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Germline control of somatic *Kras* mutations in mouse lung tumors

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Somatic *KRAS* mutations are common in human lung adenocarcinomas and are associated with worse prognosis. In mice, *Kras* is frequently mutated in both spontaneous and experimentally induced lung tumors, although the pattern of mutation varies among strains, suggesting that such mutations are not random events. We tested if the occurrence of *Kras* mutations is under genetic control in two mouse intercrosses. Codon 61 mutations were prevalent, but the patterns of nucleotide changes differed between the intercrosses. Whole genome analysis with SNPs in (A/J x C57BL/6)F4 mice revealed a significant linkage between a locus on chromosome 19 and 2 particular codon 61 variants (CTA and CGA). In (AIRmax × AIRmin) F2 mice, there was a significant linkage between SNPs located on distal chromosome 6 (around 135 Mbp) and the frequency of codon 61 mutation. These results reveal the presence of two loci, on chromosomes 6 and 19, that modulate *Kras* mutation frequency in different mouse intercrosses. These findings indicate that somatic mutation frequency and type are not simple random events, but are under genetic control.

KEYWORDS
genome-wide association study, *Kras*, lung cancer, *Pas1*, polymorphisms

1 | INTRODUCTION

The mutational profiles of lung cancer differ greatly among individuals.[1] The two most frequently mutated oncogenes in lung adenocarcinoma encode the epidermal growth factor receptor and KRAS.[2] Mutations in *KRAS* gene are associated with worse survival.[3,4] KRAS is a member of the Ras subfamily of membrane-bound GTP-binding proteins that regulate cell proliferation, differentiation and cell death through interactions with kinase signaling cascades and transcription factors.[5] *KRAS* mutations are most frequently missense mutations at codons 12, 13, and 61 that lead to constitutively active signaling.[6,7] *KRAS* mutations have also been found in adenomatous atypical

hyperplasia,[8] a pathological alteration considered to be a precursor of lung adenocarcinoma. Thus *KRAS* mutations appear to be early events in lung tumorigenesis.

Human KRAS and murine Kras proteins are homologs, with 97.3% and 98.9% sequence identity for the 4B and 4A isoforms, respectively. Murine *Kras* gene is frequently mutated in experimentally induced lung adenomas and adenocarcinomas.[9] *Kras* mutations in mice affect almost exclusively codons 12 and 61, depending on the chemical carcinogen used to induce tumors.[10–12] Several studies have shown that *Kras*-activating mutations induce an early inflammatory response, consisting in a reactive lymphocytic infiltration of lungs, that enhances tumorigenesis in mice.[13,14] These observations point at the close interaction between inflammation and cancer.

Olga C.M. Ibañez and Giacomo Manenti contributed equally to the study.

Different mouse strains vary in susceptibility to spontaneous or chemically induced lung tumors.[15] We previously crossed the susceptible A/J and resistant C3H/He mouse lines and used linkage analysis to reveal a major effect on lung tumorigenesis by the *Pas1* (*Pulmonary adenoma susceptibility 1*) locus on chromosome 6.[16] This locus encompasses the *Kras* gene. Other chromosomal regions also contain loci that modulate lung tumor susceptibility, revealing the complex nature of the genetic control of lung tumorigenesis in mice.[17] *Pas1* was identified as a quantitative trait locus (QTL) for lung tumor susceptibility [16] and inflammatory response.[18,19] We later further characterized it as an expression QTL (eQTL) modulating *Kras* expression in lung tumors as well as in normal tissue.[20] Recently, we described a role of *Pas1* as a major regulator of transcription in lung tumor nodules.[21] Thus *Kras* is considered a good candidate for the active element of *Pas1*.

The pattern of somatic *Kras* mutations in lung tumors varies among mouse strains regarding the codons affected and the specific nucleotide changes.[11,12,22–24] This variability, in which differences in epistasis may play a role,[23] may be due either to a genetic control or to random processes, and the mechanisms generating or selecting for certain *Kras* mutations are unknown.

