A public resource facilitating clinical use of genomes

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Rapid advances in DNA sequencing promise to enable new diagnostics and individualized therapies. Achieving personalized medicine, however, will require extensive research on highly reidentifiable, integrated datasets of genomic and health information. To assist with this, participants in the Personal Genome Project choose to forgo privacy via our institutional review board-approved "open consent" process. The contribution of public data and samples facilitates both scientific discovery and standardization of methods. We present our findings after enrollment of more than 1,800 participants, including whole-genome sequencing of 10 pilot participant genomes (the PGP-10). We introduce the Genome-Environment-Trait Evidence (GET-Evidence) system. This tool automatically processes genomes and prioritizes both published and novel variants for interpretation. In the process of reviewing the presumed healthy PGP-10 genomes, we find numerous literature references implying serious disease. Although it is sometimes impossible to rule out a late-onset effect, stringent evidence requirements can address the high rate of incidental findings. To that end we develop a peer production system for recording and organizing variant evaluations according to standard evidence guidelines, creating a public forum for reaching consensus on interpretation of clinically relevant variants. Genome analysis becomes a two-step process: using a prioritized list to record variant evaluations, then automatically sorting reviewed variants using these annotations. Genome data, health and trait information, participant samples, and variant interpretations are all shared in the public domain—we invite others to review our results using our participant samples and contribute to our interpretations. We offer our public resource and methods to further personalized medical research.

genome interpretation | genomic medicine | human genetics

As whole genome DNA sequencing costs plummet below the cost of standard diagnostic genetic testing, personal genomes promise dramatic changes for science, medicine, and society. A genome sequence can be a clinical diagnostic that lasts a lifetime, and personal genomes for every individual are likely to become standard components of health care. We now face challenging questions: How do we interpret genome data? Can we and should we regulate access to personal genetic data and/or interpretations? Can whole-genome data truly be considered anonymizable—even if not combined with other personal data? How strictly should a promise of privacy made to research subjects limit our ability to scientifically share their data with other researchers? The fact that combined genetic and phenotype data are so personal and reidentifiable creates a tension between standard commitments ensuring research subject privacy and the scientific need for verification and reproducibility of research findings (1).

The Personal Genome Project (PGP) explores one solution to these issues in its creation of a public resource where participants acknowledge and agree to the potential risk of reidentification. This public resource not only shares genome data publicly but brings these together with publicly shared phenotype information, genetic interpretations, and cell lines; such integrated data means the PGP can provide common ground for many types of genome research. Sharing reidentifiable data requires new instruments for informed consent, as participants explicitly waive their expectation of privacy to make personal biological and

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Data deposition: The sequences reported in this paper are made available through the Personal Genome Project (http://www.personalgenomes.org/data/PGP12.05/).

See Profile on page 11893.

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health information public (2). This process, now called "open consent" (3), places a high value on the autonomy of individuals and on their ability to give open-ended consent for unknown risks. Our informed consent materials extensively discuss both risks associated with loss of privacy and the limited options for restoring privacy once data and cell lines are made public.

Although our goal is to have the broadest possible participation in the PGP, because of the novel nature of the risks and research the Committee on Human Studies (Boston, MA) encouraged us to initially enroll individuals with a master's-level degree or equivalent training in genetics. The "PGP-10" pilot group was chosen in 2006 from 10 such individuals who volunteered for the project. These individuals have chosen to publicly associate their names with their PGP accounts—participants may voluntarily self-identify in this way, but this is not required. Samples from these 10 individuals have since been used to pilot a variety of technologies within our groups and others, including whole-genome sequencing, induced pluripotent stem (iPS) cell line generation and genome engineering, allele-specific expression profiling, epigenetic profiling, and microbiome profiling (4–11). These data go beyond the genome sequence itself to create additional layers of information that move into the realm of associated environmental and trait profiling.

Beyond generating an initial public resource of linked genotype and phenotype data, a key goal of our pilot was to develop and prototype methods for interpreting genome information and making these interpretations public. Early versions of our methods have already been used by other groups in their own genome research and interpretations (9, 12–14). Unlike many published genome interpretation efforts, which have focused on discovery of novel pathogenic variants in patients with genetic disease (15–19), this pilot focuses on 10 individuals not believed to have such diseases. As cohorts with heritable medical conditions join the PGP, our research will extend to disease-focused interpretations. Nevertheless, interpretation methods for individuals not suspected of having genetic disease will be essential for integrating genome data into clinical practice as genome sequencing becomes increasingly routine.

Results

More than 1,000 Participants Enrolled Through Open Consent with Public Health Records. The PGP has piloted the use of an open consent format for collection of combined genome and phenotype data, allowing data to be shared publicly. PGP participants must understand and agree to the following: (*i*) any genome and health record data provided to us could be included in an open-access public database, (*ii*) no guarantees are made regarding anonymity, privacy, and confidentiality, (*iii*) participation may involve a risk of harm or privacy loss to themselves and their relatives, (*iv*) participation does not promise to benefit participants in any tangible way, and (*v*) withdrawal from the study is possible at any time, but complete removal of data that have been available in the public domain may not be possible. This process of making data public means that results are also returned to participants, and an ongoing relationship with these participants is maintained to monitor outcomes of participation prospectively.

On the basis of our experiences with the PGP-10, we created an enrollment system for volunteers that ensures they understand the risks entailed (Fig. 1). Volunteers are provided with a study guide to inform them of genetic concepts and privacy risks and are required to pass an entrance examination testing their understanding of human subjects research, PGP protocols, and basic genetics. Of volunteers meeting minimum eligibility criteria, 44% drop out at this step; 87% of those who successfully complete the examination go on to sign the full consent form and enroll in the project (*SI Appendix*, Fig. S1).

The examination and consent form are completed through an Internet-based system and are electronically signed by volunteers; more than 1,800 participants have enrolled through this process as of May 2012. Because participants are a self-selected group, they are not representative of the general population (*SI Appendix*, Fig. S2); however, we may prioritize participants from underrepresented groups or who have particular traits and/or familial relationships to other participants. Participants are able to extend their profiles with a variety of personal data, including self-collected genetic data, listing enrolled relatives, health records and trait information, and answers to trait and ancestry surveys. These data are made publicly available immediately. As of May 2012, more than 1,000 participants have imported electronic health record data. In addition, as of May 2012, more than 800 participants have DNA samples derived from blood or saliva. These health record data and DNA samples represent the seed of a public resource integrating phenotype data with genotype data and include both common and rare diseases (phenotype data in *SI Appendix*, Dataset S1).

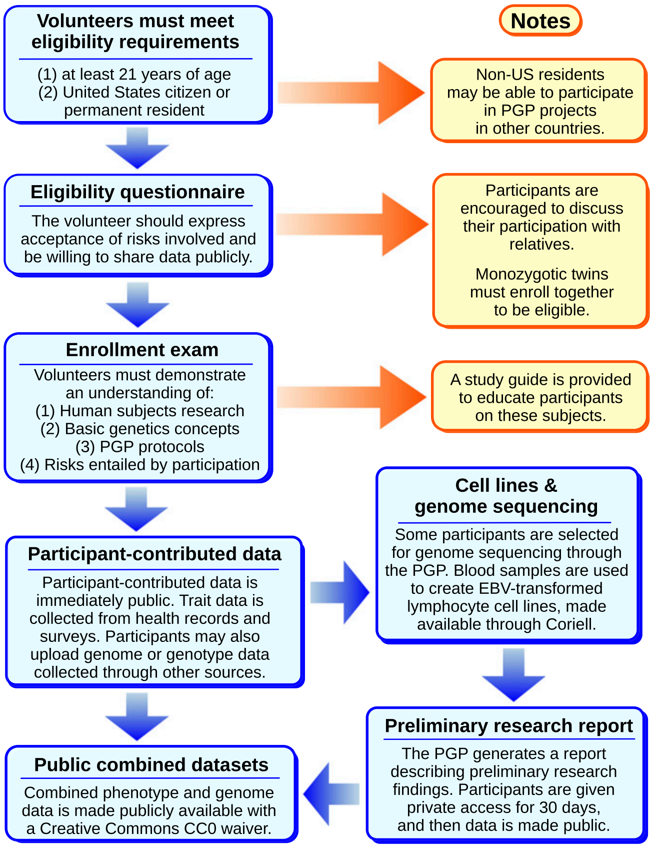

Volunteers must meet eligibility requirements
(1) at least 21 years of age
(2) United States citizen or permanent resident
Notes
Non-US residents may be able to participate in PGP projects in other countries.
Eligibility questionnaire
The volunteer should express acceptance of risks involved and be willing to share data publicly.
Participants are encouraged to discuss their participation with relatives.
Monozygotic twins must enroll together to be eligible.
Enrollment exam
Volunteers must demonstrate an understanding of:
(1) Human subjects research
(2) Basic genetics concepts
(3) PGP protocols
(4) Risks entailed by participation
A study guide is provided to educate participants on these subjects.
Cell lines & genome sequencing
Some participants are selected for genome sequencing through the PGP. Blood samples are used to create EBV-transformed lymphocyte cell lines, made available through Coriell.
Participant-contributed data
Participant-contributed data is immediately public. Trait data is collected from health records and surveys. Participants may also upload genome or genotype data collected through other sources.
Preliminary research report
The PGP generates a report describing preliminary research findings. Participants are given private access for 30 days, and then data is made public.
Public combined datasets
Combined phenotype and genome data is made publicly available with a Creative Commons CC0 waiver.

Fig. 1. PGP enrollment and data collection process. Enrollment in the PGP involves a series of steps meant to ensure informed consent for the public release of personal, reidentifiable genome and trait data. Current and historical copies of our consent forms are publicly available at http://www.personalgenomes.org/consent/.

PGP-10 Pilot Cell Lines and Genome Sequence Data. To enable follow-up functional studies and genome sequence confirmation by third parties, cell lines are established for PGP participants and shared publicly alongside whole-genome data. Fibroblast and EBV-transformed lymphocyte cell lines were established with samples collected from the PGP-10 pilot cohort and have been made available through Coriell Cell Repositories (*SI Appendix*, Table S1). The PGP-10 genome data were produced using DNA purified from these cell lines, sequenced by Complete Genomics, Inc. (CGI) using their 2.0 pipeline (software version 2.0.1.5, matched against the build 37 reference genome). These genome data files have been shared publicly via our site (http://www.personalgenomes.org/data/PGP12.05/).

In addition to calling variants, CGI's genome files report which regions of the genome are confidently called as matching reference and which are "no-call" gaps that are insufficiently covered (and therefore not called as either variant or reference).

Using these data, we are able to assess what fraction of the genome has been successfully genotyped. On average, 96.5% of assembled reference genome positions were called homozygously in the CGI var files for the PGP-10 (*SI Appendix*, Table S2). Coverage is subject to systematic biases: positions called in one genome are much more likely to have been called in the other nine genomes (*SI Appendix*, Fig. S3). A position called in any given genome has a 92% chance of also being called in the other nine genomes, whereas a position not covered in that genome only has a 12% chance of being covered in all of the other nine.

The high quality of our pilot data is evident from analysis of several genomes derived from the same individual. PGP1 genomes were produced using DNA from three different cell lines: EBV-transformed lymphocytes, fibroblasts, and fibroblast-derived iPS cells. We use these data to assess overlap in variant calls because the underlying DNA sequences are expected to be mostly identical. When analysis is limited to positions explicitly called reference or variant in all three genomes (2,993,691 variant positions, 2.65 Gb total), 98.5% of variant positions are shared in all three genomes (Fig. 2*A*). When reference positions are taken into account, the three genomes have matching calls for 99.998% of these positions.

Which positions are sufficiently covered, and thus explicitly called as reference or variant, varies between genomes. Within one of the three PGP1 genomes 87% of variant positions, on average, are also called by the other two genomes. From this set of positions we can estimate the error rate due to random (rather than systematic) causes within a given genome: 99.6% of these variant positions are also called variant by at least one of the other two genomes (i.e., called variant by at least two out of three). When reference positions are included in analysis, 96% of positions called in one genome are called in all three, and 99.9994% of genotype calls in that genome match the call made by at least two out of the three genomes.

In total, 3,815,237 different variant positions were reported in the three genomes, 77% of which were called in all three (Fig. 2*B*). When these diagrams are constructed separately by variant type, we find that more complex length-changing variant calls also have high consistency, with 99.0% of such variants in a given genome called as variant by at least two out of three (*SI Appendix*, Fig. S4). In Fig. 2*B*, most positions where variant calls do not match are due to differences in coverage or base call quality that result in a "no-call" in one or more of the three sequences, as opposed to actual inconsistency in the variant vs. reference calls. This demonstrates the importance of respecting the logical inequivalence between the predicates "is not called as variant" and "is called as reference" and the need for correspondingly precise bookkeeping, possibly through the use of three or four valued logics (20, 21).

