Assessment of Genetic Susceptibility to Multiple Primary Cancers through Whole-Exome Sequencing in Two Large Multi-Ancestry Studies

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ABSTRACT

Up to one of every six individuals diagnosed with one cancer will be diagnosed with a second primary cancer in their lifetime. Genetic factors contributing to the development of multiple primary cancers, beyond known cancer syndromes, have been underexplored. To characterize genetic susceptibility to multiple cancers, we conducted a pan-cancer, whole-exome sequencing study of individuals drawn from two large prospective cohorts (6,429 cases, 165,853 controls). We created two groupings of individuals diagnosed with multiple primary cancers: 1) an overall combined set with at least two cancers across any of 36 organ sites; and 2) cancer-specific sets defined by an index cancer at one of 16 organ sites with at least 50 cases from each study population. We then investigated whether variants identified from exome sequencing were associated with these sets of multiple cancer cases in comparison to individuals with one and, separately, no cancers. We identified 22 variant-phenotype associations, 10 of which have not been previously discovered and were significantly overrepresented among individuals with multiple cancers, compared to those with a single cancer. Overall, we describe variants and genes that may play a fundamental role in the development of multiple primary cancers and improve our understanding of shared mechanisms underlying carcinogenesis. Further investigation of these findings may lead to new screening strategies for individuals at risk for multiple primary cancers.
INTRODUCTION

The substantial global burden of cancer coupled with increasing survival due to improved screening, surveillance, and treatments has yielded a growing number of cancer survivors who are at risk of developing a second primary cancer in their lifetime. The prevalence of multiple primary cancers globally is estimated to be between 2 and 17%, with the wide range likely due to differences in cancer registration practices, case definitions, population characteristics, and follow-up times. Cancer predisposition syndromes, such as Li-Fraumeni, Lynch, and hereditary breast and ovarian cancer, are known to increase the risk of multiple primary cancers; however, less than 2% of all cancers are attributed to hereditary cancer syndromes. Genetic risk factors for multiple primary cancers beyond known syndromes are not well understood.

Genome-wide association studies (GWAS) have implicated many common, low penetrance variants in 5p15 (TERT-CLPTM1L), 6p21 (HLA), 8q24, and other loci in the risk of several cancer types. Additional studies have investigated pleiotropy in these regions or characterized cross-cancer susceptibility variants. A pleiotropic locus has the potential to not only affect risk of many different cancer types, but also increase the likelihood that a single individual develops multiple primary cancers. In our prior work, we discovered that the rare pleiotropic variant \( \text{HOXB13 G84E} \) had a stronger association with the risk of developing multiple primary cancers than of a single cancer. This suggests that there may be increased power to detect pleiotropic variation in individuals with multiple primary cancers relative to those with only a single cancer. Identifying widespread pleiotropic signals is informative for understanding shared genetic mechanisms of carcinogenesis, toward the identification of informative markers for cancer prevention and precision medicine.

In this study, we survey the landscape of rare and common variation in individuals with multiple primary cancers, single cancers, and cancer-free controls through whole-exome sequencing.
(WES) in two large, multi-ancestry studies. We evaluate associations previously discovered in studies of individuals with a single cancer and find novel pleiotropic variation in individuals with multiple primaries.

**MATERIAL AND METHODS**

**Study Populations and Phenotyping**

Our study included ancestrally diverse individuals with multiple primary cancers or no cancer from two large prospective studies: the Kaiser Permanente Research Bank (KPRB) and the UK Biobank (UKB). From the KPRB, we included individuals who were previously genotyped through the Research Program on Genes, Environment and Health (RPGEH) and the ProHealth Study. For the UKB, we specifically studied participants from the 200K release of WES data, which also included individuals diagnosed with a single cancer\(^{10}\).

For both study populations, ascertainment of cancer diagnoses has been previously described\(^{11,12}\). Both studies included prevalent and incident diagnoses of malignant, borderline, and in situ primary tumors\(^{12}\). ICD codes indicating non-melanoma skin cancer or metastatic cancer were not considered primary tumors. Cancers were primarily defined according to the SEER site recode paradigm\(^{13}\). However, for hematologic cancers, we incorporated morphology following WHO classifications\(^{14}\), placing cancers into three major subtypes: lymphoid neoplasms, myeloid neoplasms, and NK- and T-cell neoplasms (Table S1). Cases were individuals with ICD-9 or ICD-10 codes for primary tumors at two or more distinct organ sites. In the KPRB, controls without a cancer diagnosis were matched 1:1 to cases on age at specimen collection, sex, genotyping array (which matched on self-reported race/ethnicity), and reagent kit. In the UKB, controls included all individuals without a cancer diagnosis.
In both study populations, we excluded duplicates/twins and first-degree relatives, retaining the individual from each related pair who had higher coverage at targeted sites. Following quality control (QC) of WES data (described below), the KPRB and UKB study populations used in this project included 3,111 and 3,318 cases with multiple primary cancers and 3,136 and 162,717 cancer-free controls, respectively. The UKB also contributed 29,091 individuals with a single cancer diagnosis. While our study was primarily unselected for cancer type, prostate cancer cases were oversampled in the KPRB due to inclusion of individuals from the ProHealth Study.

Genetic Ancestry and Principal Components Analysis

Genetic ancestry was defined using genome-wide, imputed array data that underwent extensive QC, as previously described. Ancestry principal components (PCs) were computed using flashPCA by projecting our study samples onto PCs defined by 1000G phase 3 reference populations. Individuals were assigned to the closest reference population using distance from the top 10 PCs. Individuals with ancestral PCs greater than five standard deviations from the reference population mean were excluded. The final analytic dataset included individuals of European, African, East Asian, South Asian, and Hispanic/Latino ancestry (Figure S1). A total of N = 646 (10.2%) and N= 8,739 (5.26%) individuals were of non-European ancestry in the KPRB and UKB, respectively (Table 1).

