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Genetic ancestry contributes to somatic mutations in lung cancers from admixed Latin American populations

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Abstract

Inherited lung cancer risk, particularly in non-smokers, is poorly understood. Genomic and ancestry analysis of 1153 lung cancers from Latin America revealed striking associations between Native American ancestry and their somatic landscape, including tumor mutational burden (TMB),

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and specific driver mutations in *EGFR*, *KRAS*, and *STK11*. A local Native American ancestry risk score was more strongly correlated with *EGFR* mutation frequency compared to global ancestry correlation, suggesting that germline genetics (rather than environmental exposure) underlie these disparities.

Introduction:

Lung cancer causes over 1.7 million deaths per year world-wide(1), and kills more people than any other malignancy in Latin America(2). Lung adenocarcinoma (LUAD) is the most common subtype of lung cancer that is typically driven by genomic alterations of genes in the receptor tyrosine kinase (RTK)/RAS/RAF pathway(3) often allowing effective therapeutic targeting by RTK and other pathway inhibitors. It is well-known, but mysterious, that the frequency of somatic *EGFR* mutations is higher in LUADs from patients in East Asia (~45%) compared to LUADs from patients in Europe or patients of European (EUR) and/or African (AFR) descent in North America (~10%)(4-8). In Latin American countries, the frequency of somatic *EGFR* mutations in LUAD ranges from roughly 14% in Argentina, to 25-34% in Colombia, Brazil and Mexico, to 51% in Peru(9-11)(Fig. 1A). Moreover, recent genomic studies from East Asian (EAS) and African (AFR) populations have suggested different distributions of tumor mutation burden (TMB) and levels of somatic copy number alteration (SCNA)(12,13), compared to LUAD patients of European (EUR) ancestry.

Despite the differences in patterns of somatic mutation between LUAD from patients of different ethnicity, the landscape of ancestry effects on the lung cancer genomes for the Latin American populations has not been comprehensively described; and it remains unknown whether the differences are due to ancestry-specific germline variation, or rather to population-specific environmental exposures (Fig. 1B). This is of particular importance as Native American (NAT) ancestry -- which includes components of East Asian (EAS) ancestry derived through waves of migration(14) – is present to varying degrees in modern populations in Latin America, along with EUR and AFR ancestry(15).

Results:

To explore the landscape of somatic cancer mutation in lung cancers from Latin America and to assess the influence of germline ancestry of genetically admixed patient populations on these somatic alterations, we performed genomic analysis of 601 lung cancer cases from Mexico and 552 from Colombia, including 499 self-reported non-smokers (Table S1). Next-generation sequencing targeting a panel of 547 cancer genes plus intronic regions of 60 cancer genes(16) was used to identify single nucleotide variants (SNVs), indels, SCNAs) and gene fusions. This gene panel covers all currently known lung cancer drivers(3), which are the focus of this work. Because we do not have matched germline samples, we applied a custom script to identify known, hotspot lung cancer driver mutations for the full 1153 samples to ensure the sensitivity for low coverage samples, as well as to avoid potential germline contamination (Methods). We found that 552 (48%) of all samples harbored oncogenic mutations in *EGFR*, *KRAS*, *BRAF*, *ERBB2*, or *MET*, or fusions in *ALK*, *ROS1*, or *RET*; 785 of 1153 samples harbored at least one detectable alteration in a broader set of known lung cancer driver genes also including *TP53, STK11, KEAP1, SMARCA4, SETD2, MYC,* and *MDM2* (Fig. 2, Table S2-S3). The detected mutation frequencies of *EGFR* and *KRAS* were 30% and 10%, respectively, in the tested lung cancer samples from Mexican patients, and 23% and 13%, respectively, in the tested lung cancer samples from Colombian patients. SCNA analysis (Methods) identified 9% and 2% cases with high-level amplifications in *MYC* and *MDM2*, respectively. We did not observe novel amplification or deletion peaks in this Latin American lung cancer cohort as assessed by GISTIC analysis.

