Novel germline mutation in lung cancer pedigrees establishes *BCAR1* as a human cancer susceptibility gene: a case report

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Background: Lung cancer is the most prevalent malignancy worldwide. Most cases are sporadic and carry somatic mutations in hotspot genes. However, accumulating studies have identified several germline mutations that predispose patients to lung cancer at present.

Case Description: In this report, 2 siblings diagnosed with lung squamous cell carcinoma and lung adenocarcinoma were sequenced by whole exome sequencing (WES) and Sanger sequencing. In this context, we reported a novel frameshift germline mutation of breast cancer anti-estrogen resistance protein 1 (*BCAR1*) in exon 4 (NM_001170717: c.942delinsAATGCCAGGGC), causing a frameshift and introducing a premature stop codon, which was detected in both siblings. Screening across other family members revealed their presence in 2 affected individuals. The *BCAR1* gene was previously demonstrated to be associated with lung cancer. The variant detected in this report would impair the regulation and functions of *BCAR1* in some extent, thus may promote the tumorigenesis of lung cancer.

Conclusions: In conclusion, our findings suggest that *BCAR1* is a possible susceptibility gene for lung cancer, and its functional analyses in lung cancer need further investigation. In this study, we first reported a novel causative mechanism of lung cancer: an insertion of 11 bp in *BCAR1* gene, which can be helpful in the genetic diagnosis of this disease.

Keywords: Lung cancer; germline mutation; BCAR1; susceptibility gene; case report

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Introduction

Lung cancer is the most prevalent malignancy worldwide, in which non-small cell lung cancer (NSCLC) is the predominant histologic type, mainly including adenocarcinoma and squamous cell carcinoma (1). Smoking is the primary non-inherited risk factor for lung cancer; however, approximately 25% of lung cancer cases worldwide are not attributable to tobacco use (2). Multiple risk factors, including environment, hormones, and genetic predisposition, have been associated with the pathogenesis of lung cancer in never smokers. Among these factors, familial lung cancer has been reported (3), and people with family history of lung cancer have double the risk of developing the disease (4,5). In the past decades, numerous somatic mutations have been identified in lung cancer tissues and contributed to the establishment of an oncogene-centric molecular classification paradigm in this disease. In contrast, relatively few germline mutations have been identified. Although familial forms of lung cancer are rarely described, several susceptibility genes have been identified, such as *EGFR* (3,6), *HER2* (7), *BAP1* (8), and *BRCA2* (9), among which *EGFR* germline mutations have been the most reported.

In this report, we have presented 2 siblings with lung cancer harboring germline mutation in breast cancer anti-estrogen resistance protein 1 (*BCAR1*). Somatic mutations and germline mutations including *BCAR1* and

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FAM20C were identified in the 2 siblings through whole exome sequencing (WES). Further frameshift germline mutation of *BCAR1* was confirmed in the proband by Sanger sequencing, and screening across other family members revealed their presence in 2 affected individuals. To our knowledge, this is the first time that frame-shift germline mutations of *BCAR1* were found by WES and Sanger sequencing in lung cancer patients. Furthermore, the *BCAR1* gene, encoding a scaffold protein, has been shown to be overexpressed and linked to adverse features in lung cancer (10-12). Therefore, we suspected that *BCAR1* germline mutation potentially predisposes patients to lung cancer. We present the following article in accordance with the CARE reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-21-7017/rc).

Case presentation

Tumor tissues and blood from 2 patients were collected in 2018. According to the digestive system tumor classification of the World Health Organization (WHO) in 2010, tumor samples were identified by hematoxylin and eosin (H&E) stained slides. The tumor tissue samples were histologically confirmed as lung squamous cell carcinoma and lung adenocarcinoma respectively by 2 molecular pathologists, and the tumor cell content exceeded 70%. All procedures performed in this study were in accordance with the ethical standards of the institutional and or national research committee(s) and with the Helsinki Declaration (as revised in 2013) and ethical standards of the Medical Ethics Committee of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology and got its approval (ethical approval No. S412). Written informed consent was obtained from both patients for publication of this case report and accompanying images. A copy of the written consent is available for review by the editorial office of this journal.