Whole-Exome Sequencing and Quality Control

The Regeneron Genetics Center used the Illumina NovaSeq 6000 platform to perform WES for both study populations. Sample preparation and QC were performed using a high-throughput, fully-automated process that has been previously described in detail. Briefly, following sequencing, reads were aligned to the GRCh38 reference genome and variants were called with WeCall for the KPRB and DeepVariant for the UKB. Samples with gender discordance, 20x coverage at less than 80% of targeted sites, and/or contamination greater than 5% were excluded.
Additional QC was applied to filter low quality variants and related individuals. First, genotype calls with low depth of coverage (DP) were updated to missing (DP < 7 for SNPs and DP < 10 for indels). Then, sites with low allele balance (AB) were removed. Specifically, variants without at least one sample having AB ≥ 15% for SNPs or AB ≥ 20% for indels were excluded. Additionally, variants with missingness > 10% and HWE p-value < 10^{-15} were excluded. Following these steps, a total of ~3.51M high-quality sites were retained for the KPRB and ~15.92M were retained for the UKB; excluding singletons, there were ~1.36M and ~8.22M variants, respectively. In the UKB, the larger number of variants observed was due to rare variation present in the larger sample size; when restricting to common variants (MAF > 1%), there were ~186K and ~137K variants, respectively for the KPRB and UKB.

**Association Analyses in Individuals with Multiple Cancers versus Cancer-Free Controls**

Genetic association analyses of single variants and genes investigated the following cancer phenotypes: (1) diagnosis with at least two primary cancers across any of the 36 organ sites ("any 2+ primary cancers") and (2) groupings of individuals defined by a shared index cancer at one of 16 organ sites with at least 50 cases from each study population. ("cancer-specific analyses"). Primary analyses compared multiple cancer cases to cancer-free controls. Within our cancer-specific analyses of 16 organ sites, there were cases shared across our index cancer groupings. For example, the set of individuals with at least one diagnosis of breast cancer overlaps with those having at least one ovarian cancer diagnosis.

Single-variant and gene-based association analyses were performed using REGENIE v2.2.4, a machine-learning approach for performing whole-genome regression that adjusts for case-control imbalance by applying saddlepoint approximation when the standard case-control p-value is less than 0.05^{19}. We assessed single-variant associations for high-quality variants with minor allele...
count (MAC) > 2. WES variants were functionally annotated using SnpEff v5.0\textsuperscript{20} and dbNFSP v3.5\textsuperscript{21} accessed through ANNOVAR\textsuperscript{22}. Missense variants were classified using five algorithms: (1) SIFT (“D”); (2) HDIV from Polyphen2; (3) HVAR from Polyphen2; (4) LRT (“D”); and (5) MutationTaster (“A” or “D”). For our gene-based burden analyses, we used three minor allele frequency cut-offs (MAF < 0.5%, 1%, or 5%), including singletons, computed within each population. Following previous work, three gene-based models were evaluated\textsuperscript{23}: (1) all rare variants with predicted loss-of-function (pLOF) by SnpEff, (2) pLOF and missense rare variants predicted to be deleterious by the above five classification algorithms, and (3) pLOF and missense rare variants predicted to be deleterious by at least one algorithm. Out of all allele frequency and burden combinations, we report the burden test with the lowest p-value. In the case of ties, we report the most restrictive grouping (fewest number of variants included). In our gene-based and single-variant analyses, we adjusted for covariates including age, top 10 PCs, and sex (except for sex-specific index cancers of the breast, cervix, ovary, uterus, other female genital organ, and prostate). In the KPRB population, we additionally adjusted for genotyping array and reagent kit, as they were used to perform case-control matching. In the UKB, we adjusted for flow cell (S2 vs S4), which differed for the initial 50K and subsequent 150K release of WES samples.

Single-variant and gene-based burden analyses for each phenotype were combined across study populations in a fixed-effects meta-analysis using METASOFT\textsuperscript{24} and metafor v3.0.2\textsuperscript{25}, respectively. For our single-variant analyses, we report all suggestive, independent [linkage disequilibrium (LD) $r^2 < 0.2$] associations with $p < 5 \times 10^{-6}$. For our gene-based analyses, we report all associations adjusted for the number of genes tested ($p < 2.65 \times 10^{-6} = 0.05/18,842$). We report meta-analysis p-values (Main Text), except when a variant was unique to a single study population (Supplements).

**Distinguishing Susceptibility Signals for Multiple Cancers versus Single Cancers**

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We also evaluated whether the variants and genes associated with the diagnosis of multiple primary cancers (versus non-cancer controls) remained associated when comparing individuals with multiple cancers to those diagnosed with a single cancer. These analyses assessed whether the variants or genes were pleiotropic for developing multiple cancers or general markers of susceptibility to a specific cancer. We undertook these analyses in the UKB sample only, since individuals diagnosed with a single primary cancer were not sequenced in the KPRB. Single-variant and gene-level analyses were implemented as described above. For each variant or gene of interest identified in our case-control analyses, we performed a case-case analysis comparing individuals diagnosed with multiple cancers to those diagnosed with a single cancer. For our cancer-specific analyses, we compared individuals diagnosed with the index cancer plus any other cancer to those diagnosed with the index cancer only. For example, for a finding discovered in our cancer-specific analysis of prostate cancer, we performed a case-case analysis comparing individuals diagnosed with prostate cancer plus any other cancer to individuals with only a prostate cancer diagnosis.

