The Pedigree Analysis of EGFR p.V1010M Germline Mutation in a Family with a Family History of Non-Small-Cell Lung Cancer (NSCLC)

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Research Article

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Abstract

Background: The causes of tumor can be divided into genetic factors and environmental factors, but previous studies have shown that genetic factors contribute less to lung cancer. *EGFR* is the most common driver gene in non-small-cell lung cancer (NSCLC), but most variations are found as somatic variations. In this study, we reported a pedigree of *EGFR* p.V1010M germline mutation for the first time, and explored the correlation between V1010M and NSCLC disease occurrence. Furthermore, the effect of the V1010M on the treatment of EGFR-TKIs was investigated through the treatment of the proband with simultaneous somatic mutation of *EGFR* p.L858R.

Methods: The families were screened by NGS and Sanger sequencing, and the pedigree was drawn to investigate the relationship between *EGFR* p.V1010M and the occurrence of NSCLC disease. Schrodinger software was used to predict the structural function of mutant amino acid sequence proteins.

Results: A total of 10 blood samples were collected from four generations of the family members, many of whom suffered from lung cancer. And 6 carriers of *EGFR* p.V1010M were detected. Pedigree analysis showed that there was still no evidence of correlation between *EGFR* p.V1010M and disease occurrence. Meanwhile, the proband detected the somatic mutation of *EGFR* p.L858R, and the response after the treatment of gifitinib was SD, which turned to PD 4 months later. Schrodinger software showed that the 1010th amino acid valine was located near the C terminal, and the variation to methionine had little effect on the structure of *EGFR* dimer.

Conclusion: This study is the first report of a pedigree with *EGFR* p.V1010M germline mutation, which might be a pathogenic mutation and associated with EGFR-TKIs resistance in NSCLC.

Introduction

The occurrence of malignant tumor is mainly caused by genetic susceptibility, environmental factors and others. It's closely related to gene variation and epigenetics[1], which may be caused by a single driver gene variation or the cumulative effect of multiple gene variations. Moreover, both germline and somatic mutations are likely to cause cancer. Cancers can show high familial aggregation, but genetic factors may not play a dominant role in tumorigenesis as previously thought. A study in the NEMJ in 2000 showed that the heritability of breast cancer, colorectal cancer and prostate cancer was only 27-42%[2], meaning that environmental factors accounted for as much as 58-73% of the incidence of tumors.

Lung cancer has the highest morbidity and mortality rate in the world, and has obvious familial aggregation. However, studies have shown that the heritability of lung cancer is even lower, about 26%[2], and the gene variation spectrum is completely different from other tumor species with high heritability. Lung cancer is more likely caused by single driver genes, such as *EGFR*, *ALK*, *ROS1* and *KRAS*, etc. 80-85% of lung cancer is non-small-cell lung cancer (NSCLC), which is the most successfully studied tumor species in the current "precision therapy" of treatment selection based on molecular typing[1].

EGFR is the most common driver gene of NSCLC, with a mutation rate of about 49.3% in the Asian NSCLC population[3]. Its variation types are various, mostly located at exon 18-21. There have been three generations of EGFR-TKIs, with the most accurate efficacy for exon 19 (19Del) and exon 21 (L858R), and generally poor efficacy for the variation of exon 18 and 20. The response to EGFR-TKIs varies greatly from site to site and each site needs to be analyzed separately. Most of *EGFR*-mutations are somatic mutations, only a few *EGFR* germline mutations have been reported, and most of them lack data on the occurrence, development and drug sensitivity of diseases[4]. In this study, *EGFR* germline mutation p.V1010M was reported for the first time, and its pathogenicity and drug sensitivity were discussed through family investigation and clinical treatment process, so as to provide data for the development mechanism and treatment plan of lung cancer.

1. Clinical Data

1.1 General Information of the Proband

A female, 54 years old, never smoked, was admitted to the local hospital on June 5, 2019 due to "cough for 3 days". The mass in the posterior basal segment of the lower lobe of the right lung was detected, as well as diffuse nodules and thickened interlobular septum in both lungs. It was considered that there was a high possibility of right lower lobe peripheral lung cancer with bilateral pulmonary blood flow and lymph node metastasis, and obstructive pneumonia in right lower lobe. She visited our department on June 10. Bronchofiberscope biopsy revealed lung adenocarcinoma. Brain MRI, whole body bone scan showed no metastatic lesion, and the final diagnosis was right lung adenocarcinoma (T4N3M0, IIIc).

