Quantifying the impact of rare and ultra-rare coding variation across the phenotypic spectrum

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Abstract

Protein truncating variants (PTVs) are likely to modify gene function and have been linked to hundreds of Mendelian disorders[1,2]. However, the impact of PTVs on complex traits has been limited by the available sample size of whole-exome sequencing studies (WES) [3]. Here we assemble WES data from 100,304 individuals to quantify the impact of rare PTVs on 13 quantitative traits and 10 diseases. We focus on those PTVs that occur in PTV-intolerant (PI) genes, as these are more likely to be pathogenic. Carriers of at least one PI-PTV were found to have an increased risk of autism, schizophrenia, bipolar disorder, intellectual disability and ADHD (P-value (p) range: $5x10^{-3}$-$9x10^{-12}$). In controls, without these disorders, we found that this burden associated with increased risk of mental, behavioral and neurodevelopmental disorders as captured by electronic health record information. Furthermore, carriers of PI-PTVs tended to be shorter ($p=2x10^{-5}$), have fewer years of education ($p=2x10^{-4}$) and be younger ($p=2x10^{-7}$); the latter observation possibly reflecting reduced survival or study participation. While other gene-sets derived from *in vivo* experiments did not show any associations with PTV-burden, gene sets implicated in GWAS of cardiovascular-related traits and inflammatory bowel disease showed a significant PTV-burden with corresponding traits, mainly driven by established genes involved in familial forms of these disorders. We leveraged population health registries from 14,117 individuals to study the phenome-wide impact of PI-PTVs and identified an increase in the number of hospital visits among PI-PTV carriers. In conclusion, we provide the most thorough investigation to date of the impact of rare deleterious coding variants on complex traits, suggesting widespread pleiotropic risk.

We assembled whole-exome sequencing data from 100,304 individuals, drawing from a combination of cohort and case/control disease studies with phenotypic information on a total of 13 quantitative traits and 10 diseases (**Supplementary Table 1-3**). We used a common pipeline to process, annotate and analyze the data (**Supplementary Notes** and **Supplementary Figure 1** for principal components plots).

We began by focusing our analysis on PTVs that occur in a set of 3,172 PI genes (**Supplementary Table 4** reports all gene sets used in this study). Our motivation for focusing on the PI-PTVs was two-fold. First, this gene class was identified through an unbiased approach that leveraged the observed frequency distribution in ExAC [4] without relying on information from model organisms or *in vitro* experiments. Second, PI-PTVs have been shown to associate with early onset neurodevelopmental and psychiatric disorders, and are likely to result in reproductively disadvantageous phenotypes [5 6,7]. To focus on those variants that are most likely to be subject to purifying selection, we considered only rare (allele frequency < 0.1%) and ultra-rare (observed in less than 1 in 201,176 individuals) variants (**Supplementary Notes**).

After excluding participants diagnosed with a psychiatric or neurodevelopmental disorder, we observed an average of 7.72 and 0.30 rare PTVs per individual, across all genes and in PI genes respectively (**Figure 1a**); one or more ultra-rare PI-PTV was observed in 11% of the individuals. The number and frequency of rare variants differs across populations, reflecting the degree of selection compounded by recent demography, including bottlenecks, split times, and migration between populations [8]. The ratio of deleterious to neutral alleles per individual increases as humans migrated out of Africa, consistent with negative selection against deleterious variants and serial founder effects that reduce the effective population size [9]. Conditional on a variant being ultra-rare, we observe a higher ratio of PTV to synonymous variants (**Figure 1b**); recently arisen ultra-rare variants have had less time to be purged by negative selection, which is further magnified in populations that have undergone a recent bottleneck. For example, we observed a higher ratio among Ashkenazi Jewish and Finnish populations as compared to non-Finnish Europeans, reflecting the more recent population-specific bottlenecks [10,11].

We tested the association between a burden of PI-PTVs and the 13 traits and 10 disease diagnoses (**Figure 2**) by performing study and ethnicity-specific linear or logistic regression analysis adjusting for potential confounders such as overall mutation rate (**Supplementary Table 5**). The results of these separate analyses were then meta-analyzed (**Supplementary Notes**). We used an experiment-wise p-value threshold of $2x10^{-3}$ to account for multiple testing (0.05/23 traits tested). Among the quantitative traits, we found that carriers of at least one rare PI-PTV tended to be shorter (-0.2 cm, $p=3x10^{-4}$), have fewer years of education (-2.2 months, $p=4x10^{-4}$) and be younger (-3.7 months, $p=2x10^{-7}$).

