LETTER

doi:10.1038/nature14135

Large-scale discovery of novel genetic causes of developmental disorders

The Deciphering Developmental Disorders Study*

Despite three decades of successful, predominantly phenotype-driven discovery of the genetic causes of monogenic disorders[1], up to half of children with severe developmental disorders of probable genetic origin remain without a genetic diagnosis. Particularly challenging are those disorders rare enough to have eluded recognition as a discrete clinical entity, those with highly variable clinical manifestations, and those that are difficult to distinguish from other, very similar, disorders. Here we demonstrate the power of using an unbiased genotype-driven approach[2] to identify subsets of patients with similar disorders. By studying 1,133 children with severe, undiagnosed developmental disorders, and their parents, using a combination of exome sequencing[3–11] and array-based detection of chromosomal rearrangements, we discovered 12 novel genes associated with developmental disorders. These newly implicated genes increase by 10% (from 28% to 31%) the proportion of children that could be diagnosed. Clustering of missense mutations in six of these newly implicated genes suggests that normal development is being perturbed by an activating or dominant-negative mechanism. Our findings demonstrate the value of adopting a comprehensive strategy, both genome-wide and nationwide, to elucidate the underlying causes of rare genetic disorders.

We established a network to recruit 1,133 children (median age 5.5 years, Extended Data Fig. 1a) with diverse, severe undiagnosed developmental disorders, through all 24 regional genetics services of the UK National Health Service and Republic of Ireland. Among the most commonly observed phenotypes (Extended Data Fig. 1b and Supplementary Table 1) were intellectual disability or developmental delay (87% of children), abnormalities revealed by cranial MRI (30%), seizures (24%), and congenital heart defects (11%). These children are predominantly (~90%) of northwest European ancestry (Extended Data Fig. 1c), with 47 pairs of parents (4.1%) exhibiting kinship equivalent to, or in excess of, second cousins (Extended Data Fig. 1d and Supplementary Information). In most families (849 of 1,101) the child was the only affected family member, but 111 children had one or more parents with a similar developmental disorder, and 124 had a similarly affected sibling (Supplementary Information). Prior clinical genetic testing would have already diagnosed many children with easily recognized syndromes, or large pathogenic deletions and duplications, enriching this research cohort for less distinct syndromes and novel genetic disorders.

We sequenced the exomes of 1,133 children with developmental disorders and their parents, from 1,101 families, representing 1,071 unrelated children and 30 sibships. We also performed exome-focused array comparative genomic hybridization (exome-aCGH) on the children ($n = 1{,}009$) and UK controls ($n = 1{,}013$), and genome-wide genotyping on the trios ($n = 1{,}006$) to identify deletions, duplications, uniparental disomy and mosaic large chromosome rearrangements. From our exome sequencing and exome-aCGH data, we detected an average of 19,811 coding or splicing single nucleotide variants (SNVs), 491 coding or splicing insertions and deletions (indels) and 148 copy number variants (CNVs) per child (Supplementary Information). From analyses of the genotyping array data[12] we identified six children with uniparental disomy and five children with mosaic large chromosomal rearrangements (Supplementary Information). The SNVs, indels and CNVs were analysed jointly in the following analyses, allowing, for example, the identification of compound heterozygous CNVs and SNVs affecting the same gene.

We discovered 1,618 *de novo* variants (1,417 SNVs, 114 indels and 87 CNVs) in coding and non-coding regions (Supplementary Tables 2 and 3), of which 1,596 (98.6%) were validated using a second, independent assay, and the remainder were validated clinically. This represents an average of 1.12 *de novo* SNVs and 0.09 *de novo* indels in coding or splicing regions per child, which is within the range of similar studies[3–11]. The distribution of *de novo* SNVs and indels per child closely approximated the Poisson distribution expected for random mutational events (Extended Data Fig. 2).

We classified 28% ($n = 317$) of children with probable pathogenic variants (Supplementary Table 4 and ref. 13) in 1,129 robustly implicated developmental disorder genes (published before November 2013), or with pathogenic deletions or duplications. Most of these diagnoses involved *de novo* SNVs, indels or CNVs (Table 1). Females had a significantly higher diagnostic yield of autosomal *de novo* mutations than males ($P = 0.01$, Fisher's exact test). Among the single-gene diagnoses, most genes linked to developmental disorders (95 out of 148) were only observed once, although eight (*ARID1B*, *SATB2*, *SYNGAP1*, *ANKRD11*, *SCN1A*, *DYRK1A*, *STXBP1*, *MED13L*) each accounted for 0.5–1% of children in our cohort (Extended Data Fig. 3). For seventeen of these children we identified two different genes with pathogenic variants, resulting in a composite clinical phenotype.

Analyses that assess the enrichment in patients of a particular class of variation, so-called 'burden analyses', both highlight classes of variants for detailed analysis and enable estimation of the proportion of a particular class of variant that is likely to be pathogenic. We observed a significant ($P = 0.0004$) burden of 87 *de novo* CNVs in the 1,133 children with developmental disorders compared to 12 in 416 controls (Scottish Family Health Study[14]) despite most children (77%) having previously had clinical microarray testing (Extended Data Fig. 4).

We used gene-specific mutation rates that account for gene length and sequence context[15] to assess the burden of different classes of *de novo* SNVs and indels (Supplementary Information). We observed no significant excess of any functional class of *de novo* SNVs or indels in

Table 1 | Breakdown of diagnoses by mode and by sex

	Female (%)	Male (%)	Total (%)
Undiagnosed	383 (69.6)	433 (74.3)	816 (72.0)
Diagnosed	167 (30.4)	150 (25.7)	317 (28.0)
De novo mutation	124 (22.5)	80 (13.7)	204 (18.0)
Chr X*	24 (4.4)	5 (0.9)	28 (2.6)
Autosomal*	100 (18.2)	75 (12.9)	176 (15.5)
Autosomal dominant†	9 (1.6)	11 (1.9)	20 (1.8)
Autosomal recessive	20 (3.6)	26 (4.5)	46 (4.1)
X-linked inherited	1 (0.2)	19 (3.3)	20 (1.8)
UPD/mosaicism	4 (0.7)	6 (1.0)	10 (0.9)
Composite	9 (1.6)	8 (1.4)	17 (1.5)
Total	550	583	1,133

UPD, uniparental disomy.
* Chromosome X (Chr X) and autosomal values are subsets of '*De novo* mutation'.
† Inherited from a parent.

*Lists of participants and their affiliations appear at the end of the paper.

autosomal-recessive developmental-disorder-linked genes (Extended Data Fig. 5), suggesting that few of these mutations are causally implicated. By contrast, we observed a highly significant excess of all 'functional' classes (coding and splice site variants excepting synonymous changes) of *de novo* SNVs and indels in the dominant and X-linked developmental-disorder-linked genes (Extended Data Fig. 5) within which *de novo* mutations can be sufficient to cause disease. Not all protein-altering mutations in known dominant and X-linked developmental disorder genes will be pathogenic, and these burden analyses inform estimates of positive predictive values for different classes of mutations. The remaining genes (that is, those not linked to developmental disorder) in the genome also exhibit a more modest, but significant, excess of functional, but not silent, *de novo* SNVs and indels (Extended Data Fig. 5).

We observed 96 genes with recurrent, functional mutations (Fig. 1a), a highly significant excess compared to the expected number derived from simulations (median = 55; Supplementary Information). This enrichment is even more pronounced (observed, 29; expected, 3) for recurrent loss-of-function mutations (Fig. 1b). Among undiagnosed children, we observed an excess of 22 genes (observed: 45, expected: 23) with recurrent functional mutations (Fig. 1a) and an excess of 8 genes (observed, 9; expected, 1) with recurrent loss-of-function mutations (Fig. 1b), implying that an appreciable fraction of these recurrently mutated genes are novel developmental-disorder-linked genes.