1.2 Treatment Process

Gefitinib tablets were taken orally 0.25g, QD (Figure 1), with no *EGFR* mutation detected in peripheral blood sample by amplification refractory mutation system (ARMS), using ADx-ARMS test kit (Amoy Diagnostics Co., Ltd. Xiamen, China.) on CFX96 Touch Real-Time PCR Detection System (Bio-Rad, USA). But on July 15, *EGFR* p.L858R & p.V1010M were detected in peripheral blood sample by 3D Medicines Inc. (Shanghai, China) using next-generation sequencing (NGS) and digital droplets PCR (ddPCR). Recombinant human endostatin (Endostar), pemetrexed and nedaplatin were added because the efficacy was not significant. The lesion showed a tendency to progress even with afatinib replacement. Systemic bone imaging showed new lesions: the left side of T11 vertebral, the left sacroiliac joint, and the right lower femur. Bilateral adrenal nodules (25×20mm on the left, 63×37mm on the right), and nodules in the upper inner quadrant of the left breast (16×11mm) were found. *MET* amplification and *EGFR* amplification were detected in peripheral blood sample on Feb 19, 2020, and afatinib was discontinued after that. In the end, the patient died on June 20.

2. Pedigree Analysis

2.1 Proband

The patient had no chance for surgical treatment at the time of diagnosis. Due to the patient refused needle biopsy, and there were not enough previous tissue sample, peripheral blood was used for ARMS assay (*EGFR*, *ALK* and *ROS1* gene hotspots detection), all of which were negative. After that, peripheral blood was used for NGS detection on the 189-gene panel and ddPCR detection.

The *EGFR* p.L858R (mutant allele frequency, MAF: NGS: 0.07%; ddPCR: 4.64%) and *EGFR* p.V1010M (MAF: 49.59%) were detected by NGS and ddPCR (Figure 2). Given the high MAF of V1010M, germline mutation was considered, which was confirmed as a hybrid germline mutation by family analysis.

2.2 Family Analysis

Many members have been suffered from lung cancer in their families, with obvious familial aggregation. After obtaining the approval of the Ethics Committee of the Second Affiliated Hospital of Chongqing Medical University and obtaining the consent of the family members and signing the informed consent, we collected the blood samples of 9 relatives of the family and sent them to 3D Medicines Inc. for Sanger sequencing.

Verification sites of Sanger method: *EGFR* p.V1010M. Chromosomal location: chr7: 55268962-55268962: G>A□DNA range: chr7: 55268952-55268972□CATGGACGACG (>A) TGGTGGATGC; PCR primers sequence: F: ACAGGCACCTGCTGGCAATA, R: ATAGTGGACCTAAAAGGCTTACAATC.

It was found that a sister (III1), the daughter (IV4) and the granddaughter (V1) of proband (III3), the daughter (IV1) and son (IV2) of the proband's sister were heterozygous for *EGFR* p. V1010M respectively (Figure 3).

3. Protein Structure And Function Prediction

The influence of DNA level variation on the organism is finally manifested by the change of protein structure and function. *EGFR* c.3028G>A lead to the No.1010 amino acids in *EGFR* protein changed from valine (V) to methionine (M). Using Schrodinger software to simulate the *EGFR* structure, the results showed that the 1010th amino acid was located out of kinase binding domain and near the C terminal (Figure 4), and the variation to methionine had little effect on the structure of *EGFR* dimer, suggesting that there might be other reasons for drug resistance (Figure 5).

4. Discussion

Lung cancer is the most common cause of cancer death in the world, with 1.38 million deaths every year, accounting for 18.2% of the total number of cancer deaths[5]. It is also the cancer with the highest morbidity and mortality in China, with about 781,000 new cases and 626,000 deaths reported in 2014 [6]. Lung cancer has obvious familial aggregation, but it is not clear whether the same environment, similar habits and the inherent genetic factors that all contribute to familial aggregation of lung cancer.

