Dear Dylan,

Thanks for the reminders.

Yesterday I finally found the time to go through the “Quantifying prior probabilities…” paper. I found it to be a very interesting manuscript and approach. In a way I’m quite surprised these aspects haven’t been taken into account previously. I made a number of comments, but they are all naïve and generally boil down to style suggestions of where I think specific text sections should be placed.

…

I especially liked sections 3.1-3.6, and felt these could be built off further (I gave one example of how from a metabolic perspective).

…

I did struggle about with the transition to 3.7, where the link to the previous sections was not obvious to me. Sections 3.7-3.12 felt like a different paper.

Quantifying prior probabilities for disease-causing variants reveals the top genetic contributors in inborn errors of immunity

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1

1 **Abstract**

2 We present a framework to quantify the prior probability of observing 3 known disease-causing variants across all genes and inheritance modes. First, 4 we computed genome-wide occurrence probabilities by integrating population 5 allele frequencies, variant classifications, and Hardy-Weinberg expectations 6 under autosomal dominant, recessive, and X-linked inheritance. Second, both 7 pathogenic variants and missing causal candidates were tested to identify 8 the most likely genetic disease determinant and provide a clear confidence 9 range for the overall diagnosis. This provided a complete and interpretable 10 summary of evidence for genetic diagnosis. Third, we summarised variant

11 probabilities for 557 genes responsible for inborn errors of immunity (IEI), now 12 integrated into a public database. Fourth, we derived new data-driven IEI 13 classifications using protein-protein interactions and curated clinical features, 14 aligned to immunophenotypes. Finally, we validated the framework in national 15 scale cohorts, showing close concordance with observed case numbers. The 16 resulting datasets supported causal variant interpretation and evidence-aware

decision-making in clinical genetics.1

17

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**Availability:** This data is integrated in public panels at https://iei-genetics.github.io. The source code are accessible as part of the variant risk estimation project at https://github.com/ DylanLawless/var\_risk\_est and IEI-genetics project at https://github.com/iei-genetics/ iei-genetics.github.io. The data is available from the Zenodo repository: https://doi.org/ 10.5281/zenodo.15111583 (VarRiskEst PanelAppRex ID 398 gene variants.tsv). VarRiskEst is available under the MIT licence.

2

18

19 Graphical abstract.

3

20 **Acronyms**

21 **ACMG** American College of Medical Genetics and Genomics. . . . . . . . . . . . . . . . . . .40 2223 **ACAT** Aggregated Cauchy Association Test . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 40 2425 **AD** Autosomal Dominant. . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .6 2627 **AF** Allele Frequency. . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 6 2829 **ANOVA** Analysis of Variance . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 19 3031 **AR** Autosomal Recessive . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 6 3233 **BMF** Bone Marrow Failure . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 29 3435 **CD** Complement Deficiencies . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 30 3637 **CI** Confidence Interval. . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .27 3839 **CrI** Credible Interval . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 13 4041 **CF** Cystic Fibrosis . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 17 4243 ***CFTR*** Cystic Fibrosis Transmembrane Conductance Regulator. . . . . . . . . . . . . . . . . .8 44

45 **CVID** Common Variable Immunodeficiency. . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 15 4647 ***DCLRE1C*** DNA Cross-Link Repair 1C . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 8 4849 **dbNSFP** database for Non-Synonymous Functional Predictions . . . . . . . . . . . . . . . . . 8 5051 **GE** Genomics England . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 7 5253 **gnomAD** Genome Aggregation Database . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 8 5455 **gVCF** genomic variant call format . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 13 5657 **HGVS** Human Genome Variation Society . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 8 5859 **HPC** High-Performance Computing. . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .12 6061 **HSD** Honestly Significant Difference . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 19 6263 **HWE** Hardy-Weinberg Equilibrium . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 6 6465 **IEI** Inborn Errors of Immunity. . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .6 6667 **Ig** Immunoglobulin . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 33 6869 ***IL2RG*** Interleukin 2 Receptor Subunit Gamma. . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 8 7071 **InDel** Insertion/Deletion . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 8 7273 **IUIS** International Union of Immunological Societies . . . . . . . . . . . . . . . . . . . . . . . . . . . . 6 74

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75 **LD** Linkage Disequilibrium . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 32 7677 **LOEUF** Loss-Of-function Observed/Expected Upper bound Fraction . . . . . . . . . . . 19 7879 **LOF** Loss-of-Function . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 19 8081 **MOI** Mode of Inheritance . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 6 8283 ***NFKB1*** Nuclear Factor Kappa B Subunit 1 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 8 8485 **OMIM** Online Mendelian Inheritance in Man . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 37 8687 **PID** Primary Immunodeficiency . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 6 8889 **PPI** Protein-Protein Interaction . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 8 9091 **pLI** Probability of being Loss-of-function Intolerant . . . . . . . . . . . . . . . . . . . . . . . . . . . . 19 9293 **QC** Quality Control . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 13 9495 ***RAG1*** Recombination activating gene 1 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 8 9697 **SCID** Severe Combined Immunodeficiency . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 8 9899 **SNV** Single Nucleotide Variant . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 6 100

101 **SKAT** Sequence Kernel Association Test. . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .40 102

103 **STRINGdb** Search Tool for the Retrieval of Interacting Genes/Proteins. . . . . . . . .8 104

105 **TP** true positive. . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .6 106

107 **FP** false positive. . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .6 108

109 **TN** true negative . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 6 110

111 **FN** false negative. . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 6 112

113 ***TNFAIP3*** Tumor necrosis factor, alpha-induced protein 3 . . . . . . . . . . . . . . . . . . . . . . 8 114

115 **UMAP** Uniform Manifold Approximation and Projection . . . . . . . . . . . . . . . . . . . . . . 20 116

117 **UniProt** Universal Protein Resource. . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 7 118

119 **VCF** variant call format . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 13 120

121 **VEP** Variant Effect Predictor. . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .8 122

123 **VRE** variant risk estimate . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 9 124

125 **XL** X-Linked . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 6 126

5

127 **1 Introduction**

128 Accurately determining the probability that a patient harbours a disease-causing 129 genetic variant remains a foundational challenge in clinical and statistical genetics. 130 For over a century, the primary focus has been on identifying true positive (TP)s, 131 pathogenic causal variants observed in affected individuals. Peer review and classifi 132 cation frameworks also work to suppress false positive (FP)s. However, two critical 133 components of the genetic landscape have received far less attention: false nega 134 tive (FN)s, where pathogenic variants are missed due to technical or interpretive 135 limitations, and true negative (TN)s, which represent the vast majority of benign 136 or non-causal variants. TNs are more commonly used in contexts such as cancer 137 screening, where a negative result can provide reassurance that a panel of known 138 actionable variants has been checked. Yet outside these specific uses, their broader 139 statistical and clinical value is rarely leveraged. From a statistical perspective, FNs 140 and TNs are an untapped goldmine. They hold essential information about what is 141 not observed, what should be expected under baseline assumptions, and how confident 142 one can be in the absence of a pathogenic finding. Yet these dimensions are rarely 143 quantified, leaving a bias in current variant interpretation frameworks towards known 144 TPs and lacking principled priors for genome-wide disease probability estimation.

145 In this study, we focused on reporting the probability of disease observation 146 through genome-wide assessments of gene-disease combinations. Our central hypoth 147 esis was that by using highly curated annotation data including population Allele 148 Frequency (AF)s, disease phenotypes, Mode of Inheritance (MOI) patterns, and vari 149 ant classifications and by applying rigorous calculations based on Hardy-Weinberg 150 Equilibrium (HWE), we could accurately estimate the expected probabilities of ob 151 serving disease-associated variants. Among other benefits, this knowledge can be used 152 to derive genetic diagnosis confidence by incorporating these new priors.

153 We focused on known Inborn Errors of Immunity (IEI) genes, sometimes called 154 the “Primary Immunodeficiency (PID) or Monogenic Inflammatory Bowel Disease 155 genes” (1–3), to validate our approach and demonstrate its clinical relevance. This 156 application to a well-established genotype-phenotype set, comprising over 500 gene 157 disease associations, underscores its utility. The most recent update on the classification 158 of IEI from the International Union of Immunological Societies (IUIS) expert committee 159 was reported by Poli et al. (1), with an accompanying diagnostic guide (4).

160 Quantifying the risk that a patient inherits a disease-causing variant is a fun 161 damental challenge in genomics. Classical statistical approaches grounded in HWE 162 (5; 6) have long been used to calculate genetic MOI probabilities for Single Nucleotide 163 Variant (SNV)s. However, applying these methods becomes more complex when 164 accounting for different MOI, such as Autosomal Recessive (AR) versus Autosomal 165 Dominant (AD) or X-Linked (XL) disorders. In AR conditions, for example, the 166 occurrence probability must incorporate both the homozygous state and compound 167 heterozygosity, whereas for AD and XL disorders, a single pathogenic allele is sufficient 168 to cause disease. Advances in genetic research have revealed that MOI can be even

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169 more complex (7). Mechanisms such as dominant negative effects, haploinsufficiency, 170 mosaicism, and digenic or epistatic interactions can further modulate disease risk and 171 clinical presentation, underscoring the need for nuanced approaches in risk estimation. 172 Karczewski et al. (8) made significant advances; however, the remaining challenge lies 173 in applying the necessary statistical genomics data across all MOI for any gene-disease 174 combination, which our current work aims to address. Similar approaches have been 175 reported for diseases such Wilson disease, mucopolysaccharidoses, primary ciliary 176 dyskinesia, and treatable metabolic diseasse, (9; 10), as reviewed by Hannah et al. 177 (11).

178 To our knowledge, all approaches to date have been limited to single MOI, specific 179 to the given disease, or restricted to a small number of genes. We argue that our 180 integrated approach is highly powerful because the resulting probabilities can serve 181 as informative priors in a Bayesian framework for variant and disease probability 182 estimation; a perspective that is often overlooked in clinical and statistical genetics. 183 Such a framework not only refines classical HWE-based risk estimates but also has 184 the potential to enrich clinicians’ understanding of what to expect in a patient and to 185 enhance the analytical models employed by bioinformaticians. The dataset also holds 186 value for AI and reinforcement learning applications, providing an enriched version of 187 the data underpinning frameworks such as AlphaFold (12) and AlphaMissense (13).

188 This gap is not only due to conceptual limitations, but to the historical absence of 189 large, harmonised reference datasets. Only recently have resources become available to 190 support rigorous genome-wide probability estimation. These include high-resolution 191 population allele frequencies (e.g. gnomAD v4 (8)), curated variant classifications 192 (e.g. ClinVar (14)), functional annotations (e.g. UniProt (15)), and pathogenicity 193 prediction models (e.g. AlphaMissense (13)). We previously introduced PanelAppRex 194 to aggregate gene panel data from multiple sources, including Genomics England (GE) 195 PanelApp, ClinVar, and Universal Protein Resource (UniProt), thereby enabling 196 advanced natural searches for clinical and research applications (2; 3; 14; 15). This 197 earlier work relied on expert-curated panels, such as those from the NHS National 198 Genomic Test Directory and the 100,000 Genomes Project, converted into machine 199 readable formats for rapid variant discovery and interpretation. Together, these 200 resources now make it possible to model the expected distribution of variant types, 201 frequencies, and classifications across the genome.

202 By reframing variant interpretation as a problem of calibrated expectation rather 203 than solely reactive confirmation, our framework empowers clinicians and researchers to 204 anticipate both observed and unobserved pathogenic burdens. This scalable, genome 205 wide approach promises to streamline diagnostic workflows, reduce uncertainty in 206 inconclusive cases, inform statistical models and genetic epidemiology studies, and 207 accelerate the integration of genetic insights into patient care.

7

208 **2 Methods**

209 **2.1 Dataset**

210 Data from Genome Aggregation Database (gnomAD) v4 comprised 807,162 indi 211 viduals, including 730,947 exomes and 76,215 genomes (8). This dataset provided 212 786,500,648 SNVs and 122,583,462 Insertion/Deletion (InDel)s, with variant type 213 counts of 9,643,254 synonymous, 16,412,219 missense, 726,924 nonsense, 1,186,588 214 frameshift and 542,514 canonical splice site variants. ClinVar data were obtained from 215 the variant summary dataset (as of: 16 March 2025) available from the NCBI FTP site, 216 and included 6,845,091 entries, which were processed into 91,319 gene classification 217 groups and a total of 38,983 gene classifications; for example, the gene *A1BG* contained 218 four variants classified as likely benign and 102 total entries (14). For our analysis 219 phase we also used database for Non-Synonymous Functional Predictions (dbNSFP) 220 which consisted of a number of annotations for 121,832,908 SNVs (16). The Pan 221 elAppRex core model contained 58,592 entries consisting of 52 sets of annotations, 222 including the gene name, disease-gene panel ID, diseases-related features, confidence 223 measurements. (2) Protein-Protein Interaction (PPI) network data was provided by 224 Search Tool for the Retrieval of Interacting Genes/Proteins (STRINGdb), consisting 225 of 19,566 proteins and 505,968 interactions (17). The Human Genome Variation 226 Society (HGVS) nomenclature is used with Variant Effect Predictor (VEP)-based 227 codes for variant IDs. AlphaMissense includes pathogenicity prediction classifications 228 for 71 million variants in 19 thousand human genes (13; 18). We used these scores 229 to compared against the probability of observing the same given variants. **Box 2.1** 230 list the definitions from the IUIS IEI for the major disease categories used throughout 231 this study (1).

232 The following genes were used for disease cohort validations and examples. We 233 used the two most commonly reported genes from the IEI panel Nuclear Factor Kappa 234 B Subunit 1 (*NFKB1*) (19–22) and Cystic Fibrosis Transmembrane Conductance 235 Regulator (*CFTR*) (23–25) to demonstrate applications in AD and AR disease genes, 236 respectively. We used Severe Combined Immunodeficiency (SCID)-specific genes 237 AR DNA Cross-Link Repair 1C (*DCLRE1C*), AR Recombination activating gene 238 1 (*RAG1*), XL Interleukin 2 Receptor Subunit Gamma (*IL2RG*) to demonstrate a 239 IEI subset disease phenotype of SCID. We also used AD Tumor necrosis factor, 240 alpha-induced protein 3 (*TNFAIP3*) for other examples comparable to *NFKB1* since it 241 is also causes AD pro-inflammatory disease but has more known ClinVar classifications 242 at higher AF then *NFKB1*.

