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Comparison of Causative Variant Prioritization Tools Using Next-generation Sequencing Data in Japanese Patients with Mendelian Disorders

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ABSTRACT

Background During the investigation of causative variants of Mendelian disorders using next-generation sequencing, the enormous number of possible candidates makes the detection process complex, and the use of multidimensional methods is required. Although the utility of several variant prioritization tools has been reported, their effectiveness in Japanese patients remains largely unknown.

Methods We selected 5 free variant prioritization tools (PhenIX, hiPHIVE, Phen-Gen, eXtasy-order statistics, and eXtasy-combined max) and assessed their effectiveness in Japanese patient populations. To compare these tools, we conducted 2 studies: one based on simulated data of 100 diseases and another based on the exome data of 20 in-house patients with Mendelian disorders. To this end we selected 100 pathogenic variants from the "Database of Pathogenic Variants (DPV)" and created 100 variant call format (VCF) files that each had pathogenic variants based on reference human genome data from the *1000 Genomes Project*. The later "in-house" study used exome data from 20 Japanese patients with Mendelian disorders. In both studies, we utilized 1-5 terms of "Human Phenotype Ontology" as clinical information.

Results In our analysis based on simulated disease data, the detection rate of the top 10 causative variants was 91% for hiPHIVE, and 88% for PhenIX, based on 100 sets of simulated disease VCF data. Also, both software packages detected 82% of the top 1 causative variants. When we used data from our in-house patients instead, we found that these two programs (PhenIX and hiPHIVE) produced higher detection rates than the other three systems in our study. The detection rate of the top 1 causative variant was 71.4% for PhenIX, 65.0% for hiPHIVE.

Conclusion The rates of detecting causative variants in two Exomizer software packages, hiPHIVE and PhenIX, were higher than for the other three software systems we analyzed, with respect to Japanese patients.

Key words computational biology; databases; genetic; whole exome sequencing

Next-generation sequencing (NGS) can exhaustively analyze genes in one sequencing process, and this new capability has dramatically altered the fields of genomic research and medical genetics. In particular, many undiagnosed patients have been receiving benefit from genetic diagnoses by exhaustive gene analysis. Our previous study showed the utility of genetic diagnoses for patients with Mendelian disorders using NGS.[1] Research methods of nationwide large-scale genomic studies, such as Genomics England and the Initiative of Rare and Undiagnosed Diseases project in Japan, have also been based on NGS technology.

Variant prioritization plays a central role in the genetic diagnosis of patients with Mendelian disorders when using NGS techniques, including whole genome sequencing and whole exome sequencing (WES). WES can detect some 30,000 more variants compared to human reference sequences, and approximately 10,000 of these represent nonsynonymous amino acid substitutions, alterations of conserved splice site residues, or small insertions or deletions.[2] Therefore, to detect the causative variant among an enormous number of possibilities, subsequent prioritization steps are required. For example, to interpret variants, we utilize several reference databases and software systems, including a common variant database, pathogenic variant databases, and in-silico prediction tools. Ultimately, detailed consideration by clinicians and bioinformaticians is needed for detecting the causative variant.[1] In such

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Abbreviations: DPV, Database of Pathogenic Variants; HPO, human phenotype ontology; NGS, next-generation sequencing; SNPs, single nucleotide polymorphisms; SNVs, single nucleotide variants; VCF, variant call format; WES, whole exome sequencing

Table 1. Comparison of phenotype-based variant detection tools[8, 10, 12]

Software	Availability	Population,Disease-Specific, and Sequence Databases	In Silico Predictive Algorithms	Framework Algorithm
eXtasy-order statistics eXtasy-combined max	Website and Command line	1000 Genomes Project dbNSFP database HGMD	Polyphen2 SIFT MutationTaster CAROL LRT PhastCons Phylop	Phenomiser algorithm Endeavour algorithm Random Forest learning Haploinsufficiency prediction score
Phen-Gen	Website and Command line	HGMD	Polyphen2 SIFT	Bayesian framework Unifying framework Genomewide approach Phenomiser algorithm Random-walk-with-restart algorithm
PhenIX	Website and Command line	1000 Genomes Project ESP 6500	Polyphen2 SIFT MutationTaster	Phenomiser algorithm
hiPHIVE	Website and Command line	1000 Genomes Project ESP 6500 MGD IMPC	Polyphen2 SIFT MutationTaster	Phenomiser algorithm PhenoDigm algorithm Random-walk analyses Random-walk-with-restart algorithm

CAROL, Calculated Combined Annotation Scoring Tools; dbNSFP, database for nonsynonymous SNPs' functional predictions; ESP 6500, Exome Server Project; HGMD, Human Gene Mutation Database; IMPC, International Mouse Phenotyping Consortium; LRT, Likelihood-Ratio Test; MGD, Mouse Genome Database; SIFT, Sorting Intolerant from Tolerant. Software version: eXtasy (Sifrim et al. 2013)[8] ver.0.1, Phen-Gen (Javed et al. 2014)[10] ver.1.0, PhenIX and hiPHIVE (Smedley et al. 2015)[12] ver. 10.0.1.

variant prioritization processes, clinical information is essential. Specifically, we use gene lists that have been made by clinicians for each specific analysis. Such records can help select related variants for a given patient's symptoms, but in those cases where the causative gene is omitted from such a list, we would not be able to detect the causative variant. Identification of disease-causing variants for patients with Mendelian disorders is very complicated and easily qualifies as a "needle in a haystack" challenge.[3]

Several phenotype-driven software tools are now available to help select differential diagnoses.[4] Additionally, to rank the candidate variants in the context of the enormous number of variants that can be detected by NGS, several software systems use both the patient's phenotypic information and the NGS-derived genotypic data.[5–12] These software packages are also able to directly reference a variety of diverse databases and ancillary software systems (Table 1). Comparative evaluations of these software products using patient data from Western countries has already been published,[13] and their utility for causative variant detection is well described. However, the utility of these variant detection software systems using data from Japanese patients' data is unknown.[11] The genetic basis of differences among ethnic groups has been under-investigated. However, we cannot rule out that differences in individual single nucleotide variants (SNVs) in each ethnic group can affect genetic testing results.[14] Therefore, we proceeded to undertake the first effort to evaluate their capabilities with regard to Japanese patients.

MATERIALS AND METHODS

To our knowledge, 11 software packages have been established as variant prioritized tools using Human Phenotype Ontology (HPO).[15] In this research, software was selected using the following criteria using exome sequencing data and available as downloaded packages. We finally selected five variant prioritization software packages PhenIX,[12] hiPHIVE,[12] Phen-Gen,[10] eXtasy-order statistics,[8, 16] and eXtasy-combined max.[8] These products, except for the Phen-Gen package, have already been compared in a previous report.[13] To test these software packages, we selected 1–5 HPO terms corresponding to specific variant call format (VCF) files.[15] The HPO provides a common lexicon of phenotypic abnormalities found in human diseases, and has been utilized in many databases and NGS projects.[17] For our analyses using PhenIX and hiPHIVE, we settled on a cut-off of allele frequency at 1%. We ran all the software programs in the form we received them via downloads. In this research, we performed two different

analyses, one using artificially simulated data and the other using actual data from in-house Japanese patients (Fig. 1). We retained the default parameter settings in all the software products. In addition to the comparison of variant detection rates across the various software products, we also compared variant types and detection rates. To classify each variant type, we utilized the guidelines of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology, which were developed in 2015. In that statement, null variants including nonsense, frameshift and splice sites variants, were classified as very strong pathogenic criteria.[18]

Simulated disease data analysis

For this part of our study, we created 100 VCF files using reference genome bam data (1000 Genomes Project,[19] http://www.internationalgenome.org/home). In addition, we randomly selected 100 pathogenic variants from the "Database of Pathogenic Variants (DPV), http://dpv.cmg.med.keio.ac.jp/dpv-pub/variants)." This database contains germline pathogenic variants in Japanese patients with Mendelian disorders. We then created 100 simulated patient VCF files using each pathogenic variant.