Because of the importance of *Kras* mutations in the pathogenesis of lung cancer and of the major role of genetic factors in modulating strain susceptibility to mouse lung tumorigenesis, we investigated whether *Kras* mutation frequency and type in lung tumors are under genetic control. This study employed two established murine models of lung disease that we have already used to study the genetics of lung tumorigenesis: The first model consisted of a population generated by crossing lung-cancer-susceptible A/J inbred mice with lung-cancer-resistant C57BL/6J inbred mice for four generations.[20] The second was a population generated by intercrossing AIRmax and AIRmin noninbred mice for two generations.[18] AIRmax mice are resistant while AIRmin animals are susceptible to chemically induced lung tumorigenesis,[19,25] even though these mouse lines were originated by phenotypic selection for having an intense (AIRmax) or weak (AIRmin) acute inflammatory response to the subcutaneous injection of a neutral substance.[26] Taking advantage of existing phenotype and whole-genome genotype data as well as genomic DNA from animals of these two crosses, this study examined the association of genome variations with somatic *Kras* mutations in chemically induced lung tumors.

2 | MATERIALS AND METHODS

2.1 | Ethics statement

The animal materials used in this study derive entirely from previous studies by our group, both already reported[18,20] and never reported (data on the urethane treatment of ABF1 and female ABF4 mice). No additional animals were used for this study. The original study protocols had received institutional ethical approval.

In Italy, animals received humane care according to the criteria outlined in a protocol approved on December 21, 2006, by the institutional ethical committee for animal use (CESA) at the Fondazione IRCCS Istituto Nazionale dei Tumori, and sent to the Ministry of Health as required by the Italian regulation (d.lgs. 116/92) in force at the time. In Brazil, animal procedures had been approved by the Institutional Animal Care and Use Committee of Butantan Institute (protocol number: 671/09) where animals received humane care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals."[27] Animal experiments complied with the ARRIVE guidelines and were carried out in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments, or the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978).

2.2 | Mouse crosses, lung tumor induction, and DNA extraction

As previously reported,[20] (A/J × C57BL6) F1 hybrids and (A/J × C57BL6) F4 progeny (called ABF1 and ABF4 mice, respectively) had been obtained by crossing female A/J with male C57BL/6J mice, and breeding the animals up to four generations. Genomic DNA was purified from tail tips of all ABF4 mice (male and female) at weaning.

At 4 weeks of age, 140 ABF1 mice (77 male, 63 female), and 396 ABF4 mice (183 male, 213 female) received urethane (1 g/kg body weight) by intraperitoneal injection to induce lung tumors (only data for male ABF4 mice were previously reported.[20]) At 40 weeks of age, these mice were killed and their lungs were infused with RNAlater solution (Invitrogen, Thermo Fisher Scientific) and then removed. Surface tumors were counted and, whenever possible, one tumor per mouse of about 1-1.5 mm was excised for the extraction of tumor DNA.

AIRmax and AIRmin lines (formally designated IBut:AIRH and IBut:AIRL, respectively) at the Institute for Laboratory Animals Research, Washington, DC, USA) and their crosses (AIRmax × AIRmin) F1 (*n* = 61) and (AIRmax × AIRmin) F2 (*n* = 693) were developed and maintained at the animal facilities of the Laboratory of Immunogenetics of the Butantan Institute. As previously reported,[18] F1 and F2 mice of both sexes received urethane (300 mg/kg body weight, subcutaneously) 7 days after birth and were euthanized at 240 days of age; the urethane treatment of AIRmax and AIRmin mice was done at early age in order to evaluate the genetics of tumor development in other organs.[28] Lung tumors were counted and tumor DNA was extracted from one tumor per mouse (*n* = 528 tumors). Genomic DNA was extracted from tail tips of all F2 mice.

2.3 | *Kras* mutations analysis

Kras activating mutations at codons 12-13 (exon 1) and 61 (exon 2) were identified in tumor DNA by DNA pyrosequencing on a PSQ96MA system (Biotage, Uppsala, Sweden) using primers reported in Supplementary Table S1. The nucleotide sequence of *Kras* was determined from the signal peaks in the pyrogram trace. The mutations at nucleotide level were expressed as "present" or "absent" for use as binary phenotypes in genome-wide linkage analysis and single-point association analysis with specific SNPs.