To ensure the robustness of the age result, we performed a series of quality control analyses to guard against the impact of technical confounders or specific study designs that might bias the results. We first confirm that the signal was not observed among PTVs in non-PI genes and synonymous variants in PI genes, our negative controls (**Supplementary Figure 2**). We further found that the effect was consistent across ethnicities and study cohorts (**Supplementary Figure 3**), when INDELs and SNPs were considered separately, when mutations possibly caused by cytosine deamination (C ->T or G -> A) were excluded and when a highly stringed QC was used (**Supplementary Figure 4**). Similarly, the association did not change after adjusting for 8 QC metrics capturing most of the sample properties (**Supplementary Figure 5**). Although we could not exclude the impact of unmeasured confounder, we find this result consistent with reduced survival, detrimental health or decreased study participation over time among PI-PTVs carries. If reduced survival or detrimental health effects drive this association, we see it as a signature of purifying selection, overall in the population.

We then focused on dichotomous traits (**Figure 2**). We observed significant associations with all psychiatric disorders that were tested – intellectual disability (ID) (odds ratio [OR]=1.7, $p=4x10^{-8}$), autism (OR=1.3,$p=1x10^{-14}$), schizophrenia (OR=1.2,$p=5x10^{-8}$), ADHD (OR=1.2, $p=5x10^{-10}$) and bipolar disorder (OR=1.2, $p=8x10^{-4}$). We did not however find PI-PTV burden to be associated with later onset, non-brain-related diseases such as type 2 diabetes, early onset myocardial infarction, inflammatory bowel disease, ulcerative colitis or Crohn's disease. Across all significantly associated phenotypes, the effect size was stronger among the subset of ultra-rare PI-PTV carriers, confirming that rarer PTVs are, on

average, more deleterious.

The association with these five neurodevelopmental/psychiatric disorders and three quantitative traits was only observed for PI-PTVs and not for PTVs in non-PI genes nor for synonymous variants in PI genes. These results suggest that the association to PI-PTVs is not driven by population stratification or technical bias (**Supplementary Figures 6**).

Our approach so far focuses on assuming that all PI-PTVs act on the phenotype in the same direction, that is, they are all either protective or risk conferring. We relaxed this hypothesis, allowing rare PI-PTVs to have different directions as well as different magnitudes of effects, and repeated these tests using SKAT [12]. We did not identify any additional associations, suggesting that PI genes do not account for a substantial fraction of variability in the traits for which no PTV burden was identified. Further, the observed burden of PI-PTVs for neurodevelopmental/psychiatric disorders, height, educational attainment and age suggests that the majority of those PI genes that have an effect, do so in the same direction (**Supplementary Figure 7**).

We also evaluated whether damaging missense variants, which are on average more common and less severely deleterious than PTVs, showed a similar signal. Damaging missense variants have been associated with complex disorders such as coronary heart disease and inflammatory bowel disease[13 14]. We found an independent signal for damaging missense variants in PI genes for all disorders and traits that were also associated with PI-PTVs. Furthermore, the strength of the association increased as a function of the number of prediction algorithms that confidently classified a missense variant as 'damaging' (**Supplementary Figure 8**) suggesting that these missense mutations are similar to PTVs in biological effect, potentially abrogating gene function. We note that this effect was particularly strong for ultra-rare variants, reinforcing the observation that variant frequency is a marker of selection and aids in the identification of pathogenic damaging missense variation[15 16].

Information about gene expression might help identifying which subset of PI genes is driving the observed signals. We tested the association between rare PTVs and significant disorders and traits in the top 500 most expressed PI genes in each of 14 different tissues. PI genes highly expressed in the frontal cortex carried the stronger signal for education attainment, ADHD, autism and the second stronger signal for schizophrenia, in line with the brain-related nature of these disorders and traits (**Supplementary Figure 9**). However, significant signals were also observed in brain-unrelated tissues. For example, blood and stomach carried the largest association with bipolar disorder and intellectual disability, respectively. Given that there was a consistent number of genes that were highly expressed in multiple tissues, we wonder if the signal was driven by PI genes highly expressed in >80% of the tissues rather than by genes that were specifically expressed in few tissues (< 20% of the tissues). PI genes highly expressed in multiple tissues modestly increased the association between rare PTVs and neurodevelopmental and psychiatric disorders, as compared to tissue-specific PI-genes (**Supplementary Figure 10**).
These results suggest that gene-expression information provides moderate additional value for the identification of driving PI genes.

