Integrating healthcare and research genetic data empowers the discovery of 49 novel developmental disorders

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Summary

De novo mutations (DNMs) in protein-coding genes are a well-established cause of developmental disorders (DD). However, known DD-associated genes only account for a minority of the observed excess of such DNMs. To identify novel DD-associated genes, we integrated healthcare and research exome sequences on 31,058 DD parent-offspring trios, and developed a simulation-based statistical test to identify gene-specific enrichments of DNMs. We identified 299 significantly DD-associated genes, including 49 not previously robustly associated with DDs. Despite detecting more DD-associated genes than in any previous study, much of the excess of DNMs of protein-coding genes remains unaccounted for. Modelling suggests that over 500 novel DD-associated genes await discovery, many of which are likely to be less penetrant than the currently known genes. Research access to clinical diagnostic datasets will be critical for completing the map of dominant DDs.

14 **Introduction**

15 It has previously been estimated that ~42-48% of patients with a severe developmental
16 disorder (DD) have a pathogenic *de novo* mutation (DNM) in a protein coding gene[1,2]. However,
17 over half of these patients remain undiagnosed despite the identification of hundreds of
18 dominant and X-linked DD-associated genes. This implies that there are more DD relevant
19 genes left to find. Existing methods to detect gene-specific enrichments of damaging DNMs
20 typically ignore much prior information about which variants and genes are more likely to be
21 disease-associated. However, missense variants and protein-truncating variants (PTVs) vary in
22 their impact on protein function[3–6]. Known dominant DD-associated genes are strongly enriched
23 in the minority of genes that exhibit patterns of strong selective constraint on heterozygous
24 PTVs in the general population[7]. To identify the remaining DD genes, we need to increase our
25 power to detect gene-specific enrichments for damaging DNMs by both increasing sample sizes
26 and improving our statistical methods. In previous studies of pathogenic Copy Number Variation
27 (CNV), utilising healthcare-generated data has been key to achieve much larger sample sizes
28 than would be possible in a research setting alone[8,9].

29

30 **Improved statistical enrichment test identifies over 300 significant DD-associated genes**

31 Following clear consent practices and only using aggregate, de-identified data, we
32 pooled DNMs in patients with severe developmental disorders from three centres: GeneDx (a
33 US-based diagnostic testing company), the Deciphering Developmental Disorders study, and
34 Radboud University Medical Center. We performed stringent quality control on variants and
35 samples to obtain 45,221 coding and splicing DNMs in 31,058 individuals (**Supplementary Fig.
36 1**; **Supplementary Table 1**), which includes data on over 24,000 trios not previously published.
37 These DNMs included 40,992 single nucleotide variants (SNVs) and 4,229 indels. The three
38 cohorts have similar clinical characteristics, male/female ratios, enrichments of DNMs by
39 mutational class, and prevalences of known disorders (**Supplementary Fig. 2**).

40 To detect gene-specific enrichments of damaging DNMs, we developed a method
41 named DeNovoWEST (*De Novo* Weighted Enrichment Simulation Test,
42 https://github.com/queenjobo/DeNovoWEST). DeNovoWEST scores all classes of sequence
43 variants on a unified severity scale based on the empirically-estimated positive predictive value
44 of being pathogenic (**Supplementary Fig. 3-4**). We then applied a Bonferroni multiple testing
45 correction with independent hypothesis weighting[10] to incorporate a gene-based weighting using
46 the selective constraint against heterozygous PTVs in the general population (s_{het}[11]), which is
47 strongly correlated with the likelihood of being a dominant disease gene[7,11].

48 We first applied DeNovoWEST to all individuals in our cohort and identified 299
49 significant genes, 35 more than when using our previous method[1] (**Supplementary Fig. 5**; **Fig.
50 1a**). The majority (181/299; 61%) of these significant genes already had sufficient evidence of

DD-association to be considered of diagnostic utility (as of September 2018) by all three centres, and we refer to them as “consensus” genes. 69/299 of these significant genes were previously considered diagnostic by one or two centres (“discordant” genes). Applying DeNovoWEST to synonymous DNMs, as a negative control analysis, identified no significantly enriched genes (**Supplementary Fig. 6**). To discover novel DD-associated genes with greater power, we then applied DeNovoWEST only to DNMs in patients without damaging DNMs in consensus genes (we refer to this subset as ‘undiagnosed’ patients) and identified 118 significant genes (**Fig. 1b; Supplementary Fig. 7; Supplementary Table 2**). While 69 of these genes were discordant genes, we identified 49 ‘novel’ DD-associated genes, which had a median of 10 nonsynonymous DNMs in our dataset (**Fig. 1c**; **Supplementary Table 3**). There were 500 patients with nonsynonymous DNMs in these 49 genes (1.6% of our cohort); all DNMs in these genes were inspected in IGV[12] and, of 198 for which experimental validation was attempted, all were confirmed as DNMs in the proband. The DNMs in these novel genes were distributed approximately randomly across the three datasets (no genes with $p < 0.001$, heterogeneity test). Fourteen of the 49 novel DD-associated genes have been further corroborated by recent OMIM entries or publications. In particular, seven of these 14 genes (*PPP2CA*[13], *ZMIZ1*[14], *CDK8*[15], *VAMP2*[16], *KMT2E*[17], *KDM6B*[18], and *TAOK1*[19]) have had genotype-phenotype studies recently published.

