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Joanna Kaplanis, Kaitlin E. Samocha, Laurens Wiel, Zhancheng Zhang ...+31 more authors

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

Joanna Kaplanis[1]*, Kaitlin E. Samocha[1]*, Laurens Wiel[2,3]*, Zhancheng Zhang[4]*, Kevin J. Arvai[4], Ruth Y. Eberhardt[1], Giuseppe Gallone[1], Stefan H. Lelieveld[2], Hilary C. Martin[1], Jeremy F. McRae[1], Patrick J. Short[1], Rebecca I. Torene[4], Elke de Boer[5], Petr Danecek[1], Eugene J. Gardner[1], Ni Huang[1], Jenny Lord[1,6], Iñigo Martincorena[1], Rolph Pfundt[5], Margot R. F. Reijnders[2], Alison Yeung[7,8], Helger G. Yntema[2], DDD Study, Lisenka E. L. M. Vissers[5], Jane Juusola[4], Caroline F. Wright[9], Han G. Brunner[5,10], Helen V. Firth[1,11], David R. FitzPatrick[12], Jeffrey C. Barrett[1], Matthew E. Hurles[1#]†, Christian Gilissen[2#], Kyle Retterer[4#]

[1] Wellcome Sanger Institute, Wellcome Genome Campus, Hinxton, UK
[2] Department of Human Genetics, Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, Nijmegen, 6525 GA, the Netherlands
[3] Centre for Molecular and Biomolecular Informatics, Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, Nijmegen, 6525 GA, the Netherlands
[4] GeneDx, Gaithersburg, Maryland, USA
[5] Department of Human Genetics, Donders Institute for Brain, Cognition and Behaviour, Radboud University Medical Center, Nijmegen, 6525 GA, the Netherlands
[6] Human Development and Health, Faculty of Medicine, University of Southampton, UK
[7] Victorian Clinical Genetics Services, Melbourne, Australia
[8] Murdoch Children's Research Institute, Melbourne, Australia
[9] Institute of Biomedical and Clinical Science, University of Exeter Medical School, Research, Innovation, Learning and Development building, Royal Devon & Exeter Hospital, Barrack Road, Exeter EX2 5DW, UK
[10] Department of Clinical Genetics, GROW school for oncology and developmental biology, and MHENS school for mental health and neuroscience, Maastricht University Medical Centre, Maastricht, 6202 AZ, the Netherlands
[11] Department of Clinical Genetics, Cambridge University Hospitals NHS Foundation Trust, Cambridge, UK
[12] MRC Human Genetics Unit, MRC IGMM, University of Edinburgh, Western General Hospital, Edinburgh, UK

* contributed equally
# contributed equally
† To whom correspondence should be addressed: meh@sanger.ac.uk

1 Summary

2 *De novo* mutations (DNMs) in protein-coding genes are a well-established cause of

3 developmental disorders (DD). However, known DD-associated genes only account for a

4 minority of the observed excess of such DNMs. To identify novel DD-associated genes, we

5 integrated healthcare and research exome sequences on 31,058 DD parent-offspring trios, and

6 developed a simulation-based statistical test to identify gene-specific enrichments of DNMs. We

7 identified 299 significantly DD-associated genes, including 49 not previously robustly associated

8 with DDs. Despite detecting more DD-associated genes than in any previous study, much of the

9 excess of DNMs of protein-coding genes remains unaccounted for. Modelling suggests that

10 over 500 novel DD-associated genes await discovery, many of which are likely to be less

11 penetrant than the currently known genes. Research access to clinical diagnostic datasets will

12 be critical for completing the map of dominant DDs.

