Improving the diagnostic yield of exome-sequencing, by predicting gene-phenotype associations using large-scale gene expression analysis

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Abstract

Clinical interpretation of exome and genome sequencing data remains challenging and time consuming, with many variants with unknown effects found in genes with unknown functions. Automated prioritization of these variants can improve the speed of current diagnostics and identify previously unknown disease genes. Here, we used 31,499 RNA-seq samples to predict the phenotypic consequences of variants in genes. We developed GeneNetwork Assisted Diagnostic Optimization (GADO), a tool that uses these predictions in combination with a patient's phenotype, denoted using HPO terms, to prioritize identified variants and ease interpretation. GADO is unique because it does not rely on existing knowledge of a gene and can therefore prioritize variants missed by tools that rely on existing annotations or pathway membership. In a validation trial on patients with a known genetic diagnosis, GADO prioritized the causative gene within the top 3 for 41% of the cases. Applying GADO to a cohort of 38 patients without genetic diagnosis, yielded new candidate genes for seven cases. Our results highlight the added value of GADO (www.genenetwork.nl) for increasing diagnostic yield and for implicating previously unknown disease-causing genes.

Introduction

With the increasing use of whole-exome sequencing (WES) and whole-genome sequencing (WGS) to diagnose patients with a suspected genetic disorder, diagnostic yield is steadily increasing [1]. Although our knowledge of the genetic basis of Mendelian diseases has improved considerably, the underlying cause remains elusive for a substantial proportion of cases. The diagnostic yield of genome sequencing varies from 8% to 70% depending on the patient's phenotype and the extent of genetic testing [2]. Sequencing all ~20,000 protein-coding genes by WES and entire genomes by WGS usually increases sensitivity but decreases specificity: it results in off-target noise and reveals many variants of uncertain

50 clinical significance. In a study by Yang *et al.*, proband-only WES identified approximately
51 875 variants in each patient, even after removing low quality variants [3].

52 One strategy to manage the list of genetic variants is to perform trio analysis of samples
53 from the proband and both of his or her biological parents to ascertain, for instance,
54 whether a variant has *de novo* status [4]. Another strategy is to limit the analyses to a gene
55 panel of Online Mendelian Inheritance in Men (OMIM) disease-annotated genes [5] or genes
56 known to be directly related to the patient's phenotype. However, determining the actual
57 disease-causing variant requires further variant filtering based on information about its
58 predicted functional consequence, population frequency data, conservation, disease-specific
59 databases (such as the Human Gene Mutation Database [6]), literature, and segregation
60 analysis [7].

61 Several tools have been developed that aid in variant filtering and prioritization [8,9].
62 Annotation tools, such as VEP [10] and GAVIN [9], offer additional functionality that allows
63 variants to be filtered according to their population frequency and variant class. Other tools
64 use phenotype descriptions to rank potential candidates genes [11]. The phenotypes are
65 typically described in a structured manner, e.g. using Human Phenotype Ontology (HPO)
66 terms [12]. AMELIE (Automatic Mendelian Literature Evaluation), for example, prioritizes
67 candidate genes by their likelihood of causing the patient's phenotype based on automated
68 literature analysis [13]. However, this focus on what is known may inadvertently filter out
69 variants in potential novel disease genes. Alternatively, the causative gene defect could be
70 missed if a patient's phenotype differs from the features previously reported to be
71 associated to a disease gene. Tools like Exomiser can identify novel human disease genes,
72 as it prioritizes variants based on semantic phenotypic similarity between a patient's
73 phenotype described by HPO terms and HPO-annotated diseases, Mammalian Phenotype
74 Ontology (MPO)-annotated mouse and Zebrafish Phenotype Ontology (ZPO)-annotated fish
75 models associated with each exomic candidate and/or its neighbors in an interaction

76 network [14]. However, most available algorithms are based on existing knowledge on
77 human disease genes, their orthologues in animal models, or well-described biological
78 pathways (for a detailed review see [11]).

79 To overcome this, we hypothesized that co-regulation of expression data could be used to
80 prioritize variants, including those in less well studied genes. We assumed that if a gene or
81 a gene set is known to cause a specific disease or disease symptom, these genes will often
82 have similar molecular functions or be involved in the same biological process or pathway.
83 We reasoned that variants in genes with yet unknown function that are involved in the same
84 biological pathway or co-regulated with known disease genes likely result in the same
85 phenotype. In order to identify groups of genes with a related biological function, we used
86 an expansive compendium of 31,499 RNA-sequencing (RNA-seq) gene expression samples
87 to predict functions for genes with high accuracy.

88 We then developed a user-friendly tool that can prioritize variants in known *and* unknown
89 genes based on our functional predictions, which we designated GeneNetwork Assisted
90 Diagnostic Optimization (GADO). GADO ranks variants based on gene co-regulation in
91 publicly available expression data of a wide range of tissues and cell types using HPO terms
92 to describe a patient's phenotype. To validate our prioritization method, we tested how well
93 our method predicts disease-causing genes based on features described for each of the
94 genes in the OMIM database. We then used exome sequencing data of patients with a
95 known genetic diagnosis to benchmark GADO. Finally, we applied our methodology to
96 previously inconclusive WES data and identified several genes that contain variants that
97 likely explain the phenotype of the respective patients. Thus, we show that our methodology
98 is successful in identifying variants in novel, potentially relevant genes explaining the
99 patient's phenotype.

Results

Gene prioritization using GADO

We have developed GADO to perform gene prioritizations using the phenotypes observed in patients denoted as HPO terms [15]. In combination with a list of candidate genes (i.e. genes harboring rare and possibly damaging variants), this results in a ranked list of genes with the most likely candidate genes on top (**Figure 1**a). The gene prioritizations are based on the predicted involvement of the candidate genes for the specified set of HPO terms. These predictions are made by analyzing public RNA-seq data from 31,499 samples (**Figure 1**b), resulting in a gene prediction score for each HPO term. These predictions are solely based on co-regulation of genes annotated to a certain HPO term with other genes. This makes it possible to also prioritize genes that currently lack any biological annotation.