To identify individual genes enriched for damaging *de novo* mutations (Supplementary Information), we tested for a gene-specific overabundance of either *de novo* loss-of-function mutations or clustered functional *de novo* mutations in 1,130 children (excluding one twin from each of three identical twin pairs). To increase power to detect genes associated with developmental disorder, we also meta-analysed our data with published *de novo* mutations from 2,347 developmental disorder trios with intellectual disability[4,9], epileptic encephalopathy[3], autism[6–8,10], schizophrenia[5], or congenital heart defects[11] (the 'meta-DD' data set). These analyses (Fig. 2) successfully re-discovered 20 known genes linked to developmental disorder at genome-wide significance ($P < 1.31 \times 10^{-6}$, a Bonferroni P value of 0.05 corrected for 38,504 tests (Supplementary Information)). Thus, despite the broad phenotypic ascertainment in these data sets, we can robustly detect developmental-disorder-linked genes solely on statistical grounds.

To increase our power to detect novel genes linked to developmental disorder, we repeated the gene-specific analysis described above excluding the 317 individuals with a known cause of their developmental disorder. In this analysis the statistical genetic evidence was integrated with phenotypic similarity of patients, available data on model organisms and functional plausibility. We identified twelve novel disease genes with compelling evidence for pathogenicity (Table 2), nine of which

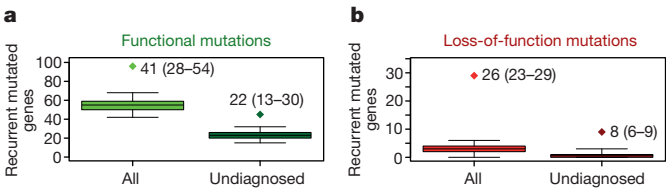

Figure 1 | Excess of recurrently mutated genes. Each panel shows the observed number of recurrently mutated genes (diamond) and the distribution of the number of recurrently mutated genes in 10,000 simulations (box indicates interquartile range, whiskers indicates 95% confidence interval) under a model of no gene-specific enrichment of mutations. **a**, All protein-altering mutations in all DDD children and undiagnosed DDD children. **b**, All loss-of-function mutations in all DDD children and undiagnosed DDD children. Each diamond is annotated with the median excess of recurrently mutated genes, with 95% confidence intervals in brackets. P value of observed excess is <0.0001 for all four tests. No statistical methods were used to predetermine sample size.

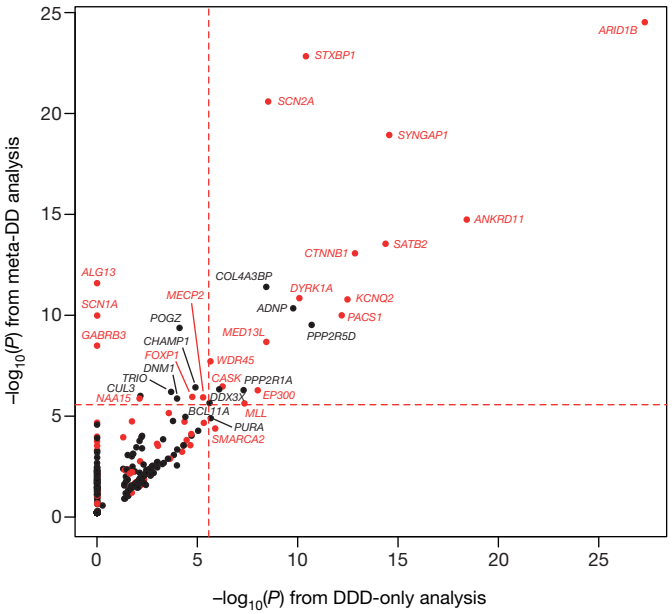

Figure 2 | Gene-specific significance of enrichment for *de novo* mutations. The $-\log_{10}(P)$ value of testing for mutation enrichment is plotted only for each gene with at least one mutation in DDD children. On the x axis is the P value of the most significant test in the DDD data set; on the y axis is the minimal P value from the significance testing in the meta-analysis data set. Red indicates genes already known to be associated with developmental disorders (in DDG2P). Only genes with a P value of less than 0.05/18,272 (red lines) (where 18,272 is the number of genes tested) are labelled.

exceeded the genome-wide significance threshold of 1.36×10^{-6} (Supplementary Information), with the remaining three genes (*PCGF2*, *DNM1* and *TRIO*) just below this significance threshold. The two children with identical Pro65Leu mutations in *PCGF2*, which encodes a component of a Polycomb transcriptional repressor complex, share a strikingly similar facial appearance representing a novel and distinct dysmorphic syndrome. *DNM1* was previously identified as a candidate gene for epileptic encephalopathy[3]. Two of the three children that we identified with *DNM1* mutations also had seizures, and a heterozygous mouse mutant manifests seizures[16]. In addition to two *de novo* missense SNVs in *TRIO*, we identified an intragenic *de novo* 82-kilobase (kb) deletion of 16 exons. For several of these novel developmental-disorder-linked genes, the meta-DD analysis increased the significance of enrichment. For example, a total of five *de novo* loss-of-function variants in *POGZ* were identified, two from our cohort, two from recent autism studies[6,7] and one from a recent schizophrenia study[5]. We also identified six genes with suggestive statistical evidence of being novel genes associated with developmental disorder, defined as having a P value for mutation enrichment less than 1×10^{-4} and being plausible from a functional perspective (Extended Data Table 1). We anticipate that most of these genes will eventually accrue sufficient evidence to meet the stringent criteria we defined above for declaring a novel developmental-disorder-linked gene.

Notably, we observed identical missense mutations in unrelated, phenotypically similar patients for four of these novel developmental-disorder-linked genes (*PCGF2*, *COL4A3BP*, *PPP2R1A* and *PPP2R5D*), and for a fifth gene, *BCL11A*, we identified highly significant clustering of non-identical missense mutations (Fig. 3). We hypothesize that the mutations in some of these genes may be operating by either dominant-negative or activating mechanisms. This hypothesis is supported by previous functional evidence for several of the mutated amino acids. The three identical Ser132Leu mutations in *COL4A3BP*, which encodes an intracellular transporter of ceramide, remove a serine that when phosphorylated downregulates transporter activity from the ER to the

Table 2 | Novel genes with compelling evidence for a role in developmental disorder

Evidence	Gene	*De novo* DDD (missense, LOF)	*De novo* meta (missense, LOF)	*P* value	Test	Mutation clustering	Predicted haploinsufficiency (%)
De novo enrichment	*COL4A3BP*	3 (3,0)	5 (5,0)	4.10×10^{-12}	Meta	Yes	14.7
	PPP2R5D	4 (4,0)	5 (5,0)	6.01×10^{-12}	DDD	Yes	19.7
	ADNP	4 (0,4)	5 (0,5)	4.59×10^{-11}	Meta	No	9.8
	POGZ	2 (0,2)	5 (0,5)	4.31×10^{-10}	Meta	No	30.0
	PPP2R1A	3 (3,0)	3 (3,0)	2.03×10^{-8}	DDD	Yes	23.5
	DDX3X	4 (3,1)	5 (3,2)	2.26×10^{-7}	DDD	No	12.7
	CHAMP1	2 (0,2)	3 (0,3)	4.58×10^{-7}	Meta	No	52.9
	BCL11A	3 (3,0)	4 (3,1)	1.03×10^{-6}	DDD	Yes	0.6
	PURA	3 (1,2)	3 (1,2)	1.14×10^{-6}	DDD	No	9.4
De novo enrichment plus additional evidence	*DNM1*	3 (3,0)	5 (5,0)	1.43×10^{-6}	Meta	No	13.5
	TRIO	2 (2,0)	7 (7,0)	5.16×10^{-6}	Meta	Yes	25.7
	PCGF2	2 (2,0)	2 (2,0)	1.08×10^{-5}	DDD	Yes	37.7