We also investigated whether some synonymous DNMs might be pathogenic by disrupting splicing. We annotated all synonymous DNMs with a splicing pathogenicity score, SpliceAI[20], and identified a significant enrichment of synonymous DNMs with high SpliceAI scores (≥ 0.8, 1.56-fold enriched, $p = 0.0037$, Poisson test; **Supplementary Table 4**). This enrichment corresponds to an excess of ~15 splice-disrupting synonymous mutations in our cohort, of which six are accounted for by a single recurrent synonymous mutation in *KAT6B* known to disrupt splicing[21].

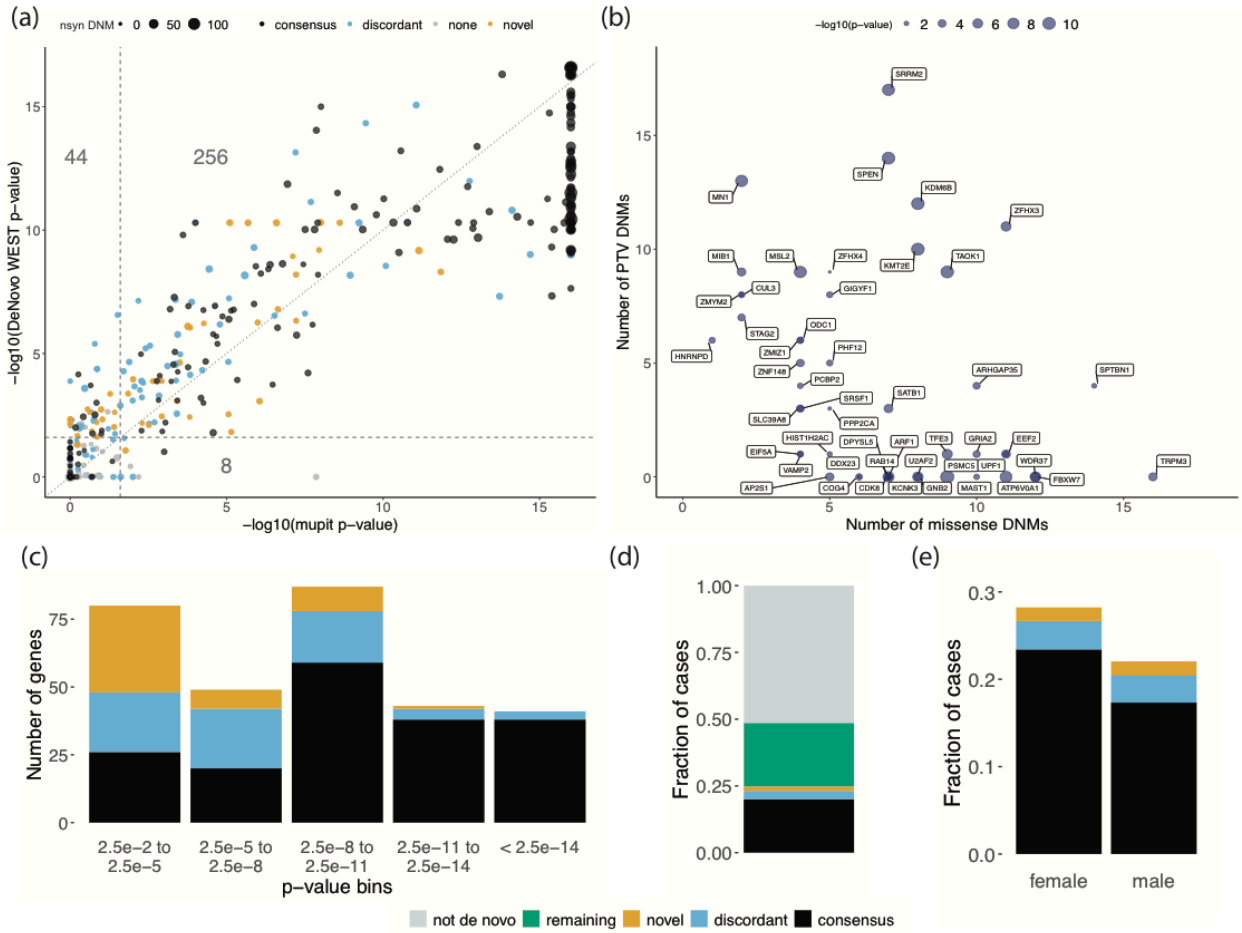


Figure 1: Results of DeNovoWEST analysis. (a) Comparison of p-values generated using the new method (DeNovoWEST) versus the previous method (mupit)[1]. These are results from DeNovoWEST run on the full cohort. The dashed lines indicate the threshold for genome-wide significance (the p-values have already been corrected for multiple testing). The size of the points is proportional to the number of nonsynonymous DNMs in our cohort (nsyn). The numbers describe the number of genes that fall into each quadrant (43 in the top left, 256 in the top right, and 8 in the bottom right). (b) The number of missense and PTV DNMs in our cohort in the 49 novel genes. The size of the points are proportional to the log10(-p-value) from the analysis on the undiagnosed subset. (c) The histogram depicts the distribution of p-values from the analysis on the undiagnosed subset for discordant and novel genes; p-values for consensus genes come from the full analysis. The number of genes in each p-value bin is coloured by diagnostic gene group. (d) The fraction of cases with a nonsynonymous mutation in each diagnostic gene group. (e) The fraction of cases with a nonsynonymous mutation in each diagnostic gene group split by sex. In all figures, black represents the consensus known genes, blue represents the discordant known genes, and orange represents the novel genes. In (c), green represents the remaining fraction of cases expected to have a pathogenic *de novo* coding mutation ("remaining") and grey is the fraction of cases that are likely to be explained by other genetic or nongenetic factors ("not de novo").

