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**GeVIR is a continuous gene-level metric that uses variant distribution patterns to
prioritize disease candidate genes**

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With large scale population sequencing projects gathering pace, there is a need for strategies that advance disease gene prioritization^{1,2}. Metrics that provide information about a gene and its ability to tolerate protein-altering variation can aid clinical interpretation of human genomes and advance disease gene discovery¹⁻⁴. Previous methods analyzed total variant load in a gene¹⁻⁴, but not their distribution pattern within a gene. Using data from 138,632 exome/genome sequences², we developed Gene Variation Intolerance Rank (GeVIR) to produce a continuous gene-level metric for 19,361 genes that is able to prioritize both dominant and recessive Mendelian disease genes⁵, outperforming missense constraint metrics³ and comparable, but complementary, to loss-of-function (LoF) constraint metrics². GeVIR is also able to prioritize short genes, for which LoF constraint cannot be confidently estimated². A majority of the most intolerant genes identified here have no defined phenotype and are candidates for severe dominant disorders.

The increasing size of publicly available variant databases from large predominantly healthy populations^{1,2} provides us with an intriguing opportunity to analyze small gene-coding regions devoid of variants, as an alternative to existing methods that measure variant deficiency at a gene level^{1-4,6} or within large coding regions^{7,8}. Since some disease-causing variants are dominant, highly penetrant, and tend to cluster in functionally important coding regions⁹, it is reasonable to hypothesize that genes important for human development might have regions that are so sensitive to variation they are never observed in healthy individuals. These regions were recently studied in the context of variant prioritization¹⁰; however, a continuous gene-level variation intolerance metric utilizing variant distribution was not developed.

To illustrate the hypothesis behind GeVIR, which is based on analyzing the length, evolutionary conservation, and number of Variant Intolerant Regions (VIR, a region between two protein-altering variants) in gnomAD (v2.0.1), we analyzed ~3.5 million VIRs in 19,361 protein-coding genes and selected 2,935 genes harboring at least one VIR overlapping with known pathogenic variants from

41 ClinVar¹¹. Although ~91% of the VIRs were small (1-5 amino acids), only ~53% of pathogenic
42 missense variants were located within them (Fig. 1a,b). Moreover, pathogenic missense variants were
43 observed ~3.7 times (two-sided Fisher's exact test $P < 2.2 \times 10^{-308}$, Supplementary Table 1) more often
44 inside the largest VIRs (>20 amino acids), a trend that was not observed for LoF variants (Fig. 1c).
45 There was also a positive correlation between VIR length and evolutionary conservation, measured by
46 mean GERP++ score¹² among 1,938,102 VIRs (length ≥ 1 amino acid) in 18,491 genes (Fig. 1d).

47 To create GeVIR, we counted the number of VIRs of each length (only with high coverage) in
48 the canonical transcripts of 19,361 genes. Examples of variant distribution patterns in four disease
49 genes with different modes of inheritance and length are demonstrated in Figure 2a. We calculated the
50 weight of each VIR length based on how frequently it was observed among all genes (Fig. 2b-d). A
51 score for each gene was calculated as a sum of all its VIR weights, multiplied by average GERP++
52 score and divided by the total number of VIRs in a gene (including those with low coverage) (Fig. 2e).
53 High evolutionary conservation supported region importance and increased the weight, whereas low
54 conservation was penalized because lack of variation could be a result of sequencing or variant filtering
55 errors (scores between -1 and 1 were rounded to avoid extreme penalties from multiplication). Finally,
56 genes were sorted using this score to create GeVIR percentiles (Supplementary Table 2).

57 To evaluate GeVIR performance, we compared its ability to rank genes that are expected to
58 have different levels of intolerance to variation with recently released gnomAD gene constraint
59 metrics: Missense z-score³, **missense** and **loss-of-function observed/expected upper bound fraction**
60 **called MOEUF and LOEUF** (a recommended replacement for the probability of being LoF intolerant
61 score, pLI)², respectively (Table 1). GeVIR outperformed both missense metrics at prioritizing
62 autosomal dominant (AD)⁵, human orthologs of mouse heterozygous lethal knockouts^{13,14}, and cell
63 essential¹⁵ genes by ~4-6% AUC, whereas LOEUF showed a similar performance to GeVIR in the first
64 and the second assays, but was ~4% lower in the last assay. GeVIR ranked genes with homozygous
65 LoF variants in healthy populations (Null)¹ and cell non-essential¹⁵ genes ~2% less effectively than

