Supplementary material

de Magalhães and Wang, *The fog of genetics: Known unknowns and unknown unknowns in the genetics of complex traits and diseases*

Contents

In this file

Supplementary Methods
Supplementary Results
Supplementary Table 1. Gene length in GWAS-hit genes (GHGs) vs non-GWAS-hit genes (non-GHG)
Supplementary Table 2. GD in GWAS-hit genes (GHGs) vs non-GWAS-hit genes (non-GHG)
Supplementary Figure 1. GD of genes with hits in GWAS and genes without GWAS hits
Supplementary Figure 2. Comparison of GD between five major populations
Acknowledgements
References

In accompanying Excel file

Dataset S1. Full list of genes with GD and GWAS hits

Supplementary Methods:

Calculation of genetic diversity

Variant calls were retrieved from the final phase (phase 3) of the 1000 Genomes Project (http://ftp.1000genomes.ebi.ac.uk/vol1/ftp/release/20130502/) (Auton *et al.* 2015). Genetic Diversity (GD), which was calculated from the 1000 Genomes Project global population data, refers to the nucleotide diversity between genes. The normalised (by length) minor allele frequency (MAF) of SNPs in each gene was used to represent GD (see below). Because GRCh37 is the reference genome used by the 1000 Genomes Project phase 3 variants, GRCh37 was used as the reference genome for genetic variants mapping. We calculated GD for genic regions including introns, given that most genetic associations are found in non-coding regions and intronic regions specifically (Freedman *et al.* 2011). As the regions in upstream and downstream often contain regulatory elements which could affect functions of the gene, the flanking regions 1000bp upstream and downstream of each gene, which tends to be under strong LD, were also included when mapping variants to genes and calculating GD . The start and stop positions of genic and flanking regions were retrieved from GRCh37 using Ensembl BioMart. Then, the genetic variants from the 1000 Genomes Project were mapped to genes containing genic and flanking regions. The calculation of GD was performed as follows:

1. Consider a cohort consisting of n individuals, for a given SNP at position i, the total number of minor alleles (representing nucleotide changes) at position i in the population is:
$$n \times MAF_i$$
2. If there are m SNPs located in the genic region and two flank regions, the total number of minor alleles within the genic and flank regions can be calculated by:
$$\sum_{i=0}^{m} n \times MAF$$
3. As n is a constant, the above formula can be simplified as:
$$\sum_{i=0}^{m} MAF$$
4. Then the normalised minor alleles in every 1000bp DNA is:

$$GD = \frac{1000 \times \sum_{i=0}^{m} MAF}{length_{genic} + length_{upstream} + length_{downstream}}$$

In total, 54,849 genes were obtained from the GRCh37 assembly, including protein coding gene, lincRNA, antisense, misc_RNA, snRNA, pseudogene. See Dataset S1 for the full dataset, including GD values.

Nucleotide diversity (π) data

Data on nucleotide diversity (π) per gene was obtained for the whole genic region of protein-coding genes from PopHuman, a genomics browser based on the 1000 Genomes Project (Casillas *et al.* 2018). To focus on individual populations, π was obtained from three populations: Utah residents with Northern and Western European ancestry (CEU), Han Chinese in Beijing, China (CHB) and Yoruba in Ibadan, Nigeria (YRI).

Counting the number of traits reported in the GWAS-Catalog per gene

Data files were downloaded from the GWAS-Catalog website on 31/01/2018 (MacArthur *et al.* 2017). Then, in each entry, the reported SNPs were mapped to genes (with 1000bp in each upstream and downstream flanks, as for the GD calculations) in the GRCh37 assembly using rsIDs. SNPs mapping to two or more adjacent genes are counted separately. Through this method, connections between studied traits and genes (where reported SNPs located in) were recovered. By counting the number of unique *Mapped Traits* that are associated with any given gene, the corresponding number of associated traits of a gene was obtained.