95

96 Taken together, 24.8% of individuals in our combined cohort have a nonsynonymous
97 DNM in one of the consensus or significant DD-associated genes (**Fig. 1d**). We noted
98 significant sex differences in the autosomal burden of nonsynonymous DNMs (**Supplementary**
99 **Fig. 8**). The rate of nonsynonymous DNMs in consensus autosomal genes was significantly
100 higher in females than males (OR = 1.17, $p = 1.1 \times 10^{-7}$, Fisher's exact test; **Fig. 1e**), as noted
101 previously[1]. However, the exome-wide burden of autosomal nonsynonymous DNMs in all genes
102 was not significantly different between undiagnosed males and females (OR = 1.03, p = 0.29,
103 Fisher's exact test). This suggests the existence of subtle sex differences in the genetic
104 architecture of DD, especially with regard to known and undiscovered disorders.

105

106 **Characteristics of the novel DD-associated genes and disorders**

107 Based on semantic similarity[22] between Human Phenotype Ontology terms, patients with
108 DNMs in the same novel DD-associated gene were less phenotypically similar to each other, on
109 average, than patients with DNMs in a consensus gene ($p = 9.5 \times 10^{-38}$, Wilcoxon rank-sum test;
110 **Fig. 2a**). This suggests that these novel disorders less often result in distinctive and consistent
111 clinical presentations, which may have made these disorders harder to discover via a
112 phenotype-driven analysis or recognise by clinical presentation alone. Each of these novel
113 disorders requires a detailed genotype-phenotype characterisation, which is beyond the scope
114 of this study.

115 Overall, novel DD-associated genes encode proteins that have very similar functional
116 and evolutionary properties to consensus genes, e.g. developmental expression patterns,
117 network properties and biological functions (**Fig. 2b; Supplementary Table 5**). Despite the
118 high-level functional similarity between known and novel DD-associated genes, the
119 nonsynonymous DNMs in the more recently discovered DD-associated genes are much more
120 likely to be missense DNMs, and less likely to be PTVs (discordant and novel; $p = 3.3 \times 10^{-21}$,
121 chi-squared test). Sixteen of the 49 (33%) of the novel genes only had missense DNMs, and
122 only a minority had more PTVs than missense DNMs. Consequently, we expect that a greater
123 proportion of the novel genes will act via altered-function mechanisms (e.g. dominant negative
124 or gain-of-function). For example, the novel gene *PSMC5* (DeNovoWEST $p = 6.5 \times 10^{-10}$) had
125 one inframe deletion and nine missense DNMs, eight of which altered one of two amino acids
126 that interact within the 3D protein structure: p.Pro320Arg and p.Arg325Trp (**Supplementary**
127 **Fig. 9a-b**), and so is likely to operate via an altered-function mechanism. Additionally, we
128 identified one novel DD-associated gene, *MN1*, with *de novo* PTVs significantly ($p = 1.6 \times 10^{-7}$,
129 Poisson test) clustered at the 3' end of its transcript (**Supplementary Fig. 9c**). This clustering of
130 PTVs indicates the transcript likely escapes nonsense mediated decay and potentially acts via a

gain-of-function or dominant negative mechanism[23], although this will require functional confirmation.