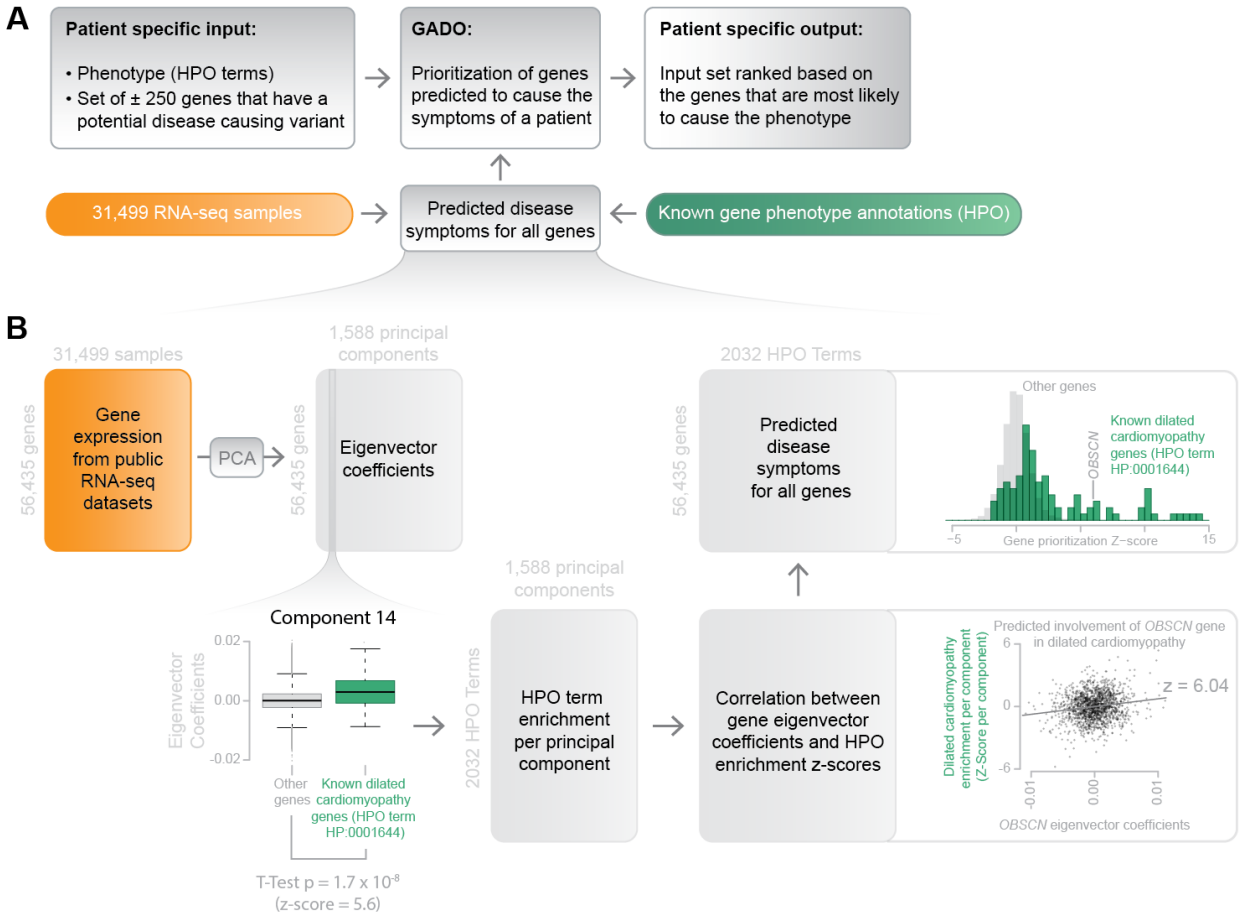


Figure 1: Schematic overview of GADO. *(a) Per patient, GADO requires a set of phenotypic features and a list of candidate genes (i.e. genes harboring rare alleles that are predicted to be pathogenic) as input. It then ascertains whether genes have been predicted to cause these features, and which ones are present in the set of candidate genes that has been provided as input. The predicted HPO phenotypes are based on the co-regulation of genes with sets of genes that are already known to be associated with that phenotype. (b) Overview of how disease symptoms are predicted using gene expression data from 31,499 human RNA-seq samples. A principal component analysis on the co-expression matrix results in the identification of 1,588 significant principal components. For each HPO term we investigate every component: per component we test whether there is a significant difference between eigenvector coefficients of genes known to cause a specific phenotype and a background set of genes. This results in a matrix that indicates which principal components are informative for every HPO term. By correlating this matrix to the eigenvector coefficients of every individual gene, it is possible to infer the likely HPO disease phenotype term that would be the result of a pathogenic variant in that gene.*

Public RNA-seq data acquisition and quality control

To predict functions of genes and HPO term associations, we downloaded all human RNA-seq samples publicly available in the European Nucleotide Archive (accessed June 30, 2016) (supplementary table 1) [16]. We quantified gene-expression using Kallisto [17] and removed samples for which a limited number of reads are mapped. We used a principal

131 component analysis (PCA) on the correlation matrix to remove low quality samples and
132 samples that were annotated as RNA-seq but turned out to be DNA-seq. In the end, we
133 included 31,499 samples and quantified gene expression levels for 56,435 genes (of which
134 22,375 are protein-coding).

135 Although these samples are generated in many different laboratories, we previously
136 observed that, after having corrected for technical biases, it is possible to integrate these
137 samples into a single expression dataset [18]. We validated that this is also true for our new
138 dataset by visualizing the data using t-Distributed Stochastic Neighbor Embedding (t-SNE).
139 We labeled the samples based on cell-type or tissue and we observed that samples cluster
140 together based on cell-type or tissue origin (**Figure 2**a). Technical biases, such as whether
141 single-end or paired-end sequencing had been used, did not lead to erroneous clusters,
142 which suggests that this heterogeneous dataset can be used to ascertain co-regulation
143 between genes and can thus serve as the basis for predicting the functions of genes.

144 **Prediction of gene HPO associations and gene functions**

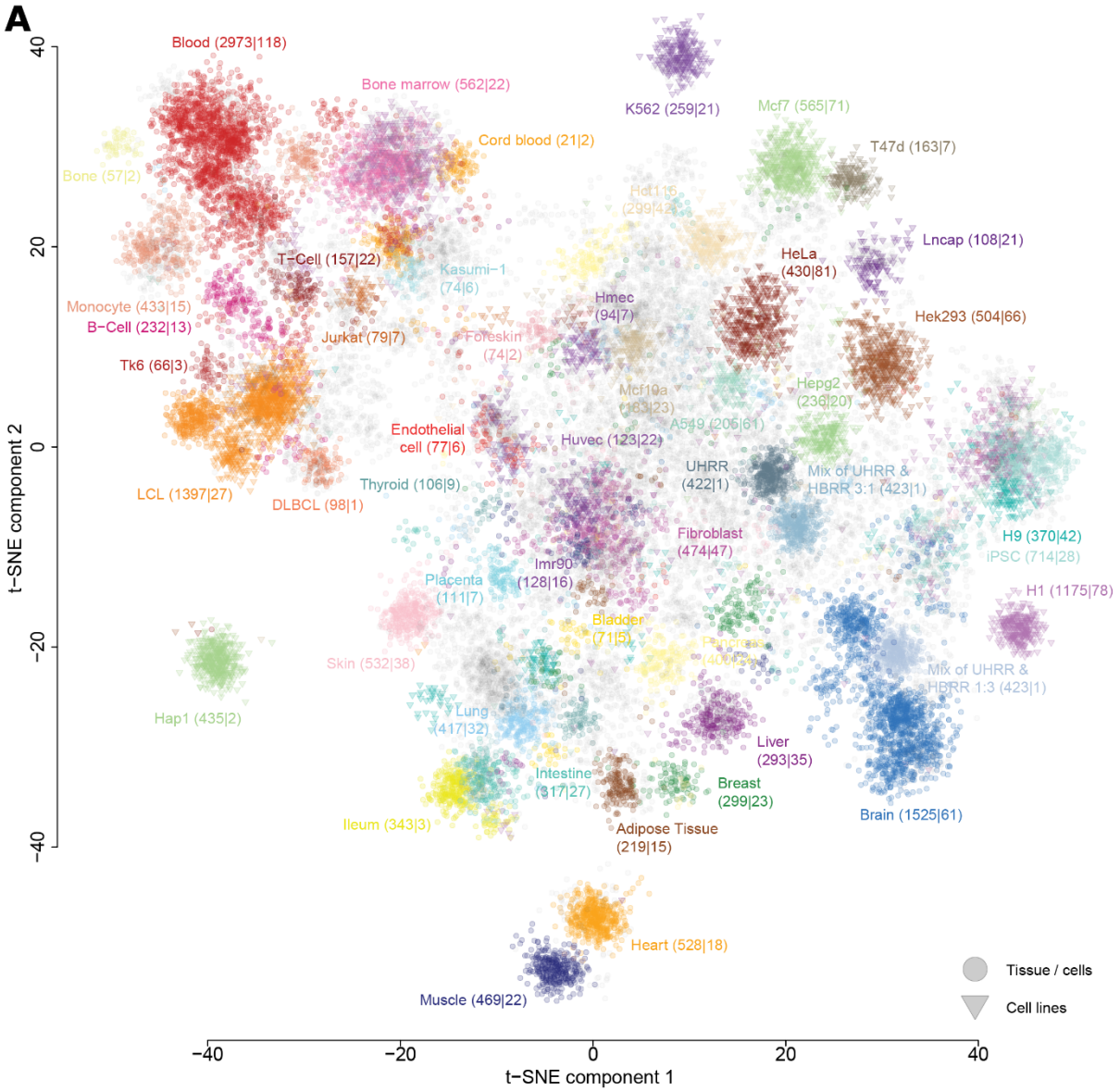
145 To predict HPO term associations and putative gene functions using co-regulation (**Figure**
146 **1**b), we used a method that we had previously developed and applied to public expression
147 microarrays [19]. Since these microarrays only cover a subset of the protein-coding genes
148 (n = 14,510), we decided to use public RNA-seq data instead. This allows for more accurate
149 quantification of lower expressed genes and the expression quantification of many more
150 genes, including a large number of non-protein-coding genes. [20].

151 We applied this prediction methodology [19] to the HPO gene sets and also to Reactome
152 [21], KEGG pathways [22], Gene Ontology (GO) molecular function, GO biological process
153 and GO cellular component [23] gene sets. For 5,088 of the 8,657 gene sets (59%) with at
154 least 10 genes annotated, the gene function predictions had significant predictive power
155 (see materials and methods). For the 8,657 gene sets with at least 10 genes annotated, the

156 median predictive power, denoted as Area Under the Curve (AUC), ranged between 0.73

157 (HPO) to 0.87 (Reactome) (**Figure 2**b).

A



B

Database	Number of gene sets	Gene sets ≥ 10 genes	Gene sets with significant predictive power	Median AUC
Reactome	2,143	1,388	1,150	0.87
GO molecular function	4,070	726	398	0.82
GO biological process	11,753	2,576	1,115	0.82
GO cellular component	1,609	500	370	0.84
KEGG	186	186	168	0.84
HPO	7,920	3,281	1,887	0.73

159 ***Figure 2: A compendium of gene expression profiles that can be used for gene function***
160 ***prediction*** *(a) 31,499 RNA-seq samples derived from many different studies show coherent clustering*
161 *after correcting for technical biases. Generally, samples originating from the same tissue, cell-type or*
162 *cell-line cluster together. The two axes denote the first t-SNE components. (b) Gene co-expression*
163 *information of 31,499 samples is used to predict gene functions. We show the prediction accuracy for*
164 *gene sets from different databases. AUC, Area Under the Curve, GO, Gene Ontology, HPO, Human*
165 *Phenotype Ontology.*

166 **Prioritization of known disease genes using the annotated HPO terms**

167 Once we had calculated the prediction scores of HPO disease phenotypes, we leveraged
168 these scores to prioritize genes found by sequencing the DNA of a patient. For each
169 individual HPO term–gene combination, we calculated a prediction z-score that can be used
170 to rank genes. In practice, however, patients often present with not one feature but a
171 combination of multiple features. Therefore, we combined the z-scores for each HPO term
172 [24] to generate an overall z-score that explains the full spectrum of features in a patient.
173 GADO uses these combined z-scores to prioritize the candidate genes: the higher the
174 combined z-score for a gene, the more likely it explains the patient's phenotype.

