No impact of passive smoke on the somatic profile of lung cancers in never-smokers

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ABSTRACT   *EGFR* and *HER2* mutations and *ALK* rearrangement are known to be related to lung cancer in never-smokers, while *KRAS*, *BRAF* and *PIK3CA* mutations are typically observed among smokers. There is still debate surrounding whether never-smokers exposed to passive smoke exhibit a "smoker-like" somatic profile compared with unexposed never-smokers.

Passive smoke exposure was assessed in the French BioCAST/IFCT-1002 never-smoker lung cancer cohort and routine molecular profiles analyses were compiled.

Of the 384 patients recruited into BioCAST, 319 were tested for at least one biomarker and provided data relating to passive smoking. Overall, 219 (66%) reported having been exposed to passive smoking. No significant difference was observed between mutation frequency and passive smoke exposure (*EGFR* mutation: 46% in never exposed *versus* 41% in ever exposed; *KRAS*: 7% *versus* 7%; *ALK*: 13% *versus* 11%; *HER2*: 4% *versus* 5%; *BRAF*: 6% *versus* 5%; *PIK3CA*: 4% *versus* 2%). We observed a nonsignificant trend for a negative association between *EGFR* mutation and cumulative duration of passive smoke exposure. No association was found for other biomarkers.

There is no clear association between passive smoke exposure and somatic profile in lifelong, never-smoker lung cancer.

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Introduction

Approximately one-third of adults and 40% of children are exposed to second-hand smoke worldwide, which accounts for 1% of all-cause mortality [1]. This exposure is estimated to cause >21 000 lung cancer-related deaths annually. Passive smoking is a well-known risk factor for lung cancer in never-smokers (LCINSs) [2]. Lifelong never-smokers living with a smoker have an estimated 26% increased risk of lung cancer (95% CI 7–47%), with both a dose–response and dose–duration relationship [3]. Similar results have been found for exposure in the workplace and during childhood [4, 5]. In a recent French study, we reported that 66% of LCINSs had been exposed to passive smoke, especially women and those exposed in domestic settings [6, 7].

The somatic mutation pattern in non-small cell lung cancers (NSCLCs) can vary widely according to smoking status. Never-smokers are known to carry a higher frequency of *EGFR* (epidermal growth factor receptor) and *HER2* (human epidermal growth factor receptor 2)/*ERBB2* (v-Erb-b2 avian erythroblastic leukaemia viral oncogene homologue 2) mutations, and *ALK* (anaplastic lymphoma kinase) rearrangement. In contrast, *KRAS* (Kirsten rat sarcoma viral oncogene homologue), *BRAF* (v-Raf murine sarcoma viral oncogene homologue B) [2, 8–12] and *PIK3CA* (phosphatidylinositol-3-kinase, catalytic subunit α) [8] mutations are thought to be linked to active smoking.

We thus hypothesised that the profile of somatic mutation in patients who were never-smokers and exposed to passive smoking could represent a "smoker-like" pattern, potentially differing from the profile of never-smokers who had never been exposed to passive smoking. Controversial results have been reported regarding an *EGFR* mutation profile of never-smokers exposed to passive smoking that proved similar to those observed in smokers [13–16]. It was our belief that in never-smokers, somatic alterations (*KRAS*, *BRAF* and *PIK3CA*) were more frequent, while *EGFR*, *HER2*/*ERBB2* and *ALK* alterations were not.

Here, we report the profile of the principal somatic alterations (*EGFR* and *KRAS* mutations, and *ALK* alteration) in a never-smoker BioCAST study, involving a large cohort of French LCINSs [17], according to their exposure to passive smoking, as well as for the profile of low-frequency mutations (*HER2*/*ERBB2*, *BRAF* and *PIK3CA*). Our study applied a multivariate approach and, overall, we found no significant difference.

Method
Population

For this study, we used data from the nationwide BioCAST/IFCT-1002 cohort of LCINSs in France. The study design has been reported elsewhere [18] and our principal results ~~were recently published~~ [7]. Briefly, BioCAST is a prospective, multicentre, observational study designed to describe the clinical, pathological and molecular epidemiology of LCINSs in a French population. This study enrolled consecutive, newly diagnosed NSCLC patients who professed themselves to be never-smokers (smoked <100 cigarettes in their lifetime). Patients were surveyed using a standardised questionnaire during a pre-planned phone interview with a member of the study team. This 17-page questionnaire requested information on demographics, occupational exposure [19], exposure to domestic pollution, and personal and familial medical history, as well as some lifestyle, hormonal and reproductive factors (women only). Additional medical data were collected directly from participating physicians.