Statistical analyses

Non-parametric tests were employed for the statistical analyses. The Mann–Whitney U-test was used to compare different groups of genes (e.g., genes with GWAS hits versus genes without GWAS hits). Correlation analyses were performed using Kendall's rank correlation (similar results were obtained using Spearman's rank-order correlation). Statistical analyses were performed using SPSS version 22 (IBM), R version 3.4.2 (R Core Team 2017) and RStudio version 1.1.383 (RStudio Team 2015).

Supplementary Results:

We estimated genetic diversity for each human gene based on the number of alternative alleles using data from the 1000 Genomes Project (Auton *et al.* 2015), normalized by gene length (see Supplementary Methods). We then counted the number of GWAS hits from the GWAS catalog (MacArthur *et al.* 2017) and analyzed the relationship between the number of GWAS hits and genetic diversity. As expected, genes with GWAS hits tend to be longer (Supplementary Table 1) and there is a correlation between gene size and number of GWAS hits (Kendall's tau = 0.392 for protein-coding genes; tau = 0.260 for non-coding genes; p-value < 0.001 for both). In a way, this means that genetic association studies will be biased towards finding associations in larger genes, presumably because these have more genetic variants, even though larger genes will not *a priori* be the most important biologically. Larger genes also tend to have a slightly higher genetic diversity (Kendall's tau = 0.057 for protein-coding genes; tau = 0.034 for non-coding genes; p-value < 0.001 for both), we speculate because perhaps longer genes have a slightly lower chance of mutations being deleterious. Importantly, we found that genes with GWAS hits have a greater genetic diversity (Supplementary Figure 1). Indeed, genes with a greater genetic diversity have more GWAS hits (Kendall's tau = 0.106 for protein-coding genes; tau = 0.073 for non-coding genes; p-value < 0.001 for both), an effect that is still observed when accounting for the potentially confounding effects of gene length (p-value < 0.001). The differences observed are not huge (Supplementary Figure 1; Supplementary Table 2), but given that most genetic variants are thought to be neutral (Kimura 1983), this is to be expected.

Similar results were obtained using nucleotide diversity (π), another measure of DNA polymorphisms (Nei 1987), and in individual populations. After merging π to the number of traits associated with each gene, we obtained a dataset with 21,020 genes. The analyses for GD reported above were repeated using π from CEU, CHB and YRI with very similar results. For all three populations, genes with a greater π have more GWAS hits (Kendall's tau for CEU = 0.127; tau for CHB = 0.114; tau for YRI = 0.087; p-value < 0.001 for all three); the correlation between π and number of GWAS hits in a gene is still statistically significant after accounting for the effects of gene length (p-value < 0.001 for all three populations). Besides, genes with GWAS hits (GHGs) have a greater π than genes without GWAS hits (non-GHG): GHGs = 0.0006781 vs non-GHG = 0.0005552 for CEU; GHGs = 0.0006222 vs non-GHG = 0.0005142 for CHB; GHGs = 0.00088535 vs non-GHG = 0.0007907 for YRI (using a Mann-Whitney test, differences are highly statistically significant at p-value < 0.001 for all populations).

Overall, these results demonstrate that genes with greater diversity have a higher probability of being associated with human phenotypes. Given that the power to detect a genetic association increases with the allelic frequency (Hong & Park 2012), our results are in line with theoretical expectations and crucially show that GWAS hits are biased towards particular types of genes.