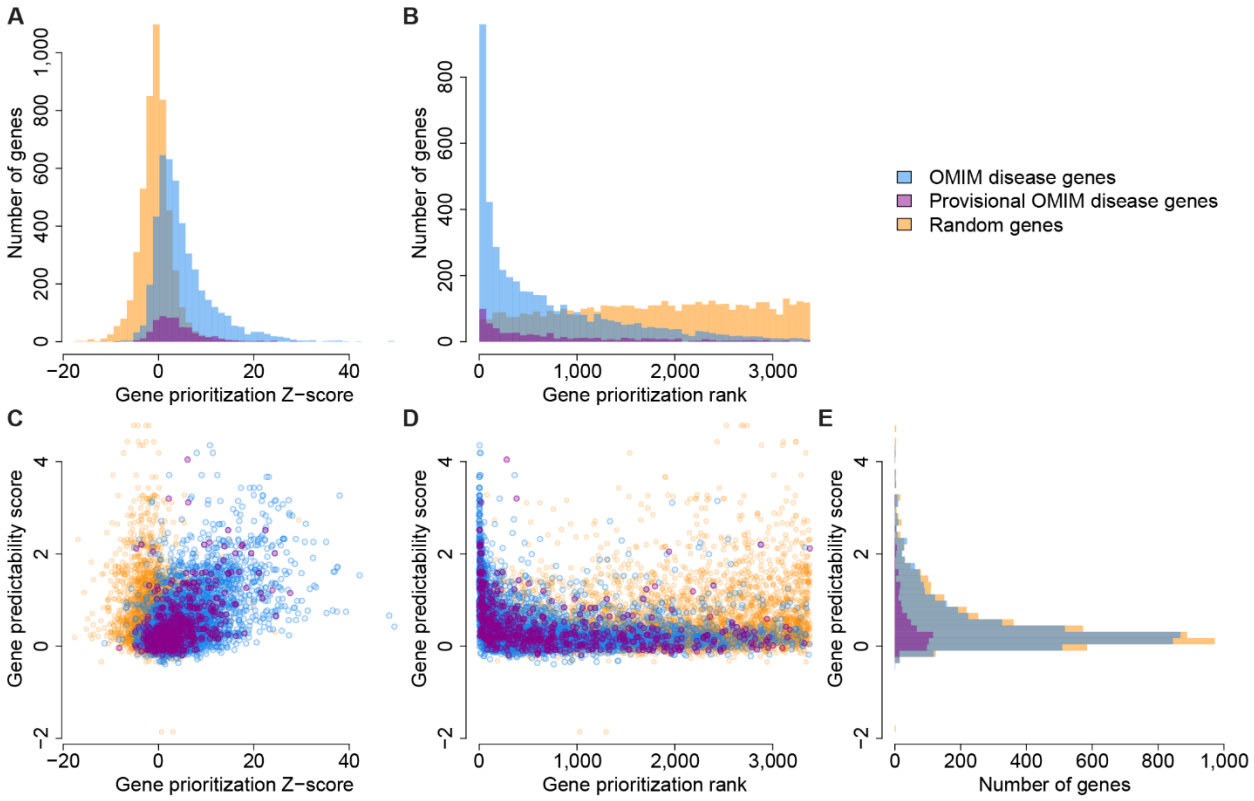
175 Because many HPO terms have fewer than 10 genes annotated, and since we were unable
176 to make significant predictions for some HPO terms, certain HPO terms are not suitable to
177 use for gene prioritization. We solved this problem by taking advantage of the way HPO
178 terms are structured. Each term has at least one parent HPO term that describes a more
179 generic phenotype and thus has also more genes assigned to it. Therefore, if an HPO term
180 cannot be used, GADO will make suggestions for suitable parental terms (supplementary
181 figure 1).

182 To benchmark our prioritization method, we used the OMIM database [5]. We tested how
183 well our method was able to retrospectively rank disease-causing genes listed in OMIM
184 based on the annotated symptoms of these diseases. We took each OMIM disease gene (n
185 = 3,382) and used the associated disease features (15 per gene on average) as input for
186 GADO. What we found was that for 49% of the diseases GADO ranks the causative gene in
187 the top 5% (**Figure 3**a, b). Moreover, we observed a statistically significant difference

188 between the performance of GADO on true gene-phenotype combinations and its

189 performance using a random permutation of gene-phenotype combinations (p-value = 2.16

190 × 10^{-532}).



191

192 ***Figure 3: Performance of disease gene prioritization compared to random permutation.*** *(a)*
193 *OMIM disease genes and provisional disease genes have significantly stronger z-scores compared to*
194 *permuted disease genes (T-test p-values: 2.16×10^{-532} & 5.38×10^{-80}, respectively). We also observe*
195 *that the predictions of the provisional OMIM genes are, on average, weaker than the other OMIM*
196 *disease genes (T-test p-value: 1.89×10^{-7}). (b) Ranking the disease based on z-scores shows GADO's*
197 *ability to prioritize the causative gene for a disease among all OMIM genes. For 49% of the disorders*
198 *the causative gene is ranked in the top 5%. (c) We observe a clear relation between the prioritization*
199 *z-scores and the gene predictability scores (Pearson r = 0.54). We don't observe this relation in the*
200 *permuted results. (d) GeneNetwork performs best for genes with high predictability scores. (e) The*
201 *different groups have similar distributions of gene predictability scores.*

202 **Gene predictability scores explains performance differences between genes**

203 For some combinations of genes and HPO terms listed in OMIM, GADO could not establish

204 the gene-phenotype combination (**Figure 3**). For example, variants in *SLC6A3* are known to

205 cause infantile Parkinsonism-dystonia (MIM 613135) [25–27], but GADO was unable predict

206 the annotated HPO terms related to the Parkinsonism-dystonia for this gene. This may,

207 however, be due to very low expression levels of *SLC6A3* in most tissues except specific
208 brain regions [28].

209 To better understand why we can't predict HPO terms for all genes, we used the Reactome,
210 GO and KEGG prediction scores. Jointly these databases comprise thousands of gene sets.
211 Since these databases describe such a wide range of biology, we assumed that if a gene
212 does not show any prediction signal for any gene set in these databases, gene co-
213 expression is probably not informative for this gene. To quantify this, we calculated, per
214 gene, the average skewness of the z-score distribution of the Reactome, GO and KEGG gene
215 sets. From this we were able to derive a 'gene predictability score' for every gene that is
216 independent of whether this gene is already known to play a role in any a disease or
217 pathway (**Figure 3**c, d, e). We then ascertained whether these 'gene predictability scores'
218 are correlated with the prediction z-score of the OMIM diseases, and found a strong
219 correlation (Pearson r = 0.54, p-value = 1.14×10^{-332}) between the gene predictability
220 scores and GADO's ability to identify a known disease gene (**Figure 3**c).

221 To investigate why some genes have a high 'gene predictability score' but low prediction
222 performance, we scored a set of genes known to cause cardiomyopathy (CM) for the
223 amount of literature evidence that these genes cause CM. We found several genes for which
224 the prediction score for the CM phenotype is lower than expected based on the gene
225 predictability scores (supplementary figure 2a). Pathogenic variants in the *TTR* gene
226 implicated in hereditary amyloidosis (MIM 105210) [29], for instance, cause accumulation of
227 the transthyretin protein in different organ systems, including the heart, resulting in CM.
228 However, this gene is primarily expressed in the liver. Therefore, its disease mechanism is
229 different from other mechanisms resulting in CM, as many inherited CMs are caused by
230 deleterious variants in genes highly expressed in the heart and directly affecting the
231 function of the cardiac sarcomere. Therefore, the phenotypic function prediction for this
232 gene may be worse than we would expect based on the predictability score. We performed a

233 similar analysis using the HPO term 'dilated cardiomyopathy' and observed a low prediction
234 performance for the *TMPO* gene, despite a high gene predictability score (supplementary
235 figure 2b). Previously, this gene was reported to be related to dilated cardiomyopathy
236 (DCM) and listed as such by OMIM. However, recent reclassification of the reported variants
237 using the ExAC data revealed that the reported variant was far too common to be causative
238 for DCM [30].