We used WES to analyse the formalin-fixed paraffin-embedded (FFPE) tissues and blood samples of the 2 siblings. Somatic mutations were obtained from tumor tissues and blood leukocytes, and germline mutations were obtained from blood leukocytes. As for individual mutational differences, mutations can be divided into somatic mutations and germline mutations. Somatic mutations are analysed using WES data of tumor samples with comparison to paired normal tissue for each individual. Somatic mutations are tumour-specific mutations after filtering out germline mutations which can be observed in the paired normal control samples as well. Therefore, it reflects individual differences between tumors. Germline mutations are defined as a gene alteration found in both tumor and paired normal control samples. They are individual-specific mutations in healthy tissues with comparison to reference genomes, reflecting individual differences and gene polymorphism in the population. The DNA was isolated from tumor FFPE tissues and blood leukocytes of blood samples using the DNeasy Blood and Tissue Kit (69504, QIAGEN, Venlo, Netherlands) according to the manufacturer's instructions. The DNA quantity was assessed by Agilent's Bioanalyzer (Agilent Technology, Santa Clara, CA, USA). The targeted capture pulldown and exon-wide libraries from genomic DNA were generated through the TruePrep DNA Library Prep Kit V2 for Illumina (#TD501, Vazyme, Nanjing, China). The sequences of captured libraries were performed as pair-end reads on sequences on the Illumina HiSeq 2500 platform. Variants were called using the Genome Analysis ToolKit (GATK; https://gatk.broadinstitute.org/hc/en-us) and functional annotation was performed through Annovar (https://annovar.openbioinformatics.org/en/latest/) and data were filtered by public databases, such as ExAC, 1000 Genomes Project, and Huma Gene Mutation Database (HGMD).

The blood samples of the 2 siblings and the 17 family members (except for family II: 6, 8 and family III: 6) were analysed by Sanger sequencing. The DNA was isolated from blood leukocytes of blood samples, germline *BCAR1* and *FAM20C* mutations of the 2 siblings were further confirmed by Sanger sequencing, and screening across all 17 family members by Sanger sequencing revealed their presence in family members. Resequencing of *BCAR1* c.942delinsAATGC CAGGGC (NM_001170717) was performed using the primers 5'-AAGTCTGTGCCTCTGCTT-3' (forward) and 5'-TTCCTCATCTGTCAACCATC-3' (reverse), amplified by polymerase chain reaction (PCR) at an annealing temperature of 58 ℃. Resequencing of *FAM20C* c.951delinsGGACAGGTGAGCCCTTCCTTCCTC CCTC CATCCGC (NM_020223) was performed using the primers 5'-CATATGAGGAAC CCAGCACGTC-3' (forward) and 5'-AAAGGTGACGATGACATACAGGA-3' (reverse), amplified by polymerase chain reaction at an annealing temperature of 60 ℃. Multiple sequencing reactions were loaded onto multiwell plates, which were injected into capillaries for electrophoresis on the instrument. Fluorescent-labeled modified (dideoxy)

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nucleotides were used that terminate the formation of a new DNA strand as they encounter their complementary nucleotides in the target sequence. This resulted in DNA strands of variable length, which were separated on a gel by electrophoresis and reflected the sequence being analyzed. Each capillary was separately calibrated for the dyes used in the sequencing reactions so that the software could perform the multi-component analysis to identify each of the dye-labeled fragments. These sequencing reactions were then analyzed using Variant Reporter V1.1.

Patient 1

Patient 1, a 68-year-old male with smoking history, sought medical consultation for a 1-week history of cough and hemoptysis without any inducing factors, and was diagnosed with space-occupying lesions of the left upper lung in a local hospital. He was transferred to our department in May 2018 for further management. The chest computed tomography (CT) and positron emission tomography (PET)-CT confirmed a 6.4 cm × 5.2 cm mass in the left upper lung (*Figure 1A*), and multiple nodules were also found. A CT-guided lung biopsy revealed a NSCLC with a histological type of squamous cell (*Figure 1B*). The patient was diagnosed with stage IIIc lung squamous cell carcinoma (cT4N3M0) based on clinical-pathologic-radiographic correlation. Panel detection of cancer driver genes revealed 3 common somatic mutations associated with lung cancer including *EGFR*, *PIK3CA*, and *TP53*. He then underwent 2 cycles of chemotherapy using albumin paclitaxel and nedaplatin on 30 May 2018 and 22 June 2018, followed by radiotherapy (IMRT, PTV 60 Gy/30 F) on 12 July 2018. The subsequent CT revealed that the tumor had shrunk (*Figure 1C*). However, the patient was readmitted for cough, chest distress, and asthma in January 2019. The repeated CT scan revealed tumor recurrence (*Figure 1D*). The patient refused further treatment, and he died in July 2019.