8

Box 2.1 Definitions for IEI Major Disease Categories

**Major Category Description**

1. CID Immunodeficiencies affecting cellular and humoral immunity 2. CID+ Combined immunodeficiencies with associated or syndromic features 3. PAD - Predominantly Antibody Deficiencies

4. PIRD - Diseases of Immune Dysregulation

5. PD - Congenital defects of phagocyte number or function

6. IID - Defects in intrinsic and innate immunity

7. AID - Autoinflammatory Disorders

8. CD - Complement Deficiencies

9. BMF - Bone marrow failure

243

244 **2.2 Variant classification occurrence probability**

To quantify the likelihood that an individual harbours a variant with a given disease classification, we compute the variant-level occurrence probability (variant risk estimate (VRE)) for each variant. As a starting point, we considered the classical HWE for a biallelic locus:

*p*2 + 2*pq* + *q*2 = 1*,*

where *p* is the allele frequency, *q* = 1 *− p*, *p*2

245 represents the homozygous dominant, 2*pq* the heterozygous, and *q*2

246 the homozygous recessive genotype frequencies. For 247 disease phenotypes, particularly under AR MOI, the risk is traditionally linked to the homozygous state (*p*2

248 ); however, to account for compound heterozygosity across 249 multiple variants, we allocated the overall gene-level risk proportionally among variants.

250 Our computational pipeline estimated the probability of observing a disease 251 associated genotype for each variant and aggregated these probabilities by gene and 252 ClinVar classification. This approach included all variant classifications, not limited 253 solely to those deemed “pathogenic”, and explicitly conditioned the classification on 254 the given phenotype, recognising that a variant could only be considered pathogenic 255 relative to a defined clinical context. The core calculations proceeded as follows:

256 **1. Allele frequency and total variant frequency.** For each variant *i* in a gene, 257 . For each gene (any genomic region or set), we

the allele frequency was denoted as *pi*

258 defined the total variant frequency (summing across all reported variants in that gene) 259 as:

*P*tot =X *i∈*gene

*pi.*

9

260 Note that, because each calculation is confined to one gene, no additional scaling 261 was required for our primary analyses (*P*tot). However, if this same unscaled summation 262 is applied across regions or variant sets of differing size or dosage sensitivity, it can 263 bias burden estimates. In such cases, normalisation by region length or incorporation 264 of gene- or region-specific dosage constraints is recommended.

265 If any of the possible SNV had no observed allele (*pi* = 0), we assigned a minimal 266 risk:

*pi* =1

max(*AN*) + 1

267 where max(*AN*) was the maximum allele number observed for that gene. This 268 adjustment ensured that a nonzero risk was incorporated even in the absence of 269 observed variants in the reference database.

270 **2. Occurrence probability based on MOI.** The probability that an individual 271 is affected by a variant depends on the MOI. For **AD** and **XL** variants, a single 272 pathogenic allele suffices:

*p*disease*,i* = *pi.*

273 For **AR** variants, disease manifests when two pathogenic alleles are present, either 274 as homozygotes or as compound heterozygotes. We use:

*p*disease*,i* = *pi P*tot*.*

275 Under HWE, the overall gene-level probability of an AR genotype is

*P*AR = *P*2tot =X *i*

where *P*tot =P*ipi*

*p*2*i* + 2X *i<j*

*pi pj,*

276 . A naïve per-variant assignment

*p*2*i* + 2 *pi* (*P*tot *− pi*)

277 would, when summed over all *i*, double-count the compound heterozygous terms. To 278 partition *P*AR among variants without double counting, we allocate risk in proportion 279 to each variant’s allele frequency:

10

*p*disease*,i* =*pi*

*P*tot*× P*2tot = *pi P*tot*.*

280 This ensures

X *i*

*p*disease*,i* =X *i*

*pi P*tot = *P*2tot*,*

281 recovering the correct AR risk while attributing each variant its fair share of 282 homozygous and compound-heterozygous contributions.

283 More simply, for AD or XL conditions a single pathogenic allele suffices, so the 284 classification risk (e.g. benign, pathogenic) equals its population frequency. For AR 285 conditions two pathogenic alleles are required - either two copies of the same variant 286 or one copy each of two different variants, so we divide the overall recessive risk among 287 variants according to each variant’s share of the total classification frequency in that 288 gene.

289 **3. Expected case numbers and case detection probability.** Given a popu 290 lation with *N* births (e.g. as seen in our validation studies, *N* = 69 433 632), the 291 expected number of cases attributable to variant *i* was calculated as:

*Ei* = *N · p*disease*,i.*

292 The probability of detecting at least one affected individual for that variant was 293 computed as:

*P*(*≥* 1)*i* = 1 *−*1 *− p*disease*,i* *N.*

294 **4. Aggregation by gene and ClinVar classification.** For each gene and for each 295 ClinVar classification (e.g. “Pathogenic”, “Likely pathogenic”, “Uncertain significance”, 296 etc.), we aggregated the results across all variants. The classification grouping can 297 be substituted by any alternative score system. The total expected cases for a given 298 group was:

*E*group =X *i∈*group

*Ei,*

11

299 and the overall probability of observing at least one case within the group was 300 calculated as:

*P*group = 1 *−*Y *i∈*group

(1 *− p*disease*,i*)*.*

301 **5. Data processing and implementation.** We implemented the calculations 302 within a High-Performance Computing (HPC) pipeline and provided an example for a 303 single dominant disease gene, *TNFAIP3*, in the source code to enhance reproducibility. 304 Variant data were imported in chunks from the annotation database for all chromosomes 305 (1-22, X, Y, M).

306 For each data chunk, the relevant fields were gene name, position, allele number, 307 allele frequency, ClinVar classification, and HGVS annotations. Missing classifications 308 (denoted by “.”) were replaced with zeros and allele frequencies were converted to 309 numeric values. Subsequently, the variant data were merged with gene panel data from 310 PanelAppRex to obtain the disease-related MOI mode for each gene. For each gene, if 311 no variant was observed for a given ClinVar classification (i.e. *pi* = 0), a minimal risk 312 was assigned as described above. Finally, we computed the occurrence probability, 313 expected cases, and the probability of observing at least one case of disease using the 314 equations presented.

315 The final results were aggregated by gene and ClinVar classification and used to 316 generate summary statistics that reviewed the predicted disease observation probabili 317 ties. We define the *VRE* as the prior probability of observing a variant classified as 318 the cause of disease

319 **6. Score-positive-total.** For use as a simple summary statistic on the resulting 320 user-interface, we defined the *score-positive-total* as the total number of positively 321 scored variant classifications within a given region (gene, locus, or variant set). Using 322 the ClinVar classification assigned to a scale from*−*5 (benign) to +5 (pathogenic), 323 we included only scores *>* 0, corresponding to some evidence of pathogenicity. The 324 score-positive-total yields a non-normalised estimate of the prior probability that a 325 phenotype is explained by known pathogenic variants.

326 **7. Classification scoring system.** Each ClinVar classification was assigned an 327 integer score: pathogenic = +5, likely pathogenic = +4, pathogenic (low penetrance) 328 = +3, likely pathogenic (low penetrance) = +2, conflicting pathogenicity = +2, 329 likely risk allele/risk factor/association = +1, drug response/uncertain significance/no 330 classification/affects/other/not provided/uncertain risk allele = 0, protective = –3, 331 likely benign = –4, benign = –5. No further normalisation was applied. The resulting 332 distribution (**Figure S1 A-B**) is naturally comparable to a zero-centred average 333 rank (**C-D**). This straightforward, modular approach can be readily replaced by

12

334 any comparable evidence-based classification system. Variants with scores *≤* 0 were 335 omitted, since benign classifications do not inform disease likelihood in the score 336 positive-total summary.

337 **2.3 Integrating observed true positives and unobserved false** 338 **negatives into a single, actionable conclusion**

339 In this section, we detail our approach to integrating sequencing data with prior classi 340 fication evidence (e.g. pathogenic on ClinVar) to calculate the posterior probability of 341 a complete successful genetic diagnosis. Our method is designed to account for possible 342 outcomes of TP, TN, and FN, by first ensuring that all nucleotides corresponding 343 to known variant classifications (benign, pathogenic, etc.) have been accurately se 344 quenced. This implies the use of genomic variant call format (gVCF)-style data which 345 refer to variant call format (VCF)s that contain a record for every position in the 346 genome (or interval of interest) regardless of whether a variant was detected at that 347 site or not. Only after confirming that these positions match the reference alleles 348 (or novel unaccounted variants are classified) do we calculate the probability that 349 additional, alternative pathogenic variants (those not observed in the sequencing data) 350 could be present. Our Credible Interval (CrI) for pathogenicity thus incorporates 351 uncertainty from the entire process, including the tally of TP, TN, and FN outcomes. 352 We ignore the contribution of FPs as a separate task to be tackled in the future.

353 We estimated, for every query (e.g. gene or disease-panel), the posterior probability 354 that at least one constituent allele is both damaging and causal in the proband. The 355 workflow comprises four consecutive stages.

356 **(i) Data pre‑processing.** We synthesized an example patient in a disease cohort 357 of 200 cases. We made several scenarios where a causal genetic diagnosis based on 358 the available data is either simple, difficult, or impossible. Our example focused 359 on a proband two representative genes for AD IEI: *NFKB1* and *TNFAIP3*. All 360 coding and canonical splice‑region variants for *NFKB1* were extracted from the gVCF. 361 We assumed a typical Quality Control (QC) scenario, where sites corresponding to 362 previously reported pathogenic alleles were checked for read depth *≥*10 and genotype 363 quality *≥*20. Positions that failed this check were labelled *missing*, thus separating 364 true reference calls from non-sequenced or uninformative sequence.

365 **(ii) Evidence mapping and occurrence probability.** PanelAppRex variants 366 were annotated with ClinVar clinical significance. Each label was converted to 367 an ordinal evidence score *Si ∈* [*−*5*,* 5] and rescaled to a pathogenic weight *Wi* = 368 ; *−*5*,* 5 *→* 0*,* 1). This scoring system can be replaced with any comparable

rescale(*Si*

369 alternative. The HWE-based pipeline of Section 2.2 supplied a per‑variant occurrence 370 . The adjusted prior was

probability *pi*

13

*p∗i* = *Wi pi,* and flag*i ∈ {*present*,* missing*}.*

371 **(iii) Prior specification.** In a hypothetical cohort of *n* = 200 diploid individuals 372 the count of allele *i* follows a Beta-Binomial model. Marginalising the Binomial yields 373 the Beta prior

*πi ∼* Beta*αi, βi**, αi* = round(2*np∗i*) + ˜*wi, βi* = 2*n −* round(2*np∗i*) + 1*,* 374 where *w*˜*i* = max(1*, Si* +1) contributes an additional pseudo‑count whenever *Si >* 0.

375 **(iv) Posterior simulation and aggregation.** For each variant *i* we drew *M* = 10 000 realisations *π*(*m*)

376 and normalised within each iteration,

*i*

*i* =*π*(*m*)

*π*˜(*m*)

~~P~~

*i*

*.*

*jπ*(*m*)

*j*

377 Variants with *Si >* 4 were deemed to have evidence as *causal* (pathogenic or likely 378 pathogenic). We note that an alternative evidence score or conditional threshold can be substituted for this step. Their mean posterior share *π*¯*i* = *M−*1 P*m π*˜(*m*)

379 and 95%

*i*

380 CrI were retained. The probability that a damaging causal allele is physically present 381 was obtained by a second layer:

*P*(*m*) =X *i*: *Si>*3

*π*˜(*m*)

*i G*(*m*)

*i, G*(*m*)

*i ∼* Bernoulli(*gi*)*,*

382 for missing

with *gi* = 1 for present variants, *gi* = 0 for reference calls, and *gi* = *pi* variants. The gene‑level estimate is the median of *{P*(*m*)*}M*

383

384 percentiles.

*m*=1 and its 2*.*5th/97*.*5th

385 **(v) Scenario analysis.** The three scenarios were explored for a causal genetic 386 diagnosis that is either simple, difficult, or impossible given the existing data. The 387 proband spiked data had either: (1) known classified variants only, including only one 388 known TP pathogenic variant, *NFKB1* p.Ser237Ter, (2) inclusion of an additional 389 plausible yet non-sequenced splice‑donor allele *NFKB1* c.159+1G>A (likely pathogenic) 390 as a FN, and (3) where no known causal variants were present for a patient, one rep 391 resentative variant from each distinct ClinVar classification was selected and marked 392 as unsequenced to emulate a range of putative FNs. The selected variants were:

14

393 *TNFAIP3* p.Cys243Arg (pathogenic), p.Tyr246Ter (likely pathogenic), p.His646Pro 394 (conflicting interpretations of pathogenicity), p.Thr635Ile (uncertain significance), 395 p.Arg162Trp (not provided), p.Arg280Trp (likely benign), p.Ile207Leu (benign/- 396 likely benign), and p.Lys304Glu (benign). All subsequent steps were identical.

397 **2.4 Validation of autosomal dominant estimates using *NFKB1***

398 To validate our genome-wide probability estimates in an AD gene, we focused on 399 *NFKB1*. Our goal was to compare the expected number of *NFKB1*-related Common 400 Variable Immunodeficiency (CVID) cases, as predicted by our framework, with the 401 reported case count in a well-characterised national-scale PID cohort.

402 **1. Reference dataset.** We used a reference dataset reported by Tuijnenburg et al. 403 (19) to build a validation model in an AD disease gene. This study performed whole 404 genome sequencing of 846 predominantly sporadic, unrelated PID cases from the 405 NIHR BioResource-Rare Diseases cohort. There were 390 CVID cases in the cohort. 406 The study identified *NFKB1* as one of the genes most strongly associated with PID. 407 Sixteen novel heterozygous variants including truncating, missense, and gene deletion 408 variants, were found in *NFKB1* among the CVID cases.

409 **2. Cohort prevalence calculation.** Within the cohort, 16 out of 390 CVID cases 410 were attributable to *NFKB1*. Thus, the observed cohort prevalence was

Prevalencecohort =16

390*≈* 0*.*041*,*

411 with a 95% confidence interval (using Wilson’s method) of approximately (0*.*0254*,* 0*.*0656).

412 **3. National estimate based on literature.** Based on literature (19; 20; 22), the 413 prevalence of CVID in the general population was estimated as

PrevalenceCVID =1

25 000*.*

414 For a UK population of *N*UK *≈* 69 433 632*,* the expected total number of CVID 415 cases was

*E*CVID *≈*69 433 632

416 ~~25 000~~ *≈* 2777*.*

417 Assuming that the proportion of CVID cases attributable to *NFKB1* is equivalent to 418 the cohort estimate, the literature extrapolated estimate is Estimated NFKB1 cases *≈* 419 2777 *×* 0*.*041 *≈* 114*,* with a median value of approximately 118 and a 95% confidence 420 interval of 70 to 181 cases (derived from posterior sampling).

