

Gene-level heritability analysis explains the polygenic architecture of cancer

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Abstract

It is unknown how high-frequency single nucleotide polymorphisms (SNPs) affect the risk of developing cancer in the broader population. Understanding the contribution of these variants is crucial to use genetic information in risk assessment. More importantly, identifying genes harbouring SNPs explaining a large proportion of the heritable risk could provide new insights in the aetiology of this disease and opportunities for treatment of patients. We addressed these questions by developing a new method to estimate genome-wide and gene-level cancer heritability. Using data from the UK Biobank, we identified a new class of 1,430 genes, which we called cancer heritability genes, whose SNPs account for a significant contribution to the heritable risk of 35 malignancies. Functional analysis and comparison with cancer genomics data showed that cancer heritability genes are mostly tumour suppressors and control cell proliferation, invasion and metastasis. Our study suggests that SNPs in cancer heritability genes could provide a selective advantage for tumour proliferation and metastasis, thus representing a potential target for personalised treatment.

Decades of research have shown that inherited genomic mutations affect the risk of individuals to develop cancer [28, 25]. In familial studies, inherited mutations in cancer susceptibility genes, such as *TP53* [18], *BRCA1* and *BRCA2* [21, 33], confer up to an 8-fold increase in cancer risk in first degree relatives [28]. However, this evidence is limited to rare, highly penetrant inherited mutations, which explain only a small fraction of the relative risk for all cancers [19].

It has been hypothesised that cancer heritability could be apportioned to high-frequency low-penetrant variants, such as single nucleotide polymorphism (SNPs), which are co-inherited and synergistically modulate the risk of cancer in the broader population. Genome-Wide Association Studies (GWAS) have been instrumental in discovering SNPs associated with an increased risk of many diseases, including many types of cancers, such as breast [8, 30, 11], prostate [29, 9], testicular[16, 32, 17], chronic lymphocytic leukaemia [7, 27, 15], acute lymphocytic leukaemia [31,

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23] and several lymphomas [6, 10]. However, less than thousand cancer susceptibility SNPs have been identified, each accounting for a limited increase in cancer risk (odds-ratio, $OR < 1.5$). In general, the vast majority of SNPs show only subtle effects and their contribution is usually cancelled by multiple hypotheses correction procedures applied in GWAS analysis [24].

Although most SNPs show only weak, non statistically significant effects, they still contribute to the heritable risk, suggesting that cancer heritability will likely be polygenic; thus, accurate estimates of cancer heritability should take into account the contribution of all common SNPs in the genome. Moreover, since the functional impact of SNPs depends on the region where they are located [26], quantifying the amount of heritability apportioned to SNPs in protein-coding genes and nearby regulatory regions could provide new insights into the aetiology of this disease and identify new intervention points for treatment. Here we developed a new method, called BAyesian Gene HERitability Analysis (BAGHERA), which implements a new hierarchical Bayesian extension of Linkage Disequilibrium (LD) score regression [3], to obtain estimates of the heritability explained by all genotyped SNPs (genome-wide heritability) and SNPs in or around protein coding genes (gene-level heritability) from summary statistics.

We used BAGHERA to analyse 35 self-reported cancers in the UK Biobank [4], a large-scale prospective study aiming at systematically screening and phenotyping more than 500,000 individuals. In our study, we used available UK Biobank summary statistics for $N = 337,159$ patients [22]; each cancer has 798 cases on average and a relative prevalence ranging from $< 0.1\%$ to 2%, with higher estimates for common malignancies in European populations, such as breast and prostate cancer [2]. A custom pipeline was developed for pre-processing summary statistics to apply filters for SNP quality control, assign LD scores and annotate SNPs to genes, with overlapping genes merged into non-overlapping genic regions (see Online Methods).

We then estimated the genome-wide heritability of each cancer by computing the median of the posterior distribution of h_{SNP}^2 , and transforming this value on to the liability scale, h_{SNPL}^2 , to obtain estimates independent from prevalence and comparable across malignancies. We observed heritability values ranging from 4% for ovarian cancer up to 44% for adnexal cancer (Supplementary Table 1). Overall, 22 out of 35 cancers show heritability higher than 10%, consistent with other recent estimates and suggesting a significant contribution of SNPs to the heritable risk of cancer [25]. It is important to note that BAGHERA provided accurate estimates even for low-heritability cancers, whereas LD score regression returns negative values (see Supplementary Materials).