13

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

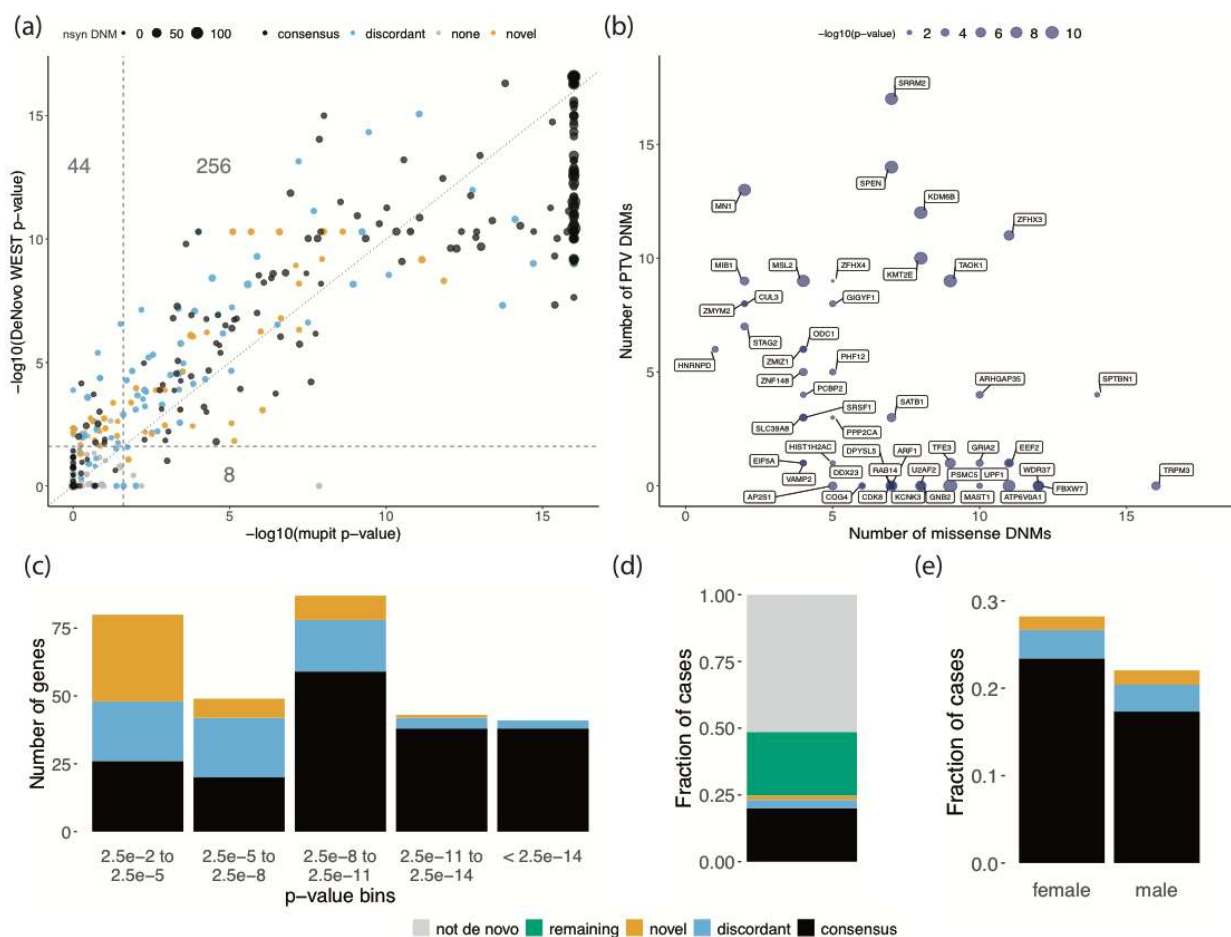39   mutational class, and prevalences of known disorders (**Supplementary Fig. 2**).

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

51 DD-association to be considered of diagnostic utility (as of September 2018) by all three

52 centres, and we refer to them as "consensus" genes. 69/299 of these significant genes were

53 previously considered diagnostic by one or two centres ("discordant" genes). Applying

54 DeNovoWEST to synonymous DNMs, as a negative control analysis, identified no significantly

55 enriched genes (**Supplementary Fig. 6**). To discover novel DD-associated genes with greater

56 power, we then applied DeNovoWEST only to DNMs in patients without damaging DNMs in

57 consensus genes (we refer to this subset as 'undiagnosed' patients) and identified 118

58 significant genes (**Fig. 1b; Supplementary Fig. 7; Supplementary Table 2**). While 69 of these

59 genes were discordant genes, we identified 49 'novel' DD-associated genes, which had a

60 median of 10 nonsynonymous DNMs in our dataset (**Fig. 1c**; **Supplementary Table 3**). There