All of the PGP-10 had genome data produced from EBV-transformed lymphocyte cell lines, and these are used in all remaining genome analyses. Because the cost difference between exomes and whole genomes is already small, and may eventually vanish entirely, we preferred whole-genome sequencing over targeted approaches. On average these genomes have 3.2 million substitution variant calls relative to the build 37 reference genome and 300,000 short length-changing variants (*SI Appendix*, Table S2). Each individual has on average 8,250 single base substitution variants predicted to be nonsynonymous in a canonical transcript from University of California, Santa Cruz Known Genes (Table 1) (22). Of these, almost all (99.97%) are found in either dbSNP (build 132) or Exome Variant Server data (ESP5400) (23, 24). Notably, this novel variant rate (0.03%) is lower than the rate of random error we would predict on the basis of PGP1 genome comparisons (Fig. 2 and *SI Appendix*, Fig. S4); this may be due to increased accuracy in coding regions or due to common errors shared by both our data and other databases.

Genome variant statistics can vary depending on a given genome's coverage and the stringency used to identify variations, but our data are generally similar to whole-genome sequencing numbers reported elsewhere. Our counts for the number of missense variants in a single individual are somewhat lower than in other publications: this may be due to differences in coverage, stringency in variant calls, or the transcript annotations used for predictions (25, 26). MacArthur et al. (27) reported, on average, 304 nonsense and frameshift variants per individual with European ancestry (compared with our average of 166); their count was reduced to 64 after filtering to increase both variant confidence

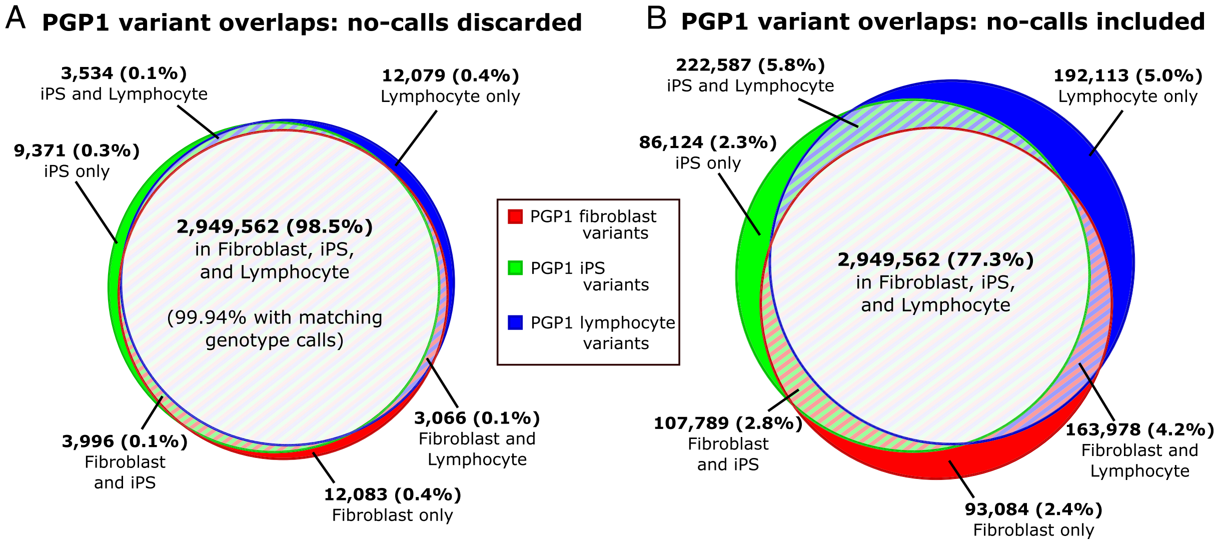


Fig. 2. Venn diagram comparisons of variant calls in PGP1 genomes. Analysis of PGP1 genome variant calls from three different tissues: fibroblast cells, fibroblast-derived iPS cells, and EBV-transformed lymphocyte cells. (*A*) Overlap of all variant calls, limited to positions that are explicitly called as reference or variant in all three genomes. Positions where any of the three genomes have a no-call (lacking coverage to make a confident call) are discarded from analysis. The low residual discordance consists of sequencing errors or real differences between these three tissues and indicates high sequence quality in each of these samples. (*B*) Overlap of all variant calls; positions not called in other genomes are included in the analysis. Most locations that were called as "variant" by one genome and not by other genomes were due to a lack of coverage in the other genomes. Reporting the regions confidently called as matching reference (as opposed to regions lacking sufficient coverage) is critical to genome interpretation and data comparisons.

Table 1. PGP-10 variants with potential functional consequences

Individual (huID)	No. of nonsyn. single base substitution (nsSNPs) variants	No. of nsSNPs not present in dbSNP132 or ESP5400	No. of nonsyn. with "probably damaging" Polyphen 2 prediction	No. of variants in PharmGKB or HuGENet	No. of nonsyn. variants in genes with clinical testing (GeneTests)	No. of nonsyn. variants matching OMIM entries	No. of nonsense and frameshift mutations	No. with prioritization score of 4 or more
PGP1 (hu43860C)	7,781	4	610	1,853	773	34	170	23
PGP2 (huC30901)	8,170	5	618	1,747	809	46	148	29
PGP3 (huBEDA0B)	7,899	1	582	1,793	780	46	169	35
PGP4 (huE80E3D)	8,042	2	606	1,820	829	47	147	26
PGP5 (hu9385BA)	8,312	1	652	1,865	873	53	172	27
PGP6 (hu04FD18)	8,008	3	596	1,810	832	46	167	29
PGP7 (hu0D879F)	8,380	4	649	1,917	868	48	167	25
PGP8 (huAE6220)	8,551	3	694	1,879	876	50	157	30
PGP9 (hu034DB1)	7,542	2	575	1,740	752	41	166	27
PGP10 (hu604D39)	9,810	2	769	1,723	956	42	199	37
Average	8,250	2.7	635	1,815	835	45	166	29

nonsyn., nonsynonymous; nsSNP, nonsynonymous SNP.

and likelihood of functional effect (i.e., not terminal or rescued by splice variants; this latter filtering was not performed by us).

Prioritization of Variants with Potential Clinical Relevance. Creating public methods for genome interpretation and returning interpreted results to participants are core goals of the PGP. Our system facilitates interpretation of whole-genome data by prioritizing variants for review. Preliminary versions of the system have been used in previous publications (9, 12–14). Here we apply the system to our pilot PGP-10 genomes.

To assist discovery of variants with potential phenotypic effects, potential amino acid changes are predicted for all variants occurring within gene coding regions. Variants are then matched against a variety of publicly available datasets: allele frequency data from 1,000 Genomes Project and Exome Variant Server data (24, 28), Polyphen 2 predictions (29), Human Genome Epidemiology Network (HuGENet) (30), Pharmacogenetics Knowledge Base (PharmGKB) (31), GeneTests (32), and Online Mendelian Inheritance in Man (OMIM) (33). After processing, there are many variants that potentially have clinically important consequences (Table 1). On average 635 variants are predicted as "probably damaging" by Polyphen 2, and another 166 are predicted to be severely disruptive nonsense or frameshift variants. When matching variants against our imported databases, each genome on average was found to have 1,815 variants with dbSNP IDs matched to a PharmGKB or HuGENet entry, 45 nonsynonymous variants matched to an OMIM entry, and 835 nonsynonymous variants occurring within genes that have clinical testing available (GeneTests). In total, these variants represented thousands of locations of potential significance when searching a presumed-healthy genome for clinically significant findings.

More complete evaluation of these variants requires incorporating information from the literature, but there are too many variants to do this comprehensively; variant interpretation is inefficient because automatic literature interpretation is computationally refractory—literature analysis requires human attention. To address this, we sought to prioritize variants for review. Review prioritization is implemented through an automatic "prioritization score" heuristic that uses these data to score variants in three categories: computational information, published gene-specific information, and published variant-specific information (*SI Appendix*, Table S3). Each category assigns up to two points, for a total of up to six points for a given variant. On average we found that each of the PGP-10 genomes had 29 variants with prioritization scores of 4 or more, and 131 variants with scores of 3 or more. Because our system accumulates data (see below), the burden of variant review drops dramatically when evaluations from prior genome interpretations can be reused: after 64 genomes we find that there are on average only 8 variants with a prioritization score of 4 or more, and 44 with a score of 3 or more (Fig. 3).

To test how well prioritization scores performed in prioritizing known disease-causing variants, we evaluated the prioritization scores that would be assigned to variants taken from a variety of disease-causing mutation databases (34–38) (lists downloaded September 2011). Although the findings reported in these databases may also be found in the databases used by our prioritization calculation (OMIM, Genetests, PharmGKB, and HuGENet), they are otherwise independent and are not themselves used in generating prioritization scores. We compared the prioritization scores assigned to variants from these databases with scores given to all nonsynonymous variants in PGP genomes (Fig. 4). On average, 44.0% of variants from these disease databases had prioritization scores or 4 or more, and 90.2% had scores of 3 or more. In contrast, only 0.22% of nonsynonymous variants in the PGP-10 have scores of 4 or more, and 1.1% have scores of 3 or more.

We applied our prioritization score system to prioritize genetic variants within the PGP-10 genomes for review. Our analysis focused on the discovery of unexpected variants predicted to have clinically significant consequences with moderate or high penetrance, because these potentially actionable variants were seen as the most important to return. Using the prioritization scores and presence in databases to guide our review of rare variants, we found 10 variants predicted to cause notable traits or pathogenic effects with moderate or high penetrance (*SI Appendix*, Table S4) and 21 variants predicted to cause moderate or severe disease in a recessive manner (*SI Appendix*, Table S5).

Follow-Up of Findings in the PGP-10. In the course of our review of the PGP-10 variants we observed multiple instances in which literature reports suggested that highly penetrant pathogenic phenotypes were caused by, or associated with, variants in the PGP-10 genomes. We found that such reports must be carefully appraised. Although some of these can be discarded because of clear phenotype discordance or unusual allele frequencies, some variants are rare and predict severe late-onset disease: participants could have undetected early stages of possibly clinically serious conditions. Because the PGP-10 genome analyses were not driven by medical or family history, follow-up evaluation of such findings entails issues very similar to follow-up of "incidental" findings; this potentially leads participants to incur unnecessary medical procedures, risks, and costs (39). However, after considerable discussion within the PGP team, we pursued additional communications and noninvasive clinical testing, with the thought that the public nature of our data and interpretations would inform researchers and clinicians who have similar findings in the future.

Focused follow-up was performed for one of the first variants found, MYL2-A13T in PGP6, which has been reported to cause familial hypertrophic cardiomyopathy in a dominant manner (40–44). Because this disease is potentially lethal and because there

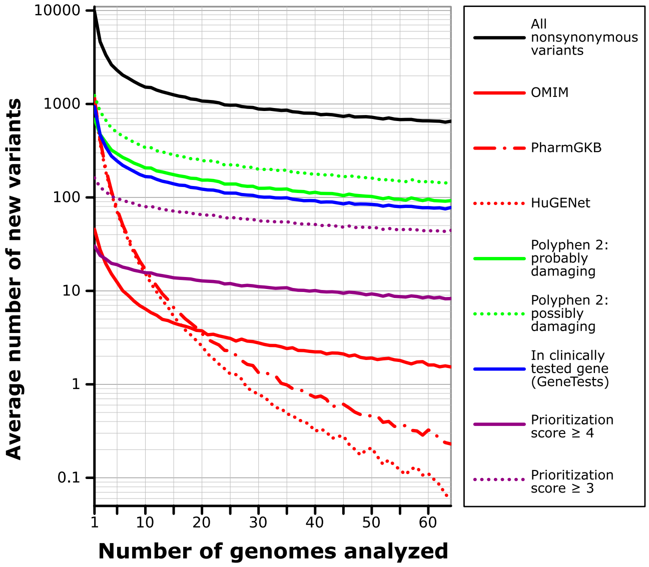


Fig. 3. Drop in number of new variants in each additional genome. For each new genome that is analyzed, the number of new variants not already seen in a previous genome falls dramatically. If editors record variant evaluations, the process of genome evaluation becomes easier as the number of new variants that are prioritized within each new genome is reduced. Data represent the average of 1,000 simulations using random orderings of a combined set of 64 genomes (the PGP-10 and 54 unrelated public genomes released by CGI).

were several publications supporting a pathogenic effect for the variant (*SI Appendix*, Fig. S5*A*), we confirmed the presence of this variant in a Clinical Laboratory Improvement Amendments-approved laboratory and consulted with researchers at the Laboratory for Molecular Medicine (LMM). LMM's internal data contained an additional pedigree of hypertrophic cardiomyopathy involving this variant (*SI Appendix*, Fig. S5*B*). Combined with published pedigrees the familial evidence is weak: both this pedigree and one of the published pedigrees each had one affected individual who was not a carrier of the MYL2-A13T variant, demonstrating that segregation of the variant was inconsistent with disease and significantly weakening the pathogenic hypothesis.

