Cancer Genetics 230 (2019) 13–20

Cancer Genetics

ORIGINAL ARTICLE

# Novel rearrangements involving the *RET* gene in papillary thyroid carcinoma

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**Abstract**

*Background:* In the field of gene fusions driving tumorigenesis in papillary thyroid carcinoma (PTC), rearrangement of the proto-oncogene *RET* is the most frequent alteration. Apart from the most common rearrangement of *RET* to *CCDC6*, more than 15 partner genes are yet reported. The landscape of *RET* rearrangements in PTC ("RET-PTC") can notably be enlarged by modern targeted next-generation sequencing, indicating similarities between oncogenic pathways in other cancer types with identical genetic alterations.

*Methods:* Targeted next-generation sequencing was performed for two cases of *BRAF*-wild type PTC with confirmation of the results by Sanger sequencing. A "UniProt" database research was performed to assess protein alterations resulting from *RET* rearrangements.

*Results:* *RUFY2-RET* and *KIAA1468-RET* were detected. The fusion genes were not present in normal tissue of the index patients. The rearrangement *RUFY2-RET* lead to a fusion of the *RET* tyrosine kinase domain to a RUN domain and a coiled-coil domain. For *KIAA1468-RET,* a fusion to a LisH domain and two coiled-coil domains resulted.

*Conclusions:* *RUFY2-RET* and *KIAA1468-RET* are novel RET/PTC rearrangements. The fusions were previously described in non–small cell lung cancer. The rearrangement results in a fusion of the *RET* tyrosine kinase to regulatory domains of RUFY2 and KIAA1468.

**Keywords** RET/PTC, *RET* gene, RUFY2, KIAA1468, Papillary thyroid carcinoma.



## Introduction

Differentiated thyroid carcinoma is a malignant tumor characterized by a high incidence. In the United States of America, from 1975 to 2014, the yearly incidence rose from 4.9 to 14.3 per 100,000 inhabitants [1,2]. Differentiated thyroid carcinoma usually is associated with a favorable outcome, following adequate surgical therapy. Still, up to 10% of carcinomas show recurrence and up to 30% develop distant metastases [3]. Within the category of differentiated thyroid carcinoma, papillary thyroid carcinoma (PTC) is represented with a frequency of 80–85% [4]. In sporadic PTC, oncogenic fusions are present in 4–46%, involving *RET, BRAF, NTRK1, NTRK3, ALK, PPARG* and *THADA* [5–9]. Most gene fusions in PTC involve the *RET* ("rearranged during transfection") gene [5,10–12]. The proto-oncogene *RET* encompasses 21 exons and is located on chromosome 10q11.21. It encodes a transmembrane receptor tyrosine kinase [13,14]. In most cases, the breakpoint within the *RET* gene is located in intron 11 [15–17]. The underlying pathological mechanism was reported as follows: a fusion to a promoter belonging to a gene, which is expressed in thyroid follicular cells, leads to an expression of *RET*, which otherwise remains clinically silent. A loss of the transmembrane region of *RET* is caused by its

Received August 28, 2018; received in revised form October 7, 2018; accepted November 7, 2018

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https://doi.org/10.1016/j.cancergen.2018.11.002

common rearrangements. The resulting cytosolic location of *RET*, and the fusion to dimerization motifs – usually coiled-coil regions – deriving from the fusion partner gene, lead to a constitutive activation of the encoded tyrosine kinase [18,19]. This unselected activation of the tyrosine kinase positively influences downstream pathways including RAS-MAPK and PI3K-AKT cascades, which are involved in the regulation of cell survival, proliferation, migration, and differentiation [18,20–23].