Ancestry effects on somatic cancer genomes are understudied(17,18), and few genomic data sets have been developed from lung cancer patients with admixed ancestry. One potential source of samples for analysis of ancestry effects is discarded tissue or nucleic acids, left over after the clinical analysis of cancer samples. Here, we developed an analytical pipeline (https://github.com/jcarrotzhang/ancestry-from-panel) that offers the advantage of simultaneous measurement of global and local ancestry from sequencing tumor DNA only, without requiring matched germline samples (Fig. S1A-C). Briefly, we called the genotype of germline single nucleotide polymorphisms (SNP) using on-target and off-target reads from the sequencing panel, and measured global ancestry based on principal component analysis (PCA)(19) of the germline SNPs, in which principal components (PCs) 1, 2 and 3 captured prominently the axis of AFR, EUR and NAT ancestry, respectively (Fig. S2A). We then imputed missing SNPs using an external haplotype reference panel(15), and assigned local ancestry to each genomic region(20), based on the imputed variants. We validated our approach to ancestry analysis by performing whole-genome sequencing on a subset of 44 tumor samples, and SNP genotyping on 12 paired tumor and normal samples (Fig. S2B-C). We found a high accuracy of our off-target reads approach based on panel sequencing of tumor DNA, by comparing tumor and normal ancestry estimations (Pearson's r>0.99), and by comparing panel sequencing to whole-genome sequencing (Pearson's r>0.96). As panel testing of cancer genes has emerged as a practical diagnostic tool in clinical care, our off-target reads-based analytical method opens new avenues to explore the association of germline variants and somatic alterations by re-analyzing large, existing somatic sequencing datasets without matched germline sequencing data.

Having obtained data on both somatic alteration and genetic ancestry, our next step was to assess the correlation of these features, using multivariable regression controlling for self-reported smoking status and country of sample collection (Methods). As previous work focused on differences *between* populations, these associations with ancestry *within* a single admixed population provide more direct evidence of a putative genetic cause. First, we found a significant anti-correlation between TMB and PC3 representing the NAT ancestry ($P=9\times10^{-7}$, coef.=−0.02), in line with previous study of lung cancers from EAS patients(12); no correlation was found with the total SCNA burden, or with aneuploidy.

Evaluation of ancestry-mutation association, adjusting for sample-specific TMB, in each gene from Fig 2 showed that NAT ancestry was positively correlated with mutations in *EGFR* (FDR corrected $p=9\times10^{-5}$, coef.=0.005), and anti-correlated with mutations in *KRAS* (FDR corrected $p=9\times10^{-5}$, coef.=−0.007), and mutations in *STK11* (FDR corrected $p=7\times10^{-4}$, coef.=−0.013), in line with previous studies focusing on EAS patients(12,21).

Each feature (TMB, *EGFR*, *KRAS*, or *STK11* mutation) was independently associated with NAT ancestry in a joint model (Table S4). In never smoking patients, the TMB and NAT ancestry association was stronger in *EGFR*-mutant (P=0.002, coef.=−0.031) than *EGFR*-wild type (P=0.038, coef.=−0.013). Moreover, the interaction of *EGFR* and NAT ancestry was significantly associated with TMB (P=0.04, coef.=−0.022) in a joint model (TMB ~ NAT ancestry + *EGFR* + *EGFR**NAT ancestry), suggesting that the association of TMB and NAT ancestry is different in *EGFR*-mutant and *EGFR*-wild type samples. Furthermore, we demonstrated that NAT ancestry was predominantly associated with oncogenic, driver mutations in *EGFR*, but not with non-oncogenic, passenger mutations (Fig. S3). In addition, we did not observe SCNA of any lung cancer driver gene associated with ancestry (Methods). The observed correlations held in separate analyses of the Mexican and Colombian cohorts (Fig. S4A-B).