Patient 2

Patient 2, a 51-year-old female without a smoking history, was the younger sister of patient 1. She had experienced intermittent coughing for 1 month and sought medical consultation at a local hospital. Auxiliary examination revealed the presence of a nodule in the right parahilar region, as well as an increased number and enlarged size of mediastinal lymph nodes. She was transferred to our department in June 2018. Subsequent PET-CT and enhanced CT showed a 2.7 cm × 2.2 cm mass in the right lower lung (*Figure 2A*). Multiple enlarged lymph nodes were also identified in the right parahilar region and mediastinum. A CT-guided lung biopsy was performed on 12 June 2018. Pathological examination indicated lung adenocarcinoma (*Figure 2B*) with pleural metastasis, clinical stage was IVA (cT4N3M1a). The patient received 1 cycle of chemotherapy (pemetrexed disodium 800 mg/dL + carboplatin 0.5 g/dL) on 17 June 2018, and then started treatment with crizotinib. A follow-up CT scan demonstrated partial response with the indication of tumor shrinkage (*Figure 2C*). On 28 August 2019, lung lesions were stable; however, brain magnetic resonance imnaging (MRI) revealed potential metastasis (*Figure 2D*). The patient is currently under treatment with alectinib.

Discussion

With the development of next-generation sequencing (NGS) technology, gene mutations and dynamic epigenetic changes have further deepened our understanding of lung cancer and provided clues for new therapeutic targets. Molecular testing has become an integral part of lung cancer management. The detection of *EGFR*, *BRAF* and *MET* mutations as well as the analysis of *ALK*, *ROS1*, *RET* and *NTRK* translocations have already been incorporated into the diagnostic criteria for NSCLC (13). These oncogenic drivers strongly influence the formation and progression of lung cancer (6,14,15). Epigenetic regulation of genes is divided into different aspects, including DNA methylation, histone modifications and regulation of non-coding RNAs. Unlike direct gene mutations, epigenetic alterations of genes often lead to changes in intracellular RNA and protein expression levels leading to cancer. For example, aberrant DNA methylation promotes carcinogenesis through promoter methylation of tumor suppressor genes (TSG) to suppress their expression (16,17). The epigenetic disruption of tumor suppressor miRNA or oncomirs by promoter CpG methylation is common and can promote pro-tumoral capacities. Post-translational modifications of histones are also associated with lung carcinogenesis (18,19). Many studies have shown that key TSG or oncogenes are regulated by aberrant expression of miRNA, resulting in the acquisition of the characteristics of lung cancer (20-22). Overall, these data all emphasize the critical role of both gene mutations and multiple epigenetic events in the development of lung cancer.

In recent years, the developing technology of WES

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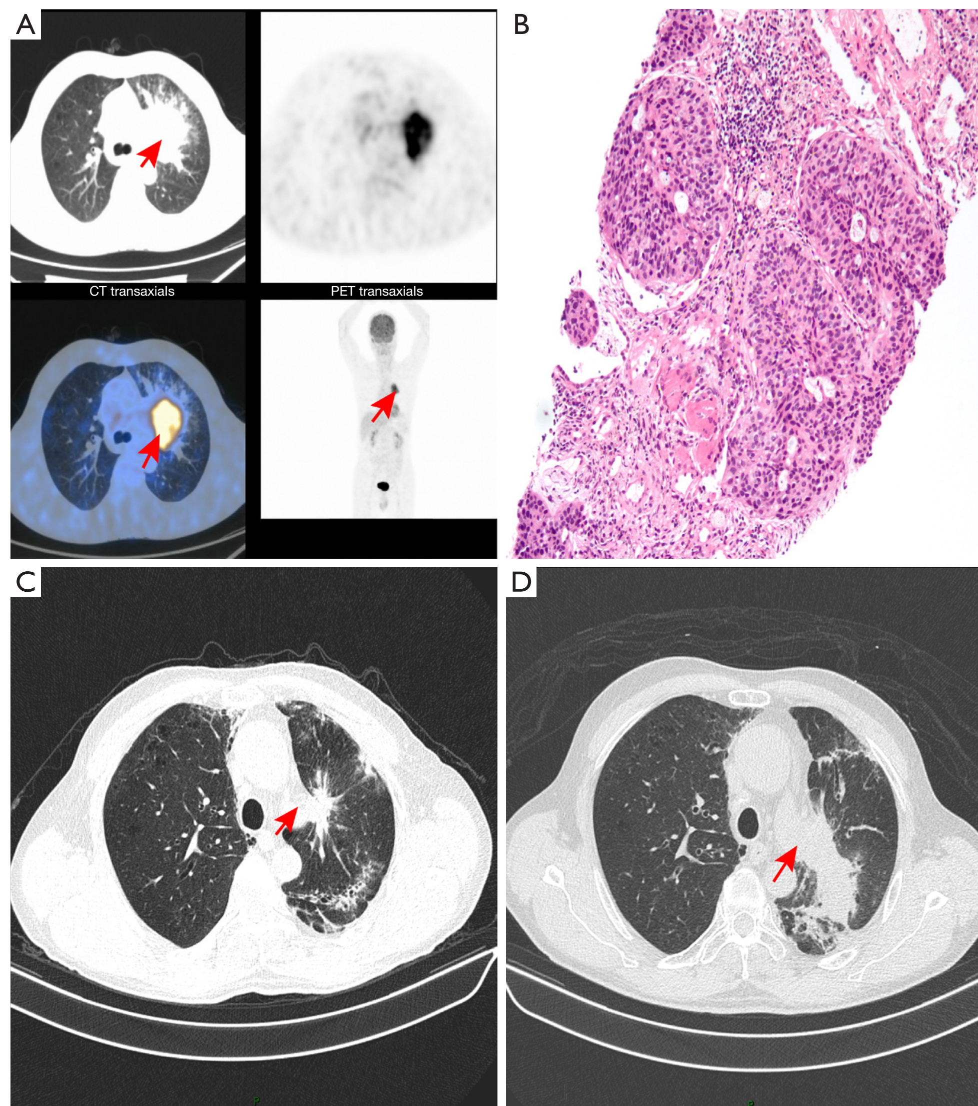

