Supplemental Material

Variant selection from gnomAD

Sequence variants from 125,748 exomes in the gnomAD v2 database were annotated with the functional consequence on the canonical transcript by the Ensembl Variant Effect Predictor (VEP) (McLaren et al., *Genome Biology*. 2016. [PMID 27268795]). Predicted loss-of-function (LoF) variants (nonsense, frameshift, and essential splice site variants) were annotated with LOFTEE (Karczewski et al., *Nature*. 2020. [PMID 32461654]), and only high confidence pLoF variants were retained for analysis. In order to exclude variants with allele frequencies too high to be compatible with disease, only variants with a global allele frequency and "popmax" allele frequency (the highest continental allele frequency) ≤1%, and an allele frequency in any population that is not included in popmax of ≤5% were included in the analysis. All variants were annotated with the ClinVar classification and review status if reported.

Approach 1. Expected LSD NGS screen positives based on observed numbers in gnomAD.

Using the selected variants (see above), the number of individuals with a homozygous pLoF variant, at least two heterozygous pLoF variants (unphased), a heterozygous pLoF plus a heterozygous missense variant (unphased), a homozygous missense variant, and/or at least two heterozygous missense variants (unphased) were counted gene-wise from the individual level gnomAD exome call set (internal use only).

For the results presented in the main text (Table 1), all ClinVar benign (B) and likely benign (LB) variants associated with a review status of 2-stars or more (i.e. two diagnostic laboratories report the variant as B or LB with no conflicting reports) were removed from the analysis. These gnomAD counts, stratified by variant function, are also displayed in Supplemental Table 1. Supplemental Table 2 contains the same estimates without removing ClinVar B/LB 2-star variants. Though calculated on the same data, these counts are slightly lower than those displayed in the gnomAD browser. This is due to our exclusion of 2-star review status ClinVar B/LB variants and due to counting individuals with two rare heterozygous and/or homozygous variants together to prevent double counting individuals with both.

Supplemental Table 1: Individuals in gnomAD carrying two rare pLoF and/or missense variants by gene and variant consequence, excluding all ClinVar B/LB variants with 2-star review status and above.

		ARSA	*CYP27A1*	*GALC*	*IDUA*	*GAA*
Individuals (n)	homozygous pLoF	0	0	0	0	0
	≥2 heterozygous pLoF	0	0	0	0	1
	heterozygous pLoF plus heterozygous missense	2	0	15	4	10
	homozygous missense	15	8	10	9	10
	≥2 heterozygous missense	32	25	30	24	133
	Total with ≥2 pLoF and/or missense	49	33	54	37	150
Frequency	homozygous pLoF	0	0	0	0	0
	≥2 heterozygous pLoF	0	0	0	0	7.95E-06
	heterozygous pLoF plus heterozygous missense	1.59E-05	0	1.19E-04	3.18E-05	7.95E-05
	homozygous missense	1.11E-04	6.36E-05	7.95E-05	7.16E-05	7.95E-05
	≥2 heterozygous missense	2.39E-04	1.99E-04	2.39E-04	1.91E-04	1.06E-03
	Total with ≥2 pLoF and/or missense	3.66E-04	2.94E-04	4.29E-04	2.94E-04	1.19E-03

Supplemental Table 2: Estimated number of NGS NBS screen positive newborns per year based on gene variants found in gnomAD, including all ClinVar B/LB variants with 2-star review status and above.

LSD (protein)		Estimated number of First-Tier NGS Screen Positives Per Year		
	gnomAD (125,748 exomes)	CA (500,000 births/yr)	WA (85,000 births/yr)	USA (4,000,000 births/yr)
MLD (ARSA)	49	195	33	1,559
CTX (CYP27A1)	37	147	25	1,177
Krabbe (GALC)	54	215	37	1,718
MPS-I (IDUA)	41	163	28	1,304
Pompe (GAA)	447	1,777	302	14,219

Approach 2. Expected LSD NGS screen positives based on Hardy-Weinberg estimates.

Using the same selected variants as in Approach 1, the global allele frequencies of variants were used to calculate Hardy-Weinberg estimates of genetic prevalence (q^2), an approach that is currently available through public access of the gnomAD data. From these estimates, we calculated the expected number of newborns born per year with positive NGS screens in a large population NBS laboratory (California), medium-population NBS laboratory (Washington) and across the USA.

The results of this analysis, excluding all ClinVar B/LB variants with 2-star review status and above, are displayed in Supplemental Table 3.

Supplemental Table 3: Estimated number of NGS NBS screen positive newborns per year based on gene variants found in gnomAD, excluding all ClinVar B/LB variants with 2-star review status and above.

LSD (protein)	Estimated genetic prevalence	Estimated number of First-Tier ES-NGS Screen Positives Per Year		
		CA (500,000 births/yr)	WA (85,000 births/yr)	USA (4,000,000 births/yr)
MLD (ARSA)	1 / 7,003	71	12	571
CTX (CYP27A1)	1 / 7,679	65	11	521
Krabbe (GALC)	1 / 4,819	104	18	830
MPS-I (IDUA)	1 / 3,513	142	24	1,139
Pompe (GAA)	1 / 4,091	122	21	978