The study was conducted in 75 centres throughout metropolitan France, from November 1, 2011, to January 31, 2013, and sponsored by the French Intergroup for Thoracic Oncology (IFCT). The IFCT research staff was in charge of administrative management and quality assurance in compliance with international research standards.

The Sud-Est IV (Lyon, France) ethics committee approved the study protocol on September 13, 2011. The Advisory Committee on Information Processing for Health Research (CCTIRS) authorised the use of a computerised database on September 8, 2011, and the National Commission for Data Protection (CNIL) was consulted on September 23, 2011, in accordance with French law. The BioCAST study was registered on the US National Institutes of Health website, at www.clinicaltrials.gov, under the identifier NCT01465854.

Within the BioCAST dataset used for the study, we determined patients who were self-declared as having ever been exposed to passive smoking, and had at least one biomarker test available for *EGFR*, *HER2*, *KRAS*, *BRAF*, *PIK3CA* and ALK.

Passive smoking exposure

Passive smoking exposure was self-declared by patients and reported on the standardised questionnaire. Exposure to passive smoke in a domestic setting was defined as "living in the same house as at least one

smoker for at least 1 year". Passive smoking exposure at the workplace was defined as "working with at least one smoker in the same closed room for at least 1 year". For this category, we retained only those who declared having worked with at least one smoker for the entire day, excluding exposure during breaks only or part-time days. Overall exposure was defined as a patient who reported being "ever exposed" to passive smoking either in a domestic setting or the workplace, or both. We also asked if patients had been exposed in a domestic setting before the age of 18 years old, usually considered as childhood age, or not.

For each patient declared as ever exposed, we additionally asked for the number of index smokers and the number of years of exposure to each index smoker. We then proceeded to calculate two additional variables for each domestic and workplace exposure: 1) the cumulative duration of exposure (CDE), computed as the sum of years exposed to passive smoking from each identified index-smoker; and 2) passive smoker-years (PSYs), computed as number of index smokers multiplied by the number of years of exposure to each index smoker. The corresponding variables for overall exposure to passive-smoke were obtained from a sum of the values calculated in a domestic setting and in the workplace.

Tumour somatic mutation analysis

The French National Cancer Institute (INCa) launched a network of 28 molecular genetics platforms, which provide routine cancer molecular testing for all patients [20]. Despite the variability of sample processing and analysis techniques in the corresponding laboratories, previous studies have reported the high quality of these analyses in thoracic oncology [21]. Each participating BioCAST physician was asked to systematically order tests for somatic mutations in *EGFR* and *KRAS*, as well as in the *ALK* fusion gene, to be recorded on its local labelled platform. Investigator sites were also encouraged to additionally request *BRAF*, *HER2* and *PI3KCA* mutation analyses, which are also routinely performed on these platforms. All centres were allowed to forego further mutation testing if one mutation known to exclude the others was found. The final, detailed results of these analyses were collected for each patient. We consulted the Catalogue of Somatic Mutations in Cancer for the purposes of categorising observed KRAS mutations in transversion (G>T or G>C) or transition (G>A or T>C). Given that most somatic mutations are mutually exclusive in the majority of lung cancer cases [11], we considered patients tested for at least *EGFR* and *KRAS* mutations, as well as *ALK* alterations, as "wild type", which was also optional for *HER2*, *BRAF* and *PIK3CA* tests, and for no mutation found in any tested biomarkers.

Statistics

Categorical variables are presented as percentages. Proportion comparison was conducted with the Chi-squared test, if the expected count in each category was at least 5, or with Fisher's exact test if not. To express the uncertainty of estimated probabilities, we have reported 95% Wilson confidence intervals. We used the one-sample Kolmogorov–Smirnov test to assess the plausibility of normal distribution assumption for continuous variables. Normally distributed continuous variables were expressed in mean and standard deviation. Means comparisons were performed applying the two-sided Student's t-test. Differences in distribution of continuous variables between two independent samples were assessed by the Mann–Whitney U-test. Continuous variables were also classed into tertile categories. We applied a binary logistic regression model to assess the risk of mutation for each considered gene. We generated three models: 1) unadjusted (crude odds ratios); 2) adjusted for sex and age only; and 3) comprehensively adjusted for the majority of potential confounding factors. Missing values were reported as such and all tests were two-sided. All statistics were performed by means of the SPSS V20 software (IBM SPSS Statistics, New York, USA).