To better understand the relationship of ancestry and exposure-induced mutagenesis in the risk of developing LUADs through the activation of RTK/RAS/RAF pathway, we tested the ancestry associations with RTK/RAS/RAF pathway oncogene alterations adjusting for mutational signatures (Table S5). The positive correlation of NAT ancestry with *EGFR* mutation (OR=1.23 in every 10% increase of NAT ancestry, 95% CIs 1.12-1.35), and negative correlation of NAT ancestry with *KRAS* mutation (OR=0.85 in every 10% increase of NAT ancestry, 95% CIs 0.77-0.95) remained significant (Fig. 3A-B). The association of NAT ancestry with *EGFR* mutation was also observed in an analysis restricted to patients who reported themselves as never smokers (OR=1.46 in every 10% increase of NAT ancestry, 95% CIs 1.25-1.70, Fig. S5). This association was also observed in an analysis restricted to patients who reported themselves as smokers (OR=1.45 in every 10% increase of NAT ancestry, 95% CIs 1.08-1.94, Fig. 3B). When including smokers, *KRAS* mutation rate increased with the proportion of smoking signature (OR=1.27 in every 10% increase of smoking signature, 95% CIs 1.04-1.56, Fig. 3B). The ancestry effect on *KRAS* mutations in reported never smokers trended toward significance but was not significant (P=0.08) in this study, perhaps due to sample size (n=387). Moreover, the interaction of smoking signature and NAT ancestry did not modify the effect size of ancestry on *KRAS* (P=0.34, Methods). Gender and the APOBEC signature were not associated with mutations of any lung cancer oncogenes. Age of diagnosis was negatively associated with the risk of *ALK*-translocated cases (P=3x10^{-5}, OR=0.97 in every 10-year increase of age, 95% CIs 0.96-0.99). Together, we conclude that NAT ancestry was associated with genomic differences in Latin American LUAD patients that are independent of smoking activity.

To assess whether the observed association with *EGFR* and *KRAS* mutations is due to NAT ancestry itself or to an environmental exposure/socioeconomic status related to the NAT ancestry, we next investigated the influence of local ancestry. Previous work has shown that associations between local ancestry and phenotype (while accounting for global ancestry) provide evidence of a genetically driven phenotype, as local ancestry is not expected to be causally associated with environmental exposure or socioeconomic status(22,23). We used RFMix(20) to map local ancestry, producing 5425 genomic regions with an assignment of AFR, EUR or NAT ancestral population for each parental chromosome (Table S6-S7). We performed a multivariable logistic regression of NAT ancestry for each genomic region correlating with the *EGFR*-mutant or *KRAS*-mutant samples, controlling for the global ancestry (Methods). We did not identify any region where correlation reached genome-wide significance of $P<5x10^{-5}$ (Fig. 4A, S6).

We next evaluated whether local ancestries across multiple sub genome-wide significance threshold regions ($5x10^{-5}<P<0.05$) were associated with the somatic mutation phenotype by constructing a polygenic ancestry score. This approach is conceptually similar to previous work leveraging local ancestry to quantifying phenotypic heritability(22), but we employ a risk score rather than variance partitioning as the former is more stable at low sample sizes. The local ancestry risk score was defined as the sum of NAT ancestry across each associated region weighted by the Z-score for the association of that region with the given mutation (Methods). To guard against overfitting, Z-scores and local ancestry risk scores were computed by cross-validation: splitting the dataset into ten subsets, obtaining Z-scores for the mutation-ancestry associations using nine subsets, and then calculating the local ancestry risk score on the held-out subset (Fig. S7). We then performed another logistic regression including both the cross-validated local ancestry risk score and global ancestry as covariates, and found that the local ancestry risk score was significantly associated with *EGFR* and *KRAS* mutations, respectively, whereas global ancestry was no longer significant in the joint model (Fig. 4B). In contrast, the local ancestry risk score was not associated with TMB and *STK11* mutations in a joint model with global ancestry. Finally, although previous work suggested associations between *cis* alleles and *EGFR* mutations(24), we did not observe an association between the local ancestry of the *EGFR* locus and somatic mutations in *EGFR* (P=0.8). Moreover, when including the local ancestry of the *EGFR* locus as a covariate, the association of *EGFR* mutations and the local ancestry risk score remained significant ($P=4x10^{-6}$). Our finding suggests that one or more genetic loci specific to NAT ancestry may modulate the evolution of lung cancer tumors to harbor *EGFR* or *KRAS* mutations in the Latin American populations.

Discussion:

In summary, the genomic landscape of LUADs is strikingly varied in Latin American patients with mixed ancestries. In our study of 1153 lung cancers, we demonstrated that NAT ancestry was correlated with somatic driver alterations, including *EGFR* and *KRAS* mutations that can be effectively targeted by small molecule inhibitors to prolong survival(4,25), and TMB and *STK11* that are potential prognostic biomarkers in lung cancer patients(26,27). The ancestry and TMB association was independent of smoking-related mutational processes, and therefore, further investigation on the impact of ancestry-related TMB differences on the response to checkpoint inhibitors is needed(28). Of note, our TMB estimates may be susceptible to germline contaminations due to the lack of matched normal samples. If germline variants specific to the Mexican or Colombian population could not be sufficiently filtered (due to smaller germline reference panels), individuals with higher NAT ancestry would have more germline contamination and the anti-correlation between TMB and NAT ancestry may thus be even more significant than we have observed. Furthermore, due to the lack of matched germline samples and the use of panel sequencing data, we only tested known, hotspot mutations and protein truncating mutations in lung cancer driver genes. Future studies will be needed to comprehensively characterize lung cancer genomes from Latin American patients.