61 were 500 patients with nonsynonymous DNMs in these 49 genes (1.6% of our cohort); all DNMs

62 in these genes were inspected in IGV[12] and, of 198 for which experimental validation was

63 attempted, all were confirmed as DNMs in the proband. The DNMs in these novel genes were

64 distributed approximately randomly across the three datasets (no genes with p < 0.001,

65 heterogeneity test). Fourteen of the 49 novel DD-associated genes have been further

66 corroborated by recent OMIM entries or publications. In particular, seven of these 14 genes

67 (*PPP2CA*[13], *ZMIZ1*[14], *CDK8*[15], *VAMP2*[16], *KMT2E*[17], *KDM6B*[18], and *TAOK1*[19]) have had

68 genotype-phenotype studies recently published.

69   We also investigated whether some synonymous DNMs might be pathogenic by

70 disrupting splicing. We annotated all synonymous DNMs with a splicing pathogenicity score,

71 SpliceAI[20], and identified a significant enrichment of synonymous DNMs with high SpliceAI

72 scores ($\geq$ 0.8, 1.56-fold enriched, p = 0.0037, Poisson test; **Supplementary Table 4**). This

73 enrichment corresponds to an excess of ~15 splice-disrupting synonymous mutations in our

74 cohort, of which six are accounted for by a single recurrent synonymous mutation in *KAT6B*

75 known to disrupt splicing[21].

76

77   **Figure 1: Results of DeNovoWEST analysis.** (a) Comparison of p-values generated using the

78   new method (DeNovoWEST) versus the previous method (mupit)[1]. These are results from

79   DeNovoWEST run on the full cohort. The dashed lines indicate the threshold for genome-wide

80   significance (the p-values have already been corrected for multiple testing). The size of the

81   points is proportional to the number of nonsynonymous DNMs in our cohort (nsyn). The

82   numbers describe the number of genes that fall into each quadrant (43 in the top left, 256 in the

83   top right, and 8 in the bottom right). (b) The number of missense and PTV DNMs in our cohort in

84   the 49 novel genes. The size of the points are proportional to the log10(-p-value) from the

85   analysis on the undiagnosed subset. (c) The histogram depicts the distribution of p-values from

86   the analysis on the undiagnosed subset for discordant and novel genes; p-values for consensus

87   genes come from the full analysis. The number of genes in each p-value bin is coloured by

88   diagnostic gene group. (d) The fraction of cases with a nonsynonymous mutation in each

89   diagnostic gene group. (e) The fraction of cases with a nonsynonymous mutation in each

90   diagnostic gene group split by sex. In all figures, black represents the consensus known genes,

91   blue represents the discordant known genes, and orange represents the novel genes. In (c),

92   green represents the remaining fraction of cases expected to have a pathogenic *de novo* coding

93   mutation ("remaining") and grey is the fraction of cases that are likely to be explained by other

94   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 x 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

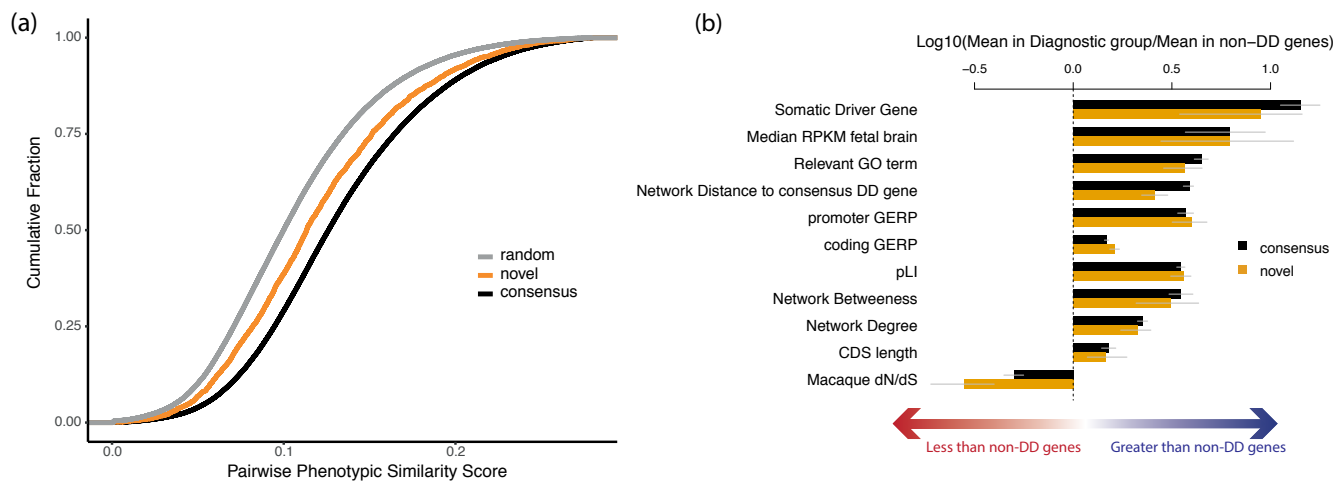104 architecture of DD, especially with regard to known and undiscovered disorders.