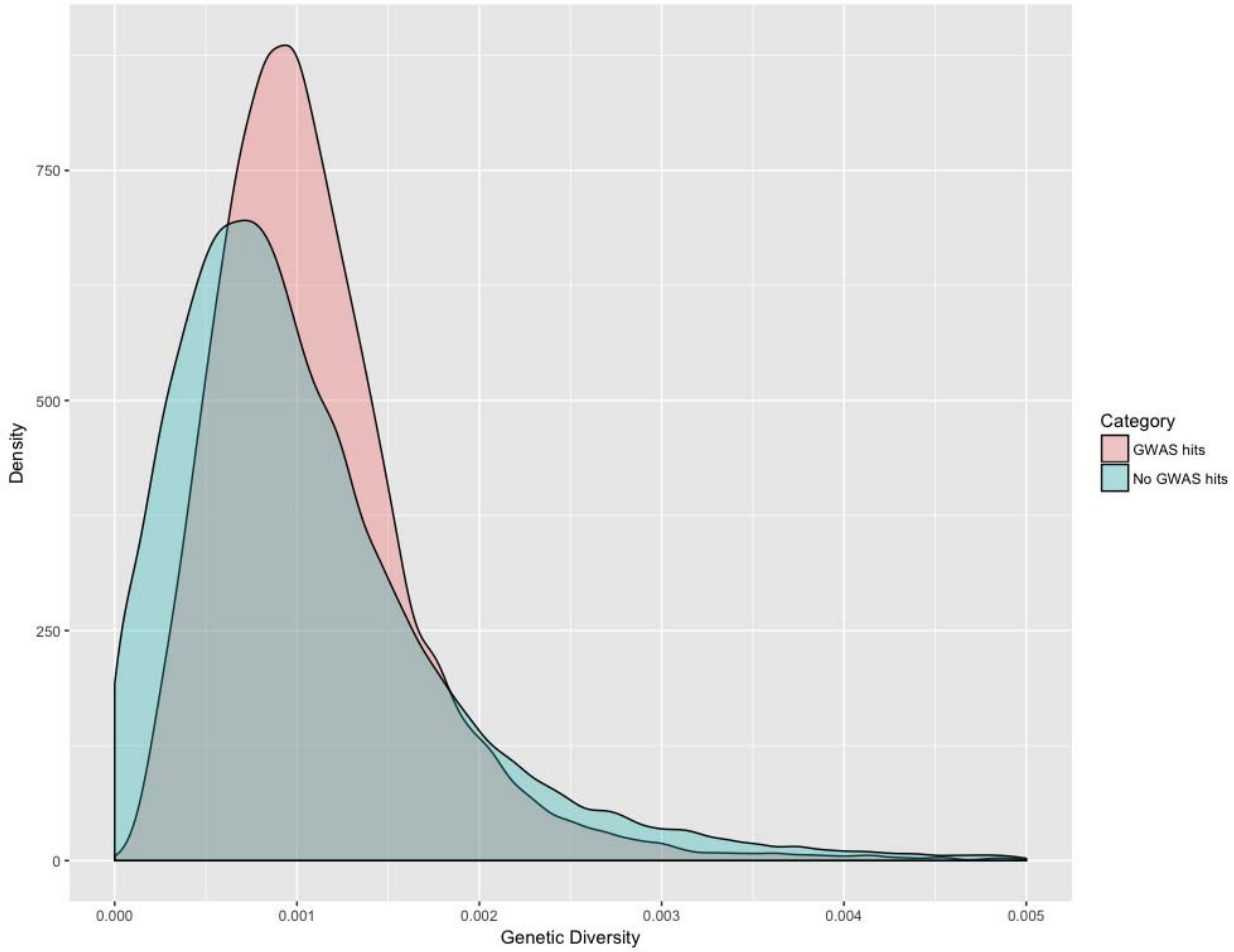
Supplementary Table 1: *Gene length in GWAS-hit genes (GHGs) vs non-GWAS-hit genes (non-GHG).*

Gene class	n	Min.	Median	Mean	Max.
Genome-Wide	54849	2008	4913	32270	2306638
GHGs	11869	2047	47011	100576	2306638
non-GHGs	42980	2008	3291	13408	1231306
Protein-coding GHGs	8196	2186	59864	118152	2306638
Protein-coding non-GHGs	11234	2059	16368	30762	1231306
Non-coding GHGs	3673	2047	19777	61357	1538213
Non-coding non-GHGs	31759	2008	2688	7266	456257