The table summarizes the 12 genes with compelling evidence to be novel developmental-disorder-linked genes. The number of unrelated patients with independent functional or loss-of-function (LOF) mutations in the Deciphering Developmental Disorders (DDD) cohort or the wider meta-analysis (meta) data set including DDD patients is listed. The *P* value reported is the minimum *P* value from the testing of the DDD data set and the meta-analysis data set. The data set that gave this minimal *P* value is also reported. Mutations are considered to be clustered if the *P* value of clustering of functional SNVs is less than 0.01. Predicted haploinsufficiency is reported as a percentile of all genes in the genome, with ~0% being highly likely to be haploinsufficient and 100% very unlikely to be haploinsufficient, based on the prediction score described in ref. 26 updated to enable predictions for a higher fraction of genes in the genome. During submission, a paper was published describing a novel developmental disorder caused by mutations in *ADNP* (ref. 27).

Golgi[17], presumably resulting in intra-cellular imbalances in ceramide and its downstream metabolic pathways. The two mutated amino acids (Arg182Trp and Pro179Leu) in *PPP2R1A*, which encodes the scaffolding A subunit of the protein phosphatase 2 complex, have been previously identified as sites of driver mutations in endometrial and ovarian cancer[18]. It has previously been shown that mutating either of these two residues results in impaired binding of B subunits of the complex[18]. Intriguingly, *PPP2R5D* encodes one of the possible B subunits of the same protein phosphatase 2 complex, suggesting that the clustered missense mutations (Pro201Arg and Glu198Lys) in this gene may similarly perturb interactions between subunits of this complex. Further functional studies will be required to confirm this hypothesis.

We assessed transmission biases of potentially pathogenic inherited SNVs in our probands (Supplementary Information) and observed a genome-wide trend ($P = 0.015$) towards over-transmission to probands of very rare (minor allele frequency (MAF) <0.0005%) loss-of-function variants, but not damaging missense variants. We also observed a 1.8-fold enrichment ($P = 0.04$) of rare (MAF <5%) biallelic loss-of-function variants (Supplementary Table 5) among probands without a likely dominant cause of their disorder, compared to those with either a diagnostic *de novo* mutation or an affected parent. Again we saw no enrichment in biallelic damaging missense variants (Extended Data Table 2), consistent with a similar observation in children with autism[19]. These observations suggest that although inherited loss-of-function variants (both monoallelic and biallelic) are probably contributing to developmental disorder in our patients, much larger sample sizes will be required to pinpoint specific developmental-disorder-linked genes in this way.

To direct future, detailed functional experiments on the developmental role of a subset of candidate genes from this study we used two approaches. First, knockdown-induced phenotypes were recorded in early zebrafish development. Second, we performed a systematic review of perturbed gene function in human, mouse, *Xenopus*, zebrafish and *Drosophila*. In both approaches the animal phenotypes were compared to those seen in individuals in our cohort.

We undertook an antisense-based loss-of-function screen in zebrafish to assess 32 candidate developmental-disorder-linked genes with *de novo* loss-of-function, *de novo* missense or biallelic loss-of-function variants from exome sequencing (Supplementary Information and Supplementary Table 6). These candidate genes corresponded to 39 zebrafish orthologues. Knockdowns of these zebrafish genes were repeated at least twice and all morpholinos were co-injected with *tp53* morpholino to eliminate off-target toxicity. Successful knockdown of the targeted messenger RNA could be confirmed using polymerase chain reaction with reverse transcription (RT–PCR) for 82.4% of genes (28 out of 34), and 9 out of 11 (82%) of genes that were tested gave an equivalent phenotype when knocked down by a second, independent morpholino. Knockdown of at least one or a pair of zebrafish orthologues of 65.6% of candidate developmental-disorder-linked genes (21 out of 32) resulted in perturbed embryonic and larval development (Fig. 4, Extended Data Table 3, Supplementary Data and Supplementary Table 7). Large-scale mutagenesis[20] and morpholino[21] studies suggest that knockout or knockdown of 6–12% of genes give developmental phenotypes, suggesting at least a fivefold enrichment of developmentally non-redundant genes among the 32 selected for modelling. We then compared the phenotypes of the zebrafish morphants to those of the patients with *de novo* mutations or biallelic loss-of-function variants in the orthologous genes (Extended Data Table 3). Eleven out of twenty-one (52.4%) of the genes were categorized as strong candidates based on phenotypic similarity (Fig. 4a). Seven out of eleven were potential microcephaly genes, the knockdown of which in zebrafish gives significant reductions in both head measurements and neural tissue (Fig. 4b and Supplementary Information). Six out of twenty-one (28.6%) genes resulted in severe morphant

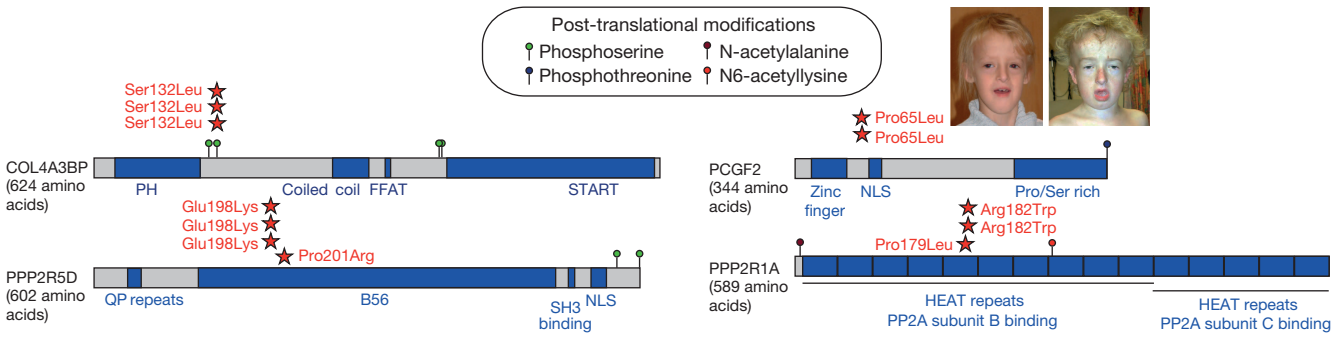

Figure 3 | Four novel genes with clustered mutations. The domains (blue), post-translational modifications, and mutation locations (red stars) are shown for four proteins with highly clustered *de novo* mutations in unrelated children with severe, undiagnosed developmental disorders. For the protein PCGF2, where all observed mutations are identical, photos are shown to highlight the facial similarities of patients carrying the same mutation.