Given the high degree of shared comorbidities across neurodevelopmental/psychiatric disorders, we leveraged information from the Danish National Psychiatric registry to evaluate whether the signal was driven by a specific disorder or shared across multiple disorders. Individuals with multiple neurodevelopmental/psychiatric disorders, and especially those with ID, showed a stronger enrichment of PI-PTVs (**Supplementary Figure 11**). Nevertheless, among those without comorbidities, the signal remained significant and remarkably similar across disorders. We further found that carriers of ultra-rare PI-PTVs had earlier onset of ADHD (-4.0 months, p =0.008; **Supplementary Table 6**). However, this was partially explained by the fact that individuals with earlier diagnosis of ADHD were also more likely to be diagnosed with ID (14.7 vs. 15.5 years for ADHD patients with and without ID, T-test P-

value=0.009). Indeed when we considered ADHD cases without major comorbidities, the effect was attenuated (-2.9 months, p=0.12). Finally, in controls with none of these psychiatric diagnoses, we still observed a significant association with the broader ICD-10 category of mental, behavioral and neurodevelopmental disorders, suggesting that PI-PTVs influence the broader cognitive spectrum (**Supplementary Table 7**).

Since previous studies have shown a higher rate of PI *de novo* PTVs in autism-affected females as compared to males [17 18], we wondered if sex played a role here. In this study however, we did not have parent-offspring subjects needed to distinguish *de novo* variants from those that have recently arisen in the population, the latter being the majority of observed rare variants. This would potentially dilute the sex-specific effect if it is in fact a property of *de novo* variants but not of rare variants more generally. We found both weak and insignificant differences between males and females in the effect of PI-PTVs on 4 neurodevelopmental/psychiatric disorders (**Supplementary Table 8**). Interestingly, we did not observe differences in ADHD-affected males and females, in contrast with the hypothesis that affected females might be enriched for rare deleterious variants[19]. We cannot exclude that differences in the diagnostic criteria used in these European studies compared to those of previous studies, which were mostly conducted in the U.S., might explain these results.

We also assessed whether the observed burden of PTVs was specific to PI genes, or such a burden could be identified for other gene sets that are likely to contain functionally relevant genes. First, we examined other experimental and literature-based gene sets linked to severe phenotypes. Specifically, we considered all genes (i) reported in ClinVar [2], (ii) that resulted in lethal or subviable phenotypes in mice [20], (iii) that were required for proliferation and survival in a human cancer cell line [21] and those (iv) that were categorized as haploinsufficient by ClinGen (**Supplementary Table 4** and **Supplementary notes**). Except for the haploinsufficient genes, which showed a significantly stronger association with autism alone, none of the other gene sets tested showed the PTV burden that was captured by PI genes (**Supplementary Figure 12**). This suggests that the degree of natural selection against PTVs in a gene is indeed an important indicator of whether such PTVs are likely to be implicated as strong effects for neurodevelopmental/psychiatric disorders, height, educational attainment and age.

We reasoned that a single variant approach, rather than a gene-based test, might provide increased resolution. We considered all high quality ClinVar variants (0.76 on average per individual) and a set of variants deemed to be recessive lethal (0.03) (**Supplementary Notes**). Carriers of these variants were not enriched in any of the disorders or traits examined here (**Supplementary Table 9**).

Second, we examined whether results from GWAS conducted on the same phenotypes as those included in this study could implicate genes containing an aggregate PTV burden. We used DEPICT[22] to link genome-wide significant hits to candidate genes (**Supplementary Table 10** and **Supplementary Notes**) and, within each GWAS-derived gene set, we studied the association between rare PTVs and the phenotypes using the SKAT test. GWAS-derived gene-sets captured associations between rare PTVs and different classes of lipids (**Figure 3** and **Supplementary Table 11**). For example, the association between rare PTVs and HDL-cholesterol was captured by gene sets derived from GWAS of HDL ($p=2x10^{-9}$), total cholesterol ($p=3x10^{-8}$) and triglycerides ($p=4x10^{-9}$), but not by those of coronary heart diseases (p=0.25), consistent with previous observations about non-causality of HDL-cholesterol on coronary heart diseases [23]. The inclusion of both rare damaging missense and PTVs resulted in additional signal co-localization between inflammatory bowel disease, early onset myocardial infarction and the corresponding GWAS-derived gene-sets. However, it appeared that all these signals were being driven by well-known genes, involved in rare familial forms of these diseases. Specifically, when Mendelian lipid genes and *NOD2* were removed from the cardiovascular and inflammatory bowel disease-related GWAS gene-sets respectively, no signal remained (**Supplementary Figure 13**). This might reflect a lack of power (despite

