Gene-gene interaction of AhR with and within the Wnt cascade affects susceptibility to lung cancer

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Abstract

Background: Aberrant *Wnt* signalling, regulating cell development and stemness, influences the development of many cancer types. The Aryl hydrocarbon receptor (*AhR*) mediates tumorigenesis of environmental pollutants. Complex interaction patterns of genes assigned to *AhR/Wnt*-signalling were recently associated with lung cancer susceptibility. Aim: To assess the association and predictive ability of *AhR/Wnt*-genes with lung cancer in cases and controls of European descent. Methods: Odds ratios (OR) were estimated for genomic variants assigned to the Wnt agonist and the antagonistic genes *DKK2*, *DKK3*, *DKK4*, *FRZB*, *SFRP4* and *Axin2*. Logistic regression models with variable selection were trained, validated and tested to predict lung cancer, at which other previously identified SNPs that have been robustly associated with lung cancer risk could also enter the model. Further, decision trees were created to investigate variant x variant interaction. All analyses were performed for overall lung cancer and for subgroups. Results: No genome-wide significant association of *AhR/Wnt*-genes with overall lung cancer was observed, but within the subgroups of ever smokers (e.g. maker rs2722278 *SFRP4*; OR=1.20; 95%-CI: 1.13-1.27; $p=5.6\ 10^{-10}$) and never smokers (e.g. maker rs1133683 *Axin2*; OR=1.27; 95%-CI: 1.19-1.35; $p=1.0\ 10^{-12}$). Although predictability is poor, *AhR/Wnt-variants* are unexpectedly overrepresented in optimized prediction scores for overall lung cancer and for small cell lung cancer. Remarkably, the score for never-smokers contained solely two *AhR/Wnt-variants*. The optimal decision tree for never smokers consists of 7 *AhR/Wnt-variants* and only two lung cancer variants Conclusions: The role of variants belonging to *Wnt*/*AhR*-pathways in lung cancer susceptibility may be underrated in main-effects association analysis. Complex interaction patterns in individuals of European descent have moderate predictive capacity for lung cancer or subgroups thereof, especially in never smokers.

Key words: susceptibility, association, gene-gene integration, prediction, polygenic risk score, decision trees, never smoker, small cell lung cancer

Background

Lung cancer (LC) is the most common cancer worldwide since 1985. It is the leading cause of cancer related death around the world. [1] It was estimated for 2020, that globally 2.2 million new LC-cases were diagnosed, which are 11.4% of all new cancer cases. In the same year 1.8 million LC-cases died, which are 18% of all cancer related deaths. [2] The lifetime risk of developing a clinical manifest lung cancer (from birth to age 74) is higher in men (3.78%) than in women (1.77%).

The *Wnt* signalling pathway is a multi-regulator of e.g. cell proliferation, differentiation, genetic stability, and much more. It is crucial in the development of embryos and in the dynamic balance of adult tissues, so also that of the lung. With respect to LC, changes of the *Wnt* signalling pathway have been observed for *Wnt* ligands, frizzled, TCF/LEF (T cell factor/lymphoid enhancer factor)-dependent transcription, and *Wnt* inhibitor silencing. [3]

Genome-wide association studies (GWAS) have identified dozens of susceptibility loci throughout the genome that are associated with the susceptibility to lung cancer or one of its histological subtypes. [4–11] Genes related to *Wnt* signalling, one of the key pathway regulating cell development and stemness, were not detected as being associated to LC susceptibility in individuals of European descent so far, unlike *TERT* (5p15.33) that was one of the first for which a robust association was observed. [12] Aberrant *Wnt* signalling is often observed in expression profiles of many cancers, but to date no association of *Wnt/Ahr* genes with susceptibility to cancer of any type has been observed. [13–15] Administration of RNAi against *Wnt* was shown to reduce tumour burden in lung adenocarcinoma (adenoLC). [16] In non-small cell lung cancer (NSCLC), overexpressed *miR-582-3p* maintains stemness features by negatively targeting the regulators of *Wnt* signalling *Axin2, DKK3* and *SRP1* for degradation, thereby increasing β-catenin mediated *Wnt* activity. [17] *TERT* expression was found to be directly enhanced by binding of β-catenin to its promoter region and thereby links telomerase activity to *Wnt* signalling. [13] This is inasmuch important, as *TERT* is one of the first and most robust susceptibility genes for LC identified by GWAS. [18, 19] The tight regulatory machinery of the *Wnt* pathway has several major antagonists, such as Secreted Frizzled related protein (*sFRP*), Dickopff 5 (*DKK*) protein and *Axin2* protein. [20] Evidence also exists for a crosstalk between *AhR* and *Wnt* signalling. [21]

Aryl hydrocarbon receptor (7p21.1; *AhR*) is a ligand induced transcription factor, which is translocated into the nucleus. It is known to mediate the toxicity and tumorigenesis of a variety of environmental pollutants, including for

NSCLC. *AhR* upregulates the enzyme CYP1A1 when cells are exposed to carcinogenic metabolites, such as some polycyclic aromatic hydrocarbons (PAHs) found in cigarette smoke. The CYP1A1 coding gene is discussed as a susceptibility gene for LC. *AhR* is a major determinant in the process of smoking driven LC. [22–24] The complexity of both the *AhR* signalling pathway and the *Wnt* signalling cascade is reflected by interaction effects of genomic variants within genes, which control their function. [25] Recently, the association of the *Wnt*-genes *DKK4* (8p11.21), *DKK3* (11p15.3), *DKK2* (4q25), *FRZB* (2q32.1, also known as *sFRP*3), *SFRP4* (7p14.1), *Axin2* (17q24.1) and a potential interaction with *AhR* was investigated with respect to the susceptibility to LC in a sample of 600 subjects from North India. [25, 26] A notable association with LC, e.g. for the *SFRP4* variant rs1802073 (OR=3.19; 95%-CI 1.81-5.63), was reported. Classification And Regression Tree (CART) analysis revealed an interaction of *DKK2* and *SFRP4* polymorphisms to be the best (off all investigated) predictors for LC; especially within smokers. They also reported to have identified several high-risk subgroups in smokers, e.g. characterised by *DKK2* (rs17037102 / rs419558) and *Axin2* (rs9915936). A similar picture was observed in a sample of 270 subjects from Istanbul, Turkey. [27] A two-way interaction between *DKK*3 (rs3206824) and *SFRP4* (rs1802074) was found to be predictive of LC.

We aimed to assess a possible association of *AhR* pathway and *Wnt* signalling cascade with LC within the large-scale series of cases and controls of European descent hold by the International Lung Cancer Consortium (ILCCO) / Integrative analysis of Lung Cancer Etiology and Risk (INTEGRAL). To do this, we also evaluated the contribution of these genes to genetic prediction of LC as a complement to known LC-related markers.

Methods

The work presented has been reviewed and approved by the ILCCO Steering Committee.

Cases and Controls

Phenotype and genotype data of 58,181 entries of the data repository of ILCCO were extracted. Details of the repository is described previously. [4, 28] QC control samples, individuals without information on smoking status or age, and samples of poor genotyping quality or sex discrepancies, were excluded. To avoid population stratification, this analysis is focused on European-ancestry population (defined as more than 95% probability of being of European descent). 14,068 incident LC-cases and 12,390 cancer-free controls of European descent remained for analysis. Those

genotyped with other genome-wide array in addition to OncoArray were separated to form an independent validation set (2nd validation set) of size (n=4,359, including 2,360 LC-cases and 1,999 controls).