66 Missense z-scores, which achieved the best results in both assays. Importantly, however, GeVIR and
67 LOEUF showed a much higher trend to prioritize autosomal recessive (AR)⁵ genes closer to the middle
68 of the ranked list (~1.7-1.8 fold enrichment (FE) at the 40th-60th percentiles) than missense metrics
69 (Missense z-scores ~1.1 FE, $P = 0.015$; MOEUF ~1.3 FE, $P = 8.86 \times 10^{-7}$; Fig 3a and Supplementary
70 Table 3). Considering that Null and cell non-essential (i.e. low intolerant) genes could also be disease
71 causing (Supplementary Table 4) and from results of previous assays (Table 1), the distribution of AR
72 genes showed that GeVIR and LOEUF outperformed missense metrics in prioritizing essential and
73 disease genes. A good ranking method should also prioritize highly intolerant (e.g. AD) over medium
74 intolerant (e.g. AR) genes; therefore, the performance of all metrics at distinguishing AD (true positive)
75 from AR (false positive) genes was evaluated by calculating F1 scores for the AD class cumulatively at
76 each percentile. GeVIR achieved a 62.3% peak F1 score and surpassed missense metrics by ~2-3%, but
77 was outperformed by LOEUF by ~2%.

78 To clarify the impact of evolutionary conservation on GeVIR, scores were recalculated without
79 GERP++ adjustments and assessed in the same assays (Table 1). Despite a decreased performance, it
80 still outperformed missense metrics in prioritizing essential and disease genes, but performed worse in
81 ranking Null and cell non-essential genes, which probably had shorter VIRs and consequently relied
82 more on GERP++ adjustments. Nevertheless, this highlights that, for estimating missense intolerance,
83 GeVIR is a better alternative to state-of-the-art methods that are based on observed/expected ratios of
84 variants^{2,3}.

85 Although GeVIR and LOEUF showed a comparable performance and, unlike missense metrics,
86 demonstrated a potential to rank AR genes, they represent gene intolerance to different types of
87 variation (i.e. missense and LoF, respectively). Moreover, LOEUF was significantly more biased
88 towards longer genes (Spearman $r = -0.54$) than GeVIR ($r = -0.26$), especially in the first decile
89 (median protein length ~1.91 and ~1.15 times longer than expected 425 amino acids, respectively) (Fig.
90 3b). To rank genes by intolerance to both missense and LoF, GeVIR and LOEUF were combined into

VIRLoF (rank summation with re-sorting), which resulted in a more optimal ranking that outperformed other metrics in most assays (Table 1).

To investigate this difference, we selected 2,989 genes (~15.4%) with the lowest LOEUF (<0.35; a recommended hard threshold for most intolerant genes; L. Francioli, G. Tiao, K. Karczewski, M. Solomonson and N. Watts from <https://macarthurlab.org/2018/10/17/gnomad-v2-1/>), and the same number of genes ranked as the most intolerant by GeVIR and VIRLoF. At this threshold, GeVIR and LOEUF showed a similar performance (F1 = ~56.5%) and were outperformed by VIRLoF (F1 = ~60.2%); however, only ~55.9% (1,637) of the genes prioritized by GeVIR were the same as those prioritized by LOEUF (Fig. 4a,d), highlighting a need for both metrics. This group of both missense and LoF most intolerant genes contained ~31.7% (250/790) of known AD genes, and only ~2.2% (35/1,585) of known AR genes (~14.3 fold enrichment, two-sided Fisher's exact test $P = 1.0 \times 10^{-72}$) (Fig. 4b,c). Moreover ~70% of these genes (1,164/1,637) are not yet linked to any phenotype in OMIM⁵, but they are significantly enriched (~3.8 FE, $P = 7.9 \times 10^{-10}$) with mouse heterozygous lethal genes (36/1,164) compared with unknown genes in the remaining ~91.4% (115/14,174). This suggests that they might be crucial for organism development, and heterozygous *de novo* deleterious variants in them are likely to result in pathogenic phenotypes.

Missense and LoF intolerant genes (1,637) were enriched in histone modification and dopaminergic synapse function, a trend not observed with genes prioritized by either GeVIR or LOEUF alone ("functional annotation term" enrichment analysis performed using DAVID v6.8^{16,17}, Supplementary Note and Supplementary Tables 5 and 6). Genes prioritized only by GeVIR (1,317; i.e. intolerant to missense variants) were enriched in spliceosome complex function, similar to those prioritized by both metrics (1,672), but the former were generally shorter, with 221 and 559 amino acid median protein length, respectively. This trend was not observed with genes prioritized only by LOEUF and highlights that potentially important short genes could be missed without GeVIR, possibly due to an insufficient gnomAD population size, an acknowledged limitation of LOEUF².