The HPO of each disease was randomly selected using the "Phenomizer[15, 20] system (http://compbio.charite.de/phenomizer)." With this tool, we can pick up causative disorders using the HPO. Additionally, the HPO terms with regard to each disease are described on this site. We analyzed data on 100 simulated patients with the five software packages, using each VCF file and between 1 to 5 selected HPO terms.

In-house patient data analysis

In the second part of this research project, we analyzed 20 exome samples collected from Japanese patients with Mendelian disorders. All samples were sequenced using the TruSight One sequencing panel (Illumina, San Diego, CA). Sequencing and variant detection methods have already been described in our previous research.[1] We performed WES using the Ion AmpliSeq TM Exome RDY kit (Thermo Fisher Scientific, Waltham, MA) for undiagnosed patients despite having performed TruSightOne sequencing. All causative variants were validated by Sanger sequencing. Clinical geneticists selected 1 to 5 HPO of each patient based upon a review of their medical records. Using both the HPO and VCF information, we evaluated the detection rate of each software program. This study was approved by the ethics committee at Tottori University (dated September 22, 2014, approval number G152).

RESULTS

Simulated disease data analysis

We analyzed 100 simulated disease VCF data using the five different software packages (Fig. 2a). The detection rates of causative variants revealed that the best two systems at detecting the top ten variants were hiPHIVE at 91%, and PhenIX at 88%. In addition, both products detected 82% of the top 1 variant. We also note that both of the eXtasy software packages (eXtasy-order statistics and eXtasy-combined max) have limitations on their HPO terms available for analysis and that they could not analyze 20 VCF data items. The detection rate of the top 10 causative variants in eXtasy-order statistics was 19.0%, and in eXtasy-combined max product was 21.0%. In addition, the percentage of variants that could be detected as the chief cause was 6.0% in the order statistics system and 10.0% in combined max product. In Phen-Gen software, the detection rate of the top 5 causative variants was 29%, the top 10 was 33%, and the top 1 was zero percent. Detailed results from the simulated disease data analysis are presented in Supplementary Table S1.

PhenIX and hiPHIVE could detect a total of 82 causative variants as being the most critical. They also produced higher detection rates than the other three software products (Phen-Gen, eXtasy-order statistics and eXtasy-combined max), which correctly detected a total of only 12 variants as being the most critical. We confirmed that the difference in productivity between PhenIX or hiPHIVE and the three other products was quite statistically significant ($P < 0.001$, by Fisher's exact test). Similarly, PhenIX and hiPHIVE detected 87 causative variants as being the first to fifth main factors and showed a higher detection rate than the other three systems (Phen-Gen, eXtasy-order statistics and eXtasy-combined max), which only detected 40 variants as being in the first to fifth factors. We also confirmed the significance of this difference between the PhenIX or hiPHIVE systems and the three other software packages using the detection rates regarding the top first to fifth data ($P < 0.001$, by Fisher's exact test).

Subsequently, we compared the results of PhenIX and hiPHIVE against each other. A total of 79 variants were detected as the top causative factors by both products. The hiPHIVE system detected 7 causative variants as having a higher priority than did the PhenIX product (ID 3, 7, 30, 60, 72, 88, 96), but the PhenIX software detected 6 causative variants as higher priority than did the hiPHIVE product (ID 29, 38, 59, 70, 71, 73).

In this simulated disease aspect of our study, the analyzed variants consisted of 51 missense mutation variants (Fig. 2b) and 44 null variants (Fig. 2c). Because

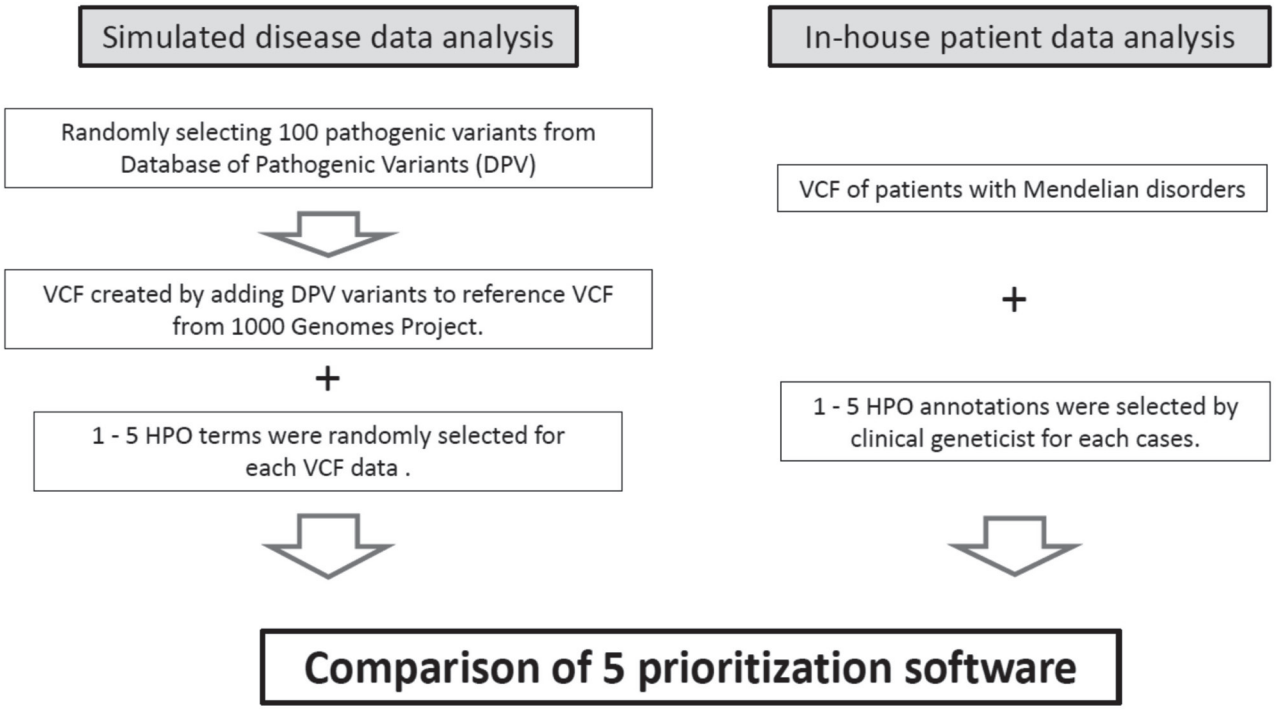

Fig. 1. Overview of the prioritization software comparison study.
Two separate investigations were performed, one using data from in-house patients, and the other based on simulated data. We compared 5 different software products (PhenIX, hiPHIVE, Phen-Gen, eXtasy-order statistics and eXtasy-combined max). These systems use variant call format (VCF) files and the human phenotype ontology (HPO). In simulated disease analyses, we created 100 virtual patient VCF files, and added pathogenic variants based on reference to the VCF files in the 1000 Genome Project. We selected 100 pathogenic variants from the Japanese pathogenic variant database (http://dpv.cmg.med.keio.ac.jp/dpv-pub/variants). Additionally, we selected several HPO files for each VCF from the Phenomizer site (http://compbio.charite.de/phenomizer). For the in-house patient analysis, we used VCF files and HPO for specific patients with Mendelian disorders.

five variants were deleted from the DPV site, we excluded these from our comparison of the detection rates and variant types. We found that Exomiser's two kinds of software (PhenIX and hiPHIVE) correctly detected 80% of the VCF files as the top 1 through 5 for both the missense mutations and null variants. For Phen-Gen, no remarkable difference was found in the detection rate in distinguishing between these variant types. For the eXtasy-order statistics package, the "not ranked" detection rate was 64% (28/44) of the null variants and only 11.8% (6/51) for the missense variants. "Not ranked" means that the software did not remarkably detect any causative variants within the top 1–100. This tendency was also observed in the eXtasy-combined max program, where 64% (28/44) of the null variants and 11.8% (6/51) of the missense variants were also not detected.