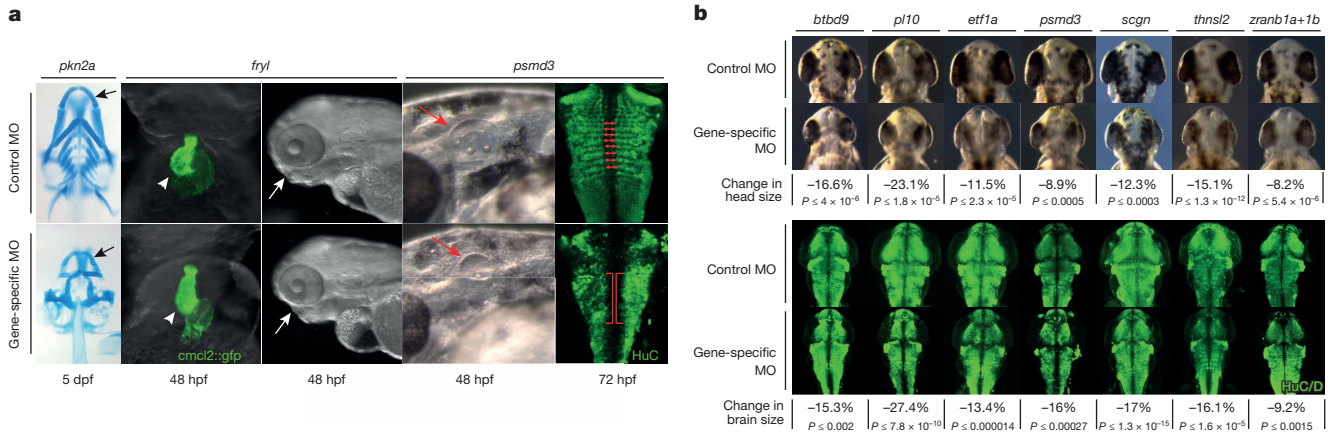

Figure 4 | Candidate gene loss-of-function modelling in zebrafish reveals enrichment for developmentally important proteins. **a**, Examples of developmental phenotypes: knockdown of *pkn2a* results in reduced cartilaginous jaw structures (black arrows); knockdown of *fryl* results in cardiac and craniofacial defects (white arrowheads and arrows, respectively); while knockdown of *psmd3* results in smaller ear primordia (red arrows), and mis-patterned CNS neurons (compare red double arrows and brackets). MO, morpholino. **b**, Knockdown outcomes of seven genes with variants present in microcephaly patients: interocular measurements of bright-field images from control and loss-of-function embryos reveal significant decreases in head size. A neuronal antibody stain (anti-HuC/D, green channel) labels the brains of control and morphant zebrafish. Measurements taken across the widest extent of the midbrain identify significant reductions in brain size, probably underlying the concomitant head-size reductions seen in bright-field images. In **b**, tables show average percentage reduction in head and brain width, and *P* values of a *t*-test. Original magnifications: **a**, 5× (*pkn2a*), 10× (*fryl* and *psmd3*, bright-field) and 40× (psmd3, green channel); **b**, 10× (brightfield) and 20× (green channel).

phenotypes which could not be meaningfully linked to patient phenotypes. As many of our candidate developmental disorder genes carried heterozygous loss-of-function variants (*de novo* mutations), it is to be expected that the severity of loss-of-function phenotypes in zebrafish may exceed that observed in our patient cohort. The genes with proven non-redundant developmental roles can reasonably be assigned higher priority for downstream functional investigations and genetic analyses.

Our systematic review of gene perturbation in multiple species sought both confirmatory and contradictory (for example, healthy homozygous knockout) evidence from other animal models for these 21 apparently developmentally important genes. We identified 16 genes with solely confirmatory data, often from multiple different organisms, none with solely contradictory data, two with both confirmatory and contradictory evidence, and three with no evidence either way (Supplementary Table 8).

In summary, our analyses validate a large-scale, genotype-driven strategy for novel developmental-disorder-linked gene discovery that is complementary to the traditional phenotype-driven strategy of studying patients with very similar presentations, and is particularly effective for discovering novel developmental disorders with highly variable or indistinct clinical presentations. Our meta-analysis with previously published developmental disorder studies increased power to detect novel developmental-disorder-linked genes and highlights the shared genetic aetiologies between diverse neurodevelopmental disorders such as intellectual disability, epilepsy, autism and schizophrenia[22]. We identified significantly more pathogenic autosomal *de novo* mutations in females compared to males. An increased burden of monogenic disease among females with neurodevelopmental disorders has become more apparent[23,24], and our observations strengthen this proposition. Further investigations are required to assess whether males might be enriched for poly/oligogenic causation.

The 35 patients with pathogenic mutations in the 12 novel developmental-disorder-linked genes we discovered increased our diagnostic yield from 28% to 31%. This raises the question of what are the causes of the developmental disorders in the other 69% of patients. The undiagnosed patients are not obviously less severely affected than the diagnosed patients (for example, fewer phenotype terms, older age of recruitment). We anticipate that there are many more pathogenic, monogenic, coding mutations in these undiagnosed patients that we have detected, but for which compelling evidence is currently lacking. This hypothesis is supported by four strands of evidence: (1) modelling statistical power suggests that studying ~1,000 trios has only 5–10% power to detect an averagely mutable haploinsufficient developmental-disorder-linked gene (Extended Data Fig. 6a and Supplementary Information); (2) the expectation that our power to detect novel developmental-disorder-linked genes that operate recessively or by gain-of-function mechanisms will be lower than for haplosufficient genes; (3) the significant enrichment in undiagnosed patients of functional mutations in genes predicted to exhibit haploinsufficiency (Extended Data Fig. 6b); and (4) the strong enrichment for developmental phenotypes in the zebrafish knockdown screen.

Given our limited power to detect pathogenic mutations that act through dominant-negative or activating mechanisms, it was notable that in four of our novel genes (*COL4A3BP*, *PPP2R1A*, *PPP2R5D* and *PCGF2*) we observed identical *de novo* mutations in unrelated trios. Two hypotheses might explain this observation. First, that there is a vast number of different gain-of-function mutations, of which we are just scratching the surface in this study, or second, that these particular variants are enriched in our cohort owing to these mutations conferring a positive selective advantage in the germ line[25]. Analysis of larger data sets will be required to assess these hypotheses, although they are not necessarily mutually exclusive.

These considerations of the limited power of even nationwide studies such as ours motivate the international sharing of minimal genotypic and phenotypic data, for example through the DECIPHER web portal (http://decipher.sanger.ac.uk), to provide diagnoses for patients who would otherwise remain undiagnosed. Plausibly pathogenic variants observed in undiagnosed patients in our study (*de novo* SNVs, indels and CNVs, and biallelic loss of function in genes not yet associated with disease) are shared through DECIPHER, and we encourage other, comparable studies to adopt a similar approach.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

Received 3 July; accepted 4 December 2014.
Published online 24 December 2014.

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Supplementary Information is available in the online version of the paper.

Acknowledgements We dedicate this paper to J. Tolmie and L. Brueton for their unwavering and enthusiastic support of the Deciphering Developmental Disorders project. We thank the families for their participation and patience. We thank M. Daly and K. Samocha for access to unpublished mutation rate estimates. We are grateful to S. Saunders, D. Smedley, D. Conrad, A. Ramu and N. Huang for access to data and algorithms. We thank the UK National Blood Service and the Generation Scotland: Scottish Family Health Study for access to DNA from controls. Generation Scotland has received core funding from the Chief Scientist Office of the Scottish Government Health Directorates CZD/16/6 and the Scottish Funding Council HR03006. The Deciphering Developmental Disorders study presents independent research commissioned by the Health Innovation Challenge Fund (grant number HICF-1009-003), a parallel funding partnership between the Wellcome Trust and the Department of Health, and the Wellcome Trust Sanger Institute (grant number WT098051). The views expressed in this publication are those of the author(s) and not necessarily those of the Wellcome Trust or the Department of Health. The study has UK Research Ethics Committee approval (10/H0305/83, granted by the Cambridge South REC, and GEN/284/12 granted by the Republic of Ireland REC). The research team acknowledges the support of the National Institute for Health Research, through the Comprehensive Clinical Research Network.

