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Hereditary lung cancer syndrome targets never smokers with germline EGFR gene T790M mutations

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Introduction

Lung carcinomas that initially respond to tyrosine kinase inhibitors (TKIs) often harbor somatic gain of function mutations in the tyrosine kinase domain of the epidermal growth factor receptor (*EGFR*) gene.^{1,2} These mutations are more frequently associated with adenocarcinoma histology, female gender, East Asian ethnicity and low or absent tobacco exposure.³ Despite initial response to TKI therapy, the tumors usually recur or progress by development of acquired resistance.

The major mechanism of acquired resistance is the presence of a second *EGFR* point mutation T790M in exon 20.^{4,5} T790M mutations usually occur with a common activating mutation, and it greatly increases the ATP-binding affinity of the initial mutation.^{4,6-9} T790M mutations may occur in treatment naïve tumors and cell lines^{10,11} and the mutant peaks in sequencing electropherograms are present in a minority of cells and may require sensitive detection methods. By contrast, the common activating mutations are often equal to or greater than the wild type peak, a phenomenon we have termed as mutant allele specific imbalance (MASI).¹² T790M germline mutations are present in 1% of lung cancers undergoing *EGFR* gene sequencing with the mutant allele occurring in about equal proportion to the wild type allele.¹³

In this report we describe the development of lung cancer in a young woman with a germline T790M mutation, and we combined an extensive study of her family pedigree with a review of the existing literature.

Materials and methods**Literature search**

We searched the PubMed database using combinations of the terms “Lung Neoplasms”, “Receptor, Epidermal Growth Factor” “Germ-Line” and “T790M”.

Investigation of a family with germline T790M mutation

Using an IRB approved protocol and after informed consent, we were able to track a germline T790M mutation in the *EGFR* gene present in the proband, as well as relevant history through five generations of her family. The family pedigree was constructed with the assistance of the proband and several family members. The smoking histories of deceased individuals were confirmed by at least two close relatives. Blood samples were obtained from 17 family members representing three generations. Genetic testing of the T790M mutation was performed by a CLIA approved laboratory utilizing *EGFR* exon 20-specific primers to sequence the region containing T790, using a standardized Sanger sequencing protocol (BigDye® Terminator v3.1 Cycle Sequencing Kit Protocol, Applied Biosystems, 2002).

Detection of *EGFR* and germline T790M mutations in Japanese patients

Paired tumor and non-malignant lung samples from 629 Japanese patients with resected lung cancers were examined for *EGFR* mutations using previously published methods.¹⁴⁻¹⁶ The study received local IRB permission and all patients gave informed consent for mutation testing. Testing was performed for therapy selection. Two patients were excluded because they had neuroendocrine tumors, leaving 627 patients with NSCLC.

Results

Literature search

A search of the PubMed database yielded five references for germline T790M mutations.^{13,17-20}

The proband

The proband was a 29-year-old female with a total tobacco exposure of 0.1 pack years. She presented with a 4.4 cm. left upper lobe adenocarcinoma and biopsy proven bilateral preneoplastic and preinvasive lesions (Supplemental Fig. 2). Analysis of tumor DNA for *EGFR* exons 18-21 revealed an L858R mutation in exon 21 (minor peak in comparison to the wild type peak), and a T790M mutation (equivalent in height to the wild type peak) (Fig. 1). Mutation analysis of her blood mononuclear cells indicated a T790M mutation, with equivalent heights of the mutant and wild type peaks, confirming the presence of a germline T790M mutation. No L858R mutation was detected in the mononuclear cells.