239 **Benchmarking GADO using solved cases with realistic phenotyping**

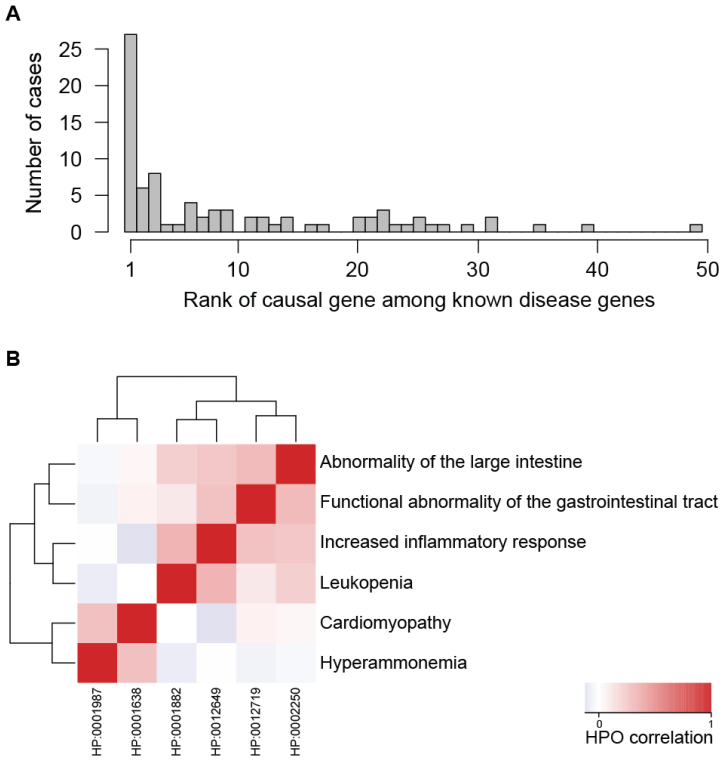
240 Although *in silico* benchmarking demonstrated the potential of GADO, it used all annotated
241 HPO terms for a disease. In practice, however, patients may only present with a limited
242 number of the annotated features. To perform a validation that was a more realistic
243 reflection of clinical practice, we used exome sequencing data of 83 patients with a known
244 genetic diagnosis. We used their phenotypic features as listed in their medical records prior
245 to the genetic diagnosis (supplementary table 2). On average, per patient, GADO yielded 56
246 possible disease-causing genes with variants that are rare and predicted to be deleterious.
247 In 41% of the patients the actual causative gene was ranked in the top 3 and in 50% of the
248 cases it was in the top 5 (mean rank 10) (**Figure 4**a).

249 **Clustering of HPO terms**

250 In addition to ranking potentially causative genes based on a patient's phenotype, we
251 observed that GADO can be used to cluster HPO terms based on the genes that are predicted
252 to be associated to these HPO terms. This can help identify pairs of symptoms that often occur
253 together, as well as symptoms that rarely co-occur, and we actually observed this for a patient
254 suspected of having two different diseases. This patient is diagnosed with a glycogen storage
255 disease, GSD type Ib, caused by compound heterozygous variants in *SLC37A4* (MIM 602671)
256 and DCM that is probably caused by a truncating variant in *TTN* (MIM 188840). Clustering of
257 the assigned HPO terms placed the phenotypic features related to GSD type Ib ('leukopenia'
258 (HP:0001882) and 'inflammation of the large intestine' (HP:0002037)) together, while

259 Cardiomyopathy (HP:0001638) was only weakly correlated to these specific features (**Figure**

260 **4**b).



261

262 ***Figure 4: Performance of GeneNetwork on solved cases*** *(a) Rank of the known causative gene*
263 *among the candidate disease causing variants. (b) Our cohort contained a case with two distinct*
264 *conditions, and clustering showed the HPO terms of the same disease are closest to each other. Note,*
265 *the HPO term "Inflammation of the large intestine" did not yield a significant prediction profile and*
266 *therefore the parent terms "Abnormality of the large intestine", "Increased inflammatory response"*
267 *and "Functional abnormality of the gastrointestinal tract" where used for this case.*

268 **Reanalysis of previously unsolved cases**

269 To assess GADO's ability to discover new disease genes, we applied it to data from 38

270 patients who are suspected to have a Mendelian disease but who have not had a genetic

271 diagnosis. All patients had undergone prior genetic testing (WES with analysis of a gene

272 panel according to their phenotype, supplementary table 3). On average three genes had a

273 z-score ≥ 5 (which we used as an arbitrary cut-off and that correspond to a p-value of 5.7 X

274 10^{-7}) and were further assessed. In seven cases, we identified variants in genes not

275 associated to a disease in OMIM or other databases, but for which we could find literature or

276 for which we gained functional evidence implicating their disease relevance (**Table 1**). For
277 example, we identified two cases with DCM with rare compound heterozygous variants in
278 the *OBSCN* gene (MIM 608616) that are predicted to be damaging. In literature, inherited
279 variant(s) in *OBSCN*, encoding obscurin, are associated with hypertrophic CM [31] and DCM
280 [32]. Furthermore, obscurin is a known interaction partner of titin (TTN), a well-known
281 DCM-related protein [31]. Another example came from a patient with ichthyotic peeling skin
282 syndrome, which is caused by a damaging variant in *FLG2 (*MIM 616284). We recently
283 published this case where we prioritized this gene using an alpha version of GADO [33].

HPO terms used	Number of genes with candidate variant	Number of genes with z ≥ 5	Candidate gene	Variants	CADD scores	GnomAD minor allele frequency	Supporting papers	Expression in relevant tissue
HP:0001644	247	5	*OBSCN*	NM_001098623.2: c.[15037C>T]; [20963delC]	24.8 25.2	8.0×10^{-5} 1.7×10^{-3}	[31, 32]	Yes
HP:0001644	226	3	*OBSCN*	NM_001098623.2: c.[5545C>T]; [22384+3_22384+21del]	14.7 7.8	3.2×10^{-4} 0	[31, 32]	Yes
HP:0008066 HP:0008064	359	3	*FLG2*	NM_001014342.2: c.[632C>G]; [632C>G]	35.0 35.0	1.1×10^{-5} 1.1×10^{-5}	[34]	Yes
HP:0001263 HP:0001249 HP:0000717 HP:0000708 HP:0002167 HP:0002360 HP:0000664	206	12	*INO80*	NM_017553.2: c. [898C>T]	34	0	[35, 36]	Yes
HP:0001644	346*	2	MB	NM_00203377.1: c.[214G>A]	22.4	3.6×10^{-5}	[37]	Yes
HP:0001644	126*	1	*SYNPO2L***	NM_001114133.2: c.[473G>A]	24.1	5.4×10^{-4}	[38]	Yes
HP:0001638	336	4	*NRAP***	NM_001261463.1: c.[4648C>T]	20.4	8.7×10^{-4}	[39]	Yes

Table 1: unsolved cases with new candidate genes. *Out of the 38 unsolved patients investigated, we identified candidate genes in seven patients. For these genes we have found literature that indicates these genes fit the phenotype of these patients or for which we gained functional evidence implicating their disease relevance. *These variants where pre-filtered for family segregation. **The variants in these genes do not fully explain the phenotype but are likely contributing to the phenotype.*

www.genenetwork.nl

All analyses described in this paper can be performed using our online toolbox at www.genenetwork.nl. Users can perform gene prioritizations using GADO by providing a set of HPO terms and a list of candidate genes (**Figure 5**a). Per gene, it is also possible to download all prediction scores for the HPO terms and pathways. Our co-regulation scores between genes can be used for clustering. Furthermore, the predicted pathway and HPO

295 annotations of genes can be used to perform function enrichment analysis (**Figure 5**b). We

296 also support automated queries to our database.

A

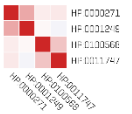
GENE NETWORK

Search here or paste a list of multiple genes (Ensembl IDs or HGNC symbols)

HOME API

PHENOTYPE	ANNOTATED GENES	HPO-TERM
Abnormality of the face	1948	HP:0000271
Intellectual disability	1289	HP:0001249
Neoplasm of the endocrine system	88	HP:0100568
Abnormality of the anterior pituitary	186	HP:0011747

PHENOTYPE CORRELATION



Genes not found
LOC101448202

Gene prioritization

RANK	GENE	Z-SCORE	NETWORK	HP:0000271	HP:0001249	HP:0100568	HP:0011747
1	MT-CYB	3.3		0.2	-2	-1.5	9.9
2	EHMT1	3		2.6	1.5	0.6	1.3
3	ZNF236	2.9		1.6	2.6	1	0.6
4	OCAF5	2.5		1.7	1	1.1	1.1
5	SLC25A5	2.3		0.8	4.1	0.7	-0.8
6	GCC2	2.3		1.4	0.7	1.5	1
7	TYMS	2.3		1.3	2.6	0.7	-0.1
8	IPO11	2.2		-0.7	1.7	1.6	1.9
9	ASH1L	2.2		1.7	1.7	1.5	-0.5
10	EIF4ENIF1	2.1		1.1	3	0.1	0
11	SOCS5	2		2.3	-0.1	0.9	0.9
12	DNA2	2		0.1	1.7	1.5	0.5
13	RNPEP	1.9		1.5	0.5	1.1	0.7
14	ATP50	1.9		0.5	0.9	0.7	1.7
15	MAML2	1.9		1	1.1	0.9	0.8
16	OMXL2	1.8		0.9	1.7	1.1	0
17	GRK5	1.8		0.4	0.3	1.4	1.5
18	PALM	1.8		1.3	-0.2	1.5	1
19	LRCH2	1.8		0.8	1.7	-0.1	1.1
20	PKHD1L1	1.8		0.3	1.7	1.1	0.4
21	SMG6	1.7		2.7	2	-0.4	-0.9

B

GENE NETWORK

Search here or paste a list of multiple genes (Ensembl IDs or HGNC symbols)

