Germ-line exon 21 *EGFR* mutations, V843I and P848L, in nonsmall cell lung cancer patients

To the Editor:

Somatic epidermal growth factor receptor (*EGFR*) mutations are now routinely integrated in the molecular diagnosis of nonsmall cell lung cancers (NSCLC) [1, 2]. Thus, germ-line *EGFR* mutations are rarely mentioned or looked for in the context of patients with a family history of cancer, as their association with NSCLC familial cancer risk is not well established. Moreover, the predictive value of these mutations for response to EGFR tyrosine kinase inhibitors (TKIs) is not well known [3]. We report here two different heterozygous germ-line *EGFR* variants identified in two Caucasian NSCLC patients, who demonstrated different responses to EGFR-TKI.

A 63-year-old Caucasian, male former smoker with no family history of cancer (fig. 1a), was admitted for dyspnoea in August 2011, leading to discovery of a lower left lobe lung tumour and pleural effusion. Transthoracic biopsy diagnosed an invasive, acinar-predominant adenocarcinoma, classified cT2a N3 M1a (stage IV). Molecular analyses of the tumour by direct sequencing identified two concomitant heterozygous *EGFR* exon 21 mutations, L858R and V843I, confirmed in two independent experiments (fig. 1b). The V843I variant, but not L858R, was also detected in DNA obtained from a blood sample, with written informed consent, confirming a germ-line mutation (fig. 1c). Following treatment with cisplatin and pemetrexed, the patient relapsed in December 2012 with vertebral metastasis. An EGFR-TKI (erlotinib) was initiated, resulting in a stable disease for 9 months.

A 31-year-old Caucasian, female current smoker with a history of throat cancer in her maternal grandfather (who smoked) (fig. 1d), was admitted in February 2012 for intracranial hypertension due to a brain tumour, associated with a right upper lobe lung nodule. Surgical treatment of the two sites diagnosed an invasive, acinar-predominant adenocarcinoma, classified pT4 N0 M1b (stage IV). Molecular analyses of the primary lung tumour and the brain metastasis using direct sequencing revealed *EGFR* mutation P848L in exon 21 (fig. 1e), which was also detected in DNA obtained from healthy lung tissue and a blood sample, with written informed consent, confirming the germ-line mutation (fig. 1f). In July 2012, the patient relapsed, with psoas muscle metastasis. Despite treatment with cisplatin plus pemetrexed chemotherapy, the muscular lesion developed, prompting a change to erlotinib treatment in March 2013. After 4 months, the muscular metastasis progressed and erlotinib was discontinued.

Germ-line *EGFR* variants have rarely been described (<1 in 1000 *EGFR* mutations), and concern four *EGFR* mutations in two exons: the T790M [4–7] and R776X [3, 8] in exon 20, and the V843I [9, 10] and P848L [11, 12] in exon 21. Although somatic T790M mutations are common in patients with acquired resistance to EGFR-TKIs (50%), germ-line *EGFR* T790M mutations are rare, even in never-smokers (0.54%) [7]. The three other known *EGFR* mutations, R776X, V843I and P848L, belong to a group of very rare *EGFR* mutations with constitutive characteristics that are not always demonstrated. A recent study found only one *EGFR* germ-line mutation (R766G) among 71 lung tumour samples which was not found in 954 alleles from healthy individuals studied, leading to the conclusion that it is not a polymorphism responsible for NSCLC [3].

Germ-line mutations in *EGFR* are rare but may contribute to oncogenesis. T790M has a moderate effect on EGFR function but, when combined with other *EGFR* mutations it shows a remarkable enhancement of EGFR activity. *In vitro* studies revealed that tyrosine autophosphorylation is enhanced in R776G-mutant *EGFR* when compared with wild-type *EGFR* and may be associated with a proliferative advantage. Germ-line *EGFR* mutations are often associated with another somatic *EGFR* mutation, such as the common L858R mutation. Among the five T790M germ-line mutated cases, three are described with L858R [7]. One of our cases harboured the V843I mutation combined with L858R; there are two other V843I mutated cases in the literature, one associated with L858R or L861Q [9] and another with L858R [10]. The mechanism through which V843I mutation confers predisposition remains unknown, but the acquisition of a second mutation must be essential for the development of lung adenocarcinoma. One possibility is that V843I causes genetic instability, thereby predisposing cells to additional mutations within the gene. This hypothesis is supported