Figure 1 Image and histological findings of patient 1. (A) PET/CT scan performed in May 2018, showing pulmonary nodules (red arrow). (B) Histopathological result of lung lesion (HE, ×100). (C) Lung CT image in October 2018, revealing a shrunken lung lesion. Plain CT scan of the lung (red arrow) demonstrates an irregular 2.1 cm × 1.7 cm mass with marginal burrs beside the mediastinum in the upper lobe of the left lung. Multiple lymph nodes in the mediastinum and left hilum with cancerous lymphangitis. (D) Lung CT image (red arrow) in January 2019, showing an irregular mass with marginal burrs beside the mediastinum in the upper lobe of the left lung. Multiple lymph nodes in the mediastinum and left hilum, accompanied by cancerous lymphangitis, and more surrounding obstructive pneumonia than before. PET-CT, positron emission tomography-computed tomography.

has shown enormous potential. This technology is now rapidly entering the clinical laboratories and changing the landscape of clinical testing and diagnostics, including to search for potential oncogenes to provide reference for molecular diagnosis of tumors (23,24). WES has achieved great success in identifying pathogenic variants for rare diseases and continued to contribute to the discovery of novel genes as well as the expansion of the phenotypic spectrum of known genes (25,26). The best drug targets related to tumor gene mutations can be obtained through WES technology, thereby changing the corresponding metabolic pathways, which may become the gold standard for individualized tumor therapy in the future (27,28). In addition, obtaining tumor gene mutations and DNA

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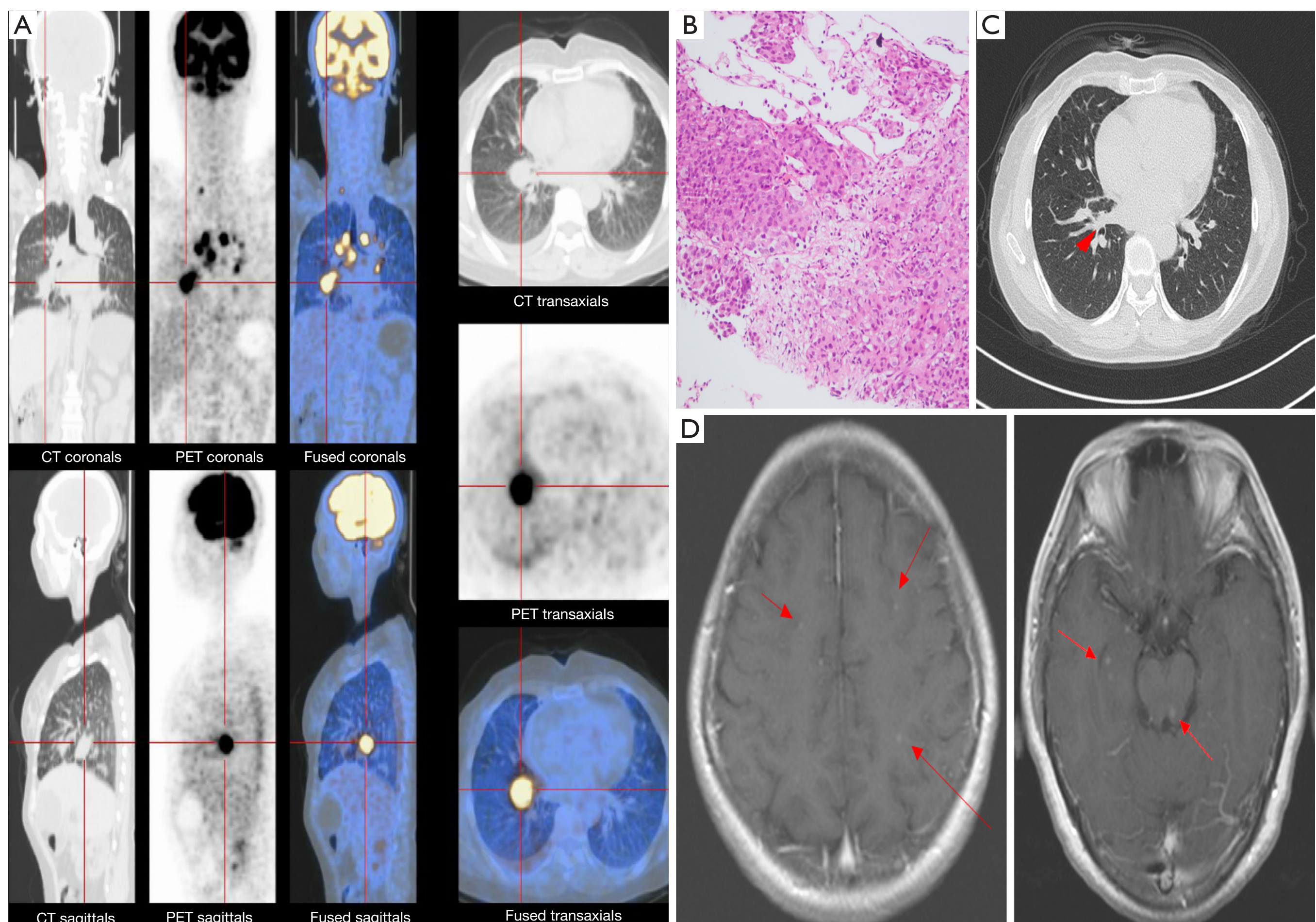