A study involving 44,788 twins found that the heritability of lung cancer was about 26%[2]. This indicates that genetic factors only play a small part in the incidence of lung cancer, and most of the reasons can be attributed to environmental factors. The results of a genome-wide association study (GWAS) also confirmed that the penetrance rate of gene variation was in direct proportion to the genetic risk of cancer: common SNPs with low penetrance were associated with low genetic risk; infrequent moderate penetrance genetic variants were associated with moderate genetic risk, such as ataxia telangiectasia mutation (*ATM*) and checkpoint kinase 2 (*CHEK2*); rare genetic variants with high penetrance are associated with high genetic risk, such as *BRCA1/2* are associated with hereditary breast and ovarian cancer, *MLH1*, *MSH2* and other MMR pathway gene variants are associated with Lynch syndrome[7]. What is in common is that *ATM*, *CHEK2*, *BRCA1/2*, *MLH1*, *MSH2* and so on are all belonged to DNA damage repair (DDR) pathway genes. This type of genetic variation causes errors in the DNA replication process to go uncorrected, so more genes are mutated and tumors eventually form. GWAS has shown that the genetic risk of most common cancers is mostly polygenic[7], so the DDR pathway gene germline mutations become the strongest genetic factor in tumor formation. However, the incidence of DDR pathway variation in lung cancer is low, and only a few are germline mutations, so the role of genetic factors in the etiology of lung cancer remains to be fully elucidated[8].

EGFR is the most common driver gene of NSCLC, suggesting that *EGFR* plays an important role in the occurrence of NSCLC. However, most of the pathogenic *EGFR* mutations are somatic mutations and are not passed on to the next generation. At present, very few *EGFR* germline mutations have been reported, including G724S, K757R, V786M, T790M, L792F, R831H, V843I, L844V and D1014N[4, 9]. Among those studies, only T790M has a significant family history, but the relationship between T790M germline mutation and the occurrence of lung cancer has not been determined through the family pedigree[4]. In another family with V843I germline mutation, only 1 developed lung cancer in 20 blood relatives from two generations, so the relationship between V843I germline mutation and the occurrence of lung cancer could not be confirmed either[9]. Very similar to this study, the V843I mutation was also resistant to erlotinib, and the protein structure analysis also found that the V843I mutation did not change the affinity of ATP pocket region to EGFR-TKIs, and the mechanism of resistance remains unknown[9].

Germline mutation of *EGFR* p.V1010M was first identified in this family. This mutation has not been reported so far, and it is defined as variant of unknown significance (VOUS) according to American College of Medical Genetics (ACMG). In the Catalogue of Somatic Mutations in Cancer (COSMIC) web site, functional analysis through hidden Markov models (FATHMM) prediction of *EGFR* p.V1010M is pathogenic (score 0.72). In the pedigree (Figure 6), it can be seen that among the five generations of the proband's maternal relatives, seven are known to have developed into lung cancer, with significant familial aggregation characteristics. Six of the ten tested were found to be germline *EGFR* p.V1010M carrier. However, except for the proband (III3), no lung cancer was found in III1, IV1, IV2, IV4 and V1. Considering that IV1, IV2, IV4 and V1 are all under the age of 40, whether they will develop into lung cancer in the future still needs long-term observation. Therefore, current evidence does not confirm

whether there is a causal relationship between germline *EGFR* p.V1010M and the incidence of lung cancer.

Somatic mutations of *EGFR* p.L858R and *KRAS* p.G12V (Figure 2D) were found in the proband and II4, respectively, which are obvious oncogenic driver gene variations. Since somatic variations is usually attributed to environmental influences in the later period, environmental factors are the main cause of lung cancer, which has been confirmed once again.

According to different *EGFR* mutation sites, the efficacy of EGFR-TKIs is also different, and mutation sites can be divided into sensitive sites and resistance sites[1]. In this case, somatic mutation of *EGFR* p.L858R was simultaneously detected beyond *EGFR* p.V1010M, which is a common sensitive mutation. However, after the use of geffitinib and afatinib, the optimal efficacy was SD as assessed by RECIST 1.1, and it showed a progression trend 4 months after the use of the drug, suggesting that *EGFR* p.V1010M may be the resistance site of EGFR-TKIs.