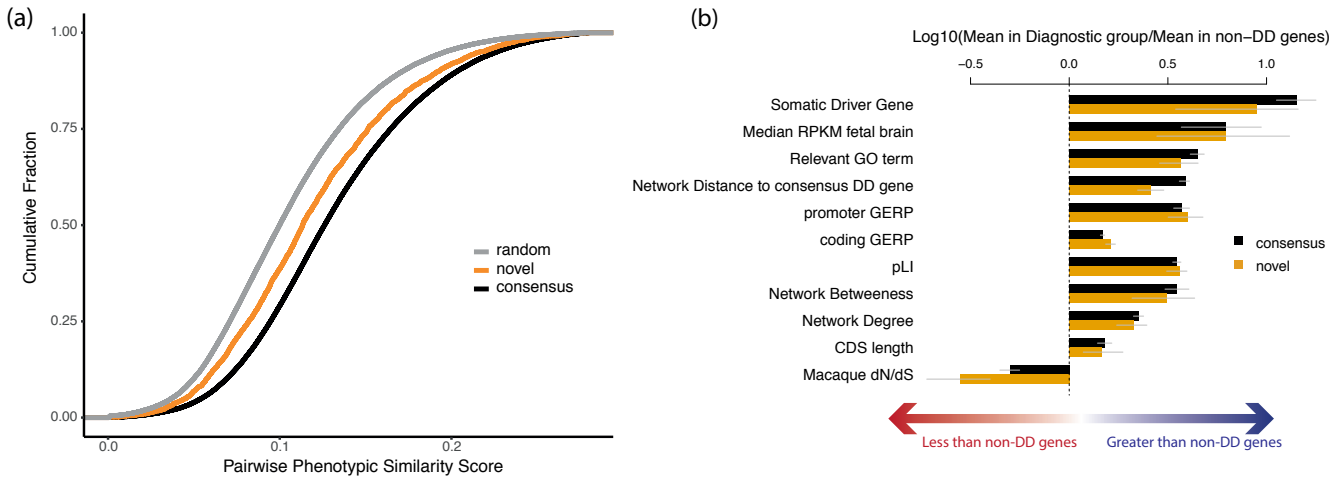


Figure 2: Functional properties and mechanisms of novel genes. (a) Comparing the phenotypic similarity of patients with DNMs in novel and consensus genes. Random phenotypic similarity was calculated from random pairs of patients. Patients with DNMs in the same novel DD-associated gene were less phenotypically similar than patients with DNMs in a known DD-associated gene ($p = 9.5 \times 10^{-38}$, Wilcoxon rank-sum test). (b) Comparison of functional properties of consensus known and novel DD genes. Properties were chosen as those known to be differential between consensus and non-DD genes.

We observed that missense DNMs were more likely to affect functional protein domains than other coding regions. We observed a 2.76-fold enrichment ($p = 1.6 \times 10^{-68}$, G-test) of missense DNMs residing in protein domains among consensus genes and a 1.87-fold enrichment ($p = 1.4 \times 10^{-4}$, G-test) in novel DD-associated genes, but no enrichment for synonymous DNMs (**Supplementary Table 6**). Three protein domain families in consensus genes were specifically enriched for missense DNMs (**Supplementary Table 7**): ion transport protein (PF00520, $p = 3.9 \times 10^{-7}$, G-test), ligand-gated ion channel (PF00060, $p = 6.7 \times 10^{-7}$, G-test), and protein kinase domain (PF00069, $p = 4.4 \times 10^{-2}$, G-test). Missense DNMs in all three enriched domain families, have previously been associated with DD (**Supplementary Table 8**)[24].

We observed a significant overlap between the 299 DNM-enriched DD-associated genes and a set of 369 previously described cancer driver genes[25] ($p = 1.7 \times 10^{-46}$, logistic regression correcting for s_{het}), as observed previously[26,27], as well as a significant enrichment of nonsynonymous DNMs in these genes (**Supplementary Table 9**). This overlap extends to somatic driver mutations: we observe 117 DNMs at 76 recurrent somatic mutations observed in at least three patients in The Cancer Genome Atlas (TCGA)[28]. By modelling the germline

158 mutation rate at these somatic driver mutations, we found that recurrent nonsynonymous
159 mutations in TCGA are enriched 21-fold in the DDD cohort ($p < 10^{-50}$, Poisson test,
160 **Supplementary Fig. 9**), whereas recurrent synonymous mutations in TCGA are not significantly
161 enriched (2.4-fold, $p = 0.13$, Poisson test). This suggests that this observation is driven by the
162 pleiotropic effects of these mutations in development and tumourigenesis, rather than
163 hypermutability.
164
165 ***Recurrent mutations and potential new germline selection genes***
166 We identified 773 recurrent DNMs (736 SNVs and 37 indels), ranging from 2-36
167 independent observations per DNM, which allowed us to interrogate systematically the factors
168 driving recurrent germline mutation. We considered three potential contributory factors: (i)
169 clinical ascertainment enriching for pathogenic mutations, (ii) greater mutability at specific sites,
170 and (iii) positive selection conferring a proliferative advantage in the male germline, thus
171 increasing the prevalence of sperm containing the mutation[29]. We observed strong evidence
172 that all three factors contribute, but not necessarily mutually exclusively. Clinical ascertainment
173 drives the observation that 65% of recurrent DNMs were in consensus genes, a 5.4-fold
174 enrichment compared to DNMs only observed once ($p < 10^{-50}$, proportion test). Hypermutability
175 underpins the observation that 68% of recurrent *de novo* SNVs occurred at hypermutable CpG
176 dinucleotides[30], a 1.8-fold enrichment over DNMs only observed once ($p = 1.1 \times 10^{-59}$,
177 proportion test**)**. We also observed a striking enrichment of recurrent mutations at the
178 haploinsufficient DD-associated gene *MECP2*, in which we observed 11 recurrently mutated
179 SNVs within a 500bp window, nine of which were G to A mutations at a CpG dinucleotide.
180 *MECP2* exhibits a highly significant twofold excess of synonymous mutations within the
181 Genome Aggregation Database (gnomAD) population variation resource[5], suggesting that
182 locus-specific hypermutability might explain this observation.
183 To assess the contribution of germline selection to recurrent DNMs, we initially focused
184 on the 12 known germline selection genes, which all operate through activation of the RAS-
185 MAPK signalling pathway[31,32]. We identified 39 recurrent DNMs in 11 of these genes, 38 of
186 which are missense and all of which are known to be activating in the germline (see
187 supplement). As expected, given that hypermutability is not the driving factor for recurrent
188 mutation in these germline selection genes, these 39 recurrent DNMs were depleted for CpGs
189 relative to other recurrent mutations (9/39 vs 450/692, $p = 0.0067$, chi-squared test).
190 Positive germline selection has been shown to be capable of increasing the apparent
191 mutation rate more strongly[29] than either clinical ascertainment (10-100X in our dataset) or
192 hypermutability (~10X for CpGs). However, only a minority of the most highly recurrent
193 mutations in our dataset are in genes that have been previously associated with germline
194 selection. Nonetheless, several lines of evidence suggested that the majority of these most