We then identified heritability genes for each cancer, defined as those with per-SNP heritability higher than the genome-wide estimate (see Online Methods). We found 13 heritability genes per malignancy on average, ranging from 2 genes for Non-Hodgkin's lymphoma to 246 for breast cancer (Figure 1A and Supplementary Table 2). Gene-level heritability across the 35 cancers has a long-tail distribution (Figure 1B), with a median 22.6-fold change compared to the genome-wide estimate, ranging from 4.5-fold for *SDK1* in breast cancer to 374.1-fold for *KITLG* in testicular cancer. Interestingly, 95% of heritability genes show heritability 10-fold higher than the genome-wide estimate. Heritability genes represent less than 1% of all the genes in the genome across all cancers; this result is consistent with cancer heritability being polygenic. However, heritability genes account for up to 43% of all the heritable risk (breast cancer), suggesting that a small set of genes has

the largest contribution to cancer heritability (Figure 1A). Consistent with our hypotheses, when we looked at the contribution of SNPs outside our protein-coding regions, we did not observe any difference compared to the genome-wide estimate. We then tested whether higher heritability could be explained by the presence of genome-wide significant SNPs ($P < 5 \times 10^{-8}$) nearby protein-coding regions. For each cancer, we identified genes harbouring at least 1 genome-wide significant SNP, and denoted this set as minSNPs; significant SNPs falling outside genic regions are assigned to the non-coding nuisance gene, for consistency with our analysis. We found 67 (56 in coding regions) minSNPs in total, with at least 1 minSNP in only 8 of the 35 cancers (Supplementary Table 3). This is a striking difference compared to the 13 heritability genes found on average in our analysis (Figure 1C and Supplementary Table 3); interestingly, our method was able to recover 47 (70%) of the minSNPs (84% of the coding ones), suggesting that it can detect heritability genes regardless of the association strength of their SNPs. Taken together, our analysis found 976 non-overlapping genic regions, which identify 1,430 genes in the genome, having a significant contribution to the heritable risk in at least 1 cancer. We denoted these 1,430 genes as Cancer Heritability Genes (CHGs).

We then characterised the functional role of CHGs to understand whether they are involved in biological processes and molecular functions mediating cancer phenotypes. We performed gene ontology enrichment analysis, using the slim gene ontology for human, finding 1,324 out of 1,430 CHGs to be annotated with at least one term. We found a statistically significant enrichment for 10 terms (False Discovery Rate, FDR $< 10\%$, Figure 2A and Supplementary Table 4). CHGs are enriched in cell morphogenesis ($OR : 1.62, P : 8.76 \times 10^{-6}$), cell division ($OR : 1.59, P : 2.78 \times 10^{-4}$) and cell adhesion ($OR : 1.35, P : 1.23 \times 10^{-3}$), which are biological processes underpinning tumor proliferation, invasion and metastasis; these classes include heritability genes such as *EGFR*, *FGFR2*, *SMAD3* and *NF1*. We also observed a significant enrichment of genes associated with cytoskeleton organization ($OR : 1.33, P : 1.64 \times 10^{-3}$) and anatomical structure development ($OR : 1.19, P : 2.68 \times 10^{-3}$), which includes members of the SWI/SNF complex, such as *ARID1B*. We further validated this finding by testing whether cancer heritability genes are associated with any hallmark of cancer [12]. Interestingly, we found 30 CHGs associated with at least one hallmark ($OR : 1.98, P : 1.1 \times 10^{-3}$); in particular, invasion, metastasis, growth and cell death are controlled by cancer heritability genes, such as *APC*, *CDKN2A*, *EGFR*, *ESR1*, *SMAD2* and *SMAD3* (2C and Supplementary Table 5). Taken together, we demonstrated that CHGs are directly implicated in biological processes underpinning tumorigenesis.