116 Although GeVIR is based on the same principle as the Conservative Coding Regions (CCR)
117 map¹⁰ (i.e. analyzed distance between variants in gnomAD), it is important to differentiate between
118 gene (GeVIR) and variant (a potential application of CCR) prioritization methods. For example,
119 *TARDBP*, associated with AD diseases, is among the top 1% of the genes ranked by GeVIR since it
120 contained many long VIRs, but all associated ClinVar pathogenic variants would be misclassified by
121 CCR since they are located outside long CCRs/VIRs (Fig. 2a). Havrilla et al.¹⁰ compared their method
122 with other gene constraint metrics by ranking genes based on the number of CCRs $\geq 95\%$. To allow
123 comparisons, we used the same approach (7,000 genes with at least one CCR $\geq 95\%$ and a GeVIR
124 rank) and found that GeVIR, even without GERP++ adjustments, was less biased by gene length
125 (Spearman $r = 0.03/0.04$) and more efficiently distinguished AD from AR genes (peak F1 =
126 62.38/61.68%), than CCR ($r = -0.29$, F1 = 57.25%) (Extended Data Fig. 1). Moreover, GeVIR provides
127 a continuous metric of intolerance for 19,361 genes that can be used for AR gene prioritization,
128 whereas CCR is limited to ~7,000 genes and therefore can only be used for AD gene/variant
129 prioritization¹⁰.

130 Here we present a novel method to measure gene variation intolerance by analyzing variant
131 distribution patterns within genes. GeVIR mostly represents gene intolerance to missense variation,
132 outperforming existing gnomAD missense gene constraint metrics and complementing the LoF metric,
133 due to its ability to estimate intolerance of short genes (e.g. *LITAF*, associated with AD Charcot-Marie-
134 Tooth disease, length = 162 amino acids, ranked by GeVIR ~35%, but by gnomAD metrics ~67-72%,
135 Fig. 2a). GeVIR, like other gene constraint metrics derived from gnomAD data, might underestimate
136 intolerance of genes associated with phenotypes that could be present in the gnomAD population (e.g.
137 late onset diseases, cancer)¹⁸, but is not biased towards more studied genes (a criticism associated with
138 gene scores that rely on gene networks^{19,20}). GeVIR scores are available online (www.gevirank.org),
139 with additional metrics to facilitate prediction of the inheritance mode (AD/AR) for potential disease

140 genes (Supplementary Note and Supplementary Fig. 5), and should aid interpretation of genome
141 sequencing data in a clinical setting and advance human disease gene discovery.

142

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148 contributing groups can be found at <https://gnomad.broadinstitute.org/about>.

149

150 **Author Contributions**

151 N.A., M.T., and A.B. conceived and designed the research. N.A. executed the analysis. N.A. and M.T.
152 performed the primary writing. M.T. and A.B. supervised all aspects of the research, and reviewed and
153 edited the manuscript.

154

155 **Competing interests**

156 The authors declare no competing interests.

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159 **Figure legends**

160 **Fig. 1 | Correlation between length of variant intolerant regions (VIRs), location of pathogenic**
161 **variants, and evolutionary conservation.** **a**, Distances in amino acids between two non-synonymous
162 variants (i.e. VIRs) from gnomAD in 2,935 genes containing at least one missense or LoF (stop gained
163 or frameshift) pathogenic variant. **b,c**, Distribution of pathogenic missense and LoF variants inside
164 VIRs (**b**) and normalized by cumulative region length (**c**). In **c**, the central values represent pathogenic
165 variants per amino acid ratio computed on 2,935 genes, whereas error bars represent 95% confidence
166 interval from bootstrapping ($n = 10,000$ iterations of resampling with replacement on 50% of 2,935
167 genes (i.e. $n = 1,467$); each iteration result is shown on overlaid dot plots). **d**, Correlation between VIR
168 length and evolutionary conservation (GERP++ scores) of 1,938,102 VIRs with high coverage in
169 18,491 genes.

170
171 **Fig. 2 | GeVIR workflow.** **a**, Comparison of distribution of pathogenic missense, stop gained and
172 frameshift variants from ClinVar with non-synonymous protein coding variants from gnomAD in 4
173 genes (*TCF4*, *LITAF*, *ARSA* and *TARDBP*) associated with Mendelian diseases. Variant Intolerant
174 Regions (VIR) can be seen as “gaps” in the genes. **b**, Boundaries of nucleotide regions used to
175 calculate VIR properties. **c**, Two genes (A and B) containing a total of 5 variants (orange boxes), which
176 divide genes A and B into 4 and 3 VIRs, respectively, 6 of which are valid (5 shown in green and 1
177 (VIR4) with zero length). In gene B, VIR2 has low mean sequence coverage (48, shown in white) and
178 is classified as non-valid. **d**, Calculation of valid region length weights based on their frequency in all
179 genes (longer regions are rare and have a larger weight). **e**, Gene score calculations for A and B: each
180 valid region weight in a gene is multiplied by its GERP++ score and their sum is divided by the total
181 number of regions in the gene (valid and non-valid). GeVIR ranking is based on these gene scores
182 sorted in descending order. According to GeVIR, gene A is more intolerant to variants than gene B.