195 highly recurrent mutations are likely to confer a germline selective advantage. Based on the
196 recurrent DNMs in known germline selection genes, DNMs under germline selection should be
197 more likely to be activating missense mutations, and should be less enriched for CpG
198 dinucleotides. **Table 1** shows the 16 *de novo* SNVs observed nine or more times in our DNM
199 dataset, only two of which are in known germline selection genes (*MAP2K1* and *PTPN11*). All
200 but two of these 16 *de novo* SNVs cause missense changes, all but two of these genes cause
201 disease by an altered-function mechanism, and these DNMs were depleted for CpGs relative to
202 all recurrent mutations. Two of the genes with highly recurrent *de novo* SNVs, *SHOC2* and
203 *PPP1CB,* encode interacting proteins that are known to play a role in regulating the RAS-MAPK
204 pathway, and pathogenic variants in these genes are associated with a Noonan-like
205 syndrome[33]. Moreover, two of these recurrent DNMs are in the same gene *SMAD4*, which
206 encodes a key component of the TGF-beta signalling pathway, potentially expanding the
207 pathophysiology of germline selection beyond the RAS-MAPK pathway. Confirming germline
208 selection of these mutations will require deep sequencing of testes and/or sperm[32].
209

Symbol	Chr	Position	Ref	Alt	Consequence	Recur	Likely mechanism	CpG	Somatic Driver Gene	Germline Selection Gene	DD status
PACS1	11	65978677	C	T	missense	36	activating	Yes	-	-	consensus
PPP2R5D	6	42975003	G	A	missense	22	dominant negative	-	-	-	consensus
SMAD4	18	48604676	A	G	missense	21	activating	-	Yes	-	consensus
PACS2	14	105834449	G	A	missense	13	dominant negative	Yes	-	-	discordant
MAP2K1	15	66729181	A	G	missense	11	activating	-	Yes	Yes	consensus
PPP1CB	2	28999810	C	G	missense	11	all missense/in frame	-	-	-	consensus
NAA10	X	153197863	G	A	missense	11	all missense/in frame	Yes	-	-	consensus
MECP2	X	153296777	G	A	stop gain	11	loss of function	Yes	-	-	consensus
CSNK2A1	20	472926	T	C	missense	10	activating	-	-	-	consensus
CDK13	7	40085606	A	G	missense	10	all missense/in frame	-	-	-	consensus
SHOC2	10	112724120	A	G	missense	9	activating	-	-	-	consensus
PTPN11	12	112915523	A	G	missense	9	activating	-	Yes	Yes	consensus
SMAD4	18	48604664	C	T	missense	9	activating	Yes	Yes	-	consensus
SRCAP	16	30748664	C	T	stop gain	9	dominant negative	Yes	-	-	consensus
FOXP1	3	71021817	C	T	missense	9	loss of function	Yes	-	-	consensus
CTBP1	4	1206816	G	A	missense	9	dominant negative	Yes	-	-	discordant

210
211
212 **Table 1: Recurrent Mutations.** *De novo* single nucleotide variants with more than 9
213 recurrences in our cohort annotated with relevant information, such as CpG status, whether the
214 impacted gene is a known somatic driver or germline selection gene, and diagnostic gene group
215 (e.g. consensus known). “Recur” refers to number of recurrence. “Likely mechanism” refers to
216 mechanisms attributed to this gene in the published literature.

Evidence for incomplete penetrance and pre/perinatal death

Nonsynonymous DNMs in consensus or significant DD-associated genes accounted for half of the exome-wide nonsynonymous DNM burden associated with DD (**Fig. 1b**). Despite our identification of 299 significantly DD-associated genes, there remains a substantial burden of both missense and protein-truncating DNMs in unassociated genes (those that are neither significant in our analysis nor on the consensus gene list). The remaining burden of protein-truncating DNMs is greatest in genes that are intolerant of PTVs in the general population (**Supplementary Fig. 11**) suggesting that more haploinsufficient (HI) disorders await discovery. We estimated that our statistical power to detect the gene enrichment for protein-truncating DNMs expected for an HI disorder was lower in unassociated genes compared to the novel DD-associated genes ($p = 2.9 \times 10^{-6}$ Wilcox rank-sum test; **Fig. 3a**). However, the novel genes do not have significantly less power compared to the consensus genes ($p = 0.059$, Wilcox rank-sum test).

A key parameter in the above power analysis is the fold-enrichment of *de novo* PTVs expected in as yet undiscovered HI disorders, which we assumed above to be 37-fold, based on the average enrichment observed in known HI DD-associated genes. However, we observed that novel DD-associated HI genes had significantly lower PTV enrichment compared to the consensus HI genes ($p = 1.6 \times 10^{-5}$, Poisson test; **Fig. 3b**). Two additional factors that could lower DNM enrichment, and thus power to detect a novel DD-association, are reduced penetrance and increased pre/perinatal death, which here covers spontaneous fetal loss, termination of pregnancy for fetal anomaly, stillbirth, and early neonatal death. To evaluate incomplete penetrance, we investigated whether HI genes with a lower enrichment of protein-truncating DNMs in our cohort are associated with greater prevalences of PTVs in the general population. We observed a significant ($p = 0.031$, weighted linear regression) negative correlation between PTV enrichment in our cohort and the ratio of PTV to synonymous variants in the gnomAD dataset of population variation[5], suggesting that incomplete penetrance does lower *de novo* PTV enrichment in our cohort (**Fig. 3c**).