2.4 | SNP genotyping

Genome-wide genotype data for male ABF4 mice[20] and (AIR-max × AIRmin) F2 male and female mice[18] had been obtained using the GoldenGate Genotyping Assay (Illumina, San Diego, CA) with the Mouse MD Linkage Panel, which represents 1449 mouse SNPs over the whole genome. After quality control filtering, genotypes were obtained for 548 informative (polymorphic) non-redundant SNPs in ABF4 mice and for 879 informative SNPs in (AIRmax × AIRmin) F2 mice. Genome maps are based on Genome assembly GRCm38, Ensembl release 71.

Female ABF4 mice were genotyped for six SNPs (rs13483540, rs13459194, rs13483612, rs3656289, rs3023496, rs13483682) on chromosome 19 in a 39.696 Mbp region, spanning from 15.466 to 55.162 Mbp, and comprising markers rs13483612 and rs3655407. This work was done by pyrosequencing with primers reported in Supplementary Table S2. Variations in nucleotide sequence were determined from the signal peaks in the pyrogram trace.

2.5 | Statistical analysis

In ABF4 mice, quantitative trail loci (QTLs) modulating Kras mutation frequency were identified by crossing data on the phenotypes of each Kras mutation with data on SNP genotypes. This analysis was done by simple interval mapping using the scanone function in R/qtl package with a model for binary traits and the expectation maximization (EM) algorithm R/qtl.[29] Logarithm of odds (LOD) scores were considered significant or suggestive when greater than the 95% (α = 0.05) or 90% (α = 0.1) LOD threshold, respectively, calculated by 10 000 permutations. In (AIRmax × AIRmin) F2 mice, genome-wide association analysis was carried out between genotype and the Kras mutational status; this analysis was done using the "assoc" command (which compares frequencies of alleles between mice carrying and not carrying the mutation) in PLINK.[30] The genome-wide statistical threshold for significance was calculated by 10 000 permutations. Single-point association analyses between specific SNPs and Kras mutation phenotypes were carried out using Fisher's exact test.

3 | RESULTS

3.1 | Linkage analysis for somatic Kras mutations in lung tumors of urethane-treated (A/J × C57BL/6) mice

Kras codons 12, 13 and 61 were sequenced to identify mutations in lung tumor DNA from 140 urethane-treated (A/J × C57BL/6) F1 (ABF1) mice and from 280 urethane-treated (A/J × C57BL/6) F4 (ABF4) mice. Single point mutations in codons 12 and 61, but not 13, were found in the majority of animals, as only about 10% of animals had the complete wild-type sequence (Table 1). Mutations in codon 61 were prevalent. In particular, at the second base of codon 61, the transition (A to G) leading to a Glu/Arg (Q61R) substitution and the transversion (A to T) resulting in a Glu/Leu (Q61L) substitution were the most frequent. In codon 12, a single missense mutation (G12D) was observed, at a 3.6% and 10.7% frequency in ABF1 and ABF4 mice, respectively (Table 1).

To investigate whether the frequency or type of Kras somatic mutation in lung tumors is under genetic control, we carried out genetic linkage analysis in the population of male ABF4 mice, using genotype data for 548 SNPs dispersed over 19 autosomes. At a

TABLE 1 Frequencies of Kras mutations at codons 12 and 61 in lung tumors of (A/J × C57BL/6) mice treated with urethane

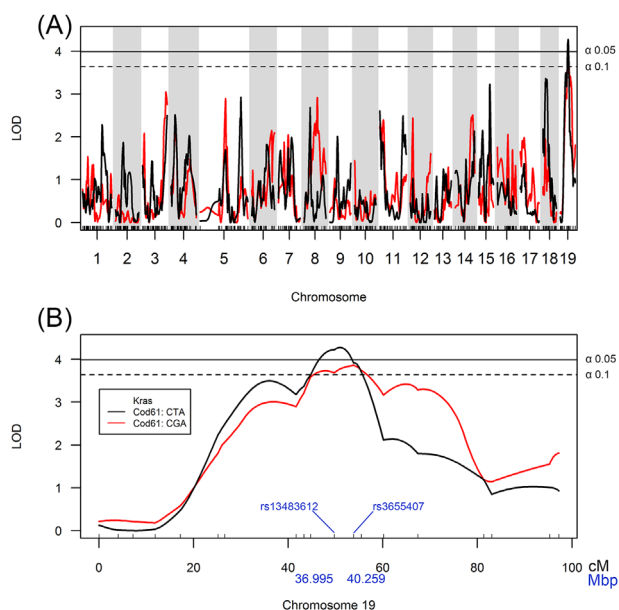|	ABF4			
Genotype	Male(n = 134)	Female (n = 146)	Total (n = 280)	ABF1[a] (n = 140)
Mutations in codon 12				
AGT (G12S)	0	0	0 (0)	0
TGT (G12C)	0	0	0 (0)	0
CGT (G12R)	0	0	0 (0)	0
GAT (G12D)	12 (9.0)	18 (12.3)	30 (10.7)	5 (3.6)
GTT (G12V)	0	0	0 (0)	0
Mutations in codon 61				
CGA (Q61R)	33 (24.6)	48 (32.9)	81 (28.9)	57 (40.7)
CTA (Q61L)	72 (53.7)	70 (47.9)	142 (50.7)	63 (45.0)
CCA (Q61P)	0	0	0 (0)	0
CAT (Q61H)	1 (0.7)	0	1 (0.4)	0
CAC (Q61H)	0	0	0 (0)	0
Wild-type at both codons[b]	16 (11.9)	10 (6.8)	26 (9.3)	15 (10.7)