Author Contributions See Supplementary Information for author contribution details.

Author Information Data can be accessed at the European Genome Phenome Archive (https://www.ebi.ac.uk/ega/) under accession number EGAS00001000775. Reprints and permissions information is available at www.nature.com/reprints. The authors declare competing financial interests: details are available in the online version of the paper. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to M.E.H. (meh@sanger.ac.uk).

The Deciphering Developmental Disorders Study

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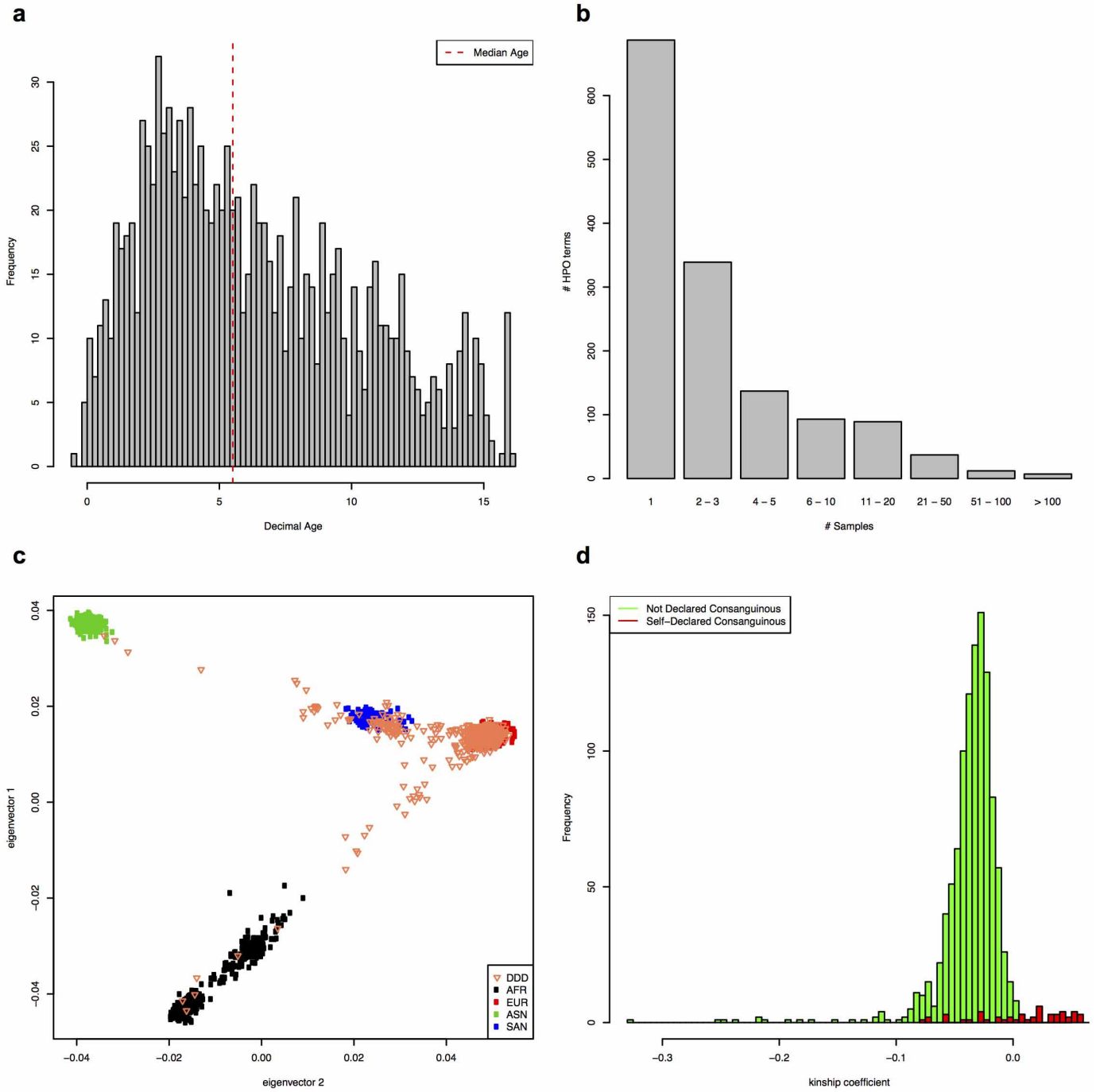
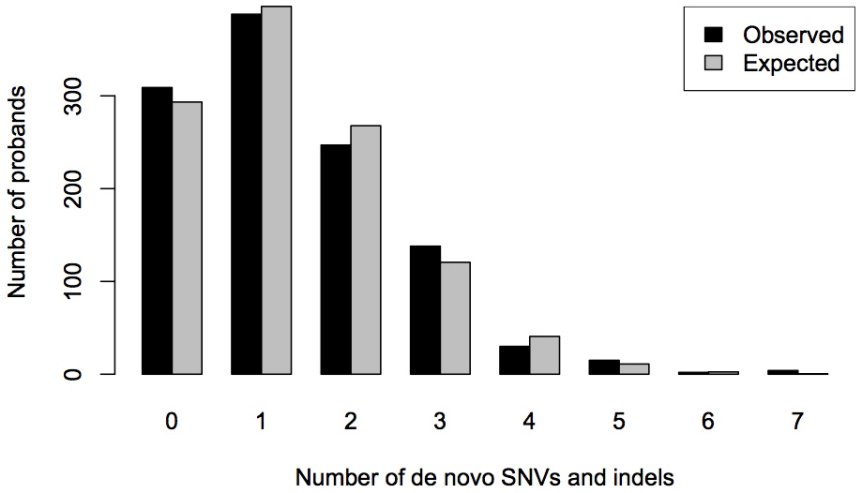
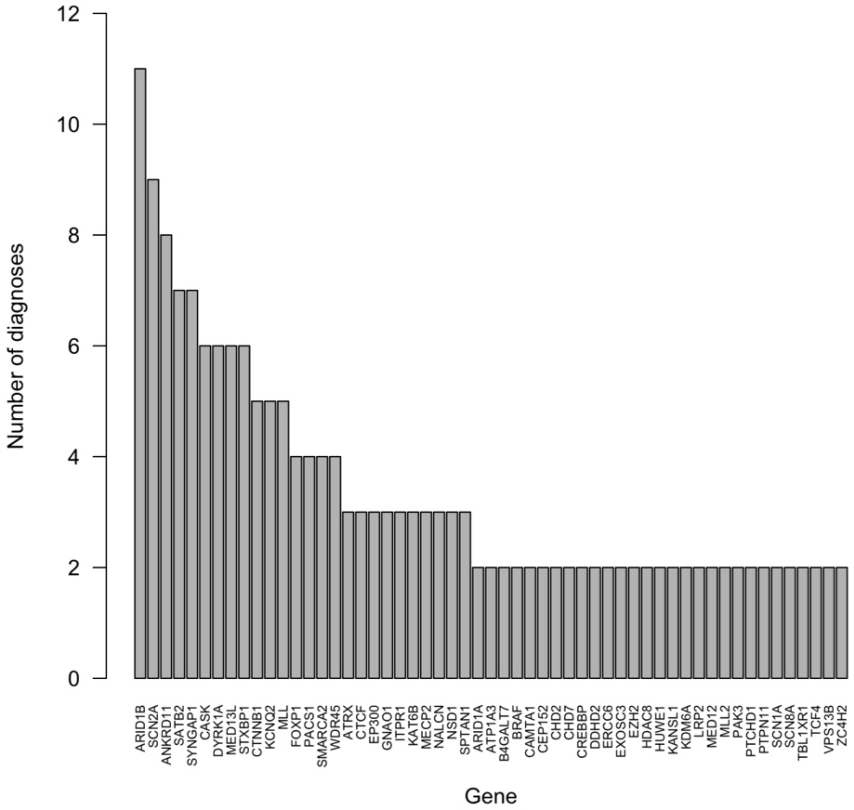