15

421 **4. Bayesian adjustment.** Recognising that the sequenced cohort cases likely 422 captures the majority of *NFKB1*‑related patients (apart from close relatives), but may 423 still miss rare or geographically dispersed variants, we combined the cohort-based and 424 literature-based estimates using two complementary Bayesian approaches:

425 1. **Weighted adjustment (emphasising the cohort,** *w* = 0*.*9**):** We assigned 426 90% weight to the directly observed cohort count (16) and 10% to the extrapo 427 lated population estimate (114), thereby accounting, illustratively, for a small 428 fraction of unobserved cases while retaining confidence in our well‑characterised 429 cohort:

Adjusted Estimate = 0*.*9 *×* 16 + 0*.*1 *×* 114 *≈* 26*,*

430 yielding a 95% CrI of roughly 21 to 33 cases.

431 2. **Mixture adjustment (equal weighting,** *w* = 0*.*5**):** To reflect greater un 432 certainty about how representative the cohort is, we combined cohort and 433 population prevalences equally. We sampled from the posterior distribution of 434 the cohort prevalence,

*p ∼* Beta(16 + 1*,* 390 *−* 16 + 1)*,*

435 and mixed this with the literature-based rate at 50% each (19; 20; 22). This 436 yields a median estimate of 67 cases and a wider 95% CrI of approximately 437 43 to 99 cases, capturing uncertainty in both under-ascertainment and over 438 generalisation.

439 **5. Predicted total genotype counts.** The predicted total synthetic genotype 440 count (before adjustment) was 456, whereas the predicted total genotypes adjusted 441 for synth\_flag was 0. This higher synthetic count was set based on a minimal risk 442 threshold, ensuring that at least one genotype is assumed to exist (e.g. accounting for 443 a potential unknown de novo variant) even when no variant is observed in gnomAD 444 (as per **section 2.2**).

445 **6. Validation test.** Thus, the expected number of *NFKB1*-related CVID cases 446 derived from our genome-wide probability estimates was compared with the observed 447 counts from the UK-based PID cohort. This comparison validates our framework for 448 estimating disease incidence in AD disorders.

16

449 **2.5 Validation study for autosomal recessive CF using *CFTR***

450 To validate our framework for AR diseases, we focused on Cystic Fibrosis (CF). For 451 comparability sizes between the validation studies, we analysed the most common SNV 452 in the *CFTR* gene, typically reported as p.Arg117His (GRCh38 Chr 7:117530975 453 G/A, MANE Select HGVSp ENST00000003084.11: p.Arg117His). Our goal was to 454 validate our genome-wide probability estimates by comparing the expected number 455 of CF cases attributable to the p.Arg117His variant in *CFTR* with the nationally 456 reported case count in a well-characterised disease cohort (23–25).

**1. Expected genotype counts.** Let *p* denote the allele frequency of the p.Arg117His variant and *q* denote the combined frequency of all other pathogenic *CFTR* variants, such that

*q* = *P*tot *− p* with *P*tot =X *i∈*CFTR

*pi.*

Under Hardy–Weinberg equilibrium for an AR trait, the expected frequencies were: *f*hom = *p*2(homozygous for p.Arg117His)

and

*f*comphet = 2*p q* (compound heterozygotes carrying p.Arg117His and another pathogenic allele)*.* For a population of size *N* (here, *N ≈* 69 433 632), the expected number of cases were: *E*hom = *N · p*2*, E*comphet = *N ·* 2*p q, E*total = *E*hom + *E*comphet*.*

457 **2. Mortality adjustment.** Since CF patients experience increased mortality, we 458 adjusted the expected genotype counts using an exponential survival model (23–25). 459 With an annual mortality rate *λ ≈* 0*.*004 and a median age of 22 years, the survival 460 factor was computed as:

*S* = exp(*−λ ·* 22)*.*

461 Thus, the mortality-adjusted expected genotype count became:

*E*adj = *E*total *× S.*

17

462 **3. Bayesian uncertainty simulation.** To incorporate uncertainty in the allele 463 frequency *p*, we modelled *p* as a beta-distributed random variable:

*p ∼* Beta(*p ·* ANeff + 1*,* ANeff *− p ·* ANeff + 1)*,*

464 using a large effective allele count (ANeff) for illustration. By generating 10,000 465 posterior samples of *p*, we obtained a distribution of the literature-based adjusted expected counts, *E*adj 466 .

467 **4. Bayesian Mixture Adjustment.** Since the national registry may not capture 468 all nuances (e.g., reduced penetrance) of *CFTR*-related disease, we further combined 469 the literature-based estimate with the observed national count (714 cases from the 470 UK Cystic Fibrosis Registry 2023 Annual Data Report) using a 50:50 weighting:

*E*Bayes = 0*.*5 *×* (Observed Count) + 0*.*5 *× E*adj*.*

471 **5. Validation test.** Thus, the expected number of *CFTR*-related CF cases de 472 rived from our genome-wide probability estimates was compared with the observed 473 counts from the UK-based CF registry. This comparison validated our framework for 474 estimating disease incidence in AD disorders.

475 **2.6 Validation of SCID-specific estimates using PID–SCID** 476 **genes**

477 To validate our genome-wide probability estimates for diagnosing a genetic variant 478 in a patient with an PID phenotype, we focused on a subset of genes implicated in 479 SCID. Given that the overall panel corresponds to PID, but SCID represents a rarer 480 subset, the probabilities were converted to values per million PID cases.

481 **1. Incidence conversion.** Based on literature, PID occurs in approximately 1 in 482 1,000 births, whereas SCID occurs in approximately 1 in 100,000 births. Consequently, 483 in a population of 1,000,000 births there are about 1,000 PID cases and 10 SCID cases. 484 To express SCID-related variant counts on a per-million PID scale, the observed SCID 485 counts were multiplied by 100. For example, if a gene is expected to cause SCID in 10 486 cases within the total PID population, then on a per-million PID basis the count is 487 10 × 100 = 1,000 cases (across all relevant genes).

18

488 **2. Prevalence calculation and data adjustment.** For each SCID-associated 489 gene (e.g. *IL2RG*, *RAG1*, *DCLRE1C*), the observed variant counts in the dataset were 490 adjusted by multiplying by 100 so that the probabilities reflect the expected number 491 of cases per 1,000,000 PID. In this manner, our estimates are directly comparable to 492 known counts from SCID cohorts, rather than to national population counts as in 493 previous validation studies.

494 **3. Integration with prior probability estimates.** The predicted genotype 495 occurrence probabilities were derived from our framework across the PID gene panel. 496 These probabilities were then converted to expected case counts per million PID cases 497 by multiplying by 1,000,000. For instance, if the probability of observing a pathogenic variant in *IL2RG* is *p*, the expected SCID-related count becomes *p ×* 106

498 . Similar 499 conversions are applied for all relevant SCID genes.

500 **4. Bayesian Uncertainty and Comparison with Observed Data.** To address 501 uncertainty in the SCID-specific estimates, a Bayesian uncertainty simulation was 502 performed for each gene to generate a distribution of predicted case counts on a per 503 million PID scale. The resulting median estimates and 95% CrIs were then compared 504 against known national SCID counts compiled from independent registries. This 505 comparison permuted a direct evaluation of our framework’s accuracy in predicting 506 the occurrence of SCID-associated variants within a PID cohort.

507 **5. Validation Test.** Thus, by converting the overall probability estimates to a 508 per-million PID scale, our framework was directly validated against observed counts 509 for SCID.

510 **2.7 Protein network and genetic constraint interpretation**

511 A PPI network was constructed using protein interaction data from STRINGdb (17). 512 We previously prepared and reported on this dataset consisting of 19,566 proteins and 513 505,968 interactions (https://github.com/DylanLawless/ProteoMCLustR). Node 514 attributes were derived from log-transformed score-positive-total values, which in 515 formed both node size and colour. Top-scoring nodes (top 15 based on score) were 516 labelled to highlight prominent interactions. To evaluate group differences in score 517 positive-total across major disease categories, one-way Analysis of Variance (ANOVA) 518 was performed followed by Tukey Honestly Significant Difference (HSD) post hoc 519 tests (and non-parametric Dunn’s test for confirmation). GnomAD v4.1 constraint 520 metrics data was used for the PPI analysis and was sourced from Karczewski et al. 521 (8). This provided transcript-level metrics, such as observed/expected ratios, Loss-Of 522 function Observed/Expected Upper bound Fraction (LOEUF), Probability of being 523 Loss-of-function Intolerant (pLI), and Z-scores, quantifying Loss-of-Function (LOF) 524 and missense intolerance, along with confidence intervals and related annotations for 525 211,523 observations.

19

526 **2.8 Gene set enrichment test**

527 To test for overrepresentation of biological functions, the prioritised genes were 528 compared against gene sets from MsigDB (including hallmark, positional, curated, 529 motif, computational, GO, oncogenic, and immunologic signatures) and WikiPathways 530 using hypergeometric tests with FUMA (26; 27). The background set consisted of 531 24,304 genes. Multiple testing correction was applied per data source using the 532 Benjamini-Hochberg method, and gene sets with an adjusted P-value *≤*0.05 and more 533 than one overlapping gene are reported.

534 **2.9 Deriving novel PID classifications by genetic PPI and** 535 **clinical features**

536 We recategorised 315 immunophenotypic features from the original IUIS IEI annota 537 tions, reducing the original multi-level descriptors (e.g. “decreased CD8, normal or 538 decreased CD4”) first to minimal labels (e.g.“low”) and second to binary outcomes 539 (normal vs. not-normal) for T cells, B cells, neutrophils, and immunoglobulins Each 540 gene was mapped to its PPI cluster derived from STRINGdb and Uniform Manifold 541 Approximation and Projection (UMAP) embeddings from previous steps. We first 542 tested for non-random associations between these four binary immunophenotypes and PPI clusters using *χ*2

543 tests. To generate a data-driven PID classification, we 544 trained a decision tree (rpart) to predict PPI cluster membership from the four 545 immunophenotypic features plus the traditional IUIS Major and Subcategory la 546 bels. Hyperparameters (complexity parameter=0.001, minimum split=10, minimum 547 bucket=5, maximum depth=30) were optimised via five-fold cross validation using 548 the caret framework. Terminal node assignments were then relabelled according to 549 each group’s predominant abnormal feature profile.

550 **2.10 Probability of observing AlphaMissense pathogenicity**

551 We obtained the subset pathogenicity predictions from AlphaMissense via the Al 552 phaFold database and whole genome data from the studies data repository(13; 18). The 553 AlphaMissense data (genome-aligned and amino acid substitutions) were merged with 554 the panel variants based on genomic coordinate and HGVSc annotation. Occurrence 555 probabilities were log-transformed and adjusted (y-axis displaying log10(occurrence 556 prob + 1e-5) + 5)), to visualise the distribution of pathogenicity scores across the 557 residue sequence. A Kruskal-Wallis test was used to compare the observed disease 558 probability across clinical classification groups.

559 **2.11 Probability model definitions**

Estimating disease risk requires accounting for both variant penetrance, *P*(*D | G*), where *D* denotes the disease state and *G* the genotype, and the fraction of cases

20

attributable to a given variant, *P*(*G | D*). In a fully penetrant single-variant model (*P*(*D | G*) = 1), the lifetime risk *P*(*D*) equals the genotype frequency *P*(*G*). For an allele with population frequency *p*, this gives *P*(*D*) = *p*2for a recessive mode of inheritance and *P*(*D*) = 2*p*(1 *− p*) *≈* 2*p* for a dominant mode. With incomplete penetrance, *P*(*D*) = *P*(*G*) *P*(*D | G*), and when multiple variants contribute to disease,

*P*(*D*) = *P*(*G*) *P*(*D | G*)

*P*(*G | D*)*.*

560 Because both *P*(*D | G*) and *P*(*G | D*) are often uncertain, we integrate ClinVar clinical 561 classifications, population allele frequencies and curated gene–disease associations, 562 assuming James-Stein shrinkage to derive robust aggregate priors. By focusing on 563 *| D*) is the probability that disease *D*

a filtered set of variants *V* where each *P*(*Gi*

is attributable to variant *i* and assuming P*i∈V P*(*Gi*

564 *| D*) *≈* 1, we obtain calibrated 565 estimates of genotype frequency *P*(*G*) despite uncertainty in individual parameters.

21

566 **3 Results**

567 **3.1 Occurrence probability across disease genes**

568 Our study integrated large-scale annotation databases with gene panels from PanelAp 569 pRex to systematically assess disease genes by MOI (2). By combining population 570 allele frequencies with ClinVar clinical classifications, we computed an expected occur 571 rence probability for each SNV, representing the likelihood of encountering a variant 572 of a specific pathogenicity for a given phenotype. We report these probabilities for 573 54,814 ClinVar variant classifications across 557 genes (linked dataset (28)).

574 We focused on panels related to Primary Immunodeficiency or Monogenic In 575 flammatory Bowel Disease, using PanelAppRex panel ID 398. **Figure 1** displays all 576 reported ClinVar variant classifications for this panel. The resulting natural scaling 577 system (-5 to +5) accounts for the frequently encountered combinations of classification 578 labels (e.g. benign to pathogenic). The resulting dataset (28) is briefly shown in 579 **Table 1** to illustrate that our method yielded estimates of the probability of observing 580 a variant with a particular ClinVar classification.

Table 1: **Example of the first several rows from our main results for 557 genes of PanelAppRex’s panel: (ID 398) Primary immunodeficiency or monogenic inflammatory bowel disease**. “ClinVar Significance” indicates the pathogenicity classification assigned by ClinVar, while “Occurrence Prob” represents our calculated probability of observing the corresponding variant class for a given phenotype. MOI shows the gene-disease-specific mode of inheritance. Additional columns, such as population allele frequency, are not shown. (28)

Gene Panel ID ClinVar Clinical Significance GRCh38 Pos HGVSc HGVSp MOI Occurrence Prob ABI3 398 Uncertain significance 49210771 c.47G>A p.Arg16Gln AR 0.000000007 ABI3 398 Uncertain significance 49216678 c.265C>T p.Arg89Cys AR 0.000000005 ABI3 398 Uncertain significance 49217742 c.289G>A p.Val97Met AR 0.000000002 ABI3 398 Uncertain significance 49217781 c.328G>A p.Gly110Ser AR 0.000000002 ABI3 398 Uncertain significance 49217844 c.391C>T p.Pro131Ser AR 0.000000015 ABI3 398 Uncertain significance 49220257 c.733C>G p.Pro245Ala AR 0.000000022 ... ... ... ... ... ... ... ...