Disparities in access to genetic testing have been observed among Hispanic lung cancer patients in the United States(29). Our study shows that while controlling for global ancestry, local ancestry is associated with mutations in *EGFR* and *KRAS*, providing the first example, to our knowledge, of a germline influence, which may or may not act together with ancestry-specific environmental exposure, on targetable somatic events in lung cancer. These findings highlight the importance of providing somatic genetic testing for Latin American lung cancer patients with admixed ancestries. Given the limited sample size, we could not determine the precise risk loci for *EGFR* and *KRAS* mutation by local ancestry mapping. As low-dose CT scans have enabled lung cancer screening that can significantly reduce lung cancer mortality(30,31), we believe that the identification of germline allele(s) predisposing to the development of *EGFR*-mutant or *KRAS*-mutant LUAD from large, Latin American lung cancer sample sets may improve our understanding of the biological causes of *EGFR* and *KRAS* mutations and the evolutionary processes in lung cancers. Such findings could therefore shed light on prevention and early detection strategies for lung cancer in Latin America and beyond, particularly for non-smokers.

Methods:

Sample collection:

The protocol of this work was approved by the ethical and scientific committees of the Instituto Nacional de Cancerologia in Mexico City, Mexico, the Foundation for Clinical and Applied Cancer Research in Bogotá, Colombia, and the Dana-Farber Cancer Institute in Boston, Massachusetts, for detecting *EGFR* mutations and further genomic analysis. Biopsies were collected for histological diagnosis by the pathology departments of Instituto Nacional de Cancerologia and Foundation for Clinical and Applied Cancer Research.

Library preparation and sequencing:

Genomic DNA was extracted from fresh-frozen, blood and paraffin-embedded samples by a standard procedure using the Wizard Genomics DNA kit (Promega, Madison, USA) according to the manufacturer’s instructions. DNA was fragmented to 250 bp and size-selected DNA was ligated to sample-specific barcodes. A custom targeted hybrid capture sequencing platform (OncoPanel) was used to assay genomic alterations in tumor DNA(16). Each library was quantified by sequencing on an Illumina MiSeq nano flow cell (Illumina, San Diego, CA). Libraries were pooled in equal mass to a total of 500 ng for enrichment using the Agilent SureSelect hybrid capture kit (Agilent Technologies, Santa Clara, CA; cat. no. G9611A). Libraries were sequenced on an Illumina HiSeq2500 or HiSeq3000. Pooled samples were demultiplexed using the Picard tools (https://broadinstitute.github.io/picard/). Paired reads were aligned to the hg19 reference genome using BWA(32) with the following parameters “-q 5 -l 32 -k 2 -o 1”. Aligned reads were sorted and duplicate-marked using Picard. In each batch, we sequenced a control DNA sample as a “plate normal”. For a subset of 44 cases, the same libraries were sequenced on Illumina NovaSeq for low-coverage whole-genome sequencing at 1X coverage.

Mutation analysis:

Mutation detection for single nucleotide variants (SNVs) was performed using MuTect v1.1.4(33) in paired mode by pairing each sample to a control DNA sample profiled with the same OncoPanel. SomaticIndelDetector(34) was used for indel calling. Mutations were annotated by Variant Effect Predictor (VEP)(35) and Oncotator(36). Called variants with a frequency greater than or equal to 0.01% that were found in the Genome Aggregation Database (gnomAD)(37) containing 25,748 exomes, were excluded. TMB was calculated by dividing the total number of coding, non-silent mutations in an individual by the target size (3 MB). Mutational signatures were called using SignatureAnalyzer(38) with SNVs classified by 96 tri-nucleotide contexts. Prior studies have shown that mutational signature analysis can be inferred based on on-target reads from clinical panel sequencing(8,39). Smoking (COSMIC signature 4) and APOBEC signature (COSMIC signature 2) activities were inferred by the estimated number of mutations in a tri-nucleotide context associated with each signature.

