Whole-genome sequencing of rare disease patients in a national healthcare system

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Most patients with rare diseases do not receive a molecular diagnosis and the aetiological variants and mediating genes for more than half such disorders remain to be discovered. We implemented whole-genome sequencing (WGS) in a national healthcare system to streamline diagnosis and to discover unknown aetiological variants, in the coding and non-coding regions of the genome. In a pilot study for the 100,000 Genomes Project, we generated WGS data for 13,037 participants, of whom 9,802 had a rare disease, and provided a genetic diagnosis to 1,138 of the 7,065 patients with detailed phenotypic data. We identified 95 Mendelian associations between genes and rare diseases, of which 11 have been discovered since 2015 and at least 79 are confirmed aetiological. Using WGS of UK Biobank[1], we showed that rare alleles can explain the presence of some individuals in the tails of a quantitative red blood cell (RBC) trait. Finally, we reported 4 novel non-coding variants which cause disease through the disruption of transcription of *ARPC1B*, *GATA1*, *LRBA* and *MPL*. Our study demonstrates a synergy by using WGS for diagnosis and aetiological discovery in routine healthcare.

Rare diseases affect approximately 1 in 20 people, but only a minority of patients receive a genetic diagnosis[2]. Approximately 10,000 rare diseases are known, but fewer than half have a resolved genetic aetiology[3]. Even when the aetiology is known, the prospects for diagnosis are severely diminished by a fragmentary approach to phenotyping and the restriction of genetic testing to a disease-specific panel of genes. On average, a molecular cause is determined after three misdiagnoses and 16 physician visits over a “diagnostic odyssey” lasting more than two years[4]. However, recent developments in WGS technology mean it is now possible to perform comprehensive genetic testing systematically in an integrated national healthcare system. The large-scale implementation of WGS for diagnosis will also enable the discovery of new genetic aetiologies, through the identification of novel causal mutations in the coding and non-coding parts of the genome.

In a pilot study for the 100,000 Genomes Project supported by the National Institute for Health Research (NIHR), we have performed WGS of 13,037 individuals enrolled at 57 National Health Service (NHS) hospitals in the United Kingdom and 26 hospitals in other countries (Fig. 1a, Extended Data Fig. 1a, Supplementary Table 1) in three batches, to clinical standard (Fig. 1b). The participants were distributed approximately equally between the sexes (Supplementary Table 1) and their distribution across ethnic groups closely matched that reported in the UK

census (Fig. 1c; https://www.ons.gov.uk/census/2011census). In total, 9,802 participants (75%) were affected with a rare disease or had an extreme measurement of a quantitative trait, of which 9,024 were probands and 778 were affected relatives. Each participant was assigned to one of 18 domains (Table 1): 7,388 individuals to one of 15 rare disease groups, 50 individuals to a control group, 4,835 individuals to a Genomics England Limited (GEL) group and 764 individuals to a group of UK Biobank participants with extreme red blood cell indices (Supplementary Information). The rare disease domains covered pathologies of a wide range of organ systems and each had pre-specified inclusion and exclusion criteria (Supplementary Information, Supplementary Table 1, Extended Data Fig. 1b). The variation in sample size across domains was primarily due to differences in recruitment rate, which limited the efficiency of the experimental design. We subsequently collected detailed phenotypic information, through web-based data capture applications, in the form of Human Phenotype Ontology (HPO) terms for 13 of the rare disease domains (Fig. 2a,b, Extended Data Fig. 1c). Patients with diverse diagnoses were enrolled to the GEL domain, together with healthy family members, but only the affection status of these participants were available for this study. In addition, HPO-coded phenotypes were not collected for Leber Hereditary Optic Neuropathy (LHON) and Ehler-Danlos and Ehler-Danlos-like Syndromes (EDS) patients. In total, 19,605 HPO terms were selected to describe patient phenotypes. Quantitative data were transcribed to HPO terms using domain-specific rules, while free text was transcribed manually.

Domain name	**Acronym**	**Participants**	**Rare diseases**	**HPO/Clinical reporting**
Bleeding, Thrombotic and Platelet Disorders	BPD	1169	Y	Y
Technical Controls	CNTRL	50	N	N
Cerebral Small Vessel Disease	CSVD	246	Y	Y
Ehler-Danlos and Ehler-Danlos-like Syndromes	EDS	17	Y	Y
100,000 Genomes Project–Rare Diseases Pilot	GEL	4835	Y	N
Hypertrophic Cardiomyopathy	HCM	252	Y	Y
Intrahepatic Cholestasis of Pregnancy	ICP	268	Y	Y
Inherited Retinal Disorders	IRD	725	Y	Y
Leber Hereditary Optic Neuropathy	LHON	71	Y	Y
Multiple Primary Malignant Tumours	MPMT	588	Y	Y
Neurological and Developmental Disorders	NDD	660	Y	Y
Neuropathic Pain Disorders	NPD	193	Y	Y
Pulmonary Arterial Hypertension	PAH	1148	Y	Y
Primary Immune Disorders	PID	1359	Y	Y
Primary Membranoproliferative Glomerulonephritis	PMG	184	Y	Y
Stem cell and Myeloid Disorders	SMD	257	Y	Y
Steroid Resistant Nephrotic Syndrome	SRNS	251	Y	Y
UK Biobank – Extreme Red Cell Traits	UKB	764	N	N

Table 1. Study domain names, acronyms, numbers of participants, whether the domain included cases with a rare disease and whether domain participants were assigned HPO terms for diagnostic reporting.

Following bioinformatic quality control (QC) and data analysis (Extended Data Fig. 2–4), we identified 172,005,610 short variants, of which 157,411,228 (91.5%) were single nucleotide variants (SNVs) and 14,594,382 (8.5%) were indels up to 50bp long (Extended Data Fig. 5). 48.6% and 40.8% of the SNVs and indels, respectively, were absent from all major variant databases (Fig. 1e). 54.8% of the variants were observed in only one member of the maximal set of 10,259 unrelated participants, of which 82.6% were novel. Only 9.08% of novel variants were observed in more than one member of the unrelated set, typically in sets of individuals with recent common ancestry (Fig. 1f). SNVs and indels common in our dataset were well represented in genetic databases but, in accordance with theory, the vast majority of the variants we observed were very rare and most were uncatalogued. We called 24,436 distinct large deletions (>50bp) by synthesising inferences from two algorithms across individuals. We also called more complicated types of structural variant, such as inversions, but they were called unreliably and could not be confidently aggregated across individuals (Supplementary Information). We used the WGS data to determine that only 13 (0.1%) individuals had non-standard sex chromosomal karyotypes (Extended Data Fig. 3e–g). Using the high quality variant calls, we inferred a wide range of bioinformatically estimated family sizes, in keeping with differences in enrolment strategies (Supplementary Information), of which most comprised singletons (Fig. 1d).

We issued clinical reports for 1,103 distinct causal variants (731 SNVs, 264 indels, 102 large deletions, 6 other structural variants) affecting 303 genes (Extended Data Fig. 5). 266 of the 995 SNVs and indels (26.7%) were absent from the Human Gene Mutation Database (HGMD) and from the set of variants in ClinVar having a pathogenic or likely pathogenic interpretation and no benign interpretations. We identified strong evidence (posterior probability (PP) > 0.75) for 99 genetic associations between rare variants and groupings of patients with similar phenotypes using the Bayesian genetic association method, BeviMed[5]. Of these 99 associations, 62 are consistent with firmly established evidence and a further 11 have been reported in the literature since 2015, either by us or by other researchers. We showed that genetic associations with the extremes of a quantitative trait can identify genes in which mutations cause Mendelian pathologies. Finally, we used a novel method, RedPop, to call cell-type specific regulatory elements (REs) from open chromatin and histone modification data. We combined these calls with cell-type specific transcription factor binding information to identify four pathogenic rare non-coding variants that cause disease by disrupting the proper regulation of gene expression.

Summary of clinical findings

For each of the 15 rare disease domains, we established a list of diagnostic-grade genes (DGGs) and lists of their corresponding transcripts on the basis of the scientific literature (Supplementary Information). The number of DGGs for each domain ranged from two for Intrahepatic Cholestasis of Pregnancy (ICP) to 1,423 for Neurological and Developmental Disorders (NDD). The DGG lists were not mutually exclusive because some genes harbour

mutations that cause pathologies compatible with the enrolment criteria of multiple domains (Fig. 2c). Twelve multidisciplinary teams (MDTs) with domain-specific expertise examined the rare variants observed in DGGs in the context of the HPO phenotypes of the carriers. They categorised a subset of the variants as *pathogenic* or *likely pathogenic* following standard guidelines[6] and assessed their allelic contribution to disease as *full* or *partial*. A variant's contribution was considered to be at least partial if, given all other known variants in the case, it was considered to have a disease determining consequence. A conclusive molecular diagnosis was returned for 1,138 of the 7,065 (16.1%) patient records reviewed and those diagnoses featured 1,103 distinct causal variants (Supplementary Table 2). One quarter of the reports featured variants in *BMPR2*, *ABCA4* and *USH2A* and a further quarter featured variants in a group of 18 DGGs. The remaining half of the clinical reports concerned variants spread across 306 DGGs, which often featured in a single report (Fig. 2d, Extended Data Fig. 6). The diagnostic yield by domain ranged from no patients out of 184 (0%) for Primary Membranoproliferative Glomerulonephritis (PMG) to 391 patients out of 725 (53.9%) for Inherited Retinal Disease (IRD). The variability of diagnostic yield can be attributed to heterogeneity in: phenotypic and genetic pre-screening before enrolment, the genetic architecture of diseases and prior knowledge of genetic aetiologies.