Figure 2 Image and histological findings of patient 2. (A) Initial diagnosis in June 2018; PET/CT scan showed a 2.3 cm × 2.2 cm lung nodule; (B) histopathological result of lung lesion (HE, ×100); (C) lung CT image (red arrow) in March 2019, revealing that the soft tissue nodules in the anterior basal segment of the lower lobe of the right lung were partial response; (D) MRI of the brain was obtained in affected individuals in August 2019, revealing multiple spotty enhanced foci can be seen in the bilateral brain, cerebellum and brainstem. The red arrows show brain metastases. PET, positron emission tomography; CT, computed tomography; MRI, magnetic resonance imaging.

methylation information through WES technology can prompt tumor prognosis and guide clinical practice (29-31). Chang *et al.* applied an exome sequencing technology to determine variations and mutations in eight commonly used cancer cell lines, the results show that exome sequencing can be a reliable and cost effective way for identifying alterations in cancer genomes (32). Varela *et al.* applied WES on a series of primary clear cell renal cell carcinoma (ccRCC) (33). *PBRM1*, encoding for SWI/SNF chromatin remodeling complex was discovered in this study. There are also several studies that have attempted to identify driver mutations of cancer including leukemias, myelomas and solid tumors (34-36). In our report, WES was used to analyze the genetic characteristics of the two siblings.

The results show that driver genes of *EGFR*, *PIK3CA*, and *TP53* were identified in patient 1. Only a few distinct somatic mutations (patient 1 and patient 2, 21 and 4 somatic mutations, respectively) were identified in the 2 siblings (*Table 1*). In contrast, analysis of WES data from leukocytes revealed 479 germline mutations between the 2 siblings (https://cdn.amegroups.cn/static/public/10.21037atm-21-7017-1.xlsx). Surprisingly, 2 shared frameshift germline mutations (*BCAR1* and *FAM20C*) were also identified by analyzing WES data from white blood cells of the 2 patients. We identified

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Table 1 Somatic mutations between patient 1 and patient 2