183
184 **Fig. 3 | Comparison of GeVIR gene ranking with gnomAD constraint metrics on 19,361 genes.** **a**,
185 Percentage of genes associated exclusively with autosomal recessive (AR) diseases out of all AR genes
186 in OMIM ($n = 1,585$) in each rank decile. **b**, Median protein length (amino acids) in each rank decile.
187 Correlation between protein length and gene rank was measured with Spearman’s rank correlation
188 coefficient.

189
190 **Fig. 4 | Comparison of GeVIR, LOEUF and VIRLoF performance on the most variant intolerant**
191 **genes.** **a-c**, 2,989/19,361 genes (~15%) with upper observed/expected loss-of-function (LOEUF) scores
192 < 0.35 (green), and the same number of genes with the highest GeVIR (red) and VIRLoF (purple)
193 scores. Venn diagrams show overlaps between all 2,989 genes (**a**), genes exclusively associated with
194 autosomal dominant (AD) (**b**) or autosomal recessive (AR) (**c**) OMIM genes. **d**, Method performance is
195 compared, considering AD genes as true positives and AR genes as false positives, using precision,
196 recall and F1 metrics of AD class.

197 **Tables**

198
199 **Table 1 | Comparison of GeVIR gene ranking with gnomAD constraint metrics on 19,361 genes.** Autosomal dominant
200 (AD) and autosomal recessive (AR) gene groups consisted of genes associated with diseases with only one mode of
201 inheritance in OMIM⁵. Human cell essential and non-essential gene groups were based on CRISPR/Cas screens¹⁵. Metrics
202 performance was measured with area under the curve (AUC). Assays in which lower AUC indicates better performance are
203 marked with *. In the AD/AR classification assay, AD class F1 score is calculated at each percentile (cumulative)
204 considering AD genes as true positives and AR genes as false positives and performance is reported as peak F1 score with
205 gene percentile in brackets. Results of metrics that outperformed others were highlighted in bold. If VIRLoF showed the
206 best results, then the second best performing metric was also highlighted in bold.

Assay	Gene number	GeVIR	GeVIR (without GERP++)	Missense z-scores	MOEUF	LOEUF	VIRLoF
Autosomal dominant (AD)	790	73.54	72.80	68.72	68.21	72.40	74.38
Mouse heterozygote lethal knockout	388	74.92	74.10	69.71	69.45	76.03	77.18
Cell essential	663	72.62	69.97	66.81	68.22	68.70	72.13
Nulls*	328	27.10	32.88	24.69	27.89	31.18	27.38
Cell non-essential*	865	29.61	37.07	27.26	27.61	27.92	27.08
AD/AR classification	790 AD, 1,585 AR	62.34 (29.94)	61.48 (25.44)	59.13 (35.40)	60.27 (32.82)	64.15 (27.17)	65.74 (25.84)

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208

209 **Online Methods**

210 **Protein-coding gene list.** The gnomAD database (version 2.0.1) was used
211 (https://github.com/macarthur-lab/gnomad_browser), which contained gene annotation from
212 GENCODE (version 19). Gene nucleotide and peptide sequences were obtained from Ensembl Biomart
213 (GRCh37/hg19) (<http://grch37.ensembl.org/biomart/martview/>) and gene constraint metrics from
214 gnomAD (<https://gnomad.broadinstitute.org/>). Only genes with canonical transcripts that start with
215 methonine, end with a stop codon, with protein coding nucleotide sequence divisible by 3, and without
216 reported issues in gnomAD gene constrain metrics were used. The gene list consisted of 19,361 genes
217 (18,326 were linked with HGNC²¹ approved symbols by Ensembl IDs), 1,009 of which were marked as
218 outliers (e.g. “too many missense variants”) in the gnomAD gene constraint metrics study².