Clinical reporting was enhanced by the use of PCR-free WGS with a mean autosomal depth >35X instead of whole-exome sequencing (WES). For example, a causal SNV encoding a start loss of *HPS6* in a case with Hermansky-Pudlak syndrome was identified by WGS but not identified by WES prior to the study. We compared the coverage obtained from the WGS samples to coverage obtained from research WES of UK Biobank samples (https://www.biorxiv.org/content/10.1101/572347v1), INTERVAL samples[7] and samples from the Columbia University exome sequencing study for chronic kidney disease[8] (Supplementary Information). Although less costly to generate per sample, all WES datasets exhibited much greater variation in coverage within and between genomic sites harbouring known pathogenic SNVs or indels than WGS (Extended Data Fig. 7). Of the 938 distinct autosomal aetiological SNVs and indels reported in this study, 25–99 (2.67%–10.5%) had insufficient coverage in WES for reliable genotyping, depending on dataset (Extended Data Fig. 7). Moreover, deletions spanning only a few short exons or part of a single exon are not reliably called by WES[9,10]. Of the 102 distinct large deletions that we reported (length range 203bp–16.80Mb, mean 786.33Kb, median 15.91Kb), 22 (21.6%) overlapped only one exon.

Our recent genetic discoveries have informed treatment decisions: 27 patients with early-onset dystonia due to variants in *KMT2B* can be treated by deep brain stimulation[11]; cases with *DIAPH1*-related macrothrombocytopenia and deafness[12] can have their platelet count restored to a safe level in a preoperative setting with Eltrombopag[13]; and a case of severe thrombocytopenia accompanied by myelofibrosis and bleeding caused by a gain-of-function variant in *SRC*[14] was cured by an allogeneic haematopoietic stem cell transplant. In addition, our diagnoses have helped stratify patient care: patients with Primary Immune Disorders (PID) due to variants in *NFKB1*, which we have shown are the commonest monogenic cause of combined variable immunodeficiency (CVID)[15], have unexplained splenomegaly and an increased risk of cancer; 27 cases from the Bleeding, Thrombotic and Platelet Disorders (BPD)

domain with isolated thrombocytopenia caused by variants in *ANKRD26*, *ETV6* or *RUNX1* have an increased risk of malignancy[16,17,18] compared to 19 cases with benign thrombocytopenia due to variants in *ACTN1*, *CYCS* or *TUBB1*[19]. Furthermore, our discoveries have improved the accuracy of prognosis, which is worse for patients with Pulmonary Arterial Hypertension (PAH) if the cause is mutations in *BMPR2*[20] or *EIF2AK4*[21], while the impact of mutations in *ATP13A3*, *AQP1*, *GDF2* and *SOX17*, genes which we have recently reported as aetiological[22], remains to be determined.

Quantitative intermediate phenotypes can contain information that is useful for understanding genetic aetiology in difficult to diagnose patients. We examined WGS read alignments for patients with complete absence of a protein encoded by a DGG but carrying an explanatory variant call on only one haplotype. Two patients with a severe unexplained bleeding disorder due to the absence of αIIbβ3 integrin on their platelet membranes carried two different complex variants in intron 9 of *ITGB3*: a tandem repeat and an SVA retrotransposon which was not called by either of the two structural variant callers we employed, but was discernible due to an excess of improperly mapped reads and confirmed by long-read nanopore sequencing (Extended Data Fig. 8a–e). The third patient had an absence of RhD and RhCE proteins on the membrane of her red cells leading to severe haemolytic anemia. This was due to a large tandem repeat in *RHAG*, which encodes the Rh-associated glycoprotein (Extended Data Fig. 8f).

Discovery of rare variants associated with rare diseases

Several cases with similar aetiologies are typically needed to make a novel discovery in rare disease genetics. Cases can be aggregated across siloed studies, using services such as Matchmaker Exchange (MME)[23]. We used MME to identify novel aetiologies for *SLC18A2*[24] and *WASF1*[25] (Supplementary Information). However, in the context of a study of a unified healthcare system, it is possible to make discoveries by statistical analyses of large patient collections.

We applied the statistical method BeviMed[5] to identify genetic associations between gene loci and rare diseases under various modes of Mendelian inheritance (Supplementary Information). We defined a set of phenotypic tags for each domain to determine a set of case/control groupings for BeviMed. Groups of cases were assigned the same tag if their phenotypes were *a priori* judged compatible with a shared genetic aetiology of disease (Supplementary Table 3). The number of unrelated cases in each tag group ranged from three for Roifman syndrome to 1,101 for PAH. For each gene-tag pair, we compared the genotypes at rare variant sites between unrelated individuals with the tag (cases) and unrelated individuals without the tag (controls). We considered a PP of association > 0.75 to be strong evidence supporting a genetic aetiology. Additionally, for each analysis BeviMed inferred a conditional PP over the mode of inheritance, a conditional PP over the molecular consequence class of variants mediating disease risk (e.g. 5' UTR variants or predicted loss-of-function variants) and conditional PPs of pathogenicity for each specific variant. These quantities were used to

compare established to inferred modes of inheritance and to estimate the number of cases attributable to variants in each gene[5].

We inferred strong evidence for an association between each of 95 genes, spanning nine domains, and one of 29 phenotypic tags. These genes included 68 established DGGs, 11 DGGs discovered since 2015[15,26,27,22,28,29,30,31,22,32,33] and 16 candidates requiring further investigation (Fig. 3; Supplementary Table 3). Thus, 79 of 95 genetic associations are confirmed, which sets a lower bound on the observed positive predictive value (PPV) of 83%, which is broadly in line with an ancestry-controlled statistical estimate of the study-wide PPV of 79% (Supplementary Information). We estimated that 611.3 cases can be explained by rare variants in the 79 genes with a confirmed association, 115.6 of which can be explained by the association between variants in *BMPR2* and PAH. 51 of the 95 genetic associations relied only on evidence from alleles carried by single cases, showing the power of joint statistical modelling of rare variants. Only three of the unconfirmed associations relied on evidence from alleles carried by more than one case, demonstrating the robustness of the results to cryptic relatedness. For one gene (*GP1BB*), the mode of inheritance inferred by BeviMed differed from that established in the literature, challenging long-held assumptions[34]. These results and other findings from this project[22,35,36,37,25,38,15,39,40,10,19,41,42,25,43,11,44,12,45] show that a unified analysis of standardised homogeneously collected genetic and phenotypic data from large cohorts of different rare disease domains is a powerful approach for genetic discovery.

Polygenic and rare variant associations with the extremes of a quantitative trait in UK Biobank

Several rare diseases (e.g. familial hypercholesterolaemia, CVID**,** thrombocytopenia, von Willebrand disease) are diagnosed and clinically characterised by reference to a quantitative trait that acts as a causal intermediate (or close proxy) for pathology and symptoms. Mutation-selection equilibrium ensures strong negative selection in the extreme tails of heritable quantitative traits, so individuals in the tails should have lower fecundity, perhaps due to greater risk of disease. We sought to identify genes likely to carry mutations causing RBC pathologies by computing a univariate quantitative summary of baseline RBC full blood count (FBC) traits in the UK Biobank participants of European ancestry. We aimed to develop a red cell phenotype capturing as much rare-variant heritability as possible. To achieve this, we used the joint distribution of estimated effect sizes from published GWAS associations between variants with MAF < 1% and four mature RBC FBC traits as a model for the effect of causal rare alleles we hoped to identify by WGS[46] (Fig 4a). We successfully sequenced 764 participants, 383 of which were extreme for the left tail of the phenotype, corresponding to a low RBC count (RBC#) and a high mean cell volume (MCV), and 381 of which were extreme for the right tail of the phenotype, corresponding to a high RBC# and a low MCV (Fig. 4b,c).