Sample	Chromosome	Position	Reference	Variant	Gene	Type	snp150Common	InterVar_ automated	cytoBand
Patient 1	chr1	78414310	–	AA	FUBP1				1p31.1
Patient 1	chr1	120612040	–	CCTCCGCCG	NOTCH2				1p11.2
Patient 1	chr2	112786523	–	T	MERTK		rs147500027		2q13
Patient 1	chr3	189507518	–	AGAG	TP63				3q28
Patient 1	chr5	56177849	CAA	–	MAP3K1	Nonframeshift deletion	rs5868032		5q11.2
Patient 1	chr6	43738449	–	GACA	VEGFA	Frameshift insertion			6p21.1
Patient 1	chr6	157100431	GGC	–	ARID1B	Nonframeshift deletion			6q25.3
Patient 1	chr6	163991700	–	T	QKI		rs555941441		6q26
Patient 1	chr7	151945071	-	T	KMT2C	Stopgain	rs150073007		7q36.1
Patient 1	chr8	145738768	G	-	RECQL4				8q24.3
Patient 1	chr9	133759490	AAG	-	ABL1	Nonframeshift deletion	rs201725154		9q34.12
Patient 1	chr11	32452241	C	-	WT1		rs76500597		11p13
Patient 1	chr12	121434630	–	TCATTCAT	HNF1A				12q24.31
Patient 1	chr13	28942678	ATG	–	FLT1		rs138306957		13q12.3
Patient 1	chr16	79628537	T	–	MAF		rs66467731		16q23.2
Patient 1	chr17	7578712	TTTT	–	TP53				17p13.1
Patient 1	chr17	11924223	GCG	–	MAP2K4	Nonframeshift deletion			17p12
Patient 1	chr17	43364293	–	G	MAP3K14	Unknown	rs56405343		17q21.31
Patient 1	chrX	47030561	GGA	–	RBM10	Nonframeshift deletion			Xp11.23
Patient 1	chrX	53222043	–	C	KDM5C		rs146836963		Xp11.22
Patient 1	chrX	66765158	–	GCAGCA	AR	Nonframeshift insertion			Xq12
Patient 2	chr5	35861068	T	C	IL7R	Nonsynonymous SNV	rs1494558	Benign	5p13.2
Patient 2	chr5	35871190	G	A	IL7R	Nonsynonymous SNV	rs1494555	Benign	5p13.2
Patient 2	chr5	176520243	G	A	FGFR4	Nonsynonymous SNV	rs351855	Benign	5q35.2
Patient 2	chr12	121437382	A	G	HNF1A	Nonsynonymous SNV		Benign	12q24.31

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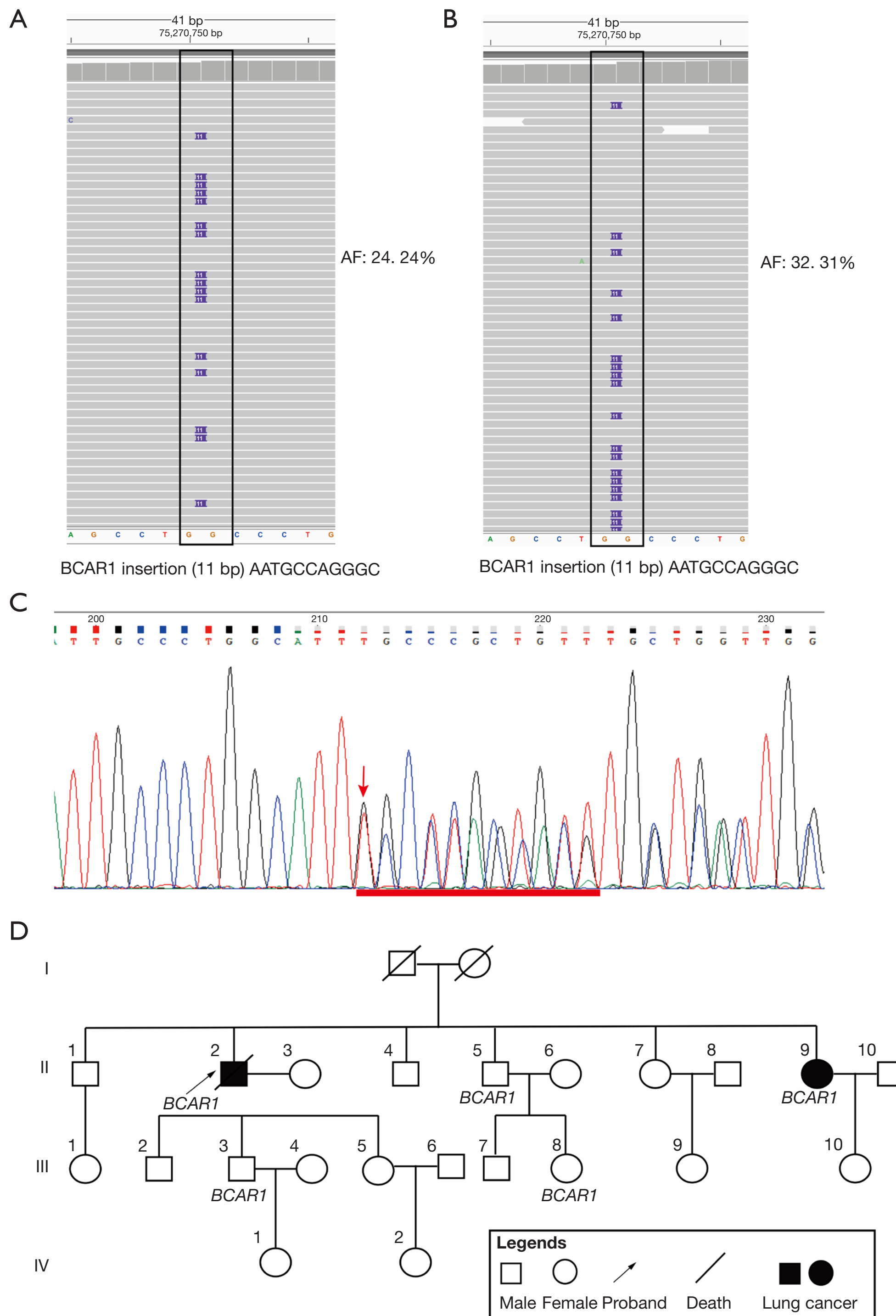