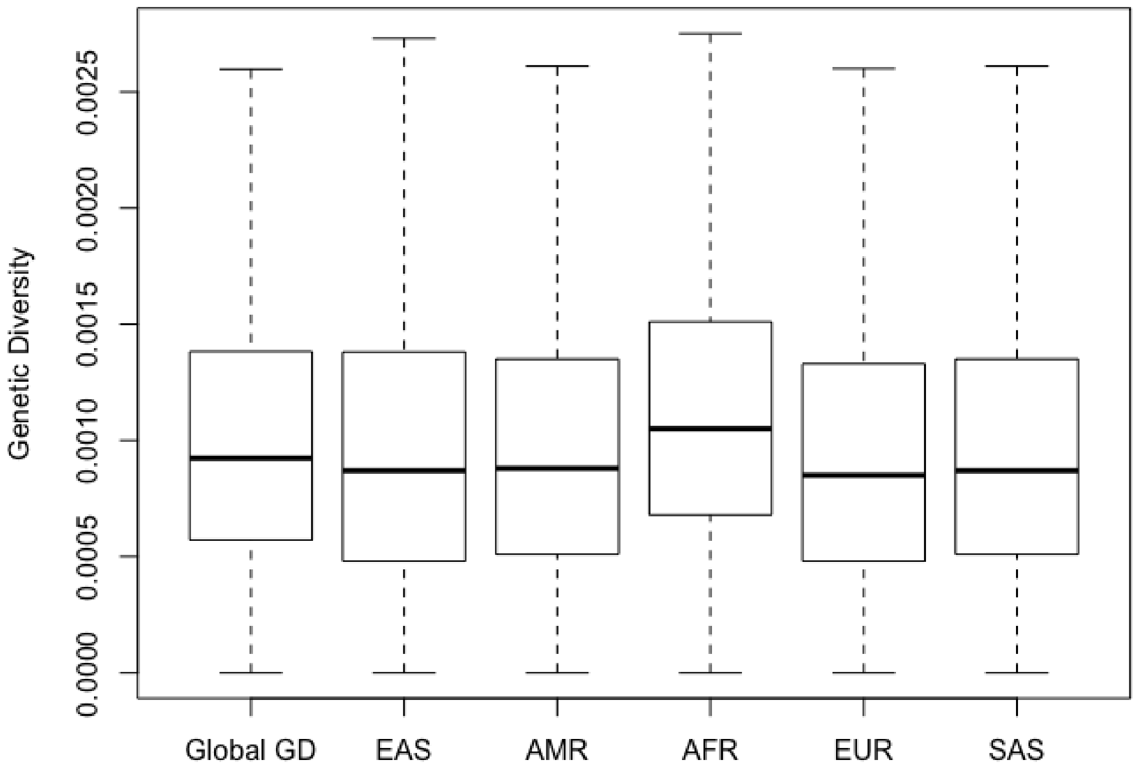
Supplementary Table 2. *GD in GWAS-hit genes (GHGs) vs non-GWAS-hit genes (non-GHG).*

Gene class	n	Min.	Median	Mean	Max.
Genome-Wide	54849	0	0.000923	0.001098	0.033518
All GHGs	11869	0.000024	0.001009	0.001158	0.033518
All non-GHGs	42980	0	0.000885	0.001075	0.019021
Protein-coding GHGs	8196	0.000061	0.000976	0.001073	0.031054
Protein-coding non-GHGs	11234	0	0.000846	0.000972	0.013428
Non-coding GHGs	3673	0.000024	0.001087	0.001347	0.033518
Non-coding non-GHGs	31759	0	0.000903	0.001112	0.019021

Supplementary Figure 1. *Density plot of genetic diversity of genes with hits in GWAS (pink; n =11,869) and genes without GWAS hits (green; n = 42,980). Only genetic diversity <0.005 were displayed. The difference between the genetic diversity of genes with hits in GWAS (median = 0.001009) and genes without GWAS hits (median = 0.000885) is highly statistically significant (p-value < 0.001; Mann-Whitney test). Please refer to Supplementary Table 2 for further details of the genetic diversity of the two groups.*



Supplementary Figure 2. *Comparison of GD between five major populations: AFR, African; AMR, Ad Mixed American; EAS, East Asian; EUR, European; SAS, South Asian.*



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