The distribution of a polygenic predictor of the quantitative phenotype, derived from genetic variants known to be associated with RBC# and MCV exhibits left and right shifts from the population distribution in the respectively named tails (Fig. 4d). However, these shifts are not as strong as those predicted by Gaussian variance components modelling, a discrepancy which could be explained partly by rare alleles generating excess density in the tails (kurtosis 6.9). A

WGS GWAS of an ordinal outcome (left tail, unselected, right tail) did not yield novel associations. Consequently, we treated each of the tail groups as a set of cases in a BeviMed analysis, identifying 12 genes with PP evidence for an association stronger than 0.4, a liberal threshold (Fig. 4e). *HBB* and *TFRC* can be considered positive controls, as they are known to carry mutations causing Mendelian microcytic anaemias. Other genes, including *CUX1* and *ALG1* are biologically plausible candidates. These results (Supplementary Table 3) indicate that the analysis of quantitative extremes in apparently healthy population samples may identify medically relevant loci unidentified by GWAS for quantitative traits[46,47].

Aetiological variants in regulatory elements

Recent statistical modelling suggests that only a small proportion of the burden of heritable neurodevelopmental disorders can be attributed to *de novo* pathogenic SNVs in non-coding elements[48]. Nevertheless, rare variants in REs are known to cause disease by disrupting transcription or translation[49,50,51]. We searched for aetiological variants in the REs of 246 DGGs implicated in recessive haematopoiesis-related disorders. Firstly, we defined a set of active REs we named a 'regulome' for each of six blood progenitor and mature blood cell types. We achieved this by merging transcription factor binding sites identified by ChIP-seq with genomic regions called by RedPop, a new detection method exploiting the anti-covariance of ATAC-seq and H3K27ac ChIP-seq coverage in REs (Supplementary Information). We linked the REs to genes on the basis of genomic proximity and promoter capture Hi-C data[52]. Secondly, we assigned each regulome to one or more of the BPD, PID and Stem Cell and Myeloid Disorders (SMD) domains, depending on the relevance of the corresponding cell types to these domains (Supplementary Table 3). Finally, we searched for cases carrying a rare homozygous or hemizygous deletion of an RE active in a cell type assigned to the domain of the case and which was linked to a DGG of that domain. We also searched for heterozygous deletions meeting these criteria that were in compound heterozygosity with a rare coding variant in a DGG linked to the deleted element (Fig. 5a). These approaches explained three cases: a PID patient carrying a deletion overlapping the 5' UTR region of *ARPC1B* in compound heterozygosity with a frameshift variant in the same gene (Thaventhiran *et al*, under review), a nine year old boy with autism spectrum disorder and thrombocytopenia carrying a hemizygous deletion of a *GATA1* enhancer on the X chromosome, and a male with several autoimmune-mediated cytopenias carrying a homozygous deletion of intronic CTCF binding sites[53] of *LRBA*.

The X-linked deletion in the boy with autism (Extended Data Fig. 9a–b) removed an element regulating *GATA1* as well as exons 1-4 of *HDAC6*. He had a persistently low platelet count (52×10^9/l), a mean platelet volume in the 99.9th percentile of the distribution for UK Biobank males (Fig. 5b)[54] and normal RBC parameters except for mild dyserythropoiesis. Electron microscopic imaging of his platelets showed reduced α-granule content (Extended Data Fig. 9c–e). Culture of his stem cells recapitulated ineffective formation of platelets by megakaryocytes (Extended Data Fig. 9f–k). Macrothrombocytopenia, reduced α-granule content, ineffective platelet formation and dyserythropoiesis are all characteristic of patients with pathogenic coding mutations of *GATA1*[55,56]. His platelets contained reduced GATA1 (Fig. 5g), consistent with reduced transcription due to deletion of the *GATA1* enhancer[57]. HDAC6 is the major deacetylase for removing the acetyl group from Lys40 of α-tubulin, which is located in

polymerized microtubules[58]. The absence of HDAC6 in the child was accompanied by extremely high expression levels of acetylated α-tubulin in his platelets (Fig. 5e), concordant with observations of *Hdac6* knockout mice[59]. This aberrant acetylation is associated with bleeding[59] and altered emotional behaviour[60] in mice. Thus, the reduced expression of *GATA1* and the absence of HDAC6 jointly cause a new syndrome of macrothrombocytopenia accompanied by neurodevelopmental problems.

The patient with a homozygous deletion of a CTCF binding site in the first intron of *LRBA* presented with a pancytopenia, characterised mostly by neutropenia and anaemia, and complicated by periods of thrombocytopenia. These cytopenias were mediated by autoantibodies due to a loss of tolerance for multiple autoantigens, which is characteristic of patients with reduced *LRBA* function[61].

We adapted our approach to solving cases caused by non-coding deletions to search for non-coding SNVs with a CADD[62] score > 20, in the presence of a high-impact coding variant in compound heterozygosity in the assigned DGG. This approach identified two potentially aetiological SNVs in elements assigned to *AP3B1* and *MPL*, and we studied the 10 year old male patient carrying the latter mutation in more detail. *MPL* encodes the receptor for the megakaryocyte growth factor thrombopoietin[63]. Loss of *MPL* causes chronic amegakaryocytic thrombocytopenia in humans[64] and *Mpl* knockout mice have severe thrombocytopenia[65,66]. The SNV (chr1:43803414 G>A) was in an RE detected by RedPop, the activity of which is specific to megakaryocytes in blood cell physiology (Extended Data Fig. 10), had a CADD score of 21.8, was absent from gnomAD, and was in compound heterozygosity with a deletion of exon 10 of *MPL,* which was inherited from the patient's mother (Extended data, Fig. 10a,b,c). A luciferase reporter assay showed approximately 50% reduced promoter activity for the A allele compared to the reference allele (Extended Data Fig. 10d). As a result, platelet MPL levels were significantly reduced in the patient compared to controls (Extended Data Fig. 10e). In contrast to *MPL*-null patients[67], who are extremely thrombocytopenic because their bone marrow is almost devoid of megakaryocytes and eventually suffer haematopoietic stem cell exhaustion, this boy had platelet counts which stabilised around 45×10^9/l and a marrow that was only moderately depleted of megakaryocytes. As the regulatory SNV does not abolish *MPL* transcription completely (Extended Fig. 10c), the boy has a milder clinical phenotype than *MPL*-null cases.

Discussion

Before now there has been limited integration between clinical genetic testing services and aetiological studies of rare diseases on a national scale. We have shown that WGS in a universal national healthcare system can tackle these two objectives concurrently (Fig. 1a). This synergy can only be achieved if sequencing data from explained cases (Fig. 2), unexplained cases and unaffected individuals are analysed jointly and if consent to contact participants for follow-up studies has been obtained at enrolment. We have shown that long-read sequencing can aid the identification of complex structural variants, which can still be called unreliably by short-read WGS. We have demonstrated the utility of data aggregation and sharing through the number of genetic associations we have found across a diversity of rare diseases (Fig. 3). This study follows on from large-scale whole-exome and shallow genome sequencing studies in the

UK[68, 69] and has been the blueprint for the UK's 100,000 Genomes Project, which recently completed sequencing. The NHS plans to increase provision of WGS-based diagnostics from 8,000 to 30,000 samples per month. To achieve this aim, it has reduced the number of clinical genomics laboratories to seven, each servicing approximately 8 million people. It has also introduced a unified and consistent WGS and informatics infrastructure for these seven hubs and is providing training in genomics to NHS staff. We have initiated WGS of UK Biobank participants to study individuals with extreme values for a quantitative phenotype. Extreme trait values may be the result of measurement error, extreme polygenic loads[47] or rare genetic variation and such individuals are typically excluded from GWAS studies. We have shown that genetic associations with the tail of a quantitative distribution can identify genes mediating Mendelian pathologies in the same domain of human biology (Fig. 4). The forthcoming WGS of 0.5 million UK Biobank participants provides an opportunity to study other traits following similar approaches. Finally, we have provided examples of rare variants causing disease by disrupting non-coding REs of the genome. The reliability and affordability of WGS and the availability of cell-type specific epigenetic data make the exploration of the non-coding genome (Fig. 5, Extended Data Fig. 10) a promising focus for future research in unresolved rare disorders for which the aetiological cell types are known.

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Methods

Enrolment, research ethics and consent Patients with rare diseases and their close relatives were enrolled to the NIHR BioResource (NBR) as part of a pilot study for the 100,000 Genomes Project. For this study, 15 rare disease domains were approved after review by the Sequencing and Informatics Committee of the NBR. Enrolment of participants for this pilot study was coordinated by the University of Cambridge, started in December 2012 and was completed in March 2017. In addition, samples from a second rare diseases pilot study, coordinated by GEL, are included together with a number of control samples and samples from the UK Biobank cohort[70]. The NBR–Rare Diseases study was coordinated by the University of Cambridge. Participants were recruited mainly at NHS Hospitals in the UK, but also at hospitals overseas (Supplementary Table 1, Extended Data Fig. 1a)**.** All 13,187 participants provided written informed consent, either under the East of England Cambridge South national research ethics committee (REC) reference 13/EE/0325 or under alternative REC-approved studies. Obtaining consent for overseas samples was the responsibility of the respective principal investigators at the hospitals where enrollment took place. The NBR retained blank versions of the consent forms from overseas participants and a material transfer agreement was applied to regulate the exchange of samples and data between the donor institutions and the University of Cambridge.