Figure 3 Detection of the *BCAR1* frameshift germline mutation using WES and visualized using the Integrative Genome Viewer software tool. (A,B) The insertion sites of *BCAR1* mutation in patient 1 and patient 2, respectively. (C) The c.942delinsAATGCCAGGGC change at nucleotide position 212 was confirmed by Sanger sequencing as indicated by the arrow. (D) Family tree showing affected in individuals with germline mutation in *BCAR1* participating in linkage study. WES, whole exome sequencing.

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the frameshift germline mutations of *BCAR1* in exon 4 (NM_001170717: c.942delinsAATGCCAGGGC) (*Figure 3A,3B*) and *FAM20C* in exon 4 (NM_020223: c.951delinsGGACAGGTGAGCCCTTCCTTCCTCCCTCCATCCGC). In addition, the 2 siblings with *BCAR1* and *FAM20C* variants were both identified as heterozygote mutations. Mutations in *FAM20C* cause an osteosclerotic bone dysplasia in humans known as Raine syndrome (37). However, another novel germline mutation of *FAM20C* was found in our report, encoding a novel frameshift germline mutation c.951delinsGGACAGGTGAGCCCTTCC TTCC TCCCTCCATCCGC, which showed a benign mutation (https://www.ncbi.nlm.nih.gov/clinvar/variation/VCV000402845.3). To further verify the hypothesis that germline mutations in *BCAR1* may be associated with lung cancer susceptibility gene in patients, the mutation sites of the 2 genes were sequenced in the 2 siblings by Sanger sequencing. Both patients harbored a germline insertion of 11 bp (NM_001170 717: c.942delinsAATGCCAGGGC) producing a frameshift at codon 314 (*Figure 3C*). The *BCAR1* mutation was confirmed by Sanger sequencing, and screening across all the 17 family members (except for family II: 6, 8 and family III: 6) revealed its presence in 2 affected individuals participating in the linkage study and in a further 3 relatives (*Figure 3D*). Several investigations have revealed that *BCAR1* is an oncogene involved in metastasis and progression of multiple cancer types (38,39). Li *et al.* found that more deleterious *BCAR1* mutations were detected in the *HER2*+ breast cancer subtype than in the *HER2*-breast cancer subtype, suggesting that *BCAR1* mutations lead to its overactivation and are associated with *ERBB2* overexpression (40), becoming resistant to the anti-proliferative effects of tamoxifen and leading to aggressive tumor progression (41-43). Overexpression of *BCAR1* was associated with activation of p38 in NSCLC cases, and *BCAR1* knockdown caused reduction of phospho-p38 levels in A549 cells (44). Multiple mutations in the *BCAR1* protein have also been reported to contribute to ovarian cancer susceptibility (45). Huang *et al.* evaluated the predictive power of *BCAR1* as a marker for poor prognosis in NSCLC cases (44). The results show that higher *BCAR1* levels were strongly associated with more poorly differentiated NSCLC and predicted poorer prognosis. Multiple mutations of *BCAR1* may affect its or different pathway-related genes expression levels, which may lead to different clinical syndromes. In order to clarify the clinical significance of *BCAR1* expression in circulating tumor cells (CTCs) in the peripheral blood and tumor tissues in patients with early stage lung adenocarcinoma (ES-LUAD), Jiang *et al.* analyzed the predictive power of *BCAR1* expression in CTCs and tumor tissues for disease-free survival (DFS). The results found that *BCAR1* may have a "dual impact" on markers of epithelial-mesenchymal transition (EMT) in tumor tissues and CTCs due to micro-environmental disparities, resulting in important clinical significance, which can potentially guide accurate treatment of early stage lung adenocarcinoma (46). Frameshift germline mutation of *BCAR1* is exceedingly rare, which is not presented in any online databases of human genetic variation, including the Exome Aggregation Consortium (ExAC) database, indicating it as a novel variant. In this study, frameshift germline mutation of *BCAR1* was confirmed in the proband by Sanger sequencing, and screening across other family members revealed their presence in 2 affected individuals. The *BCAR1* variant was identified in family *II* (*Figure 3D*), indicating that the allele frequency (AF) of *BCAR1* variant was approximately 50% (heterozygote). The heterozygous *BCAR1* mutation was identified in a heterozygous state in both patients. The *BCAR1* gene encodes p130cas protein which possesses an amino-terminal Src-homology 3 domain, a substrate binding domain, proline-rich and serine-rich regions, and a bipartite Src-binding domain in carboxy-terminal sequence (10,47). The p130Cas protein itself has no intrinsic enzymatic or transcriptional activity, but they could control signaling events via phosphorylation and dephosphorylation and their association with effector proteins in multimolecular complexes. Tyrosine phosphorylation is the major post-translational modification of p130Cas, mainly occurring in the substrate binding domain, which helps to open binding sites for a variety of effector proteins (11). Many studies have demonstrated that p130Cas plays a vital role in cell migration (48,49), apoptosis (50,51), and cell transformation (52). The p130Cas protein has also been reported as involved in the development and progression of several human cancers, including breast cancer (53,54), prostate cancer (55,56), and lung cancer (44). In lung cancer, *BCAR1* is usually overexpressed, and increased *BCAR1* predicts poorer prognosis in NSCLC (44,57). High *BCAR1* expression induced EMT in lung cancer cells to promote lung cancer cell migration and invasion (44,57). Mao *et al.* confirmed that the higher *BCAR1*-mRNA and protein expression levels in lung cancer were associated with poorer patient prognosis (58). The MTT assay and cell plate clone formation assay confirmed that *BCAR1* plays an important role in cell proliferation, survival and clone formation in lung cancer.