this being the largest WES study for the majority of the traits), inaccurate links between genome-wide significant hits and the corresponding candidate genes or PTVs and common variants acting on partially distinct pathways. Nevertheless, we observed similar results when including SNPs below genome-wide significance to increase power and when using different methods to link SNPs with corresponding candidate genes to increase precision, including gene-based testing [24] and eQTL mapping **(Supplementary Figures 13** and **Supplementary Notes).**

Finally, we leveraged national population health registries to increase the scope of disorders we could examine. These well-studied and validated registries [25,26] include diagnostic codes from 14,117 individuals in Finland and Sweden, recorded between 1968 to 2015 (**Supplementary Table 12**). Individuals with psychiatric disorders were excluded from our analyses. To maximize the validity of the diagnoses, we used a curated list of disease definitions aggregating related ICD codes (**Supplementary Table 13**). We studied the association between rare PI-PTVs and 101 diseases with at least 50 cases, using a survival analysis model. The only association that surpassed the significance threshold for multiple testing was with chronic kidney failure (hazard ratio=1.9, $p=3x10^{-6}$; number of cases=120; **Supplementary Figure 14**). The association was strong among the Finnish data, but only significant when considering ultra-rare PI-PTVs in the Swedish data (**Supplementary Table 14**).

We speculated that this association might reflect a burden of underlying comorbidities that were too rare to be included in this analysis. Using registry information from 28,709 Finnish individuals, we found that patients with chronic kidney failure also have a higher rate of cardiovascular-related comorbidities, as well as skin infections, kidney cancer and other abnormalities of the renal system (**Supplementary Table 15**). Therefore, it is challenging, to determine whether it is the chronic kidney failure or some more rare comorbid condition that drives the association with PI-PTVs.

We also examined whether the association between PI-PTVs and diminished cognition and detrimental health would result in a higher number of hospital visits, counting the number of in-patient visits associated with a unique ICD codes. In both the Swedish and Finnish datasets, we observed a significant increase in the rate of hospital visits with a greater burden of PI-PTVs (+7.6% per additional PI-PTV, p=0.0002). We used different strategies to model the outcome and observed similar results (**Supplementary Table 16** and **Supplementary Figure 15**).

By aggregating WES data on more than 100,000 individuals for 23 different traits and disorders, we have gained insight into the role of PTVs in conferring risk for these conditions. First, PTVs occurring in PI genes had a remarkably similar effect on autism, schizophrenia, bipolar disorder and ADHD, which was not driven by major underlying comorbidities. This suggests that these PI-PTVs are likely to be pleiotropic, influencing some core intermediate phenotypes that relate to risk across many psychiatric disorders. Further, this burden suggests that both PI genes will be eventually discovered conclusively for each of these disorders, not just autism, but such associations will need to be interpreted in the light of this shared effect across disorders. The strong enrichment of PI-PTVs in individuals with neurodevelopmental/psychiatric disorders does not exclude the existence of non-PI genes involved in the etiology of these disorders. These genes, however are more likely to have weaker and, possibly, trait-specific effect.

Second, we detected a significant association between PI-PTVs and decreased human height. In contrast to this, a recent large-scale study using the exome-chip has shown a similar numbers of height-increasing and height-decreasing rare variants [27]. This discrepancy could be because, by using a more stringent frequency cut-off and focusing on a subset of genes likely to cause early onset severe disease, we effectively considered variants related to a burden of (incompletely) penetrant Mendelian-type disorders, often characterized by reduced growth. Such an interpretation is consistent with a tighter link to directional selection on stronger impact mutations for human height.