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 x 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 x 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 x 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 x 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

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

132   confirmation.

133

134   **Figure 2: Functional properties and mechanisms of novel genes.** (a) Comparing the

135   phenotypic similarity of patients with DNMs in novel and consensus genes. Random phenotypic

136   similarity was calculated from random pairs of patients. Patients with DNMs in the same novel

137   DD-associated gene were less phenotypically similar than patients with DNMs in a known DD-

138   associated gene (p = 9.5 x 10^(-38), Wilcoxon rank-sum test). (b) Comparison of functional

139   properties of consensus known and novel DD genes. Properties were chosen as those known to

140   be differential between consensus and non-DD genes.

141

142   We observed that missense DNMs were more likely to affect functional protein domains

143   than other coding regions. We observed a 2.76-fold enrichment (p = 1.6 x 10^(-68), G-test) of

144   missense DNMs residing in protein domains among consensus genes and a 1.87-fold

145   enrichment (p = 1.4 x 10^(-4), G-test) in novel DD-associated genes, but no enrichment for

146   synonymous DNMs (**Supplementary Table 6**). Three protein domain families in consensus

147   genes were specifically enriched for missense DNMs (**Supplementary Table 7**): ion transport

148   protein (PF00520, p = 3.9 x 10^(-7), G-test), ligand-gated ion channel (PF00060, p = 6.7 x 10^(-7), G-

149   test), and protein kinase domain (PF00069, p = 4.4 x 10^(-2), G-test). Missense DNMs in all three

150   enriched domain families, have previously been associated with DD (**Supplementary Table**

151   **8**)[24].

152   We observed a significant overlap between the 299 DNM-enriched DD-associated genes

153   and a set of 369 previously described cancer driver genes[25] (p = 1.7 x 10^(-46), logistic regression

154   correcting for s_{het}), as observed previously[26,27], as well as a significant enrichment of

155   nonsynonymous DNMs in these genes (**Supplementary Table 9**). This overlap extends to

156   somatic driver mutations: we observe 117 DNMs at 76 recurrent somatic mutations observed in

157   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 x 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

