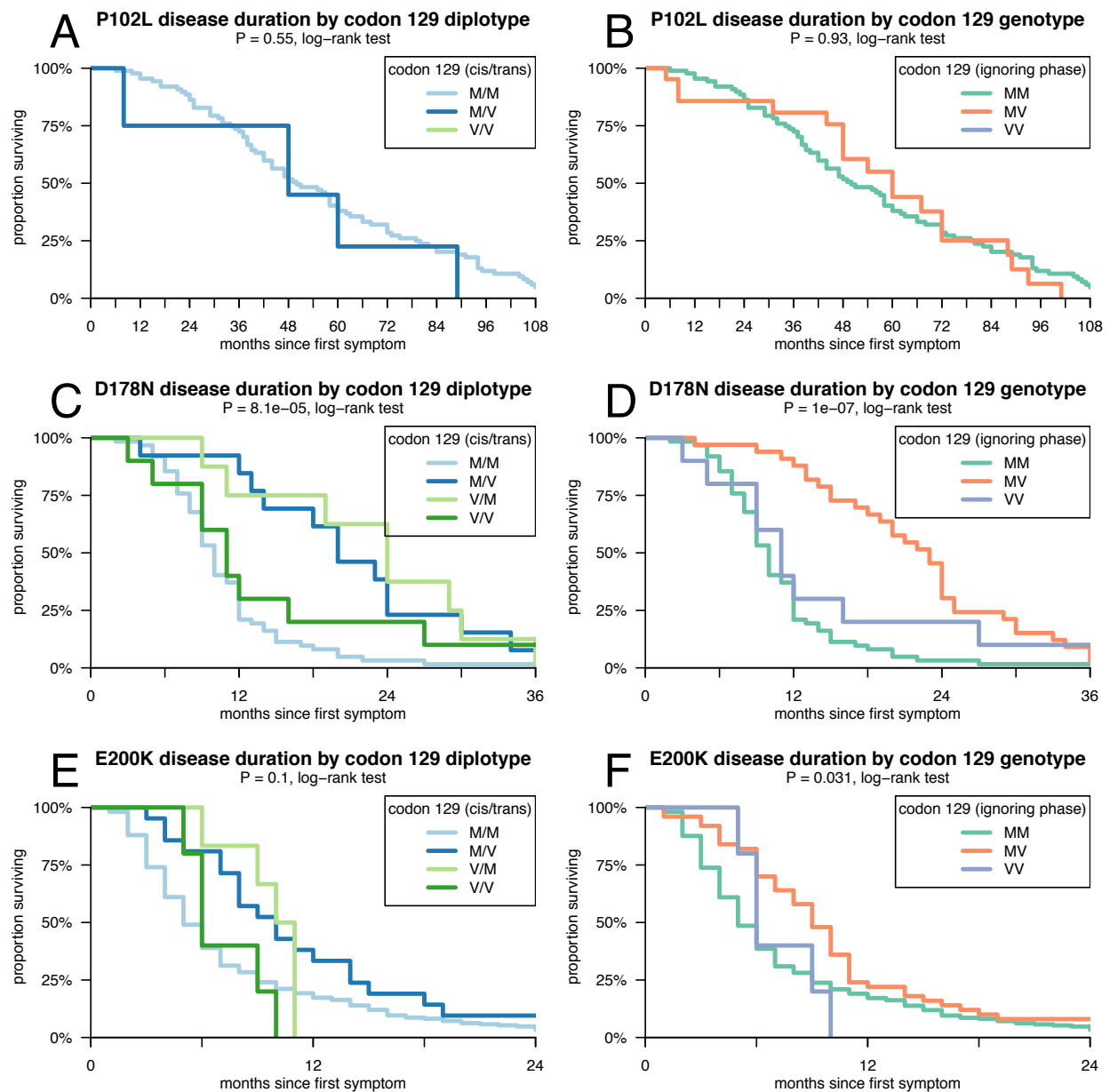
**Figure S4. Age of onset and codon 129**

Survival curves for age onset or death in P102L (A-B), D178N (C-D), and E200K (E-F) genetic prion disease stratified by codon 129 diplotype (A, C, E) or phaseless genotype (B, D, F).



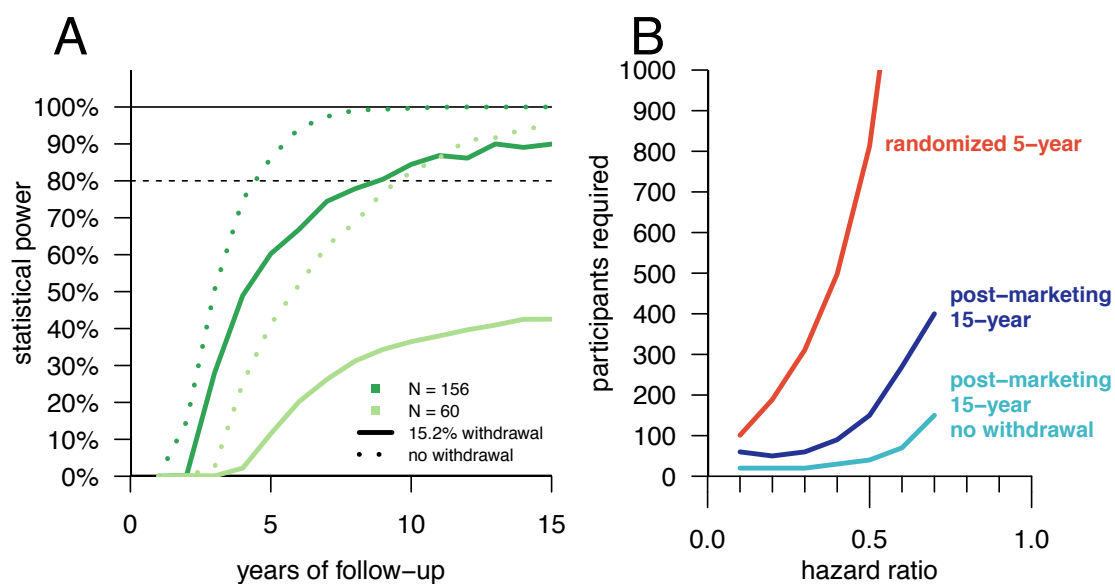
**Figure S5. Disease duration and codon 129**

Survival curves for disease duration (time from first symptom to death) in P102L (A-B), D178N (C-D), and E200K (E-F) genetic prion disease stratified by codon 129 diplotype (A, C, E) or phaseless genotype (B, D, F).



**Figure S6. Power increases with long follow-up periods in simulations using historical controls.**

A) Simulated trial power under the Cox proportional hazards model as a function of the number of individuals randomized and the number of years of follow-up with (solid line) or without (dotted line) modeling withdrawal, assuming a hazard ratio of 0.5 and a run-in period of one year. B) Number of participants required for 80% power at  $P < 0.05$ , as a function of hazard ratio (x axis) and trial design (different curves). Numbers for randomized trials (red curve) are taken directly from Table 2, while numbers for post-marketing studies (dark and light blue curves) are obtained by simulation (Supplementary Discussion).



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