Values are number (percentage) of tumors with that specific mutation.
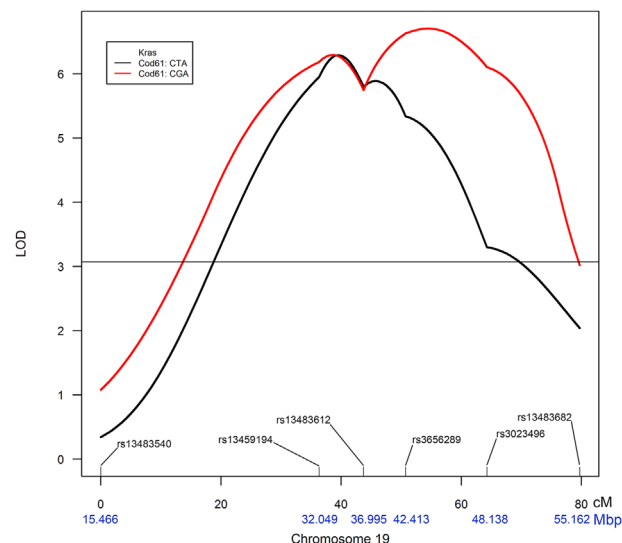[a]Males (n = 77) and females (n = 63) together.
[b]Codon 12, GGT; codon 61, CAA.

genome-wide α < 0.05 level of significance (LOD threshold, 3.99), no locus was found to link to mutations at codon 12. Regarding codon 61, we identified a significant linkage between the CTA sequence and a region on chromosome 19 (peak LOD = 4.3) (Figure 1A). At α < 0.1 (LOD threshold, 3.64), a suggestive linkage was found between the CGA sequence at codon 61 and the same region of chromosome 19 (peak LOD = 3.86). The locus associated with CTA was near the marker rs13483612 (at 36.995 Mbp), while that associated with CGA was near rs3655407 (at 40.260 Mbp) (Figure 1B).

These animals were all male (discovery group), but we also had tumor DNA (but not genome-wide SNP data) from 146 urethane-treated females of the same intercross that we have used as validation group. Therefore, we genotyped this DNA for *Kras* mutations on chromosome 6 and for six SNPs on chromosome 19 in a region spanning markers rs13483612 and rs3655407. The pattern of *Kras* mutations in females was similar to that observed in males (Table 1). An association analysis was then carried out using genotype data for these six SNPs for males and females together (280 lung nodules). This analysis confirmed the linkage between the CTA sequence of codon 61 and chromosome 19 (peak LOD = 6.29), near marker rs13459194 at 32.049 Mbp (Figure 2). It also revealed significant linkage for the CGA sequence of codon 61 and chromosome 19 (peak LOD = 6.30), near the same marker; for this mutation, the curve is bimodal with an even higher peak near marker

FIGURE 2 Linkage map for *Kras* codon 61 mutations in a region of chromosome 19 spanning from 15.466 to 55.162 Mbp. Mutation data are from lung tumor DNA from 280 urethane-treated ABF4 mice (males and females together). On the x-axis, inside tick marks show the positions of the six genotyped markers (see **Table S2**). Distances are expressed in both centimorgan (cM) and Mbp (in blue). Solid horizontal line, 95% LOD threshold

rs3656289 at 42.413 Mbp (LOD = 6.71). No significant association was found between any SNP and chromosome 6.