HOME API

SHOW PREDICTED GENES ANNOTATED GENES NETWORK

ALL GENES	100
CLUSTER 1	31
CLUSTER 2	28
CLUSTER 3	22
CLUSTER 4	19
MY SELECTION	0

ANALYSE CLUSTER 1

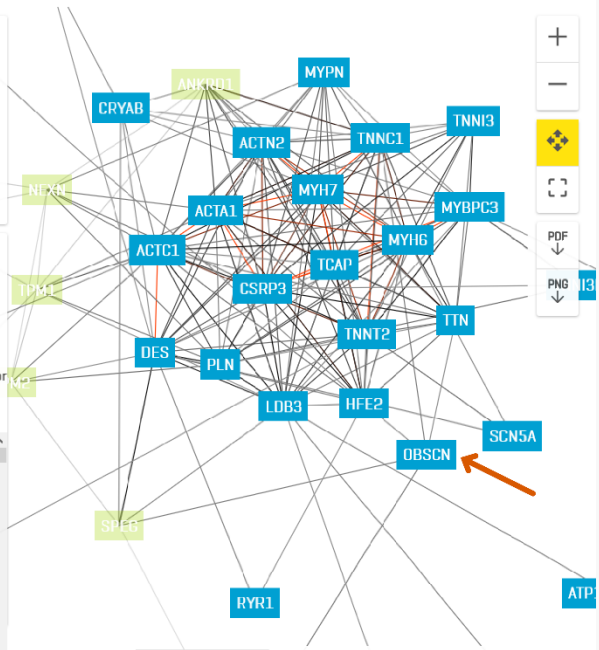
PATHWAYS & PHENOTYPES GENES

REACTOME HPO GO P GO F GO C KEGG

This is REACTOME pathway enrichment for Cluster 1.

filter

PATHWAY	P-VALUE
Cardiac conduction	1.0×10^{-14}
Ion homeostasis	4.5×10^{-14}
Striated Muscle Contraction	5.4×10^{-14}
Muscle contraction	7.1×10^{-14}
Formation of RNA Pol II elongation complex	6.0×10^{-11}
RNA Polymerase II Transcription Elongation	6.0×10^{-11}
HIV Transcription Elongation	6.7×10^{-11}
Formation of HIV-1	



COLOR GENES BY CLUSTER Cluster 1 Cluster 2 Cluster 3 Cluster 4

297

298 ***Figure 5: www.genenetwork.nl*** *(a) Prioritization results of one of our previously solved cases. This*
299 *patient was diagnosed with Kleefstra syndrome. The patient only showed a few of the phenotypic*
300 *features associated with Kleefstra syndrome and additionally had a neoplasm of the pituitary (which is*
301 *not associated with Kleefstra syndrome). Despite this limited overlap in phenotypic features, GADO*
302 *was able to rank the causative gene (EHMT1) second. Here, we also show the value of the HPO*
303 *clustering heatmap, the two terms related to the neoplasm cluster separately from the intellectual*
304 *disability and the facial abnormalities that are associated to Kleefstra syndrome. (b) Clustering of a set*
305 *of genes allowing function / HPO enrichment of all genes or specific enrichment of automatically*
306 *defined sub clusters. Here we loaded all known DCM genes and OBSCN, and we focus on a sub-cluster*
307 *of genes containing OBSCN (highlighted by the arrow). We see that it is strongly co-regulated with*
308 *many of the known DCM genes. Pathway enrichment of this sub-cluster reveals that these genes are*
309 *most strongly enriched for the muscle contraction Reactome pathway. DCM, Dilated Cardiomyopathy.*

310 # Discussion

311 Prioritizing genes from WES or WGS data remains challenging. To meet this challenge, we
312 developed GADO, a novel tool to prioritize genes based on the phenotypic features of a
313 patient. Since the classification of variants is labor-intensive, prioritization of the most likely
314 candidate variants saves time in the diagnostic process.

315 Importantly, GADO can also aid in the discovery of currently unknown disease genes. The
316 main advantage of our methodology is that it does not rely on any prior knowledge about
317 disease-gene annotations. Instead, we used predicted gene functions based on co-
318 expression networks extracted from a large compendium of publicly available RNA-seq
319 samples. RNA-seq has previously shown to be very helpful to accurately quantify expression
320 levels of lowly expressed genes and non-coding genes [18]. To evaluate our diagnostic
321 algorithm, we developed a testing scenario based on simulated patients presenting with all
322 clinical features listed in OMIM for a certain disease or syndrome. This validation test
323 showed that for 49% of the diseases the causative gene ranks in the top 5%. We also
324 investigated the OMIM "provisional" category of genes for which there is limited evidence.
325 Both the OMIM disease-gene annotation and the provisional annotations perform
326 significantly better than a random permutation. While we do find a small but significant
327 difference in prediction performance between the provisionally annotated genes and the
328 more established disease associated genes, we conclude, based on our findings, that these

329 provisional OMIM annotations are generally of similar reliability to the other OMIM disease
330 annotations.

331 Benchmarking on sequence data of patients with a known genetic diagnosis revealed that
332 GADO returned the real causative variant within the top 3 results for 41% of the samples,
333 indicating the potential power of GADO for a large number of diseases. Finally, in seven
334 patients, GADO was able to identify potential novel disease genes that are strong candidates
335 based on literature or functional evidence. For other cases we have identified genes with a
336 strong prediction score harboring variants that might explain the phenotype. However, since
337 very little is known about these genes it is not yet possible to draw firm conclusions.
338 Hopefully this will become possible in the near future through initiatives like Genematcher
339 [40].

340 **Potential to discover novel human disease genes**

341 Over the last decade, several computational tools have been developed to prioritize variants
342 in genes. Some, such as GAVIN, focus on variant filtering and prioritization based on
343 deleteriousness scores, allele frequency and inheritance model [9]. Other methods measure
344 the similarity between the clinical manifestations observed in a patient and those
345 representing each of the diseases in a database or literature. Exomiser is closely related to
346 GADO as it prioritizes genes based on specified HPO terms and also infers HPO annotation
347 for unknown genes [14]. The gene prioritization by Exomiser is based on the effects of
348 orthologs in model organisms and applies a guilt-by-association method using protein-
349 protein associations provided by STRING [41]. Exomiser performs better than GADO in
350 ranking known disease-causing genes (supplementary figure 3, supplementary table 4) and
351 is also able to identify potential new genes in human disease. However, Exomiser has a
352 limitation in that only a subset of the protein-coding genes has orthologous genes in other
353 species for which a knockout model also exists. Additionally, the used STRING interactions
354 are biased towards well studied genes and rely heavily on existing annotations to biological

355 pathways (supplementary figure 4). There are however, still 3,922 protein-coding genes
356 that are not currently annotated in any of the databases we used, and there are even more
357 non-coding genes for which the biological function or role in disease is unknown. Since
358 GADO does not rely on prior knowledge, it can be used to prioritize variants in both coding
359 *and* non-coding genes (for which no or limited information is available). GADO thus enables
360 the discovery of novel human disease genes and can complement existing tools in analyzing
361 the genomic data of patients who have a broad spectrum of phenotypic abnormalities.

362 **Limitations**

363 The gene predictability score indicates for which genes we can reliably predict phenotypic
364 associations and for which genes we cannot based on gene co-regulation. This score gives
365 insight into which genes are expected to perform poorly in our prioritization. We found
366 strong correlation between these gene predictability scores and the gene prioritization z-
367 scores. Thus, genes with a high predictability score have more accurate HPO term
368 predictions. However, since our predictions primarily rely on co-activation patterns that we
369 identified from RNA-seq data, our method does not perform well for genes where gene-
370 expression patterns are not informative of their function. This could, for instance, be the
371 case for proteins relying heavily on post-translation modifications for regulation or genes for
372 which different transcripts have distinct functions. This last limitation can potentially be
373 overcome by predicting HPO-isoform associations by using transcript-based expression
374 quantification.

375 Insufficient statistical power to obtain accurate predictions may be another explanation for
376 the low predictability scores of certain genes. This may be true for genes that are poorly
377 expressed or expressed in only a few of the available RNA-seq samples. The latter issue we
378 expect to overcome in the near future as the availability of RNA-seq data in public
379 repositories is rapidly increasing. Initiatives such as Recount enable easy analysis on these

380 samples [42], allowing us to update our predictions in the future, thereby increasing our
381 prediction accuracy.

382 For some genes we are unable to predict annotated disease associations despite having a
383 high gene predictability scores. Some genes, such as *TTR,* simply act in a manner unique to
384 a specific phenotype. Other genes, such as *TMPO,* turned out to be false positive disease
385 associations. These examples show that our gene predictability score has the potential to
386 flag genes acting in a unique manner as well as genes that might be incorrectly assigned to
387 a certain disease or phenotype.

388 We noted that the median prediction performance of HPO terms is lower compared to the
389 other gene sets databases used in our study, such as Reactome. This may be due to the
390 fact that phenotypes can arise by disrupting multiple distinct biological pathways. For
391 instance, DCMs can be caused by variants in sarcomeric protein genes, but also by variants
392 in calcium/sodium handling genes or by transcription factor genes [43]. As our methodology
393 makes guilt-by-association predictions based on whether genes are showing similar
394 expression levels, the fact that multiple separately working processes are related to the
395 same phenotype can reduce the accuracy of the predictions (although it is often still
396 possible to use these predictions as the DCM HPO phenotype prediction performance AUC =
397 0.76).