We then studied whether CHGs mediate cancer processes by acting either as tumour suppressor genes (TSGs) or oncogenes. To do that, we used the Precision Oncology Knowledge Base (OncoKB, [5]), an expert-curated list of 519 genes, including 197 tumour suppressor genes (TSGs), 148 oncogenes and other cancer genes of unknown function. We found that 58 CHGs (4%) are also cancer genes ($OR : 10.3, P : 0.0012$). Specifically, 30 CHGs are tumour suppressors ($OR : 1.83, P : 0.024$), whereas only 14 are reported as oncogene ($OR : 0.66, P : 0.92$) (Figure 2C). Tumour suppressor CHGs include well-known cancer driver genes, such as *CDKN2A*, *CDKN2B* and *CDKN2C*, which regulate cell growth, and DNA repair genes, such as *MUTYH* and *FANCA*. Our results suggest a functional role for cancer heritability genes consistent with a two-hit model [14]; while inherited mutations associated with oncogene activation are likely to be under purifying selection, mutations in tumour suppressor genes can be observed at higher frequency because

deleterious effects are only observed upon complete loss of function. We hypothesised that SNPs impairing the function of tumour suppressor genes could provide a selective advantage for tumour development.

Since CHGs are predominantly involved in common cell development functions, they may be thought unlikely to be specific for a particular cancer. Thus, we estimated the amount of heritability explained by CHGs across all cancers to test for pleiotropic effects. We observed that CHGs explain 22% of heritability on average, ranging from 43% for breast cancer to 2% for leukaemia (Figure 3A). However, the contribution of the heritability genes specific to each cancer was consistently higher than the CHGs contribution, suggesting the lack of pleiotropic effects. This observation is consistent with results from tumour sequencing studies, which have shown that most genes are mutated in only one type of cancer [1] and pleiotropic effects are limited to few master regulators, such as *TP53*, *PIK3CA* or potent oncogenes, such as *KRAS*. Analogously, of the 1,430 CHGs, $\approx 15.7\%$ show a significant heritability enrichment in at least 2 cancers, and only $\approx 2\%$ in 3 or more (Figure 3B-C and Supplementary Table 5). Our analysis suggests that while cancer heritability is controlled by mechanisms underpinning all cancers, the specific contribution of each gene is cancer specific; this could be explained by the fact that the same molecular function is accomplished by different genes in a tissue-specific way.

Our study provides new fundamental evidence demonstrating a strong contribution of high-frequency inherited mutations to the heritable risk of cancer. To do that, we developed a new method, called Bayesian Gene Heritability Regression Analysis (BAGHERA), which enabled the identification of 1,430 genes with a significant contribution to the heritable risk of 35 malignancies. We showed that these loci are tumor suppressors controlling cell development, adhesion and motility, which are fundamental processes required for tumorigenesis. Ultimately, our results support a two-hit model, where inherited mutations in tumour suppressor genes could create a favourable genetic background for tumorigenesis. It is therefore conceivable that SNPs make normal cells more likely to evade the cell-cell contact inhibition of proliferation, to elude the anatomical constraints of their tissue and to achieve more easily independent motility in presence of other early oncogenic events. Preliminary support for this model has been provided by studies in hereditary diffuse gastric cancer (HDGC) [20]; specifically, a germline mutation causing loss of E-Cadherin function, a crucial gene for cell adhesion, has been found to be a causal factor in up to 50% of all HDGC patients [13].

We also recognise the limitations of our work; while our method provides accurate estimates of genome-wide heritability, extremely low heritability diseases could lead to negative gene-level heritability estimates; this was a trade-off to ensure reasonable computational efficiency, although a rigorous model is provided as part of our software. Our analysis does not incorporate functional information, such as gene expression, which limits our power of detecting tissue-specific contributions. On this point, as the genes may be expressed in different cellular compartments, they may contribute to the stromal niches in which cancers develop and their role in tissue specificity of mutations will be of interest to analyse experimentally.

Taken together, our study provides a new view of the genetic architecture of cancer with gene-level resolution. We anticipate that the availability of genome editing techniques will enable testing of the functional mechanisms mediated by cancer heritability genes. We also expect that integrating our results with tumour sequencing data will provide new venues for personalised treatment and patients stratification.