In PTC, *RET* fusion genes received the acronym RET/PTC, which were numbered consecutively [24]. Nowadays, due to the multiplicity of RET/PTC rearrangements, numerous authors prefer a nomenclature according to the genes involved [5]. 19 oncogenic *RET* rearrangements in PTC have yet been described, involving *AFAP1L2, AKAP13, CCDC6, ERC1, FKBP15, GOLGA5, HOOK3, KTN1, NCOA4, PCM1, PPFIBP2, PRKAR1A, RFG9, SPECC1L, TBL1XR, TRIM24, TRIM27, TRIM33* and *UEVLD* [5,25,26]. Of these, RET/PTC1 (*CCDC6-RET*) and RET/PTC3 (*NCOA4-RET*) were discovered to have the highest frequency (approximately 90% of all RET rearrangements). In sporadic PTC, the frequency of RET/PTC1 is 2 times higher than of RET/PTC3 [5,27–29].

Yet, it is uncertain, how many different rearrangements of the *RET* gene exist in papillary thyroid carcinoma, and whether different RET/PTC rearrangements lead to a variable course of disease. Modern next-generation sequencing methods are able to comprehensively reveal the identity and characteristics of RET/PTC fusion genes, which will notably enlarge the spectrum of the yet known *RET* rearrangements, offering the basis to address the question.

## Material and methods

### Patients and material

Tumor tissue was obtained from two patients who underwent surgery for papillary thyroid carcinoma at the University Medical Center Mainz. Routine histologic and molecular genetic assessment already excluded a BRAF-V600E mutation. The methods are described by Musholt et al. [30]. Absence of the BRAF mutation was confirmed in the present next-generation sequencing analysis.

The first tumor sample was obtained from index patient I, a 40-year-old female patient, who underwent total thyroidectomy and central lymph node dissection (level VI) with transcervical thymus resection for papillary thyroid carcinoma, follicular variant, pT2, pN1b (5/9), M0, R0 (Fig. 1(A)). Primary tumor was used for next-generation sequencing. Postoperatively, the patient was treated with radioactive iodine (RAI) therapy. In the last follow-up, four years after diagnosis, the patient was free of disease.

The second tumor sample was retrieved from index patient II, a 28 year-old male, who underwent secondary surgery at our hospital for the recurrence of PTC. The patient had previously undergone subtotal thyroidectomy with incomplete central lymph node dissection (pT3, pN1b (11/19), L1, V0, R0) and RAI therapy. After an interval of one year following primary surgery, a bilateral multifocal recurrence had been registered in scintigraphy and sonography, which is why he presented in our outpatient clinic. We performed a secondary cervical exploration with central lymph node dissection (level VI), transcervical thymus resection and selective lymph node dissection of the lateral lymph node compartments. Thereby, a total of 32 lymph nodes was resected, of which 13 were affected by PTC, along with metastases in the perithyroidal tissue (Fig. 1(B)). Lymph node metastasis was used for next-generation sequencing. Until now, six months following secondary surgery, the patient is free of disease.

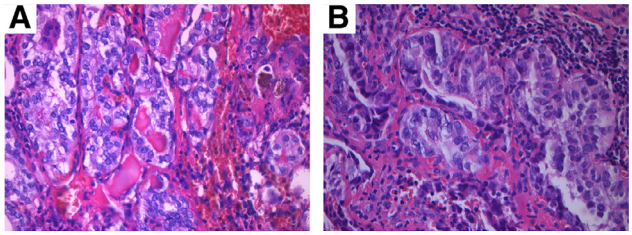


**Fig. 1** Corresponding histologic slide of the specimen used for extraction of DNA and RNA. (A) Histology. Index patient I, harboring *RUFY2-RET*: follicular variant of papillary thyroid carcinoma with irregularly developed follicular structures, partly atrophic epithelium and condensed colloid. Tumor cells display pleomorphic, vacuolated and distorted nuclei. Formalin-fixed, paraffin-embedded tumor tissue, hematoxylin and eosin stain x400. (B) Index patient II, harboring *KIAA1468-RET*: lymphatic tissue with vast invasion of a classical papillary thyroid carcinoma. Pleomorphic, enlarged tumor cell nuclei are densely assembled. Formalin-fixed, paraffin-embedded tumor tissue, hematoxylin and eosin stain x400.