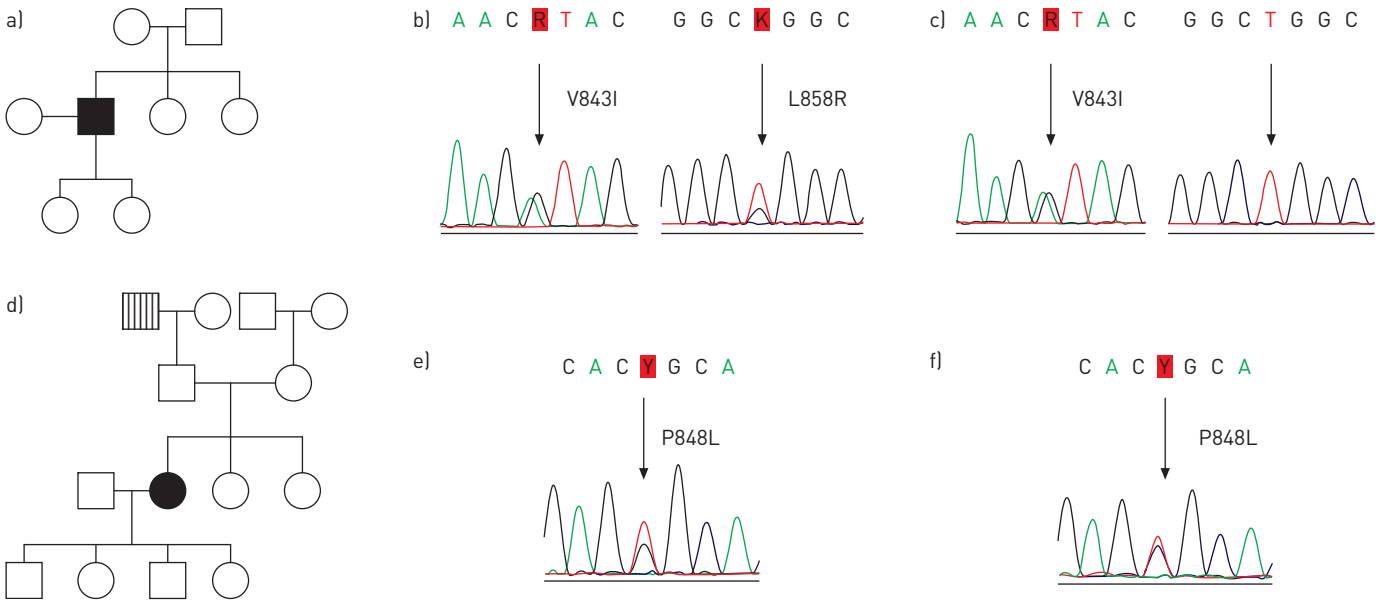

FIGURE 1 a–c) Case 1, with epidermal growth factor receptor (*EGFR*) exon 21 mutations (V843I and L858R). a) Pedigree chart. The black case corresponds to the index patient. b) DNA sequencing electrophoretograms for DNA obtained from lung tumour tissue identifying *EGFR* exon 21 mutations; both mutations are present. c) DNA sequencing electrophoretograms for DNA obtained from blood, identifying one *EGFR* exon 21 mutation, the V834I variant, is present and confirming it is germ-line. d–f) Case 2, with *EGFR* exon 21 mutation (P848L). d) Pedigree chart. The black case corresponds to the index patient. The hatched box represents a case with head and neck carcinoma. e) DNA sequencing electrophoretograms for DNA obtained from lung tumour tissue identifying an *EGFR* exon 21 mutation (P848L). f) DNA sequencing electrophoretograms for DNA obtained from blood identifying the *EGFR* exon 21 P848L variant is present and confirming it is germ-line.

by the identification that the somatic L858R mutation nonrandomly occurred *cis* to the germ-line V843I or R776X mutations [3, 10]. R776C has also been described but as a somatic mutation in conjunction with L858R [13]. Other secondary somatic *EGFR* mutations have been reported with *EGFR* germ-line mutations, for example G719A with T790M or R776H germ-line mutations [3, 4, 7].

While cases of familial lung cancer have been occasionally reported, their genetic backgrounds remain largely unknown. Germ-line T790M mutation has been documented in five cases of four families with lung cancer susceptibility, including one family with six family members in three generations having lung cancer [4–7]. Unlike one of our cases, the germ-line V843I mutation has been reported in a family with lung cancer [10] and in a family with other cancer susceptibility [9]. Unlike somatic *EGFR* mutations, reported more often in Asians, females and non-smokers, few data for patients harbouring EGFR germ-line mutations are available. Five cases with T790M germ-line mutation are known, one Asian and three Caucasian (one unknown), of whom three were male and two female [7]. Three cases with R776G germ-line mutations were Caucasian, one male and two females [3, 8]. Our V848I patient was a Caucasian male, while the two previously reported cases of V848 germ-line mutations were Asian females [9, 10]. Our cases were a current and a former smoker. T790M germ-line mutations were found in three never-smokers and one smoker [7]. R776X mutations have been reported both in nonsmokers as well as in smokers, but no data are available for patients with V843I germ-line mutations [3, 8–10]. Within large cohorts of *EGFR*-mutated lung carcinomas, the vast majority are classified as adenocarcinomas. *EGFR* germ-line mutations are also more often reported in lung adenocarcinoma, as in our two cases, but one case of lung cancer harbouring R776H germ-line mutation has been reported with squamous differentiation [8]. As yet, is hard to envisage how mutations that merely activate the kinase domain of *EGFR* might have differential effects on tumour-cell differentiation.

The predictive value of rare *EGFR* mutations is not well known. Growth inhibition assays using cell lines established from patient tissue harbouring V843I and L858R showed resistance to EGFR-TKI as well as the resistance observed in the clinical case [8]. It is widely accepted that while lung adenocarcinomas with the L858R mutation alone are susceptible to TKI therapy, gain of an additional T790M mutation overrides the effect of L858R and confers resistance to TKIs. Nevertheless, in our case, V848I was not a resistance mutation for TKI therapy, as in another case of lung adenocarcinoma with V848I *EGFR* mutation but without information about somatic or constitutive mutation [14]. In our second case, the unique germ-line P848L mutation is a TKI resistance mutation. R776X plus G719X mutated cell lines retain sensitivity to EGFR-TKIs [8].

We should look for germ-line *EGFR* mutations in cases of NSCLC with a familial history of cancers or with such very rare *EGFR* mutations. EGFR germ-line mutations must be confirmed in normal tissues or blood samples with written informed consent. The role of *EGFR* germ-line mutations in NSCLC and the familial occurrence of lung cancer or other cancers are still unclear. Further analyses are needed in order to precisely identify clinical and biological characteristics as well as the susceptibility of germ-line *EGFR* mutations to TKIs, particularly from the perspective of next-generation sequencing, which is able to identify such rare mutations, for giving genetic advice to the index patients and their relatives.

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Germ-line *EGFR* mutations are rare with various predictive values for response to EGFR inhibitors
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