History and mutation status of Proband's family

Information about the proband's family pedigree is presented in Fig. 2 and Supplemental Table 1. Eight of 17 family members tested were positive for the mutation, including the proband's mother and brother. All family members tested received genetic counseling prior to testing and after the results were completed. Two of the family members were tested to establish lineage (IV:12, III:13), and were subsequently discarded from further analysis. Eight of 15 maternally related family members tested were mutation positive (53.3%), consistent for Mendelian inheritance of an autosomal gene. As documented in Figure 2 there are 14 family members that are tested, obligate or assumed carriers of the T790M mutation. Four of these 14 (including the proband) developed lung cancer. CT scans, were available on 5 mutation carriers. All scans showed one or multiple small pulmonary lesions of uncertain diagnosis (Supplemental data).

Germline T790M mutations in lung cancer cases and controls

From our proband's family pedigree and the five reports in the literature there are a total of 29 mutation carriers. Lung cancer developed in 19 of these carriers (referred to as lung cancer cases (Table 1). The remaining 10 T790M germline cases without lung cancer are referred to as controls. However, the gender and smoking status of some of the cases and controls from the literature is lacking or ambiguous. Thus subgroup analysis contains varying number of cases and controls. (Table 2). It should be noted that, as with other germline mutation analyses, some of the controls may ultimately developed cancer.

Characteristics of Lung cancers arising in T790M germline carriers

As demonstrated in Table 1, there are 19 cancer cases known to have arisen in germline carriers including our pedigree. All but one of the probands had a family history of lung cancer, and the germline mutation status of 11 was confirmed by sequencing, one was an obligate carrier, and seven family members are assumed to be carriers. In one case (case 8) both parents had lung cancer, and the parental inheritance could not be determined. Twelve of the 14 patients with known ethnicity were Caucasian, and two were East Indian. Their ages ranged from 29-81, and there were 13 females, five males, and one of unknown gender. The median age of the cancer cases was about 63 years, with our proband at 29 years being the youngest case identified. Tumor histology was available for 14 patients, all of who had NSCLC, and all of who had one or more adenocarcinomas (case 9 also had a large cell neuroendocrine carcinoma). Three patients had multiple pathology documented lung cancers, and three had documented invasive cancers as well as multiple bilateral nodules (biopsy proven to be microinvasive cancers in one of the cases).²¹

Of the 22 apparently individual tumors arising in 11 patients that were tested for EGFR gene mutations and 16 (73%) had a second activating mutation in addition to the T790M germline mutation.

A logistic regression model was used to test the association between smoking status and lung cancer among the germline T790M carriers adjusting for sex, as there are more female cases whereas more males are represented in the mutation carriers that have not developed lung cancer (controls) (Table 2). Without adjusting for sex, the odds ratio for a 2-by-2 table can be calculated as ad/bc , which is $2*4 / (5*11) = 0.15$. After taking gender into consideration, it became 0.31 (95% confidence interval (CI):((0.04, 2.25)). Although this OR itself was not significant ($P=0.34$) given the small sample size, it is significantly smaller ($P=6.0E-05$ by a two-sample mean test) than the OR of 40.4 (95% CI: (21.8,79.6)) estimated from the general US population.²² There was an excess of never smokers in lung cancer cases arising in germline T790M carriers. The proportions of smokers in all lung cancer cases were estimated to be 0.91 in males and 0.81 in females.²³ In contrast, only 2 out of 13 germline T790M derived cases (with both sex and smoking status known) were ever smokers, which was significantly smaller ($P=6.2E-07$) than the expectation - a conservative estimate 0.81-in the general lung cancer cases. The p-value remained significant ($P=7.4E-06$) even if the two cases of smokers with uncertain gender were taken into account, i.e., 4 out of 15 cases were ever smokers.

Prevalence of T790M germline mutations in lung cancer patients and the general population

We estimated the overall penetrance of the germline mutation and its penetrance in both ever smokers (ES) and never smokers (NS) without regard to age by modeling the conditional probability of family phenotypes and genotypes given the proband's phenotype. The overall penetrance was 0.23, and 0.15 in ESs and 0.31 in NSs.