Selected Markers

For this investigation we extracted the genotypes of 113 genomic variants (markers) assigned to 58 genes, previously associated with the risk for LC in European decent people or one of its histological subtypes through a wide variety of approaches [4–11] or proxies thereof (called *LC-marker*), and 296 markers assigned to 7 genes involved in *Wnt* signalling and listed in Bahl et al. [25, 26] and Yilmaz et al. [27] (called *AhR/Wnt-marker*). Thus, we focused this analysis to genes previously investigated with respect to LC. Fifty of these 409 markers were eliminated before analysis due to a MAF<1% (minor allele frequency), or departure from HWE (Hardy–Weinberg equilibrium) in genotypes (unaffected $p<10^{-7}$, affected $p<10^{-12}$), or low imputation accuracy (info<0.8). Seventy-eight of the remaining *LC-markers* were genotyped with the OncoArray (44 thereof are proxy SNPs identified using LDlink [29]) and 32 had to be imputed. Two-hundred twenty-one of the remaining *AhR/Wnt-markers* were genotyped and 28 have been imputed. A list of these markers extracted from ILCCO OncoArray repository is given in the appendix.

Association analysis

We first performed association analysis for each marker separately using the program PLINK. [30, 31] Crude (model 1) and adjusted odds ratios (ORs) were estimated along with 95%-confidence intervals within log-additive models. Sex, age and smoking status and the first 3 principal components (PCs) to adjust for population stratification (model 2); and in addition the 6 most significantly associated *LC-markers* (rs55781567, 15q25.1 *CHRNA5;* rs11780471, 8p21.2 *CHRNA2;* rs7705526, 5p15.33 *TERT;* rs56113850, 19q13.2 *CYP2A6;* rs71658797, 1p31.1 AK5; rs11571833, 13q13.1 *BRCA2*) (model 3) were included in adjusted models. ORs were estimated for overall LC, small cell LC (SCLC), squamous cell LC (SqCLC), adenocarcinoma LC (adenoLC), ever smokers, never smokers and individuals aged ≤55 years (early onset LC) as subgroups. We generated QQ-plots for the *AhR/Wnt-markers* and estimated the genomic inflation factor λ. To account for multiple testing, genome-wide statistical significance was considered to correspond to a p-value of 10^{-7} or lower, suggestive significance to a p-value between 10^{-5} and 10^{-7} and nominal significance to a p-value between 0.05 and 10^{-5}.

Logistic Regression - Predicting models with model selection

We fitted logistic regression models with variable selection to find appropriate polygenic risk scores (PRS) in order to predict the disease (LC) status (affected or unaffected). Any *AhR/Wnt-marker* or the *LC-marker* could be included in the model without preference. To avoid multi-collinearity we removed one of two SNPs in LD to another (R^2>0.8, pruning). The remaining entered the models as potential predictors. We performed forward selection until the Bayesian information criterion (BIC, most stringent selection), the *Akaike* information criterion (AIC, less stringent selection, contains in general more predictors) or the sample size corrected AIC (AICC) indicate a best solution (and 10 more selection steps). The resulting PRSs are called BIC-, AIC- and AICC-scores. Note, that for the purpose of model building, the AIC-selection is asymptotically equivalent to cross-validation (CV). [32, 33] To avoid overfitting, we assigned individuals to a training or a validation set (to build a score) and a testing set (to examine the score performance) with a 1/3 probability each. For comparison, we also generated a BIC^{LC}-score with at least one marker, only allowing *LC-markers* to enter the model building. To compare the importance for LC prediction of the sets g of *LC-maker*s and *AhR/Wnt-markers, respectively,* we contrasted the importance-values defined as $I_g = \sum_{m \exists g} |\beta_m| \cdot MAF_m$ for each score (MAF_m the minor allele frequency and β_m the logistic regression coefficient of marker m). The superiority of the AIC-scores over the BIC^{LC}-score and the BIC-score was tested applying the nonparametric test of DeLong, DeLong, and Clarke-Pearson 1988 (1-sided) on AUCs of ROC (area under the receiver operation characteristic curve). [34] In addition, a corresponding precision-recall plot was created for the SCLC.

Decision trees

Decision trees were created to examine marker x marker interaction with respect to the LC prediction. Any *AhR/Wnt-marker* or the *LC-marker* could be included in a tree without preference. This was accomplished in the entire sample and in all subgroups defined above. The R packages *rpart* and *DescTools* were used. [35, 36] To avoid trees being formed by spurious epistasis we removed one of two SNPs in LD to another (R^2>0.8, pruning). Since overfitting is a point of concern when building decision trees, the complexity parameter was first optimized applying 10-fold cross-validation, grading the performance on the validation set by Somers' D (concordance of true and predicted LC-status). The ability of the optimal trees to predict the LC-status was then tested within the independent sample of 4,359 cases and controls. True positive (TP) and true negative (TN) rates are given.

All statistical analyses were performed with SAS® 9.4, PLINK 1.90 and 2.0 or R 4.0.2.

Gene Expression

We extracted information on gene expression from the *Human Protein Atlas* [37, 38] and *LungGENS* [39, 40].

Results

Sample description

The analysed sample consists of 14,068 LC-cases and 12,390 controls with median age of 63. Sixty-three percent were male, 52% of cases and 28% of controls were current smokers. The most frequent histological subtype is adenocarcinoma (38%), followed by squamous cell carcinoma (SqCLC) (26%) and small cell lung cancer (SCLC) (10%). The proportion of never-smokers was largest within the subgroup of adenocarcinoma cases (14%), but almost the same between those cases aged ≤55 years (10%) and aged >55 years (9%). Details on smoking status and histological subtypes are presented in Table 1.

Table 1 Smoking by LC status and subgroups

Association analysis

We first performed association analysis for each *Wnt/AhR-marker* separately. The p-values for an association of *AhR/Wnt-markers* with LC range from 0.005 (rs12115174; 8p11.21 *DKK4*; OR=0.9211) to 1 (model 2; adjusted for sex, age, smoking status and population stratification); with a negligible genomic inflation (λ=1.02). A nominally significant association ($10^{-5} < p \leq 0.05$) was observed for only 8 of the 249 markers (~3%). The corresponding point estimates of OR range from 0.88 (rs1053070054; 8p11.21 *DKK4*; p=0.007) to 1.12 (rs74596148; 7p14.1 *SFRP4*; p=0.25). A QQ-plot indicates that achieved p-values almost perfectly agree with the expectation of no associated marker (see Figure 1). P-values and OR are in moderate agreement between the models (e.g. model 2 to model 3; additionally adjusted by *LC-markers*: Kendall's rho_p=0.75, rho_{OR}=0.78).

Figure 1: Association of *AhR/Wnt-marker*

Subgroup analysis: When dividing the cases according to histological subtypes (SCLC; SqCLC and adenoLC) the observation of no detectable association for *WNT/AhR-markers* remains. Merely the number of nominally significant association ($10^{-5} < p \leq 0.05$) increases to 12 (5%) or 21 (8%) of the 249 markers for SqCLC and SCLC, respectively,

hence close to the expected type 1 error. (Additional file 1: S-Table 2). When dividing the cases and controls according to their smoking behaviour (ever and never smokers), genome-wide significance ($p \leq 10^{-7}$) was achieved for 7 and 8 markers, respectively. Another 12 and 3 markers, respectively, were found suggestively significant ($10^{-7} < p \leq 10^{-5}$) (see Additional file 1: S-Figure 1) for ever and never smokers. Those markers found associated among ever smokers have mainly been directly genotyped and are assigned to *SFRP4* and *DKK4*. E.g. for marker rs2722278 we estimated an OR=1.20 (95%-CI: 1.13-1.27), yielding a p-value of $5.6\ 10^{-10}$. Those markers found associated among never smokers have mainly been imputed and are mostly assigned to *Axin2,* but also to *AHR*, *FRZB* and *DKK2*. Marker rs17037102, assigned to *DKK2*, was the only one found associated with LC by Bahl et al. and in this analysis (see Table 2 and Additional file 1: S-Table 3). Interestingly, the ORs of these markers estimated by model 3 (additionally adjusted for selected *LC-marker*) differ from that estimated by model 2. They are closer to one and no more significant. E.g. for rs1133683 (*Axin2*) we observe an OR=1.27 (95%-CI: 1.19-1.35, $p=1x10^{-12}$) fitting model 2, but OR=0.95 (95%-CI: 0.86-1.06, p=0.3586) fitting model 3.