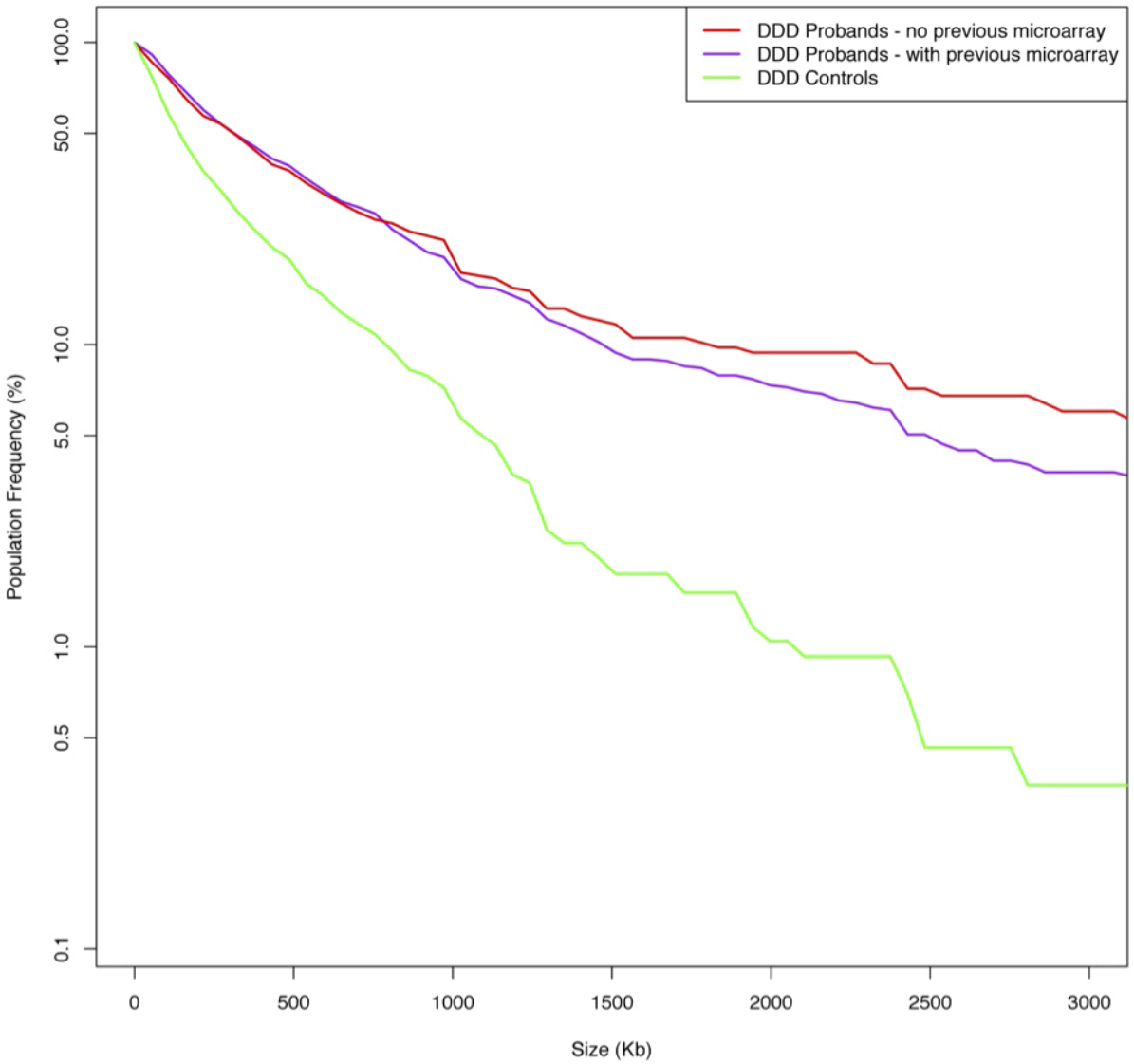
Extended Data Figure 1 | Characteristics of the families. **a**, Gestation-adjusted decimal age (years) at last clinical assessment. The histogram shows the distribution of the gestation-adjusted decimal age at last clinical assessment across the 1,133 probands. The dashed red line shows the median age. **b**, Frequency of human phenotype ontology (HPO) term usage. Bar plot showing, for each used HPO term, the number of times it was observed across the 1,133 proband patient records. **c**, Projection PCA plot of the 1,133 probands. PCA plot of 1,133 DDD probands projected onto a PCA analysis using four different HapMap populations from the 1000 genomes project. Black, African; red, European; green, east Asian; blue, south Asian; and the 1,133 DDD probands are represented by orange triangles. **d**, Self-declared and genetically defined consanguinity. Overlaid histogram showing the distribution of kinship coefficients from KING comparing parental samples for each trio. Green, trios where consanguinity was not entered in the patient record on DECIPHER; red, trios consanguinity was declared in the patient record on DECIPHER.