581 **3.2 Integrating observed true positives and unobserved false** 582 **negatives into a single, actionable conclusion**

583 Having established a probabilistic framework for estimating the prior probability 584 of observing disease-associated variants under different inheritance modes, we then 585 applied this model to an example patient to demonstrate its potential for clinical 586 genetics. The algorithm first verified that all known pathogenic positions have been 587 sequenced and observed as reference (true negatives), and identified any positions 588 that were either observed as variant (true positives) or not assessable due to missing

22

A

Classification

Pathogenic

Likely pathogenic

Pathogenic, low penetrance

likely pathogenic, low penetrance

Conflicting

classifications of pat...

risk factor

likely risk allele

association

uncertain risk allele

Uncertain

significance

other

not provided

no classifications from unflagged ... no classification for the single v...

drug response Affects

protective

Likely benign

Benign

B D

Gene name Gene name

(every 100th) (Top 15 pathogenic count)

C4A

FBRS

CDCA7 VPS45 NLRP3 BRCA2

ADA

AIRE

ERCC2 NBAS

RECQL4 G6PD

PMS2

RAG1

BRCA1 VPS13B NPC1

BRCA2 COL7A1 ATM

CFTR

C

0 250 500 750 1000 1250

C4A

FBRS CDCA7 VPS45 NLRP3 BRCA2

0% 25% 50% 75% 100%

Score

5.0

2.5

0.0

−2.5

−5.0

−5.0 −2.5 0.0 2.5 5.0

0 250 500 750 1000 1250

Score count

Figure 1: **Summary of ClinVar clinical significance classifications in the PID gene panel.** (A) Shows the numeric score coding for each classification (i.e. -5 benign to +5 pathogenic) as defined in methods Section 2.2. (B) Displays the stacked absolute count of classifications per gene. The same counts are shows as percentages per gene in (C). (D) For demonstration, the top 15 genes ranked by absolute count of pathogenic (score 5) variant classifications, indicating those most frequently occurring in the population as disease-causing.

589 sequence data of failed QC. These missing sites represent potential false negatives. By 590 jointly modelling the observed and unobserved space, the method yielded a calibrated, 591 evidence-weighted probability that at least one damaging causal variant could be 592 present within a gene.

593 **3.3 Scenario one - simple diagnosis**

594 We present the results from three scenarios for an example single-case patient being 595 investigated for the genetic diagnosis of IEI. **Figure S2** shows the results of the first 596 simple scenario, in which only one known pathogenic variant, *NFKB1* p.Ser237Ter, 597 was observed and all other previously reported pathogenic positions were successfully 598 sequenced and confirmed as reference. In this setting, the model assigned the full 599 posterior probability to the observed allele, yielding 100 % confidence that all present 600 evidence supported a single, true positive causal explanation. The most strongly 601 supported observed variant was p.Ser237Ter (posterior: 0.594). The strongest 602 (probability of observing) non-sequenced variant was a benign variant p.Thr567Ile 603 (posterior: 0). The total probability of a causal diagnosis given the available evidence

23

604 was 1 (95% CI: 1–1) (**Table S1**).

605 **3.4 Scenario two - complex diagnosis**

606 **Figure 2** shows the second more complex scenario, where the same pathogenic variant 607 *NFKB1* p.Ser237Ter was present, but coverage was incomplete at three additional 608 sites of known classified variants. Among these was the likely-pathogenic splice-site 609 variant *NFKB1* c.159+1G>A, which was not captured in the sequencing data. The 610 panels of **Figure 2 (A–F)** illustrate the stepwise integration of observed and missing 611 evidence, culminating in a posterior probability that reflects both confirmed findings 612 and residual uncertainty. **Table 2** demonstrates our main goal and lists the final 613 conclusion for reporting the clinical genetics results. **Table S2** lists the main metrics 614 used to reach the conclusion (as illustrated in **Figure 2**).

615 Bayesian integration of every annotated allele yielded the quantitative CrIs for 616 pathogenic attribution that (i) preserve Hardy-Weinberg expectations, (ii) accommo 617 date AD, AR, XL inheritance, and (iii) carry explicit uncertainty for non-sequenced 618 (or failed QC) genomic positions. **Figure 2 (A)** depicts the prior landscape where 619 occurrence probabilities are partitioned by observed or missing status and by causal 620 or non‑causal evidence, with colour reflecting the underlying ClinVar score. **Figure** 621 **2 (B)** shows posterior normalisation which concentrates probability density on two 622 high‑confidence (high evidence score) alleles since the benign variants are, by definition, 623 non-causal. **Figure 2 (C)** shows the resulting per‑variant probability of being simul 624 taneously damaging and causal; only the confirmed present (true positive) nonsense 625 variant p.Ser237Ter and the (false negative) hypothetical splice‑donor c.159+1G>A 626 retain substantial support. Restricting the view to causal candidates in **Figure 2** 627 **(D)** confirms that posterior mass is distributed across these two variants. **Figure 2** 628 **(E)** decomposes the total damaging probability into observed (approximately 40 %) 629 and missing (approximately 34 %) sources, whereas **Figure 2 (F)** summarises the 630 gene‑level posterior: inclusion of the splice‑site allele (scenario 2) produces a median 631 probability of 0.542 with a 95 % CrI of 0.264–0.8.

632 Numerically, the present variant p.Ser237Ter accounts for 0.399 of the posterior 633 share, and the potentially causal but missing splice‑donor allele c.159+1G>A contributes 634 0.339. The remaining alleles together explain a negligible share (**Table S2**). Thus, we 635 can report that in this patient’s scenario the probability of correct genetic diagnosis 636 due to *NFKB1* p.Ser237Ter is 0.542 (95 % CrI of 0.264–0.8) given that a likely 637 alternative remains to be confirmed for this patient. Upon confirmation that the 638 second variant is not present, the confidence will rise to 1 (95 % CrI of 1–1) as shown 639 in scenario one.

24

Table 2: Final variant report for clinical genetics scenario 2. Reported causal: p.Ser237Ter (posterior 0.377). Undetected causal: c.159+1G>A (posterior 0.364). The total probability of a causal diagnosis given the available evidence was 0.511 (95% CI: 0.237–0.774).

Parameter present missing

Gene NFKB1 NFKB1

HGVSc c.710C>G c.159+1G>A

HGVSp p.Ser237Ter .

Inheritance AD AD

Patient sex Male Male

gnomAD frequency 6.57e-06 6.57e-06

95% CI lower 0.003 NA

p(median) 0.090 NA

95% CI upper 0.551 NA

Posterior p(causal) 0.377 0.364

Interpretation Reported causal; variant observed Reported causal; variant not detected — consider follow‑up

**Summary** Overall probability of correct causal diagnosis due to SNV/INDEL given the currently available evidence:

0.511 (95% CI 0.237–0.774).

640 **3.5 Scenario three - currently impossible diagnosis**

641 **Figure S3** shows the third scenario, in which no observed variants were detected in the 642 proband for *NFKB1*. Instead, a broad range of plausible FN were detected as missing 643 for the gene *TNFAIP3*. The strongest (probability of observing and pathogenic) of 644 these non-sequenced variants was p.Cys243Arg (posterior: 0.347). However, the total 645 probability of a causal diagnosis for the patient *given the available evidence* was 0 646 (95% CI: 0–0) since these missing variants must be accounted for (**Table S3**). Upon 647 confirmation, these probabilities can update, as per scenario two.

25

Gene: NFKB1 A

p.Ser237Ter

p.Gly650Arg

p.Arg231His

c.159+1G>A

p.Val236Ile

p.Thr567Ile

5

0

0

4.5

0

−5

causal other causal other

present present missing missing

B

p.Ser237Ter

p.Gly650Arg p.Arg231His

c.159+1G>A

p.Val236Ile

p.Thr567Ile

causal other causal other

present present missing missing

C

p.Ser237Ter

p.Gly650Arg p.Arg231His

c.159+1G>A

p.Val236Ile

p.Thr567Ile

causal other causal other

present present missing missing

D

0.000 0.002

prior occurrence probability p(causal & damaging)

0.0 0.5 1.0

posterior share distribution (causal & damaging)

E

0.0 0.5 1.0 p(causal & damaging)

score

pathogenic

total

F

0.5

0.4

0.3

0.2

0.1

0.0

1000

c.159+1G>A p.Val236Ile p.Thr567Ile

49.1% 50.9% missing present

p.Ser237Ter

p.Gly650Arg p.Arg231His

p.Ser237Ter c.159+1G>A

present

causal

missing

causal

0.0 0.2 0.4 0.6 0.8 1.0 damaging−only

posterior p(causal & damaging)

unknown benign

count

median = 0.511

750

95% CI = [0.237, 0.774]

500

250

0

0.0 0.2 0.4 0.6 0.8 1.0 total p(causal & damaging & present)

Figure 2: **Quantification of present (TP) and missing (FN) causal genetic variants for disease in *NFKB1* (scenario 2).** The example proband carried three known heterozygous variants, including pathogenic p.Ser237Ter, and had incom plete coverage at three additional loci, including likely-pathogenic splice-site variant c.159+1G>A. The sequential steps towards the posterior probability of complete genetic diagnosis are shown: (A) Prior occurrence probabilities, stratified by observed/missing and causal/non-causal status. Pathogenicity scores (-5 to +5) are annotated. (B) Posterior distributions of normalised variant weights *π*˜*i*. (C) Per-variant posterior probability of being both damaging and causal. (D) Posterior distributions for causal variants only. (E) Decomposition of total pathogenic probability into observed (green) and missing (orange) sources. (F) Gene-level posterior showing the probability that at least one damaging causal allele is present; median 0.54, 95 % CrI 0.26-0.80. This result can be compared to scenarios one and three in **Figures S2** and **S3**, respectively.

26

648 **3.6 Validation studies**

649 **3.6.1 Validation of dominant disease occurrence with *NFKB1***

650 To validate our genome-wide probability estimates for AD disorders, we focused on 651 *NFKB1*. We used a reference dataset from Tuijnenburg et al. (19), in which whole 652 genome sequencing of 846 PID patients identified *NFKB1* as one of the genes most 653 strongly associated with the disease, with 16 *NFKB1*-related CVID cases attributed to 654 AD heterozygous variants. Our goal was to compare the predicted number of *NFKB1*- 655 related CVID cases with the reported count in this well-characterised national-scale 656 cohort.

657 Our model calculated 0 known pathogenic variant *NFKB1*-related CVID cases 658 in the UK with a minimal risk of 456 unknown de novo variants. In the reference 659 cohort, 16 *NFKB1* CVID cases were reported. We additionally wanted to account for 660 potential under-reporting in the reference study. We used an extrapolated national 661 CVID prevalence which yielded a median estimate of 118 cases (95% CI: 70–181), while 662 a Bayesian-adjusted mixture estimate produced a median of 67 cases (95% CI: 43–99). 663 **Figure S5 (A)** illustrates that our predicted values reflect these ranges and are closer 664 to the observed count. This case supports the validity of our integrated probability 665 estimation framework for AD disorders, and represents a challenging example where 666 pathogenic SNV are not reported in the reference population of gnomAD. Our min-max 667 values successfully contained the true reported values.

668 **3.6.2 Validation of recessive disease occurrence with *CFTR***

669 Our analysis predicted the number of CF cases attributable to carriage of the 670 p.Arg117His variant (either as homozygous or as compound heterozygous with 671 another pathogenic allele) in the UK. Based on HWE calculations and mortality 672 adjustments, we predicted approximately 648 cases arising from biallelic variants and 673 160 cases from homozygous variants, resulting in a total of 808 expected cases.

674 In contrast, the nationally reported number of CF cases was 714, as recorded 675 in the UK Cystic Fibrosis Registry 2023 Annual Data Report (23). To account for 676 factors such as reduced penetrance and the mortality-adjusted expected genotype, we 677 derived a Bayesian-adjusted estimate via posterior simulation. Our Bayesian approach 678 yielded a median estimate of 740 cases (95% Confidence Interval (CI): 696, 786) and 679 a mixture-based estimate of 727 cases (95% CI: 705, 750). **Figure S5 (B)** illustrates 680 the close concordance between the predicted values, the Bayesian-adjusted estimates, 681 and the national report supports the validity of our approach for estimating disease.

682 **Figure S4** shows the final values for these genes of interest in a given population 683 size and phenotype. It reveals that an allele frequency threshold of approximately 684 0.000007 is required to observe a single heterozygous carrier of a disease-causing 685 variant in the UK population for both genes. However, owing to the AR MOI pattern 686 of *CFTR*, this threshold translates into more than 100,000 heterozygous carriers,

27

687 compared to only 456 carriers for the AD gene *NFKB1*. Note that this allele frequency 688 threshold, being derived from the current reference population, represents a lower 689 bound that can become more precise as public datasets continue to grow. This marked 690 difference underscores the significant impact of MOI patterns on population carrier 691 frequencies and the observed disease prevalence.

692 **3.6.3 Interpretation of ClinVar variant occurrences**

693 **Figure S6** shows the two validation study PID genes, representing AR and dominant 694 MOI. **Figure S6 (A)** illustrates the overall probability of an affected birth by ClinVar 695 variant classification, whereas **Figure S6 (B)** depicts the total expected number of 696 cases per classification for an example population, here the UK, of approximately 69.4 697 million.

698 **3.6.4 Validation of SCID-specific disease occurrence**

699 Given that SCID is a subset of PID, our probability estimates reflect the likelihood of 700 observing a genetic variant as a diagnosis when the phenotype is PID. However, we 701 additionally tested our results against SCID cohorts in **Figure S8**. The summarised 702 raw cohort data for SCID-specific gene counts are summarised and compared across 703 countries in **Figure S7**. True counts for *IL2RG* and *DCLRE1C* from ten distinct 704 locations yielded 95% CI surrounding our predicted values. For *IL2RG*, the prediction 705 was low (approximately 1 case per 1,000,000 PID), as expected since loss-of-function 706 variants in this XL gene are highly deleterious and rarely observed in gnomAD. 707 In contrast, the predicted value for *RAG1* was substantially higher (553 cases per 708 1,000,000 PID) than the observed counts (ranging from 0 to 200). We attributed 709 this discrepancy to the lower penetrance and higher background frequency of *RAG1* 710 variants in recessive inheritance, whereby reference studies may underreport the true 711 national incidence. Overall, we report that agreement within an order of magnitude 712 was tolerable given the inherent uncertainties from reference studies arising from 713 variable penetrance and allele frequencies.