Clinical and laboratory phenotype data Staff at hospitals responsible for enrolment were provided with the eligibility criteria for their respective domains as described above in the domain descriptions. The clinical and laboratory phenotype data were captured through case report forms (CRF) by paper questionnaires or by online CRF data capture applications and deposited in the NBR study database. Online data capture allowed for the free entry of HPO terms[71] by staff at the enrolment centre and data from paper questionnaires were transformed into HPO terms by the study coordination office. Free text entries were transformed into HPO terms where feasible. An overview of the HPO data obtained for the 15 NBR rare disease domains is depicted in Extended Data Fig. 1c,d.

DNA sequencing Samples were received as either DNA extracted from whole blood or as whole blood EDTA samples, which were used for extraction at the NBR laboratory in Cambridge. Samples were tested for adequate concentration (Picogreen), quality controlled (QC) for DNA degradation (gel electrophoresis) and purity (OD 260/280; Trinean) before selection for WGS. DNA samples were prepared at a minimum concentration of 30 ng/µl in 110 µl, visually inspected for degradation and had to have an OD 260/280 between 1.75 and 2.04. They were then prepared in batches of 96 and shipped on dry ice to the sequencing provider (Illumina Inc, Great Chesterford, UK). Further sample QC was performed by Illumina to ensure that the concentration of the DNA was > 30 ng/µl and that every sample generated high quality genotyping results (Illumina Infinium Human Core Exome microarray). Samples with a repeated array genotyping call rate < 0.99, high levels of cross-contamination, mismatches with the declared gender that could not be resolved by further investigation, or for which consent had been withdrawn, were excluded from WGS (n=59). The genotyping data were also used for

positive sample identification and sample identity was verified before data delivery. In short 0.5 µg of the DNA sample was fragmented using Covaris LE220 (Covaris Inc., Woburn, MA, USA) to obtain an average size of 450bp DNA fragments. DNA samples were processed using the Illumina TruSeq DNA PCR-Free Sample Preparation kit (Illumina Inc., San Diego, CA, USA) on the Hamilton Microlab Star (Hamilton Robotics, Inc, Reno, NV, USA). The final libraries were checked using the Roche LightCycler 480 II (Roche Diagnostics Corporation, Indianapolis, IN, USA) with KAPA Library Quantification Kit (Kapa Biosystems Inc., Wilmington, MA, USA) for concentration. From February 2014 to June 2017 three read lengths were used: 100bp, 125bp and 150bp (377, 3,154 and 9,656 samples, respectively). Samples sequenced with 100bp and 125bp reads utilised three and two lanes of an Illumina HiSeq 2500 instrument, respectively, while samples sequenced with 150bp reads utilised a single lane of a HiSeq X instrument. At least 95% of the autosomal genome had to be covered at 15X and a maximum of 5% of insert sizes had to be less than twice the read length. Following sample and data QC at Illumina, 13,187 sets of WGS data files were received at the University of Cambridge High Performance Computing Service (HPC) for further QC.

WGS data processing pipeline The WGS data for the 13,187 samples returned by the sequencing provider underwent a series of processing steps (Extended Data Fig. 2), described in detail in the Supplementary Information. Briefly, the samples were sex karyotyped and pairwise kinship coefficients were computed. This information was used to check for repeat sample submissions and sample swaps. Additionally, four further QC checks were applied to ensure the SNVs and indels were of a high standard. Overall, 150 samples (1.1%) were removed, leaving a dataset of 13,037 samples for downstream analysis. The 13,037 individuals were assigned one of the following ethnicities: European, African, South Asian, East Asian or Other. Pairwise relatedness adjusted for population stratification was then computed and used to generate networks of closely related individuals and to define a maximal set of 10,259 unrelated individuals. The variants in the 13,037 individuals were left-aligned and normalised with bcftools, loaded into our HBase database and filtered on their overall pass rate (OPR), defined in the Supplementary Information. The sex karyotypes, the ethnicities and the relatedness estimates were used, along with enrolment information, to annotate the samples and variants. Samples were annotated with: affected/unaffected status, membership of the set of probands, membership of the maximal unrelated set, ethnicity and sex karyotype. Variants were annotated with CellBase consequence predictions, HGMD information where available and population-specific allele frequencies.

Pertinent findings For each of the 15 rare disease domains (i.e. all domains except UKB, CNTRL and GEL) a list of DGGs was generated by domain-specific experts. Genes were included in the lists if there was a high enough level of evidence in the literature for gene-disease association. The 2,497 gene/domain pairs, encompassing 2,073 unique DGGs across all domains, were manually curated and annotated with the relevant RefSeq and/or Ensembl transcript identifiers to support variant reporting. Transcripts were selected based on, by order of priority, community input, presence in the Locus Reference Genomic (LRG) resource[72] or designation as canonical in Ensembl. Variants (SNVs, indels) were shortlisted if (i) their MAF in control populations[73] was < 1/1,000 for putative novel causal variants and < 25/1,000 for

variants listed as disease-causing in HGMD, (ii) their predicted impact according to the Variant Effect Predictor[74] was “HIGH” or “MODERATE” or if the consequences with respect to the designated transcript included one of “splice_region_variant” or “non_coding_transcript_exon_variant” if the variant was in a non-coding gene, (iii) the variant affected a DGG relevant to the patient’s disease. Variants with more than 3 alleles or a MAF >= 10% in the diseases cohort were discarded to, respectively, guard against errors in repetitive regions and remove potential systematic artefacts. The above filtering criteria were applied universally to all domains, except for ICP which adopted a higher MAF threshold of 3% for both novel and previously reported variants. The higher threshold accounted for causal variants being present in the male and non-child bearing female population. This strategy reduced the number of variants for review by the MDT from about 4 million per person to fewer than 10, while confidently retaining known regulatory or moderately common pathogenic variants. For each affected participant with prioritised variants, the variant calls, HPO-coded phenotype and the relevant metadata (unique study numbers; referring clinician and hospital; self-declared gender and genetically inferred sex, ancestry, relatedness, and consanguinity level) were transferred to Congenica Inc (Cambridge, United Kingdom) for visualisation in the Sapientia™ web application during MDT meetings. MDTs brought together experts from different hospitals across the UK and abroad, and typically consisted of an experienced clinician with domain-specific knowledge, a scientist with experience in clinical genomics, a clinical bioinformatician and a member of the reporting team. Assignment of the level of pathogenicity followed the American College of Medical Genetics guidelines[6] and variants (V) were marked in Sapientia™ as pathogenic, likely pathogenic or of uncertain significance (VUS). Only pathogenic and likely pathogenic variants were systematically reported and VUSs were reported at the MDT’s discretion. As per REC-approved study protocol, secondary findings (e.g. breast cancer pathogenic variants in *BRCA1* in patients not presenting with this phenotype) were not reported.

Genetic association testing in genes We used the BeviMed statistical method[5] to identify genetic associations with rare diseases in our dataset. Each run of BeviMed requires the definition of a set of cases and controls, all of which should be unrelated with each other, and a set of rare variants to include in the inference. To achieve adequate power, the cases should be chosen such that they potentially share a common genetic aetiology (e.g. because the phenotypes are similar) and the rare variants should be chosen such that they potentially share a mechanism of action on phenotype (e.g. because they are predicted to have a similar effect on a particular gene product). BeviMed computes PP values of no association, dominant association and recessive association and, conditional on dominant or recessive association, it computes the PP that each variant is pathogenic. We can impose a prior correlation structure on the pathogenicity of the variants that reflects competing hypotheses as to which class of variant is responsible for disease. These classifications typically group variants by their predicted consequences. The class of variant responsible can then be inferred by BeviMed, thereby suggesting a particular mechanism of disease. The methodology is described in further detail in the Supplementary Information and in reference[5].