RESULTS

Characterization of Multiple Primary Cancer Diagnoses in Two Large Study Populations

Our meta-analyses included 6,429 cases with multiple primary cancers and 165,853 cancer-free controls (Table 1). All cases had at least two independent primary cancer diagnoses, and 656 cases had more than two diagnoses (Figure S2). In the KPRB, the maximum number of cancer diagnoses for an individual was 6 (n = 1) and in the UKB, the maximum number was 5 (n = 2). Overall, 36 unique cancer sites were represented across multiple cancer cases in the two study populations, with 180 unique pairs of sites (e.g., breast and melanoma) and 298 unique pairs of sites and diagnostic sequence (e.g., breast followed by melanoma) (Table S2). Only 51 of the 298 ordered pairs had at least 25 cancer cases when grouping individuals by first and second cancer diagnosis (i.e., ignoring any subsequent cancer diagnoses; Table S2, Figure 1). The top
ordered pairs represented in the combined study populations were prostate then melanoma (N = 221), cervix then breast (N = 202), melanoma then prostate (N = 180), breast then melanoma (N = 174), and prostate then colorectal (N = 170). Prostate, breast, melanoma, colorectal, and cervix were the most common sites of first cancer diagnoses (Figure 1). The prevalence of each cancer pair was similar in the KPRB and UKB (Figure S3). As most individual cancer pairs were underpowered for downstream analysis, we considered all multi-cancer cases combined, as well as groupings of individuals with a shared index cancer (16 cancers) (Figure S4, Table S3). Among those with multiple cancers, the cancers with the largest number of cases were prostate (N = 1,977; oversampled in KPRB), breast (N = 1,874), melanoma (N = 1,443), colorectal (N = 1,324), and urinary bladder (N = 829).

Exome-wide Single Variant Association Analyses

We found 22 associations (p < 5x10^{-6}) between individual variants and the multiple cancer phenotypes (i.e., either any 2+ primary cancers or cancer-specific analyses) (Figure 2, Table S4). We found an additional four associations (Figure S5) in our cancer-specific analyses of lymphoid and myeloid neoplasms; however, we assumed them to represent somatic alterations in the blood as they had low allele balance across our heterogeneous samples (Figure S6) and occur in genes known to be impacted by clonal hematopoiesis of indeterminate potential (CHIP)\textsuperscript{26}. Results were relatively homogeneous across the KPRB and UKB study populations (Table S4).

We detected two variants associated with any 2+ primary cancers, rs555607708 (OR [95% CI] = 2.72 [1.79, 4.15], p = 3.10x10^{-6}), a frameshift variant in CHEK2 known to be associated with risk at many cancer sites\textsuperscript{27}, and rs146381257 (OR [95% CI] = 7.82 [3.28, 18.62], p = 3.45x10^{-6}), a 5’upstream variant in ZNF106. The risk-increasing allele for rs555607708 (CHEK2) was most commonly found among individuals with at least one breast cancer (41.9%), prostate cancer (30.6%), melanoma (22.6%), or cervical cancer (16.1%) (Figure 2). For rs146381257 (ZNF106),
frequencies were increased in prostate cancer (33.3%), lung cancer (28.6%), breast cancer (28.6%), lymphoid neoplasms (23.8%), urinary bladder cancer (19.0%), pancreatic cancer (14.3%), and kidney cancer (14.3%).

Cancer-specific analyses identified 10 associations between previously reported risk variants for a single cancer and risk of diagnosis with that cancer plus any other cancer (Figure 2). Notably, we detected an association with the MC1R variant rs1805008 for melanoma\(^{28}\) (OR [95% CI] = 1.56 [1.35, 1.81], \(p = 2.73 \times 10^{-9}\)) when comparing all individuals with at least one melanoma diagnosis plus any other cancer diagnosis to cancer-free controls. We also replicated the previously associated prostate-specific antigen (PSA) variant, rs17632542\(^{29}\) (KLK3, OR [95% CI] = 1.49 [1.28, 1.73], \(p = 3.87 \times 10^{-7}\)) in individuals with at least one prostate cancer diagnosis. In addition, we replicated associations between missense risk variant rs6998061 (8q24 locus, POU5F1B) and multiple tumor types in both our prostate cancer-specific analysis\(^{30}\) (OR [95% CI] = 1.23 [1.13, 1.33], \(p = 4.39 \times 10^{-7}\)) and our colorectal cancer-specific analysis\(^{31}\) (OR [95% CI] = 1.25 [1.15, 1.37], \(p = 1.06 \times 10^{-7}\)).

The remaining variants demonstrating associations with multiple cancer phenotypes were not previously associated with any single cancer (Figure 2). They included a variant discovered in our breast cancer-specific analysis, rs143745791 (NCPB1, OR [95% CI] = 5.95 [2.79, 12.67], \(p = 3.76 \times 10^{-6}\)), for which 16.2% of carriers, restricted to cases, had a breast and cervical cancer diagnosis, and a variant discovered in our urinary bladder cancer-specific analysis, rs141647689 (SDK1, OR [95% CI] = 9.29 [3.63, 23.80], \(p = 3.45 \times 10^{-6}\)), for which 14.3% of carriers also had prostate cancer (Figure 2). Three variants found in our lymphoid neoplasm-specific analysis had increased frequencies in cases who also had a diagnosis of prostate cancer: rs535484207 (RANBP2, OR [95% CI] = 256.01 [26.82, 2,442.95], \(p = 1.46 \times 10^{-6}\), rs139586367 (UFL1, OR [95% CI] = 284.06 [27.95, 2,886.15], \(p = 1.79 \times 10^{-6}\), and rs191064896 (ADGRB1, OR [95% CI] = 108.36...
[15.02, 781.08], p = 3.32x10^{-6}), where 21.4%, 40.0%, and 25.0% of carriers for the risk-increasing allele, for each respective variant, had both cancers. The ADGRB1 variant was also present at increased frequencies among individuals with a lymphoid neoplasm and breast cancer diagnosis (25.0%, Figure 2).