714 **3.7 Genetic constraint in high-impact protein networks**

715 We next examined genetic constraint in high-impact protein networks across the whole 716 IEI gene set of over 500 known disease-gene phenotypes (1). By integrating ClinVar 717 variant classification scores with PPI data, we quantified the pathogenic burden per 718 gene and assessed its relationship with network connectivity and genetic constraint 719 (8; 17).

28

720 **3.7.1 Score-positive-total within IEI PPI network**

721 The ClinVar classifications reported in **Figure 1** were scaled -5 to +5 based on their 722 pathogenicity. We were interested in positive (potentially damaging) but not negative 723 (benign) scoring variants, which are statistically incidental in this analysis. We tallied 724 gene-level positive scores to give the score-positive-total metric. **Figure S9 (A)** shows 725 the PPI network of disease-associated genes, where node size and colour encode the 726 score-positive-total (log-transformed). The top 15 genes with the highest total prior 727 probabilities of being observed with disease are labelled (as per **Figure 1**).

728 **3.7.2 Association analysis of score-positive-total across IEI categories**

729 We checked for any statistical enrichment in score-positive-totals, which represents the 730 expected observation of pathogenicity, between the IEI categories. One-way ANOVA 731 revealed an effect of major disease category on score-positive-total (*F*(8*,* 500) = 732 2*.*82*, p* = 0*.*0046), indicating that group means were not identical, which we observed 733 in **Figure S9 (B)**. However, despite some apparent differences in median scores across 734 categories (i.e. 9. Bone Marrow Failure (BMF)), the Tukey HSD post hoc comparisons 735 **Figure S9 (C)** showed that all pairwise differences had 95% CIs overlapping zero, 736 suggesting that individual group differences were not significant.

737 **3.7.3 UMAP embedding of the PPI network**

738 To address the density of the PPI network for the IEI gene panel, we applied UMAP 739 (**Figure 3**). Node sizes reflect interaction degree, a measure of evidence-supported 740 connectivity (17). We tested for a correlation between interaction degree and score 741 positive-total. In **Figure 3**, gene names with degrees above the 95th percentile are 742 labelled in blue, while the top 15 genes by score-positive-total are labelled in yellow 743 (as per **Figure 1**). Notably, genes with high pathogenic variant loads segregated from 744 highly connected nodes, suggesting that LOF in hub genes is selectively constrained, 745 whereas damaging variants in lower-degree genes yield more specific effects. This 746 observation was subsequently tested empirically.

29

4

MSH6

BRCA2

Cluster 1

2

POLR3A

MVK

BRCA1

ADA

PMS2ATM

AIRE

RECQL4

2 3 4

UMAP2

0

LYN

PTPRC

NBAS RAG1

NFKBIA CFTR

G6PD

TP53

CHUK

IKBKB

5

6

7

8

9

10 11 12

PIK3R1

CD40

NFKB1

MYD88

IKBKG

13

SYK RELA TLR4

−2

LCK

CD8A

IL10

Variant Score Positive Total Interaction

14 15 16 17

−4

STAT3

IFNG

STAT1

JAK1

Degree

−2 0 2 4

UMAP1

Figure 3: **UMAP embedding of the PPI network.** The plot projects the high-dimensional protein-protein interaction network into two dimensions, with nodes coloured by cluster and sized by interaction degree. Blue labels indicate hub genes (degree above the 95th percentile) and yellow labels mark the top 15 genes by score positive-total (damaging ClinVar classifications). The spatial segregation suggests that genes with high pathogenic variant loads are distinct from highly connected nodes.

747 **3.7.4 Hierarchical clustering of enrichment scores for major disease cate** 748 **gories**

749 **Figure S10** presents a heatmap of standardised residuals for major disease categories 750 across network clusters, as per **Figure 3**. A dendrogram clusters similar disease 751 categories, while the accompanying bar plot displays the maximum absolute standard 752 ised residual for each category. Notably, (8) Complement Deficiencies (CD) shows 753 the highest maximum enrichment, followed by (9) BMF. While all maximum values 754 exceed 2, the threshold for significance, this likely reflects the presence of protein 755 clusters with strong damaging variant scores rather than uniform significance across 756 all categories (i.e. genes from cluster 4 in 8 CD).

757 **3.7.5 PPI connectivity, LOEUF constraint and enriched network cluster** 758 **analysis**

759 Based on the preliminary insight from **Figure S10**, we evaluated the relationship 760 between network connectivity (PPI degree) and LOF constraint (LOEUF upper rank) 761 Karczewski et al. (8) using Spearman’s rank correlation. Overall, there was a weak 762 but significant negative correlation (*ρ* = *−*0*.*181, *p* = 0*.*00024) at the global scale,

30

763 indicating that highly connected genes tend to be more constrained. A supplementary 764 analysis (**Figure S11**) did not reveal distinct visual associations between network 765 clusters and constraint metrics, likely due to the high network density. However 766 once stratified by gene clusters, the natural biological scenario based on quantitative 767 PPI evidence (17), some groups showed strong correlations; for instance, cluster 2 768 (*ρ* = *−*0*.*375, *p* = 0*.*000994) and cluster 4 (*ρ* = *−*0*.*800, *p <* 0*.*000001), while others 769 did not. This indicated that shared mechanisms within pathway clusters may underpin 770 genetic constraints, particularity for LOF intolerance. We observe that the score 771 positive-total metric effectively summarises the aggregate pathogenic burden across 772 IEI genes, serving as a robust indicator of genetic constraint and highlighting those 773 with elevated disease relevance.

774 **Figure S12 (C, D)** shows the re-plotted PPI networks for clusters with significant 775 correlations between PPI degree and LOEUF upper rank. In these networks, node 776 size is scaled by a normalised variant score, while node colour reflects the variant score 777 according to a predefined palette.

778 **3.8 New insight from functional enrichment**

779 To interpret the functional relevance of our prioritised IEI gene sets with the highest 780 load of damaging variants (i.e. clusters 2 and 4 in **Figure S12**), we performed 781 functional enrichment analysis for known disease associations using MsigDB with 782 FUMA (i.e. GWAScatalog and Immunologic Signatures) (26). Composite enrichment 783 profiles (**Figure S13**) reveal that our enriched PPI clusters were associated with 784 distinct disease-related phenotypes, providing functional insights beyond traditional 785 IUIS IEI groupings (1). The gene expression profiles shown in **Figure S14** (GTEx v8 786 54 tissue types) offer the tissue-specific context for these associations. Together, these 787 results enable the annotation of IEI gene sets with established disease phenotypes, 788 supporting a data-driven classification of IEI.

789 Based on these independent sources of interpretation, we observed that genes from 790 cluster 2 were independently associated with specific inflammatory phenotypes, in 791 cluding ankylosing spondylitis, psoriasis, inflammatory bowel disease, and rheumatoid 792 arthritis, as well as quantitative immune traits such as lymphocyte and neutrophil 793 percentages and serum protein levels. In contrast, genes from cluster 4 were linked 794 to ocular and complement-related phenotypes, notably various forms of age-related 795 macular degeneration (e.g. geographic atrophy and choroidal neovascularisation) and 796 biomarkers of the complement system (e.g. C3, C4, and factor H-related proteins), 797 with additional associations to nephropathy and pulmonary function metrics.

31

798 **3.9 Genome-wide gene distribution and linkage disequilib** 799 **rium**

800 **Figure 4 (A)** shows a genome-wide karyoplot of all IEI panel genes across GRCh38, 801 with colour-coding based on MOI. Figures **(B)** and **(C)** display zoomed-in locus plots 802 for *NFKB1* and *CFTR*, respectively. In **Figure 4 (B)**, the probability of observing 803 variants with known classifications is high only for variants such as p.Ala475Gly, which 804 are considered benign in the AD *NFKB1* gene that is intolerant to LOF. In **Figure 4** 805 **(C)**, high probabilities of observing patients with pathogenic variants in *CFTR* are 806 evident, reproducing this well-established phenomenon. Furthermore, the analysis of Linkage Disequilibrium (LD) using R2

807 shows that high LD regions can be modelled 808 effectively, allowing independent variant signals to be distinguished.

A

B C

Figure 4: **Genome-wide IEI, variant occurrence probability and LD by R**2**.** (A) Genome-wide karyoplot of all IEI panel genes mapped to GRCh38, with colours indicating MOI. (B) Zoomed-in locus plot example for *NFKB1* showing variant occurrence probabilities; only benign variants such exhibit high probabilities in this AD gene intolerant to LOF. (C) Locus plot example for *CFTR* displaying high probabilities for pathogenic variants; due to the dense clustering of pathogenic variants, score filter >0 was applied. Top five variants are labelled per gene.

32

809 **3.10 Novel PID classifications derived from genetic PPI and** 810 **clinical features**

811 We recategorised 315 immunophenotypic features from the original IUIS IEI anno 812 tations, reducing detailed descriptions (e.g. “decreased CD8, normal or decreased 813 CD4”) to minimal labels (e.g. “low”) and then binarising them (normal vs. not-normal) 814 for T cells, B cells, Immunoglobulin (Ig) and neutrophils (**Figure S15**). These sim 815 plified profiles were mapped onto STRINGdb PPI clusters, revealing non‑random distributions (*χ* 8162 *<* 1*e*‑13; **Figure S16**), indicating that network context captures key 817 immunophenotypic variation.

818 We next compared four classifiers under 5‑fold cross‑validation to determine which 819 features predicted PPI clustering. As shown in **Figure S17**, the fully combined model 820 achieved the highest accuracy among the four: (i) phenotypes only (33 %) (i.e. T 821 cell, B cell, Ig, Neutrophil); (ii) phenotypes+IUIS major category (50 %) (e.g. CID. 822 See **Box 2.1** for more); (iii) IUIS major+subcategory only (59 %) (e.g. CID, T-B+ 823 SCID); and (iv) phenotypes+IUIS major+subcategory (61 %). This demonstrated that 824 incorporating both traditional IUIS IEI classifications and core immunophenotypic 825 markers into the PPI‑based framework yielded the most robust discrimination of PID 826 gene clusters. Variable importance analysis highlighted abnormality status for Ig and 827 T cells were among the top ten features in addition to the other IUIS major and sub 828 categories. Per‑class specificity remained uniform across the classes while sensitivity 829 dropped.

830 The PPI and immunophenotype model yielded 17 data‑driven PID groups, whereas 831 incorporating the full complement of IUIS categories expanded this to 33 groups. For 832 clarity, we only demonstrate the decision tree from the smaller 17‑group model in 833 **Figure 5**. Each terminal node is annotated by its predominant immunophenotypic 834 signature (for example, “group 65 with abnormal T cell and B cell features”), and 835 the full resulting gene counts per 33 class are plotted in **Figure 5**. Although, less 836 user-friendly, this data‑driven taxonomy both aligns with and refines traditional IUIS 837 IEI classifications to provide a scaffold for large‑scale computational analyses. Because 838 this framework is fully reproducible, alternative PPI embeddings that incorporate 839 additional molecular annotations can readily swapped to continue building on these 840 IEI classification schemes.

33

**Ignot\_normal = 1** 3

.11 .16 .27 .02 .16 .14 .05 .03 .02 .00 .03 .02 .00 .00 .00 .00 .00

13%

**Tcellnot\_normal = 0** 2

.13 .23 .11 .14 .05 .08 .10 .05 .03 .00 .05 .00 .02 .00 .00 .01 .01

63%

**= 0**

2

.13 .25 .07 .18 .02 .07 .12 .05 .03 .00 .05 .00 .02 .00 .00 .01 .01

50%

2

.13 .19 .15 .09 .04 .16 .09 .04 .02 .01 .03 .01 .03 .00 .01 .00 .00

100%

**Bcellnot\_normal = 0** 3

.12 .14 .39 .00 .02 .07 .08 .00 .00 .07 .02 .00 .08 .02 .00 .00 .00

12%

**= 1**

6

.13 .11 .22 .00 .04 .29 .06 .02 .01 .02 .01 .02 .04 .01 .02 .00 .00

37%

**= 1**

6

.13 .09 .14 .00 .05 .40 .05 .03 .01 .00 .01 .03 .03 .01 .03 .00 .00

25%

**Neutrophilnot\_normal = 0 Neutrophilnot\_normal = 0 = 1 = 1**

**Neutrophilnot\_normal = 0 Ignot\_normal = 0**

**= 1 = 1**

6

.19 .09 .16 .00 .09

.22 .06 .04 .01 .00

.01 .04 .03 .01 .04

.00 .00

Gene count

3

.13 .13 .30 .02 .19

.13 .02 .04 .02 .00

.04 .00 .00 .00 .00

.00 .00

11%

200

150

100

50

0

2

.00 .33 .11 .00 .00 .22 .22 .00 .00 .00 .00 .11 .00 .00 .00 .00 .00

2%

2

.14 .28 .06 .19 .01 .07 .13 .05 .03 .00 .02 .00 .01 .00 .00 .00 .01

45%

11

.04 .00 .08 .04 .08 .08 .00 .12 .04 .00 .32 .00 .12 .00 .00 .08 .00

5%

3

.13 .17 .42 .00 .02 .06 .10 .00 .00 .08 .02 .00 .00 .00 .00 .00 .00

10%

13

.09 .00 .27 .00 .00 .09 .00 .00 .00 .00 .00 .00 .45 .09 .00 .00 .00

2%

6

.06 .10 .12 .00 .00 .65 .04 .02 .00 .00 .00 .00 .02 .00 .00 .00 .00

11%

**Neutrophilnot\_normal = 0**

6

.21 .08 .15 .00 .10 .23 .06 .03 .00 .00 .02 .03 .03 .02 .05 .00 .00

13%

14%

**= 1**

3

.00 .14 .29 .00 .00

.14 .00 .14 .14 .00

.00 .14 .00 .00 .00

.00 .00

1%

Group 11 Tcell

Group 12 Tcell Neutrophil

Group 14 Tcell Bcell

Group 16 Tcell Bcell Ig

Group 17

Tcell Bcell Ig Neutrophil

Group 4 Ig

Group 5 Bcell Ig Neutrophil

Group 7 all normal

Group 8 Neutrophil

Gene count

40 30 20 10 0

Grp. 39 Tcell

PID classification: PPI ~ Phenotypes

Grp. 44 Tcell Bcell

Grp. 57 Neut

Grp. 65 Tcell Bcell

Grp. 12

Grp. 13

Grp. 14

Grp. 2

Grp. 20

Grp. 52 Tcell Neut

Grp. 6

Grp. 63 Neut

Grp. 8

Grp. 21

Grp. 27

Grp. 36 Ig

Grp. 53 Ig

Grp. 61

Grp. 62

Grp. 17 Tcell Ig

Grp. 25 Tcell Ig

Grp. 41 Tcell Ig

Grp. 55 Ig

Grp. 35 Bcell Ig

Grp. 30 Tcell Bcell Ig

Grp. 42 Tcell Ig

Grp. 47 Bcell Ig

Grp. 49 Tcell Bcell Ig

Grp. 50 Tcell Bcell Ig

Grp. 51 Tcell Bcell Ig

Grp. 56 Tcell Bcell Ig

Grp. 58 Tcell Bcell Ig

Grp. 64 Tcell Bcell Ig

PID classification model:

PPI ~ Phenotypes + IUIS IEI Major/sub categories

Figure 5: **Fine-tuned model for PID classification.** (Top) In each terminal node, the top block indicates the number of genes in the node; the middle block shows the fitted class probabilities (which sum to 1); and the bottom block displays the percentage of the total sample in that node. These metrics summarise the model’s assignment based on immunophenotypic and PPI features. (Middle) Bar plot presenting the distribution of novel PID classifications, where group labels denote the predominant abnormal clinical feature(s) (e.g. T cell, B cell, Ig, Neutrophil) characterising each group. (Bottom) The complete model including the traditional IUIS IEI categories.