Third, we systematically compared the co-localization of signal between GWAS-candidate genes and rare PTVs. We found few overlaps (cardiovascular-related traits, inflammatory bowel disease) which, we revealed, were entirely driven by a few genes previously identified by both GWAS and WES studies. Other traits did not show any overlap. Schizophrenia, for example, which is highly enriched for PI-PTVs, did not show overlap with GWAS candidate genes. Even among traits where genes with low-frequency coding variants have been previously identified by exome-chip-based studies, such as height and systolic blood pressure, we found no substantial rare PTVs enrichment. These results suggest that the relationship between GWAS signal and rare coding variants is not always straightforward, and that, when interpreting WES data, other complementary approaches such as those that integrate population genetic models and large sample resources, might be more suitable to nominate gene sets of interest. The degree of overlap, and therefore the most effective strategy to identify pathogenic variants, is likely to depend on the selective pressure shaping the genetic architecture of the trait under investigation. Moreover, it cannot be overlooked that individuals carrying rare PTVs in genes implicated by common variant-based approaches might present phenotypic outcomes that deviate from those under investigation. Finally, it is interesting to notice that, while PTVs tend to have a consistent directional effect within PI gene, this is not the case for GWAS-derived gene sets, where most of signals could only be captured by assuming heterogeneity in effect direction.

In conclusion, PI genes are well suited to capture the impact of rare to ultra-rare PTVs on the cognitive, behavioral and developmental spectra. This is less the case for major later-onset complex traits with modest effect on reproductive fitness. Strategies to prioritize gene sets relevant for these traits would need to consider the role that relaxed selective pressure has been playing in shaping the frequency distribution of disease-causing PTVs.

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Figure 1

A. Average number of variants per individual in N=83,439 participants without neurodevelopmental/psychiatric disorders. We report the results separately for each ethnic group. **B.** Ratio between PTV/Synonymous for each ethnic group.