V1010 is located on exon 22 of *EGFR* gene, near the kinase binding domain and the C-terminal. The three-dimensional computer simulation of the structure of the mutant protein showed that V1010M had little effect on the spatial structure of the *EGFR* dimer, and did not affect the ATP pocket binding to the EGFR-TKIs molecule. Therefore, the biological mechanism of EGFR-TKIs resistance caused by V1010M is still unknown, and further study is needed in animal models and cell line experiments.

Somatic variation caused by environmental factors is usually not thought to be directly passed on to the next generation. However, the same environment, similar habits and even the same work content in the same family will all cause familial aggregation of cancer. Family history is still a risk factor for lung cancer. Although the risk of the disease may not be transmitted to offspring through DNA sequence changes of germline, the risk of the disease can also be increased through DNA methylation, histone modification and RNA regulation[8]. Therefore, the importance of family history assessment should not be underestimated, and the family we reported also confirmed that people with a family history of lung cancer have a higher risk of developing lung cancer.

In summary, this family is the first family to be analyzed by pedigree with *EGFR* p.V1010M germline mutation. Although it has not been proved that this mutation site is a genetic factor for the occurrence of lung cancer, it is found that it may be a potential drug-resistant mutation of EGFR-TKIs. Next, we will conduct cell line and animal model studies to explore the biological mechanism of *EGFR* p.V1010M for the development and treatment of disease resistance. At the same time, we will follow up this family for a long time to investigate the correlation between *EGFR* p.V1010M and the development of lung cancer, which will also improve the awareness of cancer prevention and health care in this family, help them diagnosis and intervene in time.

Declarations

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Conflicts of interest/Competing interests: The authors declare no potential conflicts of interest.

Availability of data and material: All data generated or analysed during this study are included in this published article.

Code availability: Not applicable.

Authors' contributions: Na Li and Depei Huang contributed equally to this manuscript. Na Li is responsible for the diagnosis and treatment of the proband, as well as the collection of pedigree. Depei Huang is responsible for the analysis of sample detection results and article writing. Chunyi Liu and Lan Xiong participated in the diagnosis and treatment of the proband and the collection of their pedigree. Xueke She, Xudong Shen and Hushan Zhang play a huge role in gene detection. Jiang Youfan is responsible for the overall coordination of the project.

Ethics approval: The study was approved by the Ethics Committee of the Second Affiliated Hospital of Chongqing Medical University.

Consent to participate: Written informed consent was obtained from individual or guardian participants.

Consent for publication: Not applicable.