We examined 627 resected Japanese NSCLC lung cancers for the presence of *EGFR* mutations prior to the onset of TKI or other therapies. Of these cases, 553 were from frozen

tissues (552 tumors, one malignant pleural effusion), and 74 from formalin fixed, paraffin embedded materials of resected tumors. *EGFR* mutations were detected in 209/627 samples (33.3%). One patient harbored both L858R and an acquired T790M mutation. We compared the reported prevalence of germline T790M mutations in lung cancer patients in the USA (5/503)¹³ with the prevalence in our Japanese cases (0/627) (Fisher's exact test p-value = 0.017).

As germline T790M mutations predispose to the development of lung cancer, the prevalence of germline mutations in lung cancer cases may exceed the prevalence in the general population. The T790M mutation was absent in the 1000 Genomes Project database²² and in the genomes of 6503 individuals from the NHLBI GO Exome Sequencing Project (<http://evs.gs.washington.edu/EVS/>). Thus, without adjusting for ethnicity, we estimate that the prevalence of germline T790M mutations in the general population is less than 1 in 7500 subjects. Only one T790M germline mutation has been identified during non-*EGFR* gene targeted sequencing - in a patient with an unrelated malignancy.²³

Discussion

As the rare germline T790M mutations predispose to lung cancer, they represent a rare but interesting lung cancer familial predisposition gene. Based on the data learned from our family, germline T790M is a major cancer predisposition gene, with an estimated 31% risk for lung cancer in never smoker carriers. We report the investigation of several cases from a single family, with the 29 year old proband inheriting the mutation maternally. As we obtained relevant family history from several family members for five generations, and tested for the germline mutation in members from three generations, our family represents the most extensively studied pedigree reported. We combined the family data with the scant published literature data to summarize our current knowledge of this familial form of lung cancer. Our review of the literature was complicated by double reporting of some cases,^{13,19} deliberate alteration of some patient data for confidentiality reasons,¹³ and by incorrect or difficult to interpret data from another report (see Supplemental data).²⁰

A comparison of lung cancers arising in patients with sporadic activating mutations in the *EGFR* gene versus cancers arising in T790M carriers demonstrates similarities and differences. Germline T790M mutations are rare, and, with the exception of family member testing, are almost always detected during analysis of lung cancers for *EGFR* mutations. As germline inheritance predisposes to lung cancer, the prevalence in lung cancer cases is likely to be higher than in controls without cancer. Vikis et al sequenced the probands of 237 families with predisposition to lung cancer, but did not identify any germline T790M mutations, confirming that these mutations are very rare, even in families with a genetic predisposition for lung cancer.⁸ While about 1% of NSCLC cases have heterozygous germline mutations, an analysis of public databases indicated that the prevalence in the general population was probably less than one in 7500. While *EGFR* mutations are more frequent in lung cancers arising in East Asian ethnicities, our data from 627 Japanese cases indicated that the prevalence of germline mutations in lung cancer cases in Japan was lower than that reported for USA cases. To date, not a single case of germline T790M mutation has been reported in East Asians.

Almost all of the lung cancers having acquired *EGFR* mutations as well as those arising in T790M carriers have adenocarcinoma histology. While, some sporadic adenocarcinomas of the lung arise as multiple apparently independent tumors and may be associated with multiple preinvasive lesions,^{21,24-26} these findings are characteristic of many inherited cancer syndromes.²⁷⁻³⁰ We obtained CT scans of six of the germline mutation carriers, including the proband and five unaffected carriers. The proband had multiple bilateral ground glass and solid nodular lesions, histologically proven to represent the entire spectrum of preneoplastic, preinvasive and microinvasive lesions associated with peripheral adenocarcinomas.²¹ One or more subcentimeter solid or ground glass nodules of uncertain etiology were identified in all the unaffected carriers (Supplemental Table 2).