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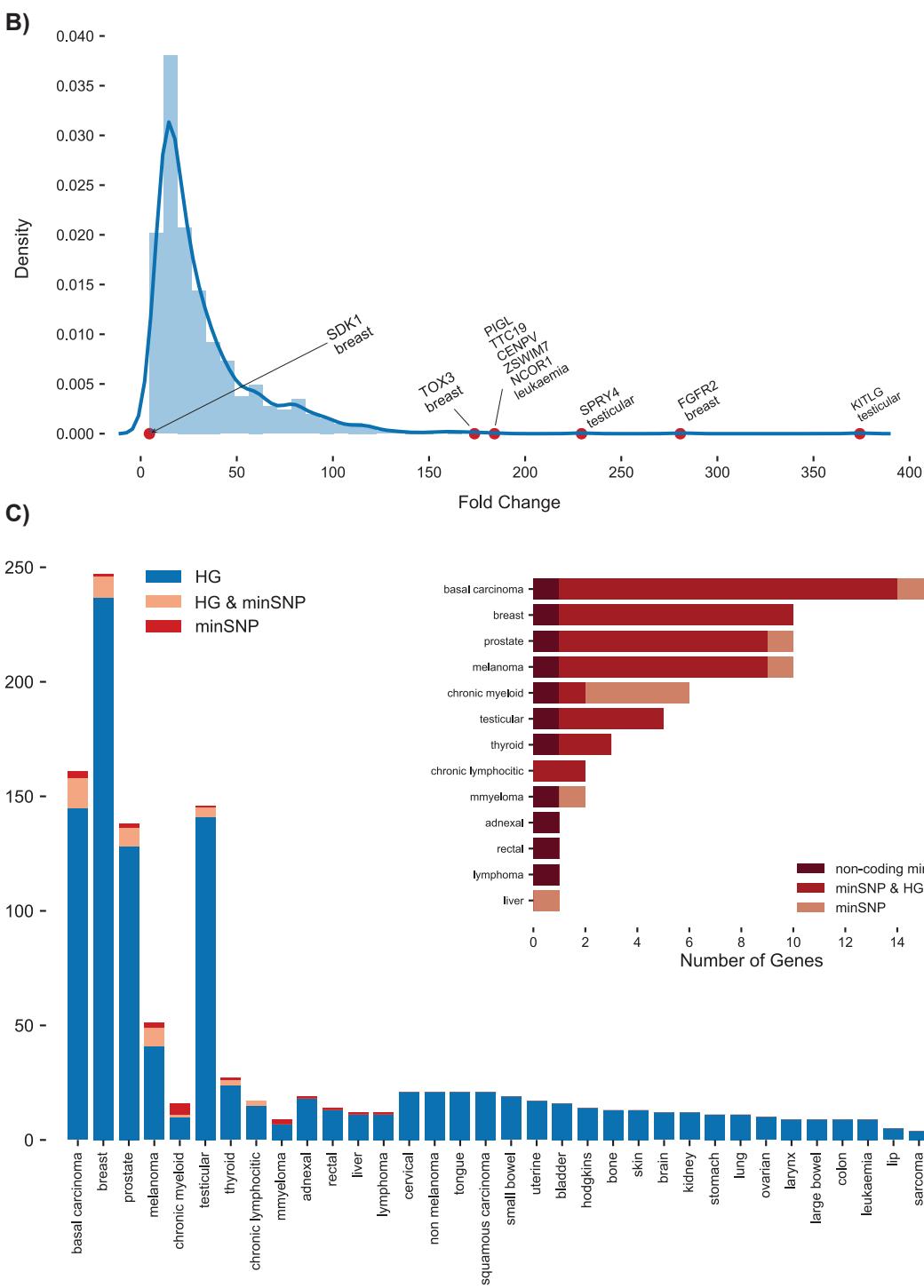
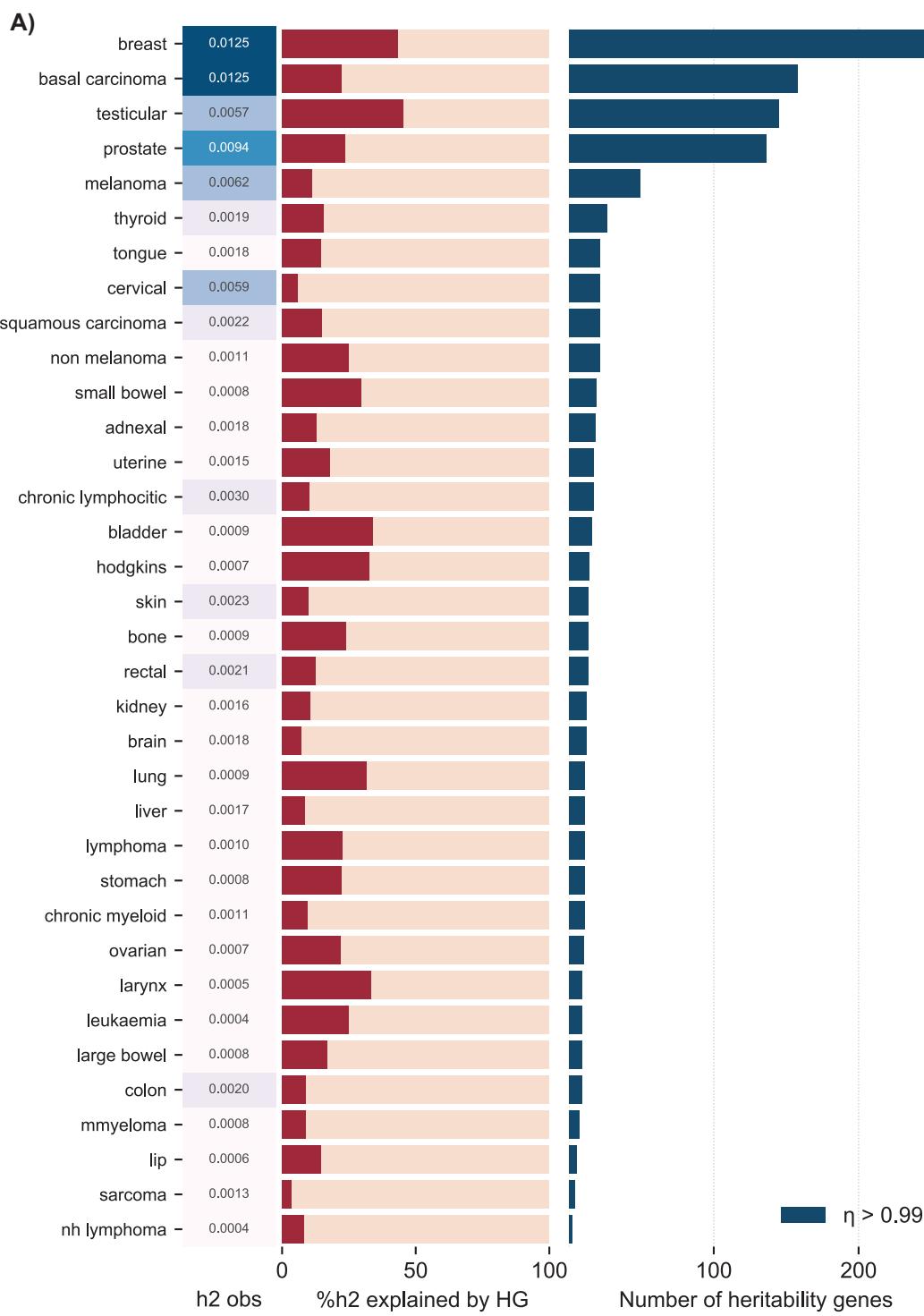
Figures