In-house patient data analysis

We also analyzed the data from our 20 in-house patients with Mendelian disorders (Fig. 3) using each of the five software products. The detection rate of the top 10 causative variants for the hiPHIVE system was 85.7%, and for the PhenIX product this was 76.2%. Also, the percentage of variants that could be detected as the chief cause was 61.9% in hiPHIVE and 71.4% in PhenIX. In our analysis of eXtasy, we found that it discovered the principal causative variant in only 5% of the cases, and its finding of the top 10 factors was only 10%. Remarkably, we also found that, for eXtasy, the combined max analysis could not detect any of the main causative variants, nor any within the top 10. Likewise, Phen-Gen also generated poor results, identifying zero of the top five causative variants, only 5% of the top 10, and zero of the chief causes. Detailed results of variant data analysis are described in Supplementary Table S2.

PhenIX could detect 15 of the top causative variants and showed a higher detection rate than the other three software products (Phen-Gen, order statistics, and combined max eXtasy), each of which only detected one top variant. We confirmed the significance of the differences between PhenIX and these three other software products ($P < 0.001$, by Fisher's exact test).

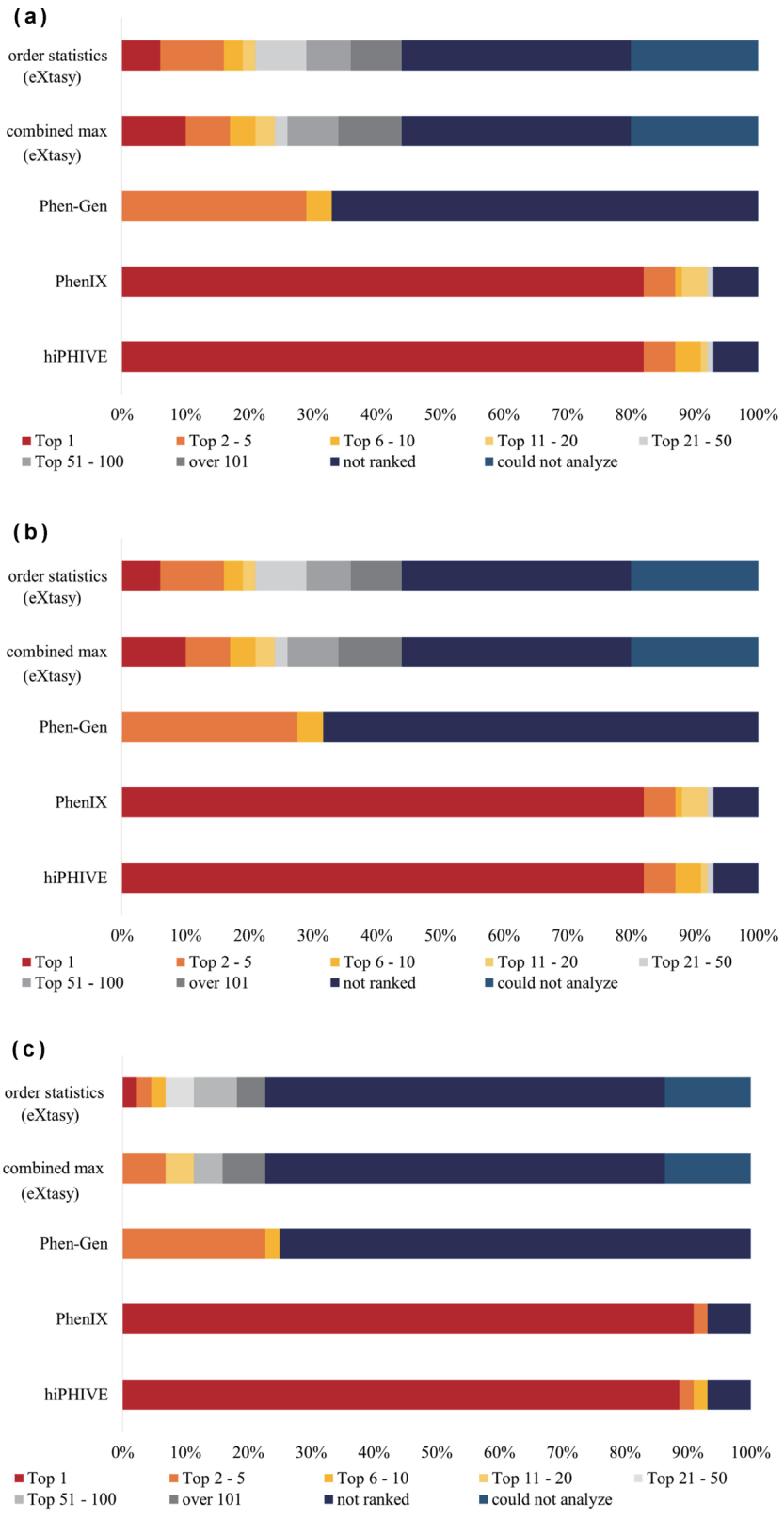

Fig. 2. Simulated disease analysis data ranked by category.
(**a**) Ranking of causative variant detection in simulated disease data analysis (n = 100). (**b**) Ranking of missense causative variants detection in simulated disease data analysis (n = 51). (**c**) Ranking of null causative variants detection in simulated disease data analysis (n = 44). The term "not ranked" means software could not detect causative variants; "could not analyze" means the software could not perform the analysis.

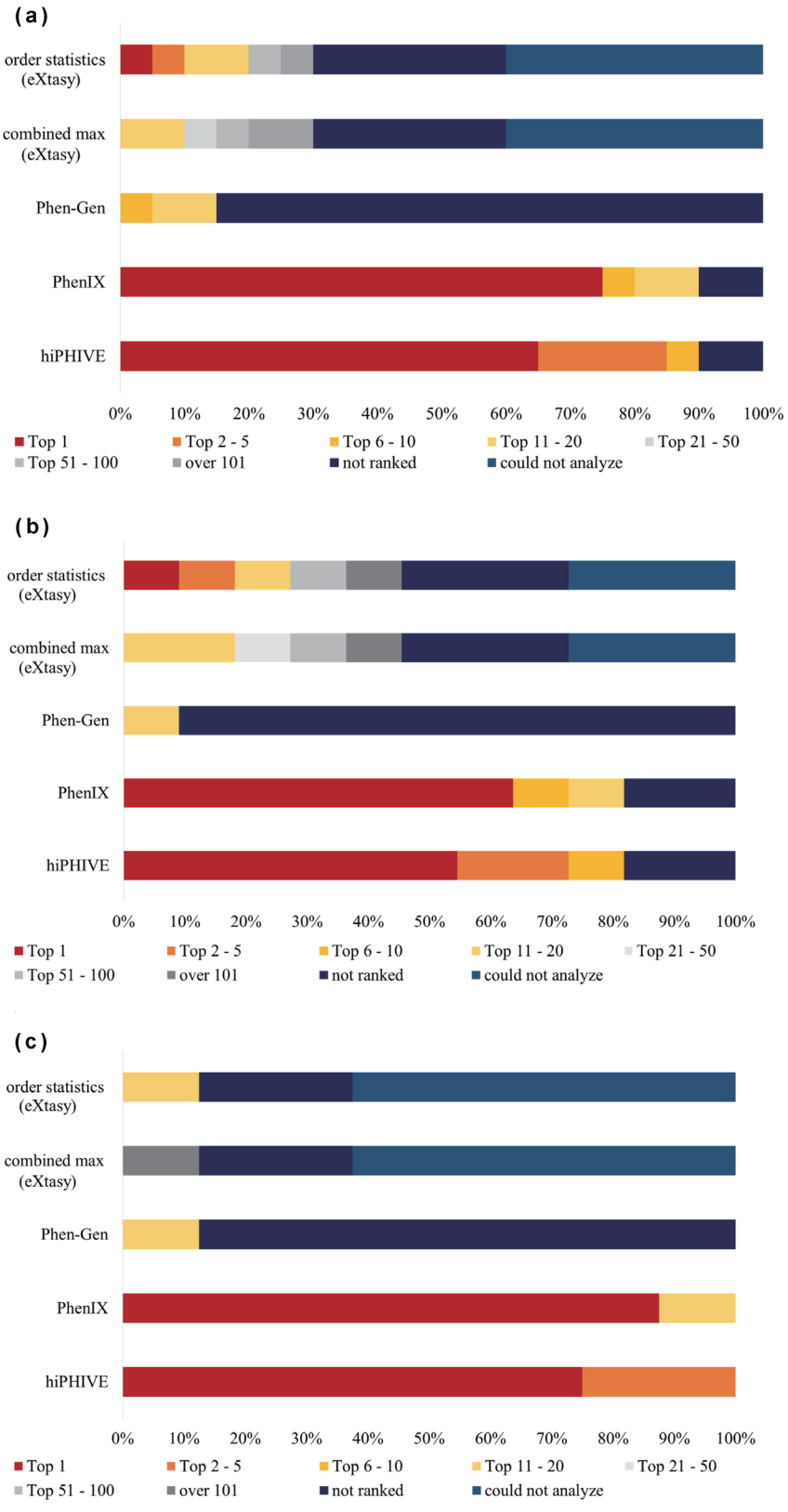

Fig. 3. In-house patient data ranked by category.
(**a**) Data from 20 patients was used in the analysis of each software product. (**b**) Ranking of missense causative variants detection regarding in-house patient data analysis (n = 11). (**c**) Ranking of null causative variants detection of in-house patient data analysis (n = 8). The term "not ranked" means software could not detect causative variants; "could not analyze" means the software could not perform the analysis.