Table 2 Significantly associated *AhR/Wnt-markers* within never and ever smokers

Logistic Regression - Predicting models with model selection

We further fit logistic regression models with variable selection to evaluate the contribution of *AhR/Wnt-markers* to a polygenic risk scores (PRS), but without postulating the usefulness of the score as such. Eight *LC-markers* from only eight *LC-genes* (*CYP2A6*, *CHRNA5*, *TERT*, *AMICA1*, *CHRNA3*, COPS2, *HCG4* and *CHRNA2*) were selected for the BIC-score (most stringent selection) to predict overall LC. Hence, the BIC-score and the BIC^{LC}-score are identical. In contrast, the AIC-score (for overall LC identical to the AICC-score) includes 20 *LC-markers* and remarkable 17 *AhR/Wnt-markers*, with *LC-markers* being more important than the *AhR/Wnt-markers* (importance ratio 0.56: 0.34) (see Figure 2, Additional file 1: S-Figure 3 and S-Table 4). The ability to distinguish cases and controls from susceptibility genes only was, as expected, poor for each of the scores (see Additional file 1: S-Table 5). In the training set the performance of the AIC/AICC-score (AUC=0.607) exceeded those of the BIC/BIC^{LC}-score (AUC=0.582) significantly (p<0.001). Within the test set (AUCs: 0.577 and 0.576) and the 2nd validation set (AUCs: 0.553 and 0.548), the higher complexity with additional *AhR/Wnt-markers* did not improve discriminability for overall LC (p=0.87 and p=0.35).

Similar score composition and performance was observed for most subgroups. The BIC-scores in the subgroups adenoLC (involved marker LC:*AhR/Wnt*=6:--), SCLC (3:--) and smokers (7:--) contained *LC-markers* only, whereas *AhR/Wnt-markers* are included even under this stringent variable selection in the subgroups SqCLC (5:1) and Early onset LC (2:2). However, between 14 and 31 *AhR/Wnt-markers* entered these subgroup's AIC-scores. For these subgroups, the importance of the LC-markers for the AIC-score is higher than that of the included *Ahr/Wnt-markers*.

Figure 2: Comparison of score composition

Most important, we observed a significantly higher predictive accuracy (larger AUCs) of the *AhR/Wnt-markers* enriched AIC-scores compared to BIC^{LC}–score in the subgroup of **SCLC** patients (p=0.019; AUC_{AIC}=0.577 AUC_{BIC}=0.546) within the test set (see Additional file 1: S-Figure 4). For this subgroup, the selected *AhR/Wnt-markers* contribute to the AIC-score more than twice as much as the *LC-markers* (importance ration 0.60: 1.49). The precision-recall plot of Figure 3 indicates that a positive SCLC prediction based on the AIC-score can be trusted more than that based on *LC-markers* alone (BIC^{LC}-score). In the 2nd validation set the score-specific AUCs were similar but no more significantly different (p=0.08; AUC_{AIC}=0.564 vs. AUC_{BIC}=0.531). The AIC-score of this SCLC-subgroup is composed of 12 *LC-markers* (assigned to *CHRNA5*, *HCG4*, *DNAJB4* (4x each), *CYP2A6*, *CHRNA3*, *CHRNA2*, *AMICA1*, *KCNJ4*, *AS1*, *BRCA2*, *EGFL8* and *WNK1* (2x each)) and 27 *AhR/Wnt-markers* (assigned to all *AhR/Wnt*-genes except *DKK3*). However, only one LC patient in the test set (n=434) and one in the 2nd validation set (n=164) was recognized as a patient at a threshold of 50% case probability.

Figure 3: ROC and precision-recall-plot: SCLC

Interestingly the BIC-score for **never smokers** was built by only two *AhR/Wnt-markers* (assigned to *Axin2* and *SFRP4*) but not a single LC-marker. Further, the *LC-markers* are the minority in the composite of the AIC-score (15:23). They also contribute less to the AIC-score than the *AhR/Wnt-markers* (importance ratio of 0.96 : 1.46). The median predicted case probability, in the test set (24.8%) and 2nd validation set (25.6%), exceeds that of controls by 1%- to 2%-points. However, AUC differed neither in the test set (p=0.13; AUC_{AIC}=0.540 AUC_{BIC}=0.514) nor in the 2nd validation set (p=0.36; AUC_{AIC}=0.535 AUC_{BIC}=0.526) significantly. Nevertheless, this observation highlights the value of the *AhR/Wnt-markers* in the subgroup of never smokers.

Decision trees

Finally, we generated decision trees to evaluate the contribution of *AhR/Wnt-markers* to LC prediction that allow for a complex interaction structure, but without postulating the usefulness of the trees as such. The decision tree for overall LC (whole sample) consists off solely a single decision node (rs55781567 assigned to *CHRNA5*), achieving a Somers' concordance index D=0.0565 in the 2nd validation set (see Additional file 1: S-Table 6 and S-Figure 2). A single-node decision-tree was also found optimal for participants aged ≤55 years (split: rs1051730 assigned to *CHRNA3*), achieving a Somers' concordance index D=0.096. These two, unsophisticated trees are characterised by balanced TP- (about 62%) and TN-rates (about 44%).

The decision trees for ever smokers, SCLC and SqCLC were more complex achieving Somers' concordance indexes D of 0.007, -0.0005 and 0.0126, respectively. The trees for SCLC and SqCLC are characterised by an extreme TP-rate <5% and TN-rate >99%; the tree for Ever Smokers by a TP-rate >99% and TN-rate <5%. Remarkably, a marker assigned to *CHRNA5* was always chosen as the first and most important split for the trees for ever smokers, for SCC and SqCLC. However, markers assigned to *AhR/Wnt-genes* (*smoker*: *DKK2*; *SCLC*: *FRZB*; *SqCLC*; *DKK2* and *DKK3*) appear at lower-level decision-nodes (Additional file 1: S-Figure 5, 6, 7 and 8). With the same program settings, no decision tree could be created for adenocarcinoma.

Most notable is the optimal decision tree for the 5,242 **never smokers** (75% LC-cases, 25% controls), the only one that does not contain a marker belonging to the CHRN (*Cholinergic receptors nicotinic subunits*) gene group (see Figure 4). The tree is built from only two *LC-markers* but 7 *AhR/Wnt-markers*, achieving a Somers' concordance index D=-0.002. One can make out three branches of this tree. Branch I covers two thirds of individuals (n=754, 66% of 1141 in the 2nd validation set): All of these are graded as "unaffected" based on only the two *LC-markers*: first decision node (rs885518 assigned to MTAP) and second decision node (rs7705526 assigned to *TERT* that links telomerase activity to *Wnt signalling*). For branch II an additional node (rs17214897 assigned to *DKK2*) is taken into account, covering a further tenth (9.9%) of never smokers. In this branch, very few subjects of the training set (1.7% within branch II eq. 0.17% of all never smokers) are graded "affected". However, one in four individuals of the 2nd validation set belonging to both branches, I and II, is truly "affected" but has not been detected (TP-rate=0%, TN-rate=100%). Rated as "affected" appears in the test set only in the third branch III, covering the remaining fourth of never smokers

(n=284 of the 2nd validation set). This third branch requires genotypes of several *AhR/Wnt-markers* assigned to *AHR*, *Axin2*, *DKK2* and/or *SFRP4*. Herein, one in three (n=97 of the 2nd validation set) is truly "affected" and is given a chance to be correctly identified, which appears in 8 LC-cases (TP-rate=9%, TN-rate=88%). We also noted that the histological subtypes are equally distributed between the branches (see Additional file 1: S-Table 7).