Females were over-represented in the germline cases. Of the 22 lung tumors arising in 11 patients whose tumors had *EGFR* gene sequencing, 16 (73%) had a second activating mutation. Thus, the important gain-of-function properties of these double mutants may explain their frequent presence in lung cancers arising in T790M carriers. The median age of the cancer cases was about 63 years, with our proband at 29 years being the youngest case identified. The median age for the T790M cases is considerably higher than for breast cancer development in BRCA1 carriers (median age 40 years), although the age ranges are similar.³¹ Female predominance is present in both lung cancers with sporadic *EGFR* mutations and for germline T790M cases.

Perhaps the most interesting and unexpected finding of our study was the effect of smoking status on the appearance of lung cancer in germline carriers. The distribution between smoking status in the germline carriers did not deviate from the expected in the US population. However, the distribution of smoking status among lung cancer cases indicated a considerable, highly significant over representation of never smokers ($p = 3,2E-07$), based on the expectation that 86% of lung cancers in the USA arise in ever smokers.³² The calculations are based on our proband being classified as an ever smoker, even though her lifetime tobacco exposure was 0.1 pack years and was highly unlikely to have contributed to the development of her lung cancer. We estimated the overall penetrance to be 0.23, and 0.15 in ever smokers and 0.31 in ever smokers. However, as we were unable to adjust for age and given the small sample size, our estimates must be interpreted with caution.

These findings are puzzling, as smoking is universally accepted as the major risk factor for lung cancer. Our hypothetical explanation is illustrated in Fig. 3. While the T790M mutation is a weak oncogene by itself, when combined with a common activating *EGFR* mutation such as L858R, the oncogenic potential of both mutations is greatly enhanced, and 73% of lung cancers arising in germline carriers contained a second activating mutation. Multiple studies have demonstrated that sporadic activating *EGFR* mutations favor never smoking status and female gender.^{3,33} *KRAS* mutations, a major driver mutation in adenocarcinomas arising in ever smokers, are mutually exclusive with *EGFR* mutations.³ Because somatic mutations of *EGFR* and *KRAS* are mutually exclusive, germline T790M mutations and *KRAS* mutations in ever smoker carriers may also be mutually exclusive. Thus second activating *EGFR* mutations in germline carriers may arise more frequently in never smokers and women and predispose to cancer development. Of interest, both lung cancers with sporadic *EGFR* mutations and the germline T790M cases favor female gender,

adenocarcinoma histology and never smoking status. However one notable difference is the absence of reported T790M germline mutations in those of East Asian ethnicity.

The management and prevention of lung cancers in germline carriers presents unusual and controversial options. The presence of the T790M mutation (with or without being accompanied by a more typical activating mutation at presentation predicts for resistance to the standard TKIs. The only germline cases reported to have received TKI therapy were the two sisters reported by Tibaldi et al,²⁰ who had partial responses. Thus in the absence of another known or suspected molecular target, conventional chemotherapy appears to be the preferred first line therapy option. Several newer third and even fourth generation TKIs have entered clinical trial, and some were designed to overcome T790M mediated resistance.^{34,35} and offer the possibility of effective targeted therapy in the future. Regular CT scans are one method for the early detection of lung cancers developing in carriers, and the carriers in the family of our proband are being offered this option. Clearly, optimal management and prevention strategies will require more information obtained from observation, prevention and therapy of many more carriers and cases. For these reasons The Dana-Farber Cancer Institute has initiated the “INHERIT EGFR study: Investigating the hereditary risk from T790M” (<http://clinicaltrials.gov/ct2/show/NCT01754025>).