We informed PGP6 of our findings, reviewed the literature with him, and recommended cardiac follow-up for a noninvasive, nonurgent, baseline echocardiogram—this echocardiogram proved to be normal. Because he seems to be unaffected and his parents had no medical history of cardiac disease, this rare variant could be interpreted as a false-positive finding. However, familial hypertrophic cardiomyopathy is known to have incomplete penetrance, and the participant reports maternal and paternal uncles with early cardiac disease—uncertainty remains regarding the effect of this variant. It remains possible that this participant will develop symptoms at some later date; because the PGP maintains ongoing relationships with participants, such health updates can be added to participant records.

Less intensive follow-up, in the form of self-reported personal and family medical history, was performed for other variants that had reported or predicted strong phenotype effects. In the case of SERPINA1 variants found in PGP1 (who is compound heterozygous for variants predicted to result in E366K and E288V substitutions), the participant would be predicted to have increased susceptibility to developing chronic obstructive pulmonary disease (COPD) in response to smoking—the participant has no history of smoking and no diagnosis of COPD. To minimize the influence of confirmation bias, the remaining findings were combined into a single questionnaire given to all participants, without any specific efforts to alert them to which variants came from which individual (*SI Appendix*, Table S6). As with other participant trait surveys, the results of this targeted questionnaire are now publicly associated with the participant profiles. None of the participants reported traits or family histories consistent with the potential findings.

The SCN5A-G615E variant predicted in PGP9 was of particular concern: although we assessed the published findings as lacking statistical significance, it is included in a commercial genetic test for Long-QT syndrome (which can cause sudden death) (45). Subsequent to the survey, we contacted the participant (a 50-y-old woman) to notify her of our findings. In addition to no personal diagnosis of Long-QT syndrome and no family history of sudden death or Long-QT syndrome, she has had electrocardiogram tests performed in 2010 and 2012, with normal results.

On the whole, despite the presumed-healthy status of our 10 pilot participants, we found many apparently erroneous hypotheses in the literature whereby rare variants were predicted to have an inconsistent (and sometimes severe) phenotype. We also found that the process of genome interpretation involved a high amount of labor, much of which could potentially be reused in later genomes. These issues led us to extend our genome interpretation system to facilitate and record standardized variant evaluations with stringent evidence requirements.

Open Software for Variant Detection, Genome Reports, and Assisted Evaluation. Our Genome-Environment-Trait Evidence (GET-Evidence) system records variant evaluations using a peer production system and is integrated with our automatic genome processing and variant prioritization. Variant interpretations can be recorded by editors, categorized, and scored according to strength of evidence and clinical effect, and relevant papers can be added using PubMed identifiers. GET-Evidence variant pages contain links to external data sources where available, including

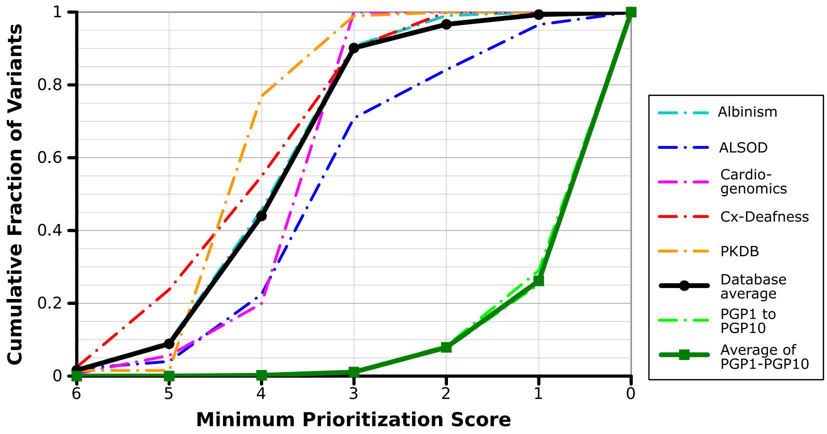


Fig. 4. Assessment of prioritization scores using disease-specific mutation databases. To demonstrate successful prioritization of variants with our prioritization score, we calculated the prioritization scores assigned to variant lists from a variety of disease-specific mutation databases: the Albinism Database (Albinism), the ALS Online Genetics Database (ALSOD), the Cardiogenomics Sarcomere Protein Gene Mutation Database (Cardiogen), the Connexins and Deafness Homepage (Cx-Deafness), and the Autosomal Dominant Polycystic Kidney Disease Mutation Database (PKDB). A variety of factors contribute to variation in performance for these lists: some diseases, for example, are more likely to be caused by severe frameshift or nonsense mutations (which we score highly), and some lists may include genes that are not yet used in clinical testing.

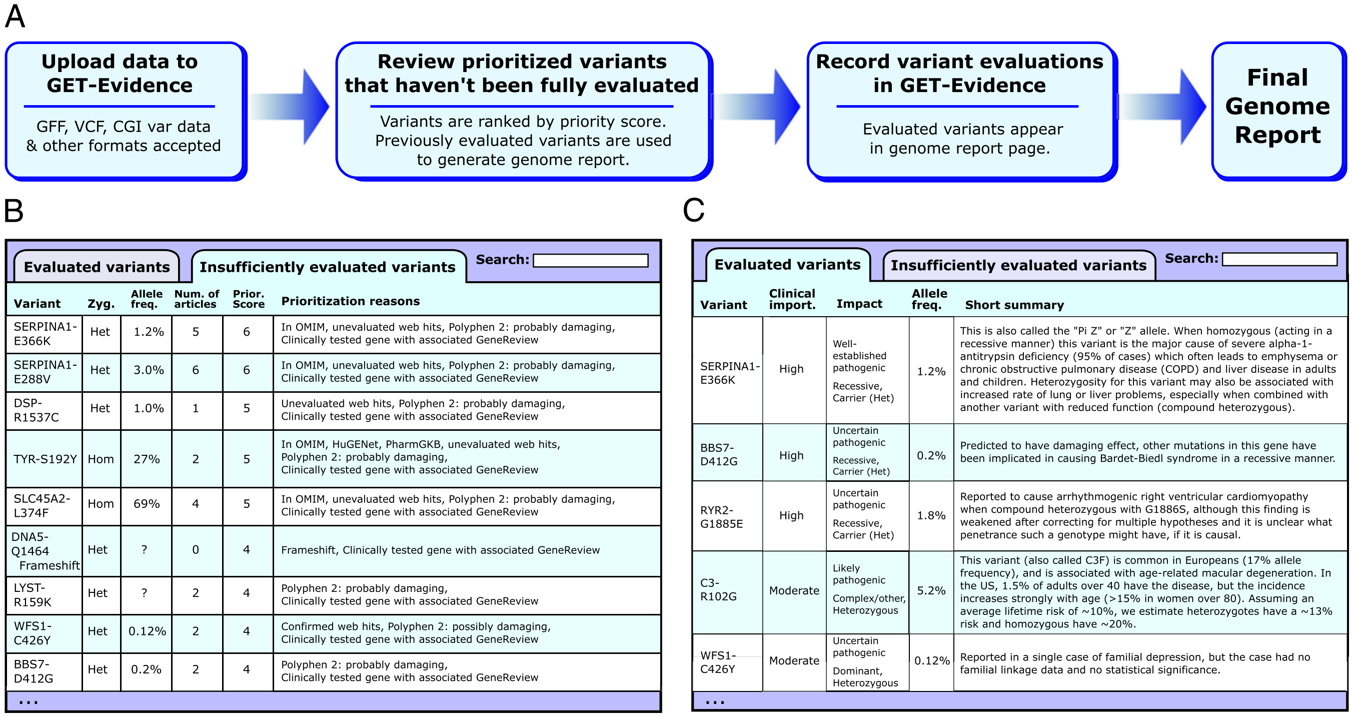
OMIM (33), GeneTests (32), dbSNP (23), PharmGKB (31), HuGENet (30), and PubMed (46).

GET-Evidence facilitates whole-genome interpretation by creating an interpretation pipeline that combines genome data processing, prioritization of variants for review, and recording of variant evaluations (Fig. 5*A*). When a genome data file is uploaded, the genome analysis system calculates the prioritization scores for all variants in an uploaded genome and matches these variants against the existing database. Two major reports are provided: an "insufficiently evaluated variants" report and a "genome report" (Fig. 5 *B* and *C*, respectively). The "genome report" lists all variants within the genome that have been sufficiently evaluated within GET-Evidence—variants initially seen here have likely been seen and evaluated in a genome previously analyzed through GET-Evidence. The "insufficiently evaluated variants" report contains all novel and unevaluated variants, sorted by prioritization score and accompanied by information that may guide evaluation (e.g., allele frequency, presence in databases, Polyphen 2 results, and number of article links added). Editors may then record or update evaluations of variants; once a variant is sufficiently evaluated, it is displayed within the genome report.

Variant evaluations record diverse information about variants that contribute to genome interpretation (Fig. 6). Editors can classify variants according to phenotypic effect (pathogenic, protective, pharmacogenetic, or benign) and inheritance pattern (dominant, recessive, or other). Papers may be added by using PubMed identifiers, creating new fields for entering case/control data and a field for adding notes regarding what evidence the paper has regarding the variant. To highlight important findings from a publication and to gather standardized information for later development of automatic interpretation, the abstracts of linked publications can be annotated through highlighting evidence features using the BioNotate platform (47). Finally, to record the overall interpretation of the variant and any additional relevant information, short summary and longer summary sections provide regions for free text summary of the variant's effect and evidence.

In addition to these classifications and text summaries, GET-Evidence uses a series of scored categories to facilitate automatic filtering and scoring of variants (*SI Appendix*, Table S7). These categories are divided into two major sections: (*i*): variant evidence scores, which assess how strongly various lines of evidence support the variant having a hypothesized effect, and (*ii*) clinical importance scores, which assess clinical aspects of the variant's hypothesized effect (Fig. 6). Variant evidence scores and clinical importance scores are used to generate an overall assessment of evidence (uncertain, likely, or well-established) and clinical importance (low, moderate, or high) (*SI Appendix*, Tables S8 and S9). Notably, variants are only considered "likely" or "well-established" if they meet minimum statistical significance requirements in either case/control or familial categories (described in *SI Appendix*, Table S9). By segregating evidence from severity we are able to distinguish between a well-established variant with a weak pathogenic effect ("well-established pathogenic, low clinical importance") from a poorly understood but potentially severe variant ("uncertain pathogenic, high clinical importance").

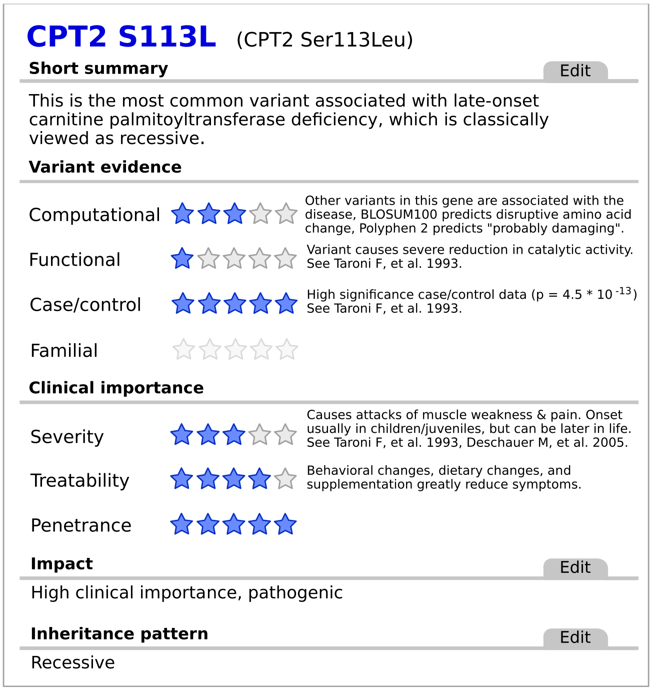
After evaluating all variants in GET-Evidence, almost all variants we found with potentially strong phenotypic consequences were evaluated as "uncertain" (Table 2 and *SI Appendix*, Table S10). Although it is always possible that one or more of these variants does cause disease with incomplete penetrance or late onset, there are clearly some erroneous associations listed in Table 2 and *SI Appendix*, Table S4. Introducing stringent evidence requirements for interpreting published data successfully addresses this issue with incidental findings. In addition, GET-Evidence's peer production model for variant evaluation assists genome interpretation by allowing the reuse of variant evaluations by later genome evaluations, thereby minimizing duplication of effort. By creating such a shared central resource for recording interpretations,


A
Upload data to GET-Evidence
GFF, VCF, CGI var data & other formats accepted
Review prioritized variants that haven't been fully evaluated
Variants are ranked by priority score. Previously evaluated variants are used to generate genome report.
Record variant evaluations in GET-Evidence
Evaluated variants appear in genome report page.
Final Genome Report
B
Evaluated variants
Insufficiently evaluated variants
Search:
...
C
Evaluated variants
Insufficiently evaluated variants
Search:
...