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BCAR1 was confirmed to promote proliferation and cell growth by immunoprecipitation and mass spectrometry (IP-MS) assay, TCGA bioinformatics analysis and protein interaction (PPI-String) database, probably through upregulation of *POLR2A* and subsequent enhancement of catalase and transferase activities. In this report, *BCAR1* variant occurred in 11 bp insertion at codon 314 which is located at the substrate binding domain containing 15 repeats of the YxxP consensus phosphorylation motif for Src family kinases (https://www.uniprot.org/uniprot/P56945#showFeaturesTable). The Src kinase binds p130Cas at its carboxy-terminal binding site, and is responsible for the direct phosphorylation of YxxP motifs in the substrate domain, which mediates the interaction of p130Cas with Crk adaptor proteins (10). The insertion of *BCAR1* variant in the substrate binding domain indicates that its association with effector proteins would be impaired in some extent, thus leading to a wide range of effects, affecting different pathways, including cardiac development, endothelial migration, and cancer. The c.942delinsAATGC CAGGGC mutation is already presented in the *BCAR1* gene, in 2 consecutive repeats, becoming triplicated in the proband family germline. The results indicate that this genetic region may be unstable and prone to duplication. Intriguingly, frameshifts often occur in repeated DNA sequences, and potential consequences of the 11 bp insertion in *BCAR1* may lead to a truncated protein.

Therefore, *BCAR1* is a possible susceptibility gene for lung cancer. Although the biological function of *BCAR1* in lung cancer remains unclear, we believe that *BCAR1* plays an important role in the development and progression of lung cancer. It is worth noting that the 2 siblings developed 2 different histologic types of lung cancer, revealing that *BCAR1* may use different mechanisms to induce different histologic types of lung cancer. To further support our hypothesis, investigation concerning the biological function and interaction protein network of *BCAR1* in the occurrence and development of lung cancer is urgent and necessary. In addition, there were another 3 carriers identified who were in good health conditions, and a long-term follow-up is of great necessity.

In summary, our report revealed a possible susceptibility gene, *BCAR1*, and further investigation of biological function of *BCAR1* in lung cancer is needed. Our study was the first to identify the frameshift germline mutation of *BCAR1* by WES and Sanger sequencing, which is beneficial for the diagnosis and in the long term precision treatment of lung cancer.

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Footnote

Reporting Checklist: The authors have completed the CARE reporting checklist. Available at https://atm.amegroups.com/article/view/10.21037/atm-21-7017/rc

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-21-7017/coif). All authors report that this work had received technical support from Shanghai Tongshu Biotechnology Co., Ltd. The authors have no other conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All procedures performed in this study were in accordance with the ethical standards of the institutional and or national research committee(s) and with the Helsinki Declaration (as revised in 2013) and ethical standards of the Medical Ethics Committee of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology and got its approval (ethical approval No. S412). Written informed consent was obtained from both patients for publication of this case report and accompanying images. A copy of the written consent is available for review by the editorial office of this journal.

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