Conclusions

Germline T790M mutations result in a very rare and unique lung cancer hereditary syndrome that targets female never smokers. The risk of lung cancer development in never smoking carriers is greater than the risk of heavy smokers with or without the mutation. Unaffected carriers with this mutation are at increased risk for developing lung cancer irrespective of their smoking status, and should be followed by increased surveillance including low dose CT scans. The resultant cancers share several features with lung cancers containing sporadic *EGFR* mutations, as well as known or suspected differences.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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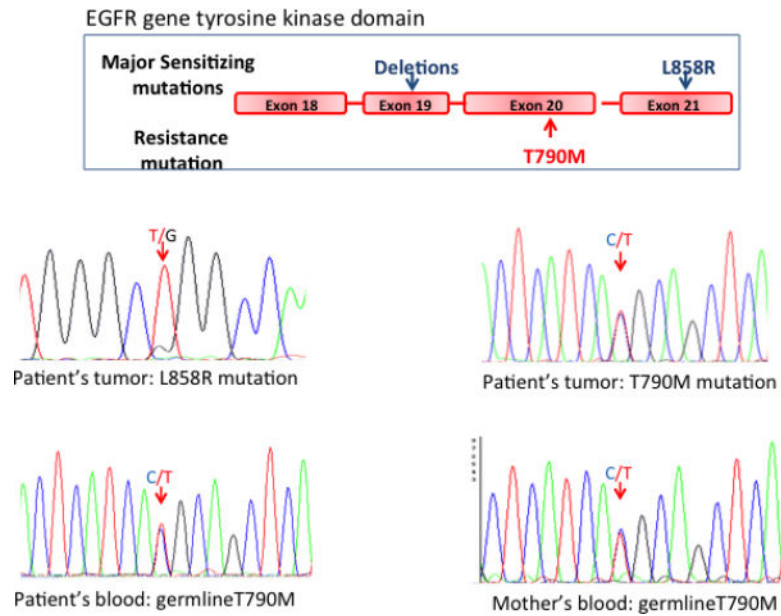


Figure 1.

Sequencing of the *EGFR* gene in the proband's adenocarcinoma revealed an activating mutation (L858R) in the *EGFR* gene (minor peak) (A) and a prominent T790M mutation, equal in size to the wild type peak (B). The T790M mutation substitutes methionine for threonine at position 790 (nucleotide c.2369). The finding in a lung cancer of a T790M mutant band equal in size to the wild type band prior to TKI therapy suggests a germline mutation. Examination of the proband's blood cells confirmed a germline T790M mutation in the patient (C) and in her mother (D). A cartoon of the location of the common mutations in the tyrosine kinase domain of the *EGFR* gene is shown in the upper part of the figure.

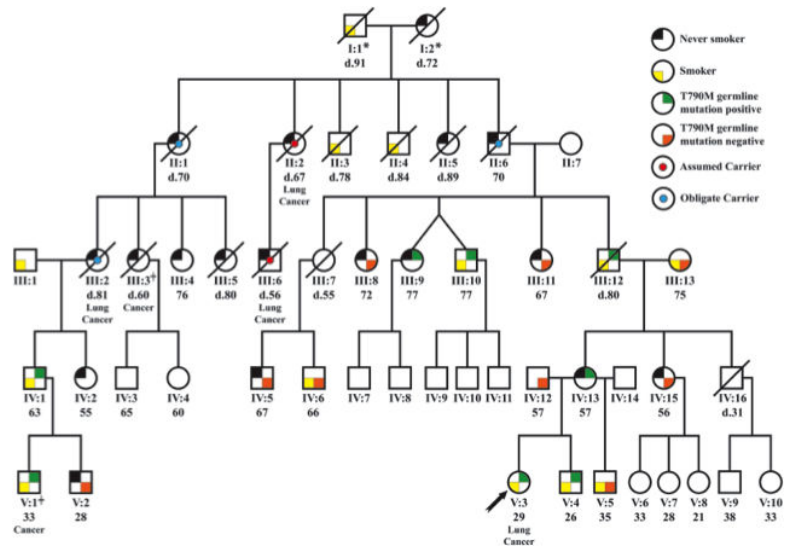


Figure 2.

Pedigree of family with germline T790M mutation. Age, smoking history, mutation status and other cancer history are recorded.