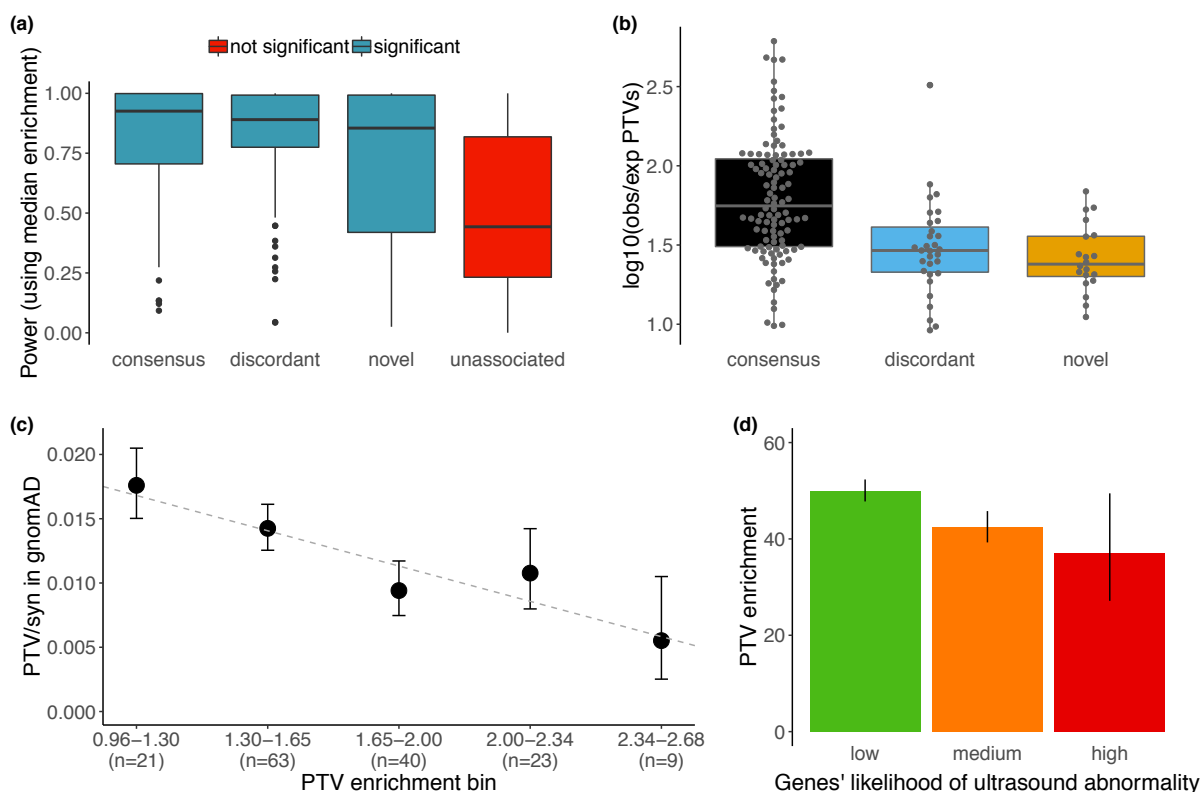216   mechanisms attributed to this gene in the published literature.

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).

250

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 x 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).

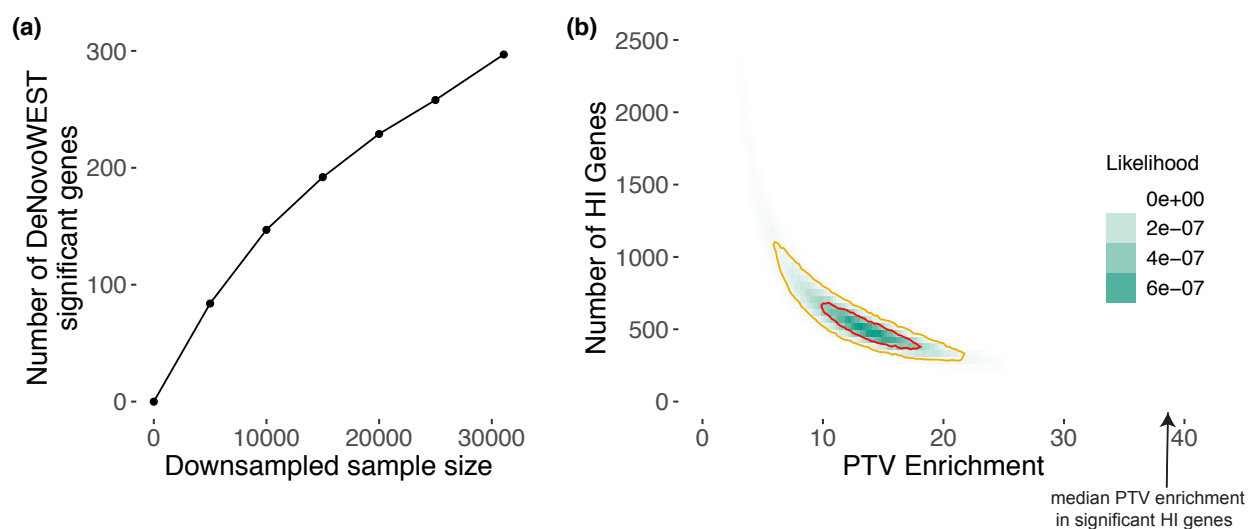**Modelling reveals hundreds of DD genes remain to be discovered**

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.

288

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.

Discussion

296

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

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)

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