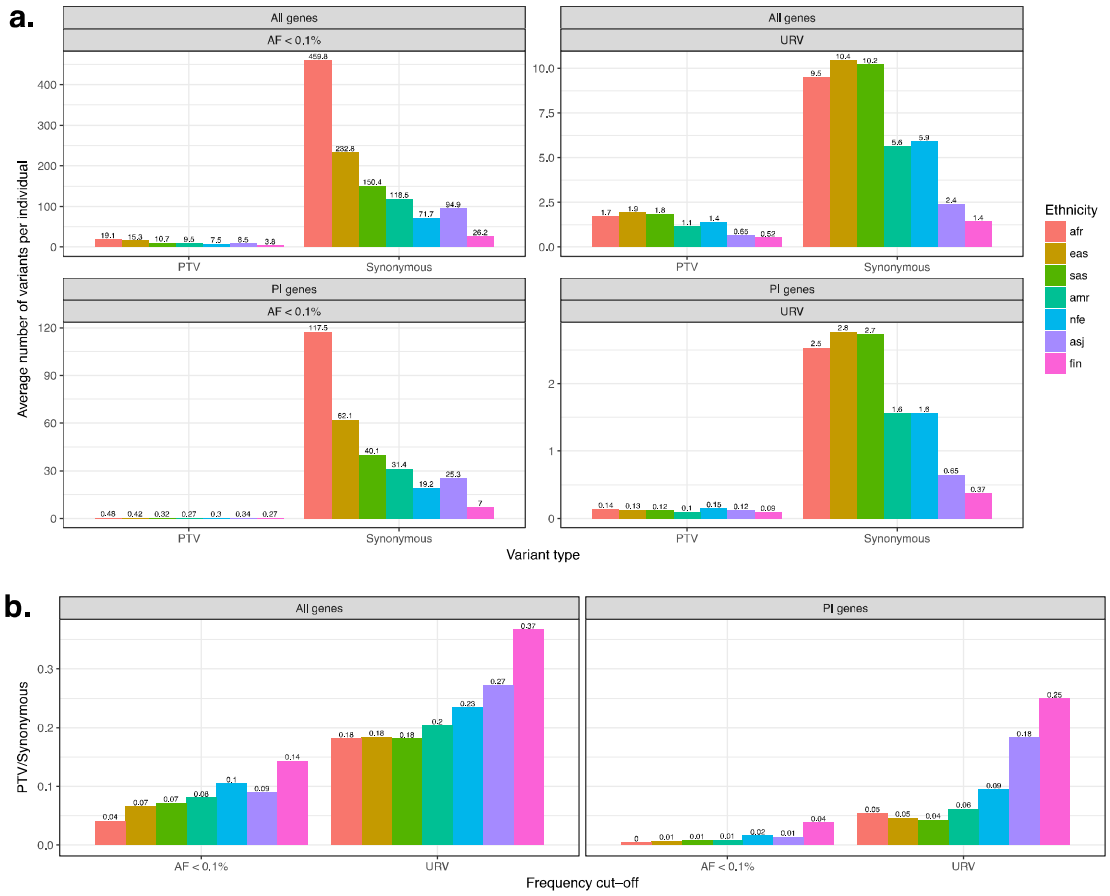

Figure 2

A. Association between PI-PTV burden and continuous traits. We reported the association in standard deviations (SD) to allow for comparison across traits. In brackets, we reported the number of individual included in the analysis for each trait. The P-values are reported only for experiment-wise significant results ($p < 2x10^{-3}$), highlighted in red. All the results are obtained from meta-analyzing study and ethnicity-specific associations. **B.** Odds ratio for association between PI-PTV burden and dichotomous traits. In brackets, we reported the number of cases and controls.

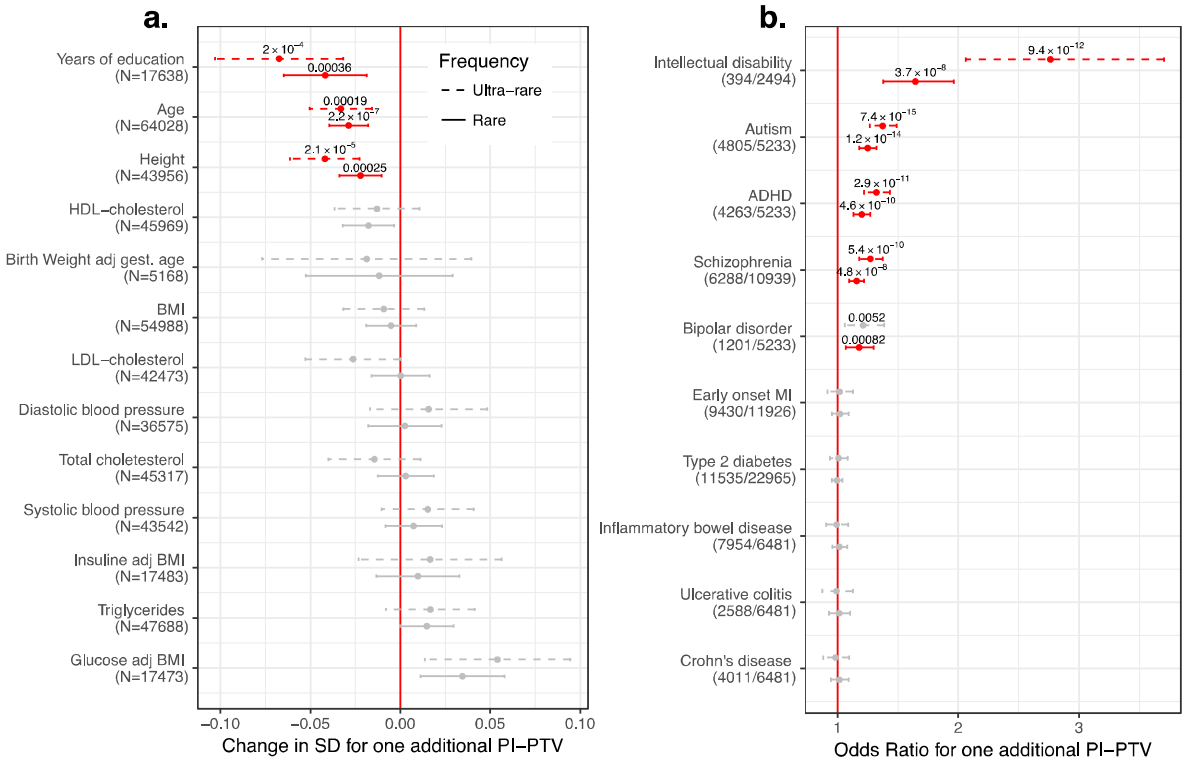

Figure 3

A. Association (SKAT test P-value) in GWAS-derived gene sets (y axis) between rare PTVs and the phenotypes reported on the x axis. Each geneset is obtained using DEPICT to link SNPs derived from GWAS with P-value < $5x10^{-8}$ and a candidate gene. In brackets we report the number of genes with at least one PTV in our dataset. P-values are reported only for experiment-wise associations ($p < 0.0003$). **B**. Association (SKAT test P-value) in GWAS-derived gene sets (y axis) between rare PTVs + damaging missense and the phenotypes reported on the x axis.