*In addition to the three obligate carriers identified in the pedigree, either individual I:1 or I:2 is a obligate carrier based on pedigree analysis. Individual III:3 is reported to have died from bladder cancer. She had lung cancer, but it is uncertain if this was a primary cancer or a metastasis. She was not considered to be a lung cancer case. Individual V:1 had a lung carcinoid tumor. There are two never smokers that developed lung cancer in the family (II:2 and III:6), given the low likelihood that they represent two sporadic cases in this family we have assumed that they are also mutation carriers, thus for a total of 14 known, obligate or assumed mutation carriers. There are two never smokers that developed lung cancer in the family (II:2 and III:6), given the low likelihood that they represent two sporadic cases in this family we have assumed that they are also mutation carriers, thus for a total of 14 known, obligate or assumed mutation carriers.

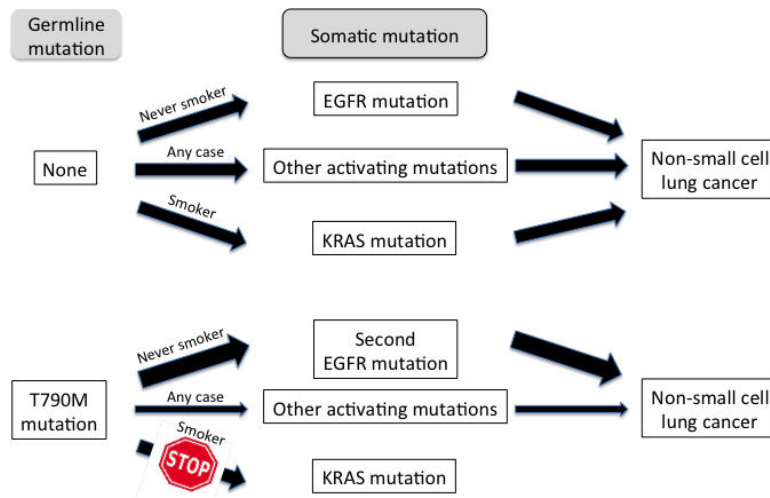


Figure 3. Hypothetical mutation driven pathways to NSCLC development in patients with and without germline T790M mutations. In subjects without the germline mutation, the major pathways are via *EGFR* mutations (in never smokers, NS) or via *KRAS* mutations (in ever smokers, ES), although other driver mutations may occur in either group. Subjects with germline T790M mutations, a weak driver mutation, usually require one or more other driver mutations for NSCLC development. In NS the second mutation is usually another *EGFR* mutation although other pathways may occasionally be activated. In ES, the presence of the germline *EGFR* gene mutation as well as the smoking status decrease or preclude the possibility of developing a *KRAS* mutation, although alternative driver mutations may occasionally occur.

Table 1

Summary of literature regarding lung cancer patients with germline T790M mutations and family member with lung cancer (mutation status known and assumed).

Reference	Case#	Family#	Other family members with lung cancer/Relationship to proband	Ethnicity	Age	Sex	Smoker	Tumor	T790M germline mutation	2 nd EGFR gene mutation	Comment
Bell et al ²¹	1	1	Proband	White	50	M	S	5 ADCs	Yes	5 tumors L858R in 2/5 delL747-T751 in 1/5	Multiple bilateral nodules
Bell et al ²¹	2	1	Yes, Brother	White	55	M	?	ADC	Yes	G719A	Widespread metastases
Bell et al ²¹	3	1	Yes, Mother	White	62	F	?	ADC	Mutation assumed	N/A	
Bell et al ²¹	4	1	Yes, Grandfather	White	72	M	?	ADC	Mutation assumed	N/A	
Girard et al ¹⁸	5	2	Proband	E. Indian	66	F	NS	ADC	yes	L858R	Multiple bilateral nodules
Girard et al ¹⁸	6	2	Yes, Father	E. Indian	41	F	NS	N/A	Mutation assumed	N/A	
Girard et al ¹⁸	7	3	Proband	White	56	M	NS	ADC	Yes	L858R	Widespread metastases
Girard et al ¹⁸	8	3	Yes, Either father or mother	White	72,80	M/F	S	N/A	Mutation Assumed	N/A	
Prudkin ¹⁹	9	4	Proband	?	72	F	NS	2 ADCs 1 LCNEC	yes	None in 3 tumors	
Prudkin	10	4	Yes, sister	?	?	F	?	ADC	Mutation unknown	N/A	
Oxnard et al ¹⁴	11-12	5,6	One case had a sibling with cancer	?	44/73	F/M	NS/S	ADC	Yes	Both had exon 19 deletions	
Oxnard et al ¹⁴	13	7	No	?	44-73	F	NS	ADC	Yes	6 Nodules- 4 had L858R, 2 exon 19 deletions.	
Tibaldi et al ²⁰	14	8	Proband	White	72	F	NS	ADC	yes	Del; E746-A750 in exon 19	Bilateral pulmonary lesions
Tibaldi et al ²⁰	15	8	Yes, Sister	White	74	F	NS	NSCLC	yes	None	
UTSW V-3	16	9	Proband	White	29	F	S	ADC	yes	L858R	
UTSW II-2	17	9	Yes, great- great aunt of proband	White	67	F	NS	N/A	Mutation assumed	unknown	
UTSW III-6	18	9	Yes, son of #17	White	56	M	NS	N/A	Mutation assumed	unknown	