Results

Population and passive smoking exposure

Of the 384 patients included in BioCAST, 334 presented complete data on overall passive smoke exposure and 219 (66%) reported being "ever exposed" to passive smoke overall (domestic or workplace). At least one biomarker was tested in 313 patients (fig. 1). The main population characteristics are presented in table 1.

Overall, 283 index smokers were responsible for the passive smoke domestic exposure reported by 198 LCINSs (122 patients reported an exposure beginning at childhood and 76 beginning during adulthood only) (table 2). Patients were exposed to a median (interquartile range) of 1 (1) index smoker. Most index smokers were parents (44%) and spouses (47%). In total, 60 patients reported exposure in their workplace. The median CDE was 24 (20) years in a domestic setting, 15 (25) years in the workplace and 26 (22) years overall.

Profile of *EGFR*, *KRAS* and *ALK* according to passive smoke exposure

Overall, 127 somatic mutations were detected in 297 tested samples of the *EGFR* gene, 18 mutations in 256 samples of *KRAS* and 20 in 171 of *ALK* rearrangement. The frequencies of somatic mutations in these biomarkers are presented according to exposure to overall passive smoke in table 3. There was no significant difference in any biomarker in terms of ever having been exposed to passive smoke compared to never having been exposed. When considering the CDE in years divided into tertiles, the frequency of *EGFR* mutation decreased as CDE increased (from 46% in never-exposed patients to 39% in highly exposed patients, defined as >30 years of cumulative exposure), though the difference was not statistically significant. Moreover, the frequency of *ALK* rearrangement appeared to increase as the CDE increased (from 5% for the lowest tertile to 18% for the highest), while the frequency of alteration in never-exposed patients was 13%.

Table 4 presents odds ratios for the likelihood of somatic mutation according to exposure to overall passive smoke for three logistic regression models. These are unadjusted, adjusted for age and sex, and additionally adjusted for body mass index, number of relatives with lung cancer, personal history of chronic bronchus diseases or respiratory infection, histology, definite occupational exposure, percentage of lifetime exposed to solid fuels for cooking and heating, and cooking dish-years (reflecting exposure to cooking oil fumes). As before, we found no significant association for any gene for ever *versus* never exposure. However, the previously found trend for *EGFR* mutation frequency decrease correlating with CDE increase remained, although still at statistically nonsignificant levels, as was the case for the *ALK* trend.

There was no significant difference between female and male patients for any variables or biomarkers. Similarly, we found no significant results when restricting analysis to solely domestic or workplace exposure, nor when considering PSYs divided into tertiles (data not shown).
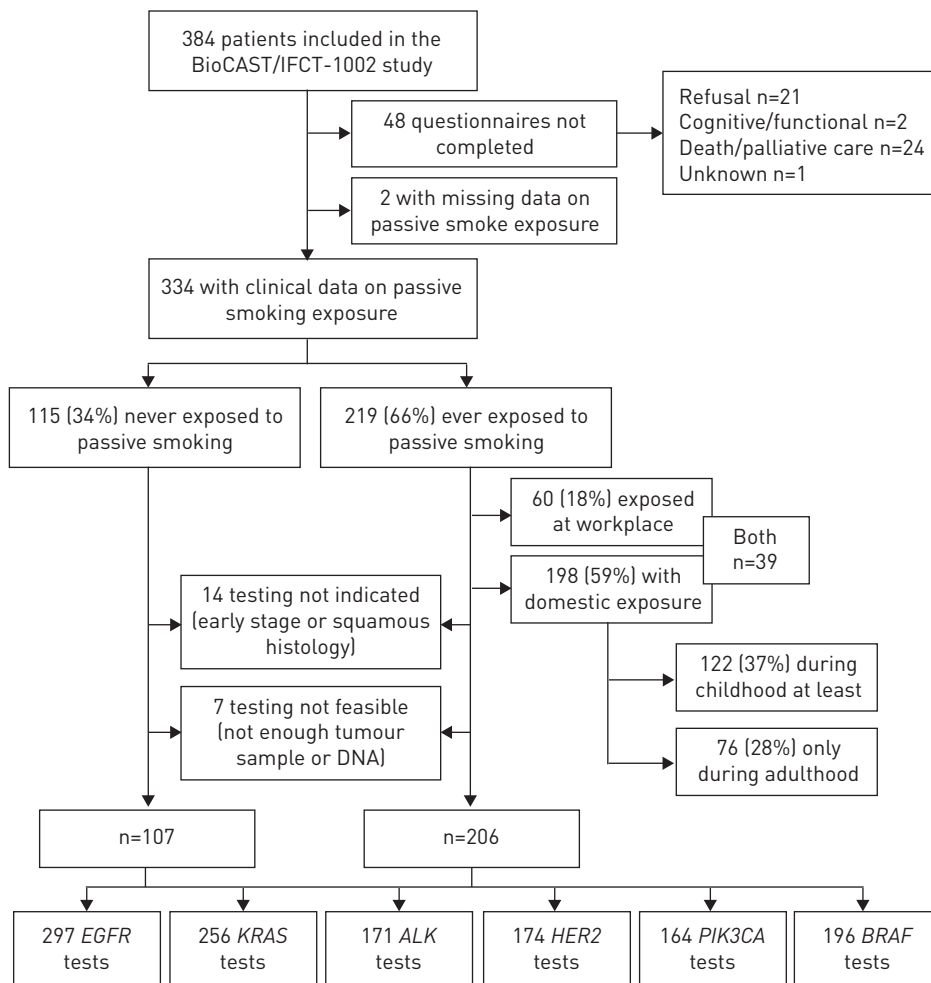