219
220 **Variant Intolerant Regions (VIRs).** Variants in canonical transcripts of 19,361 genes were analyzed
221 by looking at regions between two variants (i.e. referred to here as start and stop of a VIR) predicted by
222 Variant Effect Predictor (VEP, version 85) to be affecting protein amino acid sequence, namely:
223 “stop_gained”, “frameshift_variant”, “stop_lost”, “start_lost”, “inframe_insertion”, “inframe_deletion”,
224 “missense_variant”. Only variants with filter status “PASS”, “LCR” (low-complexity) or “SEGDPUP”
225 (segmental duplication) were analyzed. If start and stop variants were located in different exons,
226 protein-coding nucleotides from multiple exons were concatenated into a single region. To take into
227 account regions between a start codon and the first variant or the last variant and a stop codon in a
228 gene, extra “fake” variants were added at the start and stop codons of all transcripts analyzed. We
229 excluded 24,276 regions in which start or stop variant consequence predicted by VEP were inconsistent
230 with transcript exon chromosome location (e.g. “missense” variant outside exon boundaries). The
231 remaining dataset contained 4,323,481 regions, which we called Variant Intolerant Regions (VIRs).

232

233 **VIR properties.** We measured three properties of VIR: length, mean coverage (gnomAD exomes) and
234 conservation (GERP++¹²) (see Fig. 2b and Supplementary Fig. 1). VIR length and conservation were
235 measured at the amino acid level. Regions between variants in two adjacent amino acids were included
236 in the analysis, but had zero length and conservation score. VIR coverage was measured as mean
237 exome coverage of nucleotides including those affected by region start and stop variants. Exome rather
238 than genome coverage was measured because the majority of individuals in gnomAD were exome
239 sequenced (123,136 out of 138,632), and exome coverage tends to be less stable and thus could better
240 highlight potential bias in variant load. Since variant absence might be a consequence of low coverage,
241 strict filters were used to separate high and low covered VIRs. Autosomes, on average, had higher
242 coverage than allosomes and different coverage thresholds were used to mark approximately 80% of
243 the VIRs in both groups as high coverage: ≥ 50 for autosomes (3,427,010 out of 4,221,872, ~81.17%)
244 and ≥ 45 for allosomes (82,033 out of 101,609, ~80.73%). Note that 1,570,941 VIRs were formed by
245 variants in adjacent amino acids and therefore had zero length.

246
247 **Pathogenic variants and evolutionary conservation of VIRs.** Variants with known disease
248 association status (e.g. “Pathogenic”, “Likely pathogenic” or “Pathogenic/Likely pathogenic”) were
249 downloaded from ClinVar¹¹ (accessed August 2018) and re-annotated with Ensembl VEP (version 90).
250 Only variants predicted by VEP to be “missense_variant” (29,433), “stop_gained” (15,991) or
251 “frameshift_variant” (21,471), which were located in 19,361 genes with mapped VIR loci, were
252 analyzed. Firstly, 26,291 pathogenic variants were excluded as they were located in VIRs with low
253 coverage or in VIRs with zero length (i.e. regions between two variants in adjacent codons), since the
254 latter had an evolutionary conservation score of zero. We then grouped all VIRs with high coverage in
255 2,935 genes with at least one pathogenic variant by their length in 5 bins (1-5, 6-10, 11-15, 16-20, 21 or
256 more amino acids) (Fig. 1a), and counted the number and proportion of pathogenic variants that were
257 located in VIRs in each bin (Fig. 1b). Missense and loss-of-function (LoF) variants were analyzed

separately because LoF variant effect on a protein was expected to be less dependent on their location in the protein sequence. To compare pathogenic variant load in VIRs of different lengths, a “Pathogenic Variants per Amino Acid” metric was produced by dividing the number of pathogenic variants observed in VIRs in each bin by the summed length of all VIRs in that bin (Fig. 1c). To calculate 95% confidence intervals for “Pathogenic Variants per Amino Acid” metric, the analysis was repeated 10,000 times on a randomly generated set of 50% of the 2,935 genes containing pathogenic variants (i.e. bootstrapping) using resample method from python scikit-learn module²². To measure correlation between VIR length and evolutionary conservation, we grouped VIRs with high coverage in 5 bins (containing 1-5, 6-10, 11-15, 16-20, 21 or more amino acids; 1,938,102 VIRs in 18,491 genes in total) and displayed the distribution of VIR conservation scores using boxplots (Fig. 1d).

Gene Variation Intolerance Rank (GeVIR) calculation. To develop a gene-level metric based on VIRs, relative weights of VIRs based on their length were first calculated. It was assumed that longer VIRs should have larger weights, since they might be less likely to be caused by random variant distribution. The number of VIRs (with high coverage) of each length were calculated and sorted based on length, in ascending order. We then calculated the weight (W) of each length (l) based on the ratio between all VIRs and the number of VIRs with length l or longer:

$$W_l = \frac{\sum_{i=0}^{l_{max}} VIR_i}{\sum_{i=l}^{l_{max}} VIR_i}$$

For the number and weights of VIRs of each length, see Supplementary Tables 7 and 8 for autosomes and allosomes, respectively.