Additionally, we observed that the fold-enrichment of protein-truncating DNMs in consensus HI DD-associated genes in our cohort was significantly lower for genes with a medium or high likelihood of presenting with a prenatal structural malformation ($p = 0.0002$, Poisson test, **Fig. 3d**), suggesting that pre/perinatal death decreases our power to detect some novel DD-associated disorders (see supplement for details).

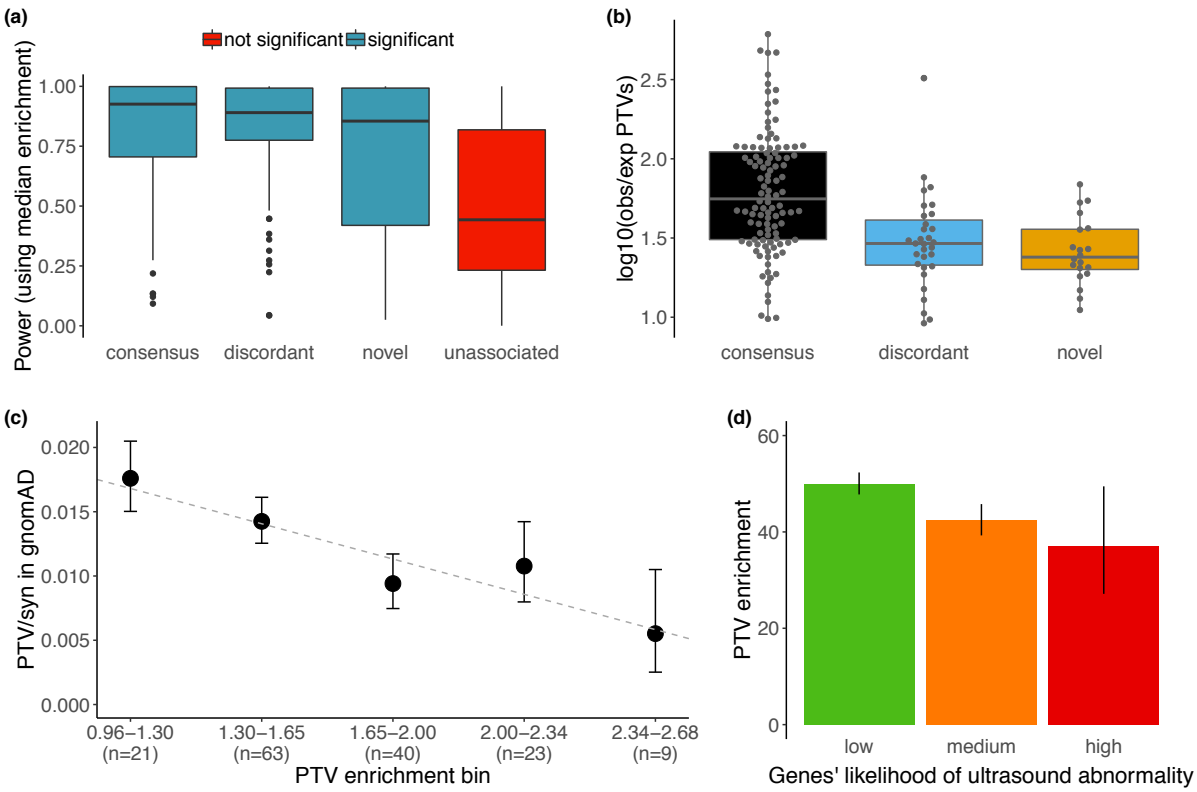
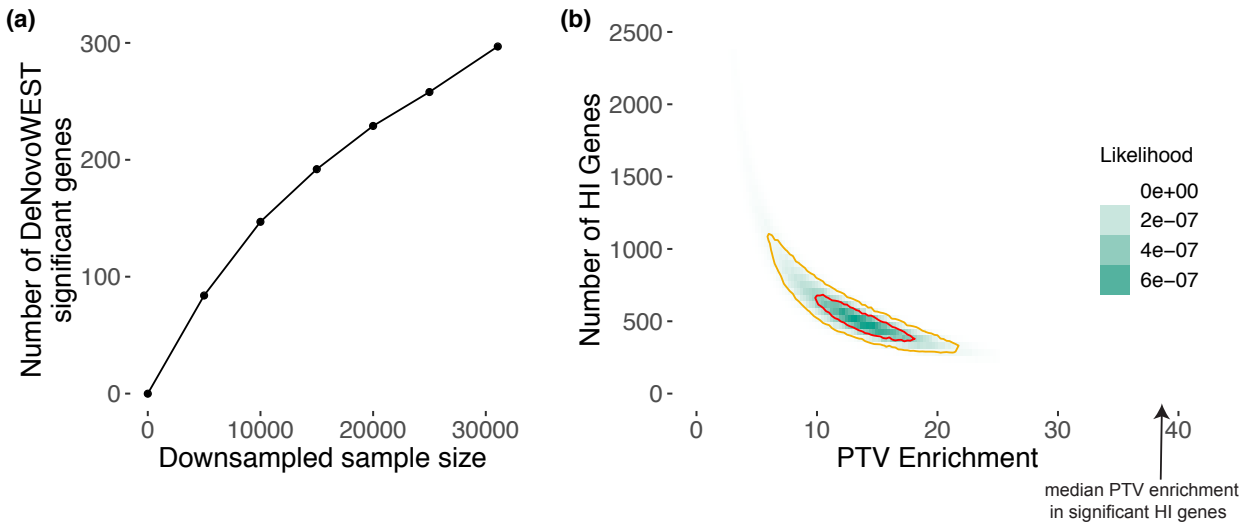