Figure 4: Decision tree for never smoker

Gene Expression

AHR, *Axin2*, *DKK3* are ubiquitously expressed, with RNA expression detected in many tissues and evidence for protein expression. *Axin2* and *DKK3* are moderately to highly expressed in normal lung tissues according to the Human Protein Atlas. [37] *AhR* is expressed at low levels in macrophage cells of the lung. No expression is reported for other *Wnt*/*AhR*-genes. (see Additional file 1: S-Figure 9 and S-Table 9). Significant differential expression is listed in *LungGENS* for *AhR*, *Axin2 DKK2*, *DKK3* and *SFRP4* [39] (see Additional file 1: S-Table 8). Further, AhR is reported to be abundantly expressed in solid lung tumours, especially in adenocarcinomas. AhR overexpression was associated with upregulation of IL-6 secretion, which is critical for lung cancer initiation. [41] Detailed information on gene expression is given in the Appendix. In addition, the *DKK*1 serum level was seen as significantly lower in NSCLC and SCLC patients compared to healthy controls. [42] Significant upregulation of *DKK2* expression was found in *APC (*adenomatous polyposis coli*)*-mutated non-SCLC lung cancers. [43]

Discussion

This investigation was intended to discover association of the *Wnt*-genes *DKK4* (8p11.21), *DKK3* (11p15.3), *DKK2* (4q25), *FRZB* (2q32.1, also known as *sFRP*3), *SFRP4* (7p14.1), *Axin2* (17q24.1) and a potential interaction with *AhR-genes,* to LC in a large sample of 26,458 individuals of European descent. No marginal association of *AhR/Wnt-markers* with overall LC was observed. Interestingly, an accumulation of associated markers was observed splitting the sample by smoking status, where respective markers in ever smokers are assigned to *SFRP4*. On the other hand, association analysis in never smokers reflects complex gene-gene interactions, as markers of several *Ahr/Wnt-genes* were found to be genome-wide associated with LC. This complexity is also visible through the decision tree analysis.

313 Our results are in line with findings from northern India [25, 26] and from Istanbul, Turkey [27], both of which are
314 based on much smaller samples (approx. 600 and 270 people, respectively). In these investigations, the interaction
315 of DKK2 and DKK3 with SFRP4 and Axin2 polymorphisms turned out to be the best (of all examined) predictors of LC,
316 especially in smokers. Axin2, but also AHR, FRZB and DKK2, were observed to be complex associated in never smok-
317 ers. Our analysis agrees with both previous studies that complex interaction patterns between the examined genes
318 contribute to overall LC susceptibility or within certain subgroups. However, we have not been able to replicate
319 reported single marker associations directly

320 To discover patterns of *Ahr/Wnt-genes* involved in LC genesis we further changed the focus from significance of
321 association to inclusion in prediction models, and followed two approaches: First, we searched for polygenic risk
322 scores (PRS). Doing so, we add up marker main effects to construct multidimensional scores, optimising model fit
323 (instead of marker preselection by p-value below some threshold), in order to discriminate cases from controls in a
324 somehow ideal way. Complex gene x gene (GxG) interactions are not modelled.

325 Nevertheless, the proportion of *Ahr/Wnt-genes* entering *some of the predictive* models was remarkable large, given
326 that these markers are not, all other candidates however genome-wide significantly associated to LC. This was par-
327 ticularly noticeable for SCLC, since *AhR/Wnt-markers* contribute more than twice as much to the prediction score as
328 *LC-markers*. It is known, that within current smokers, tobacco consumption is strongest associated to SCLC. [44]
329 Moreover, within never smokers, a stringed defined score is made up from only two *AhR/Wnt-markers*, assigned to
330 *Axin2* and *SFRP4*. However, the discriminative ability of PRSs for LC, contributing markers with significance for main
331 effect at different levels, is in general poor. The AUC of the BIC^{LC} score for overall LC (0.58 in the test set and 0.55 in
332 the 2nd validation set) corresponds to the AUC=0.54 based on four top *LC-genes* in a simulated population, as given
333 by the GWAS-ROCS Database (https://gwasrocs.ca/). This may be due to other overpowering risk factors, since mod-
334 els including e.g. age, sex and smoking variables achieve higher AUCs (0.62 to 0.79). [45]

335 Recently two polygenic risk scores (PRSs) for overall-LC had been developed, validated and assessed with respect to
336 improving eligibility to low-dose computed tomography (LDCT) as the only recommended screening test for lung
337 cancer. Jia et al. [46, 47] build a PRS on 19 genome-wide associated SNPs ($p<0.5\ 10^{-8}$). Hung et al. [48], integrated
338 their PRS on 128 SNPs (35 “known” LC-related loci, 93 suggestive associated loci selected by LASSO-regression

model) into the $PLCO_{all2014}$ risk model. Both approaches have been validated using data from the UK Biobank. For both scores, the mean PRS differed only slightly between LC cases and cancer-free controls (Jia: effect size ~ 0.19; Hung: effect size ~ 0.22). For both scores, no substantial increase in discriminability of cases from controls is reported, when adding the PRS to existing risk models (Jia: family history – AUC=0.589, family history + PRS – AUC=0.615; Hung: $PLCO_{all2014}$–AUC=0.828, $PLCO_{all2014}$+ PRS – AUC=0.832). However, both were able to show that the age at which a smoker crosses the recommended screening threshold of 1.5% for the 5-year LC risk depends on the genetic background, which is sufficiently quantified by the PRS examined. Some smokers will be eligible by <50 years of age, others by> 60 years of age. Hence, constructing reliable PRS, even with small discriminability, may help to improve the performance of LDCT.

Two- and multiway GxG interaction can also contribute to LC susceptibility, rather than just markers with observed (marginal) main effects. GxG interaction is in general less commonly investigated, not only because this requires much larger samples. However, Li et al. [49] found RGL1:RAD51B in overall LC and non-SCLC, SYNE1:RNF43 in adenocarcinoma and FHIT:TSPAN8 in SqCLC to interactively contribute to LC susceptibility. As in the presented data analysis, the impact of these genes would also have been overlooked considering main effects only. Another reason could be that LC itself is just a generic term of several subcategories that differ in terms of LC initiation and require separate PRSs. [45, 50] A third reason of the poor performance may be due to the exclusively concentration on genetic effects, rather than modelling lifelong interaction with the environment as well. E.g. GxE interaction effects for LC have been observed smoking [51], exposure to asbestos fibres [52, 53] and exposure to radon [54, 55].

With this in mind, the data analysis presented shows that the complex interaction of Wnt-related genes has the potential to be part of an adequate risk assessment for never-smokers or in relation to certain histological subtypes of LC.

As a second approach, we constructed decision trees, which mainly depict GxG interaction patterns. Although, the ability to discriminate cases from controls is again poor, CHRNA5 was in general the most important first node for overall LC and in many subgroups. *Ahr/Wnt-genes* play a complex but important role in at least one quarter of never smokers, as seen before. Remarkably, *TERT*, which links telomerase activity to *Wnt* signalling, was central in that branch and important for the remaining three quarters of never smoker. This corresponds to a concentration of

relevant genes for this subgroup in the *CLPTM1L-TERT* region on chromosome 5, as previously reported by Hung et al.. [56] Our observations confirm the suspicion, that LC in never smokers is a different entity, justified beforehand on differences in epidemiological, clinical and molecular characteristics. [50]

We would like to emphasize that this study was not intended to provide a definitive and reliable risk assessment, but rather aimed to examine in depth the LC-relevant complex interaction pattern of *AhR/Wnt-genes* hypnotized by Bahl et al.. Indeed, considering prediction instead of association provides weaker evidence for this, but is valid in view of the large amount of external evidence. The importance of the *Wnt*-signalling pathway and its antagonist's *sFRP*, *DKKs* and *Axin2* for cancer is outlined in the introduction. One can also assume a connection with the molecular functionality, since involved genes are expressed ubiquitously or in lung tissues.