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Figures

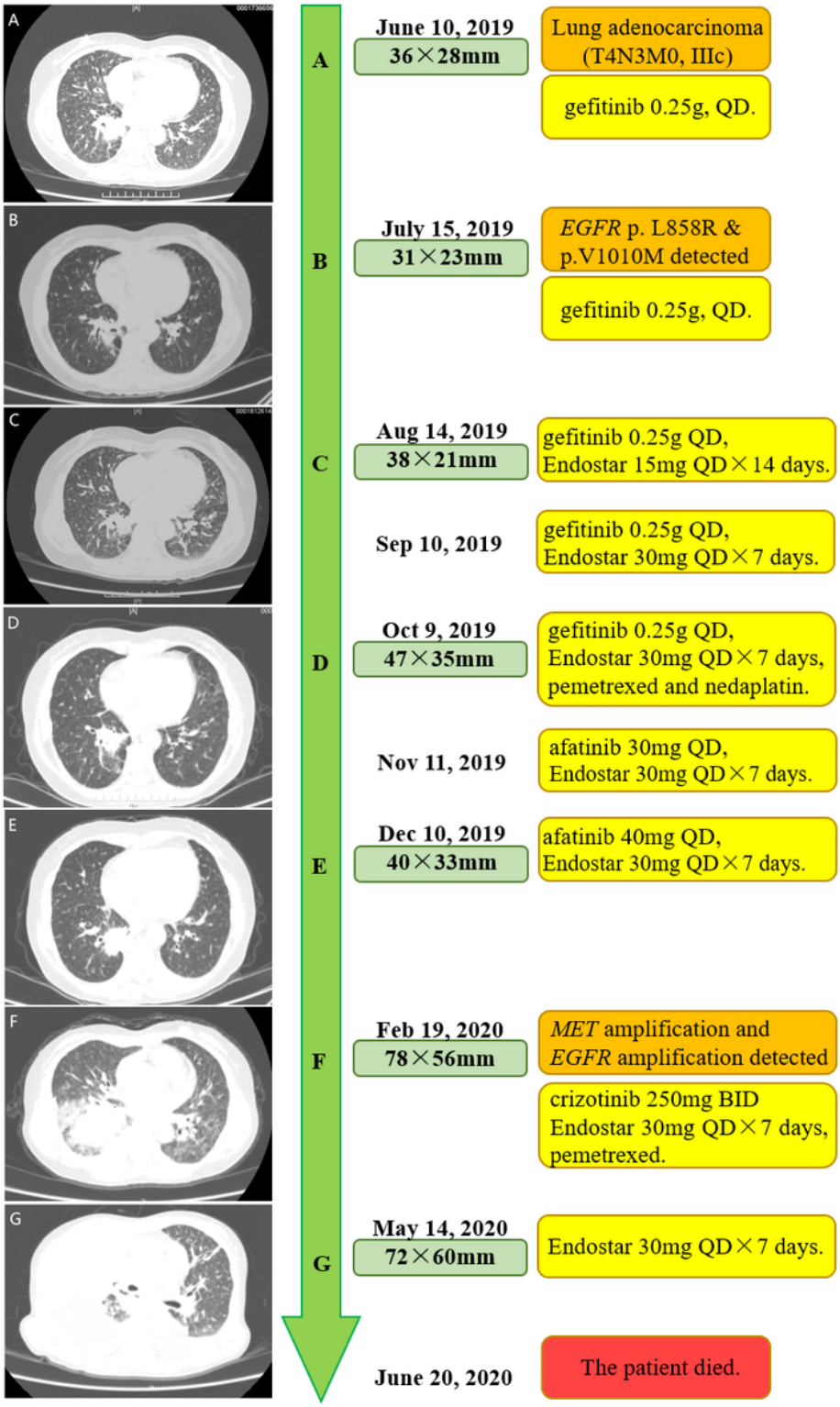

Figure 1

Chest CT and treatment process of the proband.