Figure 3: Impact of pre/perinatal death and penetrance on power. (a) Power to detect median PTV enrichment (36.6) in consensus known genes across diagnostic groups. Genes that were not significantly associated to DD in our analysis ("unassociated") have significantly lower power than the novel genes ($p = 2.9 \times 10^{-6}$, Wilcox rank-sum test). (b) Distribution of PTV enrichment in significant, likely haploinsufficient, genes by diagnostic group. (c) Comparison of the PTV enrichment in our cohort vs the PTV to synonymous ratio found in gnomAD. PTV enrichment is shown as log10(enrichment). There is a significant negative relationship ($p = 0.031$, weighted regression). (d) Overall *de novo* PTV enrichment (observed / expected PTVs) across genes grouped by their clinician-assigned likelihood of presenting with a structural malformation on ultrasound during pregnancy. PTV enrichment is significantly lower for genes with a medium or high likelihood compared to genes with a low likelihood ($p = 0.002$, Poisson test).

Modelling reveals hundreds of DD genes remain to be discovered

To understand the likely trajectory of future DD discovery efforts, we downsampled the current cohort and reran our enrichment analysis (**Fig. 4a**). We observed that the number of significant genes has not yet plateaued. Increasing sample sizes should result in the discovery of many novel DD-associated genes. To estimate how many haploinsufficient genes might await discovery, we modelled the likelihood of the observed distribution of protein-truncating DNMs among genes as a function of varying numbers of undiscovered HI DD genes and fold-

271 enrichments of protein-truncating DNMs in those genes. We found that the remaining HI burden
272 is most likely spread across ~500 genes with ~12-fold PTV enrichment (**Fig. 4b**). This fold
273 enrichment is three times lower than in known HI DD-associated genes, suggesting that
274 incomplete penetrance and/or pre/perinatal death is much more prevalent among undiscovered
275 HI genes. We modelled the missense DNM burden separately and also observed that the most
276 likely architecture of undiscovered DD-associated genes is one that comprises over 500 genes
277 with a substantially lower fold-enrichment than in currently known DD-associated genes
278 (**Supplemental Fig. 12**).
279 We calculated that a sample size of ~200,000 parent-offspring trios would be needed to
280 have 80% power to detect a 12-fold enrichment of protein-truncating DNMs for a gene with the
281 median PTV mutation rate among currently unassociated genes. Using this inferred 12-fold
282 enrichment among undiscovered HI genes, from our current data we can evaluate the likelihood
283 that any gene in the genome is an undiscovered HI gene, by comparing the likelihood of the
284 number of *de novo* PTVs observed in each gene to have arisen from the null mutation rate or
285 from a 12-fold increased PTV rate. Among the ~19,000 non-DD-associated genes, ~1,100 were
286 more than three times more likely to have arisen from a 12-fold increased PTV rate, whereas
287 ~9,000 were three times more likely to have no *de novo* PTV enrichment.



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289 **Figure 4: Exploring the remaining number of DD genes.** (a) Number of significant genes
290 from downsampling full cohort and running DeNovoWEST's enrichment test. (b) Results from
291 modelling the likelihood of the observed distribution of *de novo* PTV mutations. This model
292 varies the numbers of remaining haploinsufficient (HI) DD genes and PTV enrichment in those
293 remaining genes. The 50% credible interval is shown in red and the 90% credible interval is
294 shown in orange. Note that the median PTV enrichment in significant HI genes (shown with an
295 arrow) is 38.8.

296 **Discussion**

297 In this study, we have discovered 49 novel developmental disorders by developing an
298 improved statistical test for mutation enrichment and applying it to a dataset of exome
299 sequences from 31,058 children with developmental disorders, and their parents. These 49
300 novel genes account for up to 1.6% of our cohort, and inclusion of these genes in diagnostic
301 workflows will catalyse increased diagnosis of similar patients globally. We have shown that
302 both incomplete penetrance and pre/perinatal death reduce our power to detect novel DDs
303 postnatally, and that one or both of these factors are likely operating considerably more strongly
304 among undiscovered DD-associated genes. In addition, we have identified a set of highly
305 recurrent mutations that are strong candidates for novel germline selection mutations, which
306 would be expected to result in a higher than expected disease incidence that increases
307 dramatically with increased paternal age.

308 Our study represents the largest collection of DNMs for any disease area, and is
309 approximately three times larger than a recent meta-analysis of DNMs from a collection of
310 individuals with autism spectrum disorder, intellectual disability, and/or a developmental
311 disorder[34]. Our analysis included DNMs from 24,348 previously unpublished trios, and we
312 identified ~2.4 times as many significantly DD-associated genes as this previous study when
313 using Bonferroni-corrected exome-wide significance (299 vs 124). In contrast to meta-analyses
314 of published DNMs, the harmonised filtering of candidate DNMs across cohorts in this study
315 should protect against results being confounded by substantial cohort-specific differences in the
316 sensitivity and specificity of detecting DNMs.