FIGURE 1 Study flow chart. *EGFR*: epidermal growth factor receptor; *KRAS*: Kirsten rat sarcoma viral oncogene homologue; *ALK*: anaplastic lymphoma kinase; *HER2*: human epidermal growth factor receptor 2; *PIK3CA*: phosphatidylinositol-3-kinase, catalytic subunit α; *BRAF*: v-Raf murine sarcoma viral oncogene homologue B.

TABLE 1 Main characteristics of the population according to overall exposure to passive smoking

	All	Never exposed to passive smoking	Ever exposed to passive smoking[#]	p-value
Subjects n	334	115	219	
Males n (%)	58 (17)	30 (26)	28 (13)	0.002
Age years mean±SD	70±12	69±12	70±12	0.399
Origin n (%)				
Europe	302 (90)	105 (91)	197 (90)	0.690
Other	32 (10)	10 (9)	22 (10)	
BMI kg·m^{-2} median (IQR)	24 (5)	25 (5)	24 (6)	0.508
Relatives[¶] with lung cancer				
0–1 n (%)	252 (76)	95 (83)	157 (72)	0.022
⩾2 n (%)	80 (24)	19 (17)	61 (28)	
Missing n	2	1	1	
Personal medical history n (%)				
Any cancer	62 (19)	20 (17)	42 (19)	0.317
Chronic bronchial disease[+]	49 (15)	16 (14)	33 (15)	0.777
Lung infection[§]	112 (34)	43 (37)	69 (32)	0.279
Solid fuel exposure[f]				
>50% of lifetime n (%)	67 (26)	26 (29)	41 (24)	0.465
Missing n	75	24	51	
Cooking oil exposure				
⩾10 cooking dish-years[##] n (%)	67 (21)	21 (19)	46 (22)	0.585
Missing n	12	5	7	
Definite occupational exposure				
⩾1 carcinogenic agent[¶¶]	43 (13)	16 (14)	27 (12)	0.660
Missing n	1	1		
Age at menarche[++] years median (IQR)	13 (2)	13 (2)	13 (2)	0.871
Missing n	13	3	10	
Parity[++,§§] median (IQR)	2 (2)	2 (2)	2 (1)	0.022
Age at first live birth[++] years median (IQR)	23 (5)	24 (5)	23 (5)	0.331
Missing n	22	12	20	
Age at menopause[++] years median (IQR)	50 (7)	50 (5)	50 (7)	0.584
Missing n	40	10	30	
Hormone intake for contraception or HRT[++]				
⩾10 years n (%)	78 (30)	24 (30)	54 (30)	0.951
Missing n	17	6	11	
Histology n (%)				
Squamous cell carcinoma	29 (9)	10 (9)	19 (9)	0.317
Adenocarcinoma	285 (85)	95 (83)	190 (87)	
Other and NOS	20 (6)	10 (9)	10 (5)	
Stage				
I–IIIA n (%)	81 (25)	23 (20)	58 (27)	0.188
IIIB–IV n (%)	250 (76)	91 (80)	159 (73)	
Missing n	3	1	2	

BMI: body mass index; IQR: interquartile range; HRT: hormone-replacement therapy; NOS: not otherwise specified. [#]: domestic and or workplace; [¶]: first-degree biological relatives; [+]: includes asthma, chronic obstructive pulmonary disease, bronchiectasis and emphysema; [§]: includes pertussis, pneumonia and tuberculosis; [f]: cooking and heating; [##]: 1 cooking-dish-year was defined as frying or stir-frying one dish per day in 1 year; [¶¶]: includes asbestos, silica, chrome, diesel, paint and polycyclic aromatic hydrocarbons; [++]: among women only; [§§]: live birth.