Similarly, PhenIX detected 15 of the top 1–5 causative variants and in this regard also showed a higher detection rate than the other three software systems (Phen-Gen, order statistics, and combined max eXtasy), which only detected two variants, respectively. We again confirmed this statistical difference between PhenIX and three software ($P < 0.001$, by Fisher's exact test).

In a similar pattern, hiPHIVE could detect 13 causative variants as being the top 1, and 17 variants as being in the top 1–5. This software also produced a higher detection rate than the three other software systems (Phen-Gen, order statistics, and combined max eXtasy), which detected only one variant as a top 1 and 2 variants as being among the top 1–5 causes. We likewise confirmed this difference in top 1 and top 1–5 detection rates between hiPHIVE and three software as being statistically significant ($P < 0.001$, by Fisher's exact test).

We subsequently compared the results produced by the PhenIX and hiPHIVE systems against each other and found that the same 13 variants were detected as the top causes by both products. For three causative variants (patients 1, 16, 19), hiPHIVE identified them as having a higher priority than did PhenIX. In contrast, PhenIX detected two causative variants as having a higher priority than hiPHIVE (patients 6, 10).

Over the course of this in-house study, we analyzed 11 missense mutation variants (Fig. 3b) and 8 null variants (Fig. 3c). Because patient 3's variant types were missense mutation and null variant, we excluded the case from our comparison of the detection rates and variant types. With the Phen-Gen product, we found no remarkable difference in the detection rate despite the distinction of the variant types. In Exomiser's two types of software (PhenIX and hiPHIVE), the detection rate of causative variants within the top 10 was 100% in the analysis of the null variants, and also quite high for the missense variants: PhenIX at 72.7% (8/11) and hiPHIVE at 81.8% (9/11). In addition, the detection rates of "not ranked" for both software were 18.2% (2/11) in the missense variants analysis. With the two eXtasy software products (order statistics and combined max) the proportion of "not ranked" results was 25% (2/8) for the null variants data and 27% (3/11) for the missense variants.

DISCUSSION

Our simulated disease data analysis indicated that the detection rates with PhenIX and hiPHIVE were substantially higher than those with the remaining 3 tools; this trend was corroborated by the previously reported results.[13] However, the utility of these two software packages, specifically regarding Japanese patients, had previously been unknown. Our present research indeed showed that the two software systems generated high detection rates when using simulated Japanese patient data and also when using the data from our in-house patients. We provide detailed information available to us regarding each software product in Table 1, but only looking at this information, we do not understand why there is such a substantial difference in the detection rates across the various products. Software update frequency might be one of the most important factors in improving detection rates. In fact, frequent updates are done for Exomiser. For example, integration of a usable database and adding the most recent algorithms are done continuously.[17] The detection rate of the top 10 causative variants in hiPHIVE was 20.0% in the previous report,[13] whereas the present research detection rate of this software was 90.0%. Certain mechanisms are unknown, but frequent software updates in Exomizer might be one of the key elements of improving the identification rate.

The detection rate of the causative variants for hiPHIVE and PhenIX was higher than for the other software except for the data related to ID 71 (Supplementary Table S1). In this case, with c.755G > C, and p Arg252Pro in the *PAH* gene, we found that Phen-Gen and eXtasy-order statistics detected the causative variant as in the top 3, while eXtasy-combined max detected it among the top 7. In contrast, hiPHIVE recognized it as being among the top 10, and PhenIX identified it as among the top 14.

Similarly, other variants of the *PAH* gene in ID 70 and 72 also had relatively low detection results in hiPHIVE and PhenIX. In comparison, the detection rate of ID 73 in the *PAH* gene for hiPHIVE and PhenIX was higher than in the two eXtasy software programs. Also, Phen-Gen could detect more than could the two eXtasy products. We considered many possible reasons for this particular result regarding the *PAH* gene, but we could not reach a conclusion about the mechanism involved due to a lack of more detailed information about each software product and the methods they use. Nonetheless, this result suggests that the detection rates can differ depending on the gene.

Associations between the detection rate and the variant type were not found for hiPHIVE, PhenIX, and Phen-Gen products, except for the eXtasy software systems. Notably, in the two types of eXtasy software, usable HPOs were limited, and we could not analyze 20/100 (20%) of the VCF files. Although statistical analysis could not be performed because of our small

sample size, this result suggests that the detection rate of the 2 eXtasy software products might be inadequate with regard to the analysis of null variants.

In this simulated study, we created VCF files to simulate patient genomic data. The 100 bam data files registered in the 1000 Genome Project were randomly selected in this study. These bam data files contain not only Japanese data files but also non-Japanese data files, and we cannot deny that specific SNVs related to non-Japanese data affected the analysis results. In our subsequent analyses using this simulated data, we obtained results similar to what we found using data from actual in-house patients. As a result, we speculate that this approach of using simulated patient data might indeed be more broadly useful for the comparisons of other variant prioritization tools.

From in-house patient data analysis results involving ranked comparisons, it is clear that the causative variant detection rate of hiPHIVE and PhenIX was higher than that of the other software products. We particularly note that hiPHIVE was able to detect all the causative variants within the top 10 except for only the two *ECHS1* items. Indeed, these two *ECHS1* gene variants from patients 7 and 12 were missed by all five software products. The *ECHS1* gene was not included in our TruSight One sequencing panel, and we only detected these variants in the *ECHS1* gene using WES. We suggest that since the mitochondrial short-chain enoyl-CoA hydratase 1 deficiency due to *ECHS1* mutations was first reported only in 2014,[21] it is possible that the software packages we examined might be unable to readily detect such relatively new disorders (Fig. 3). All five software products use the Phenomizer program described in Table 1 of this paper, and we hypothesize that this relatively new gene was not listed in it. However, since these software packages and their related databases are updated daily, we have to assume that Phenomizer was updated at the time of our analyses.

Several variants cause specific phenotypes in Mendelian disorders. N540K in the *FGFR3* of hypochondroplasia and S2G in the *SHOC2* of Noonan-like syndrome with loose anagen hair are well-known variants related to specific phenotypes among clinical geneticists. We found that hiPHIVE and PhenIX could detect these variants, but the other software products remarkably could not. This failure is noteworthy because we would expect that purpose-built causative variant prioritization tools should be easily able to detect these well-known variants.

Exomiser, including its use in hiPHIVE and PhenIX, recently received the approval of the International Rare Diseases Research Consortium as a recognized resource.[17] Overall, the detection rates of causative variants with hiPHIVE and PhenIX was higher than with the other software products in our study. This result is the same found in previous research.[13]

As a technical note, we should mention that although one can find many diagnostic software tools online using HPO and VCF files,[11] in our present study, this would have required uploading sensitive patient data. Therefore, to fully adhere to the protection of personal information, in this present research, we operated all five software as downloaded packages.