**Gene-Based Analyses of Multiple Cancers**

Out of 18,842 genes tested, we found 11 significant associations (p < 2.65x10^{-6}) across our analyses of any 2+ primary cancers and our cancer-specific analyses (Figure 3, Table S5). An additional four CHIP genes (ASXL1, TET2, JAK2, and DDX41) were significantly associated with myeloid neoplasms and are likely driven by somatic alterations (Figure S7).

In our analyses of any 2+ primary cancers and our breast cancer-specific analysis, we replicated associations for known pleiotropic genes, BRCA2 (pLOF, p = 3.76x10^{-11} and 1.91x10^{-9}) and CHEK2 (pLOF + missense, p = 2.95x10^{-11} and 1.67x10^{-8}) (Figure 3). BRCA2 also emerged in our ovarian cancer-specific analysis (pLOF, p = 1.91x10^{-9}). We found associations between the known prostate cancer gene ATM and any 2+ primary cancers and in our prostate cancer-specific analysis (pLOF + missense, p = 9.84x10^{-7} and 2.56x10^{-6}). Additional associations were observed between SAMHD1 and SLC642 and any 2+ primary cancers (pLOF + missense, p = 2.40x10^{-7} and p = 5.44x10^{-7}, respectively). BRCA1 also surfaced in the breast cancer-specific analysis (pLOF, p = 6.68x10^{-6}), as did AHCTF1 in the head and neck cancer-specific analysis (pLOF + missense, p = 1.25x10-6).

Functional variants in BRCA1 and BRCA2 were present at increased frequencies in individuals with a breast cancer diagnosis and ovary as an additional cancer site (Figure 3), such that 28.6% and 13.6% of individuals, respectively, were a carrier for at least one variant in the burden set. For BRCA1, there was also an increase of carriers with an additional melanoma (9.52%) or lung...
cancer (9.52%) diagnosis. For BRCA2, there was an increase of carriers with an additional uterine
(8.47%), lung (6.78%), or colorectal cancer (6.78%).

Comparison of Mutation Burden in Individuals with Multiple versus Single Cancers

Out of the 22 associated variants (above), 10 remained associated when comparing individuals
with multiple cancers to those with single cancers (Table S6; p < 0.05). Two of these variants
were positively associated in our analysis of any 2+ primary cancers: rs555607708 (CHEK2; OR
[95% CI] = 1.57 [1.09, 2.25], p = 0.015) and rs146381257 (ZNF106; OR [95% CI] = 5.38 [1.07,
27.18], p = 0.042). The other eight variants were positively associated with diagnosis of a specific
index cancer plus any other cancer versus the specific cancer alone (Table S6). Two of these
eight variants were associated in our breast cancer-specific case-case analysis: rs7872034, a
missense variant in SMC2 (OR [95% CI] = 1.16 [1.05, 1.27], p = 0.0025) and rs143745791, a
missense variant in NCBP1 (OR [95% CI] = 3.71 [2.08, 6.61], p = 8.37x10^-6).

Of the 11 findings from the gene-level burden analyses (above), seven remained positively
associated with multiple cancers in comparison with single cancers (p<0.05; Table S7). Four of
these genes were discovered in our case-case analysis of any 2+ primary cancers: ATM (OR
[95% CI] = 1.20 [1.06, 1.36], p = 0.00399), CHEK2 (OR [95% CI] = 1.56 [1.23, 1.98], p = 2.31x10^-4),
SAMHD1 (OR [95% CI] = 1.56 [1.14, 2.13], p = 5.34x10^-3), and BRCA2 (OR [95% CI] = 1.86
[1.31, 2.65], p = 5.43x10^-4). ATM (OR [95% CI] = 1.31[1.01, 1.68], p = 0.038) was positively
associated in our prostate cancer-specific case-case analysis, and the two remaining genes were
positively associated in our breast cancer-specific case-case analysis: BRCA1 (OR [95% CI] =
2.38 [1.07, 5.30], p = 0.034) and BRCA2 (OR [95% CI] = 1.97 [1.22, 3.18], p = 0.0055).

DISCUSSION
We investigated the genetic basis of carcinogenic pleiotropy through whole exome sequencing of individuals diagnosed with multiple primary cancers from two large, multi-ancestry study populations. Comparing individuals with multiple cancers to cancer-free controls uncovered 22 independently associated variants, ten of which remained associated when comparing individuals with multiple cancers to those with a single cancer. We also found significant associations between the genes AHCTF1, ATM, BRCA1/2, CHEK2, SAMHD1, and SLC6A2 and our multiple cancer phenotypes. Other than AHCTF1 and SLC6A2, these genes remained associated with multiple cancer diagnoses when comparing to individuals with a single cancer. These findings offer insights into germline exome variants that increase an individual’s risk of developing multiple primary cancers.

Compelling findings from our analyses of all individuals with more than one cancer diagnosis include associations with the rare variant rs146381257 in ZNF106. Carriers of the rs146381257 risk allele (C) were primarily over-represented in individuals with at least one prostate, breast, lung, or urinary bladder cancer and in individuals with lymphoid neoplasms. Carriers also demonstrated an increased risk of developing multiple cancers compared to individuals with a single cancer. ZNF106 is an RNA binding protein involved in post-transcriptional regulation and insulin receptor signaling. Although germline variation in ZNF106 has not previously been associated with cancer risk, a recent study found it to be associated with worse urinary bladder cancer survival.