We observed no differences in the distribution of mutation type (based on exons in *EGFR*, and the transversion or transition mechanism for *KRAS*) according to the exposure to overall passive smoke (table 5). However, there was a nonsignificantly higher frequency of *KRAS* transversion in patients exposed to passive smoke compared with those who were never exposed (82% *versus* 60%, respectively).

Profile of HER2, BRAF and PIK3CA *according to passive smoke exposure*

The univariate analysis results for low-frequency biomarkers have been provided in the table S1. We observed no significant association between somatic profile and exposure to passive smoke. Multivariate analyses were not performed due to the very low number of mutations and the expected wide 95% confidence intervals.

TABLE 2 Exposure to passive smoking in the BioCAST population

	Domestic exposure			Workplace exposure	Overall exposure[+]
	Overall	Childhood[#]	Adulthood[¶]		
Exposure n (%)					
Never	136 (41)	212 (63)	258 (77)	274 (82)	115 (34, 95% CI 29–40)
Ever	198 (59)	122 (37)	76 (23)	60 (18)	219 (66, 95% CI 60–71)
Index smokers n median (IQR)	1 (1)			1 (1)	
Missing n				12	
PSYs median (IQR)	24 (20)	18 (6)	22 (19)	18 (24)	26 (23)
Missing n	4	5	1	12	8
CDE years median (IQR)	24 (20)	18 (6)	22 (19)	15 (25)	26 (22)
Missing n	4	5	1	1	3

n=334. IQR: interquartile range; PSY: passive smoker-year; CDE: cumulative duration of exposure. [#]: at least; [¶]: only; [+]: domestic and workplace.

TABLE 3 Main somatic mutation profile according to exposure to passive smoking in univariate analysis

	EGFR[#]		KRAS[¶]		ALK[+]	
	Mutations n (%)	p-value	Mutations n (%)	p-value	Rearrangements n (%)	p-value
Exposure to passive smoking						
Never	46 (46, 95% CI 36–56)	0.486	6 (7, 95% CI 3–15)	0.867	7 (13, 95% CI 6–26)	0.726
Ever	81 (41, 95% CI 34–49)		12 (7, 95% CI 4–13)		13 (11, 95% CI 6–19)	
Cumulative duration of exposure						
Never	46 (46)	0.866	6 (7)	NC	7 (13)	NC
⩽20 years	28 (44)		4 (7)		2 (5)	
20–30 years	28 (42)		4 (7)		4 (10)	
>30 years	25 (39)		4 (8)		6 (18)	
Missing	3		1		2	
Time of exposure						
Never	53 (45)	0.799	8 (8)	0.940	7 (11)	0.522
Childhood	45 (41)		6 (7)		10 (15)	
Adulthood only	29 (43)		4 (7)		3 (8)	
Workplace exposure						
Never	105 (43)	0.942	15 (7)	0.643[f]	18 (12)	0.442[f]
Ever	22 (42)		3 (7)		2 (8)	
Domestic exposure						
Never	53 (45)	0.542	8 (8)	0.458[f]	7 (11)	0.533[f]
Ever	74 (41)		10 (7)		13 (12)	
Childhood exposure						
Never[§]	82 (44)	0.550	12 (7)	0.784	10 (10)	0.320
Ever	45 (41)		6 (7)		10 (15)	

p-values were calculated by the Chi-squared test unless otherwise stated. EGFR: epidermal growth factor receptor; KRAS: Kirsten rat sarcoma viral oncogene homologue; ALK: anaplastic lymphoma kinase; NC: not computable. [#]: 297 tested samples; [¶]: 256 tested samples; [+]: 171 tested samples; [§]: never-exposed to passive smoking and exposed during adulthood only; [f]: Fisher test.

Overall somatic profile

Figure 2 presents the frequency of mutation for each biomarker tested, according to CDE for passive smoke and in never-smokers never exposed to passive smoke.