398 **Complexity**

399 Given that nearly 5% of patients with a Mendelian disease have another genetic disease
400 [44], it is important to consider that multiple genes might each contribute to specific
401 phenotypic effects. Clinically, it can be difficult to assess if a patient suffers from two
402 inherited conditions, which may hinder variant interpretation based on HPO terms. We
403 showed that GADO can disentangle the phenotypic features of two different diseases
404 manifesting in one patient by correlating and subsequently clustering the profiles of HPO
405 terms describing the patient's phenotype. If the HPO terms observed for a patient do not

406 correlate, it is more likely that they are caused by two different diseases. An early indication
407 that this might be the case for a specific patient can simplify subsequent analysis because
408 the geneticist or laboratory specialist performing the variant interpretation can take this in
409 consideration. GADO also facilitates separate prioritizations on subsets of the phenotypic
410 features.

411 **Conclusion**

412 Connecting variants to disease is a complex multistep process. The early steps are usually
413 highly automated, but the final most critical interpretations still rely on expert review and
414 human interpretation. GADO is a novel approach that can aid users in prioritizing genes
415 using patient-specific HPO terms, thereby speeding-up the diagnostic process. It prioritizes
416 variants in coding *and* non-coding genes, including genes for which there is no current
417 knowledge about their function and those that have not been annotated in any ontology
418 database. This gene prioritization is based on co-regulation of genes identified by analyzing
419 31,499 publicly available RNA-seq samples. Therefore, in contrast to many other existing
420 prioritization tools, GADO has the capacity to identify novel genes involved in human
421 disease. By providing a statistical measure of the significance of the ranked candidate
422 variants, GADO can provide an indication for which genes its predictions are reliable. GADO
423 can also detect phenotypes that do not cluster together, which can alert users to the
424 possible presence of a second genetic disorder and facilitate the diagnostic process in
425 patients with multiple non-specific phenotypic features. GADO can easily be combined with
426 any filtering tool to prioritize variants within WES or WGS data and can also be used in gene
427 panels such as PanelApp [45]. GADO is freely available at www.genenetwork.nl to help
428 guide the differential diagnostic process in medical genetics.

429 # Materials and Methods

430 **Gene co-regulation and function predictions**

431 We used publicly available RNA-seq samples from the European Nucleotide Archive (ENA)
432 database [46] to predict gene functions and gene-HPO term associations. After processing
433 and quality control we included 31,499 sample for which we have expression quantification
434 on 56,435 genes (supplementary methods 1). We performed a PCA on the gene correlation
435 matrix and selected 1,588 reliable principal components (PCs) (Cronbach's Alpha ≥ 0.7).

436 We used the eigenvectors of these 1,588 PCs to predict gene functions and to predict HPO
437 term associations [19]. We applied this methodology to the gene sets described by terms in
438 the following databases: Reactome and KEGG pathways, Gene Ontology (GO) molecular
439 function, GO biological process and GO cellular component terms and finally to HPO terms.
440 We excluded terms for which fewer than 10 genes are annotated because predictions for
441 smaller groups of genes are less accurate and might be misleading. Predictions were made
442 for 8,657 gene sets in total.

443 The following steps were taken to obtain the gene prediction scores per gene set (**Figure**
444 **1**). First, for each PC, a student's T-test was conducted between the eigencoefficients of the
445 genes annotated to a particular gene set and a group of genes serving as a background.
446 This background consisted of the genes annotated to any term in a specific database,
447 excluding those annotated to the current term. Second, the resulting p-values of the T-test
448 were transformed into a z-score, which indicate to which extend each PC represents a part
449 of the biology underlying a gene set. This is done for each PC, resulting in a profile how
450 important each PC is for a gene set. Finally, to predict which genes can be associated to a
451 particular gene set, we correlated the 1,588 T-test z-scores for that gene set (as calculated
452 above) with the 1,588 eigenvector coefficients of a gene. The p-value of this correlation
453 indicates the fit between a gene and a pathway / HPO term, these p-values were

454 transformed to predictions z-scores. When a gene was already explicitly annotated to a
455 gene-set and we wanted to predict whether that gene is involved in that gene set, then
456 there is a small circular bias as the predictions profile of this set was partly calculated based
457 on this gene. To remove this bias, the 1,588 z-scores for a gene set were first re-calculated
458 while assuming this gene is not involved in that gene set, after which the gene prediction
459 was made.

460 To determine the accuracy of our predictions we assessed our ability to predict back known
461 gene set annotations. For each gene-set, we calculated an Area Under the Curve (AUC),
462 using a Mann-Whitney U test, on the predictions z-scores of the genes that are part of a set
463 versus those that are not part of a set. These AUCs indicate how accurate the predictions
464 were, with an AUC of 1 indicating perfect predictions and an AUC of 0.5 indicating no
465 predictive power. The average AUC for each category was calculated based on all gene sets
466 with at least 10 annotated genes and with a p-value ≤ 0.05 (Bonferroni corrected for the
467 number of pathways in a database).

468 **Gene predictability scores**

469 To explain why for some genes we cannot predict known HPO annotation, we have
470 established a gene predictability score. We have calculated this gene predictability using the
471 prioritization z-scores based on Reactome, GO and KEGG. For each gene and for each
472 database we calculated the skewness in the distribution of the prioritization z-scores of the
473 gene sets. We used the average skewness as the gene predictability score.

474 **GADO predictions**

475 To identify potential causative variants in patients, we used HPO terms to describe a
476 patient's features. We only used the HPO terms which have significant predictive power
477 (based on the p-value of U test to calculate the AUC). If the predictions for a patient's HPO
478 term were not significant, the parent/umbrella HPO terms were used (supplementary figure
479 1). The online GADO tool suggests the parent terms from which the user can then select

480 which terms should be used in the analysis. The gene prediction z-scores for an HPO term
481 were used to rank the genes. If a patient's phenotype was described by more than one HPO
482 term, a meta-analysis was conducted. In these cases a weighted z-score was calculated by
483 adding the z-scores for each of the patient's HPO terms and then dividing by the square root
484 of the number of HPO terms [24]. The genes with the highest combined z-scores are
485 predicted to most likely candidate causative genes for a patient. This analysis can be
486 conducted at: https://www.genenetwork.nl.

487 **Validation of disease-gene predictions**

488 To benchmark our method we used the OMIM morbid map [5] downloaded on March 26,
489 2018, containing all disease-gene-phenotype entries. From this list, we extracted the
490 disease-gene associations, excluding non-disease and susceptibility entries. We extracted
491 the provisional disease-gene associations separately. For each disease in OMIM, we used
492 GADO to determine the rank of the causative gene among all genes in the OMIM morbid
493 map. For this we used all phenotypes annotated to the OMIM disease. If any of the HPO
494 terms did not have significant predictive power, the parent terms were used.

495 To determine if these distributions were significantly different from what we expect by
496 chance, we permuted the data. We replaced the existing gene-OMIM annotation but
497 assigned every gene to a new disease (keeping the phenotypic features for a disease
498 together), assuring that the randomly selected gene was not already annotated to any of
499 the phenotypes of the original gene.

500 **Cohort of previously solved cases**

501 To test if GADO could help prioritize genes that contain the causative variant, we used 83
502 samples of patients who were previously genetically diagnosed through whole exome
503 analysis or gene panel analysis. These samples encompass a wide variety of different
504 Mendelian disorders (supplementary table 2). To assess which genes harbor potentially
505 causative variants, we first called and annotated the variants from the exome sequencing

506 files (Supplementary methods 3). For 11 of the previously solved cases, GAVIN did not flag
507 the causative variant as a candidate. To be able to include these samples in our GADO
508 benchmark, we added the causative genes for these cases manually to the candidate list.

509 The phenotypic features of a patient were translated into HPO terms, which were used as
510 input to GADO. Here we only used features reported in the medical records prior to the
511 molecular diagnosis. If any of the HPO terms did not have significant predictive power, the
512 parent terms were used. From the resulting list of ranked genes, the known disease genes
513 harboring a potentially causative variant were selected. Next, we determined the rank of the
514 gene with the known causative variant among the selected genes. If a patient harbored
515 multiple causative variants in different genes, in case of di-genic inheritance or two
516 inherited conditions, the median rank of these genes was reported (supplementary table 2).

517 **Unsolved cases cohorts**

518 In addition to the patients with a known genetic diagnosis, we tested 38 unsolved cases
519 (supplementary table 3). These are patients with mainly cardiomyopathies or developmental
520 delay. All patients were previously investigated using exome sequencing, by analyzing a
521 gene panel appropriate for their phenotype. To allow discovery of potential novel disease
522 genes, we used GADO to rank genes with candidate variants (Supplementary methods 3).
523 For genes with a prediction z-score ≥ 5, a literature search for supporting evidence was
524 performed to assess whether these genes are likely candidate genes.