34

841 **3.11 Probability of observing AlphaMissense pathogenicity**

842 AlphaMissense provides pathogenicity scores for all possible amino acid substitutions; 843 however, our results in **Figure 6** show that the most probable observations in patients 844 occur predominantly for benign or unknown variants. This finding places the likelihood 845 of disease-associated substitutions into perspective and offers a data-driven foundation 846 for future improvements in variant prediction. The values in **Figure 6 (A)** can 847 be directly compared to **Figure 1 (D)** to view the distribution of classifications. 848 A Kruskal-Wallis test was used to compare the observed disease probability across 849 clinical classification groups and no significant differences were detected. In general, 850 most variants in patients are classified as benign or unknown, indicating limited 851 discriminative power in the current classification, such that pathogenicity prediction 852 does not infer occurrence prediction (**Figure S18**). Inverse correlation likely depends 853 on factors like MOI and intolerance to LOF.

35

A

log10(probability)\*

adjusted

B

NFKB1

3

2

1

0

ACDEFGHIKLMNPQRSTVWY

Pathogenicity Pathogenic

Unknown

Alternative

A

amino acid

CFTR

250

500

Residue sequence number

Benign

750

B

log10(probability)\* Alternative

adjusted amino acid

4

3

2

1

0

ACDEFGHIKLMNPQRSTVWY

500

1000

Residue sequence number

Pathogenicity Pathogenic

Unknown

Benign

Figure 6: **(A) Probabilities of observing a patient with (B) AlphaMissense derived pathogenicity scores.** Although AlphaMissense provides scores for every possible amino acid substitution, the most frequently observed variants in patients tend to be classified as benign or of unknown significance. This juxtaposition contextualises the likelihood of disease-associated substitutions and underlines prospects for refining predictive models. \*Axis scaling for visibility near zero. Higher point indicates higher probability.

36

854 **3.12 Integration of variant probabilities into IEI genetics data**

855 We integrated the computed prior probabilities for observing variants in all known 856 genes associated with a given phenotype (1), across AD, AR, and XL MOI, into our 857 IEI genetics framework. These calculations, derived from gene panels in PanelAppRex, 858 have yielded novel insights for the IEI disease panel. The final result comprised of 859 machine- and human-readable datasets, including the table of variant classifications 860 and priors available via a the linked repository (28), and a user-friendly web interface 861 that incorporates these new metrics.

862 **Figure 7** shows the interface summarising integrated variant data. We include 863 pre-calculated summary statistics and clinical significance as numerical metrics. Key 864 quantiles (min, Q1, median, Q3, max) for each gene are rendered as sparkline box 865 plots, and dynamic URLs link table entries to external databases (e.g. ClinVar, 866 Online Mendelian Inheritance in Man (OMIM), AlphaFold) as per **Section 3.1**. The 867 prepared data are available for bioinformatic application (28) as per **Section 3.2**.



Figure 7: **Integration of variant probabilities into the IEI genetics frame work.** The interface summarises the condensed variant data, with pre-calculated summary statistics and dynamic links to external databases. This integration enables immediate access to detailed variant classifications and prior probabilities for each gene.

37

868 **4 Discussion**

869 Our study presents, to our knowledge, the first comprehensive framework for calculating 870 prior probabilities of observing disease-associated variants and the first to demonstrate 871 the method for an evidence-aware genetic diagnosis with CrI (9; 11). By integrating 872 large-scale genomic annotations, including population allele frequencies from gnomAD 873 (8), variant classifications from ClinVar (14), and functional annotations from resources 874 such as dbNSFP, with classical HWE-based calculations, we derived robust estimates 875 for 54,814 ClinVar variant classifications across 557 IEI genes implicated in PID and 876 monogenic inflammatory bowel disease (1; 2). Although our results focus on IEI, the 877 genome-wide framework also supports all inheritance patterns: AD and XL require a 878 single pathogenic allele, whereas AR demands homozygous or compound heterozygous 879 states. Classical HWE-based estimates thus furnish baseline occurrence probabilities 880 and serve as robust priors for Bayesian risk models, a practice underutilised until the 881 advent of large-scale databases (2; 8; 13; 14).

882 A major deficit in current clinical genetics is the prevailing focus on confirming 883 only the presence of TP variants. Our approach yielded three key results to overcome 884 this hurdle. We generated per-variant priors across all MOI. The patient’s results of 885 observed and unobserved variants were integrated into a single posterior probability 886 of carrying a damaging causal allele. As demonstrated in **Table S2** and **Figure 2**, 887 this key result delivers a clinically applicable, interpretable probability that combines 888 both detected and potentially unobserved variants. When whole-genome sequencing 889 analyses are not yet available, the score-positive-total metric can serve as an optional 890 decision aid, enabling manual, evidence-based ranking of candidate genes to prioritise 891 diagnoses in patients with overlapping phenotypes.

892 We acknowledge that our framework is currently focused (but not restricted) on 893 SNVs and does not incorporate numerous other complexities of genetic disease, such 894 as structural variants, de novo variants, hypomorphic alleles, overdominance, variable 895 penetrance, tissue-specific expression, the Wahlund effect, pleiotropy, and others (7). 896 In certain applications, more refined estimates would benefit from including factors 897 such as embryonic lethality, condition-specific penetrance, and age of onset (11). Our 898 analysis also relies on simplifying assumptions of random mating, an effectively infinite 899 population, and the absence of migration, novel mutations, or natural selection. We 900 demonstrated the genome-wide gene distribution and MOI for the IEI panel relative 901 to LD showing that it is an important consideration and is feasible. However, LD is 902 a challenging feature that requires accurate implementation which depends on the 903 whole genome population-based pairwise genotype matrices for the given population. 904 We used the reference global population AFs, which is more generalisable but less 905 accurate than population-specific AF values.

906 In the example single-case diagnosis scenarios, our approach enabled high-confidence 907 attribution to a known pathogenic variant while also capturing the potential impact 908 of a likely-pathogenic splice-site allele that was missed by sequencing. Scenario two 909 showed a common diagnostic challenge where a strong candidate exists alongside an

38

910 unconfirmed but plausible alternative. Our method distributes confidence across both 911 possibilities. Conventional approaches focus only on detecting TP and cannot provide 912 this insight. By quantifying residual uncertainty, we can generate structured reports 913 that clearly distinguish supported, excluded, and plausible-but-unseen variants. We 914 call this “evidence-aware” interpretation. When combined with genome-wide priors 915 from the full range of disease-gene panels, this approach applies to any phenotype 916 from PanelAppRex. By combining variant classification, allele frequency, MOI, and se 917 quencing quality metrics, our method creates a scalable foundation for evidence-aware 918 diagnostics in clinical genomics.

919 Estimating disease risk in genetic studies is complicated by uncertainties in key 920 parameters such as variant penetrance and the fraction of cases attributable to specific 921 variants (7). In the simplest model, where a single, fully penetrant variant causes 922 disease, the lifetime risk *P*(*D*) is equivalent to the genotype frequency *P*(*G*). For an 923 allele with frequency *p* (ignoring LD for AR), this translates to:

Autosomal Recessive: *P*(*D*) = *p*2*,*

Autosomal Dominant: *P*(*D*) = 2*p*(1 *− p*) *≈* 2*p.*

924 When penetrance is incomplete, defined as *P*(*D | G*), the risk becomes: *P*(*D*) = 925 *P*(*G*) *P*(*D | G*)*.* In more realistic scenarios where multiple variants contribute to 926 disease, *P*(*G | D*) denotes the fraction of cases attributable to a given variant. This 927 leads to:

*P*(*D*) = *P*(*G*) *P*(*D | G*)

*P*(*G | D*)*.*

928 Because both penetrance and *P*(*G | D*) are often uncertain, solving this equation 929 systematically poses a major challenge, which we incidentally tackled in the validation 930 studies (29; 30).

931 Our framework addresses this challenge by combining variant classifications, popu 932 lation allele frequencies, and curated gene-disease associations. While imperfect on 933 an individual level, these sources exhibit predictable aggregate behaviour, supported 934 by James-Stein estimation principles (31). Curated gene-disease associations help 935 identify genes that are explainable for most disease cases, allowing us to approximate 936 *P*(*G | D*) close to one. In this way, we obtain robust estimates of *P*(*G*) (the frequency 937 of disease-associated genotypes), even when exact values of penetrance and case 938 attribution remain uncertain.

939 This approach allows us to pre-calculate priors and summarise the overall pathogenic 940 burden. By focusing on a subset *V* of variants that pass stringent filtering, where each 941 *| D*) is the probability that a case of disease *D* is attributable to variant(s) *i*,

*P*(*Gi*

942 we assume that, in aggregate,

39

X *i∈V*

*P*(*Gi| D*) *≈* 1*.*

943 Even if the cumulative contribution is slightly less than one, the resultant risk 944 estimates remain robust within the broad CrIs typical of epidemiological studies. By 945 incorporating these pre-calculated priors into a Bayesian framework, our method refines 946 risk estimates and enhances clinical decision-making despite inherent uncertainties.

947 For the IEI-specific investigation, we showed that immunophenotypic and network 948 derived features can be used to train and test models that predict PPIs. From this, we 949 derived a new, simplified classification of immune features for IEI genes. We have listed 950 the new immunophenotypic categories (e.g. T cell low) in the user database, however 951 we have not included the detailed cluster assignments (e.g. PPI groups) because 952 they are too complex for direct interpretation manually. Instead, our demonstration 953 provides worked examples that bioinformaticians can use to perform more refined 954 clustering in larger studies.

955 Moreover, because variant sets can be collapsed instead of relying on the gene 956 level, our method complements existing statistical approaches for aggregating variant 957 effects with methods like Sequence Kernel Association Test (SKAT) and Aggregated 958 Cauchy Association Test (ACAT) (32–35) and multi-omics integration techniques 959 (36; 37). It also remains consistent with established variant interpretation guidelines 960 from the American College of Medical Genetics and Genomics (ACMG) (38) and 961 complementary frameworks (39; 40), as well as QC protocols (41; 42). Standardised 962 reporting for qualifying variant sets, such as ACMG Secondary Findings v3.2 (43), 963 further contextualises the integration of these probabilities into clinical decision 964 making.

965 We compared our occurrence probabilities with AlphaMissense pathogenicity scores 966 and observed that common variants are predominantly scored as benign or of uncertain 967 significance. While this aligns with their allele frequencies, any pathogenic variant 968 seen in a patient warrants evaluation against its prior observation probability to assess 969 causality. Predictive tools such as AlphaMissense could ostensibly enhance their 970 embedding of variant features by incorporating gene-disease associations and MOI 971 data, which may not be fully represented by raw population allele frequencies.

972 Future work should incorporate the additional variant types and models to further 973 refine these probability estimates. By continuously updating classical estimates with 974 emerging data and prior knowledge, we aim to enhance the precision of genetic 975 diagnostics and ultimately improve patient care.

40

976 **5 Conclusion**

977 Our work generates prior probabilities for observing any variant classification in 978 IEI genetic disease, providing a quantitative resource to enhance Bayesian variant 979 interpretation and clinical decision-making.

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1002 **Contributions**

1003 DL performed main analysis and wrote the manuscript. SB, AS, MS, and JT designed 1004 analysis and wrote the manuscript. JF, LJS supervised the work, and applied for 1005 funding. The Quant Group members on this project were DL, SB, AS, and MS.

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1006 **Competing interest**

1007 We declare no competing interest.

1008 **References**

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1236 **6 Supplemental**

1237 Supplemental data are presented under the same headings that correspond to their 1238 relevant main text sections.

1239 **6.1 Variant class occurrence probability**

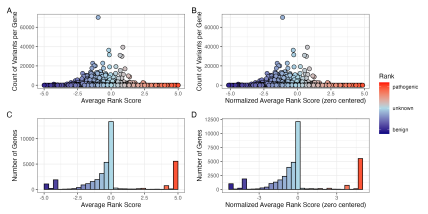
****

Figure S1: **Global distribution of ClinVar clinical-significance classification scoring.** (A) Number of variants per gene containing the assigned score for each ClinVar classification term (−5 to +5). (B) The same data after normalisation by zero centring the average rank score. (C) The tally of genes for their average rank and (D) after normalisation. No normalisation was required for the scoring system as shown by comparison of A-C and B-D.

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1240 **6.2 Integrating observed true positives and unobserved false** 1241 **negatives into a single, actionable conclusion**

Table S1: Result of clinical genetics diagnosis scenario 1 including metadata. The most strongly supported observed variant was p.Ser237Ter (posterior: 0.594). The strongest unsequenced variant was p.Thr567Ile (posterior: 0). The total probability of a causal diagnosis given the available evidence was 1 (95% CI: 1–1).