Overall, 248 patients underwent a full biomarker analysis. The mutation profile, categorised by passive smoke exposure, is presented in figure S1. Once more, we observed the same trend for a decreasing frequency of EGFR mutation correlating with CDE increase. Interestingly, "wild-type" status often tended to increase along with CDE. The frequency of multiple mutations (more than one mutation in different genes) was stable across all categories.

TABLE 4 Odds ratios for somatic mutation according to passive smoking exposure and cumulative duration of exposure

	Crude		Adjusted for age and sex		Comprehensively adjusted[#]	
	OR (95% CI)	p-value	aOR (95% CI)	p-value	aOR (95% CI)	p-value
EGFR						
Exposure to passive smoking						
Ever	0.84 (0.52–1.37)	0.49	0.81 (0.49–1.32)	0.40	0.80 (0.47–1.36)	0.41
Never	1.00		1.00		1.00	
Cumulative duration of exposure						
⩽20 years	0.96 (0.51–1.80)	0.89	0.93 (0.49–1.76)	0.83	0.90 (0.46–1.79)	0.77
20–30 years	0.88 (0.47–1.65)	0.69	0.84 (0.44–1.58)	0.59	0.87 (0.44–1.72)	0.69
>30 years	0.77 (0.41–1.45)	0.41	0.71 (0.37–1.37)	0.31	0.68 (0.34–1.37)	0.28
KRAS						
Exposure to passive smoking						
Ever	1.09 (0.40–3.01)	0.87	1.09 (0.39–3.05)	0.87	1.11 (0.34–3.60)	0.86
Never	1.00		1.00		1.00	
Cumulative duration of exposure						
⩽20 years	1.12 (0.30–4.16)	0.87	1.19 (0.32–4.46)	0.80	1.24 (0.29– 5.21)	0.77
20–30 years	1.04 (0.28–3.85)	0.96	1.03 (0.27–3.87)	0.96	0.86 (0.19–3.86)	0.85
>30 years	1.14 (0.31–4.25)	0.84	1.07 (0.28–4.13)	0.92	1.31 (0.30–5.78)	0.72
ALK						
Exposure to passive smoking						
Ever	0.84 (0.31–2.24)	0.73	0.76 (0.28–2.09)	0.60	1.35 (0.39–4.71)	0.64
Never	1.00		1.00		1.00	
Cumulative duration of exposure						
⩽20 years	0.36 (0.07–1.85)	0.22	0.35 (0.07–1.81)	0.21	0.64 (0.10–4.08)	0.64
20–30 years	0.71 (0.19–2.60)	0.60	0.64 (0.17–2.39)	0.50	1.05 (0.22–5.02)	0.95
>30 years	1.44 (0.44–4.71)	0.55	1.28 (0.38–4.39)	0.69	3.26 (0.71–14.95)	0.13

aOR: adjusted odds ratio; *EGFR*: epidermal growth factor receptor; *KRAS*: Kirsten rat sarcoma viral oncogene homologue; *ALK*: anaplastic lymphoma kinase. [#]: adjusted for sex (binary), age (continuous), body mass index (continuous), number of relatives with lung cancer (continuous), personal history of chronic bronchus diseases (binary), personal history of respiratory infection (binary), histology (adenocarcinoma, squamous cell carcinoma or other), definite exposure to any main bronchus carcinogen (binary), percentage of lifetime exposed to solid fuels for cooking and heating (continuous), and cooking dish-years (continuous).

TABLE 5 Distribution of mutation type according to exposure to overall passive smoking for *EGFR* and *KRAS*

	Never exposed	Ever exposed	p-value
EGFR			
Mutation in exon 18	0 (0)	3 (4)	NC
Mutation in exon 19	27 (60)	49 (60)	
Mutation in exon 20	2 (4)	3 (4)	
Mutation in exon 21	14 (31)	24 (30)	
Double mutation in *EGFR* gene	2 (4)	2 (2)	
Missing	1		
KRAS			
Transition[#]	2 (40)	2 (18)	0.547
Transversion[¶]	3 (60)	9 (82)	
Missing	1	1	

Data are presented as n (%) or n, unless otherwise stated. *EGFR*: epidermal growth factor receptor; *KRAS*: Kirsten rat sarcoma viral oncogene homologue; NC: not computable. [#]: G13D, G12G or G12D; [¶]: G12V, G12R or G12A.