Regulome analysis We applied the BLUEPRINT protocol for ChIP-seq data analysis (http://dcc.blueprint-epigenome.eu/#/md/chip_seq_grch37). We defined regulomes for activated

CD4+ T cells (aCD4), B cells (B), erythroblasts (EB), megakaryocytes (MK), monocytes (MON) and resting CD4+ T cells (rCD4). For each cell type, we used open chromatin data (ATAC-seq or DNAse-seq) and histone modification data (H3K27ac) to identify REs using the RedPop method (see below). Additionally, for MK and EB, we had access to the following transcription factor (TF) ChIP-seq data, which were used to call peaks (see below) and supplement the regulomes: FLI1, GATA1, GATA2, MEIS1, RUNX1, TAL1 and CTCF for MK; GATA1, KLF1, NFE2 and TAL1 for EB; and CTCF for MON and B. For each cell type, the regulome build process proceeded as follows: 1. Call RedPop regions using ATAC-seq/DNAse-seq and H3K27ac-seq data; 2. Call TF/CTCF binding peaks using ChIP-seq data if available and obtain enrichment scores; 3. Discard TF regions with an enrichment score < 10 unless they overlap between at least two different TFs; 4. Collapse overlapping features to obtain a single genomic track; 5. Merge features within 100bp of each other. Each regulome feature was assigned a gene label using either gene annotations from Ensembl (v75) or a compendium of previously published promoter capture Hi-C data (pcHi-C)[52] as follows: 1. Assign to a gene if the feature overlaps the gene or the region up to 10Kb either side of the gene body; 2. Assign to a gene if the feature overlaps the gene's pcHi-C 'blind' spot. This region is defined by three *Hind*III restriction fragments, incorporating the capture fragment overlapping target gene TSS, and 5' and 3' adjacent fragments; 3. Assign to a gene if the feature overlaps a linked promoter interacting region identified using pcHi-C in the same cell type.

Functional analysis of the *GATA1* enhancer/*HDAC6* deletion The *GATA1* enhancer/*HDAC6* deletion was confirmed by PCR using primers HDAC6-F: 5'-catcttcaagaggatcagagg and HDAC6-R: 5'-catagctagacactggtt. Electron microscopy for platelets was performed as described[55]. Immunostaining of resting and fibrinogen spread platelets was performed as described[44] and analyzed by Structured Illumination Microscopy (SIM, Elyra S.1, Zeiss, Heidelberg, D.E). Total protein lysates were obtained from platelets for immunoblot analysis as described[75]. The following antibodies were used for SIM and immunoblot analysis: rabbit anti-HDAC6 (clone D2E5, Cell Signaling technology, Danvers, MA, USA), mouse anti-acetylated tubulin antibody (clone 6-11B-1, Sigma, St Louis, MO, USA), mouse anti-alpha-tubulin (A11126, Thermo Fisher Scientific, Waltham, MA, USA), rabbit anti-VWF (Dako, Aligent Technologies, Leuven, BE), mouse anti-CD63 and rat anti-GATA1 N6 (Santa Cruz Biotechnology, Dallas, TX, USA), rabbit anti-GATA1 (NF that was produced against recombinant N-terminal zinc finger[76], rabbit anti-GAPDH (14C10, Cell Signaling) and anti-β3 integrin (sc-14009; Santa Cruz Biotechnology).

MPL expression on platelets The level of MPL protein on the platelet membrane was measured by flow cytometry (Beckman Coulter FC500) using the monoclonal antibodies: APC-labelled IgG1 against CD42b (clone HIP1, BD Pharmingen, number: 551061), PE-labelled IgG1 against CD110 (clone REA250, Miltenyi Biotec) and a PE-labelled isotype control (clone MOPC-21, BD Pharmingen, number: 555749). In short, a sample of EDTA anticoagulated blood was incubated with anti-CD110 (or control) and anti-CD42b for 30 minutes. Mean fluorescence intensity (MFI) produced by the anti-CD110 was measured by flow cytometry on cells gated on the CD42b APC signal, side and forward scatter.

Nanopore sequencing Oxford Nanopore-based sequencing of long-range PCR-amplified target DNA was performed as previously described[77] with the aim to resolve the genetic architecture of intron 9 of *ITGB3* in a case with Glanzmann's thrombasthenia. The flow cell ran for 3 hours, and the mean coverage was 863,986X**.**

Code availability Code to run HBASE is available from https://github.com/mh11/VILMAA. The RedPop software package is available from https://gitlab.haem.cam.ac.uk/et341/redpop/.

Data availability Genotype and phenotype data from the 4,835 participants enrolled in the NIHR BioResource for the 100,000 Genomes Project–Rare Diseases Pilot can be accessed by seeking access via Genomics England Limited following the procedure outlined at: https://www.genomicsengland.co.uk/about-gecip/joining-research-community/. The genotype data for the 764 UK Biobank samples will be made available through a data release process overseen by UK Biobank (https://www.ukbiobank.ac.uk/). The phenotype data from UK Biobank participants are available from UK Biobank using their normal access procedures.
The genotype data from the vast majority of the remaining 7,438 NBR participants have been deposited in the European Genome-phenome Archive (EGA) at the EMBL European Bioinformatics Institute. Deposition of genotype at EGA is grouped by rare disease domain: EGA accession codes: BPD: EGAD00001004519, CSVD: EGAD00001004513, EDS: EGAD00001005123, HCM: EGAD00001004514, ICP: EGAD00001004515, IRD: EGAD00001004520, LHON: EGAD00001005122, MPMT: EGAD00001004521, NDD: EGAD00001004522, NPD: EGAD00001004516, PAH: EGAD00001004525, PID: EGAD00001004523, PMG: EGAD00001004517, SMD: EGAD00001004524, SRNS: EGAD00001004518. Access to genotype data provided by the EGA is overseen by a Data Access Committee (DAC) (https://www.ebi.ac.uk/ega/). Access to all NBR WGS data and detailed phenotype data on the 7,438 NBR participants can be requested by completing the NBR Data Access Agreement application (dac@bioresource.nihr.ac.uk).

The ATAC-seq and H3K27ac ChIP-seq data to support the generation of the regulomes are available from GEO or EGA, or referenced to their publication as follows. H3K27ac ChIP-seq: aCD4[78], B (ERR1043004, ERR1043129, ERR928206, ERR769436), EB (EGAD00001002377), MK (EGAD00001002362), MON (ERR829362 (ERS257420), ERR829412 (ERS222466), ERR493634 (ERS214696)), rCD4[78]. ATAC-seq: aCD4 (GSE124867), B (SRR2126769 (GSE71338)), EB (SRR5489430 (GSM2594182)), MK (EGAD00001001871), MON (accession number requested), rCD4 (GEO accession will be available before publication).

MDT-reported alleles and their clinical interpretation have been deposited in ClinVar (under the name "NIHR Bioresource Rare Diseases") and DECIPHER.

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Competing Interests LHM acts as a consultant for Drayson Technologies; AMK had no competing interests at the time of the study, since the study has received an educational grant from CSL Behring to attend the ISTH meeting (2017); TJA has received consultancy payments from AstraZeneca within the last 5 years and has received speaker honoraria from Illumina Inc.; SW has received an educational grant from CSL Behring and an honorarium from Biotest, LFB; CLS has received educational grants to attend conferences from CSL Behring, Alk and Baxter; MJP has received support for attending educational events and speaker's fees from Biotest UK, Shire UK, and Baxter; TE-S has received support for attending educational events from Biotest UK, CSL and Shire UK; YMK holds a grant from Roche; ARo, CChe, CSt, EB, KTat, NLe, RPr are employees of Congenica Ltd; BTo, JFi, JK, MV, TKa are employees of GENALICE; CCol, CGe, CJBo, CRe, DRB, JFP, JHu, RJG, SHum, SHun, TSAG are employees of Illumina Cambridge Limited; CVG is holder of the Bayer and Norbert Heimburger (CSL Behring) Chair; KJM previously received funding for research and currently on the scientific advisory board of Gemini Therapeutics, Boston, USA; YMCH received free IVD diagnostic tools and reagents from companies in laboratory haemostasis for studies and/or validations (Werfen, Roche, Siemens, Stage, Nodia); MCS received travel and accommodation fees from NovoNordisk; DML serves on advisory boards for Agios, Novartis and Cerus; MIM serves on advisory panels for Pfizer, NovoNordisk, Zoe Global, has received honoraria from Pfizer, NovoNordisk and Eli Lilly, has stock options in Zoe Global, has received research funding from Abbvie, AstraZeneca, Boehringer Ingelheim, Eli Lilly, Janssen, Merck, NovoNordisk, Pfizer, Roche, Sanofi Aventis, Servier, Takeda. The remaining authors declare no competing financial interests.