Figure 1: Heritability genes across 35 cancers in the UK Biobank. A) For each malignancy we report the observed heritability (h_{SNP}^2 , blue boxes), the percentage of h_{SNP}^2 explained by heritability genes (red barplot) and the number of heritability genes (blue barplot). B) Gene-level heritability distribution across heritability genes, expressed as fold-change with respect to the genome-wide estimate, including the top 5 genes observed and the one with the lowest fold-change. C) Comparison of heritability genes (HGs) and genes harbouring genome-wide significant SNPs (minSNPs). For each cancer, we report the number of cancer heritability genes (blue) and minSNPs (red), and those that are both HGs and minSNPs. For those cancers with at least one minSNP, we show the number of minSNPs captured in our analysis. With non-coding minSNP we report if the genome-wide significant SNPs are in the non coding areas of the genome.

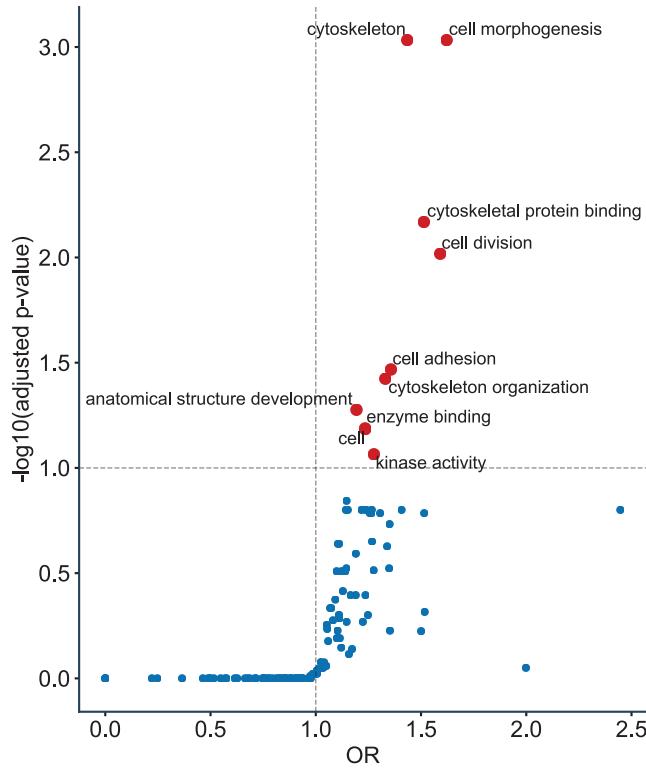
Figure 2: Cancer heritability genes are associated with the hallmarks of cancer. A) Gene ontology enrichment analysis of cancer heritability genes (CHGs). For each GO term, we report the odds ratio and its false discovery rate (FDR). Terms significant at the 10% FDR are reported in red, whereas non-significant ones in blue. B) Cancer heritability genes are associated with the hallmarks of cancer. C) Number of CHGs reported as cancer genes in the Precision Oncology Knowledge Base (OncoKB). For each cancer, we report cancer heritability genes annotated as cancer genes in red and those with uncharacterized function in blue. The inset plot shows the number of CHGs reported as tumour suppressors (TSG) or oncogenes.

Figure 3: A subset of cancer heritability genes are associated with multiple cancers. A) Percentage of heritability explained by all CHGs, with the red line representing the expected level of heritability explained by the genes assuming equal per SNP heritability contribution. B) Number of cancer heritability genes associated with multiple cancers, with more than 10% of CHGs being common to multiple malignancies. C) Cancer heritability genes associated with at least 2 malignancies.

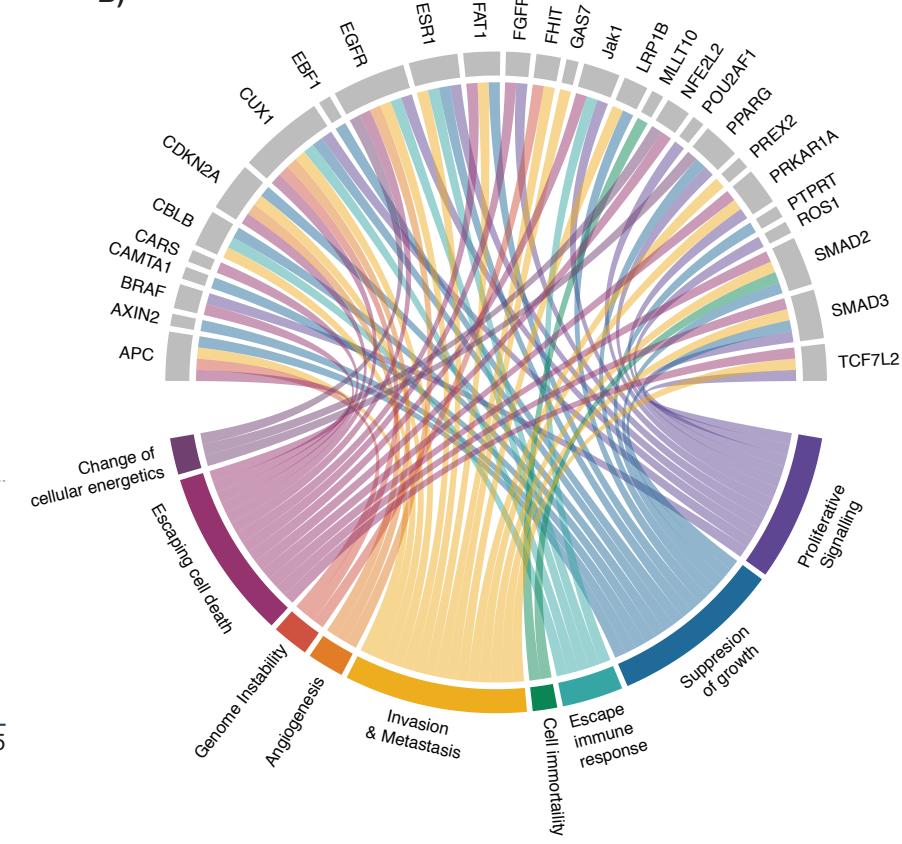
Figure 1



A)