Discussion

In this study, we demonstrated that the mutation profile was not affected by exposure to passive smoke in a French population of never-smoker lung cancer sufferers for any biomarker of the tested (*EGFR*, *KRAS*, *HER2*, *BRAF*, *PIK3CA* and *ALK*).
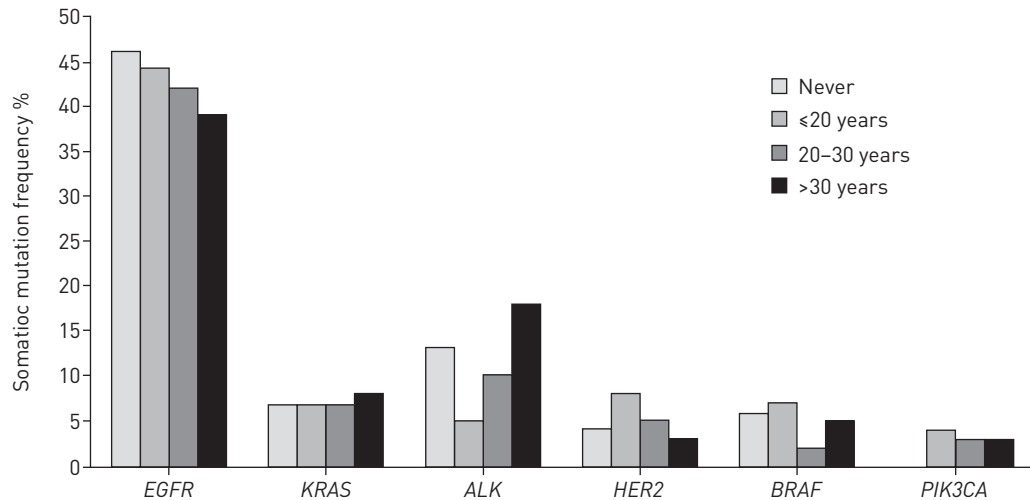
FIGURE 2 Somatic mutation pattern according to exposure to passive smoke for *EGFR* (epidermal growth factor receptor), *KRAS* (Kirsten rat sarcoma viral oncogene homologue), *ALK* (anaplastic lymphoma kinase), *HER2* (human epidermal growth factor receptor 2), *BRAF* (v-Raf murine sarcoma viral oncogene homologue B) and *PIK3CA* (phosphatidylinositol-3-kinase, catalytic subunit α).

There have been studies previously focused on this topic, such as the study by LEE *et al.* [13] involving 179 consecutive Asian LCINS patients with detailed self-reported data on environmental tobacco smoke exposure. Those authors found that passive smoke exposure negatively correlated with *EGFR* mutation frequency in a multivariate logistic regression model adjusted for sex and histology. That study also reported a trend for a dose–response relationship with PSYs, with a decreasing frequency of *EGFR* mutation observed when PSYs increased (OR for the highest quartile: 0.22, 95% CI 0.08–0.62). These results were consistent with those previously reported by TAM *et al.* [16] in 241 patients (all smoking statuses included), proving that passive smoke exposure tends to decrease the probability of *EGFR* mutation compared with never-smoking alone in univariate analysis (61.1% *versus* 74.8%, respectively; p=0.257). However, KAWAGUCHI *et al.* [14] reported contrasting findings in a population of 126 Asian LCINSs. They demonstrated that *EGFR* mutation frequency increased significantly among increasing CDE quintiles. In a multivariate model, which took into account sex, age and family history of cancer, they showed that CDE was positively linked to the incidence of an *EGFR*-activating mutation. The same team reported similar results at the 2013 World Lung Cancer Congress in 498 LCINSs of various ethnicities (425 from Asia). *EGFR* mutation was again positively associated, in a multivariate model, with CDE, though only in women (OR 1.084, 95% CI 1.003–1.171 (p=0.0422) for each 10-year CDE increase) [22]. Finally, TAGA *et al.* [15] reported their findings for 143 never- and long-term former smokers from two US cohorts. They observed a higher *EGFR* mutation frequency among patients who were not exposed to passive smoking compared with exposed patients in one of the two cohorts, while they found the opposite in the second cohort.