Additional noteworthy findings from our analyses of all multiple primary cancers combined include cancer susceptibility signals in SAMHD1 and SLC6A2. Carriers of rare and potentially deleterious variants in SAMHD1, a gene with a plausible tumor suppressor role, had a significantly higher risk being diagnosed with multiple cancers compared to single cancers. Germline SAMHD1 mutations are implicated in Aicardi-Goutieres Syndrome (AGS), an autosomal recessive
condition that results in autoimmune inflammatory encephalopathy. Most cancer-related studies have focused on the role of somatic alternations in \textit{SAMHD1}\textsuperscript{35}. However, a study of chronic lymphoid leukemia (CLL) proposed an oncogenic role of germline \textit{SAMHD1} variation mediated by DNA repair mechanisms\textsuperscript{36}. Consistent with this hypothesis, we also found increased \textit{SAMHD1} variation in individuals with lymphoid neoplasms, as well as with prostate, breast, colorectal and lung cancers. \textit{SLC6A2}, also known as \textit{NAT1}, has been found to be prognostic for colon cancer\textsuperscript{37}, and both in-vivo and in-vitro studies have linked expression to survival in many cancer types, including prostate\textsuperscript{38} and breast\textsuperscript{39}. Polymorphisms in \textit{SLC6A2} may also interact with smoking exposure to modulate risk for tobacco-related cancers\textsuperscript{40}. In our study, the increased cancer risk detected among \textit{SLC6A2} carriers was limited to comparisons with cancer-free controls.

Because we compared multiple primary cancers with both cancer-free controls and individuals diagnosed with a single cancer, we were well positioned to explore patterns of pleiotropy and disentangle variation likely to be driven by single cancers. For example, we identified two variants, rs7872034 (missense variant in \textit{SMC2}) and rs143745791 (missense variant in \textit{NCBP1}), associated with a diagnosis of at least one breast cancer (plus any other cancer) versus no cancer. These variants remained associated with a diagnosis of breast and another cancer when comparing to individuals diagnosed with a single breast cancer. While rs7872034 is in high LD (r\textsuperscript{2} = 0.98) with a known breast cancer risk variant (rs4742903; \textit{SMC2} intron)\textsuperscript{41}, it may also increase the risk of developing multiple cancers. Regarding rs143745791, germline variants in \textit{NCBP1} have not been previously associated with cancer; because it is rare (MAF < 0.2%), larger sequencing efforts may be necessary identify variation in studies of individuals with a single cancer. Expression of this gene has been found to promote lung cancer growth and poor prognosis\textsuperscript{42}, and \textit{NCBP1} is overexpressed in basal-like and triple-negative breast cancers\textsuperscript{43}. Similarly, \textit{BRCA1/2} germline variants are prevalent among these subtypes; however, in our study
populations, BRCA1/2 carriers were more common among those with an additional ovarian cancer whereas NCBP1 carriers more frequently had an additional cervical cancer.

In our prostate cancer-specific analysis comparing individuals with multiple cancers versus those with only a single cancer, we discovered an association with rs3020779, an eQTL for RNF123 (also known as KPC1), which is a gene involved in p50 mediation and downstream stimulation of multiple tumor suppressors.44 In our analysis of head and neck cancer, we detected an association with rs12253181 (eQTL for RTKN2); while this gene has not previously been associated with head and neck cancer risk, it has been shown to function as an oncogene in non-small cell lung cancer (NSCLC) and decreasing its expression may inhibit proliferation by inducing apoptosis.45

Limitations of our study included the identification of variants that were likely-somatic in our analyses of hematologic cancers due to an expansion of hematopoietic clonal populations with the same acquired mutation (i.e., CHIP). Confounding of germline testing by CHIP has been reported in TP53 and TET2, so careful interpretation is critical to avoid unnecessary clinical intervention. An additional limitation of our, and other, studies are obtaining accurate effects estimates for rare variants and the reliance on available annotations for inclusion into gene-based tests. Replication of rare findings in larger cohorts and optimization of functional impact annotations could lead to more precise results. Also, while our approach did not allow for formal replication, it was designed to identify signals for a largely understudied phenotype that were concordant in two populations. Finally, while all individuals with multiple cancers were included in our study regardless of genetic ancestry, non-European ancestries were underrepresented; larger, more diverse cohorts will be needed to fully explore the genetic basis of multiple cancers.
Strengths of this work include studying individuals of multiple ancestries who were largely unselected for specific cancer phenotypes. We also performed the first ever exome-wide study of genetic susceptibility to multiple primary cancers, using two large prospective study populations. Our study design allowed us to characterize variation across multiple primary cancers representing 36 unique sites, as well as to conduct cancer-specific analyses of 16 sites. Using this approach, we confirmed many known single-variant and gene-based findings, strengthening and supporting our novel results reported for individual cancers through our cancer-specific analyses.

In summary, by undertaking an exome-wide survey of common and rare variation in two large study populations, we identified several variant and gene-based associations that may increase the risk of developing multiple cancers within individuals. Our findings have potential implications for improving our understanding of the shared mechanisms of carcinogenesis. They may also enable screening strategies that prioritize individuals at risk for developing additional cancers. Furthermore, since many of the genes reported here have been considered as potential therapeutic targets in cancer, our work supports the use of germline information to help guide precision medicine. Future studies should aim to replicate our findings and undertake experiments that validate the functionality of the discovered pleiotropic variants. Combined with future research, our results have potential to inform genetic counseling, improve risk prediction for multiple cancers, and guide novel treatment and drug development.
SUPPLEMENTAL DATA
Supplements_Tables.xls

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WEB RESOURCES

REGENIE
Snpeff
ANNOVAR
dbNSFPv3.5
flashPCA2
bcftools
plink

DATA AVAILABILITY

All results from this study are available from the article or Supplementary Materials. The UK Biobank cohort data is publicly available from the UK Biobank access portal at https://www.ukbiobank.ac.uk. The Kaiser Permanente Research Bank data are available on dbGAP. All remaining relevant data are available in the article, supplementary information, or from the corresponding author upon reasonable request.
1. Vogt, A. et al. Multiple primary tumours: challenges and approaches, a review. *ESMO Open* 2, e000172 (2017).