HPO provides a comprehensive bioinformatic resource for the analysis of human diseases and phenotypes.[15] It has therefore been adopted as the standard for phenotypic terms in international rare affected tissues, registries, clinical laboratories, biomedical resources, and clinical software tools.[17, 22–27] The description of phenotypic variations has become critical for genomic medicine and translational research,[28–31] and our having "computable" descriptions of human diseases using HPO phenotypic profiles is a key element in the use of Phenotype-based exome analysis tools. Further studies of HPO setting methodologies for these tools are needed to enable us to detect causative variants more efficiently.

In conclusion, we confirmed the utility of causative variant prioritization tools with regard to Japanese patients. In particular, the detection rate of causative variants in two Exomizer software products, hiPHIVE and PhenIX, was higher than in that of the three other systems we analyzed.

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The authors declare no conflict of interest.

REFERENCES

1 Okazaki T, Murata M, Kai M, Adachi K, Nakagawa N, Kasagi N, et al. Clinical Diagnosis of Mendelian Disorders Using a Comprehensive Gene-Targeted Panel Test for Next-Generation Sequencing. Yonago Acta Med. 2016;59:118-25. PMID: 27493482

2 Zemojtel T, Köhler S, Mackenroth L, Jäger M, Hecht J, Krawitz P, et al. Effective diagnosis of genetic disease by computational phenotype analysis of the disease-associated genome. Sci Transl Med. 2014;6:252ra123. PMID: 25186178, DOI: 10.1126/scitranslmed.3009262

3 Cooper GM, Shendure J. Needles in stacks of needles: finding disease-causal variants in a wealth of genomic data. Nat Rev Genet. 2011;12:628-40. PMID: 21850043, DOI: 10.1038/nrg3046

4 Jalali Sefid Dashti M, Gamieldien J. A practical guide to filtering and prioritizing genetic variants. Biotechniques. 2017;62:18-30. PMID: 28118812, DOI: 10.2144/000114492

5 Robinson PN, Krawitz P, Mundlos S. Strategies for exome and genome sequence data analysis in disease-gene discovery projects. Clin Genet. 2011;80:127-32. PMID: 21615730, DOI: 10.1111/j.1399-0004.2011.01713.x

6 Gilissen C, Hoischen A, Brunner HG, Veltman JA. Disease gene identification strategies for exome sequencing. Eur J Hum Genet. 2012;20:490-7. PMID: 22258526, DOI: 10.1038/ejhg.2011.258

7 Danecek P, Auton A, Abecasis G, Albers CA, Banks E, DePristo MA, et al.; 1000 Genomes Project Analysis Group. The variant call format and VCFtools. Bioinformatics. 2011;27:2156-8. PMID: 21653522, DOI: 10.1093/bioinformatics/btr330

8 Sifrim A, Popovic D, Tranchevent LC, Ardeshirdavani A, Sakai R, Konings P, et al. eXtasy: variant prioritization by genomic data fusion. Nat Methods. 2013;10:1083-4. PMID: 24076761, DOI: 10.1038/nmeth.2656

9 Moreau Y, Tranchevent LC. Computational tools for prioritizing candidate genes: boosting disease gene discovery. Nat Rev Genet. 2012;13:523-36. PMID: 22751426, DOI: 10.1038/nrg3253

10 Javed A, Agrawal S, Ng PC. Phen-Gen: combining phenotype and genotype to analyze rare disorders. Nat Methods. 2014;11:935-7. PMID: 25086502, DOI: 10.1038/nmeth.3046

11 Smedley D, Robinson PN. Phenotype-driven strategies for exome prioritization of human Mendelian disease genes. Genome Med. 2015;7:81. PMID: 26229552, DOI: 10.1186/s13073-015-0199-2

12 Smedley D, Jacobsen JOB, Jäger M, Köhler S, Holtgrewe M, Schubach M, et al. Next-generation diagnostics and disease-gene discovery with the Exomiser. Nat Protoc. 2015;10:2004-15. PMID: 26562621, DOI: 10.1038/nprot.2015.124

13 Pengelly RJ, Alom T, Zhang Z, Hunt D, Ennis S, Collins A. Evaluating phenotype-driven approaches for genetic diagnoses from exomes in a clinical setting. Sci Rep. 2017;7:13509. PMID: 29044180, DOI: 10.1038/s41598-017-13841-y

14 Burchard EG, Ziv E, Coyle N, Gomez SL, Tang H, Karter AJ, et al. The importance of race and ethnic background in biomedical research and clinical practice. N Engl J Med. 2003;348:1170-5. PMID: 12646676, DOI: 10.1056/NEJMsb025007

15 Köhler S, Vasilevsky NA, Engelstad M, Foster E, McMurry J, Aymé S, et al. The Human Phenotype Ontology in 2017. Nucleic Acids Res. 2017;45:D865-76. PMID: 27899602, DOI: 10.1093/nar/gkw1039

16 Aerts S, Lambrechts D, Maity S, Van Loo P, Coessens B, De Smet F, et al. Gene prioritization through genomic data fusion. Nat Biotechnol. 2006;24:537-44. PMID: 16680138, DOI: 10.1038/nbt1203

17 Köhler S, Carmody L, Vasilevsky N, Jacobsen JOB, Danis D, Gourdine JP, et al. Expansion of the Human Phenotype Ontology (HPO) knowledge base and resources. Nucleic Acids Res. 2019;47:D1018-27. PMID: 30476213, DOI: 10.1093/nar/gky1105

18 Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al.; ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17:405-23. PMID: 25741868, DOI: 10.1038/gim.2015.30

19 Auton A, Brooks LD, Durbin RM, Garrison EP, Kang HM, Korbel JO, et al.; 1000 Genomes Project Consortium. A global reference for human genetic variation. Nature. 2015;526:68-74. PMID: 26432245, DOI: 10.1038/nature15393

20 Köhler S, Schulz MH, Krawitz P, Bauer S, Dölken S, Ott CE, et al. Clinical diagnostics in human genetics with semantic similarity searches in ontologies. Am J Hum Genet. 2009;85:457-64. PMID: 19800049, DOI: 10.1016/j.ajhg.2009.09.003

21 Peters H, Buck N, Wanders R, Ruiter J, Waterham H, Koster J, et al. ECHS1 mutations in Leigh disease: a new inborn error of metabolism affecting valine metabolism. Brain. 2014;137:2903-8. PMID: 25125611, DOI: 10.1093/brain/awu216

22 Ramoni RB, Mulvihill JJ, Adams DR, Allard P, Ashley EA, Bernstein JA, et al.; Undiagnosed Diseases Network. The Undiagnosed Diseases Network: Accelerating Discovery about Health and Disease. Am J Hum Genet. 2017;100:185-92. PMID: 28157539, DOI: 10.1016/j.ajhg.2017.01.006

23 Taruscio D, Groft SC, Cederroth H, Melegh B, Lasko P, Kosaki K, et al. Undiagnosed Diseases Network International (UDNI): white paper for global actions to meet patient needs. Mol Genet Metab. 2015;116:223-5. PMID: 26596705, DOI: 10.1016/j.ymgme.2015.11.003

24 Gahl WA, Mulvihill JJ, Toro C, Markello TC, Wise AL, Ramoni RB, et al.; UDN. The NIH Undiagnosed Diseases Program and Network: applications to modern medicine. Mol Genet Metab. 2016;117:393-400. PMID: 26846157, DOI: 10.1016/j.ymgme.2016.01.007

25 Gall T, Valkanas E, Bello C, Markello T, Adams C, Bone WP, et al. Defining Disease, Diagnosis, and Translational Medicine within a Homeostatic Perturbation Paradigm: The National Institutes of Health Undiagnosed Diseases Program Experience. Frontiers in Medicine. 2017;4:62. PMID: 28603714, DOI: 10.3389/fmed.2017.00062

26 Thompson R, Johnston L, Taruscio D, Monaco L, Béroud C, Gut IG, et al. RD-Connect: an integrated platform connecting databases, registries, biobanks and clinical bioinformatics for rare disease research. J Gen Intern Med. 2014;29(suppl 3):780-7. PMID: 25029978, DOI: 10.1007/s11606-014-2908-8

27 Rath A, Olry A, Dhombres F, Brandt MM, Urbero B, Ayme S. Representation of rare diseases in health information systems: the orphanet approach to serve a wide range of end users. Hum Mutat. 2012;33:803-8. PMID: 22422702, DOI: 10.1002/humu.22078