Variant ~~Flag Class Evi~~ dence

Score

Occur rence Prob

~~Adj Occ~~ Prob

Alpha ~~Beta Lower Median Upper Poste~~ rior

Share

Prob Causal

p.Ser237Ter ~~present causal 5 0.000 0 6 371 0.004 0.142 0.803 0.594 0.594~~ p.Thr567Ile missing other -5 0.002 0 1 363 NA NA NA 0.000 0.000 p.Arg231His present other 0 0.000 0 1 361 0.004 0.142 0.803 0.000 0.000 p.Gly650Arg present other 0 0.000 0 1 379 0.004 0.142 0.803 0.000 0.000 p.Val236Ile missing other 0 0.000 0 1 351 NA NA NA 0.000 0.000 Total NA NA NA NA NA NA NA 1.000 1.000 1.000 NA 1.000

Table S2: Result of clinical genetics diagnosis scenario 2 including metadata. The most strongly supported observed variant was p.Ser237Ter (posterior: 0.381). The strongest unsequenced variant was c.159+1G>A (posterior: 0.353). The total probabil ity of a causal diagnosis given the available evidence was 0.52 (95% CI: 0.248–0.787).

Variant ~~Flag Class Evi~~ dence

Score

Occur rence Prob

~~Adj Occ~~ Prob

Alpha ~~Beta Lower Median Upper Poste~~ rior

Share

Prob Causal

p.Ser237Ter ~~present causal 5.0 0.000 0 6.0 371 0.003 0.096 0.557 0.381 0.381~~ c.159+1G>A missing causal 4.5 0.000 0 5.5 367 NA NA NA 0.353 0.353 p.Thr567Ile missing other -5.0 0.002 0 1.0 365 NA NA NA 0.000 0.000 p.Arg231His present other 0.0 0.000 0 1.0 359 0.003 0.096 0.557 0.000 0.000 p.Gly650Arg present other 0.0 0.000 0 1.0 349 0.003 0.096 0.557 0.000 0.000 p.Val236Ile missing other 0.0 0.000 0 1.0 363 NA NA NA 0.000 0.000

Total NA NA NA NA NA NA NA 0.248 0.520 0.787 NA 0.520

Table S3: Result of clinical genetics diagnosis scenario 3 including metadata. No observed variants were detected in this scenario. The strongest unsequenced variant was p.Cys243Arg (posterior: 0.366). The total probability of a causal diagnosis given the available evidence was 0 (95% CI: 0–0).

Variant ~~Flag Class Evi~~ dence

Score

Occur rence Prob

~~Adj Occ~~ Prob

Alpha ~~Beta Lower Median Upper Poste~~ rior

Share

Prob Causal

p.Cys243Arg ~~missing causal 5.0 0.000 0.000 6 341 NA NA NA 0.366 0.366~~ p.Tyr246Ter missing causal 4.0 0.000 0.000 5 369 NA NA NA 0.284 0.284 p.Lys304Glu missing other -5.0 0.000 0.000 1 353 NA NA NA 0.000 0.000 p.Ile207Leu missing other -4.5 0.000 0.000 1 359 NA NA NA 0.000 0.000 p.His646Pro missing other 0.0 0.002 0.001 1 377 NA NA NA 0.000 0.000 p.Arg280Trp missing other -4.0 0.000 0.000 1 357 NA NA NA 0.000 0.000

p.Thr635Ile missing other 0.0 0.000 0.000 1 349 NA NA NA 0.000 0.000 p.Arg162Trp missing other 0.0 0.000 0.000 1 369 NA NA NA 0.000 0.000 Total NA NA NA NA NA NA NA 0 0 0 NA 0.000

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Gene: NFKB1 A

p.Ser237Ter

p.Gly650Arg

p.Arg231His

p.Val236Ile

p.Thr567Ile

5

0

0

0

−5

causal other

other

present present

missing

B

p.Ser237Ter

p.Gly650Arg p.Arg231His

p.Val236Ile p.Thr567Ile

causal other

other

present present missing

C

p.Ser237Ter

p.Gly650Arg p.Arg231His

p.Val236Ile

p.Thr567Ile

causal other

other

present present

missing

D

0.000 0.002

prior occurrence probability p(causal & damaging)

0.0 0.5 1.0

posterior share distribution (causal & damaging)

E

0.0 0.5 1.0 p(causal & damaging)

score

pathogenic

total

0.8 0.6 0.4 0.2 0.0

p.Val236Ile p.Thr567Ile

0.0%

100.0%

p.Ser237Ter

p.Gly650Arg p.Arg231His

p.Ser237Ter

causal

present

unknown benign

F

count

missing present

15000

10000

5000

0.0 0.2 0.4 0.6 0.8 1.0

damaging−only

posterior p(causal & damaging)

median = 1.000

95% CI = [1.000, 1.000]

0

0.0 0.2 0.4 0.6 0.8 1.0 total p(causal & damaging & present)

Figure S2: **Quantification of present (TP) and no missing (FN) causal ge netic variants for disease in *NFKB1* (scenario 1)**. Only one known pathogenic variant, p.Ser237Ter, was observed and all previously reported pathogenic positions were successfully sequenced and confirmed as reference (true negatives). Panels (A–F) follow the same structure as scenario 2 described in **Figure 2**, culminating in a gene-level posterior probability of 1 (95 % CrI: 0.99–1.00), with full support assigned to the observed allele given the available evidence. Pathogenicity scores (-5 to +5) are annotated.

50

Gene: TNFAIP3

A

missing

causal

p.Cys243Arg

5

B

causal

p.Cys243Arg

causal

C

missing

p.Cys243Arg

missing

p.Tyr246Ter

p.Thr635Ile p.His646Pro p.Arg162Trp p.Arg280Trp

p.Ile207Leu p.Lys304Glu

4

0

0

−4

−4.5

−5

0

other

missing

p.Tyr246Ter

p.Thr635Ile p.His646Pro p.Arg162Trp p.Arg280Trp

p.Ile207Leu p.Lys304Glu

other

missing

p.Tyr246Ter

p.Thr635Ile p.His646Pro p.Arg162Trp p.Arg280Trp

p.Ile207Leu p.Lys304Glu

other

missing

0.000 0.002

prior occurrence probability

0.0 0.5 1.0

posterior share distribution (causal & damaging)

0.0 0.5 1.0 p(causal & damaging)

score

pathogenic

p(causal & damaging)

D

1.00

0.75

total

causal

p.Cys243Arg p.Tyr246Ter p.Arg162Trp

E

100.0%

missing

p.Cys243Arg

unknown

0.50

0.25

0.00

F

p.Thr635Ile

p.His646Pro

p.Arg280Trp

p.Ile207Leu

p.Lys304Glu

missing

p.Tyr246Ter

0.0 0.2 0.4 0.6 0.8 1.0 damaging−only

posterior p(causal & damaging)

benign

count

15000 10000 5000 0

median = 0.000

95% CI = [0.000, 0.000]

0.0 0.2 0.4 0.6 0.8 1.0 total p(causal & damaging & present)

Figure S3: **Quantification of no present (TP) in *NFKB1* and only missing (FN) causal genetic variants for disease in *TNFAIP3* (scenario 3)**. No known causal variants were observed in *NFKB1*, but one representative unsequenced allele was selected from each distinct ClinVar classification and treated as a potential false negative. Panels (A–F) follow the same structure as scenario 2 described in **Figure 2**. The posterior reflects uncertainty across multiple plausible but unobserved variants, resulting in low CrI (0-0) and 100% missing overall attribution in contrast to scenarios where known pathogenic variants were observed. For this patient, we have no evidence of a causal variant since the only top candidates are not yet accounted for. Pathogenicity scores (-5 to +5) are annotated in (A).

51

1242 **6.3 Validation studies**

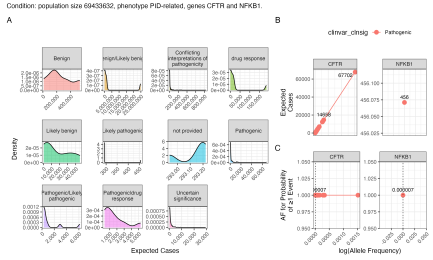
****

Figure S4: **Interpretation of probability of observing a variant classification.** The result from the chosen validation genes *CFTR* and *NFKB1* are shown. Case counts are dependant on the population size and phenotype. (A) The density plots of expected observations by ClinVar clinical significance. We then highlight the values for pathogenic variants specifically showing; (B) the allele frequency versus expected cases in this population size and (C) the probability of observing at least one event in this population size.

52

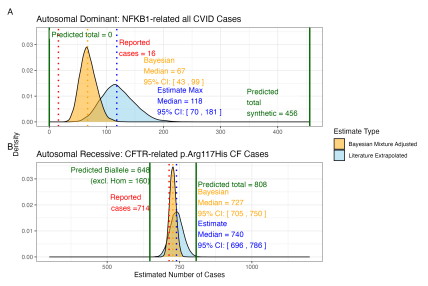


Figure S5: **Prior probabilities compared to validation disease cohort metrics.** (A) Density distributions for the number of *NFKB1*-related CVID cases in the UK. Our model (green) predicted 456 cases, which falls between the observed cohort count (red) of 390 and the upper extrapolated values. The blue curve represents maximum count of 1280, and the orange curve shows the Bayesian-adjusted mixture estimate of 835. (B) Density distributions for *CFTR*-related p.Arg117His CF cases. Our model (green) predicted 648 biallelic cases and 808 total cases. The nationally reported case count (red) was 714. The blue curve represents maximum extrapolated count of 740, and the orange curve shows the Bayesian-adjusted mixture estimate of 727. We observed close agreement among the reported disease cases and our integrated probability estimation framework.

53

1243 **6.3.1 Interpretation of ClinVar variant observations**

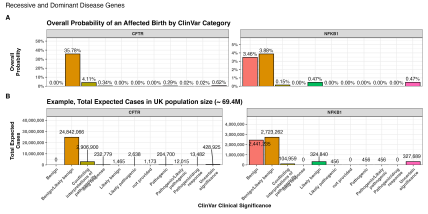
****

Figure S6: **Combined bar charts summarising the genome-wide analysis of ClinVar clinical significance for the PID gene panel**. Panel (A) shows the overall probability of an affected birth by variant classification, and (B) displays the total expected number of cases per classification, both stratified by gene. These integrated results illustrate the variability in variant observations across genes and underpin our validation of the probability estimation framework.

1244 **6.3.2 Validation of SCID-specific disease occurrence**

54

11

Prevalence (Count / Population)DCLRE1C

1e−05

4

5e−06 0e+00

0

1 1

1

6

2

1

Israel

3

0

1

0 0

1

0 0 0 0 0

3

1

0

14

8

0

0 0

1

2

IL2RG RAG1

Germany

Netherlands

Taiwan

US\_NewYork

France

Japan

Spain\_Catalonia Country

US\_California

US\_Wisconsin

Figure S7: **SCID-specific gene comparison across regions.** The bar plot shows the prevalence of SCID-related cases (count divided by population) for each gene and country (or region), with numbers printed above the bars representing the actual counts in the original cohort (ranging from 0 to 11 per region and gene).

55

A

1.00

IL2RG − related SCID

normal median: 337.75

B C

Predicted

0.75

total: 0.04

0.50

Median: 0.1

Predicted including

0.25

LP: 0.24

0.00

−500 0 500 1000 1500

RAG1 − related SCID

normal median: 108.23

1.00

Predicted

0.75

total: 552.92

0.50

Median: 712.03

Predicted including

0.25

LP: 997.2

0.00

0 500 1000

DCLRE1C − related SCID

normal median: 38.42

1.00

Predicted

0.75

total: 63.46

0.50

Median: 69.98

Predicted including

0.25

LP: 78.09

0.00

−200 0 200 400

Cases SCID per 1,000,000 PID

Legend

France

Germany

Israel

Japan

Netherlands

Spain\_Catalonia

Taiwan

US\_California

US\_NewYork

US\_Wisconsin

Predicted median Predicted incl LP

Figure S8: **Combined SCID-specific Predictions and Observed Rates per 1,000,000 PID.** The figure presents density distributions for the predicted SCID case counts (per 1,000,000 PID) for three genes: *IL2RG*, *RAG1*, and *DCLRE1C*. Country-specific rates (displayed as dotted vertical lines) are overlaid with the overall predicted distributions for pathogenic and likely pathogenic variants (solid lines with annotated medians). For *IL2RG*, the low predicted value is consistent with the high deleteriousness of loss-of-function variants in this X-linked gene, while *RAG1* exhibits considerably higher predicted counts, reflecting its lower penetrance in an autosomal recessive context.

56

1245 **6.4 Genetic constraint in high-impact protein networks** 1246 **6.4.1 Score-positive-total within IEI PPI network**

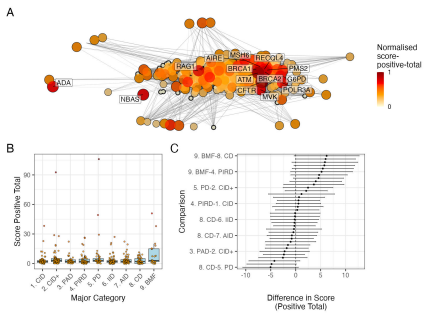
****

Figure S9: **PPI network and score-positive-total ClinVar significance vari ants**. (A) PPI network of disease-associated genes. Node size and colour represent the log-transformed score-positive-total, the top 15 genes/proteins with the highest probability of being observed in disease are labelled. (B) Distribution of score-positive total across the major IEI disease categories. (C) Tukey HSD comparisons of mean differences in score-positive-total among all pairwise disease categories. Every 5th label is shown on y-axis.