Variant	Zyg.	Allele freq.	Num. of articles	Prior. Score	Prioritization reasons
SERPINA1-E366K	Het	1.2%	5	6	In OMIM, unevaluated web hits, Polyphen 2: probably damaging, Clinically tested gene with associated GeneReview
SERPINA1-E288V	Het	3.0%	6	6	In OMIM, unevaluated web hits, Polyphen 2: probably damaging, Clinically tested gene with associated GeneReview
DSP-R1537C	Het	1.0%	1	5	Unevaluated web hits, Polyphen 2: probably damaging, Clinically tested gene with associated GeneReview
TYR-S192Y	Hom	27%	2	5	In OMIM, HuGENet, PharmGKB, unevaluated web hits, Polyphen 2: probably damaging, Clinically tested gene with associated GeneReview
SLC45A2-L374F	Hom	69%	4	5	In OMIM, unevaluated web hits, Polyphen 2: probably damaging, Clinically tested gene with associated GeneReview
DNA5-Q1464 Frameshift	Het	?	0	4	Frameshift, Clinically tested gene with associated GeneReview
LYST-R159K	Het	?	2	4	Polyphen 2: probably damaging, Clinically tested gene with associated GeneReview
WFS1-C426Y	Het	0.12%	2	4	Confirmed web hits, Polyphen 2: possibly damaging, Clinically tested gene with associated GeneReview
BBS7-D412G	Het	0.2%	2	4	Polyphen 2: probably damaging, Clinically tested gene with associated GeneReview

Variant	Clinical import.	Impact	Allele freq.	Short summary
SERPINA1-E366K	High	Well-established pathogenic Recessive, Carrier (Het)	1.2%	This is also called the "Pi Z" or "Z" allele. When homozygous (acting in a recessive manner) this variant is the major cause of severe alpha-1-antitrypsin deficiency (95% of cases) which often leads to emphysema or chronic obstructive pulmonary disease (COPD) and liver disease in adults and children. Heterozygosity for this variant may also be associated with increased rate of lung or liver problems, especially when combined with another variant with reduced function (compound heterozygous).
BBS7-D412G	High	Uncertain pathogenic Recessive, Carrier (Het)	0.2%	Predicted to have damaging effect, other mutations in this gene have been implicated in causing Bardet-Biedl syndrome in a recessive manner.
RYR2-G1885E	High	Uncertain pathogenic Recessive, Carrier (Het)	1.8%	Reported to cause arrhythmogenic right ventricular cardiomyopathy when compound heterozygous with G1886S, although this finding is weakened after correcting for multiple hypotheses and it is unclear what penetrance such a genotype might have, if it is causal.
C3-R102G	Moderate	Likely pathogenic Complex/other, Heterozygous	5.2%	This variant (also called C3F) is common in Europeans (17% allele frequency), and is associated with age-related macular degeneration. In the US, 1.5% of adults over 40 have the disease, but the incidence increases strongly with age (>15% in women over 80). Assuming an average lifetime risk of ~10%, we estimate heterozygotes have a ~13% risk and homozygous have ~20%.
WFS1-C426Y	Moderate	Uncertain pathogenic Dominant, Heterozygous	0.12%	Reported in a single case of familial depression, but the case had no familial linkage data and no statistical significance.

Fig. 5. GET-Evidence and genome reports. (*A*) Using GET-Evidence involves genome upload followed by review of prioritized insufficiently evaluated variants. Combining these reviews with previously reviewed variants produces the final genome report. (*B*) Insufficiently evaluated variants are ranked according to prioritization score and are listed with additional information of interest (allele frequency, number of associated articles, presence in databases, and computational predictions). (*C*) Sufficiently evaluated variants are presented in the genome report with summary information regarding variant effect, severity, and evidence.



CPT2 S113L (CPT2 Ser113Leu)

Short summary

This is the most common variant associated with late-onset carnitine palmitoyltransferase deficiency, which is classically viewed as recessive.

Variant evidence

Computational	★★★☆☆	Other variants in this gene are associated with the disease, BLOSUM100 predicts disruptive amino acid change, Polyphen 2 predicts "probably damaging".
Functional	★☆☆☆☆	Variant causes severe reduction in catalytic activity. See Taroni F, et al. 1993.
Case/control	★★★★★	High significance case/control data ($p = 4.5 * 10^{-13}$) See Taroni F, et al. 1993.
Familial	☆☆☆☆☆	

Clinical importance

Severity	★★★☆☆	Causes attacks of muscle weakness & pain. Onset usually in children/juveniles, but can be later in life. See Taroni F, et al. 1993, Deschauer M, et al. 2005.
Treatability	★★★★☆	Behavioral changes, dietary changes, and supplementation greatly reduce symptoms.
Penetrance	★★★★★	

Impact

High clinical importance, pathogenic

Inheritance pattern

Recessive

Fig. 6. Sample GET-Evidence variant report. Variant report pages on GET-Evidence allow editors to record and organize information relevant to variant interpretation. A scoring system is used for variant evidence and clinical importance categories to allow automatic sorting of interpreted variants. On the basis of the strong case/control evidence and high treatability and penetrance, this recessive pathogenic variant carried by PGP4 (listed in Table 2) was evaluated as well-established and high clinical importance.

GET-Evidence can act as a forum for building consensus on interpretation. The analysis system and variant interpretations, along with our public genome interpretations, are available at http://evidence.personalgenomes.org.

Discussion

With the advent of low-cost whole-genome sequencing and growing interest in personalized medicine, the research community is faced with the challenge of developing tools for interpreting genome data and using these data to inform lifestyle choices and clinical care in an effective manner. Doing so will require large, highly personal datasets: whole-genome data combined with health records, traits, and personal medical histories. Because such data are highly reidentifiable, building these datasets results in a tension between privacy protection and the desire to share and reuse data.

The approach the PGP takes is a highly public option: enrolling participants who agree to the hypothetical and unknown risks associated with making personal biological data public through an open consent format. Our public resource enables the process of scientific discovery and clinical use of genomes. In addition, we share our open consent documents and methods to enable other researchers who wish to produce public data in their own research studies.

As part of these integrated public datasets, the PGP has also created a public software tool for genome interpretation and a public database of variant interpretations. Because these records are freely editable by any registered user, the database provides a forum for achieving a public consensus interpretation of genetic variants. Other groups may freely use the GET-Evidence system, and we encourage others to contribute their interpretations of genetic variants in the public database. These edits and other data within GET-Evidence are shared, in turn, as public domain under a CC0 waiver and may be used by academic and commercial genome interpretation efforts. Future development of the GET-Evidence system should move closer toward our goal of a richly interconnected dataset of genomes, environments, and traits. Planned improvements include coded phenotypes for genetic variants as well as participant health records, genome analysis for compound heterozygosity, splicing mutations, copy number variants, and tracking the biological and computational provenance of public data.

Our genome interpretation findings highlight one of the ethical issues raised when working toward clinical utilization of whole genomes: what should be done if potentially severe pathogenic mutations are found within whole-genome sequence data? Although stringent evidence guidelines help by classifying

Table 2. Evaluation of variants reported or predicted to have strong phenotype effects

Variant (heterozygous unless otherwise noted)	Predicted phenotype	Allele frequency (%)	Prioritization score	Evidence assessment in GET-Evidence	Clinical importance assessment in GET-Evidence
SERPINA1-E366K/ SERPINA1-E288V (compound het)	Moderate α-1 antitrypsin deficiency	1.2 and 3.0	5	Well-established/ well-established	High/low
WFS1-C426Y	Familial depression	0.1	5	Uncertain	Moderate
FLG-S761fs	Palmar hyperlinearity and keratosis pilaris (ichthyosis vulgaris in recessive manner)	Unknown	4	Uncertain	Moderate (for ichthyosis vulgaris)
PKD1-R4276W	Autosomal dominant polycystic kidney disease	0.2	4	Uncertain	High
MYL2-A13T	Hypertrophic cardiomyopathy	0.02	5	Uncertain	High
SCN5A-G615E	Long-QT Syndrome	0.03	4	Uncertain	High
PKD2-S804N	Autosomal dominant polycystic kidney disease	0.3	5	Uncertain	High
SLC9A3R1-R153Q	Kidney stones	0.3	4	Uncertain	Moderate
RHO-G51A	Autosomal dominant retinitis pigmentosa	0.2	4	Uncertain	Moderate
EVC-R443Q	Ellis-van Creveld syndrome	7.9	3	Reevaluated as benign	Reevaluated as benign

Additional data regarding these variants, including PGP participant identifiers and Pubmed identifiers for related literature, are available in *SI Appendix*, Table S4.

many findings as uncertain, effects could manifest later in life. Withholding information from patients is becoming less acceptable in clinical practice and may become less acceptable for research data as well. Continuing work with PGP participants will provide insights into how genome data may be integrated more generally into both research and clinical settings.

We maintain an ongoing relationship with participants to monitor the outcomes of publicly sharing personal data. Many participants are interested in making an ongoing contribution to science—as part of our study, we can invite participants to take part in additional research. Thus, subsets of participants may choose to contribute to disease-specific research and novel profiling methods (e.g., allele-specific expression, epigenetic, metabolomic, proteomic, or microbiome profiling). In addition, biobanked tissues and cell lines may be used by researchers for additional characterization, follow-up functional studies, and genome engineering. Each additional study benefits from all previous data for the same participant, building a further-enriched dataset and contributing to the development of new personalized medical diagnostics and therapies. Currently approved for studying up to 100,000 participants, the PGP has the potential to be a widely used ongoing resource—a large, rich, public set of well-characterized individuals with extensive biological data and an ongoing interest in contributing to research.

Materials and Methods

SI Appendix, SI Materials and Methods provides full details of our enrollment process and open consent protocols. Additional details of Continuity of Care Record format health record data, cell lines, samples, genome sequencing, and quality assessment, as well as prioritization score assessment using disease-specific mutation databases, are also presented. Finally, we elaborate on the GET-Evidence data processing and editing platform; its development is facilitated through use of a shared computational and storage infrastructure (48).

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Supporting Appendix for
"A Public Resource Facilitating Clinical Use of Genomes"

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Supporting Materials and Methods

Enrollment process and open consent

Pre-enrollment screening requires that volunteers (1) be at least 21 years of age, (2) be a citizen or permanent resident of the United States, and (3) not be subject to undue influence or coercion by the Principle Investigator of this study. Volunteers with a monozygotic twin must also have their twin complete enrollment to be eligible. Individuals are asked to name two designated proxies who may be contacted in the event of death or incapacitation.

An ongoing relationship with participants exists, allowing recontact and continuing follow-up. To monitor the project for potential negative outcomes, participants are required to respond to private quarterly questionnaires where they are asked to report changes in safety, well-being, or interactions with others due to their participation in the project. If a participant has not updated their safety questionnaire at least three times in the past twelve months their account is considered lapsed, and they must update the safety questionnaire before adding data or modifying their account. Participants are invited at any point to withdraw from the project (although we do not guarantee that data, which has been public, is removable from all sources). If this recontact process fails, designated proxies are contacted, where available, to determine whether the participant is deceased or incapacitated -- depending on the decision made by the proxies, the account may be closed and removed, or updated and remain public.

Genetic data, when produced by the PGP, is initially provided privately to participants along with our preliminary interpretation and becomes public after 30 days. All genome and other public participant data are linked to the participant ID and published in the public domain under the Creative Commons CC0 waiver (1). Current and historical copies of our consent forms are provided publicly at http://www.personalgenomes.org/consent/. This enables reuse or customization, e.g. for international use. The enrollment exam is focused to assess understanding of PGP protocols, risks and benefits as well as a basic understanding of genetics as it pertains to informing family members.

CCR-formatted health record data

Health record data to date has been collected using interfaces with Google Health and Microsoft HealthVault to import Continuity of Care Record (CCR) format health record data. Specific data regarding conditions, medications, and procedures are pulled from these data and are published publicly on participant profiles. SI Dataset S1 was constructed using data from 1,021 recent Google Health records for participants, downloaded on October 27, 2011. Health condition descriptions and codes were parsed from the records and matched to PGP participant ID. Entries were pooled based on their ICD9 code or, if not present, by Google code.