A custom script (https://github.com/jcarrotzhang/ancestry-from-panel) was applied to inspect the sites of hotspot driver mutations in *EGFR*, *KRAS*, *BRAF*, *ERBB2*, *MET*, and *TP53*. For each mutation, we counted reads supporting the reference base and the altered base, after filtering out reads with base quality or mapping quality less than 20(40). A mutation was called if the total read count was greater than 5, the altered read count was greater than 2, and the mutant allele frequency was greater than 5%. Identified mutations with total coverage lower than 30X were manually inspected using IGV(41).

Copy number and rearrangements:

Read coverage was calculated at 1 MB bins across the genome and was corrected for GC content and mappability biases using ichorCNA version 0.1.0(42) using the plate normal as the matched control for each sample. GISTIC version 2.0.22(43) was applied to identify focal and arm-level SCNAs on ichorCNA generated copy number segments, with the high-level amplification defined as $\log_2$-transformed copy number ratio greater than 0.7. Rearrangement events were called by Breakmer(44) and filtered on discordant read counts and split read counts greater than 0. Total SCNA burden and the degree of aneuploidy was defined by the number of genes, or chromosomal arms affected by SCNAs, respectively (copy number ratio > 0.1 or copy number ratio > −0.1).

Ancestry analysis from genotyping array:

The Multi-Ethnic Genotyping Array (MEGA) was used for genotyping of paired fresh-frozen tumor tissue and blood samples. We used PLINK version 1.9(45) to filter out variants with missing rate greater than 2%, or failed Hardy-Weinberg equilibrium test ($p<1x10^{-6}$). Markers with allele frequency less than 1% in the 1000 Genomes dataset were also excluded. The Mexican and Colombian samples were merged with samples from the 1000 Genomes phase 3(15), and PCA was performed on the merged data set using (GCTA) version 1.91.6(46).

Ancestry analysis from sequencing:

SAMtools(47) was used to genotype germline variants after filtering out reads with base quality or mapping quality less than 20. LASER version 2.04(48) was used to estimate overall ancestry based on 637,037 germline variants from all populations in HGDP(49). We obtained principal components from LASER results that place each sample into a reference PCA space using 939 HGDP samples as reference samples. For local ancestry identification, phasing and imputation were performed using Beagle version 4.1(50) based on SAMtools genotyped variants. We imputed missing variants using phased haplotypes from 1000 Genomes(15). Ancestry was assigned to each SNP using RFMix v2(20). For each parental population (NAT, AFR and EUR), 500 samples from 1000 Genomes were used as reference samples. Local ancestry regions spanning centromeres were filtered. RFMix outputted global ancestry estimates were used as the percentage of NAT ancestry.

Association analysis:

Multivariable logistic regression or linear regression was performed using a Python module (https://www.statsmodels.org). Because the Mexican population of lung cancer patients has a higher level of NAT ancestry than the Colombian population[15], we accounted for the country of sample collection throughout our analyses. TMB was included as a covariate when associating PC3 to mutations. Total SCNA burden was included as a covariate when associating PC3 to SCNA of lung cancer genes. Gender, proportion of smoking and APOBEC signatures were included as covariates when associating the percentage of NAT ancestry with oncogenic mutations. To test whether smoking signature influence the relationship between ancestry and the *KRAS* mutations, the following model was performed:

$$KRAS \sim \text{NAT ancestry} + \text{smoking signature} + \text{NAT}^{*}\text{smoking} + \text{other covariates}$$

Where gender and country of sample collection were considered as covariates, and NAT*smoking was included as an interaction effect. If the interaction term is not significant, that means that smoking signature activity does not modify the effect of ancestry.

To identify specific genomic region(s) associated with LUAD cases harboring certain somatic alterations, a logistic regression model was applied controlling for the percentage of NAT ancestry, TMB and country of sample collection, followed by genomic control $\left(\frac{\chi 2}{\lambda}\right)$.

Ten-fold cross-validation was performed in the following steps (Fig. S7): the whole dataset was split into ten subsets. Z-scores for the mutation-ancestry associations for each genomic region were calculated using nine subsets, and a cross-validated local ancestry risk score (sum of the NAT ancestry across each associated region weighted by the Z-score of that region) was calculated for each sample on the held-out subset. These steps were repeated for ten times until a local ancestry risk score was generated for each sample:

$$\text{local ancestry risk score} = \sum_{i\,=\,0}^{n} Ai\,Zi$$

Where n is the number of regions associated with the somatic feature (P<0.05), A is the ancestry of each associated region (NAT ancestry was coded as 1, and EUR or AFR ancestry was coded as 0), and Z is the z-score of that associated region.