Because both forms of mutated codon 61 (CTA and CGA) associated with a locus near marker rs13459194, we examined the possibility that their frequencies depended on the parental strain from which the allele was inherited (Table 2). This analysis revealed a higher frequency of CTA mutations in mice that were homozygous for the A/J-derived allele than in mice that were homozygous for the C57BL/6J-derived allele (65.2% vs 28.6%). In contrast, the frequency of CGA mutations was lower in mice homozygous for the A/J-derived allele than in mice homozygous for the C57BL/6J-derived allele (13.0% vs 49.4%). These findings suggest that

FIGURE 1 Linkage analysis for codon 61 *Kras* mutations (CTA, black line; CGA, red line) in lung tumors from 134 urethane-treated male ABF4 mice. Solid horizontal lines, 95% LOD threshold (α = 0.05); dotted horizontal lines, 90% threshold (α = 0.1). Panel A, genome-wide linkage analysis. Panel B, details of linkage analysis for chromosome 19. On the x-axis, distances are expressed as centimorgan (cM, in black type) or in Mbp (in blue type); note that distances in cM are overestimated because the map was obtained using an F4 intercross (i.e., six informative meioses) rather than an F2 intercross (two informative meioses) that is the default value in R/qtl software

TABLE 2 Associations between *Kras* mutations at codon 61, expressed as binary phenotypes, and the genotype at rs13459194 on chromosome 19 in lung tumor DNA from 280 urethane-treated ABF4 mice (males and females together)

Phenotype[a]	Genotype, n (%)[b]			P-value*
	TT	TG	GG	
CTA				
0	16 (34.8)	67 (42.7)	55 (71.4)	1.6×10^{-5}
1	30 (65.2)	90 (57.3)	22 (28.6)	
CGA				
0	40 (87.0)	120 (76.4)	39 (50.6)	1.1×10^{-5}
1	6 (13.0)	37 (23.6)	38 (49.4)	

*Fisher's exact test.
[a]A phenotype of 0 means that lung tumors do not carry the particular *Kras* mutation, while 1 means that they do carry the mutation.
[b]T allele is carried by the A/J strain, and G allele is carried by the C57BL/6J strain.

germline polymorphisms modulate the frequency and type of *Kras* mutations in carcinogen-induced lung tumors in mice.

3.2 | Germline control of somatic *Kras* mutations in AIRmax x AIRmin mice

To determine if the germline control of *Kras* mutations, observed in an intercross of two inbred strains of mice, was generalizable to noninbred animals with a heterogeneous genetic background, we replicated the study using (AIRmax × AIRmin) F1 and F2 intercross mice in which lung tumors had been induced with urethane. Analysis of *Kras* mutations in lung tumor DNA revealed that about 31% of F1 animals and 18% of F2 animals had the wild-type *Kras* sequence (Table 3). Mutations were found in codons 12 and 61 but not in codon 13, and codon 61 mutations were prevalent. In codon 12, the GAT sequence was carried by about 9% of animals in both F1 and F2, whereas F2 animals also had low frequencies of four other sequences. The most frequent variant sequences of codon 61 were CGA (about 42% in F1 and 50% in F2), followed by CTA (about 17% and 12%, respectively), while three other mutations were observed at low frequency in F2.

Compared with ABF4 mice (Table 1), (AIRmax × AIRmin) F2 mice similarly had a high prevalence of codon 61 mutations, but the CGA sequence predominated instead of the CTA sequence. Moreover, in codon 12, the GAT sequence predominated in both intercrosses. In contrast, (AIRmax × AIRmin) F2 mice had a greater variety of low-frequency mutations, especially in codon 12. Overall, the pattern of *Kras* mutations in the (AIRmax × AIRmin) F2 population was more complex than in ABF4 mice.