57

1247 **6.4.2 Hierarchical Clustering of Enrichment Scores for Major Disease Cat** 1248 **egories**

A B

4627811161215191713101435

−1.2 −2.6 −0.4

1.1

−0.6 1.5

−1.3 −2.7 −0.4

1.3

−1.8 −0.4

−1.9 −3.2 −0.6

−1.6 0

−0.5

−0.2 2.7

−0.5

0.2 3.9 1.4

4

3.5

−0.4

C

Maximum

| Std Residual |

20 15

ClusterStd

−0.6 −1

−0.4 −0.8 −2.2 −0.5 −0.6 −0.4 −1.1 −1.2 −1.9 −2.8 −2.6 20.7

−0.8 −1.4 −0.6 −1.1 1.5

4.4

5.1

−0.6 −0.8 −1.1 −1.7 −1.8 3.4

−2.4

−0.6 −1

−0.4 −0.8 −2.3 −0.5 −0.6 −0.4 −0.2 −1.3 −0.7 −2.9 12.4 −1.8

−0.6 0

−0.4 0.5

0.8

−0.5 −0.6 4.8

5.5

6.4

−1.4 −3

−1.4 −1.1

−0.9 −1.5 3.3

3.7

3.3

−0.7 −0.9 −0.6 −0.3 −0.6 4

3.5

−3.7 −2.6

−0.7 −0.4 −0.5 −1

−2

−0.6 −0.7 −0.5 −1.4 −0.9 3.8

4.9

−1.4 −2.2

−0.8 7.4

−0.5 −1.1 2.1

−0.7 −0.8 −0.5 0

−1

−1.6 0.2

−3.2 −2.3

4.8

−1.5 −0.6 −1.2 −0.4 −0.7 −0.8 −0.6 −1.6 0.1

−0.4 0

−0.6 −2.5

−0.6 −1

−0.4 1.8

−2.4 −0.5 −0.6 −0.4 0.7

0.4

−1.4 0.2

−1.4 −1.8

Residual 20

051015

10 5

0

2. CID+

9. BMF

7. AID

6. IID

3. PAD

2. CID+

9. BMF

7. AID

6. IID

3. PAD

8. CD

5. PD

4. PIRD

1. CID

8. CD

5. PD

4. PIRD

1. CID

Major Category

Major Category

Figure S10: **Hierarchical clustering of enrichment scores.** The heatmap displays standardised residuals for major disease categories (x-axis) across network clusters (y-axis). A dendrogram groups similar disease categories, and the bar plot shows the maximum absolute residual per category. (8) CD and (9)BMF show the highest vales, indicating significant enrichment or depletion (residuals > |2|). Definitions in **Box 2.1**.

58

1249 **6.4.3 PPI connectivity, LOEUF constraint and enriched network cluster** 1250 **analysis**

4

PPI Degree

UMAP2

2

0

−2

−4

−2 0 2 4 UMAP1

20

40

60

LoF OE CI upper rank

15000

10000

5000

Figure S11: **Analysis of PPI degree versus LOEUF upper rank with UMAP embedding of the PPI network.** The relationship between PPI degree (size) and LOEUF upper rank (color) across gene clusters. No clear patterns are evident.

59

A

15000

Upper Rank

LOEUF

10000

5000

1

6 78

B

Spearman's

rho = −0.181

p−value = 0.00024

−log(p−value)

6 4 2

2

3

4

5

9 10

12

11

13

15

0

C

0 20 40 60 80 PPI Degree

D

Cluster 2

0

Cluster 4

0.25 0.50 0.75 Absolute Spearman's rho

TNFSF12, IRAK1, TIRAP, IL1R1, TNFSF13, TRAF3, MAP3K14, NFKBIA, TICAM1, CASP10, TNFRSF13C, REL, TLR3, RNF31, FADD, CHUK, BCL10, TLR8, CD40, TLR7, RELB, RIPK1, UNC93B1, IL17RC, ADAM17, IL17RA, NFKB2, XIAP, NLRP12, TNFSF11, MYD88, IKBKB, IL36RN, NLRC4, TNFAIP3, RHBDF2, CARD14, NLRP1, TNFRSF1A, NFKB1, IFIH1, MALT1, NLRP3, RBCK1, TRAF3IP2, RELA, IRAK4, TNFRSF11A, IKBKG, OTULIN, CARD11, AK2, TBK1, CASP8, NOD2, CARD9, ACP5, MEFV, TNFRSF13B, MVK, RIPK3, LY96, TLR4, MAPK8, NFATC2, SHARPIN, PMVK, NFATC1, IKBKE, DPP9

C8G, FCN3, CFHR5, CFHR3, C4A, CFHR4, CD59, CFB, CFHR1, C1S, C4B, CFP, C1QB, C1QA, CFI, MASP2, C1R, SERPING1, C1QC, CFD, THBD, C3, C2, C6, ELANE, C5, CD46, CD55, C7, CFH, C8A, C8B, C9

Figure S12: **Correlation between PPI degree and LOEUF upper rank. (A)** Ananlysis across all genes revealed a weak, significant negative correlation between PPI degree and LOEUF upper rank. **(B)** The cluster-wise analysis showed that clusters 2 and 4 exhibited moderate to strong correlations, while other clusters display weak or non-significant relationships. **(C) and (D)** Shows the new network plots for the significantly enriched clusters based on gnomAD constraint metrics.

60

Chronic in

ammatory diseas... Psoriasis

Cluster 2 - GWAScatalog

Gse32986 curdlan highdose v... uad male age ... Osman blood chad63 kh age 1...

Cluster 2 - Immune signature

In

ammatory bowel disease Albumin-globulin ratio

Nakaya pbmc

Neutrophil percentage of wh... Multiple sclerosis Non-albumin protein levels Lymphocyte count Serum total protein level Psoriasis vulgaris Medication use (thyroid pre... Lymphocyte percentage of wh... Crohn's disease Asthma (childhood onset) Celiac disease Rheumatoid arthritis Tonsillectomy

Asthma Blood protein levels in car... White blood cell count Immunoglobulin measurement ... Rheumatoid arthritis (acpa-... Allergic disease (asthma Eosinophil counts

1.00 0.500.75 0.000.25 proportion

0 5 10 -log10

adjusted p-value

Gse27859 macrophage vs dc up Sobolev pbmc pandemrix age ... Fuller pbmc f tularensis va... Zak pbmc mrkad5 hiv 1 gag p... uarix a... Gaucher pbmc yf vax stamari... Gse27859 macrophage vs cd11...

Haralambieva pbmc

Gse7768 ova alone vs ova wi... Gse46606 irf4 ko vs wt unst... Gse37533 pparg1 foxp3 vs fo... Gse8515 il1 vs il6 4h stim ... Gse3982 ctrl vs lps 4h mac dn umist age 18 ... Gse27241 ctrl vs digoxin tr... Gse21546 wt vs sap1a ko and...

Nakaya pbmc

Gse7348 unstim vs lps stim ... Gse8515 ctrl vs il1 4h stim... Gse25088 il4 vs il4 and ros... Gse37533 pparg2 foxp3 vs fo...

1.00 0.500.75 0.000.25 proportion

0 5 10 -log10

adjusted p-value

fl

fl

Cluster 4 - GWAScatalog

Age-related macular degener...

Advanced age-related macula...

Nephropathy

Complement c3 and c4 levels

Matrix metalloproteinase-8 ...

Early age-related macular d...

Complement factor h-related...

Disease progression in age-...

Meningococcal disease

Serum c3d:c3 ratio (systemi...

Complement c3 levels

Blood protein levels

Cluster 4 - Immune signature

Li pbmc menactra age 18 45y...

Li pbmc menomune a c y w 13...

Gse22196 healthy vs obese m...

Gse42021 cd24hi vs cd24low ...

Gse40685 treg vs foxp3 ko t...

Gse3039 nkt cell vs b2 bcel...

Gse42021 treg pln vs cd24in...

fl

Gse42021 cd24hi vs cd24int ...

uad male age ... Zak pbmc mrkad5 hiv 1 gag p...

Howard dendritic cell inact... Nakaya pbmc

Garcia pineres pbmc hpv 16 ...

Gse22140 germfree vs spf ar...

Gse37301 rag2 ko vs rag2 an...

Gse3039 cd4 tcell vs alphab...

Cholesterol ef

ux capacity...

Gse41978 id2 ko vs bim ko k...

Post bronchodilator fev1/fv... Circulating myeloperoxidase... Chronic central serous reti...

1.00 0.500.75 0.000.25 proportion

0 5 10 -log10

adjusted p-value

Gse42021 treg vs tconv pln up Gse3039 cd4 tcell vs b2 bce... Gse19888 adenosine a3r act ... Osman blood chad63 kh age 1...

1.00 0.500.75 0.000.25 proportion

0 5 10 -log10

adjusted p-value

fl

Figure S13: **Composite Enrichment Profiles for IEI Gene Sets.** We selected the top two enriched clusters (as per **Figure S12**) and performed functional enrichment

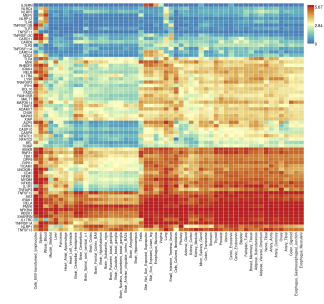
fl

analysis derived from known disease associations. For each gene set, the left panel displays the proportion of input genes overlapping with a curated gene set, and the right panel shows the *−* log10 adjusted p-value from hypergeometric testing. These profiles, stratified by cluster (Cluster 2 and Cluster 4) and by gene set category (GWAScatalog and Immunologic Signatures), highlight distinct enrichment patterns that reflect differential pathogenic variant loads in the IEI gene panels.

fl

fl

61



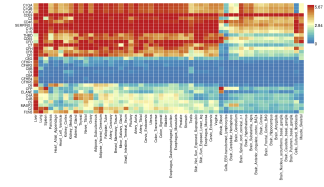


Figure S14: **Gene Expression Heatmaps for IEI Genes.** GTEx v8 data from 54 tissue types display the average expression per tissue label (log2 transformed) for the IEI gene panels. Top: Cluster 2; Bottom: Cluster 4.

62

1251 **6.5 Novel PID classifications derived from genetic PPI and** 1252 **clinical features**

A

150

Unique labels before and after cleaning Before After 140

B

300 200 100

B cell

82

11 5 4

71

249

14

155

200 150 100 50

T cell

15 31 17

82 61

197

18

170

Unique Count

100

93

0

normal

0

normal

73

Count

high

defective

borderline

decreased

low

variable

NA

high

defective

decreased

mixed

low

variable

NA

50

13 8 7 9 8

200 100

Immunoglobulin

175

103

224

Neutrophil

400

300

200

319

208

0

B cell

Neutrophil

T cell

B cell

Neutrophil

T cell

15 22

0

35

normal

17

100 0

6 4 40 2 2 normal

1 9

defective

high

mixed

variable

high

defective

mixed

variable

Immunoglobulin

Immunoglobulin

low

NA

pancytopenia

mild pancytopenia

low

NA

Metric

New category

Figure S15: **Distribution of immunophenotypic features before and after recategorisation.** The original IUIS IEI descriptions contain information such as T cell-related “decreased CD8, normal or decreased CD4 cells” which we recategorise as “low”. The bar plot shows the count of unique labels for each status (normal, not\_normal) across the T cell, B cell, Ig, and Neutrophil features.

63

A

B

Distribution of Tcell by cluster

p=4.72e−16

Distribution of Bcell by cluster p=1.48e−15

Status

not\_normal normal

23 39

19 71

40 33

0

44

7

14

52 26

11 32

4

15

1

8

4 0

2

14

3 1

8 5

2 0

3 0

0 2

0 2

Status

not\_normal normal

18 44

22 68

28 45

0

44

12 9

53 25

7

36

5

14

1

8

0 4

1

15

4 0

3

10

1 1

3 0

0 2

0 2

Count

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17

Cluster

C

D

Distribution of Ig by cluster

p=1.41e−13

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 Cluster

Distribution of Neutrophil by cluster p=1.61e−14

100 75

50

25

Status

not\_normal normal

22 40

18 72

40 33

1

43

16 5

27 51

8

35

5

14

2 7

4 0

3

13

4 0

2

11

2 0

3 0

0 2

0 2

Status

not\_normal normal

2

60

4

86

9

64

1

43

2

19

7

71

2

41

5

14

2 7

0 4

8 8

2 2

8 5

1 1

0 3

2 0

0 2

0

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 Cluster

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 Cluster

Figure S16: **Heatmaps of clinical feature distributions by PPI cluster.** The heatmaps display the count of observations for abnormality of each clinical feature (A) T cell, (B) B cell, (C) Immunoglobulin, (D) Neutrophil, in relation to the PPI clusters, with p-values from chi-square tests annotated in the titles.

A

Accuracy

0.6 0.4 0.2 0.0

C

Model Accuracy Comparison

59% 61%

50%

33%

variable importance (>10)

Bcellnot\_normal subcat3. CID... subcat9. Oth... subcat1. Con... major3. PAD subcat4. Her... subcat2. FHL... subcat5. Imm... subcat8. Oth... subcat3. Sev... subcat1. Fam... Neutrophilno... subcat3. Non... subcat1. T−B... subcat5. Hyp... subcat1. Men... Tcellnot\_normal major5. PD major7. AID subcat2. Def... Ignot\_normal major6. IID major8. CD subcat2. DNA... major9. BMF subcatNA

Phenotypes only

Phenotypes + IUIS Major

IUIS Major + Subcat

Phenotypes + IUIS Major +

B

1234567891011121314151617

only

confusion matrix

3747021401022002003581403420200310000512

Subcat

D

Class: 15

Class: 12

Class: 10

Class: 4

0 25 50 75 100 importance

E

Class: 4

Class: 17

Class: 16

Class: 14

predicted

4301110031330000

0004300000010000004160620000200000022402640101200000071550069000000010052201105101000100011000006010000000000000004000000012200100108100000000000000004000002210000000008000000000100000000100000000000000003000000000000200000000000000200000000

Freq

60

40

20

0

class

Class: 6 Class: 9 Class: 2

Class: 13 Class: 1 Class: 3

Class: 11 Class: 5 Class: 8 Class: 7

Class: 17 Class: 16 Class: 14

class

Class: 8 Class: 10 Class: 13 Class: 15

Class: 9 Class: 7 Class: 12 Class: 5 Class: 11 Class: 6 Class: 1 Class: 3 Class: 2

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 observed

0.00 0.25 0.50 0.75 1.00 Sensitivity

0.00 0.25 0.50 0.75 1.00 Specificity

Figure S17: **Performance comparison of PID classifiers.** Classification predicting PPI cluster membership from IUIS major category, subcategory, and immunological features. (A) Overall accuracy for four rpart models used to predict PPI clustering. The combined model achieves 61.4 % accuracy, exceeding all simpler approaches. Nodes were split to minimize Gini impurity, pruned by cost-complexity (cp = 0.001), and validated via 5‑fold cross‑validation. (B-E) The summary statistics from the top model are detailed.

64

1253 **6.6 Probability of observing AlphaMissense pathogenicity**

A

Observed Disease

Probability

B

NFKB1: Kruskal−Wallis chi−squared = 6.485, df = 2, p = 3.91e−02 − Not signif.

3

4

3

2

2

1

1

CFTR: Kruskal−Wallis chi−squared = 0.193, df = 2, p = 9.08e−01 − Not signif.

Benign

Unknown

Pathogenic

Pathogenic

Classification

Benign

Unknown

Figure S18: **Observed Disease Probability by Clinical Classification with AlphaMissense.** The figure displays the Kruskal-Wallis test results for NFKB1 and CFTR, showing no significant differences.

65