Reference	Case#	Family#	Other family members with lung cancer/Relationship to proband	Ethnicity	Age	Sex	Smoker	Tumor	T790M germline mutation	2 nd <i>EGFR</i> gene mutation	Comment
UTSW III-2	19	9	Yes, distant aunt of proband	White	81	F	NS	N/A	Obligate carrier	unknown	

Under "Family history of lung cancer", the number of family members with lung cancer are indicated in parentheses. The Oxnard reference only provides summary information about 5 germline mutation cases, two of which were previously reported by Girard, thus only recorded once. There is a both a paternal and maternal history of lung cancer in case #7- Girard so the lineage is unknown, however we would assume that one of the parents is a mutation carrier. Please see Supplemental Data for discussion on potential problems with the report by Tibaldi et al.²⁰ ADC = pulmonary adenocarcinoma, LCNEC = large cell neuroendocrine carcinoma, NSCLC = non-small cell lung carcinoma, not otherwise specified

Table 2

Distribution of germline T790M mutation carriers stratified by smoking status, gender, and lung cancer

Smoking Status	Sex	Case ¹ *	Control [*]
Ever Smoker	Male	1	5
	Female	1	0
Never Smoker	Male	2	1
	Female	9	3

* Case = Lung Cancer arising in germline T790M mutation carrier, Control = Germline mutation carrier that has not developed lung cancer

¹ Out of 19 lung cancer cases, there are two cases with unknown sex and four cases with unknown smoking status; overall, there are 13 cases with both sex and smoking status known (see Table 1). Although there are 10 mutation carriers, we do not know whether the proband inherited the mutation from I:1 or I:2 (see Figure 2), so we can confirm the smoking status and gender in only 9 controls.

Table 3

Comparison of lung cancers associated with sporadic and germline mutations of *EGFR* gene.

Feature	Sporadic	Germline
Frequency	Relatively common	Exceedingly rare
Lung cancer type	Mostly adenocarcinoma	Mostly adenocarcinoma
Ethnic preference	East Asian	* White
Gender preference	Female	Female
Smoking status	More frequent in never smokers	More frequent in never smokers
Field cancerization effects	Occasional	* Occasional
Presence of T790M	Rare at initial diagnosis	Present at initial diagnosis (by definition)
Presence of second activating <i>EGFR</i> mutation	Rare at initial diagnosis	Frequent at initial diagnosis.
Inheritance	Not applicable	Dominant
Penetrance	Not applicable	* May vary with smoke exposure
Response to TKIs	Frequent initial response	* Predicted to be resistant

* Given the exceedingly rare prevalence of germline T790M mutations, several of the observations (indicated by astericks) are tentative and need confirmation by study of further cases.