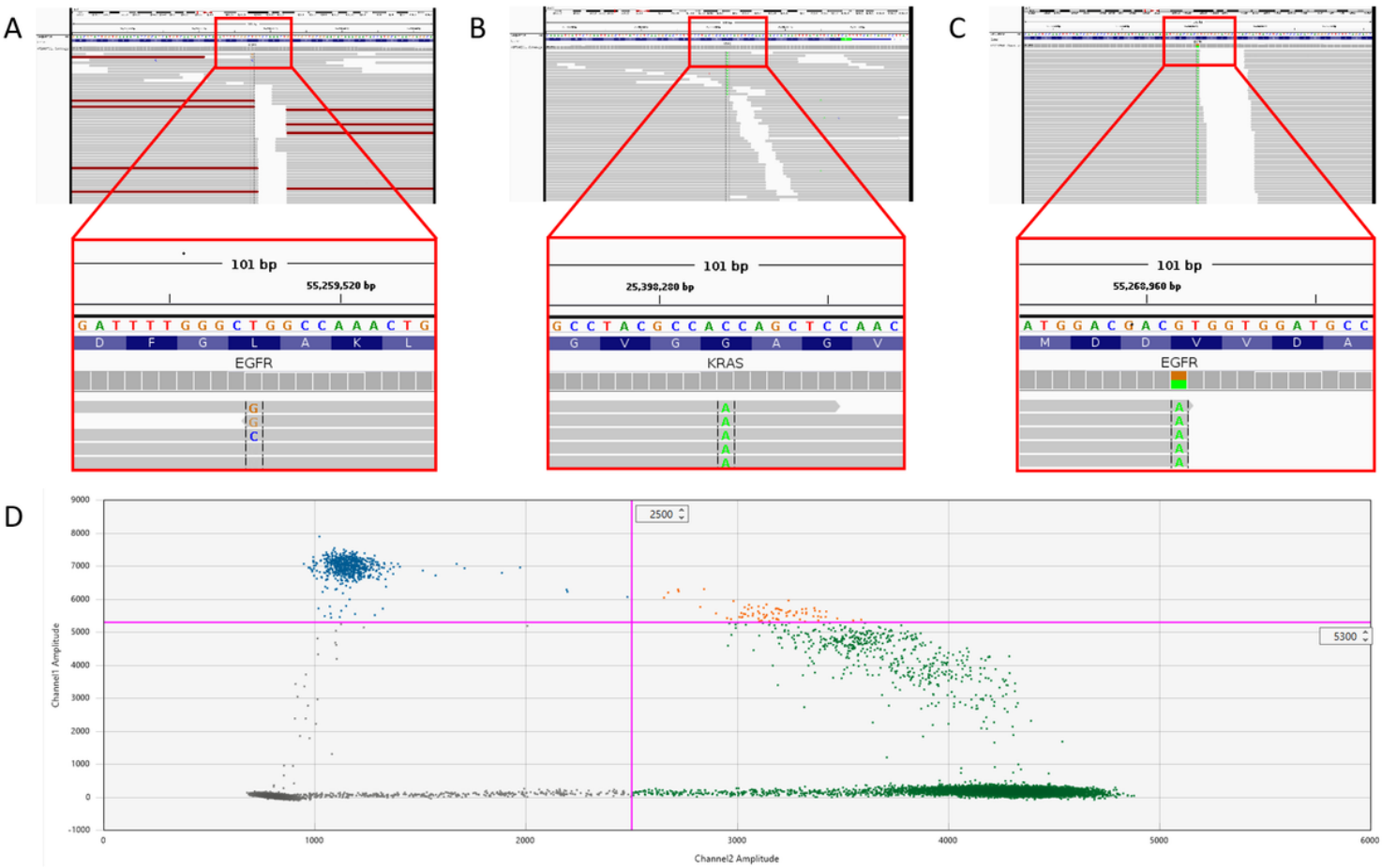

Figure 2

Detection results of NGS and ddPCR. A: somatic mutation EGFR p.L858R of the proband by NGS; B: somatic mutation KRAS p.G12V of III4 by NGS. C: germline mutation EGFR p.V1010M of the proband by NGS; D: somatic mutation EGFR p.L858R of the proband by ddPCR;