28 Biesecker LG. Phenotype matters. Nat Genet. 2004;36:323-4. PMID: 15054484, DOI: 10.1038/ng0404-323

29 Robinson PN, Webber C. Phenotype ontologies and cross-species analysis for translational research. PLoS Genet. 2014;10:e1004268. PMID: 24699242, DOI: 10.1371/journal.pgen.1004268

30 Robinson PN. Deep phenotyping for precision medicine. Hum Mutat. 2012;33:777-80. PMID: 22504886, DOI: 10.1002/humu.22080

31 Deans AR, Lewis SE, Huala E, Anzaldo SS, Ashburner M, Balhoff JP, et al. Finding our way through phenotypes. PLoS Biol. 2015;13:e1002033. PMID: 25562316, DOI: 10.1371/journal.pbio.1002033

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Supplementary Table S1. Variants details, HPO term and rank positions of causative variants in simulated disease data

ID	Disease (OMIM term)	*Gene*	HGVS notation (GRCh37/ hg19)	Inheritance	HPO ID					Rank				
										hiPHIVE	PhenIX	Phen-Gen	combined max (eXtasy)	order statistics (eXtasy)
1	Usher syndrome 1D/F	*CDH23*	g.73558142T>G	AD,DR	0000365	0000510	0001751			1	1	-	-	-
2	DEAFNESS, AUTOSOMAL DOMINANT 3A; DFNA3A	*GJB2*	g.20763323C>T	AD	0000407					1	1	-	×	×
3	DEAFNESS, AUTOSOMAL DOMINANT 3A; DFNA3A	*GJB2*	g.20763442C>T	AD	0000407					1	3	2	×	×
4	DEAFNESS, AUTOSOMAL DOMINANT 3A; DFNA3A	*GJB2*	g.20763587C>T	AD	0000407					1	1	5	×	×
5	ALZHEIMER DISEASE 3; AD	*PSEN1*	g.73640375C>T	AD	0001260	0001332	0002395	0002354	0002185	1	1	5	1	2
6	DEAFNESS, AUTOSOMAL DOMINANT 20; DFNA20	*ACTG1*	g.79478214C>T	AD	0008619	0000408				1	1	-	279	334

Supplementary Table S1 – Continued

7	PARKINSON DISEASE, LATE-ONSET; PD	*MAPT*	g.44087739A>C	AD,Mu	0000726	0001300	0000751	0002172	0001621	1	2	-	5	2
8	PULMONARY HYPERTENSION, PRIMARY, 1; PPH1	*BMPR2*	g.203329555T>C	AD	0000822	0005308	0005312	0001708	0001009	1	1	-	66	35
9	PULMONARY HYPERTENSION, PRIMARY, 1; PPH1	*BMPR2*	g.203383587_203383588delinsAAGG	AD	0011353	0004964	0005308	0005312	0001667	1	1	-	-	-
10	PULMONARY HYPERTENSION, PRIMARY, 1; PPH1	*BMPR2*	g.203384858T>C	AD	0001977	0005317	0004964	0005308	0001667	1	1	8	73	14
11	FEINGOLD SYNDROME 1; FGLDS1	*MYCN*	g.160859401C>T	AD	0004691	0004692	0000958	0000437	0000232	1	1	-	270	178
12	DEAFNESS, AUTOSOMAL RECESSIVE 9; DFNB9	*OTOF*	g.26682895A>G	AR	0008529	0004463	0000407			1	1	-	×	×
13	DEAFNESS, AUTOSOMAL RECESSIVE 9; DFNB9	*OTOF*	g.26683617T>C	AR	0008529	0004463	0000407			1	1	4	×	×

Supplementary Table S1 – Continued

14	DEAFNESS, AUTOSOMAL RECESSIVE 9; DFNB9	*OTOF*	g.2668386 5C>T	AR		0004 463	0000 407			1	1	4	×	×
15	NOONAN SYNDROME 4; NS4	*SOS1*	g.3926258 1C>A	AD	0000 028	0000 689	0000 494	0000 368	0003 010	1	1	2	2	2
16	DEAFNESS, AUTOSOMAL RECESSIVE 8; DFNB8	*TMPRSS 3*	g.4380408 8G>A	AR	0000 407					1	1	-	×	×
17	GLYCINE ENCEPHALOPATH Y; GCE	*AMT*	g.4945957 8G>A	AR	0001 298	0002 154	0000 737	0001 336	0100 247	1	1	5	8	1
18	METHYLMALONI C ACIDURIA, cblA TYPE	*MMAA*	g.1465607 24C>T	AR	0001 944	0001 987	0002 154	0001 250	0002 013	1	1	-	58	26
19	SOTOS SYNDROME 1; SOTOS1	*NSD1*	g.1766946 45G>A	AD	0002 280	0000 218	0001 943	0000 347	0001 319	1	1	-	17	9
20	CARDIOFACIOCU TANEOUS SYNDROME 1; CFC1	*BRAF*	g.1405013 36C>G	AD	0000 280	0002 750	0000 768	0004 322	0002 217	1	1	5	1	2

Supplementary Table S1 – Continued

21	LONG QT SYNDROME 1; LQT1	*KCNQ1*	g.150648632A>G	AD	0000598	0001657	0001279	0001664	0001663	-	-	-	-	-
22	ANTLEY-BIXLER SYNDROME WITH GENITAL ANOMALIES AND DISORDERED STEROIDOGENESIS; ABS1	*POR*	g.75615163delG	AR	0000818	0012385	0000047	0000054	0002650	1	1	-	14	21
23	DEAFNESS, AUTOSOMAL RECESSIVE 4, WITH ENLARGED VESTIBULAR AQUEDUCT; DFNB4	*SLC26A4*	g.107303802C>T	AR	0011387	0000376	0000407			1	1	-	×	×
24	DEAFNESS, AUTOSOMAL RECESSIVE 4, WITH ENLARGED VESTIBULAR AQUEDUCT; DFNB4	*SLC26A4*	g.107323771C>A	AR	0011387	0000376	0000407			1	1	2	×	×

Supplementary Table S1 – Continued

25	DEAFNESS, AUTOSOMAL RECESSIVE 4, WITH ENLARGED VESTIBULAR AQUEDUCT; DFNB4	*SLC26A4*	g.107334899G>A	AR	0011387	0000376	0000407			1	1	-	×	×
26	DEAFNESS, AUTOSOMAL RECESSIVE 4, WITH ENLARGED VESTIBULAR AQUEDUCT; DFNB4	*SLC26A4*	g.107350576C>T	AR	0011387	0000376	0000407			1	1	-	×	×
27	CHARGE SYNDROME	*CHD7*	g.61654385G>T	AD	0002023	0002410	0000717	0001511	0000394	1	1	-	-	-
28	BRANCHIOOTORENAL SYNDROME 1; BOR1	*EYA1*	g.72211430G>C	AD	0004742	0009798	0008551	0000410	0000104	1	1	-	5	42
29	BECKWITH-WIEDEMANN SYNDROME; BWS	*CDKN1C*	g.2906409_2906410delinsC	AD	0002060	0004742	0009795	0009797	0000113	7	5	-	-	-

Supplementary Table S1 – Continued

30	WIEDEMANN-STEINER SYNDROME; WDSTS	*KMT2A*	g.118344021delC	AD	0009796	0001374	0010628	0000691	0000384	2	13	-	-	-
31	HYPOURICEMIA, RENAL, 1; RHUC1	*SLC22A12*	g.6436035 5G>A	AR	0001919	0003537	0012611	0000791		1	1	-	×	×
32	DEAFNESS, AUTOSOMAL RECESSIVE 21; DFNB21	*TECTA*	g.120998773G>C	AR	0000407					1	1	-	×	×
33	KABUKI SYNDROME 1; KABUK1	*KMT2D*	g.49420304_49420305delAA	AD	0000164	0000175	0000218	0010314	0000508	1	1	-	-	-
34	KABUKI SYNDROME 1; KABUK1	*KMT2D*	g.49438647G>A	AD	0000592	0001680	0004736	0001212	0000403	1	1	-	-	-
35	KABUKI SYNDROME 1; KABUK1	*KMT2D*	g.49444463C>A	AD	0000365	0001382	0000252	0000358	0000535	1	1	-	-	-
36	KABUKI SYNDROME 1; KABUK1	*KMT2D*	g.49444682_49444683insGAGGCCATCCA	AD	0001007	0001249	0001382	0008897	0002650	1	1	-	-	-