2. Copur, M. S. & Manapuram, S. Multiple Primary Tumors Over a Lifetime. *Oncology (Williston Park)* 33, 629384 (2019).

3. Gaspar, T. B. et al. Telomere Maintenance Mechanisms in Cancer. *Genes* 9, 241 (2018).

4. Smedby, K. E. et al. GWAS of Follicular Lymphoma Reveals Allelic Heterogeneity at 6p21.32 and Suggests Shared Genetic Susceptibility with Diffuse Large B-cell Lymphoma. *PLoS Genet* 7, e1001378 (2011).

5. Karnes, J. H. et al. Phenome-wide scanning identifies multiple diseases and disease severity phenotypes associated with HLA variants. *Sci. Transl. Med.* 9, eaa8708 (2017).

6. Huppi, K., Pitt, J. J., Wahlberg, B. M. & Caplen, N. J. The 8q24 Gene Desert: An Oasis of Non-Coding Transcriptional Activity. *Front. Gene.* 3, (2012).

7. Rashkin, S. R. et al. Pan-cancer study detects genetic risk variants and shared genetic basis in two large cohorts. *Nat Commun* 11, 4423 (2020).

8. Lindström, S. et al. Quantifying the Genetic Correlation between Multiple Cancer Types. *Cancer Epidemiol Biomarkers Prev* 26, 1427–1435 (2017).

9. Hoffmann, T. J. et al. Imputation of the Rare HOXB13 G84E Mutation and Cancer Risk in a Large Population-Based Cohort. *PLoS Genet* 11, e1004930 (2015).

10. Szustakowski, J. D. et al. Advancing Human Genetics Research and Drug Discovery through Exome Sequencing of the UK Biobank. http://medrxiv.org/lookup/doi/10.1101/2020.11.02.20222232 (2020)
doi:10.1101/2020.11.02.20222232.

11. Rashkin, S. R. et al. Pan-Cancer Study Detects Novel Genetic Risk Variants and Shared Genetic Basis in Two Large Cohorts. *bioRxiv* 635367 (2019) doi:10.1101/635367.
12. Graff, R. E. et al. Cross-Cancer Evaluation of Polygenic Risk Scores for 17 Cancer Types in Two Large Cohorts. http://biorxiv.org/lookup/doi/10.1101/2020.01.18.911578 (2020) doi:10.1101/2020.01.18.911578.

13. Adamo, M., Groves, C., Dickie, L. & Ruhl, J. SEER Program Coding and Staging Manual 2021. National Cancer Institute, Bethesda, MD 20892. (2020).

14. Harris, N. L. et al. The World Health Organization Classification of Neoplasms of the Hematopoietic and Lymphoid Tissues: Report of the Clinical Advisory Committee Meeting – Airlie House, Virginia, November, 1997. Hematol J 1, 53–66 (2000).

15. Abraham, G., Qiu, Y. & Inouye, M. FlashPCA2: principal component analysis of Biobank-scale genotype datasets. Bioinformatics 33, 2776–2778 (2017).

16. The 1000 Genomes Project Consortium. A global reference for human genetic variation. Nature 526, 68–74 (2015).

17. Geisinger-Regeneron DiscovEHR Collaboration et al. Exome sequencing and characterization of 49,960 individuals in the UK Biobank. Nature 586, 749–756 (2020).

18. Yun, T. et al. Accurate, scalable cohort variant calls using DeepVariant and GLnexus. Bioinformatics 36, 5582–5589 (2021).

19. Mbatchou, J. et al. Computationally efficient whole-genome regression for quantitative and binary traits. Nat Genet 53, 1097–1103 (2021).

20. Cingolani, P. et al. A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of Drosophila melanogaster strain w1118; iso-2; iso-3. Fly (Austin) 6, 80–92 (2012).

21. Dong, C. et al. Comparison and integration of deleteriousness prediction methods for nonsynonymous SNVs in whole exome sequencing studies. Human Molecular Genetics 24, 2125–2137 (2015).

22. Wang, K., Li, M. & Hakonarson, H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Research 38, e164–e164 (2010).
23. Backman, J. D. et al. Exome sequencing and analysis of 454,787 UK Biobank participants. *Nature* **599**, 628–634 (2021).

24. Han, B. & Eskin, E. Random-Effects Model Aimed at Discovering Associations in Meta-Analysis of Genome-wide Association Studies. *The American Journal of Human Genetics* **88**, 586–598 (2011).

25. Viechtbauer, W. Conducting Meta-Analyses in R with the *metafor* Package. *J. Stat. Soft.* **36**, (2010).

26. Steensma, D. P. et al. Clonal hematopoiesis of indeterminate potential and its distinction from myelodysplastic syndromes. *Blood* **126**, 9–16 (2015).

27. Cybulski, C. et al. CHEK2 Is a Multiorgan Cancer Susceptibility Gene. *The American Journal of Human Genetics* **75**, 1131–1135 (2004).

28. Amos, C. I. et al. Genome-wide association study identifies novel loci predisposing to cutaneous melanoma. *Hum Mol Genet* **20**, 5012–5023 (2011).

29. Li, H., Fei, X., Shen, Y. & Wu, Z. Association of gene polymorphisms of KLK3 and prostate cancer: A meta-analysis. *Adv Clin Exp Med* **29**, 1001–1009 (2020).

30. Hazelett, D. J. et al. Comprehensive functional annotation of 77 prostate cancer risk loci. *PLoS Genet* **10**, e1004102 (2014).