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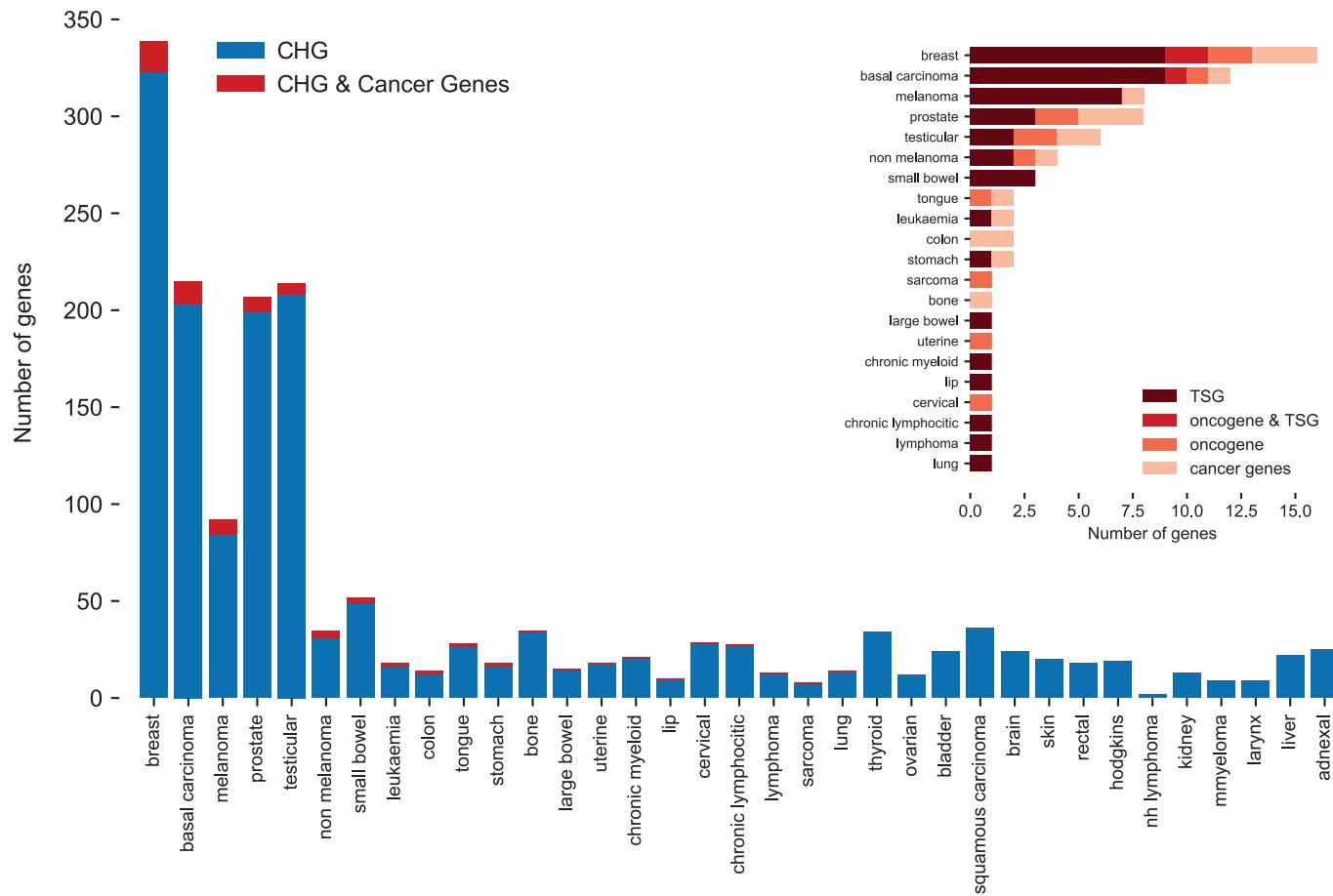


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