Although the large-scale, thoroughly quality checked, and representative sample of genetically proven European descent individuals was used for the presented analysis, some limitations must be noted. We used a rather narrow definition of *AhR/Wnt-genes* to limit the number of possible interactions. An extension to e.g. EGRF, APC, FRAT2 or the CYP-family would also be justified. We further could have chosen the random forest method as a more contemporary and robust approach than decision trees, but we would not be able to present our results so illustrative. However, the sample size allowed subgroup analyses, whereby the special importance of *AhR/Wnt-genes* for SCLC and never smokers could be shown.

Conclusions

The role of markers belonging to *Wnt* signalling and the *AhR* pathway in LC susceptibility may be underrated in main-effects association analysis. Complex interaction patterns in individuals of European decent have moderate predictive capacity for LC or subsets thereof, especially in never smokers.

List of abbreviations

AhR	Aryl hydrocarbon receptor
GWAS	genome-wide association studies
LC	lung cancer

389	NSCLC	non-small cell lung cancer
390	SCLC	small cell lung cancer
391	SqCLC	squamous cell lung cancer
392	adenoLC	adenocarcinoma lung cancer
393	OR	odds ratio
394	CART	classification and regression tree
395	AUCs of ROC	area under the receiver operation characteristic curve
396	ILCCO	International Lung Cancer Consortium
397	INTEGRAL	Integrative analysis of Lung Cancer Etiology and Risk
398	PRS	polygenic risk scores
399	BIC	Bayesian information criterion
400	AIC	Akaike information criterion
401	CV	cross validation
402	MAF	minor allele frequency
403	TP	true positive rate
404	TN	true negative rate
405	LDCT	low-dose computed tomography

Declarations

Ethics approval and consent to participate

All participants in this study signed an informed consent, approved by the local internal review board or ethics committee and administered by trained personnel. All consortium research received approval from the Dartmouth Committee for Protection of Human Subjects on 7/30/2014 with id STUDY00023602. All experimental protocols and other methods used comply with institutional, national, or international guidelines.

Consent for publication

Not applicable

Availability of data and materials

The data that support the findings of this study are available from ILCCO/INTEGRAL but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of ILCCO/INTEGRAL.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

A. R. designed the investigation, carried out parts of the formal analysis and wrote the main manuscript text. N.M. carried out parts of the formal analysis, prepared figures and critical reviewed and revised the manuscript. B.W. carried out parts of the formal analysis. R.J.H. and C.I.A. coordinate the research activity of the consortium, including

data curation and funding acquisition. H.B: supervised the investigation, including funding acquisition. R.J.H., H.B., L.LM., C.I.A. and L.A.K. critical reviewed and revised the manuscript. A.C., A.H., A.R., A.S.A., A.T., C.C., D.A., D.C.C., E.J.D., F.T., G.E.G., G.F.-T., G.L., G.R., H.B., J.A.D., J.K.F., L.A.K., L.LM., M.B.S., M.C.A., M.J., M.P.A.D., M.T.L., N.E.C., P.B., P.J.W., P.L., R.J.H., S.E.B., S.L., S.M.A., S.S.S., S.Z., T.L. and T.R.M. collected and provided study materials and data.

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Supplementary Information

"Additional file 1.pdf" contains additional information on a) which marker were extracted from ILCCO OncoArray repository, b) single marker association, c) Polygenic Risk Scores (PRS) and Decision Trees and d) gene expression in normal tissue.

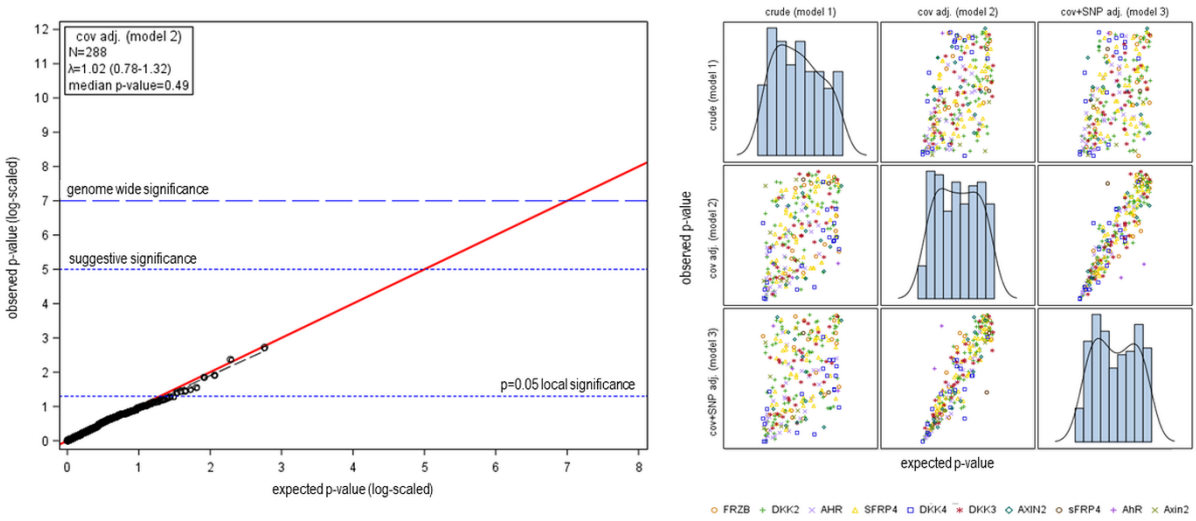
Tables and figures

Table 1 Smoking by LC status and subgroups

			Never smoker		Ever smoker					
		Total	never		former		current		ever[$]	
		N	n	%	n	%	n	%	n	%
control	age ≤ 55 yrs.	2,762	951	34%	698	25%	896	32%	217	8%
	age >55 yrs.	9,628	2,960	31%	3,572	37%	2,568	27%	528	5%
	all	12,390	3,911	32%	4,270	34%	3,464	28%	745	6%
case	SqCLC	3,692	138	4%	1,257	34%	2,158	58%	139	4%
	SCLC	1,450	48	3%	383	26%	965	67%	54	4%
	other LC	3,629	405	11%	1,200	33%	1,820	50%	204	6%
	AdenoLC	5,297	740	14%	1,989	38%	2,401	45%	167	3%
	age ≤ 55 yrs.	2,765	281	10%	452	16%	1,945	70%	87	3%
	age >55 yrs.	11,303	1,050	9%	4,377	39%	5,399	48%	477	4%
	all	14,068	1,331	9%	4,829	34%	7,344	52%	564	4%
total		*26,458*	*5,242*	*20%*	*9,099*	*34%*	*10,808*	*41%*	*1,309*	*5%*

[$] ..as recorded; SCLC: small cell lung cancer, SqCLC : squamous cell lung cancer, AdenoLC: adenocarcinoma of the lung, other LC: other histological subtypes;