All these conflicting results could be explained by the inherent difficulty in accurately reporting passive-smoke exposure [23]. In all these studies, including our own, passive smoking is only self-reported by the patients. A biological assessment of passive smoking, such as blood plasma or urine cotinine concentration measurement [4], or even urinary 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol and its glucuronides [24], may be more reliable, yet is also more costly and thus not used in our study, nor in the other previously mentioned studies. Furthermore, biological assessment is only relevant for current, not former, exposure. The frequency of overall passive-smoke exposure differs strongly between each of these studies, despite a common definition: 135 (75%) out of 179 patients in the study by LEE *et al.* [13], 73 (51%) out of 143 patients in that by TAGA *et al.*[15], 124 (98%) out of 126 patients in that by KAWAGUSHI *et al.* [14] (445 (89%) out of 498 in the latest update [22]), 19 (9%) out of 241 patients in that by TAM *et al.*[16] and 219 (66%) out of 334 patients in our study. Moreover, some bias, such as memorisation or redaction bias. could occur when reporting this exposure. In addition, dose and duration variables may also be inappropriate for accurately assessing cumulative exposure. As an example, in our cohort, a barman exposed daily to passive smoking from many smokers over 10 years was unable to enumerate the number of index smokers to whom he had been exposed. Thus, we were only able to compute the CDE, which was 10 years. In contrast, someone exposed to two index smokers at the same time would have a CDE that was twice as long (20 years) as that of the the barman. Similar concerns may occur for the PSY

variable. In addition, the population in which the studies were conducted differed from study to study, ranging from Asian only [13, 14, 16] to primarily Caucasian [7, 15], and from never-smoker only [13, 14] to all smoking statuses mixed [16]. These differences may also account for the conflicting results, as genomic susceptibility and confounding related to risk-factor exposure could differ depending on geographical origin [2, 25].

With the exception of *EGFR*, other oncogenes have been poorly investigated in this setting. *KRAS* mutation frequency was found not to differ between never-smokers and passive smokers (3.6% *versus* 0%, respectively; p=1.000) in one study [16]. In addition, KUBO *et al.* [22] and RYAN *et al.* [26] reported no association between passive smoke exposure and the frequency of *ALK* rearrangement.

Our findings suggest that passive smoking exposure is not the only risk factor for lung cancer in never-smokers, and possibly not the leading one either. This actually constitutes one of the possible reasons why mutation spectrum is not affected by indirect smoking exposure. These findings are in line with recent observations obtained by whole-genome sequencing [27]. Thus, some other risk factor may be suspected in this setting, such as environmental pollution (including natural radon and atmospheric pollution), occupational exposure, domestic pollution (cooking and heating fumes, or cooking oil), personal history of cancer or respiratory disease, or familial history of cancer supporting the role of genetic factors [2]. As BioCAST has a large dataset on exposure to these factors, we will fully explore these issues in further analysis. Electronic cigarettes should also be closely assessed for lung cancer risk in future studies.

Our study had some limitations. Firstly, the biomarker analyses were not centrally performed and used different processes depending on the centre. However, the performance and concordance of sample processing and mutation screening methods across participating centres had previously been investigated in blinded cross-validation studies, producing good results [21]. Secondly, except for *EGFR*, the number of mutations was low for all oncogenes, ranging from 18 for *KRAS* to four for *PIK3CA*. Our study may therefore lack power yet, to our knowledge, our cohort remains the largest and most comprehensive of its field in a European population. However, European descents are heterogeneous. Thirdly, we had to deal with some missing values, especially PSYs, along with the biomarker mutation testing, in which missing value rates ranged from 11% for *EGFR* to 51% for *PIK3CA* (fig. 1). Nevertheless, all missing values have been reported as such in the results section.

Our study also had some strengths. Firstly, inclusion was strictly restricted to never-smokers. In addition, we recorded a substantial amount of data about potential confounders and were able to compute a more comprehensive adjustment for most of them than other studies have. Secondly, this represents the largest study involving almost exclusively European-descent patients. It is also the first reported study investigating six of the major lung cancer oncogenes, namely *EGFR*, *KRAS*, *HER2*, *BRAF*, *PIK3CA* and *ALK*, concomitantly regarding passive smoke exposure. Thirdly, we collected all indicators of passive smoke exposure used in previous studies, such as CDE, PSYs, childhood exposure, *etc.*, in order to provide an easy and comprehensive comparison with others.

In conclusion, never-smoker patients with lung cancer exposed to passive smoke were found to not carry a smoker-like somatic mutation profile in terms of *EGFR*, *KRAS*, *HER2*, *BRAF*, *PIK3CA* and *ALK* together when compared to unexposed never-smoker patients. Passive smoking alone appeared to be insufficient to determine a somatic profile in lung cancer.

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