Data and Code availability:

Raw sequencing data from cancer gene panel sequencing are being deposited at European Genome-phenome Archive (EGA) under the accession code EGAS00001004752. Ancestry identification method from panel sequencing and custom code used in the analyses are available at https://github.com/jcarrotzhang/ancestry-from-panel. All other data supporting the findings of this study are available upon reasonable request from the corresponding authors.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Significance:

The frequency of somatic *EGFR* and *KRAS* mutations in lung cancer varies by ethnicity but we do not understand why. Our study suggests that the variation in *EGFR* and *KRAS* mutation frequency is associated with genetic ancestry and suggests further studies to identify germline alleles that underpin this association.

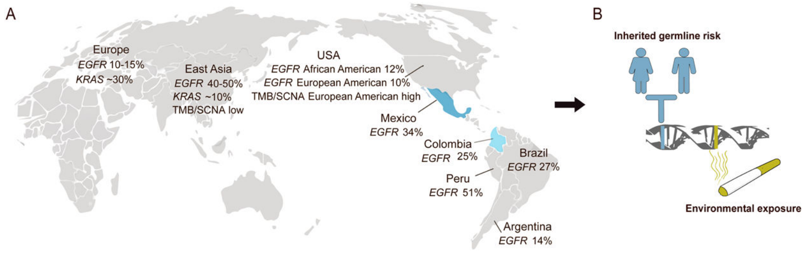


Fig. 1: Genomic differences in LUAD across patient populations.

A) TMB, SCNA burden and the frequency of *KRAS* mutations are lower, while the frequency of *EGFR* mutations is higher, in lung cancers from East Asian patients, compared to lung cancers from patients of European and/or African origin. The somatic *EGFR* mutation rate in lung cancer varies among Latin American countries. Mexican and Colombian populations have varying degrees of admixed NAT ancestry, as indicated in blue. B) Both germline variations and environmental exposures such as smoking can predispose to somatic alterations driving the development of lung cancers, that may cause the genomic differences across populations.

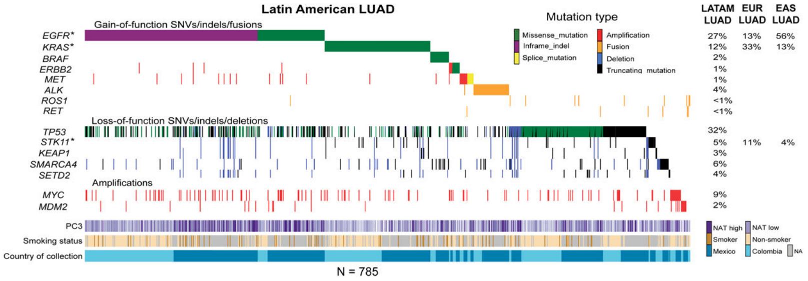


Fig 2: Somatic genome analysis of lung cancers from Mexico and Colombia.

Co-mutation plot displays alterations in known activators of the RTK/RAS/RAF pathway, tumor suppressor genes and significantly amplified genes. * indicates that oncogenic mutations in *EGFR* and *KRAS* as well as truncating mutations in *STK11* are associated with NAT ancestry, but other somatic alterations are not; correlations with mutations are controlled for TMB. LATAM: Latin American. The mutation frequency for EUR LUAD is obtained from the TCGA dataset(51). The mutation frequency for EAS LUAD is obtained from Chen et al 2020(12).