Figure 1: Association of *AhR/Wnt-marker*



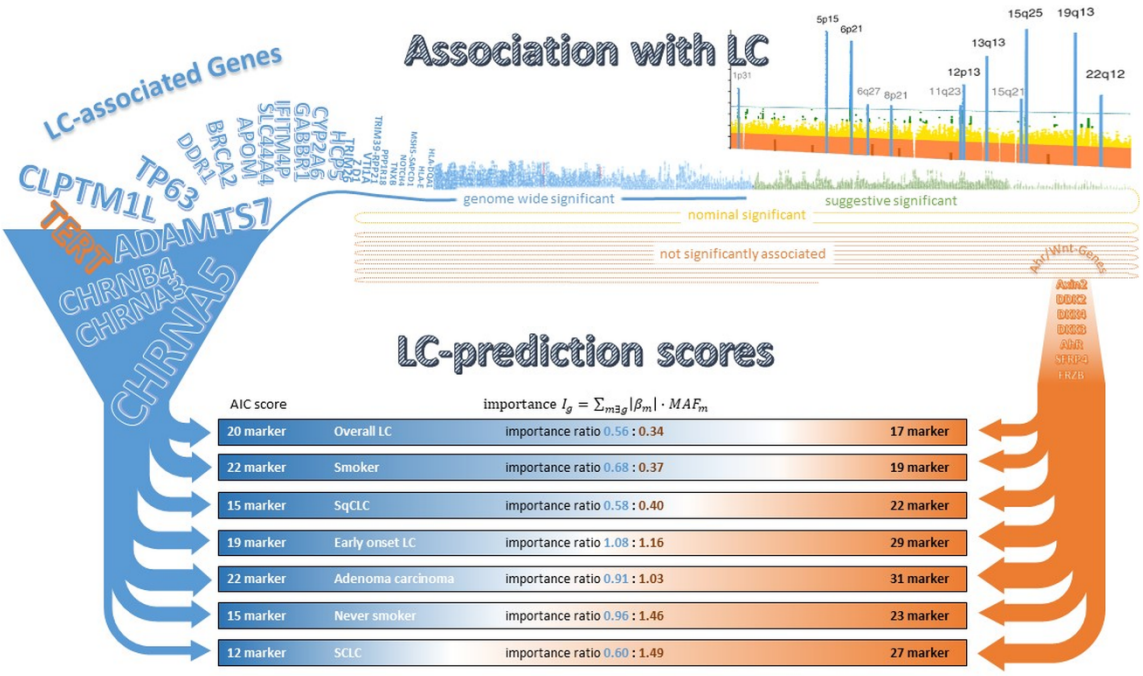
Left panel: QQ-Plot for model 2 (adjusted for sex, age and smoking status and the first three principal components); right panel: matrix of p-values generated by model 1 (crude), model 2 (adjusted for sex, age and smoking status and the first three principal components) and model 3 (additionally adjusted for 6 selected *LC-markers*), genome-wide significance: p-value ≤ 10^{-7}, suggestive significance: 10^{-7} < p-value ≤ 10^{-5}, nominal significance: 10^{-5} < p-value ≤ 0.05.

Table 2 Significantly associated *AhR/Wnt-markers* within never and ever smokers

SNP		Cyto band	MAF	gene	model 2			model 1	model 3
					p-value	OR	95%-CI	OR	OR
never smoker									
imputed	rs202198518[$]	7p21.1	14%	*AHR*	$3.4\ 10^{-13}$	0.72	0.66-0.79	0.71	0.90 [n.s.]
imputed	rs2237297[$]		14%		$9.9\ 10^{-14}$	0.71	0.65-0.78	0.71	0.90 [n.s.]
imputed	rs1133683	17q24.1	42%	*Axin2*	$1.0\ 10^{-12}$	1.27	1.19-1.35	1.27	0.95 [n.s.]
imputed	rs2240307		5%		$7.7\ 10^{-24}$	0.41	0.34-0.49	0.40	0.62 [n.s.]
imputed	rs35285779[§]		9%		$3.2\ 10^{-22}$	0.58	0.52-0.65	0.58	1.10 [n.s.]
imputed	rs35415678[§]		9%		$3.7\ 10^{-19}$	0.62	0.56-0.69	0.62	1.10 [n.s.]
imputed	rs288326	2q32.1	10%	*FRZB*	$2.5\ 10^{-8}$	1.42	1.25-1.60	1.41	0.98 [n.s.]
imputed	rs17037102	4q25	15%	*DKK2*	$7.4\ 10^{-15}$	0.69	0.63-0.76	0.69	1.09 [n.s.]
ever smoker									
genotyped	rs12532321	7p14.1	45%	*SFRP4*	$1.3\ 10^{-9}$	1.14	1.09-1.19	1.15	1.13 [s.s.]
genotyped	rs7811872		36%		$1.3\ 10^{-8}$	0.88	0.84-0.92	0.88	0.88 [gw.s.]
genotyped	rs10226308		42%		$1.8\ 10^{-8}$	0.88	0.85-0.92	0.89	0.89 [gw.s.]
genotyped	rs10488617		42%		$1.6\ 10^{-8}$	0.88	0.85-0.92	0.89	0.89 [gw.s.]
genotyped	rs2722278		16%		$5.6\ 10^{-10}$	1.20	1.13-1.27	1.16	1.20 [gw.s.]
genotyped	rs2722279		11%		$9.0\ 10^{-9}$	1.22	1.14-1.31	1.17	1.23 [gw.s.]
genotyped	rs7811420		43%		$7.9\ 10^{-8}$	0.89	0.85-0.93	0.89	0.89 [gw.s.]
imputed	rs2073664	8p11.21	9%	*DKK4*	$9.4\ 10^{-11}$	1.20	1.14-1.27	1.15	1.08 [s.s.]

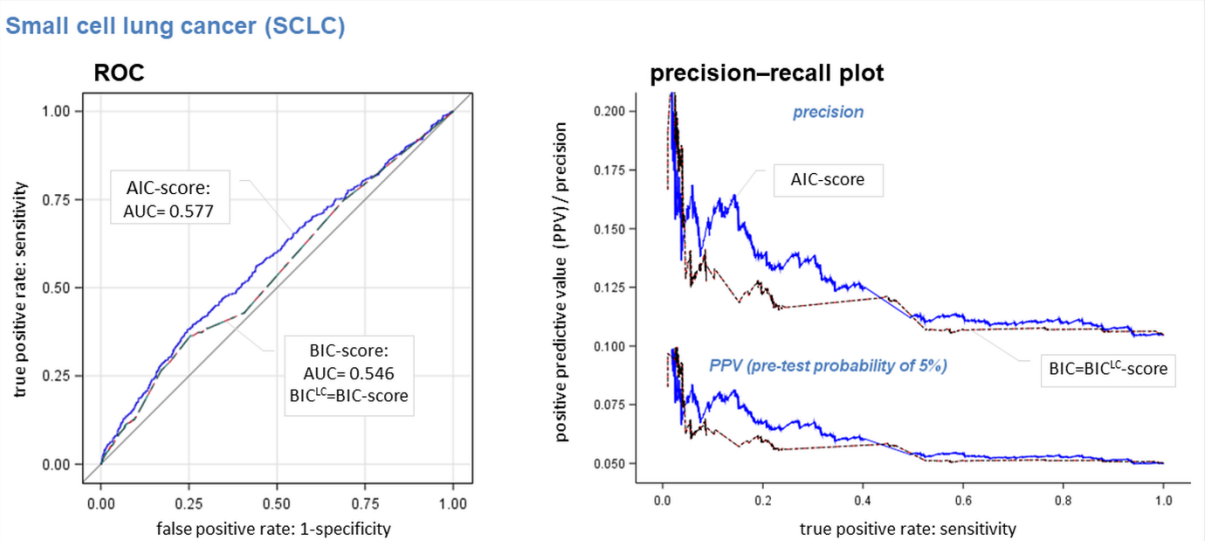
MAF: minor allele frequency; model 1: crude odds ratio (OR); model 2: adjusted for sex, age and smoking status and the first three principal components; model 3: OR additional adjusted for 6 selected *LC-markers*. [gw.s.] genome-wide significant (p-value ≤ 10^{-7}); [s.s.] suggestive significant (10^{-7} < p-value ≤ 10^{-5}); [n.s.] not significant (p>0.05). Only markers are listed for which genome-wide significance (p-value ≤ 10^{-7}) was achieved. [§, $] pair of markers in LD (R^2>0.8 in Populations of European decent)