Then, to determine if *Kras* mutational status is under genetic control in (AIRmax × AIRmin) F2 mice, we did a genome-wide association analysis using data for 890 SNPs. No statistically significant association was
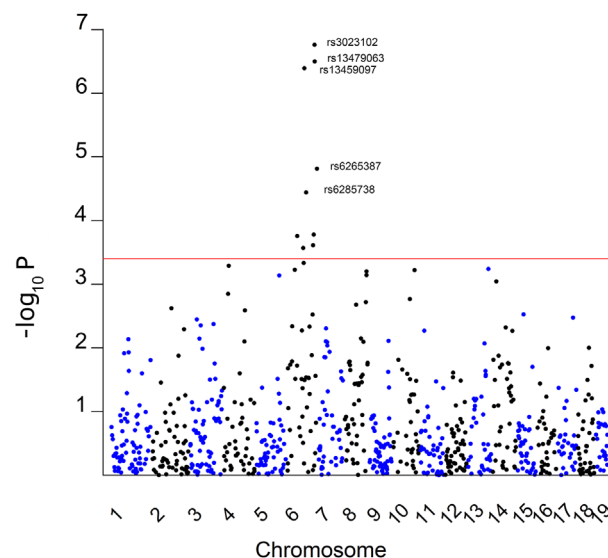
observed with codon 12 mutations. In contrast, significant associations were found between nine SNPs on distal chromosome 6 and any type of *Kras* codon 61 mutation (Figure 3). The peak association with codon 61 mutations was obtained near marker rs3023102 ($P = 1.7 × 10^{-7}$, PLINK analysis on allelic effects), which is located at 135.383 Mbp, that is, 9.8 Mbp upstream of *Kras* (145.217-145.250 Mbp). No SNP on chromosome 19 exceeded the statistical threshold for genome-wide significance in association with codon 61 mutations.

Next, we examined the association between the mutational status at codon 61 and the allele at rs3023102 on chromosome 6 (Table 4). This analysis showed that about 79% of mice homozygous for the A/J-derived allele (AA genotype) carried a *Kras* mutation at codon 61, whereas only about 44% of mice homozygous for the C57BL/6J-derived allele (GG genotype) carried such a mutation ($P = 3.0 × 10^{-7}$, Fisher's exact test). These findings indicate that genetic polymorphisms in distal chromosome 6 modulate somatic *Kras* mutations in lung tumors of (AIRmax × AIRmin) F2 mice.

4 | DISCUSSION

In this study, mutant *Kras* was found in urethane-induced lung tumors from 91% of ABF4 mice and 82% of (AIRmax × AIRmin) F2 mice. Codon 61 was the most frequently mutated in both crosses, but the pattern of mutation in this codon differed between the two populations. Indeed, in ABF4 mice, the most frequent mutation in codon 61 was an A-T transversion (from CAA to CTA), resulting in a glutamine-leucine

TABLE 3 Frequencies of *Kras* mutations at codons 12 and 61 in lung tumors of AIRmax × AIRmin mice treated with urethane

	AIRmax × AIRmin	
Genotype	**F1 (n = 139)**	**F2 (n = 527)**
Mutations in codon 12		
AGT (G12S)	0	5 (0.9)
TGT (G12C)	0	1 (0.2)
CGT (G12R)	0	4 (0.8)
GAT (G12D)	13 (9.4)	49 (9.3)
GTT (G12V)	0	1 (0.2)
Mutations in codon 61		
CGA (Q61R)	59 (42.4)	265 (50.3)
CTA (Q61L)	24 (17.3)	63 (12.0)
CCA (Q61P)	0	1 (0.2)
CAT (Q61H)	0	27 (5.1)
CAC (Q61H)	0	14 (2.7)
Wild-type at both codons[a]	43 (30.9)	97 (18.4)

Values are number (percentage) of tumors with that specific mutation.
[a]Codon 12, GGT; codon 61, CAA

FIGURE 3 Manhattan plot of genome-wide association analysis results for *Kras* codon 61 mutations in lung tumors from 527 (AIRmax × AIRmin) F2 urethane-treated mice. The figure illustrates the level of statistical significance (y-axis) as measured by the negative $\log_{10}$ of the corresponding nominal P value, for each SNP (PLINK allelic association analysis). Each genotyped SNP is indicated by a blue or black dot. SNPs are arranged by chromosomal location (x-axis). Red horizontal line: genome-wide significance level of $P ≤ 0.05$, calculated by 10 000 permutations (nominal $P = 0.0004$, $-\log_{10}P = 3.4$)