31. Hutter, C. M. et al. Characterization of the association between 8q24 and colon cancer: gene-environment exploration and meta-analysis. *BMC Cancer* **10**, 670 (2010).

32. Wu, Y. et al. Identification of the Functions and Prognostic Values of RNA Binding Proteins in Bladder Cancer. *Front. Genet.* **12**, 574196 (2021).

33. Herold, N. et al. With me or against me: Tumor suppressor and drug resistance activities of SAMHD1. *Experimental Hematology* **52**, 32–39 (2017).

34. Martinez-Lopez, A. et al. SAMHD1 deficient human monocytes autonomously trigger type I interferon. *Molecular Immunology* **101**, 450–460 (2018).
35. Mauney, C. H. & Hollis, T. SAMHD1: Recurring roles in cell cycle, viral restriction, cancer, and innate immunity. *Autoimmunity* **51**, 96–110 (2018).

36. Clifford, R. *et al.* SAMHD1 is mutated recurrently in chronic lymphocytic leukemia and is involved in response to DNA damage. *Blood* **123**, 1021–1031 (2014).

37. Shi, C. *et al.* Hypermethylation of N-Acetyltransferase 1 Is a Prognostic Biomarker in Colon Adenocarcinoma. *Front. Genet.* **10**, 1097 (2019).

38. Tiang, J. M., Butcher, N. J., Cullinane, C., Humbert, P. O. & Minchin, R. F. RNAi-Mediated Knock-Down of Arylamine N-acetyltransferase-1 Expression Induces E-cadherin Up-Regulation and Cell-Cell Contact Growth Inhibition. *PLoS ONE* **6**, e17031 (2011).

39. Minchin, R. F. & Butcher, N. J. Trimalodal distribution of arylamine N-acetyltransferase 1 mRNA in breast cancer tumors: association with overall survival and drug resistance. *BMC Genomics* **19**, 513 (2018).

40. McKay, J. D. *et al.* Sequence Variants of NAT1 and NAT2 and Other Xenometabolic Genes and Risk of Lung and Aerodigestive Tract Cancers in Central Europe. *Cancer Epidemiology Biomarkers & Prevention* **17**, 141–147 (2008).

41. kConFab Investigators *et al.* Genome-wide association study identifies 32 novel breast cancer susceptibility loci from overall and subtype-specific analyses. *Nat Genet* **52**, 572–581 (2020).

42. Zhang, H. *et al.* NCBP1 promotes the development of lung adenocarcinoma through up-regulation of CUL4B. *J Cell Mol Med* **23**, 6965–6977 (2019).

43. Wang, L. *et al.* Novel RNA-Affinity Proteogenomics Dissects Tumor Heterogeneity for Revealing Personalized Markers in Precision Prognosis of Cancer. *Cell Chemical Biology* **25**, 619-633.e5 (2018).

44. Kravtsova-Ivantsiv, Y. *et al.* Excess of the NF-κB p50 subunit generated by the ubiquitin ligase KPC1 suppresses tumors via PD-L1– and chemokines-mediated mechanisms. *Proc Natl Acad Sci USA* **117**, 29823–29831 (2020).
45. Ji, L. et al. RTKN2 is Associated with Unfavorable Prognosis and Promotes Progression in Non-Small-Cell Lung Cancer. OTT Volume 13, 10729–10738 (2020).

46. Weitzel, J. N. et al. Somatic TP53 variants frequently confound germ-line testing results. Genetics in Medicine 20, 809–816 (2018).

47. Tulstrup, M. et al. TET2 mutations are associated with hypermethylation at key regulatory enhancers in normal and malignant hematopoiesis. Nat Commun 12, 6061 (2021).
Figure 1. Cancer Diagnosis Pairs Present in the Combined Study Populations

Figure 1 Legend: Circos plot describing the pairs of first and second cancer diagnoses with at least 25 cases present in Kaiser Permanente Research Bank and the UK Biobank study populations combined. Each connection reflects the number of cases with both of the linked primary cancers, where the color of the line shows the first cancer site diagnosed.
Figure 2. Germline Single Variant Association Results for Multiple Primary Cancers Combined or Grouped by Organ Site

| 2+ Primary Cancers | SNP | Gene | EA | OR | P-value |
|--------------------|-----|------|----|----|---------|
| rs555697708*       | CHEK2 | A    | 2.72 | 3.1e-06 |
| rs146381257        | ZNF106 | C    | 7.82 | 3.45e-06 |
| rs1805008*         | MC1R | T    | 1.56 | 2.73e-09 |
| rs7195043*         | DEF8 | T    | 1.27 | 3.08e-08 |
| rs72813432*        | TCF25 | G    | 1.31 | 4.99e-07 |
| rs1800007*         | MC1R | T    | 1.41 | 2.3e-06 |
| rs369230*          | CPNE7 | G    | 1.24 | 4.45e-06 |
| rs17832542*        | KLK3 | T    | 1.49 | 3.87e-07 |

| Melanoma + | SNP | Gene | EA | OR | P-value |
|------------|-----|------|----|----|---------|
| rs6988061* | POUSF1B | G    | 1.23 | 4.39e-07 |
| rs3020779  | RNF123 | T    | 1.27 | 9.45e-06 |

| Prostate + | SNP | Gene | EA | OR | P-value |
|------------|-----|------|----|----|---------|
| rs7872034  | SMC2 | A    | 1.21 | 5.83e-07 |
| rs143745791| NCBP1 | A    | 5.95 | 3.76e-06 |
| rs12253181 | RTXN2 | G    | 1.72 | 2.84e-06 |
| rs147906750| DNA2 | A    | 87.03 | 4.4e-07 |

| Breast + | SNP | Gene | EA | OR | P-value |
|----------|-----|------|----|----|---------|
| rs2976393*| PSQA | G    | 1.34 | 1.01e-07 |
| rs141147689 | SDC1 | A    | 9.29 | 3.45e-06 |