### Main analysis: next-generation sequencing

Tumor tissue was retrieved from 4 µm formalin-fixed, paraffin-embedded (FFPE) tissue slices (80% tumor cells). Following manual microdissection, an extraction of DNA and RNA was performed using Maxwell RSC DNA and RNA FFPE Kits (Promega, Wisconsin, USA).

Anchored multiplex (AMP) polymerase chain reaction (PCR) based targeted next-generation sequencing was performed using Archer VariantPlex and Archer FusionPlex solid tumor kits (Archer, Boulder, Colorado, USA) according to the manufacturer's instructions. The panel allows for a simultaneous analysis of mutations and gene fusions in more than 60 genes, including e.g., *RET, NTRK1-3, ALK* and *RAS*. The technique allows for the detection and detailed analysis of genetic rearrangements by targeting one fusion partner involved. For VariantPlex, DNA libraries are created by two PCR reactions. The FusionPlex library preparation protocol includes the generation of cDNA from RNA template prior to PCR. In brief, each library is tagged with a unique combination of two adapter indices, which serve as molecular barcodes for sequencing. Sequencing was carried out using the MiSeq System (Illumina, San Diego, California, USA). Generated data was demultiplexed and aligned to the human reference genome hg19. The output (FASTQ format) was processed using "Archer Analysis" Software (Archer, Boulder, Colorado, USA).

For elimination of false positive results, filters for the detection of variants were set as follows: depth $\geq$ 100; allele frequency $\geq$ 10%. Inframe fusions were considered only. Called

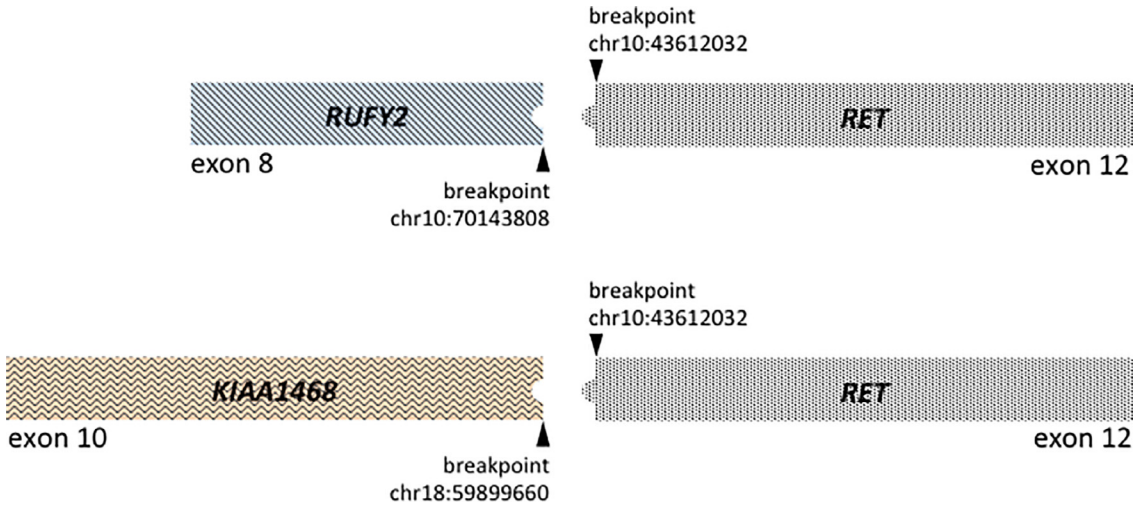


**Fig. 2** Breakpoint on RNA level. *RUFY2-RET* and *KIAA1468-RET* fusion genes: breakpoint on RNA level is between *RET*, exon 12 (both cases), and exon 8 of *RUFY2* and exon 10 of *KIAA1468*, respectively. Results according to next-generation sequencing, "Archer Analysis" Software.

variants from the Cosmic (v65) scientific database were integrated by "Archer Analysis".