Supplementary Table S1 – Continued

37	KABUKI SYNDROME 1; KABUK1	*KMT2D*	g.49447088_49447089delAG	AD	0000164	0004736	0000400	0000358	0004467	1	1	-	-	-
38	LISSENCEPHALY 3; LIS3	*TUBA1A*	g.49580430G>A	AD	0001251	0010864	0000252	0001250	0002510	2	1	-	126	31
39	DEAFNESS, AUTOSOMAL RECESSIVE 1A; DFNB1A	*GJB2*	g.20763691dupC	DR,AR	0000407	0001751				1	1	2	×	×
40	PITUITARY HORMONE DEFICIENCY, COMBINED, 6; CPHD6	*OTX2*	g.57268761C>A	AD	0011755	0004322				1	1	-	×	×
41	CARDIOFACIOCUTANEOUS SYNDROME 1; CFC1	*BRAF*	g.25380280C>G	AD	0000463	0001622	0002019	0000486	0001276	-	-	-	-	-
42	NIJMEGEN BREAKAGE SYNDROME; NBS	*NBN*	g.90983445_90983449delGTTTT	AR	0000957	0000175	0000444	0006532	0003202	1	1	-	-	-

Supplementary Table S1 – Continued

43	LOEYS-DIETZ SYNDROME 2; LDS2	*TGFBR2*	g.3071386 6C>G	AD	0002 308	0001 363	0004 955	0100 259	0002 650	1	1	4	1	36
44	MARFAN SYNDROME; MFS	*FBN1*	g.4871292 3G>A	AD	0000 494	0007 676	0007 800	0001 548	0003 179	1	1	-	3	68
45	ANGELMAN SYNDROME; AS	*UBE3A*	g.2561693 7delC	IC	0001 344	0002 286	0001 347	0005 484	0010 808	1	1	-	-	-
46	ANGELMAN SYNDROME; AS	*UBE3A*	g.2562083 7A>G	IC	0001 344	0002 019	0000 272	0000 639	0010 808	1	1	-	62	21
47	RUBINSTEIN-TAYBI SYNDROME 1; RSTS1	*CREBBP*	g.3801726 C>A	AD	0000 589	0000 028	0005 895	0002 788	0000 736	1	1	-	-	-
48	LI-FRAUMENI SYNDROME; LFS	*TP53*	g.7574031 delG	AD	0002 488	0006 744	0030 078	0002 667	0002 669	1	1	-	-	-
49	DEAFNESS, AUTOSOMAL RECESSIVE 4, WITH ENLARGED VESTIBULAR AQUEDUCT; DFNB4	*SLC26A4*	g.1073405 60_10734 0561insT	AR	0011 387	0000 376	0000 407			1	1	5	×	×
50	CHARGE SYNDROME	*CHD7*	g.6165459 5C>T	AD	0001 719	0200 021	0000 252	0000 347	0000 054	1	1	-	-	-

Supplementary Table S1 – Continued

51	CHARGE SYNDROME	*CHD7*	g.61654604C>T	AD	0000717	0001156	0000324	0001539	0004496	1	1	4	-	-
52	CHARGE SYNDROME	*CHD7*	g.61655016delA	AD	0000834	0100736	0003974	0010751	0008551	1	1	-	-	-
53	CHARGE SYNDROME	*CHD7*	g.61655027A>T	AD	0000048	0000204	0000378	0002937	0003022	1	1	3	-	-
54	CHARGE SYNDROME	*CHD7*	g.61693850_61693851delCC	AD	0000682	0010669	0000160	0000388	0001629	1	1	-	-	-
55	CHARGE SYNDROME	*CHD7*	g.61728951_61728955delATCTT	AD	0000772	0000324	0000044	0000394	0001561	1	1	-	-	-
56	CHARGE SYNDROME	*CHD7*	g.61749422C>T	AD	0000772	0002015	0000501	0000568	0004058	1	1	3	-	-
57	CHARGE SYNDROME	*CHD7*	g.61749529C>A	AD	0000772	0001360	0002901	0000066	0001171	1	1	-	-	-
58	CHARGE SYNDROME	*CHD7*	g.61763080dupT	AD	0001305	0000821	0001601	0001252	0001883	1	1	6	-	-
59	CHARGE SYNDROME	*CHD7*	g.61769239T>A	AD	0009738	0001679	0000488	0001883	0001629	5	1	3	-	-
60	GLYCINE ENCEPHALOPATHY; GCE	*GLDC*	g.6536188A>C	AR	0001274	0000718	0000737	0001336	0000711	1	2	-	191	85

Supplementary Table S1 – Continued

61	GLYCINE ENCEPHALOPATHY; GCE	*GLDC*	g.6588688G>C	AR	0003108	0001249	0000737	0100247	0001250	1	1	4	219	159
62	CORNELIA DE LANGE SYNDROME 3; CDLS3	*HDAC8*	g.71787820G>A	AD	0001263	0000218	0000527	0000545	0200055	1	1	3	60	94
63	FOCAL DERMAL HYPOPLASIA; FDH	*PORCN*	g.48368278C>T	XLD	0001274	0004334	0100559	0010622	0009381	1	1	2	340	198
64	TELANGIECTASIA, HEREDITARY HEMORRHAGIC, TYPE 2; HHT2	*ACVRL1*	g.52307534C>T	AD	0001342	0002249	0000434	0001901	0001694	1	1	-	163	76
65	MOWAT-WILSON SYNDROME; MOWS	*ZEB2*	g.145158826_145158827delCT	AD	0040082	0009748	0004415	0004322	0001636	-	-	-	-	-
66	MOWAT-WILSON SYNDROME; MOWS	*ZEB2*	g.145158871G>A	AD	0004298	0008572	0000768	0002558	0000076	-	-	-	-	-

Supplementary Table S1 – Continued

67	TELANGIECTASIA, HEREDITARY HEMORRHAGIC, TYPE 1; HHT1	*ENG*	g.130579483delC	AD	0002094	0001901	0001694	0001250	0004406	1	1	-	-	-
68	TELANGIECTASIA, HEREDITARY HEMORRHAGIC, TYPE 1; HHT1	*ENG*	g.130592008delG	AD	0001903	0100858	0002573	0001722	0011934	1	1	-	×	×
69	PHENYLKETONURIA; PKU	*PAH*	g.103234288A>C	AR	0000718	0002514	0004923	0001347	0000737	-	-	-	-	-
70	ALAGILLE SYNDROME 1; ALGS1	*PAH*	g.103245474C>A	AD	0000518	0000490	0002155	0000110	0001328	10	4	7	8	3
71	ALAGILLE SYNDROME 1; ALGS1	*PAH*	g.103246680C>G	AD	0000585	0000490	0002937	0003022	0000486	10	14	3	7	3
72	ALAGILLE SYNDROME 1; ALGS1	*PAH*	g.103246696C>G	AD	0002910	0002937	0000400	0004969	0001947	9	10	-	1	1
73	ALAGILLE SYNDROME 1; ALGS1	*PAH*	g.103306582A>G	AD	0000772	0001394	0000490	0005280	0001629	28	14	-	55	92