Additional information

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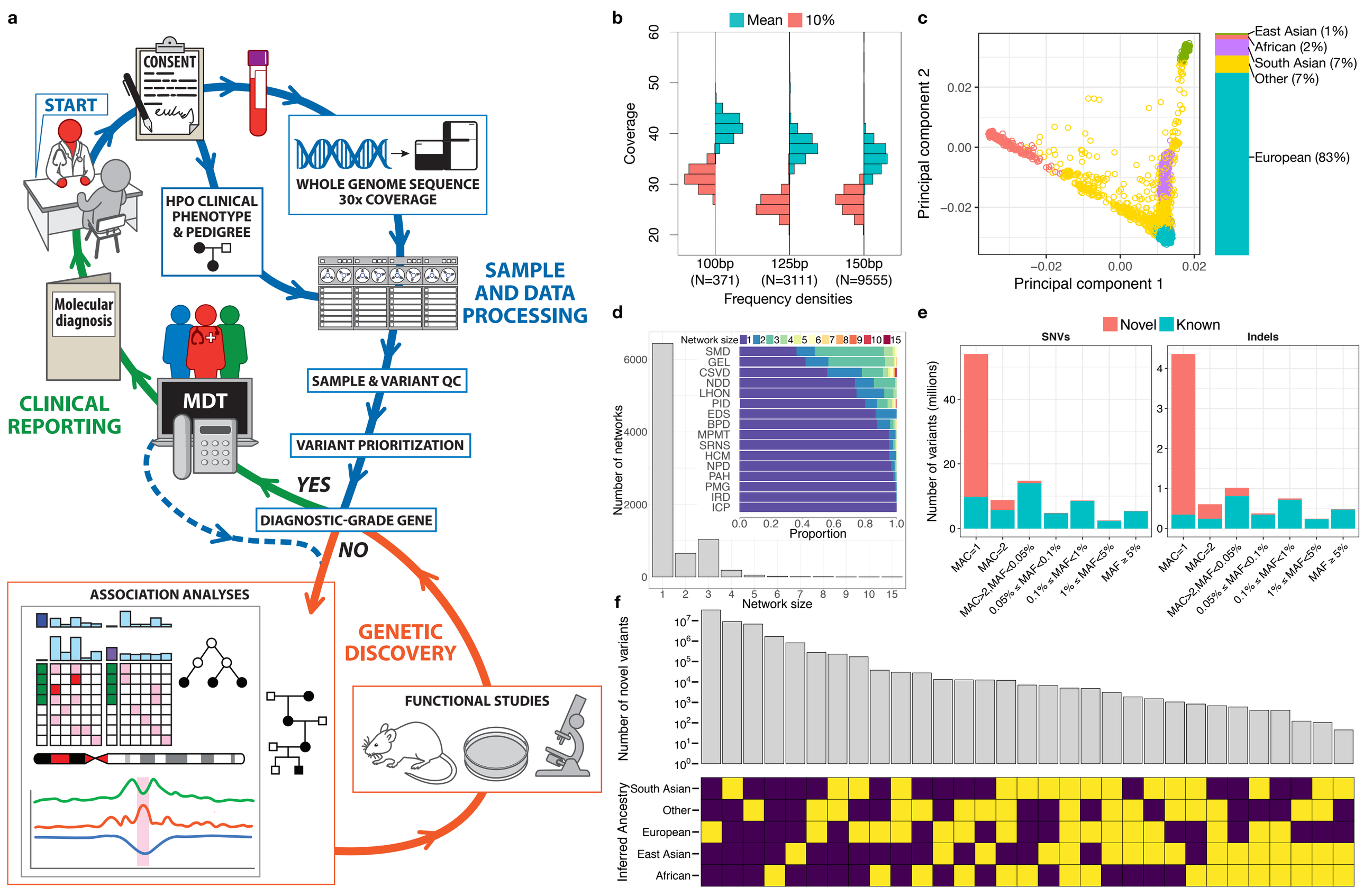
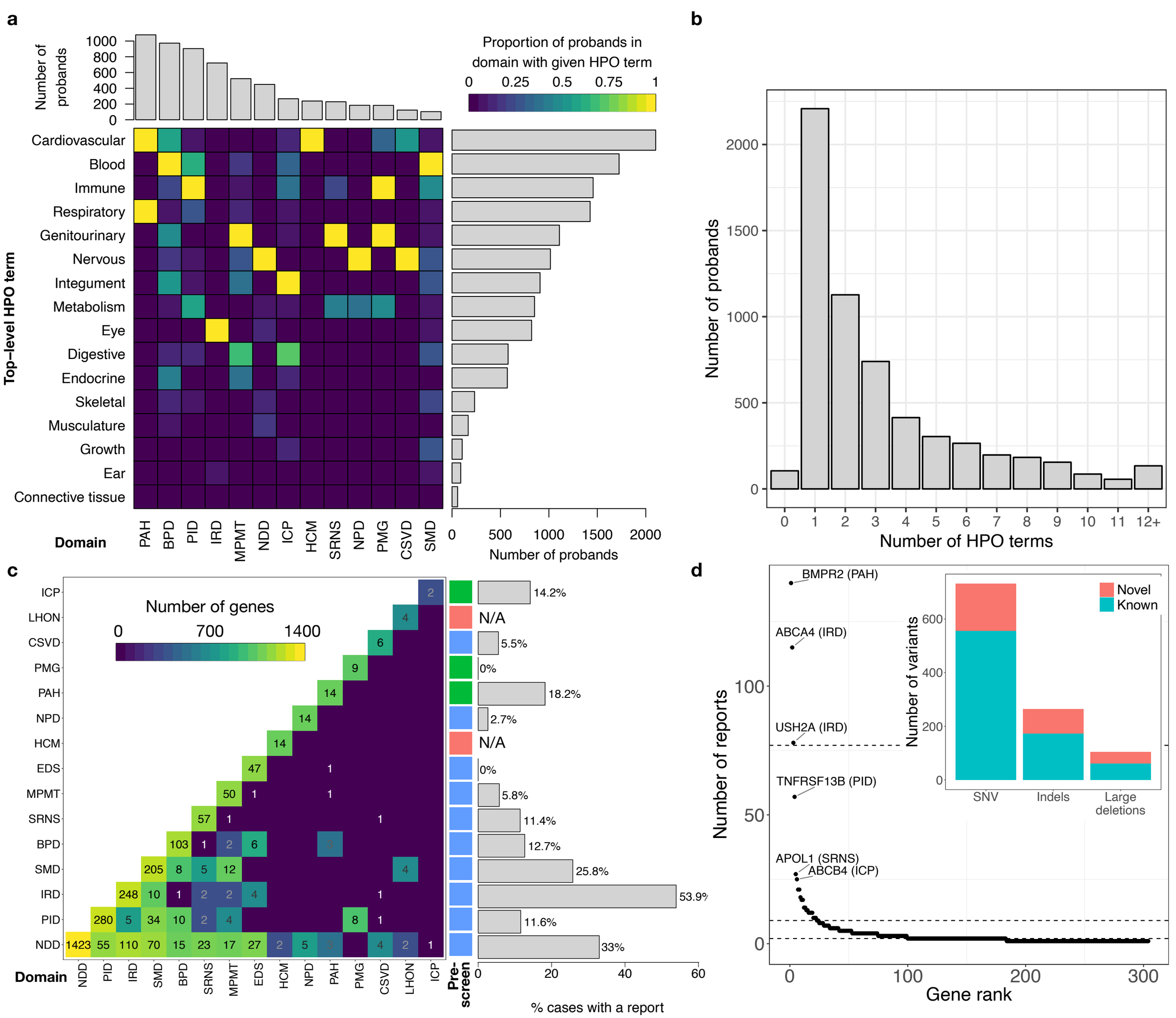
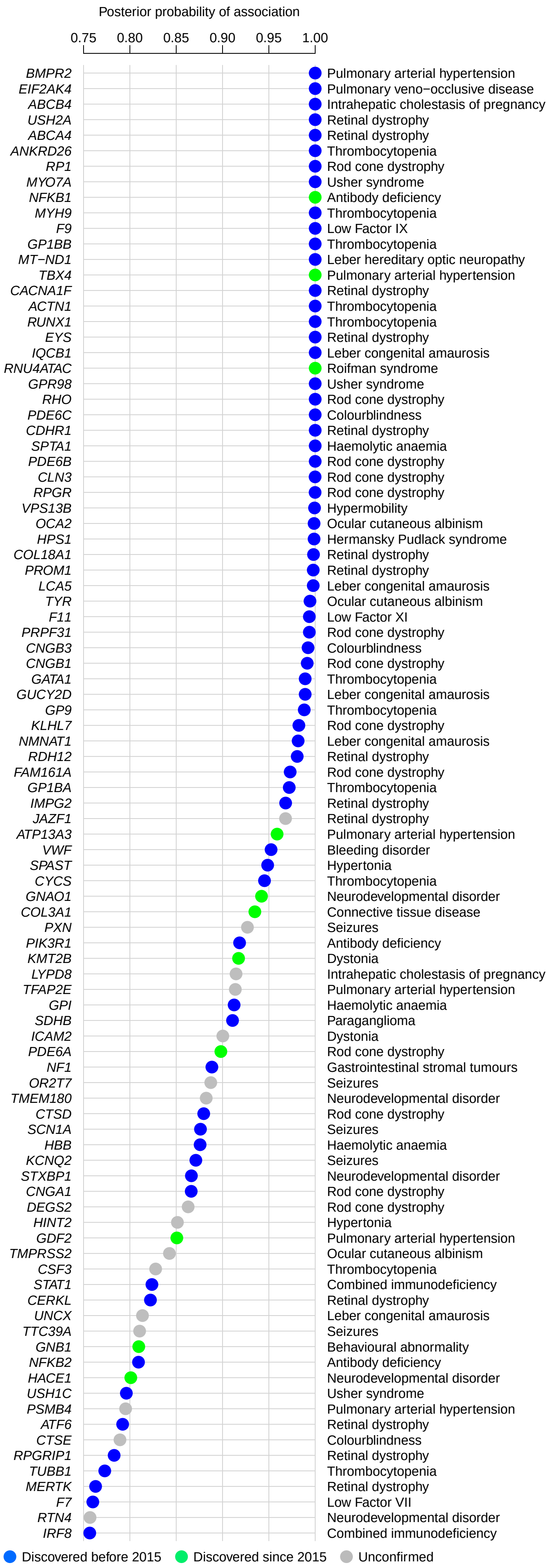
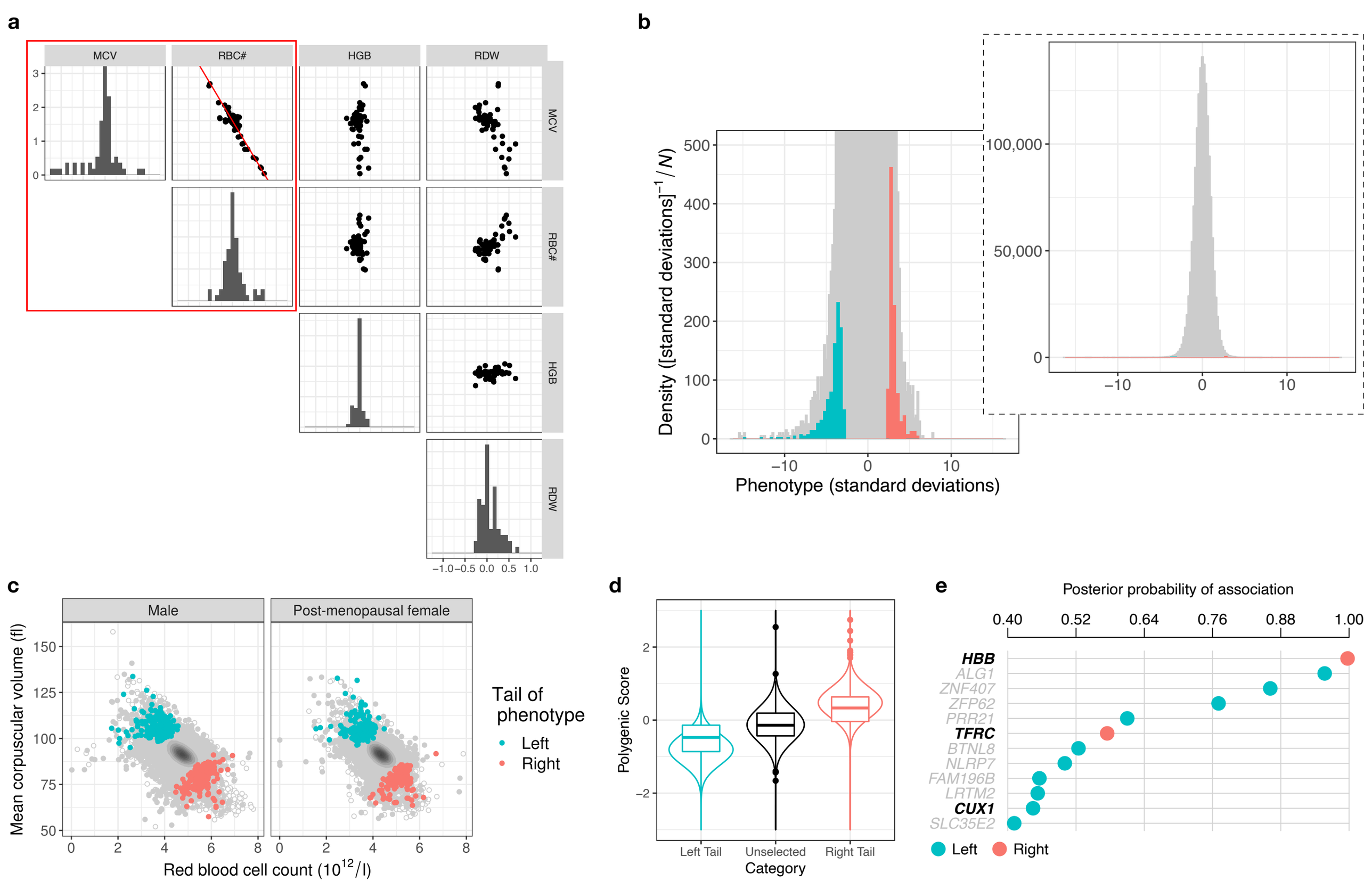
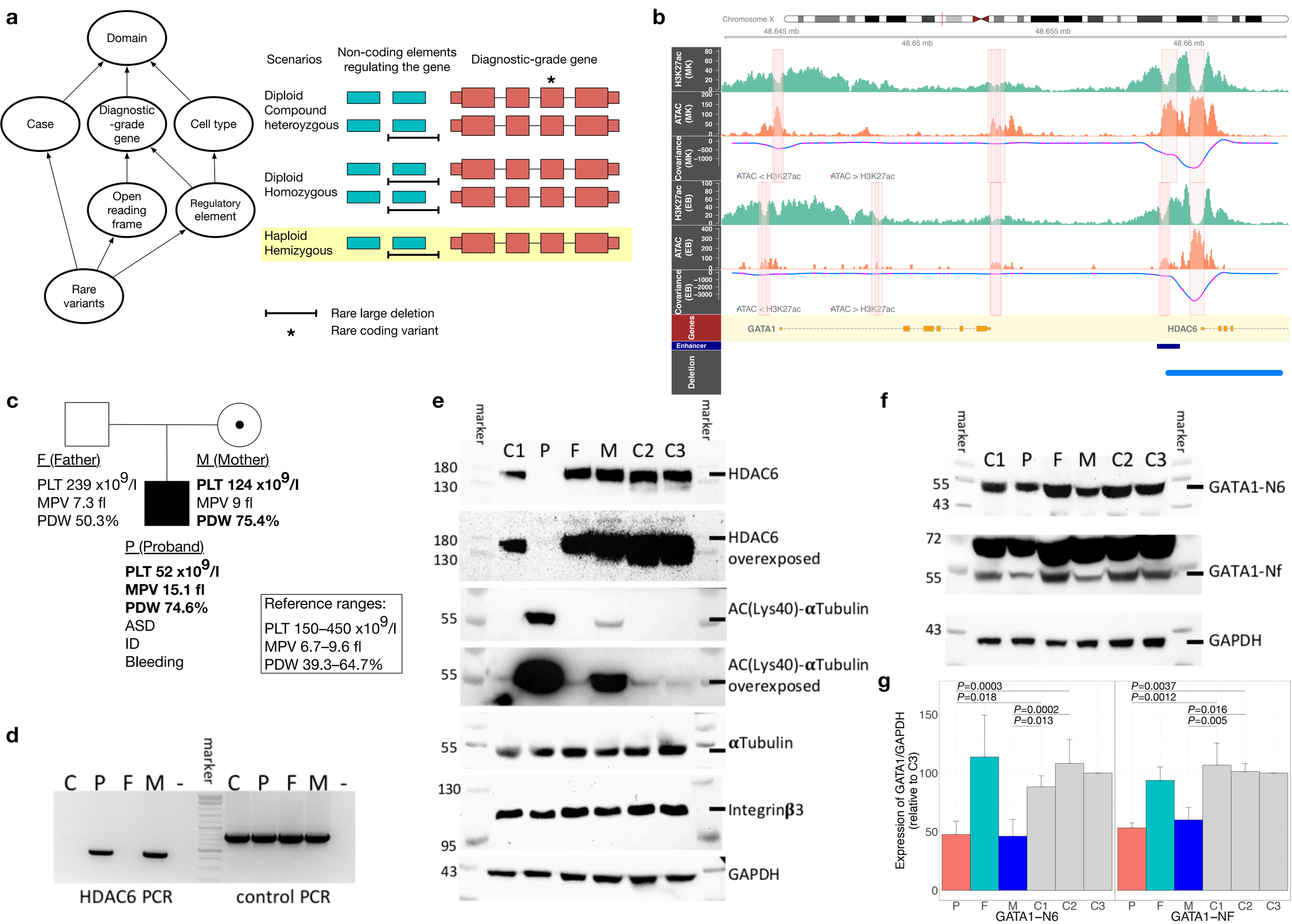