TABLE 4 Association between *Kras* mutational state at codon 61 and genotype of rs3023102 (chromosome 6, at 135.383 Mbp) in urethane-induced lung tumors of 525 (AIRmax x AIRmin)F2 mice

| | Genotype, n (%)[a] | | | |
Phenotype	AA	AG	GG	*P*-value*
CAA (wild-type)				
n = 157	44 (21.1)	72 (29.6)	41 (56.2)	3.0×10^{-7}
Not CAA				
n = 368	165 (78.9)	171 (70.4)	32 (43.8)	

[a]A allele is carried by the A/J strain, and G allele is carried by the C57BL/6J strain. Values are number (percentage) of lung tumors in each rs3023102 genotype group with the described *Kras* codon 61 sequence.
*Fisher's exact test.

substitution (Q61L, 50.7% of all mice); this was followed by an A-G transition (from CAA to CGA, or Q61R; 28.9%). In contrast, in (AIRmax × AIRmin) F2 mice the A-T transversion was detected in only 12.0% of tumors, while the A-G transition was predominant, found in 50.3% of mice. Noteworthy, the pattern of *Kras* mutation types was more variable in the intercross populations than in their F1 hybrids, in particular for (AIRmax × AIRmin)F2 mice, in which a total of 10 different *Kras* mutations were detected in codons 12 and 61 compared with only three different mutations in F1. These results support the hypothesis that genetic background modulates *Kras* mutation type in lung tumors induced by urethane, possibly due to complex genetic interactions between involved loci.

Genome-wide association analysis identified two loci associated with mutations in *Kras* codon 61. In the ABF4 intercross of inbred mice, this analysis identified a locus on chromosome 19 linked with the codon 61 mutations CAA to CGA and CAA to CTA. Determining which genetic elements of this locus are involved in the control of somatic *Kras* mutations will require fine-mapping and gene-candidate studies. In the F2 intercross of noninbred AIRmax and AIRmin mice, this analysis identified a locus in the distal region of chromosome six that associated with codon 61 mutational status (mutated vs wild-type); the LOD curve of this locus peaked 9.8 Mbp upstream of *Kras* (which lies in the *Pas1* locus). These results, from two genetically different intercrosses, indicate that somatic *Kras* mutations in mouse lung tumors can be under germline control and are not purely random events. Of note, the linkage between *Kras* mutations and distal chromosome six was observed only in (AIRmax × AIRmin)F2 mice, but not in ABF4 mice, notwithstanding the documented role of *Pas1* in modulating lung tumor multiplicity in the latter intercross.[20] These contradictory findings may be due to genetic elements on chromosome 6, distinct from *Pas1*, that differ between these two intercrosses.

In the ABF4 population, there was opposite effects of the A/J-derived allele of rs13459194 SNP (on chromosome 19) linked to *Kras* mutation control. The CTA mutation was more frequent (65.2%) in mice homozygous for the susceptible A/J-derived allele than in mice homozygous for the C57BL/6J-derived allele (65.2% vs 28.6%). In contrast, the CGA mutation was less commonly observed in mice homozygous for the same A/J-derived allele than in animals homozygous for the C57BL/6J-derived allele (49.4% vs 13.0%). These

findings show that genetic control can also be exerted on the type of *Kras* mutation, not only on the frequency of mutation. The LOD curve for the CGA mutation showed a second linkage peak at rs3656289, slightly distal to rs13459194; this bimodality may be due to a certain degree of randomness in the somatic mutation phenotype, rather than to distinct genetic elements modulating such phenotype. Regarding possible functional differences in the type of *Kras* mutation, it is not known if tumors with the CAA to CGA substitution are more or less aggressive than those carrying the CAA to CTA *Kras* variant.

It has been predicted that a large portion of somatic mutations in cancer, including *KRAS* mutations in lung cancer, are the result of a random, stochastic process.[31,32] However, different types of mutagens cause different types of mutations, pointing to mutational specificity.[33]

5 | CONCLUSIONS

Our present findings, together with previous results we obtained in another mouse model,[34] indicate that genetic background modulates (or selects for) the type of somatic mutations, at least for *Kras* mutations. Thus, this study should stimulate further research on associations between germline variations and the common somatic mutations in human lung cancer.

CONFLICTS OF INTEREST

The authors declare that no competing interests exist.

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SUPPORTING INFORMATION

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