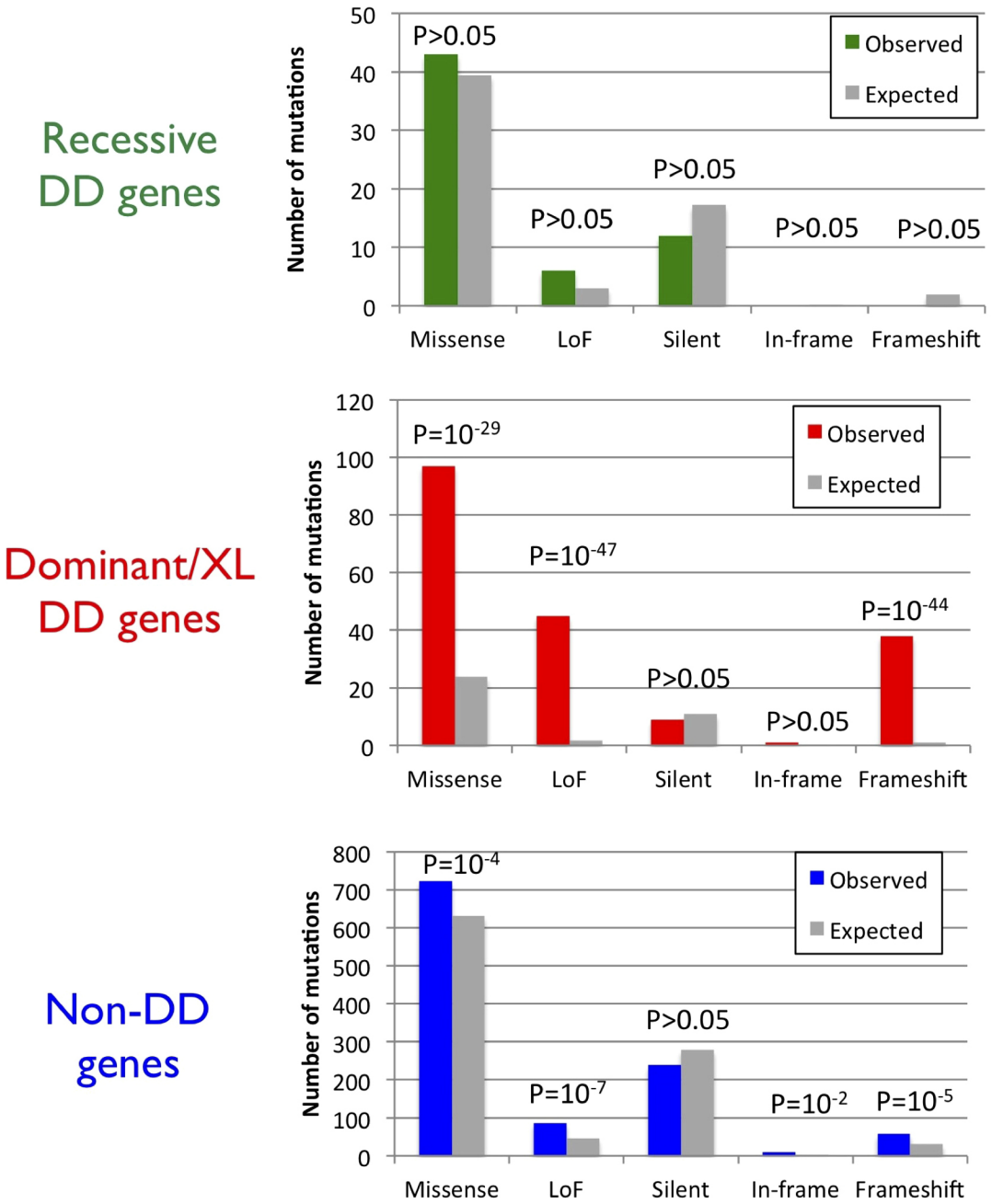
Extended Data Figure 2 | Number of validated *de novo* SNVs and indels per proband. Bar plot showing the distribution of the observed number of validated SNVs and indels per proband sample, and the expected distribution assuming a Poisson distribution with the same mean as the observed distribution.



Extended Data Figure 3 | Number of diagnoses per gene. Histogram showing the number of diagnoses per gene for genes with at least two diagnoses from different proband samples.

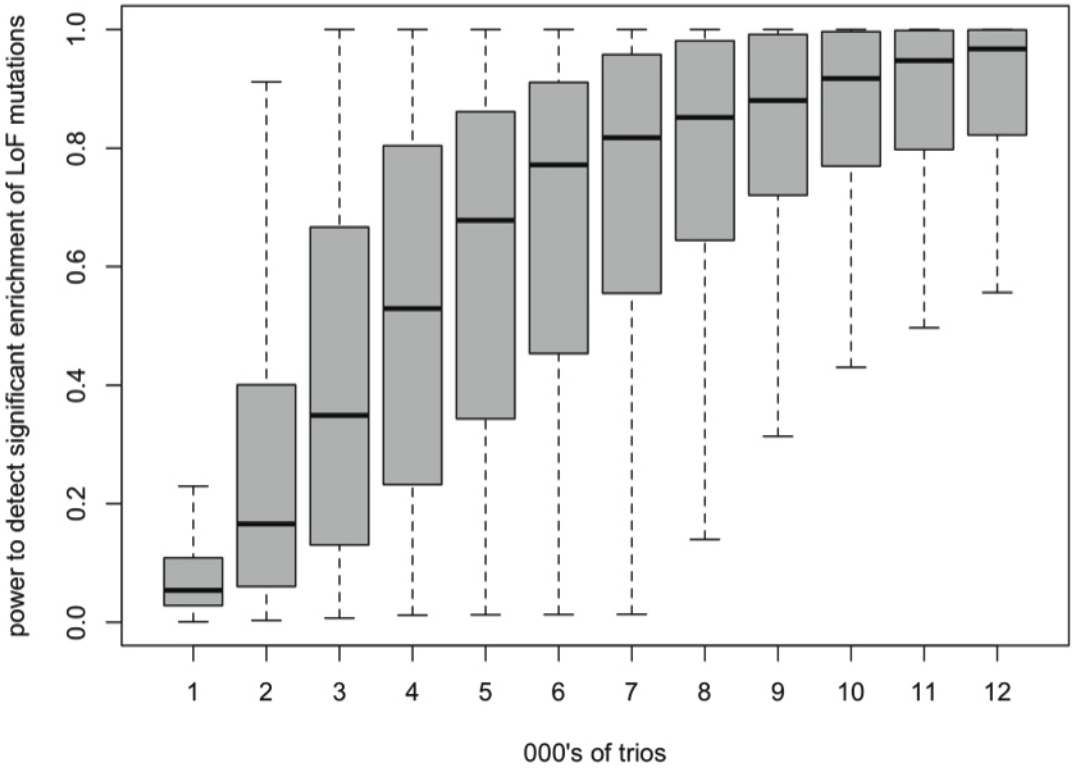


Extended Data Figure 4 | Burden of large CNVs in 1,133 DDD proband samples. Plot comparing the frequency of rare CNVs in three sample groups against CNV size. The *y* axis is on a log scale. Red, DDD probands who have not had previous microarray based genetic testing; purple, DDD probands who have had negative previous microarray-based genetic testing; green, DDD controls.



Extended Data Figure 5 | Expected and observed numbers of *de novo* mutations. The expected and observed numbers of mutations of different functional consequences in three mutually exclusive sets of genes are shown, along with the *P* value from an assessment of a statistical excess of observed mutations. The three classes of genes are described in the main text.

A



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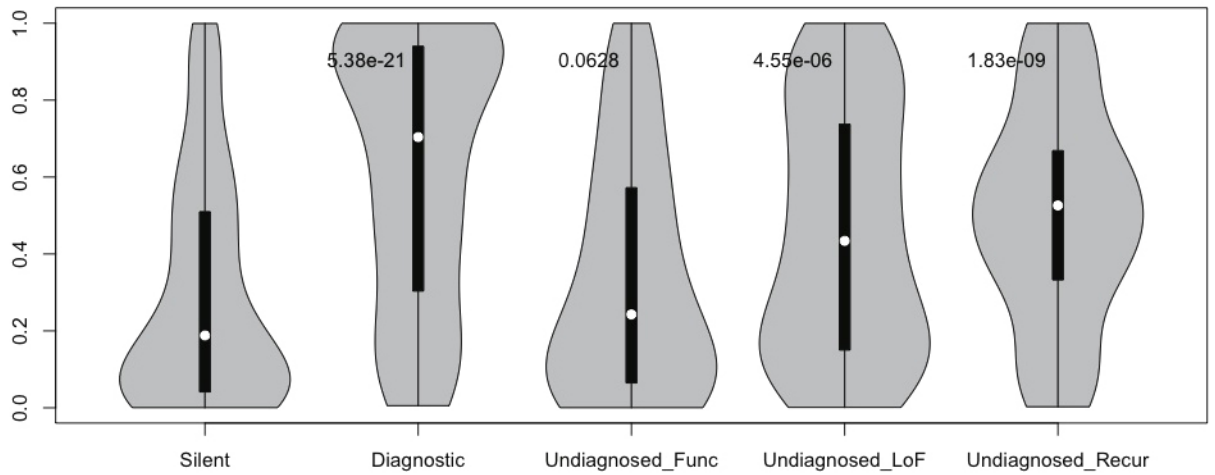