a
START
CONSENT
HPO CLINICAL PHENOTYPE & PEDIGREE
WHOLE GENOME SEQUENCE 30x COVERAGE
SAMPLE AND DATA PROCESSING
SAMPLE & VARIANT QC
VARIANT PRIORITIZATION
DIAGNOSTIC-GRADE GENE
YES
NO
MDT
Molecular diagnosis
CLINICAL REPORTING
ASSOCIATION ANALYSES
GENETIC DISCOVERY
FUNCTIONAL STUDIES
b
Mean
10%
Coverage
100bp (N=371)
125bp (N=3111)
150bp (N=9555)
Frequency densities
c
East Asian (1%)
African (2%)
South Asian (7%)
Other (7%)
European (83%)
Principal component 2
Principal component 1
d
Network size
SMD
GEL
CSVD
NDD
LHON
PID
EDS
BPD
MPMT
SRNS
HCM
NPD
PAH
PMG
IRD
ICP
Proportion
Number of networks
Network size
e
Novel
Known
SNVs
Indels
Number of variants (millions)
MAC=1
MAC=2
MAC>2,MAF≤0.05%
0.05% ≤ MAF<0.1%
0.1% ≤ MAF<1%
1% ≤ MAF<5%
MAF ≥ 5%
f
Number of novel variants
Inferred Ancestry
South Asian
Other
European
East Asian
African