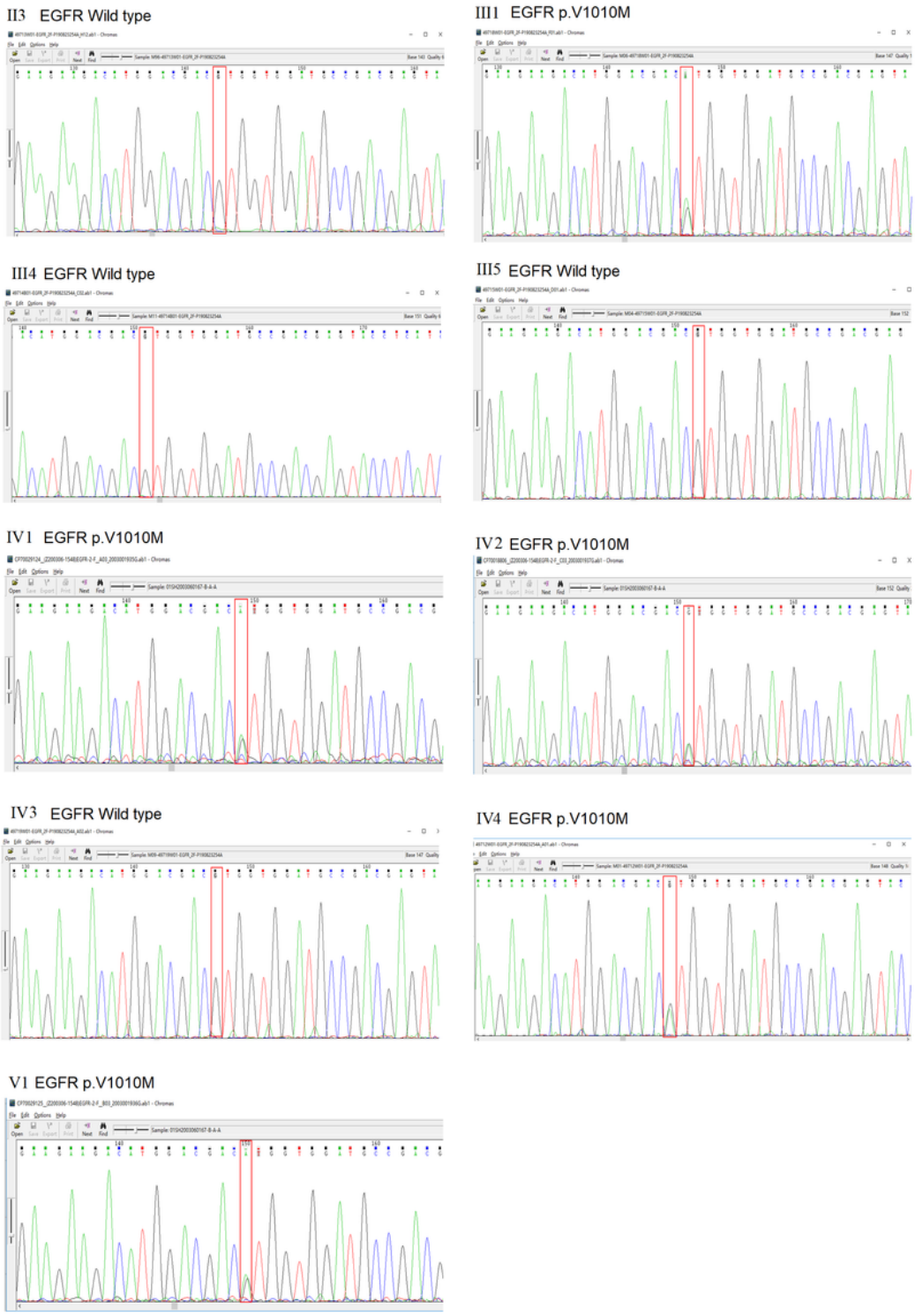

Figure 3

Detection results of familial samples by Sanger sequencing.

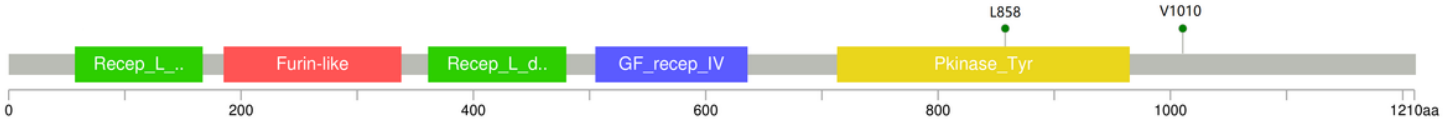

Figure 4

L858 and V1010 in relative positions of EGFR protein.

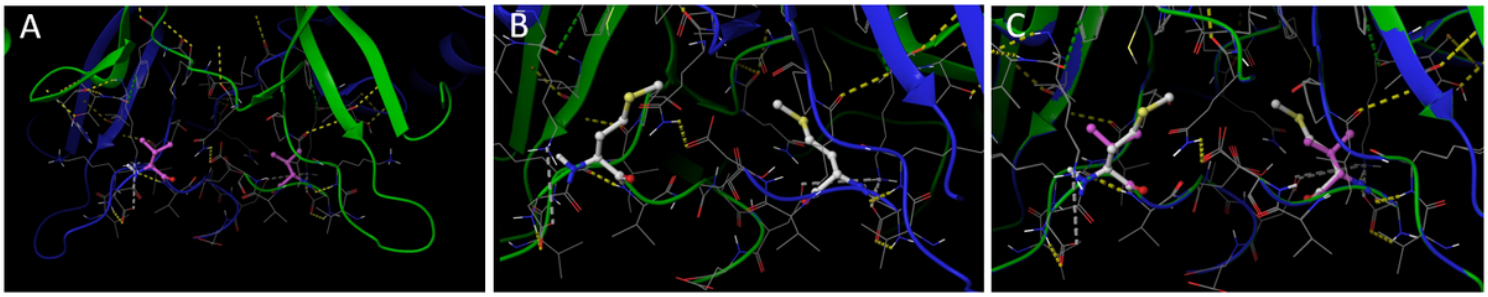

Figure 5

EGFR dimer structure simulated by Schrodinger software. A: EGFR wild-type, valine in purple; B: EGFR mutant, methionine in white; C: two structures compared in overlap.