525 **Website**

526 To make our method and data available we have developed a website available at
527 www.genenetwork.nl that can be used to run GADO, lookup gene functions predictions,
528 visualize networks using co-regulations scores and perform function enrichments of sets of
529 genes (Supplementary methods 4).

530 # Description of Supplemental Data

531 Supplementary methods 1. Processing and quality control of public RNA-seq data

532 Supplementary methods 2. Benchmark comparison with Exomiser

533 Supplementary methods 3. Variant calling and processing of benchmark samples

534 Supplementary methods 4. GeneNetwork website

535 Supplementary figure 1. Selection of parent HPO term if GADO does not have significant
536 predictive power for query term

537 Supplementary figure 2. Comparison of GADO performance with the level of evidence for
538 each cardiomyopathy-related gene

539 Supplementary figure 3. Comparison between GADO and Exomiser rankings

540 Supplementary figure 4. Correcting for biases in co-expression networks

541 Supplementary figure 5. Histogram of the gene types included in our analyses

542 Supplementary figure 6. PCA plot of 36,761 samples

543 Supplementary figure 7. Investigation of principal components capturing technical biases

544 Supplementary figure 8. Variance explained by first 1588 PCs

545 Supplementary figure 9. Visualization of PC1 to PC 10 of PCA over gene correlation matrix

546 Supplementary figure 10. Outlier genes in PC 8 and PC 9 of PCA over gene correlation
547 matrix

548 Supplementary figure 11. PC sample scores to distinguish different tissues

549 Supplementary figure 12. Outlier samples in PC sample scores of PC 8 and PC 9

550 Supplementary table 1. A list of samples annotated in the European Nucleotide Archive June
551 30, 2016

552 Supplementary table 2. A list of 83 diagnosed patients with Mendelian disorders and
553 corresponding predictions with GADO

554 Supplementary table 3. A list of 38 undiagnosed patients with suspected Mendelian
555 disorders

556 Supplementary table 4. A comparison between GADO and Exomiser predictions using a list
557 of 83 diagnosed patients with Mendelian disorders

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573 # References

574 1. Brown TL, Meloche TM. Exome sequencing a review of new strategies for rare genomic
575 disease research. Genomics. Academic Press; 2016. p. 109–14.

576 2. Wright CF, FitzPatrick DR, Firth H V. Paediatric genomics: diagnosing rare disease in
577 children. Nat Rev Genet [Internet]. Nature Publishing Group; 2018 [cited 2018 Jul
578 11];19:253–68. Available from: http://www.nature.com/doifinder/10.1038/nrg.2017.116

579 3. Yang Y, Muzny DM, Xia F, Niu Z, Person R, Ding Y, et al. Molecular findings among
580 patients referred for clinical whole-exome sequencing. JAMA [Internet]. NIH Public Access;
581 2014 [cited 2018 Jul 11];312:1870–9. Available from:
582 http://www.ncbi.nlm.nih.gov/pubmed/25326635

583 4. Wright CF, Fitzgerald TW, Jones WD, Clayton S, McRae JF, van Kogelenberg M, et al.
584 Genetic diagnosis of developmental disorders in the DDD study: a scalable analysis of
585 genome-wide research data. Lancet (London, England) [Internet]. Elsevier; 2015 [cited
586 2018 Jul 11];385:1305–14. Available from:
587 http://www.ncbi.nlm.nih.gov/pubmed/25529582

588 5. McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University. Online
589 Mendelian Inheritance in Man, OMIM [Internet]. Available from: https://omim.org/

590 6. Stenson PD, Mort M, Ball E V., Evans K, Hayden M, Heywood S, et al. The Human Gene
591 Mutation Database: towards a comprehensive repository of inherited mutation data for
592 medical research, genetic diagnosis and next-generation sequencing studies. Hum Genet
593 [Internet]. Springer Berlin Heidelberg; 2017 [cited 2018 Jul 11];136:665–77. Available
594 from: http://link.springer.com/10.1007/s00439-017-1779-6

595 7. Lek M, Karczewski KJ, Minikel E V., Samocha KE, Banks E, Fennell T, et al. Analysis of
596 protein-coding genetic variation in 60,706 humans. Nature [Internet]. Nature Research;

597 2016 [cited 2017 Jun 23];536:285–91. Available from:
598 http://www.nature.com/doifinder/10.1038/nature19057

599 8. Eilbeck K, Quinlan A, Yandell M. Settling the score: variant prioritization and Mendelian
600 disease. Nat Rev Genet [Internet]. Nature Publishing Group; 2017 [cited 2018 Jun
601 17];18:599–612. Available from: http://www.nature.com/doifinder/10.1038/nrg.2017.52

602 9. van der Velde KJ, de Boer EN, van Diemen CC, Sikkema-Raddatz B, Abbott KM,
603 Knopperts A, et al. GAVIN: Gene-Aware Variant INterpretation for medical sequencing.
604 Genome Biol [Internet]. 2017 [cited 2017 Jun 16];18:6. Available from:
605 http://genomebiology.biomedcentral.com/articles/10.1186/s13059-016-1141-7

606 10. McLaren W, Gil L, Hunt SE, Riat HS, Ritchie GRS, Thormann A, et al. The Ensembl
607 Variant Effect Predictor. Genome Biol [Internet]. BioMed Central; 2016 [cited 2018 Jul
608 11];17:122. Available from:
609 http://genomebiology.biomedcentral.com/articles/10.1186/s13059-016-0974-4

610 11. Smedley D, Robinson PN. Phenotype-driven strategies for exome prioritization of human
611 Mendelian disease genes. Genome Med [Internet]. 2015 [cited 2018 Jul 11];7:81. Available
612 from: http://www.ncbi.nlm.nih.gov/pubmed/26229552

613 12. Robinson PN, Köhler S, Bauer S, Seelow D, Horn D, Mundlos S. The Human Phenotype
614 Ontology: A Tool for Annotating and Analyzing Human Hereditary Disease. Am J Hum Genet
615 [Internet]. 2008 [cited 2018 Jul 11];83:610–5. Available from:
616 http://www.ncbi.nlm.nih.gov/pubmed/18950739

617 13. Birgmeier J, Haeussler M, Deisseroth CA, Jagadeesh KA, Ratner AJ, Guturu H, et al.
618 AMELIE accelerates Mendelian patient diagnosis directly from the primary literature. bioRxiv
619 [Internet]. Cold Spring Harbor Laboratory; 2017 [cited 2018 Jun 17];171322. Available
620 from: https://www.biorxiv.org/content/early/2017/08/02/171322

621 14. Bone WP, Washington NL, Buske OJ, Adams DR, Davis J, Draper D, et al. Computational
622 evaluation of exome sequence data using human and model organism phenotypes improves
623 diagnostic efficiency. Genet Med [Internet]. 2016 [cited 2018 Jun 27];18:608–17. Available
624 from: http://www.nature.com/articles/gim2015137

625 15. Köhler S, Vasilevsky NA, Engelstad M, Foster E, McMurry J, Aym? S, et al. The Human
626 Phenotype Ontology in 2017. Nucleic Acids Res [Internet]. 2017 [cited 2017 Jun
627 16];45:D865–76. Available from: https://academic.oup.com/nar/article-
628 lookup/doi/10.1093/nar/gkw1039

629 16. Leinonen R, Akhtar R, Birney E, Bower L, Cerdeno-Tárraga A, Cheng Y, et al. The
630 European Nucleotide Archive. Nucleic Acids Res [Internet]. Oxford University Press; 2011
631 [cited 2017 Jun 16];39:D28-31. Available from:
632 http://www.ncbi.nlm.nih.gov/pubmed/20972220

633 17. Bray NL, Pimentel H, Melsted P, Pachter L. Near-optimal probabilistic RNA-seq
634 quantification. Nat Biotechnol [Internet]. 2016 [cited 2017 Jul 5];34:525–7. Available from:
635 http://www.ncbi.nlm.nih.gov/pubmed/27043002

636 18. Deelen P, Zhernakova D V, de Haan M, van der Sijde M, Bonder MJ, Karjalainen J, et al.
637 Calling genotypes from public RNA-sequencing data enables identification of genetic variants
638 that affect gene-expression levels. Genome Med [Internet]. 2015 [cited 2015 Apr 7];7:30.
639 Available from: http://genomemedicine.com/content/7/1/30

640 19. Fehrmann RSN, Karjalainen JM, Krajewska M, Westra H, Maloney D, Simeonov A, et al.
641 Gene expression analysis identifies global gene dosage sensitivity in cancer. Nat Genet
642 [Internet]. 2015;47:115–25. Available from:
643 http://www.nature.com/doifinder/10.1038/ng.3173

644 20. Zhao S, Fung-Leung W-P, Bittner A, Ngo K, Liu X. Comparison of RNA-Seq and
645 Microarray in Transcriptome Profiling of Activated T Cells. Zhang S-D, editor. PLoS One

646 [Internet]. Public Library of Science; 2014 [cited 2018 Jun 27];9:e78644. Available from:
647 http://dx.plos.org/10.1371/journal.pone.0078644

648 21. Fabregat A, Jupe S, Matthews L, Sidiropoulos K, Gillespie M, Garapati P, et al. The
649 Reactome Pathway Knowledgebase. Nucleic Acids Res. 2018;46:D649–55.