a
Number of probands
1000
800
600
400
200
0
Proportion of probands in domain with given HPO term
0
0.25
0.5
0.75
1
Top−level HPO term
Cardiovascular
Blood
Immune
Respiratory
Genitourinary
Nervous
Integument
Metabolism
Eye
Digestive
Endocrine
Skeletal
Musculature
Growth
Ear
Connective tissue
Domain
PAH
BPD
PID
IRD
MPMT
NDD
ICP
HCM
SRNS
NPD
PMG
CSVD
SMD
0
500
1000
1500
2000
Number of probands
b
Number of probands
2000
1500
1000
500
0
0
1
2
3
4
5
6
7
8
9
10
11
12+
Number of HPO terms
c
Number of genes
0
700
1400
ICP
LHON
CSVD
PMG
PAH
NPD
HCM
EDS
MPMT
SRNS
BPD
SMD
IRD
PID
NDD
Domain
Pre-screen
N/A
14.2%
5.5%
0%
18.2%
2.7%
N/A
0%
5.8%
11.4%
12.7%
25.8%
53.9%
11.6%
33%
0
20
40
60
% cases with a report
d
BMPR2 (PAH)
ABCA4 (IRD)
USH2A (IRD)
TNFRSF13B (PID)
APOL1 (SRNS)
ABCB4 (ICP)
Number of reports
100
50
0
0
100
200
300
Gene rank
Novel
Known
Number of variants
600
400
200
0
SNV
Indels
Large deletions


Posterior probability of association
0.75
0.80
0.85
0.90
0.95
1.00
BMPR2 Pulmonary arterial hypertension
EIF2AK4 Pulmonary veno−occlusive disease
ABCB4 Intrahepatic cholestasis of pregnancy
USH2A Retinal dystrophy
ABCA4 Retinal dystrophy
ANKRD26 Thrombocytopenia
RP1 Rod cone dystrophy
MYO7A Usher syndrome
NFKB1 Antibody deficiency
MYH9 Thrombocytopenia
F9 Low Factor IX
GP1BB Thrombocytopenia
MT−ND1 Leber hereditary optic neuropathy
TBX4 Pulmonary arterial hypertension
CACNA1F Retinal dystrophy
ACTN1 Thrombocytopenia
RUNX1 Thrombocytopenia
EYS Retinal dystrophy
IQCB1 Leber congenital amaurosis
RNU4ATAC Roifman syndrome
GPR98 Usher syndrome
RHO Rod cone dystrophy
PDE6C Colourblindness
CDHR1 Retinal dystrophy
SPTA1 Haemolytic anaemia
PDE6B Rod cone dystrophy
CLN3 Rod cone dystrophy
RPGR Rod cone dystrophy
VPS13B Hypermobility
OCA2 Ocular cutaneous albinism
HPS1 Hermansky Pudlack syndrome
COL18A1 Retinal dystrophy
PROM1 Retinal dystrophy
LCA5 Leber congenital amaurosis
TYR Ocular cutaneous albinism
F11 Low Factor XI
PRPF31 Rod cone dystrophy
CNGB3 Colourblindness
CNGB1 Rod cone dystrophy
GATA1 Thrombocytopenia
GUCY2D Leber congenital amaurosis
GP9 Thrombocytopenia
KLHL7 Rod cone dystrophy
NMNAT1 Leber congenital amaurosis
RDH12 Retinal dystrophy
FAM161A Rod cone dystrophy
GP1BA Thrombocytopenia
IMPG2 Retinal dystrophy
JAZF1 Retinal dystrophy
ATP13A3 Pulmonary arterial hypertension
VWF Bleeding disorder
SPAST Hypertonia
CYCS Thrombocytopenia
GNAO1 Neurodevelopmental disorder
COL3A1 Connective tissue disease
PXN Seizures
PIK3R1 Antibody deficiency
KMT2B Dystonia
LYPD8 Intrahepatic cholestasis of pregnancy
TFAP2E Pulmonary arterial hypertension
GPI Haemolytic anaemia
SDHB Paraganglioma
ICAM2 Dystonia
PDE6A Rod cone dystrophy
NF1 Gastrointestinal stromal tumours
OR2T7 Seizures
TMEM180 Neurodevelopmental disorder
CTSD Rod cone dystrophy
SCN1A Seizures
HBB Haemolytic anaemia
KCNQ2 Seizures
STXBP1 Neurodevelopmental disorder
CNGA1 Rod cone dystrophy
DEGS2 Rod cone dystrophy
HINT2 Hypertonia
GDF2 Pulmonary arterial hypertension
TMPRSS2 Ocular cutaneous albinism
CSF3 Thrombocytopenia
STAT1 Combined immunodeficiency
CERKL Retinal dystrophy
UNCX Leber congenital amaurosis
TTC39A Seizures
GNB1 Behavioural abnormality
NFKB2 Antibody deficiency
HACE1 Neurodevelopmental disorder
USH1C Usher syndrome
PSMB4 Pulmonary arterial hypertension
ATF6 Retinal dystrophy
CTSE Colourblindness
RPGRIP1 Retinal dystrophy
TUBB1 Thrombocytopenia
MERTK Retinal dystrophy
F7 Low Factor VII
RTN4 Neurodevelopmental disorder
IRF8 Combined immunodeficiency
Discovered before 2015
Discovered since 2015
Unconfirmed


a
MCV
RBC#
HGB
RDW
b
Density ([standard deviations]$^{-1}$ / N)
Phenotype (standard deviations)
c
Male
Post-menopausal female
Mean corpuscular volume (fl)
Red blood cell count (10^{12}/l)
Tail of phenotype
Left
Right
d
Polygenic Score
Left Tail
Unselected
Right Tail
Category
e
Posterior probability of association
HBB
ALG1
ZNF407
ZFP62
PRR21
TFRC
BTNL8
NLRP7
FAM196B
LRTM2
CUX1
SLC35E2
Left
Right


a
Domain
Case
Diagnostic -grade gene
Cell type
Open reading frame
Regulatory element
Rare variants
Scenarios
Non-coding elements regulating the gene
Diagnostic-grade gene
Diploid Compound heteroyzgous
Diploid Homozygous
Haploid Hemizygous
Rare large deletion
Rare coding variant
b
Chromosome X
48.645 mb
48.65 mb
48.655 mb
48.66 mb
H3K27ac (MK)
ATAC (MK)
Covariance (MK)
H3K27ac (EB)
ATAC (EB)
Covariance (EB)
ATAC < H3K27ac
ATAC > H3K27ac
Genes
GATA1
HDAC6
Enhancer
Deletion
c
F (Father)
PLT 239 x10^9/l
MPV 7.3 fl
PDW 50.3%
M (Mother)
PLT 124 x10^9/l
MPV 9 fl
PDW 75.4%
P (Proband)
PLT 52 x10^9/l
MPV 15.1 fl
PDW 74.6%
ASD
ID
Bleeding
Reference ranges:
PLT 150–450 x10^9/l
MPV 6.7–9.6 fl
PDW 39.3–64.7%
d
C P F M -
marker
C P F M -
HDAC6 PCR
control PCR
e
marker
C1 P F M C2 C3
HDAC6
HDAC6 overexposed
AC(Lys40)-αTubulin
AC(Lys40)-αTubulin overexposed
αTubulin
Integrinβ3
GAPDH
f
GATA1-N6
GATA1-Nf
GAPDH
g
Expression of GATA1/GAPDH (relative to C3)
P=0.0003
P=0.018
P=0.0002
P=0.013
P=0.0037
P=0.0012
P=0.016
P=0.005
P F M C1 C2 C3
GATA1–N6
GATA1–NF