A score for each gene ($Gene_iScore$) was calculated as a sum of its high covered VIR weights (W_j) adjusted by their conservation ($GERP_j$) and normalized by the total number of regions in a gene including low covered regions ($Nregions_i$). GERP++ scores between -1 and 1, but not 0 (i.e. adjacent

281 variants or no evolutionary conservation data), were rounded to 1 or -1 to avoid extreme penalties in
282 VIR weight (W) adjustment due to multiplication:

$$GERP = \begin{cases} 1, & \text{if } 0 < GERP < 1 \\ -1, & \text{if } -1 < GERP < 0 \\ GERP, & \text{otherwise} \end{cases}$$

283

$$Gene_iScore = \sum_j (W_j * GERP_j) / Nregions_i$$

284
285 VIRs with low coverage were counted in normalization ($Nregions_i$) to avoid genes with a large
286 proportion of VIRs with low coverage receiving high GeneScores due to the presence of a few highly
287 covered VIRs with large weights.

288 All 19,361 genes were sorted based on their *GeneScores* in descending order and percentiles
289 calculated to generate a Gene Variation Intolerance Rank (GeVIR), where a lower rank indicates higher
290 intolerance to variation (see Supplementary Table 2).

291
292 **Disease and null and essential genes.** Disease associated genes were downloaded from the Online
293 Mendelian Inheritance in Man (OMIM) database⁵ (accessed November 2018). Only associations with
294 confident phenotype mapping number (3) were included. Only genes that were associated exclusively
295 with dominant (phenotype keywords: “autosomal dominant”) or recessive (phenotype keywords:
296 “autosomal recessive”) diseases were labeled as “AD” ($n = 790$) and “AR” ($n = 1,585$), respectively.
297 Phenotypes which were marked as unconfirmed – “?”, non-diseases – “[]” or susceptibility – “{ }” were
298 excluded, as were genes that were associated with both AD and AR diseases. A set of genes with at
299 least two different high-confidence LoF variants found in a homozygous state in at least one individual
300 in ExAC (“Null”, $n = 328$)¹, as well as cell essential ($n = 663$) and non-essential ($n = 865$) gene lists,
301 according to CRISPR/Cas studies¹⁵, were obtained from the macarthur-lab github repository

302 (https://github.com/macarthur-lab/gene_lists). Mouse essential genes were obtained from the MGI
303 database via MouseMine^{13,14} website (<http://mousemine.org/>). All genes from heterozygous mouse
304 models with a phenotype term containing the word “lethal” and that were linked to human orthologs
305 (http://www.informatics.jax.org/downloads/reports/HMD_HumanPhenotype.rpt) were exported ($n =$
306 388). Note that genes which were not present in our list of 19,361 protein-coding genes were excluded
307 from all gene datasets.

308

309 **GeVIR comparison with gnomAD gene constraint metrics.** GeVIR performance was compared with
310 three gnomAD gene constraint metrics: loss-of-function observed/expected upper bound fraction
311 (LOEUF)², missense observed/expected upper bound fraction (referred to here as MOEUF, by analogy
312 with LOEUF) and Missense z-score³. LOEUF, MOEUF and Missense z-scores for the 19,361 genes
313 were obtained from gnomAD (<https://gnomad.broadinstitute.org/downloads>). To convert constraint
314 metrics into ranked lists, gene lists were sorted (LOEUF, MOEUF in ascending, Missense z-score in
315 descending order) and gene “weak” percentiles were computed, so that the most variation intolerant
316 genes have low percentiles. To investigate possible complementarity of GeVIR and LOEUF metrics a
317 “Combined” ranked list (VIRLoF) was created, where GeVIR and LOEUF ranks were summed for
318 each gene, the list resorted and percentiles recalculated. We evaluated ranked lists on six datasets:
319 “AD”, “Mouse Heterozygous Lethal”, “Cell Essential”, “AR”, “Cell Non-essential” and “Null” (Table
320 1 and Supplementary Fig. 2a-f). Area under the curve (AUC) method from python scikit-learn²²
321 module was used to measure performance on “AD”, “Mouse Heterozygous Lethal”, “Cell Essential”,
322 “Cell Non-essential” and “Null” gene datasets. Genes from the “AR” dataset were expected to be
323 enriched in the middle of the ranked lists. To investigate this, we divided gene ranked lists into deciles
324 and calculated ratios between “AR” genes in each decile and all “AR” genes (Fig. 3a and
325 Supplementary Fig. 2f). Since a “good” ranked gene list should prioritize “AD” over “AR” genes, we
326 evaluated balance between “AD” and “AR” genes at each cumulative percentile X. We considered