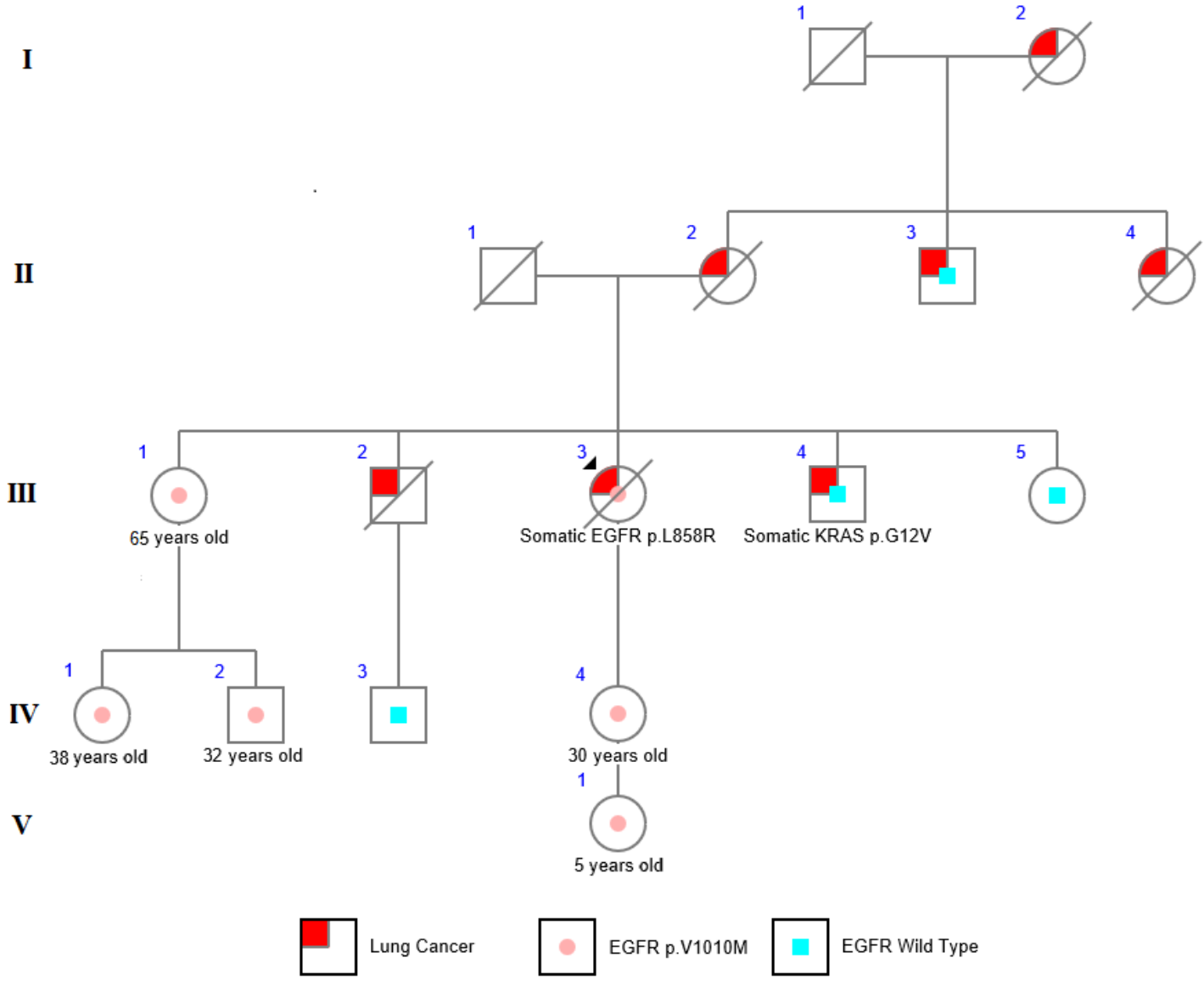

Figure 6

The pedigree analysis.