Cell lines, samples, genome sequencing and quality assessment

EBV-transformed lymphocyte cell lines were derived from whole blood and fibroblast cell lines were derived from 3mm skin punch biopsies by the Harvard Medical School Cytogenetic Core Facility. Induced pluripotent cell lines were derived from these fibroblast cell lines according to the method described in Lee et al. (2). DNA was extracted from these cell lines and sent to Complete Genomics for whole genome sequencing.

Prioritization score assessment using disease-specific mutation databases

Genetic variant lists were downloaded from five publicly available disease-specific databases (all September 2011): the Albinism Database (3), the ALS Online Genetics Database (4), the Cardiogenomics Sarcomere Protein Gene Mutation Database (5), the Connexins and deafness Homepage (6), and the Autosomal Dominant Polycystic Kidney Disease Mutation Database (7). Where appropriate, amino acid numbering in databases was adjusted to match positions predicted by Polyphen 2 and GET-Evidence’s genome analysis, which both use the canonical transcripts in the UCSC Known Genes annotation.

GET-Evidence data processing and editing platform

Imported databases were downloaded, parsed, and entered into our MySQL database and are used for variant prioritization. Databases used in our analyses include GeneTests (4,253 genes, January 2010), Online Mendelian Inheritance in Man (OMIM) (9,142 nonsynonymous substitutions extracted through custom scripts from June 2009), HuGENavigator (2,298 dbSNP IDs, January 2010), and PharmGKB (2,488 dbSNP IDs, April 2010). In addition, all nonsynonymous predictions found in the PGP genomes are entered into our database, which is then regularly updated with Polyphen 2 predictions based on the whole human proteome sequence space (downloaded July 2011). We also used web-search results, generated by the Yahoo API, to help find additional literature. Web hits are first generated when a variant is added to the database and are periodically updated. These data sources provide us with a rich set of disease, pharmacogenetic and literature predictions, and are available in the public domain or under non-commercial terms.

Genome data processing was written in Python, performed with a series of modules and originally developed as the Trait-o-matic software used in previous genome publications (8–11) and tested on other public genomes (12–21).

Processing steps involve:

1. Genome data file format is automatically detected and, if it is in CGI var file format, VCF, or 23andme genotyping data format, it is automatically translated to the GFF file format used internally by the system. Regions which are only partially called (hemizygously no-call) in the CGI var file are treated as a no-call region. Interpretation of both build 36 and build 37 genome data is supported.
2. IDs for dbSNP locations are added to variants, if not already present, based on matching positions in the latest dbSNP data.
3. Nonsynonymous amino acid change predictions are made using the knownCanonical transcripts listed in the UCSC Known Genes transcript annotations (22). This is done by predicting the variant and reference transcript nucleotide sequences, predicting amino acid sequences from these, then detecting if differences occur between variant and reference versions. Nonsynonymous predictions include multiple base substitutions and frameshift and in-frame length changing variants, in addition to single amino acid substitutions and nonsense mutations.
4. Nonsynonymous changes and dbSNP IDs are searched against a pre-loaded list of existing GET-Evidence entries (which is populated by the databases mentioned previously, previously seen public variants, and any manually added entries). If no GET-Evidence match is found, prioritization score is predicted based on existing computational and gene-specific data.
5. All nonsynonymous variants, or other variants matching existing GET-Evidence entries, are reported in GET-Evidence reports.

High variant frequency in the general population is useful for interpretation as it generally indicates that a variant is unlikely to have severe clinical consequences. To acquire this data, we downloaded and extracted allele frequency information from Exome Variant Server and 1000 Genomes data. In addition, we calculated frequencies for all variants in our combined set of 64 genomes (data for the PGP-10 and the 54 unrelated public genomes released by Complete Genomics). Exome Variant Server data was downloaded May 2012, using the ESP5400 data released in December 2011 (23). 1000 Genomes data was downloaded May 2012, using the phase 1 integrated release version 3 data released in April 2012 (24).

Users are identified in GET-Evidence using OpenID (25). All user edits are stored in the GET-Evidence MySQL database in a manner that retains records of all previous edits made to the page. These data are released under a CC0 license at http://evidence.personalgenomes.org/download (26) and the GET-Evidence software is released under the GNU AGPL version 3.0 or any later version (27).

Annotation of article abstracts was implemented using a standalone installation of BioNotate (28). When a user clicks the "annotate" button, GET-Evidence passes the variant name and the relevant article's PubMed identifier to the BioNotate instance, along with a user-identifying token. BioNotate retrieves the article abstract using NCBI's

API and allows the user to indicate the significance of words and phrases within the abstract. Upon clicking "submit", the user returns to the GET-Evidence site. GET-Evidence retrieves the newly annotated abstract from BioNotate in XML format and saves it in the edit history for the relevant variant page.

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Dataset S1: PGP participants and associated health conditions

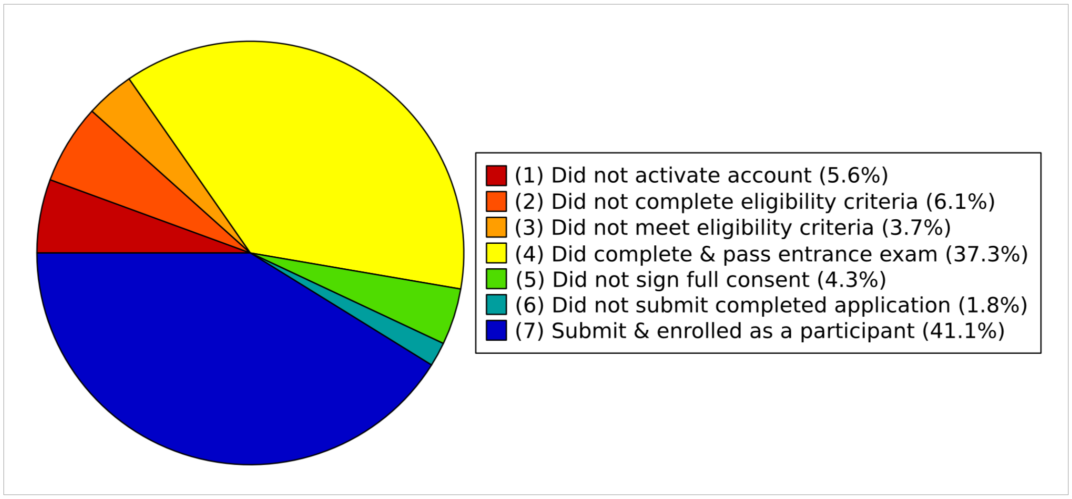
Description

Column 1: Total number of participant health records reporting this condition
Column 2: Description of condition
Column 3: ICD9 code, if available
Column 4: Google code(s) matching ICD9 code
Column 5: Participant IDs

Data from 1,021 recent Google Health records for participants was downloaded on October 27 2011. Health condition descriptions and codes were parsed from the records and matched to PGP participant ID. Entries were pooled based on their ICD9 code or, if not present, by Google code.

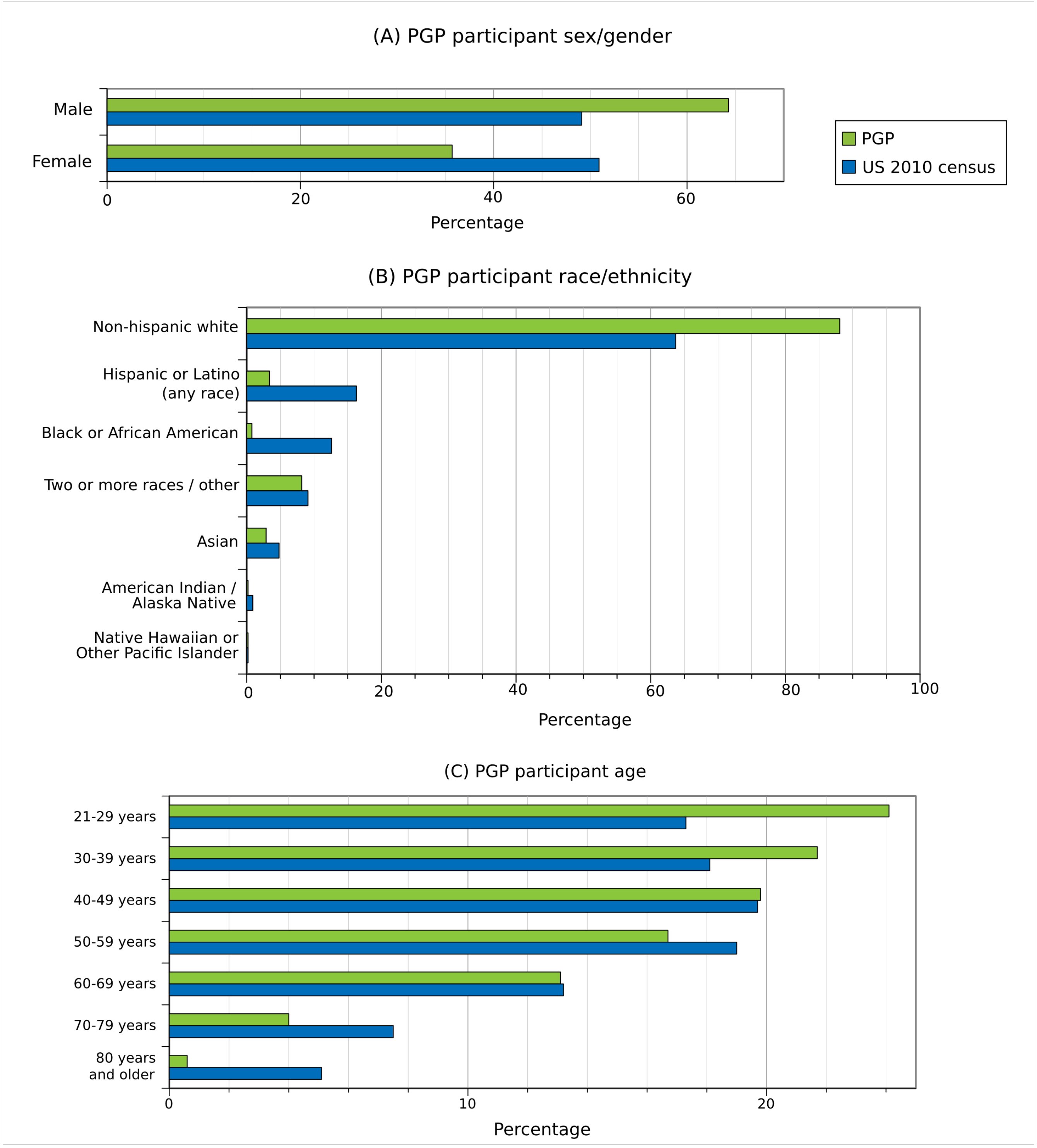
Please see supporting files for this table.

Figure S1: PGP account status



In an analysis of accounts created through the Personal Genome Project website, 41% of users complete all enrollment steps to become fully enrolled participants. Of those that do not complete all steps, the largest fraction (37%) fail to complete and pass the entrance exam. The entrance exam asks participants to demonstrate an understanding of basic genetics concepts and of the risks and potential outcomes that may result from publicly donating genome data and tissue samples. These statistics were calculated from 1,138 user log records spanning December 2010 to December 2011.

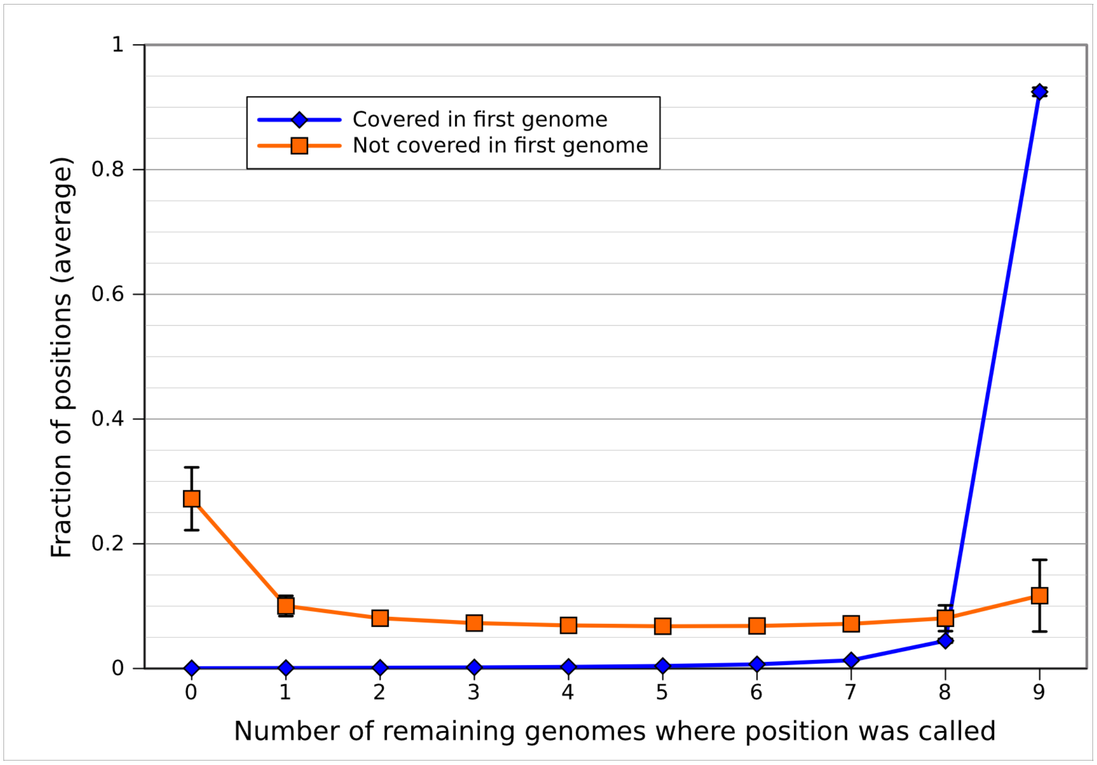
Figure S2: Demographics of PGP participants



Because participation is self-selecting and occurs through an online enrollment mechanism, the demographics of PGP participants is expected to differ from the United States population (2010 census data). Based on self-reported survey data from over 1000 participants, **(A)** Males are overrepresented and females are underrepresented, **(B)** Non-Hispanic whites are over-represented and minority groups are underrepresented, **(C)** Younger ages are overrepresented and older ages are underrepresented.