327 “AD” genes with percentiles $\leq X$ as true positives (TP), “AR” genes with percentiles $\leq X$ as false
328 positives (FP), and “AD” genes with percentiles $> X$ as false negatives (FN). Based on these values, we
329 calculated precision, recall and F_1 score for “AD” gene class:

330
$$precision = \frac{TP}{TP+FP} \quad recall = \frac{TP}{TP+FN} \quad F_1score = 2 * \frac{precision*recall}{precision+recall}$$

331

332 F_1 score could be used as a representative metric only for the first third of the genes (Supplementary
333 Fig. 2g) because, after some unknown threshold, “AR” genes had to be prioritized over “Null” genes,
334 which would result in higher numbers of FPs and thus lower precision and F_1 scores. Therefore, peak
335 F_1 score was used for gene ranking method comparisons (Table 1). Performance was measured using F_1
336 score of “AD” genes instead of accuracy or AUC, because there were ~2 times fewer “AD” than “AR”
337 genes in the dataset. Similarity between GeVIR and other ranked gene lists was investigated by
338 calculating the proportion of genes prioritized by both GeVIR and each other method at each
339 cumulative percentile (Supplementary Fig. 2h). The relationship between gene ranks and protein length
340 (based on the canonical transcript) was investigated by measuring Spearman’s rank correlation
341 coefficient (SciPy²³ package) and median protein length comparison at each decile for all ranked gene
342 lists (Fig. 3b and Supplementary Figs. 2i and 3).

343 Note that the evaluation was performed on a dataset that included 1,009 genes containing
344 extremely large or small numbers of variants (i.e. outliers) according to gnomAD gene constraint
345 metrics², and genes without constraint metrics received the lowest ranks (1 gene in Missense z-score
346 and 482 genes in LOEUF lists). However, we included outlier genes in GeVIR score calculations to
347 provide metrics for a larger number of genes, since some of them are well known disease genes (e.g.
348 *CFTR* associated with cystic fibrosis), and their presence did not decrease the performance of GeVIR.
349 Evaluation of gene scores on a dataset excluding outliers (18,352 genes) was also carried out and
350 showed the same overall trends (Supplementary Fig. 4).

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GeVIR and LOEUF comparison of most variation intolerant genes. We selected 2,989 out of 19,361 genes (~15%) that had LOEUF < 0.35 (a hard threshold suggested by the authors for most LoF intolerant genes; L. Francioli, G. Tiao, K. Karczewski, M. Solomonson and N. Watts: <https://macarthurlab.org/2018/10/17/gnomad-v2-1/>) and the same number of genes ranked at the top by GeVIR and VIRLoF. Overlapping genes were investigated by using Venn diagrams (Fig. 4) to compare all genes, “AD” and “AR” genes in the sets. To compare “prioritized gene similarity” with “method performance”, we also calculated precision, recall, and F1 score for the “AD” gene class. We then selected 1,317 genes present only in the GeVIR set, 1,317 genes present only in the LOEUF set, and 1,672 genes present in both sets, which were analyzed by DAVID 6.8^{16,17} for functional enrichment in Gene Ontology (GO) terms (biological process, cellular component, molecular function) and biological pathways (KEGG) (Supplementary Tables 5 and 6).

GeVIR comparison with Constrained Coding Regions (CCRs). Although no gene level metric was provided by the CCR study¹⁰, the authors compared it with gene constraint metrics by sorting genes based on the number of CCRs $\geq 95^{\text{th}}$ or greater percentile content within them. CCR data was downloaded from the browser (<https://s3.us-east-2.amazonaws.com/ccrs/ccr.html>) (accessed December 2018), and genes present in our study with at least one CCR at the 95th percentile were selected (7,000 genes) and sorted, based on the number of CCRs, to create a ranked gene list. The same number of genes ranked at the top by GeVIR were selected, and the cumulative number of “AD” and “AR” genes as well as cumulative F1 score for “AD” gene class and protein length of prioritized genes were compared (Extended Data Fig. 1).

Life Sciences Reporting Summary. Further information on experimental design is available in the Life Sciences Reporting Summary.

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Code availability. Code for calculating GeVIR/VIRLoF scores, data analysis and figures can be found at <https://github.com/gevirank/gevir>. Computed GeVIR/VIRLoF scores are available in Supplementary Table 2.