| Head and Neck + | SNP | Gene | EA | OR | P-value |
|-----------------|-----|------|----|----|---------|
| rs6988061*      | POUSF1B | G    | 1.25 | 1.06e-07 |

| Thyroid + | SNP | Gene | EA | OR | P-value |
|-----------|-----|------|----|----|---------|
| rs71818417 | RCBT1 | TCA | 2.46 | 1.58e-07 |

| Urinary Bladder + | SNP | Gene | EA | OR | P-value |
|-------------------|-----|------|----|----|---------|
| rs636444207       | RANBP2 | A    | 256.01 | 1.46e-06 |
| rs13966367        | UFL1 | C    | 284.06 | 1.79e-06 |
| rs191064896       | ADGR8B1 | A    | 108.36 | 3.32e-06 |
| rs115235886       | IGIP | G    | 2.55 | 4.05e-06 |

*Variant has been previously associated in single cancer studies

(+) is any additional cancer diagnosis before or after the index cancer

Figure 2 Legend: Suggestive (p < 5x10^{-6}) germline variant associations with multiple cancer phenotypes versus cancer-free controls (n = 165,853) following a fixed-effects meta-analysis of Kaiser Permanente Research Bank and UK Biobank WES data. Associations were detected for any 2+ primary cancers (n = 6,429) and with groups of cases defined by a shared index cancer, at any time point, plus any other cancer diagnosis: melanoma + (n = 1,443), prostate + (n = 1,977), breast + (n = 1,874), head and neck + (n = 283), thyroid + (n = 198), urinary bladder + (n = 829), colorectal + (n = 1,324), lymphoid neoplasms + (n = 728). Variants that have been previously associated in single cancer studies have superscript (a). The heatmap reflects the number of carriers with the risk-increasing allele for each associated variant with the index (y-axis) and additional (x-axis) cancer over the total number of carriers, restricting to cancer cases. When the index and additional cancer are the same, the heatmap value represents all carriers with the specified cancer diagnosis divided by the total number of carriers. Abbreviations: SNP – single nucleotide polymorphism; EA – effect allele; OR – odds ratio.
**Figure 3.** Germline Gene Based Association Results for Multiple Primary Cancers Combined or Grouped by Organ Site

| Gene   | OR  | P-value |
|--------|-----|---------|
| ATM    | 1.64| 9.84e-07|
| BRCA2  | 5.64| 3.76e-11|
| CHEK2  | 2.44| 2.95e-11|
| SAMHD1 | 2.74| 2.4e-07 |
| SLC6A2 | 1.98| 5.44e-07|
| ATM    | 2.29| 2.56e-06|
| BRCA1  | 46.95| 6.88e-08|
| BRCA2  | 10.26| 1.91e-09|
| CHEK2  | 3.64| 1.67e-08|
| BRCA2  | 33.52| 2.24e-06|
| AHCCTF1| 0.33| 1.25e-06|

*Results combine pLOF variants with a MAF < 0.5%, except for AHCCTF1, which uses MAF < 5%.*

*Includes missense variants predicted to be deleterious by 5/5 algorithms.*

*Includes missense variants predicted to be deleterious by at least 1/15 algorithms.*

`(†)` is any additional cancer diagnosis before or after the index cancer.

**Figure 3 Legend:** Burden tests were performed combining variants defined as pLOF with or without deleterious missense variants, defining deleteriousness by at least one (1/5) or all five (5/5) prediction algorithms used (Methods), at a MAF < 0.5%, 1%, or 5%. Following a fixed-effects meta-analysis of Kaiser Permanente Research Bank and UK Biobank data, Bonferroni significant associations ($p < 2.65 \times 10^{-6} = 0.05/18,842$) corrected for the number of genes tested were found for comparisons of cancer-free controls ($n = 165,853$) with all cases with any 2+ primary cancers ($n = 6,429$) and with groups of cases defined by an index cancer for the following phenotypes: prostate ($n = 1,977$), breast ($n = 1,874$), ovary ($n = 239$), and head and neck ($n = 283$). For each gene, the variant grouping with the smallest p-value and fewest number of variants was selected. The heatmap reflects the number of carriers of each associated variant, with the index (y-axis) and additional (x-axis) cancer over the total number of carriers, where carrier is defined as having at least one alternate allele across all variants in a given gene, restricting to cancer cases. When the index and additional cancer are the same, the heatmap value represents all carriers with the specified cancer diagnosis divided by the total number of carriers. Abbreviations: OR – odds ratio; pLOF – predicted loss of function.
# TABLES

**Table 1.** Characteristics of the Kaiser Permanente Research Bank and UK Biobank study populations by ancestry group. Cases are individuals with multiple primary cancers. Controls are those without any cancer.

| Ancestry | Population: Kaiser Permanente Research Bank | Population: UK Biobank |
|----------|---------------------------------------------|------------------------|
|          | Cases                                      | Controls               |
|          | N   | Mean Age | Female (%) | N   | Mean Age | Female (%) | N   | Mean Age | Female (%) |
| AFR      | 99  | 70.5     | 33.3       | 100 | 70.4     | 32.0       | 29  | 55.9     | 51.7       |
| EAS      | 95  | 69.7     | 49.5       | 91  | 69.5     | 49.5       | 10  | 58.8     | 80.0       |
| EUR      | 2,786 | 72.8   | 43.0       | 2,815 | 72.9    | 43.3       | 3,249 | 61.9     | 51.7       |
| LAT      | 131 | 69.5     | 46.6       | 130 | 69.5     | 45.4       | 5   | 63.8     | 80.0       |
| SAS      | -   | -        | -          | -   | -        | -          | 25  | 58.2     | 60.0       |