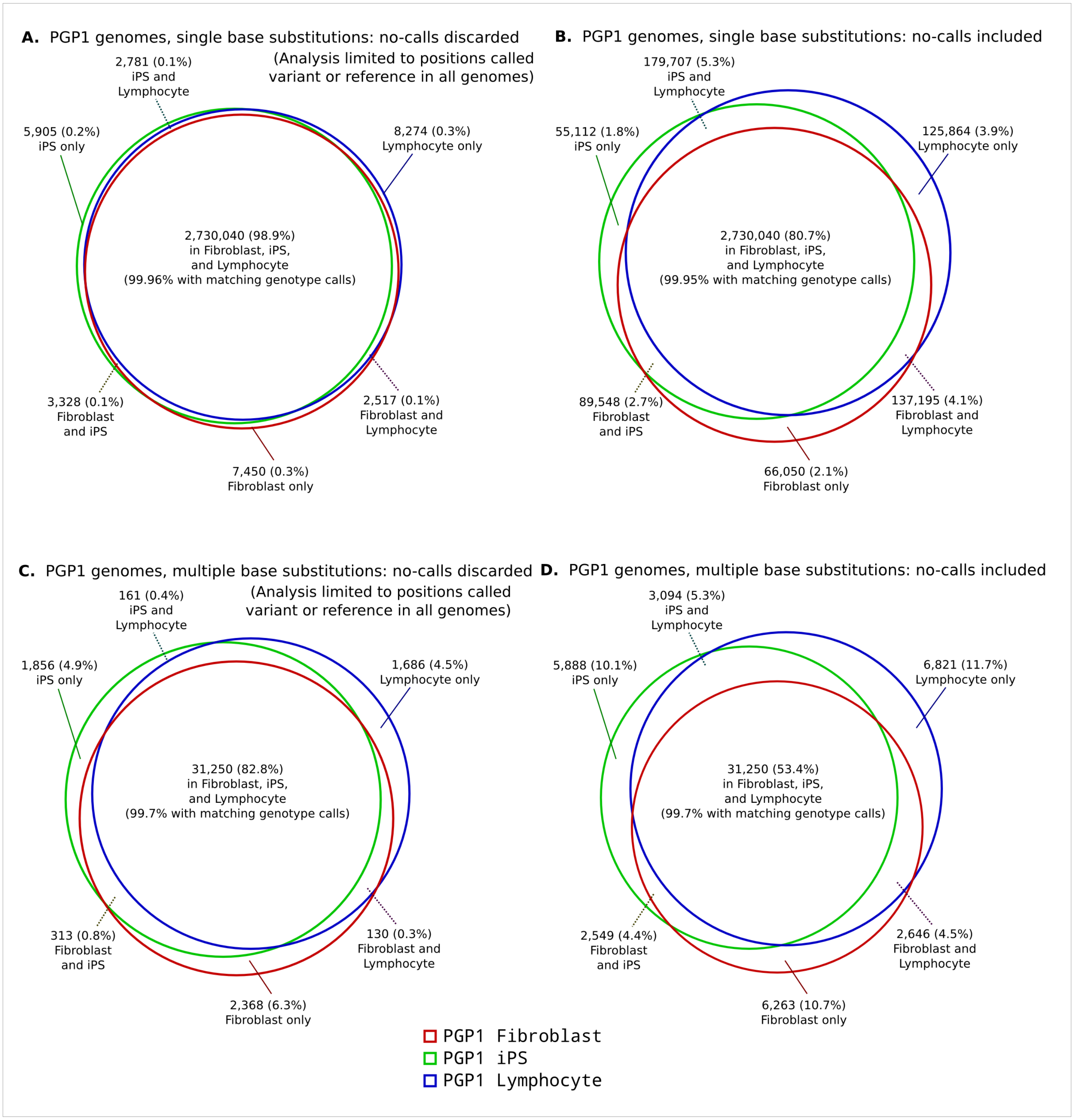
Note: For US census data, Figure 2C reports the percentages of the population within the 21 and older age range, which are the ages eligible for PGP enrollment.

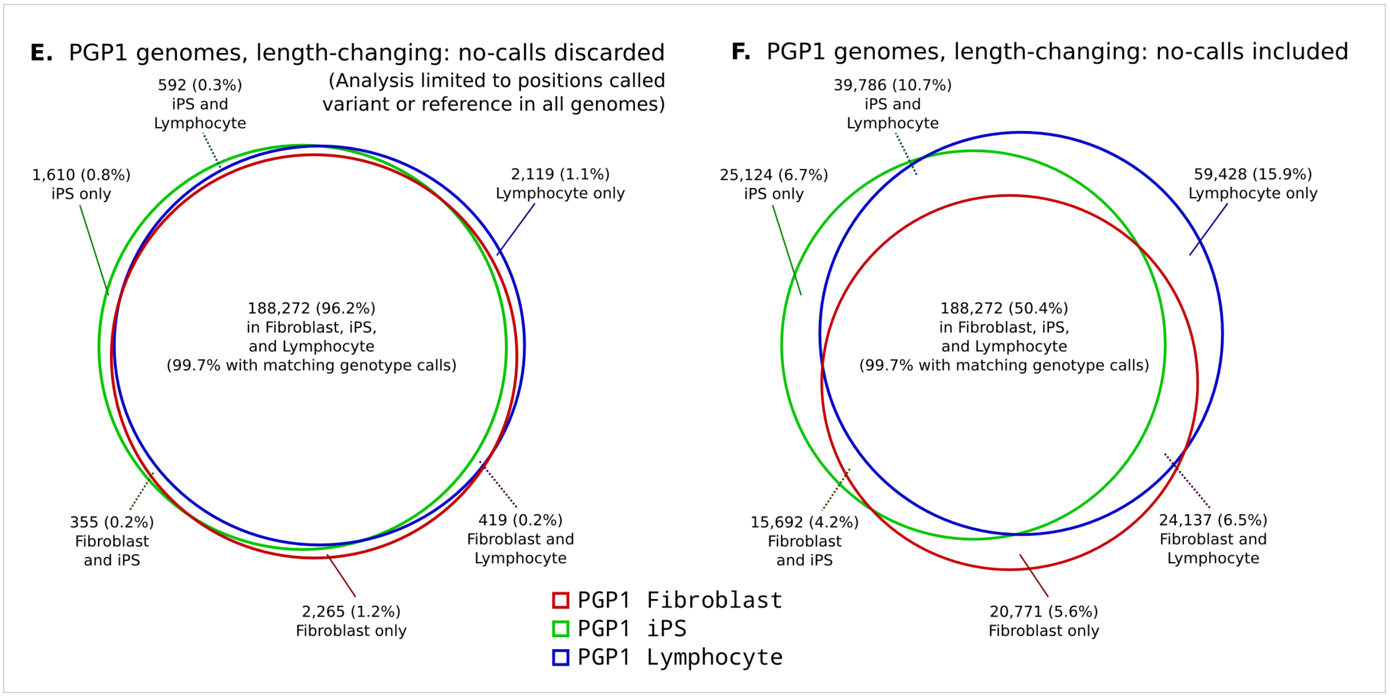
Figure S3: Histogram of call rates, split by call status in first genome



A histogram of how many of the remaining nine genomes a position was called in, split by its call status in the first genome examined. Positions which have their genotype called are highly correlated between genome data. Sites which were called in a given genome were much more likely to be called in all other genomes (blue line) – on average 92% of positions that were called in one genome were also called in the remaining nine genomes. Similarly, sites not called in a given genome were more likely to be not called in other genomes (orange line) – on average 27% of positions not called in one genome were also not called in the remaining nine genomes. Data represent the average when each of the ten genomes is used as the "first"; error bars are the standard deviation of this data.

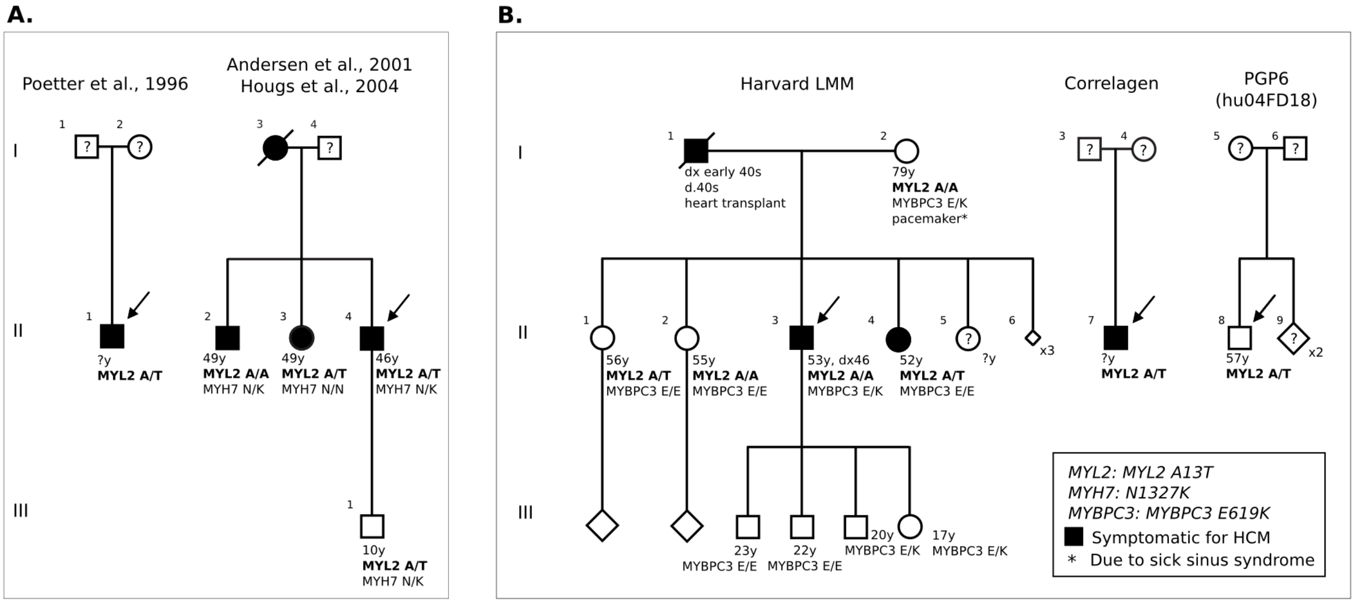
Figure S4: Venn diagrams of shared calls, split by variant type





Analysis of PGP1 genome variant calls from three different tissues (as in Figure 2), split by variant type. **(A)** Overlap of all single-base substitution variant calls, limited to positions explicitly called as variant or reference in all three genomes. On average, 99.7% called variant in one genome are called variant in at least two out of three. **(B)** Overlap of all single-base substitution variant calls, including uncalled positions lacking explicit reference or variant calls. **(C)** Overlap of all multi-base substitution variant calls, limited to positions explicitly called as variant or reference in all three genomes. On average, 94.1% called variant in one genome are called variant in at least two out of three. **(D)** Overlap of all multi-base substitution variant calls, including uncalled positions lacking explicit reference or variant calls. **(E)** Overlap of all length-changing variant calls, limited to positions explicitly called as variant or reference in all three genomes. On average, 99.0% called variant in one genome are called variant in at least two out of three. **(F)** Overlap of all length-changing variant calls, including uncalled positions lacking explicit reference or variant calls.