Figure 2: Comparison of score composition



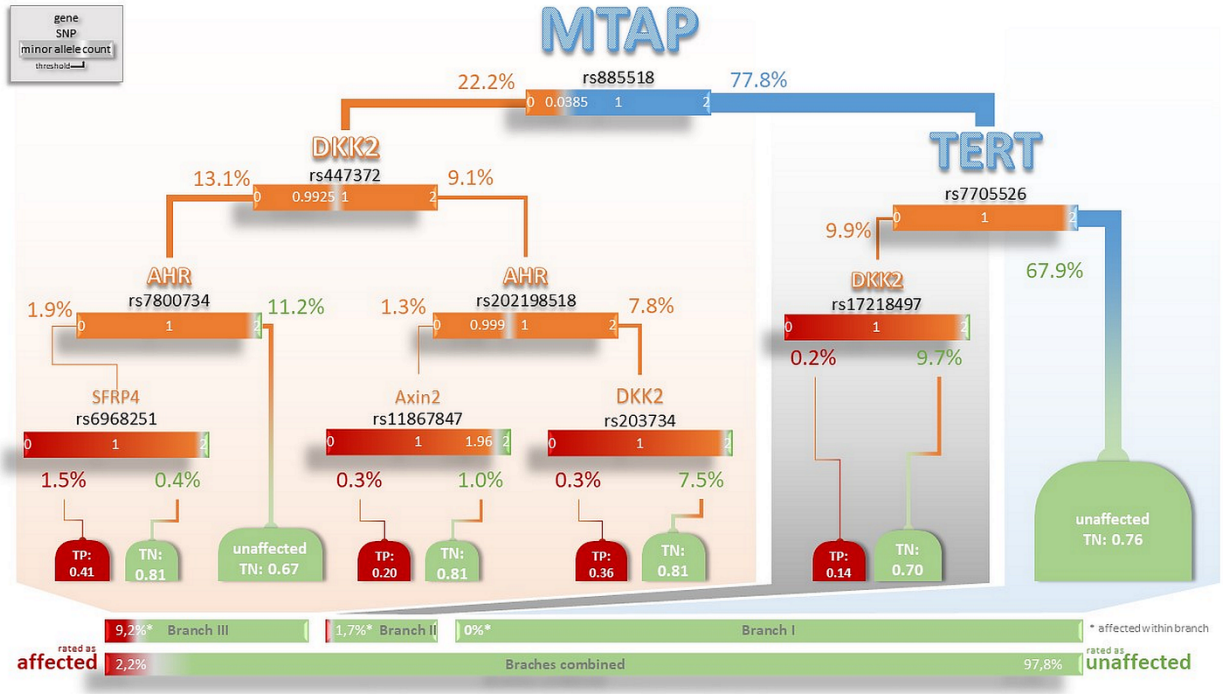
LC: lung cancer; AIC score: score of a logistic regression model with variant selection according to the Akaike information criterion (AIC); MAF_m: minor allele frequency of variant (marker) m; β_m regression parameter of variant m; LC-associated genes: previously reported as associated to LC or one of its histological subtypes; *Ahr/Wnt*-genes: selected genes assigned to Wnt-signalling, including AhR; Smoker: ever, former and current smoker; SCLC: small cell lung cancer, SqCLC : squamous cell lung cancer, Early onset LC: aged ≤55 years; TERT is framed in orange because telomerase activity is related to Wnt signalling.

Figure 3: ROC and precision-recall-plot: SCLC



The diagnostic performance of the AIC-score compared to the BIC/BIC^{LC}-score in the test-set is presented. Left panel: ROC (receiver operation characteristics); right panel: corresponding precision-recall plot; precision = (true positive cases) / (true positive cases + false positive controls), positive predictive value (PPV) = (sensitivity x pre-test-probability) / [(sensitivity x pre-test-probability) + (1-specificity x 1-pre-test-probability)] for a pre-test-probability of 5%.

Figure 4: Decision tree for never smoker



Node information: gene name, marker; split information below the node: threshold for minor allele count; blue split nodes: LC-genes, orange split nodes: Ahr/Wnt-genes, ; TERT is framed in orange because telomerase activity is related to Wnt signalling; decision nodes and bars: green for unaffected; red for affected, TN true negative rate, TP true positive red; the size of gene names, lines and decision notes is proportional to the size of the respective (sub)sample

References

1. Siegel RL, Miller KD, Jemal A (2016) Cancer statistics, 2016. CA Cancer J Clin 66:7–30

2. Ferlay J, Ervik M, Lam F, Colombet M, Mery L, Piñeros M, Znaor A, Soerjomataram I, Bray F Global Cancer Observatory: Cancer Today. https://gco.iarc.fr/today/data/factsheets/cancers/15-Lung-fact-sheet.pdf. Accessed 1 Jan 2020

3. Zhu W, Wang H, Zhu D (2021) Wnt/β-catenin Signaling Pathway in Lung Cancer. Medicine in Drug Discovery 100113

4. McKay JD, Hung RJ, Han Y, et al (2017) Large-scale association analysis identifies new lung cancer susceptibility loci and heterogeneity in genetic susceptibility across histological subtypes. Nat Genet 49:1126–1132

5. Timofeeva MN, Hung RJ, Rafnar T, et al (2012) Influence of common genetic variation on lung cancer risk: meta-analysis of 14 900 cases and 29 485 controls. Human molecular genetics 21:4980–95

6. Rosenberger A, Sohns M, Friedrichs S, et al (2017) Gene-set meta-analysis of lung cancer identifies pathway related to systemic lupus erythematosus. PLOS ONE 12:e0173339

7. Brenner DR, Brennan P, Boffetta P, et al (2013) Hierarchical modeling identifies novel lung cancer susceptibility variants in inflammation pathways among 10,140 cases and 11,012 controls. Human genetics 132:579–89

8. Ji X, Bossé Y, Landi MT, et al (2018) Identification of susceptibility pathways for the role of chromosome 15q25.1 in modifying lung cancer risk. Nature Communications 9:3221

9. Truong T, Sauter W, McKay JD, et al (2010) International Lung Cancer Consortium: coordinated association study of 10 potential lung cancer susceptibility variants. Carcinogenesis 31:625–633

10. Wang Y, Wei Y, Gaborieau V, et al (2015) Deciphering associations for lung cancer risk through imputation and analysis of 12 316 cases and 16 831 controls. European Journal of Human Genetics 23:1723–1728

11. Feng Y, Wang Y, Liu H, et al (2018) Novel genetic variants in the P38MAPK pathway gene ZAK and susceptibility to lung cancer. Mol Carcinog 57:216–224

12. Landi MT, Chatterjee N, Yu K, et al (2009) A genome-wide association study of lung cancer identifies a region of chromosome 5p15 associated with risk for adenocarcinoma. American journal of human genetics 85:679–91

13. Zhan T, Rindtorff N, Boutros M (2016) Wnt signaling in cancer. Oncogene 36:1461–1473. https://doi.org/10.1038/onc.2016.304

14. Buniello A, MacArthur JAL, Cerezo M, et al (2019) The NHGRI-EBI GWAS Catalog of published genome-wide association studies, targeted arrays and summary statistics 2019. Nucleic Acids Res 47:D1005–D1012

15. (2020) GWAS catalog. In: The NHGRI-EBI Catalog of human genome-wide association studies. https://www.ebi.ac.uk/gwas/. Accessed 22 Dec 2020

16. Kerdidani D, Chouvardas P, Arjo AR, et al (2019) Wnt1 silences chemokine genes in dendritic cells and induces adaptive immune resistance in lung adenocarcinoma. Nat Commun. https://doi.org/10.1038/s41467-019-09370-z

17. Fang L, Cai J, Chen B, et al (2015) Aberrantly expressed miR-582-3p maintains lung cancer stem cell-like traits by activating Wnt/beta-catenin signalling. Nat Commun 6:8640

18. Amos CI, Wu X, Broderick P, et al (2008) Genome-wide association scan of tag SNPs identifies a susceptibility locus for lung cancer at 15q25.1. Nat Genet 40:616–622

19. Yuan Y, Lu C, Xue L, Ge D (2014) Association between TERT rs2736100 polymorphism and lung cancer susceptibility: evidence from 22 case–control studies. Tumor Biol 35:4435–4442

20. Akiyama T (2000) Wnt/beta-catenin signaling. Cytokine Growth Factor Rev 11:273–82

21. Schneider AJ, Branam AM, Peterson RE (2014) Intersection of AHR and Wnt Signaling in Development, Health, and Disease. International Journal of Molecular Sciences 15:17852–17885