Extended Data Figure 6 | Haploinsufficiency analyses. a, Saturation analysis for detecting haploinsufficient developmental-disorder-linked genes. A box plot showing the distribution of statistical power to detect a significant enrichment of loss-of-function mutations across 18,272 genes in the genome, for different numbers of trios studied, from 1,000 trios to 12,000 trios. Line within the box shows the median, box shows the interquartile range and the whiskers show the most extreme values within 1.5 times the interquartile range from the box. **b**, Distribution of haploinsufficiency scores in selected sets of *de novo* mutations. Violin plot of haploinsufficiency scores in five sets of *de novo* mutations. Silent, all synonymous mutations; diagnostic, mutations in known developmental-disorder-linked genes in diagnosed individuals; undiagnosed_Func, all functional mutations in undiagnosed individuals; undiagnosed_LoF, all loss-of-function mutations in undiagnosed individuals; undiagnosed_recur, mutations in genes with recurrent functional mutations in undiagnosed individuals. *P* values for a Mann–Whitney *U*-test comparing each of the latter four distributions to that observed for the silent (synonymous) variants are plotted at the top of each violin. Dot indicates the median, box is interquartile range and whiskers are the most extreme values within 1.5 times the interquartile range from the box.

Extended Data Table 1 | Novel genes with suggestive evidence for a role in developmental disorder

Evidence	Gene	*de novos* DDD (Missense, LoF)	*de novos* Meta (Missense, LoF)	P Value	Test	Mutation Clustering	Predicted Haploinsufficiency
De novo enrichment + additional evidence	*NAA15*	1 (0,1)	3 (0,3)	1.64E-06	Meta	No	7.5%
	ZBTB20	3 (1,2)	3 (1,2)	4.84E-06	DDD	No	0.2%
	NAA10	2 (2,0)	3 (3,0)	8.28E-06	Meta	No	34.1%
	TRIP12	3 (1,2)	4 (2,2)	2.13E-05	Meta	No	3.8%
	USP9X	3 (1,2)	3 (1,2)	5.14E-05	DDD	No	3.8%
	KAT6A	2 (0,2)	2 (0,2)	7.91E-05	DDD	No	19.0%

Six genes with suggestive evidence to be novel developmental-disorder-linked genes. The number of unrelated patients with independent functional or loss-of-function mutations in the DDD cohort or the wider meta-analysis data set including DDD patients is listed. The *P* value reported is the minimum *P* value from the testing of the DDD data set and the meta-analysis data set. The data set that gave this minimal *P* value is also reported. Mutations are considered to be clustered if the *P* value of clustering of functional SNVs is less than 0.01. Predicted haploinsufficiency is reported as a percentile of all genes in the genome, with ~0% being highly likely to be haploinsufficient and 100% very unlikely to be haploinsufficient, based on the prediction score described in ref. 26 updated to enable predictions for a higher fraction of genes in the genome. *NAA10* is already known to cause an X-linked recessive developmental disorder in males, but here we identified missense mutations in females, suggesting a different, X-linked dominant, disorder.

Extended Data Table 2 | Biallelic loss of function and damaging functional variants

Biallelic Variant Types	Untransmitted Diplotypes (n=1080)	Likely Dominant Probands (n=270)	Other Probands (n=810)
LoF/LoF (Genome-wide)	110	17	86
LoF/Dam (Genome-wide)	87	21	71
Dam/Dam (Genome-wide)	312	90	264
LoF/LoF (DDG2P Biallelic)	1	1	3
LoF/Dam (DDG2P Biallelic)	2	0	6
Dam/Dam (DDG2P Biallelic)	26	7	25

Rare (MAF <5%) biallelic loss of function and damaging functional variants in uninherited diplotypes and probands. 'Likely dominant probands' refers to probands with a reported *de novo* mutation or affected parents, and 'other probands' refers to all remaining probands. 'DDG2P biallelic' refers to confirmed and probable DDG2P genes with a biallelic mode of inheritance. See Supplementary Methods for details of variant processing.

Extended Data Table 3 | Zebrafish modelling identifies 21 developmentally important candidate genes

Gene	# patients	Variant	Patient phenotypes	Phenotypic concordance	Relevant knockdown phenotypes
BTBD9	2/1	Biallelic LoF/*De novo* Missense	Seizures, microcephaly, hypertonia	Strong	Reduced head size, brain volume
CHD3	1/2	*De novo* LoF/Missense	CNS and craniofacial defects	Strong	Abnormal head shape
DDX3X	1/3	*De novo* LoF/Missense	Moderately short stature, microcephaly, CNS defects	Strong	Reduced head size, brain volume
ETF1	1	*De novo* LoF	CNS and craniofacial defects, seizures, microcephaly, hypertelorism	Strong	Reduced head size, brain volume
FRYL	1	*De novo* LoF	Short stature, craniofacial and cardiac defects	Strong	Cardiac defects, reduced axis length
PKN2	1	*De novo* Missense	CNS, cardiac, ear, and craniofacial defects, growth retardation	Strong	Cardiac, craniofacial cartilage, and growth defects
PSMD3	1	*De novo* Missense	Microcephaly, muscular hypotonia, seizures, growth abnormality	Strong	Reduced head size and neural defects
SCGN	1	Biallelic LoF	Seizures, microcephaly, CNS defects	Strong	Reduced head size, brain volume
SETD5	1	*De novo* LoF	Seizures, CNS and cardiac defects, poor motor coordination	Strong	Reduced head size, cardiac defects, abnormal locomotion
THNSL2	2	Biallelic LoF	Microcephaly, CNS and ear defects	Strong	Reduced head size, brain volume, neural defects
ZRANB1	2	*De novo* Missense	Microcephaly, muscle defects, seizures	Strong	Reduced head size and neural defects
DPEP2	1	Biallelic LoF	CNS defects, growth retardation	Moderate	Growth reduction
PSD2	1	*De novo* LoF	CNS defects, hypertonia, seizures	Moderate	Abnormal musculature, CNS and locomotion
SAP130	1	*De novo* LoF	Short stature, hypotonia, hypotelorism	Moderate	Abnormal locomotion
CNOT1	1/1	*De novo* LoF/Missense	Short stature, cardiac, CNS, ear and craniofacial defects	Weak	Multisystem
DTWD2	1	*De novo* LoF	CNS defects, seizures	Weak	Multisystem
ILVBL	1	*De novo* LoF	CNS and craniofacial defects	Weak	Multisystem
NONO	1	*De novo* LoF	CNS and ear defects, hypotonia, growth retardation	Weak	Multisystem, with otic and growth defects
POGZ	2	*De novo* LoF	CNS and ear defects, hypotonia, seizures, coloboma	Weak	Multisystem
SMARCD1	1/1	*De novo* LoF/Missense	CNS defects, hypotonia	Weak	Multisystem
WWC1	1	*De novo* Missense	CNS defects, hypertelorism	None	None

This table summarizes the 21 genes for which knockdown results in developmental phenotypes in zebrafish. The '# patients' column indicates how many patients were identified as carrying variants in these genes. Split numbers indicate the breakdown of variant types (for example, for *BTBD9*, 2/1 is two biallelic loss of function and one *de novo* missense carrying patients). A summary of the patient phenotypes is listed, as well as the relevant phenotypes observed in zebrafish knockdown experiments. Phenotypic concordance categories indicate the degree of overlap between the zebrafish phenotyping and the patient phenotypes. Weak concordance typically is the result of severe, multisystem phenotypes in zebrafish. See Supplementary Information for more detailed phenotype information.