Data Availability

The GERP++ file can be found at http://mendel.stanford.edu/SidowLab/downloads/gerp/hg19.GERP_scores.tar.gz. The ClinVar files can be found at ftp://ftp.ncbi.nlm.nih.gov/pub/clinvar/tab_delimited/variant_summary.txt.gz and ftp://ftp.ncbi.nlm.nih.gov/pub/clinvar/tab_delimited/var_citations.txt. The Constrained Coding Regions (CCRs) files can be found at <https://s3.us-east-2.amazonaws.com/ccrs/ccrs/ccrs.autosomes.v2.20180420.bed.gz> and <https://s3.us-east-2.amazonaws.com/ccrs/ccrs/ccrs.xchrom.v2.20180420.bed.gz>. The OMIM genemap2.txt file can be found, after registration, at <https://omim.org/downloads/>. The gnomAD gene constraint metric file can be found at https://storage.googleapis.com/gnomad-public/release/2.1.1/constraint/gnomad.v2.1.1.lof_metrics.by_transcript.txt.bgz. The gnomAD exomes variants and coverage files can be found at <https://storage.googleapis.com/gnomad-public/release/2.0.2/vcf/exomes/gnomad.exomes.r2.0.2.sites.vcf.bgz> and https://storage.googleapis.com/gnomad-public/release/2.0.2/coverage/combined_tars/gnomad.exomes.r2.0.2.coverage.all.tar, respectively. The gnomAD genomes variants files can be found at https://storage.googleapis.com/gnomad-public/release/2.0.2/vcf/genomes/gnomad.genomes.r2.0.2.sites.coding_only.chr1-22.vcf.bgz and https://storage.googleapis.com/gnomad-public/release/2.0.2/vcf/genomes/gnomad.genomes.r2.0.2.sites.coding_only.chrX.vcf.bgz. The gnomAD genes, transcripts and exons files can be found at http://broadinstitute.org/~konradk/exac_browser/exac_browser.tar.gz. The Ensembl coding and peptide sequences from build GRCh37/hg19 can be found at <https://grch37.ensembl.org/biomart/martview/> (Dataset: Human genes (GRCh37.p13); Attributes→ Sequences → “Coding sequence” and “Peptide”). The homozygous Loss-of-function tolerant genes (i.e. Nulls) can be found at https://github.com/macarthur-lab/gene_lists/blob/master/lists/homozygous_lof_tolerant_twohit.tsv. The cell essential and non-essential genes from CRISPR/Cas experiments can be found at https://github.com/macarthur-lab/gene_lists/blob/master/lists/CEGv2_subset_universe.tsv and https://github.com/macarthur-lab/gene_lists/blob/master/lists/NEGv1_subset_universe.tsv, respectively. The mouse heterozygous lethal genes can be obtained from <http://mousemine.org/> by quiring the database with following constraints: path="OntologyAnnotation.ontologyTerm" type="MPTerm"; path="OntologyAnnotation.subject" type="SequenceFeature"; path="OntologyAnnotation.evidence.baseAnnotations.subject" type="Genotype"; path="OntologyAnnotation.evidence.baseAnnotations.subject.zygosity" op="=" value="ht" code="B"; path="OntologyAnnotation.ontologyTerm.name" op="CONTAINS" value="lethal". The human-mouse ortholog mapping file can be found at

http://www.informatics.jax.org/downloads/reports/HMD_HumanPhenotype.rpt. The HGNC approved gene symbols can be found at <https://www.genenames.org/download/statistics-and-files/>.

Method-only References

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VIR weights table

Length	Number*	Weight**
0	1	$6 / (1+1+3+1) = 1$
1	1	$6 / (1+3+1) = 1.2$
3	3	$6 / (3+1) = 1.5$
5	1	$6 / 1 = 6$

* VIRs with mean coverage < 50 (45 for X and Y chromosomes, analysed separately) are not counted.

** Weight is calculated as total number of VIRs divided by number of VIRs with this length or less

e

Gene score calculation

$$Gene_i Score = \sum_j (W_j * GERP_j) N_{regions_i}$$

$$GERP = \begin{cases} 1, & \text{if } 0 < GERP < 1 \\ -1, & \text{if } -1 < GERP < 0 \\ GERP, & \text{otherwise} \end{cases}$$

VIRs include low coverage (in white)

$$Gene_a Score = \frac{1.5 \times (-1) + 6 \times 3 + 1.2 \times 2 + 1 \times 0}{4} = 4.725$$

$$Gene_b Score = \frac{1.5 \times 3 + 1.5 \times 3}{3} = 1.5$$

a **b**

a **b** **c** **d**