Figure S5: MYL2-A13T pedigrees



(A) MYL2-A13T has been implicated in causing hypertrophic cardiomyopathy (HCM) in a dominant fashion. This variant was initially reported in a study which implicated nonsynonymous variants in MYL2 and MYL3 (myosin essential and regulatory light chains) as causing HCM (1). The A13T variant was seen in one of four HCM cases with nonsynonymous variants in MYL2 in a screen of 399 unrelated cases. All four variants were reported to have strong evolutionary conservation. The association of this variant with HCM was later reported on in a case with two out of three affected siblings—the third sibling was initially thought to be a phenocopy due to concurrent obesity and hypertension (2) and then later suspected to have disease due to another variant identified in the MYH7 gene (3). Functional studies also reported that the product of MYL2-A13T bound calcium significantly differently to wild-type (4).

(B) To check for additional unpublished clinical data, we contacted all four laboratories in the United States (Harvard-Partners Laboratory for Molecular Medicine (LMM), Correlagen, GeneDx, PGxHealth) offering CLIA–approved diagnostic sequencing of MYL2 for cardiomyopathy. Only two had observed this variant. Correlagen reported finding the variant in one patient with HCM , though no further clinical or family history data was available. The LMM studied a family with two siblings and their father with HCM; the variant was found in only one of the two affected siblings (although the father was deceased in his early 40’s from cardiomyopathy treated with heart transplant, the absence of the variant in the mother indicates that the father was likely positive). Another variant, MYBPC3 Glu619Lys, was initially considered causal in the other sibling, but their 79-year-old mother (who also carries this variant) had a normal echocardiogram.

Additionally, we noted that PGP6 is Ashekenazi Jewish (AJ). The LMM family is also AJ and, when contacted, P. Andersen reported that the Andersen/Hougs pedigree was AJ. This raises the possibility that the variant is a polymorphism within the AJ population. To test this we screened an AJ DNA panel and did not detect the variant in any of the 116 controls individuals we examined.

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Table S1: PGP-10 participants and associated cell lines

Participant ID	PGP Nickname	Full name	Coriell repository ID	Cell type
hu43860C	PGP1	George M. Church	GM20431	EBV-transformed lymphocyte
			GM23248	Fibroblast
huC30901	PGP2	John D. Halamka	GM21070	EBV-transformed lymphocyte
huBEDA0B	PGP3	Esther Dyson	GM21660	EBV-transformed lymphocyte
huE80E3D	PGP4	Misha Angrist	GM21667	EBV-transfomed lymphocyte
			GM23249	Fibroblast
hu9385BA	PGP5	Kirk Michael Maxey	GM21687	EBV-transformed lymphocyte
			GM23250	Fibroblast
hu04FD18	PGP6	Steven Pinker	GM21730	EBV-transformed lymphocyte
hu0D879F	PGP7	Keith F. Batchelder	GM21731	EBV-transformed lymphocyte
huAE6220	PGP8	Stanley N. Lapidus	GM21781	EBV-transformed lymphocyte
hu034DB1	PGP9	Rosalynn D. Gill	GM21833	EBV-transformed lymphocyte
			GM23251	Fibroblast
hu604D39	PGP10	James Louis Sherley	GM21846	EBV-transformed lymphocyte

Table S2: PGP-10 genome data statistics

Individual (huID)	Ungapped build 37 locations called homozygously	# of substitution variants vs. build 37 reference	# of short insertions and deletions vs. build 37 reference
PGP1 (hu43860C)	96.7%	3,216,092	310,621
PGP2 (huC30901)	96.5%	3,212,647	315,289
PGP3 (huBEDA0B)	95.9%	3,082,457	272,251
PGP4 (huE80E3D)	96.0%	3,148,580	277,661
PGP5 (hu9385BA)	96.8%	3,259,173	321,731
PGP6 (hu04FD18)	96.1%	3,161,062	279,947
PGP7 (hu0D879F)	97.2%	3,318,280	352,538
PGP8 (huAE6220)	97.2%	3,328,192	347,134
PGP9 (hu034DB1)	95.5%	3,057,821	257,667
PGP10 (hu604D39)	95.2%	3,611,748	284,696
Average:	**96.5%**	**3,239,605**	**301,954**

Ungapped build lengths were taken from:
http://www.ncbi.nlm.nih.gov/projects/genome/assembly/grc/human/data/

Table S3: Prioritization score calculation

Each variant can score a maximum of two points in each category, for a total of up to six points.

Category	Points	Criteria
Computational	2 points	* Variant has an allele frequency of < 5% and Polyphen 2 predicts "probably damaging" (score > 0.85) * Variant has an allele frequency of < 5% and is predicted to have a severely disruptive effect on protein sequence (nonsense or frameshift mutation)
	1 point	* Variant is predicted to be "probably damaging" by Polyphen 2, but allele frequency is >= 5% * Variant is predicted to cause a frameshift or nonsense mutation, but allele frequency is >= 5% * Variant is nonsynonymous, has an allele frequency < 5%, and Polyphen 2 score is unknown or predicted to be "possibly damaging"
	0 points	* Variant is synonymous * Variant is nonsynonymous, but does not meet above criteria
Variant-specific databases	2 points	* Variant is seen in OMIM * Variant is seen in any two of the following lists: -- PharmGKB -- HuGENet -- confirmed or unevaluated online web page hits
	1 point	* Variant is seen in any one of the following lists: -- PharmGKB -- HuGENet -- confirmed or unevaluated online web page hits
	0 points	* Variant is not matched to any databases and has no confirmed or unevaluated online web page hits.
Gene-specific databases	2 points	* Variant is nonsynonymous and occurs in a gene with clinical testing available (as recorded by the GeneTests database) and an associated GeneReviews article.
	1 point	* Variant is nonsynonmyous and occurs in a gene with clinical testing available
	0 points	* Variant is synonymous or occurs within a gene which is not clinically tested.

Table S4:
Variants reported or predicted to have significant phenotypic consequences

Participant	**Variant** (heterozygous unless otherwise noted)	**Predicted phenotype**	**Supporting publications** (PMIDs)	**Confirmed by participant phenotype?**
PGP1 (hu43860C)	SERPINA1-E366K/ SERPINA1-E288V (compound heterozygous)	Moderate alpha-1 antitrypsin deficiency (increased susceptibility to liver and lung disease – the latter generally for emphysema rather than infections)	6976856, 8970361, 18565211	Participant reports frequent lung infections, but no diagnosis of COPD and no history of smoking.*
PGP1 (hu43860C)	WFS1-C426Y	Familial depression**	11244483	Participant reports mild sypmtoms.*
PGP2 (huC30901)	FLG-S761fs	Palmar hyperlinearity and keratosis pilaris	None variant specific. [11244483] predicts mild phenotype in carriers of a similar Ichthyosis Vulgaris mutation.	Participant reports not having this phenotype.
PGP5 (hu9385BA)	PKD1-R4276W	Autosomal dominant polycystic kidney disease	10200984	Participant reports no personal or family history of this disease or associated symptoms.
PGP6 (hu04FD18)	MYL2-A13T	Hypertrophic cardiomyopathy	8673105, 11102452, 11748309, 12668451, 14594949	Participant evaluated, echocardiogram was normal. Family history is ambiguous (parents healthy, but siblings of both parents had early mortality attributed to cardiac disease).
PGP9 (hu034DB1)	SCN5A-G615E	Long-QT Syndrome	11997281, 15840476, 18071069, 19716085	Participant reports no personal or family history of this disease. An unrelated EKG examination in 2010 produced normal results.
PGP10 (hu604D39)	PKD2-S804N	Autosomal dominant polycystic kidney disease	17582161	Participant reports no personal or family history of this disease or associated symptoms.
PGP10 (hu604D39)	SLC9A3R1-R153Q	Kidney stones	18784102	Participant reports no personal or family history of these symptoms.
PGP10 (hu604D39)	RHO-G51A	Autosomal dominant retinitis pigmentosa	8317502	Participant reports no personal or family history of this disease and associated symptoms.
PGP10 (hu604D39)	EVC-R443Q***	Ellis-van Creveld syndrome or related symptoms	10700184	Participant phenotype not consistent with this prediction.

* Participant reported traits after return of personal results reporting the genetic trait and putative associated phenotype.

** Although most reports for WFS1 involve it causing Wolfram syndrome in a recessive manner, this publication suggested that rare substitution variants in the gene carried heterozygously (including the one listed here) may be associated with increased risk for psychiatric disease.

*** Although Ellis-van Creveld syndrome is generally recessive, this publication reported a father–daughter pair with symptoms similar to Ellis-van Creveld syndrome. Both were heterozygous for this variant, implying that it was acting in a dominant manner.

Table S5:
Heterozygous variants with reported or potential severe recessive effects

Participant	Variant	Predicted phenotype (a "?" denotes a variant with no supporting published findings)	Supporting publications (Listed as PMID when available. Only variant-specific publications are noted)	Computational evidence
PGP1 (hu43860C)	RYR2-G1885E	Arrhythmogenic right ventricular cardiomyopathy (when compound het. with G1886S)	16769042, 16769042	Rare*, PPH2: unknown, BLOSUM100 predicts Gly to Glu is disruptive.
PGP1 (hu43860C)	FIG4-K278fs	Charcot-Marie-Tooth Disease Type 4J?	None	Rare*, Frameshift
PGP2 (huC30901)	RP1-T373I	Retinitis pigmentosa	11095597, 11095597	PPH2: Prob damaging
PGP2 (huC30901)	FLG-S761fs	Ichthyosis vulgaris?	None	Rare*, Frameshift
PGP2 (huC30901)	TGM1-Y312fs	Congenital ichthyosis?	None	Rare*, Frameshift
PGP2 (huC30901)	SLC4A1-E40K	Hemolytic anemia	8471774	Rare*, PPH2: Benign
PGP3 (huBEDA0B)	ABCA4-P1780A	Stargardt disease	10746567	Rare*, PPH2: Prob damaging
PGP3 (huBEDA0B)	LRP5-V667M	Osteoporosis-pseudoglioma syndrome	11719191	Rare*, PPH2: Prob damaging
PGP4 (huE80E3D)	SPG11-K1013E	Spastic paraplegia	Mention in meeting abstract: Boukhris et al., 19th Meeting of the European Neurological Society (2009)	Rare*, PPH2: unknown
PGP4 (huE80E3D)	CPT2-S113L	Late-onset carnitine palmitoyltransferase deficiency	8358442	Rare*, PPH2: Prob damaging
PGP4 (huE80E3D)	SLC6A5-T425M	Hyperekplexia	16751771	Rare*, PPH2: Prob damaging
PGP5 (hu9385BA)	SERPINA1-R247C	Alpha-1 antitrypsin deficiency	CCHMC Molecular Genetics Laboratory Mutation DB (online)	Rare*, PPH2: Prob damaging
PGP5 (hu9385BA)	CBS-T460M	Homocystinuria	Mention in meeting abstract: Redonnet-Vernhet et al., Annual Symposium of the Society for the Study of Inborn Errors of Metabolism (2010)	Rare*, PPH2: Prob damaging
PGP5 (hu9385BA)	ACADVL-R385W	Very long chain acyl-coenzyme A dehydrogenase deficiency	CCHMC Molecular Genetics Laboratory Mutation Database (online)	Rare*, PPH2: Prob damaging
PGP5 (hu9385BA)	TGM1-E520G	Lamellar ichthyosis	11348475, 19241467	Rare*, PPH2: Prob damaging
PGP5 (hu9385BA)	SLC7A9-A182T	Cystinuria	10471498, 11157794	Rare*, PPH2: Benign
PGP5 (hu9385BA)	SLX4-G1396fs	Fanconi Anemia (complementation group P)?	None	Rare*, Frameshift
PGP10 (hu604D39)	SPG7-G199fs	Hereditary spastic paraplegia?	None	Rare*, Frameshift
PGP10 (hu604D39)	SLC26A4-I300L	Pendred Syndrome	http://www.healthcare.uiowa.edu/labs/pendredandbor/slcMutations.htm	Rare*, PPH2: Prob damaging
PGP2 & PGP10	PEX1-I696M	Peroxisome biogenesis disorders	11389485	PPH2: Benign