Supplementary Table S1 – Continued

74	MUCOPOLYSACCHARIDOSIS, TYPE II; MPS2	*IDS*	g.148564339G>A	XLR	0002159	0002180	0000648	0001085	0001761	1	1	-	95	79
75	EXOSTOSES, MULTIPLE, TYPE II	*EXT2*	g.44151637C>A	AD	0003068	0003276	0003406	0000896	0000918	1	1	2	×	×
76	MARFAN SYNDROME; MFS	*FBN1*	g.48729201G>A	AD	0002647	0000494	0100775	0000565	0003088	4	1	-	22	88
77	MARFAN SYNDROME; MFS	*FBN1*	g.48729982C>A	AD	0001659	0000268	0007676	0000189	0004927	1	1	3	1	6
78	MARFAN SYNDROME; MFS	*FBN1*	g.48757764C>T	AD	0001166	0000268	0002816	0000501	0004927	1	1	2	-	-
79	MARFAN SYNDROME; MFS	*FBN1*	g.48780384T>C	AD	0001635	0001002	0000494	0000275	0000278	1	1	-	1	1
80	Loeys-Dietz syndrome 2	*TGFBR2*	g.30691785C>T	AD	0001156	0000175	0000316	0001634	0000278	1	1	-	1	2
81	Loeys-Dietz syndrome 2	*TGFBR2*	g.30713345C>T	AD	0002631	0001647	0001519	0009473	0002650	1	1	-	2	114
82	Loeys-Dietz syndrome 2	*TGFBR2*	g.30713705T>C	AD	0000766	0005807	0004955	0001388	0000347	1	1	3	1	1
83	Loeys-Dietz syndrome 2	*TGFBR2*	g.30732918C>T	AD	0000766	0004944	0001634	0001643	0100259	1	1	3	3	5
84	Loeys-Dietz syndrome 2	*TGFBR2*	g.30732969C>T	AD	0001631	0004955	0009473	0000939	0001762	-	-	-	-	-

Supplementary Table S1 – Continued

85	Loeys-Dietz syndrome 2	*TGFBR2*	g.30732970G>A	AD	0001166	0002308	0012385	0004944	0001363	1	1	6	12	108
86	Loeys-Dietz syndrome 1	*TGFBR1*	g.101911535G>A	AD	0005182	0001363	0000272	0000977	0001762	1	1	3	58	12
87	Mental retardation, X-linked, syndromic, Christianson type	*SLC9A6*	g.135080642_135080643delGA	XLD	0000717	0100543	0001263	0000400	0000020	-	-	-	-	-
88	Schizophrenia	*NOS1AP*	g.162337012G>A	AD	0000746	0002353	0000738	0100753	0007086	5	36	-	964	1111
89	Pseudoxanthoma elasticum	*ABCC6*	g.162569404G>A	AR	0001635	0001034	0004417	0001718	0000573	1	1	3	134	39
90	Pseudoxanthoma elasticum	*ABCC6*	g.162596709A>G	AR	0004943	0007663	0000083	0100817	0000573	1	1	-	-	-
91	Pseudoxanthoma elasticum	*ABCC6*	g.162919600C>T	AR	0000153	0004417	0000608	0001718	0001723	1	1	-	25	6
92	Hypertrophic osteoarthropathy, primary, autosomal recessive, 2	*SLCO2A1*	g.133672567C>T	AR	0002829	0001217	0030314			1	1	-	496	382
93	Ciliary dyskinesia, primary, 3	*DNAH5*	g.13777315delC	-	0012265	0002205	0001696			1	1	-	-	-
94	Ciliary dyskinesia, primary, 3	*DNAH5*	g.138307804G>A	-	0012265	0002205	0001696			1	1	-	3	1

Supplementary Table S1 – Continued

95	DEAFNESS, AUTOSOMAL RECESSIVE 4, WITH ENLARGED VESTIBULAR AQUEDUCT; DFNB4	*SLC26A4*	g.107340538C>G	AR	0011387	0000376	0000407			1	1	5	×	×
96	THROMBOTIC THROMBOCYTOPENIC PURPURA, CONGENITAL; TTP	*ADAMTS13*	g.136307568A>T	AR	0011387	0000376	0000407			12	20	-	×	×
97	Fabry disease	*GLA*	g.100653458A>G	XL	0003119	0002376	0000962	0005144	0003326	1	1	-	1	1
98	Fabry disease	*GLA*	g.100653816A>G	XL	0001131	0100820	0003394	0003401	0000407	1	1	3	10	5
99	Fabry disease	*GLA*	g.100653930T>C	XL	0001369	0000708	0003326	0001658	0000093	1	1	-	1	2
100	Fabry disease	*GLA*	g.100656798delC	XL	0001681	0001014	0001369	0100543	0012702	1	1	-	-	-

‘-’ - not ranked. ‘×’ - could not analyze.

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Supplementary Table S2. Rank positions of causative variants in patients' data

Patient	Disease (OMIM term)	*Gene*	HGVS notation (GRCh37/ hg19)	Inheritance	HPO ID					Rank				
										hiPHIVE	PhenIX	Phen-Gen	combined max (eXtasy)	order statistics (eXtasy)
1	CONGENITAL DISORDER OF GLYCOSYLATION, TYPE 1K	*ALG1*	g.5121993 G>A, g.5128843 C>T	AR	0003256	0002014	0001250	0000252	0001263	7	10	13	62	108
2	SOTOS SYNDROME	*NSD1*	g.176715832C>T	AD	0000256	0001548	0011622	0001643	0001263	1	1	-	25	2
3	JOUBERT SYNDROME 3	*AHI1*	g.135754326G>A, g.135784353C>A	AR	0002419	0000639	0001263			1	1	6	-	-
4	NEUROFIBROMATOSIS TYPE 1	*NF1*	g.29664880G>A	AD	0000957					1	1	-	×	×
5	DEAFNESS, AUTOSOMAL DOMINANT 6	*WFS1*	g.6303911 G>A	AD	0000365					1	1	-	×	×
6	HYPOCHONDROPLASIA	*FGFR3*	g.1807371 C>A	AD	0000256	0007359	0002882	0004322		2	1	-	15	1

Supplementary Table S2 – Continued

7	MITOCHONDRIAL SHORT-CHAIN ENOYL-CoA HYDRATASE 1 DEFICIENCY	*ECHS1*	g.135186833G>A, g.135186837T>C	AR	0007146	0002151	0000639	0001263	0001250	-	-	-	-	-
8	SOTOS SYNDROME	*NSD1*	g.176719045C>T	AD	0000256	0001263				1	1	-	16	13
9	AORTIC ANEURYSM FAMILIAL THORACIC 6	*ACTA2*	g.90699299C>T	AD	0002622					1	1	-	×	×
10	TELANGIECTASIA HEREDITARY HEMORRHAGIC TYPE 1	*ENG*	g.130587554A>AG	AD	0000421					3	1	-	×	×
11	JOUBERT SYNDROME 5	*CEP290*	g.884565556C>T, g.88462434A>T	AR	0002419	0001337	0000496	0001249		1	1	-	-	-
12	MITOCHONDRIAL SHORT-CHAIN ENOYL-CoA HYDRATASE 1 DEFICIENCY	*ECHS1*	g.135184174T>C, g.135186833G>A	AR	0007146	0002072	0001250	0001263		-	-	-	-	-

Supplementary Table S2 – Continued

13	MARFAN SYNDROME	*FBN1*	g.4893692 4CG>C	AD	0001 166	0002 622				1	1	-	×	×
14	NEUROFIBROMATOSIS TYPE1	*NF1*	g.2955052 1CAG>C	AD	0000 957					1	1	-	×	×
15	RETT SYNDROME CONGENITAL VARIANT	*FOXG1*	g.2923693 8C>CG	AD	0005 484	0001 263	0007 359	0002 072		1	1	-	-	-
16	MIGRAINE FAMILIAL HEMIPLEGIC1	*CACNA1A*	g.1341900 3C>G	AD	0006 846	0001 249	0001 250			4	14	-	-	-
17	NOONAN SYNDROME-LIKE DISORDER WITH LOOSE ANAGEN HAIR1	*SHOC2*	g.1127241 20A>G	AD	0001 249	0000 316	0004 322			1	1	-	198	97
18	NEUROFIBROMATOSIS TYPE1	*NF1*	g.2966508 0GC>G	AD	0000 957	0001 263				1	1	-	×	×
19	NIEMANN-PICK DISEASE TYPE C1	*NPC1*	g.2112447 0T>TAG, g.2114147 9G>A	AR	0002 505	0001 730	0001 260	0100 543	0000 130	2	16	19	149	18
20	NEUROFIBROMATOSIS TYPE 1	*NF1*	g.2956301 6T>TA	AD	0000 957					1	1	-	×	×

‘-’ - not ranked. ‘×’ - could not analyze.