317 Here we inferred indirectly that developmental disorders with higher rates of detectable
318 prenatal structural abnormalities had greater pre/perinatal death. The potential size of this effect
319 can be quantified from the recently published PAGE study of genetic diagnoses in a cohort of
320 fetal structural abnormalities[35]. In this latter study, genetic diagnoses were not returned to
321 participants during the pregnancy, and so the genetic diagnostic information itself could not
322 influence pre/perinatal death. In the PAGE study data, 69% of fetal abnormalities with a
323 genetically diagnosable cause died perinatally or neonatally, with termination of pregnancy, fetal
324 demise and neonatal death all contributing. This emphasises the substantial impact that
325 pre/perinatal death can have on reducing the ability to discover novel DDs from postnatal
326 recruitment alone, and motivates the integration of genetic data from prenatal, neonatal and
327 postnatal studies in future analyses.

328 To empower our mutation enrichment testing, we estimated positive predictive values
329 (PPV) of each DNM being pathogenic on the basis of their predicted protein consequence,
330 CADD score[3] and presence in a region or gene under missense constraint in the general
331 population[4]. These PPVs should also be highly informative for variant prioritisation in the
332 diagnosis of dominant developmental disorders. Further work is needed to see whether these

333 PPVs might be informative for recessive developmental disorders, and in other types of
334 dominant disorders. More generally, we hypothesise that empirically-estimated PPVs based on
335 variant enrichment in large datasets will be similarly informative in many other disease areas.
336 We adopted a conservative statistical approach to identifying DD-associated genes. In
337 two previous studies using the same significance threshold, we identified 26 novel DD-
338 associated genes[1,36]. All 26 are now regarded as being diagnostic, and have entered routine
339 clinical diagnostic practice. Had we used a significance threshold of FDR < 10% as used in
340 Satterstrom, Kosmicki, Wang et al[37], we would have identified 737 DD-associated genes.
341 However, as the FDR of individual genes depends on the significance of other genes being
342 tested, FDR thresholds are not appropriate for assessing the significance of individual genes,
343 but rather for defining gene-sets. There are 150 consensus genes that did not cross our
344 significance threshold in this study. It is likely that many of these cause disorders that were
345 under-represented in our study due to the ease of clinical diagnosis on the basis of distinctive
346 clinical features or targeted diagnostic testing. These ascertainment biases are, however, not
347 likely to impact the representation of novel DDs in our cohort.
348 Our modelling also suggested that likely over 1,000 DD-associated genes remain to be
349 discovered, and that reduced penetrance and pre/perinatal death will reduce our power to
350 identify these genes through DNM enrichment. Identifying these genes will require both
351 improved analytical methods and greater sample sizes. We anticipate that the variant-level and
352 gene-level weights used by DeNovoWEST will improve over time. As reference population
353 samples, such as gnomAD[5], increase in size, gene-level weights based on selective constraint
354 metrics will improve. Gene-level weights could also incorporate more functional information,
355 such as expression in disease-relevant tissues. For example, we observe that our DD-
356 associated genes are significantly more likely to be expressed in fetal brain (**Supplementary**
357 **Fig. 13**). Furthermore, novel metrics based on gene co-regulation networks can predict whether
358 genes function within a disease relevant pathway[38]. As a cautionary note, including more
359 functional information in the gene-level weights may increase power to detect some novel
360 disorders while decreasing power for disorders with pathophysiology different from known
361 disorders. Variant-level weights could be further improved by incorporating other variant
362 prioritisation metrics, such as upweighting variants predicted to impact splicing, variants in
363 particular protein domains, or variants that are somatic driver mutations during tumorigenesis.
364 Finally, the discovery of less penetrant disorders can be empowered by analytical
365 methodologies that integrate both DNMs and rare inherited variants, such as TADA[39].
366 Nonetheless, using current methods, we estimated that ~200,000 parent-child trios would need
367 to be analysed to have ~80% power to detect HI genes with a 12-fold PTV enrichment.
368 Discovering non-HI disorders will need even larger sample sizes. Reaching this number of
369 sequenced families will be impossible for an individual research study or clinical centre,

370 therefore it is essential that genetic data generated as part of routine diagnostic practice is
371 shared with the research community such that it can be aggregated to drive discovery of novel
372 disorders and improve diagnostic practice.
373
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385
386 **Data Access**
387 Sequence and variant level data and phenotypic data for the DDD study data are available
388 through EGA study ID EGAS00001000775
389 RadboudUMC sequence and variant level data cannot be made available through EGA due to
390 the nature of consent for clinical testing
391 GeneDx data cannot be made available through EGA due to the nature of consent for clinical
392 testing. GeneDx has contributed deidentified data to this study to improve clinical interpretation
393 of genomic data, in accordance with patient consent and in conformance with the ACMG
394 position statement on genomic data sharing (see Supplementary Note for details).
395 Clinically interpreted variants and associated phenotypes from the DDD study are available
396 through DECIPHER (https://decipher.sanger.ac.uk)
397 Clinically interpreted variants from RUMC are available from the Dutch national initiative for
398 sharing variant classifications (https://www.vkgl.nl/nl/diagnostiek/vkgl-datashare-database)
399 Clinically interpreted variants from GeneDx are deposited in ClinVar
400 (https://www.ncbi.nlm.nih.gov/clinvar)
401

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