* Rare variants were only seen once in 64 PGP10 + public CGI genomes

Table S6: PGP-10 trait questionnaire and responses

Predicted amino acid change: Potential phenotype (dominance) [individual w/ variant]	Prioritization reasons (PMIDs are listed for published findings)	Question(s)	Participant responses
SLC9A3R1-R153Q: Kidney stones (dom) [PGP10 het]	Rare*, PPH2: prob damaging, OMIM, Published findings (18784102)	Have you ever had kidney stones? Have any of your first degree relatives (parents, siblings, or children) had kidney stones?	All PGP10 reported "no" to the first question. For the second question: PGP1 reported gallstones in his mother, all others (including PGP10) reported "no".
PKD1-G3300R: Polycystic kidney disease (dom) [PGP7: het]	Rare*, PPH2: prob damaging, Clinically tested gene	Have you or a relative been diagnosed with polycystic kidney disease?	All PGP10 reported "no".
PKD2-S804N: Polycystic kidney disease (dom) [PGP10: het]	Rare*, PPH2: prob damaging, Published findings (17582161, 20881056), Clinically tested gene	Have you or a relative been diagnosed with polycystic kidney disease?	All PGP10 reported "no".
AARS-K967M: Charcot-Marie Neuropathy (dom) [PGP4: het]	Rare*, PPH2: prob damaging, Clinically tested gene	Have you or a relative been diagnosed with Charcot-Marie Neuropathy?	All PGP10 reported "no".
MYO1A-S797F: Hearing loss (dom) [PGP1: het]	Rare*, PPH2: poss damaging, Published findings (12736868)	Do you have profound hearing loss/deafness or use hearing aids?	All PGP10 reported "no".
SCN5A-G615E: Long QT syndrome (dom) [PGP9: het]	Rare*, Published findings (11997281, 15840476, 18071069, 19716085, 20486126), Clinically tested gene	Have you or a relative been diagnosed with long-QT syndrome? Do you have a relative who has died suddenly due to cardiac failure at an unusually young age?	For the first question: PGP3 reported she may have an affected relative, all others (including PGP9) reported "no". For the second question: PGP5 reported that his paternal grandfather died of what was believed to be MI at the age of 58, while PGP4 and PGP6 also reported "yes". All others (including PGP9) reported "no".
MYL2-A13T: Hypertrophic cardiomyopathy (dom) [PGP6: het]	Rare*, OMIM, Published findings (8673105, 11102452, 11748309, 12668451, 14594949, 15483641), Clinically tested gene	Have you or a relative been diagnosed with hypertrophic cardiomyopathy? Have you or a first-degree relative been diagnosed with cardiovascular disease before the age of 50? Do you have a relative who has died suddenly due to cardiac failure at an unusually young age?	All PGP10 reported "no" to the first question. For the second question: PGP9 reported that she had a first degree relative affected by cardiovascular disease, and PGP4 reported grandparents affected. All others (including PGP6) reported "no". For the third question: PGP4 and PGP6 reported "yes". PGP6 reported both maternal and paternal uncles who died of myocardial infarctions at ages of 41, 56, and 59 and were believed to have had coronary artery disease.
LDLR-V827I: Hypercholesterolemia (dom) [PGP6: het]	Rare*, PPH2: Prob damaging, Clinically tested gene	Have you or a relative been diagnosed with hypercholesterolemia (high cholesterol)?	PGP1 and PGP5 reported high cholesterol, PGP4 and PGP6 reported borderline high.
PCSK9-R237W: Hypocholesterolemia (dom) [PGP9: het]	Rare*, PPH2: Prob damaging, Published findings (15358785, 16424354, 16571601, 17765244), Clinically tested gene	Have you or a relative been diagnosed with hypocholesterolemia (abnormally low cholesterol)?	All PGP10 reported "no".

FLG-S761fs: Palmar hyperlinearity / Keratosis pilaris** (dom) [PGP2: het]	Rare*, Frameshift predicted, Clinically tested gene	Some subtle skin phenotypes can be caused by heterozygous variants would would cause severe skin disorder if homozygous. These can include palmar hyperlinearity (causing a hand to look unusually old). Do you have palmar hyperlinearity? Some subtle skin phenotypes can be caused by heterozygous variants would would cause severe skin disorder if homozygous. These can include keratosis pelaris (bumps on the skin on the upper arms, cheeks, or thighs), or fine scale on the skin. Do you have keratosis pilaris?	All PGP10 reported "no" to the first question. For the second question: PGP4 reported "maybe", all others (including PGP2) reported "no".
NF1-Q2721R: Neurofibromatosis 1 (dom) [PGP8: het]	Rare*, PPH2: Prob damaging, Clinically tested gene	Do you have cafe au lait spots (light brown birthmarks)? If so, please describe how many and whether they are larger than 15mm in any direction (a dime is 17mm).	PGP4 reported one spot that is 25mm at its longest. PGP5 reported a son with cafe au lait spots. All others (including PGP8) reported "no".
ALK-L1033P: Neuroblastoma (dom) [PGP3: het]	Rare*, PPH2: Prob damaging, Clinically tested gene	Have you or a relative been diagnosed with neuroblastoma?	All PGP10 reported "no".
WFS1-C426Y: Psychiatric disease (dom) [PGP1: het]	Rare*, PPH2: Poss damaging, Published findings (11244483), Clinically tested (but for unrelated disease)	Have you been diagnosed with any of the following psychiatric diseases? • Major depression • Bipolar disorder • Schizoaffective disorder • Schizophrenia	PGP4 reported depression/anxiety, no others reported psychiatric disease.
KCNQ3-R777Q: Benign neonatal seizures (dom) [PGP5: het]	Rare*, PPH2: Prob damaging, Clinically tested	Did you or a relative have benign seizures when an infant, during the first month of life, that went away?	PGP1 reported a second-degree relative with this condition. All others (including PGP5) reported "no".
SEPT9-R355W: Neuralgic amyotrophy (dom) [PGP6: het]	Rare*, PPH2: Prob damaging, Clinically tested	Neuralgic amyotrophy is a rare disease characterized by sudden onset of severe pain in shoulder or upper limbs, and subsequent muscle atrophy. Have you or a relative been diagnosed with neuralgic amyotrophy?	All PGP10 reported "no".
RHO-G51A: Retinitis pigmentosa (dom) [PGP10: het]	Rare*, PPH2: Prob damaging, OMIM, Published findings (8317502, 9380676, 16962629), Clinically tested gene	Autosomal dominant retinitis pigmentosa is characterized by progressive late onset vision loss, beginning with loss of night vision and peripheral vision. Do you or a relative have retinitis pigmentosa or similar symptoms?	All PGP10 reported "no".

* Variants are called "Rare" if only seen once in 64 PGP10 & public individuals (at least 100 chromosomes).

** Disruptive variants in this gene are reported to cause ichthyosis vulgaris in a recessive manner. Some literature implicates these genes in causing mild phenotypes (palmar hyperlinearity and keratosis pilaris) when heterozygous (see Table 2).

Table S7: GET-Evidence: Variant evidence and clinical importance scoring

Variant evidence: Computational	Add points for every consistent prediction, subtract points for contradicting evidence. * Other reports for this gene implicate it in same disease: +1 * Polyphen 2 prediction: +1 * SIFT prediction: +1 * Presence in conserved domain: +1 * Disruptive amino acid substitution (BLOSUM100 score): +1 * Nonsense or frameshift mutation: +2 -1 point total if overall evidence contradicts proposed effect
Variant evidence: Functional	Add points for each different functional observation. * Change in enzyme activity: +1 * Change in binding affinity: +1 * Change in cellular localization: +1 * Change in gene expression: +1 * Change in protein expression: +1 * Phenotype effect in animal models: +2
Variant evidence: Case/control	Significance scores for case/control data should derive from a single publication thought to be best representative of the variant's effect. Allele frequencies from other studies should only be used when an extremely high discordance contradicts the paper's hypothesis. -1 point total if case/control data and/or allele frequency strongly contradicts predicted effect 0 points if no evidence or significance > 0.1 1 point if significance < 0.1 2 points if significance < 0.05 3 points if significance < 0.025 4 points if significance < 0.01 5 points if significance < 0.0001
Variant evidence: Familial	-1 point total if familial data strongly contradicts predicted effect 0 points if no familial data or LOD < 0.5 1 point if LOD >= 0.5 2 points if LOD >= 1.0 3 points if LOD >= 1.5 and seen in at least 2 unrelated individuals 4 points if LOD >= 3 and seen in at least 2 unrelated individuals 5 points if LOD >= 5 and seen in at least 2 unrelated individuals
Clinical importance: Severity	0 points for benign 1 point for rarely having any effect on health (e.g. small increased susceptibility to infections -- either choose this or a low penetrance score, not both) 2 points for mild effect on quality of life and/or usually not symptomatic (Cystinuria) 3 points for moderate effect on quality of life (e.g., Familial Mediterranean Fever) 4 points for severe effect: causes serious disability or reduces life expectancy (e.g., Sickle-cell, Stargardt's disease) 5 points for very severe effect, lethal by early adulthood (e.g., Lethal junctional epidermolysis bullosa, Adrenoleukodystrophy)
Clinical importance: Treatability	0 points for no clinical evidence supporting intervention (e.g., PAF acetylhydrolase deficiency) 1 point for incurable: Treatment only to alleviate symptoms 2 points for potentially treatable: Treatment is in development or controversial 3 points for somewhat treatable: Standard treatment, but only a small or moderate improvement of mortality/morbidity 4 points for treatable: Standard treatment significantly reduces the amount of mortality/morbidity, but does not eliminate it 5 points for extremely treatable: Well-established treatment essentially eliminates the effect of the disease (e.g., PKU)
Clinical importance: Penetrance	0 points if < 0.1% attributable risk (extremely low penetrance) 1 point if ≥ 0.1% attributable risk (very low penetrance) 2 points if ≥ 1% attributable risk (low penetrance) 3 points if ≥ 5% attributable risk (moderate penetrance) 4 points if ≥ 20% attributable risk (moderately high penetrance) 5 points if ≥ 50% attributable risk (complete or highly penetrant)

Table S8: GET-Evidence: Assessment of strength of evidence

well-established	* At least 4 points in either “Case/control evidence” or “Familial evidence” **and** * At least eight points total in evidence categories
likely	* At least 3 points in either “Case/control evidence” or “Familial evidence” **and** * At least five points total in evidence categories
uncertain	Any variants which do not meet the above requirements.

Table S9: GET-Evidence: Assessment of clinical importance

high clinical importance	* At least 4 points in penetrance (high-moderate penetrance / >= 20% attributable risk) **and either**: * At least 3 stars in severity and at least 4 stars in treatability **or** * At least 4 stars in severity
moderate clinical importance	* At least 3 points in penetrance (high-moderate penetrance / >= 5% attributable risk) **and either**: * At least 2 stars in severity and at least 4 stars in treatability **or** * At least 3 stars in severity
low clinical importance	Any variants which do not meet the above requirements.

Table S10: GET-Evidence information regarding variants from Table S5

Variant	Predicted phenotype (a "?" denotes a variant with no supporting published findings)	Allele frequency	Priorit-ization score	Evidence assessment in GET-Evidence	Clinical importance assessment in GET-Evidence
RYR2-G1885E	Arrhythmogenic right ventricular cardiomyopathy (when compound het. with G1886S)	1.8%	4	Uncertain	High
FIG4-K278fs	Charcot-Marie-Tooth Disease Type 4J?	unknown	3	Uncertain	Moderate
RP1-T373I	Retinitis pigmentosa	1.2%	5	Uncertain	High
FLG-S761fs	Ichthyosis vulgaris?	unknown	4	Uncertain	Moderate
TGM1-Y312fs	Congenital ichthyosis?	unknown	4	Uncertain	Moderate
SLC4A1-E40K	Hemolytic anemia	1.2%	3	Uncertain	Moderate
ABCA4-P1780A	Stargardt disease	0.04%	5	Uncertain	High
LRP5-V667M	Osteoporosis-pseudoglioma syndrome	4.15%	6	Uncertain	High
SPG11-K1013E	Spastic paraplegia	1.0%	4	Uncertain	High
CPT2-S113L	Late-onset carnitine palmitoyltransferase deficiency	0.1%	6	Well-established	High
SLC6A5-T425M	Hyperekplexia	0.01%	5	Uncertain	Moderate
SERPINA1-R247C	Alpha-1 antitrypsin deficiency	0.3%	4	Uncertain	High
CBS-T460M	Homocystinuria	unknown	4	Uncertain	High
ACADVL-R385W	Very Long Chain Acyl-Coenzyme A Dehydrogenase Deficiency	unknown	4	Uncertain	High
TGM1-E520G	Lamellar ichthyosis	0.6%	5	Uncertain	Moderate
SLC7A9-A182T	Cystinuria	0.3%	4	Uncertain	Moderate
SLX4-G1396fs	Fanconi Anemia (complementation group P)?	unknown	4	Uncertain	High
SPG7-G199fs	Hereditary spastic paraplegia?	unknown	4	Uncertain	High
SLC26A4-I300L	Pendred Syndrome	0.4%	5	Uncertain	Moderate
PEX1-I696M	Peroxisome biogenesis disorders	2.7%	4	Uncertain	High