650 22. Kanehisa M, Furumichi M, Tanabe M, Sato Y, Morishima K. KEGG: new perspectives on
651 genomes, pathways, diseases and drugs. Nucleic Acids Res [Internet]. 2017 [cited 2018 Jul
652 12];45:D353–61. Available from: https://academic.oup.com/nar/article-
653 lookup/doi/10.1093/nar/gkw1092

654 23. The Gene Ontology Consortium. Expansion of the Gene Ontology knowledgebase and
655 resources. Nucleic Acids Res [Internet]. 2017 [cited 2018 Jul 12];45:D331–8. Available
656 from: http://www.ncbi.nlm.nih.gov/pubmed/27899567

657 24. Zaykin D V. Optimally weighted Z-test is a powerful method for combining probabilities
658 in meta-analysis. J Evol Biol [Internet]. NIH Public Access; 2011 [cited 2018 May
659 29];24:1836–41. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21605215

660 25. Kurian MA, Zhen J, Cheng S-Y, Li Y, Mordekar SR, Jardine P, et al. Homozygous loss-of-
661 function mutations in the gene encoding the dopamine transporter are associated with
662 infantile parkinsonism-dystonia. J Clin Invest [Internet]. 2009 [cited 2018 Jun
663 28];119:1595–603. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19478460

664 26. Puffenberger EG, Jinks RN, Sougnez C, Cibulskis K, Willert RA, Achilly NP, et al. Genetic
665 Mapping and Exome Sequencing Identify Variants Associated with Five Novel Diseases.
666 Janecke AR, editor. PLoS One [Internet]. 2012 [cited 2018 Jun 28];7:e28936. Available
667 from: http://www.ncbi.nlm.nih.gov/pubmed/22279524

668 27. Kurian MA, Li Y, Zhen J, Meyer E, Hai N, Christen H-J, et al. Clinical and molecular
669 characterisation of hereditary dopamine transporter deficiency syndrome: an observational

670 cohort and experimental study. Lancet Neurol [Internet]. 2011 [cited 2018 Jun 28];10:54–
671 62. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21112253

672 28. The Gtex Consortium. The Genotype-Tissue Expression (GTEx) project. Nat Genet
673 [Internet]. 2013 [cited 2014 Jan 20];45:580–5. Available from:
674 http://www.ncbi.nlm.nih.gov/pubmed/23715323

675 29. Benson MD. Inherited amyloidosis. J Med Genet [Internet]. BMJ Publishing Group; 1991
676 [cited 2018 Jul 10];28:73–8. Available from:
677 http://www.ncbi.nlm.nih.gov/pubmed/1848299

678 30. Nouhravesh N, Ahlberg G, Ghouse J, Andreasen C, Svendsen JH, Haunsø S, et al.
679 Analyses of more than 60,000 exomes questions the role of numerous genes previously
680 associated with dilated cardiomyopathy. Mol Genet genomic Med [Internet]. Wiley-
681 Blackwell; 2016 [cited 2018 Jul 10];4:617–23. Available from:
682 http://www.ncbi.nlm.nih.gov/pubmed/27896284

683 31. Arimura T, Matsumoto Y, Okazaki O, Hayashi T, Takahashi M, Inagaki N, et al.
684 Structural analysis of obscurin gene in hypertrophic cardiomyopathy. Biochem Biophys Res
685 Commun [Internet]. Academic Press; 2007 [cited 2018 Jun 28];362:281–7. Available from:
686 https://www.sciencedirect.com/science/article/pii/S0006291X07015963?via%3Dihub

687 32. Marston S, Montgiraud C, Munster AB, Copeland O, Choi O, dos Remedios C, et al.
688 OBSCN Mutations Associated with Dilated Cardiomyopathy and Haploinsufficiency.
689 Thangaraj K, editor. PLoS One [Internet]. Public Library of Science; 2015 [cited 2018 Jun
690 28];10:e0138568. Available from: http://dx.plos.org/10.1371/journal.pone.0138568

691 33. Bolling MC, Jan SZ, Pasmooij AMG, Lemmink HH, Franke LH, Yenamandra VK, et al.
692 Generalized Ichthyotic Peeling Skin Syndrome due to FLG2 Mutations. J Invest Dermatol
693 [Internet]. 2018 [cited 2018 Jul 10]; Available from:
694 http://www.ncbi.nlm.nih.gov/pubmed/29505760

695 34. Alfares A, Al-Khenaizan S, Al Mutairi F. Peeling skin syndrome associated with novel
696 variant in *FLG2* gene. Am J Med Genet Part A [Internet]. Wiley-Blackwell; 2017 [cited 2018
697 Jul 11];173:3201–4. Available from: http://doi.wiley.com/10.1002/ajmg.a.38468

698 35. Alazami AM, Patel N, Shamseldin HE, Anazi S, Al-Dosari MS, Alzahrani F, et al.
699 Accelerating novel candidate gene discovery in neurogenetic disorders via whole-exome
700 sequencing of prescreened multiplex consanguineous families. Cell Rep [Internet]. Elsevier;
701 2015 [cited 2018 Jul 24];10:148–61. Available from:
702 http://www.ncbi.nlm.nih.gov/pubmed/25558065

703 36. Runge JS, Raab JR, Magnuson T. Identification of Two Distinct Classes of the Human
704 INO80 Complex Genome-Wide. G3 (Bethesda) [Internet]. G3: Genes, Genomes, Genetics;
705 2018 [cited 2018 Jul 24];8:1095–102. Available from:
706 http://www.ncbi.nlm.nih.gov/pubmed/29432129

707 37. Meeson AP, Radford N, Shelton JM, Mammen PP, DiMaio JM, Hutcheson K, et al.
708 Adaptive mechanisms that preserve cardiac function in mice without myoglobin. Circ Res
709 [Internet]. 2001 [cited 2018 Jul 20];88:713–20. Available from:
710 http://www.ncbi.nlm.nih.gov/pubmed/11304494

711 38. van der Harst P, van Setten J, Verweij N, Vogler G, Franke L, Maurano MT, et al. 52
712 Genetic Loci Influencing Myocardial Mass. J Am Coll Cardiol [Internet]. Elsevier; 2016 [cited
713 2018 Jul 20];68:1435–48. Available from:
714 https://www.sciencedirect.com/science/article/pii/S0735109716346642?via%3Dihub

715 39. Truszkowska GT, Bilińska ZT, Muchowicz A, Pollak A, Biernacka A, Kozar-Kamińska K, et
716 al. Homozygous truncating mutation in NRAP gene identified by whole exome sequencing in
717 a patient with dilated cardiomyopathy. Sci Rep [Internet]. Nature Publishing Group; 2017
718 [cited 2018 Jul 20];7:3362. Available from: http://www.nature.com/articles/s41598-017-
719 03189-8

720 40. Sobreira N, Schiettecatte F, Valle D, Hamosh A. GeneMatcher: A Matching Tool for
721 Connecting Investigators with an Interest in the Same Gene. Hum Mutat [Internet]. 2015
722 [cited 2018 Jul 12];36:928–30. Available from:
723 http://www.ncbi.nlm.nih.gov/pubmed/26220891

724 41. Szklarczyk D, Morris JH, Cook H, Kuhn M, Wyder S, Simonovic M, et al. The STRING
725 database in 2017: quality-controlled protein–protein association networks, made broadly
726 accessible. Nucleic Acids Res [Internet]. 2017 [cited 2018 Jul 12];45:D362–8. Available
727 from: http://www.ncbi.nlm.nih.gov/pubmed/27924014

728 42. Collado-Torres L, Nellore A, Jaffe AE. recount workflow: Accessing over 70,000 human
729 RNA-seq samples with Bioconductor. F1000Research [Internet]. 2017 [cited 2018 Jul
730 12];6:1558. Available from: https://f1000research.com/articles/6-1558/v1

731 43. Posafalvi A, Herkert JC, Sinke RJ, van den Berg MP, Mogensen J, Jongbloed JDH, et al.
732 Clinical utility gene card for: dilated cardiomyopathy (CMD). Eur J Hum Genet [Internet].
733 Nature Publishing Group; 2013 [cited 2018 Jun 22];21. Available from:
734 http://www.ncbi.nlm.nih.gov/pubmed/23249954

735 44. Posey JE, Harel T, Liu P, Rosenfeld JA, James RA, Coban Akdemir ZH, et al. Resolution
736 of Disease Phenotypes Resulting from Multilocus Genomic Variation. N Engl J Med
737 [Internet]. NIH Public Access; 2017 [cited 2018 Jun 27];376:21–31. Available from:
738 http://www.ncbi.nlm.nih.gov/pubmed/27959697

739 45. Genomics England. PanelApp [Internet]. Available from:
740 https://panelapp.genomicsengland.co.uk

741 46. Silvester N, Alako B, Amid C, Cerdeño-Tárraga A, Cleland I, Gibson R, et al. Content
742 discovery and retrieval services at the European Nucleotide Archive. Nucleic Acids Res.
743 2015;43:D23–9.

744