22. Chang JT, Chang H, Chen PH, Lin SL, Lin P (2007) Requirement of aryl hydrocarbon receptor overexpression for CYP1B1 up-regulation and cell growth in human lung adenocarcinomas. Clin Cancer Res 13:38–45

23. Lin P, Chang H, Tsai WT, Wu MH, Liao YS, Chen JT, Su JM (2003) Overexpression of aryl hydrocarbon receptor in human lung carcinomas. Toxicol Pathol 31:22–30

24. Wang CK, Chang H, Chen PH, Chang JT, Kuo YC, Ko JL, Lin P (2009) Aryl hydrocarbon receptor activation and overexpression upregulated fibroblast growth factor-9 in human lung adenocarcinomas. Int J Cancer 125:807–15

25. Bahl C, Singh N, Behera D, Sharma S (2017) High-order gene interactions between the genetic polymorphisms in Wnt and AhR pathway in modulating lung cancer susceptibility. Personalized Medicine. https://doi.org/10.2217/pme-2017-0018

26. Bahl C, Singh N, Behera D, Sharma S (2017) Association of polymorphisms in Dickopff (DKK) gene towards modulating risk for lung cancer in north Indians. Future Oncol 13:213–232

27. Yilmaz M, Donmez G, Kacan T, Sari I, Akgül Babacan N, Sari M, Kilickap S (2015) Significant Association Between Polymorphisms of Wnt Antagonist Genes and Lung Cancer: Journal of Investigative Medicine 1

28. Amos CI, Dennis J, Wang Z, et al (2017) The OncoArray Consortium: A Network for Understanding the Genetic Architecture of Common Cancers. Cancer Epidemiol Biomarkers Prev 26:126–135

29. Machiela MJ, Chanock SJ (2015) LDlink: a web-based application for exploring population-specific haplotype structure and linking correlated alleles of possible functional variants. Bioinformatics 31:3555–3557

30. Chang CC, Chow CC, Tellier LC, Vattikuti S, Purcell SM, Lee JJ (2015) Second-generation PLINK: rising to the challenge of larger and richer datasets. GigaScience 4:1–16

31. Purcell S, Neale B, Todd-Brown K, et al (2007) PLINK: a tool set for whole-genome association and population-based linkage analyses. American journal of human genetics 81:559–75

32. Stone M (1977) An Asymptotic Equivalence of Choice of Model by Cross-Validation and Akaike's Criterion. Journal of the Royal Statistical Society Series B (Methodological) 39:44–47

33. Fang Y (2009) Asymptotic Equivalence between Cross-Validations and Akaike Information Criteria in Mixed-Effects Models. JDS 15–21

34. DeLong ER, DeLong DM, Clarke-Pearson DL (1988) Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. Biometrics 44:837–845

35. Andri S (2021) DescTools: Tools for Descriptive Statistics.

36. Therneau T, Atkinson B, Ripley B (2019) rpart: Recursive partitioning for classification, regression and survival trees.

37. Uhlén M, Fagerberg L, Hallström BM, et al (2015) Tissue-based map of the human proteome. Science. https://doi.org/10.1126/science.1260419

38. The Human Protein Atlas. https://www.proteinatlas.org/. Accessed 5 Feb 2021

39. LungGENS. https://research.cchmc.org/pbge/lunggens/. Accessed 5 Feb 2021

40. Du Y, Guo M, Whitsett JA, Xu Y (2015) "LungGENS": a web-based tool for mapping single-cell gene expression in the developing lung. Thorax 70:1092–1094

41. Xue P, Fu J, Zhou Y (2018) The Aryl Hydrocarbon Receptor and Tumor Immunity. Front Immunol. https://doi.org/10.3389/fimmu.2018.00286

42. Xu H, Wu J, Chen B, et al (2014) Serum Dickkopf-1 (DKK1) is significantly lower in patients with lung cancer but is rapidly normalized after treatment. Am J Transl Res 6:850–856

43. Shen T, Chen Z, Qiao J, Sun X, Xiao Q (2019) Neutralizing monoclonal antibody against Dickkopf2 impairs lung cancer progression via activating NK cells. Cell Death Discovery 5:1–12

44. Lee PN, Forey BA, Coombs KJ (2012) Systematic review with meta-analysis of the epidemiological evidence in the 1900s relating smoking to lung cancer. BMC cancer 12:385

45. Katki HA, Kovalchik SA, Petito LC, Cheung LC, Jacobs J, Jemal A, Berg CD, Chaturvedi AK (2018) Implications of Nine Risk Prediction Models for Selecting Ever-Smokers for Computed Tomography Lung Cancer Screening. Annals of internal medicine. https://doi.org/10.7326/M17-2701

46. Jia G, Lu Y, Wen W, Long J, Liu Y, Tao R, Li B, Denny JC, Shu X-O, Zheng W (2020) Evaluating the Utility of Polygenic Risk Scores in Identifying High-Risk Individuals for Eight Common Cancers. JNCI Cancer Spectr. https://doi.org/10.1093/jncics/pkaa021

47. Jia G, Wen W, Massion PP, Shu X-O, Zheng W (2021) Incorporating Both Genetic and Tobacco Smoking Data to Identify High-Risk Smokers for Lung Cancer Screening. Carcinogenesis. https://doi.org/10.1093/carcin/bgab018

48. Hung RJ, Warkentin MT, Brhane Y, et al (2021) Assessing Lung Cancer Absolute Risk Trajectory Based on a Polygenic Risk Model. Cancer Res 81:1607–1615

49. Li Y, Xiao X, Bossé Y, et al (2019) Genetic interaction analysis among oncogenesis-related genes revealed novel genes and networks in lung cancer development. Oncotarget 10:1760–1774

50. Sun S, Schiller JH, Gazdar AF (2007) Lung cancer in never smokers--a different disease. Nature reviews Cancer 7:778–90

581 51. Saccone NL, Culverhouse RC, Schwantes-An T-H, et al (2010) Multiple Independent Loci at Chromosome
582 15q25.1 Affect Smoking Quantity: a Meta-Analysis and Comparison with Lung Cancer and COPD. PLOS Genet-
583 ics 6:e1001053

584 52. Liu CY, Stucker I, Chen C, Goodman G, McHugh MK, D'Amelio AM Jr, Etzel CJ, Li S, Lin X, Christiani DC (2015)
585 Genome-wide Gene-Asbestos Exposure Interaction Association Study Identifies a Common Susceptibility Var-
586 iant on 22q13.31 Associated with Lung Cancer Risk. Cancer Epidemiol Biomarkers Prev 24:1564–73

587 53. Wei S, Wang L-E, McHugh MK, Han Y, Xiong M, Amos CI, Spitz MR, Wei QW (2012) Genome-wide gene-envi-
588 ronment interaction analysis for asbestos exposure in lung cancer susceptibility. Carcinogenesis 33:1531–1537

589 54. Rosenberger A, Hung RJ, Christiani DC, et al (2018) Genetic modifiers of radon-induced lung cancer risk: a
590 genome-wide interaction study in former uranium miners. Int Arch Occup Environ Health.
591 https://doi.org/10.1007/s00420-018-1334-3

592 55. Lorenzo-González M, Ruano-Ravina A, Torres-Durán M, et al (2019) Residential radon, genetic polymorphisms
593 in DNA damage and repair-related. Lung Cancer 135:10–15

594 56. Hung RJ, Spitz MR, Houlston RS, et al (2019) Lung Cancer Risk in Never-Smokers of European Descent is Asso-
595 ciated With Genetic Variation in the 5p15.33 TERT-CLPTM1Ll Region. J Thorac Oncol 14:1360–1369

596 57. Du Y, Kitzmiller JA, Sridharan A, et al (2017) Lung Gene Expression Analysis (LGEA): an integrative web portal
597 for comprehensive gene expression data analysis in lung development. Thorax 72:481–484