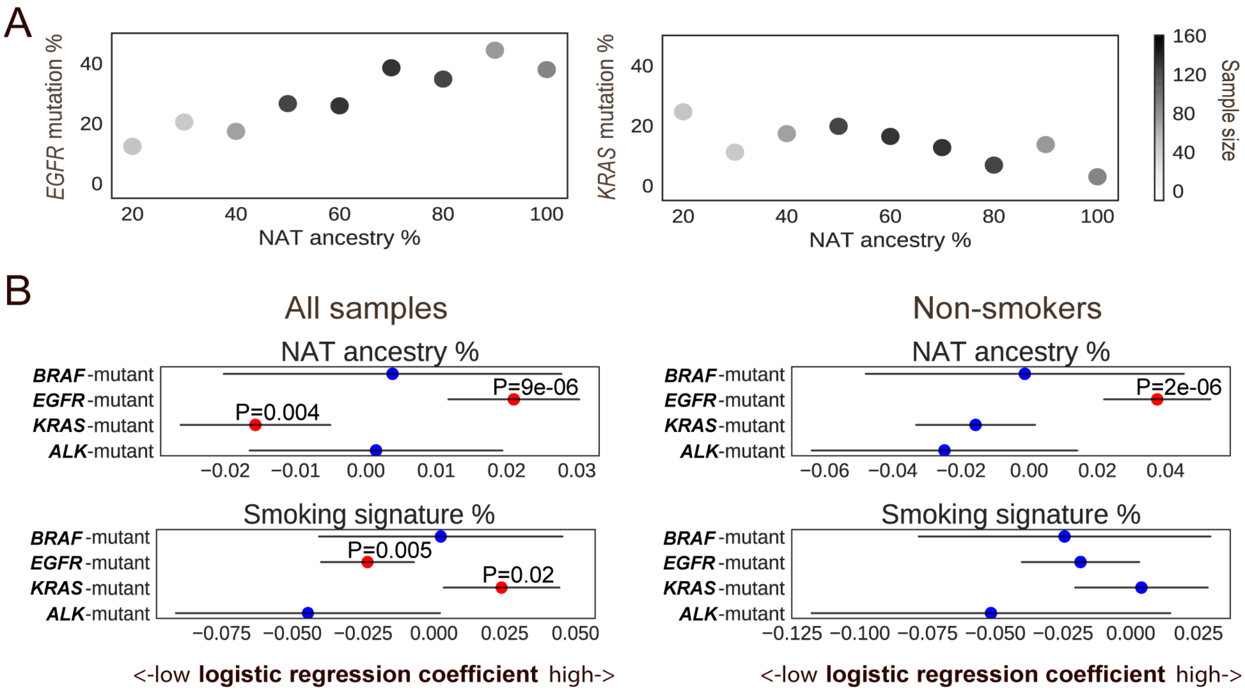
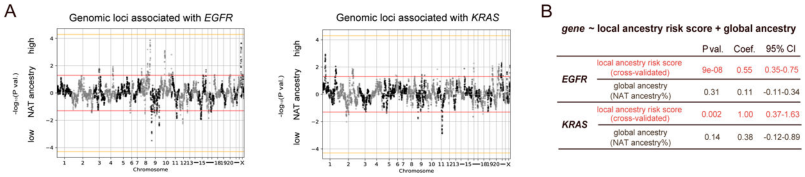


Fig. 3: Targetable LUAD driver genes associated with genetic ancestry.

A) The percentage of NAT germline ancestry is positively correlated with the percentage of somatic *EGFR* mutations, and negatively correlated with the percentage of somatic *KRAS* mutations. Color bar represents the number of samples in the NAT ancestry percentage range. B) Association of targetable LUAD driver genes with NAT ancestry, mutational signature and gender (n=705) (left), and the association in never smokers only (n=387) (right). Multivariable logistic regression P values are shown, with NAT ancestry percentage, gender, smoking and APOBEC signature as covariates. Red dots represent P value <0.05. Lines represent 95% confidence intervals.



***gene* ~ local ancestry risk score + global ancestry**

		P val.	Coef.	95% CI
EGFR	local ancestry risk score (cross-validated)	9e-08	0.55	0.35-0.75
	global ancestry (NAT ancestry%)	0.31	0.11	-0.11-0.34
KRAS	local ancestry risk score (cross-validated)	0.002	1.00	0.37-1.63
	global ancestry (NAT ancestry%)	0.14	0.38	-0.12-0.89

Fig 4: Germline local ancestry in association with somatic *EGFR* and *KRAS* mutations.

A) Genome-wide association of local NAT ancestry with *EGFR* (left) and *KRAS* (right). “NAT ancestry high” indicates positive association, whereas “NAT ancestry low” indicates negative association. Red line indicates P=0.05. Orange line indicates genome-wide significance threshold ($P<5x10^{-5}$). B) Association of local ancestry risk score with somatic *EGFR* or *KRAS* mutations, controlling for global ancestry (proportion of overall NAT ancestry).