## Confirmation of results: RT-PCR and Sanger sequencing

To confirm the newly identified RET/PTC fusion genes *RUFY2-RET* and *KIAA1468-RET*, RNA deriving from tumor and normal thyroid/thymus tissue of the index patients underwent RT-PCR based fusion-specific amplification. cDNA was generated by reverse transcription polymerase chain reaction (RT-PCR) (annealing with random primers (C1181, Promega, Wisconsin, USA) at 70 °C; elongation at 40° for 60 min (mastermix: M-MLV Reverse Transcriptase M1701, Promega, Wisconsin, USA; RNAsin Plus RNAse inhibior N2615, Promega, Wisconsin, USA; dNTP set 100 mM, Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA)). For PCR amplification of *RUFY2-RET*, the following primers were used: *RUFY2*_fw2: ATGAAAACACAGCAGCACCT and *RET*_rv2: TGTACCCTGCTCTGCCTTTC (biomers.net GmbH, Ulm, Germany). For *KIAA1468-RET*, primers *RET*_rv2: TGTACCCTGCTCTGCCTTTC and *KIAA1468*_fw2: TGTCTCCTGCATTCCATCAA were applied. 2.5 µl of cDNA template were processed (program: 95° 5 min, 96° 5 s, 52° 5 min, 68° 7 s, 72° 1 min, 36 cycles, master mix: Qiagen Fast Cycling PCR Mix (Qiagen, Hilden, Germany). Sequence lengths of 145 bp (*RUFY2-RET*) and 288 bp (*KIAA1468-RET*) were calculated for the primer combinations. Gelectrophoresis (agarose gel 2%, peqGREEN) was performed.

In addition, the PCR products of the tumor samples underwent Sanger sequencing (GenomeLab DTCS Quick Start Kit (Beckman Coulter life sciences, Brea, USA), primers: *RET*_Ex12_rv2: TGTACCCTGCTCTGCCTTTC for *RUFY2-RET* and *KIAA1468*_fw2: TGTCTCCTGCATTCCATCAA for *KIAA1468-RET*, PCR program 96° 20 s, 50° 20 s, 60° 4 min, 30 cycles). Beckman Coulter CEQ8000 analyzer and software was used for analysis.

## Prediction of protein expression

The common transcripts of the genes involved in *RET* rearrangements were retrieved from Ensembl platform (http://ensemblgenomes.org, [31]). Based on the results, full sequences of fusion genes were retraced. Translation to amino acid sequence was performed for original and fusion gene sequences using ExPASy database (https://www.expasy.org/, [32]. For amino acid sequences, a "UniProt" (https://www.uniprot.org, [33]) database research was performed to identify the protein domains present in the original protein sequences, and newly identified fusion proteins RUFY2-RET and KIAA1468-RET.

## Results

The tumors investigated were papillary thyroid carcinomas and derived from a 40-year old female (index patient I) and a 28 year-old male (index patient II). In the tumor tissue of index patient I, which belonged to the follicular subtype of PTC, *RUFY2-RET* was detected. The tissue of index patient II, the classical type of PTC, harbored *KIAA1468-RET*.

Using the Archer Variant and Fusion Plex Gene Panel (> 60 genes including e.g. *RET, NTRK1-3, ALK* and *RAS*), there were no other concomitant genetic variants to be detected in the present tumor samples.

## Index patient I: *RUFY2-RET*

*RUFY2-RET* was detected with 144 Reads (%Reads: 73). Breakpoint of *RET* on RNA level was at the initial sequence of exon 12 (chr10:43612032). In RUFY2, it was in exon 8 (chr10:70143808). Fig. 2 depicts a schematic of the resulting hybrid oncogene with the breakpoint on RNA level.

Fusion gene-specific amplification following RT-PCR confirmed the existence of the newly described RET/PTC gene

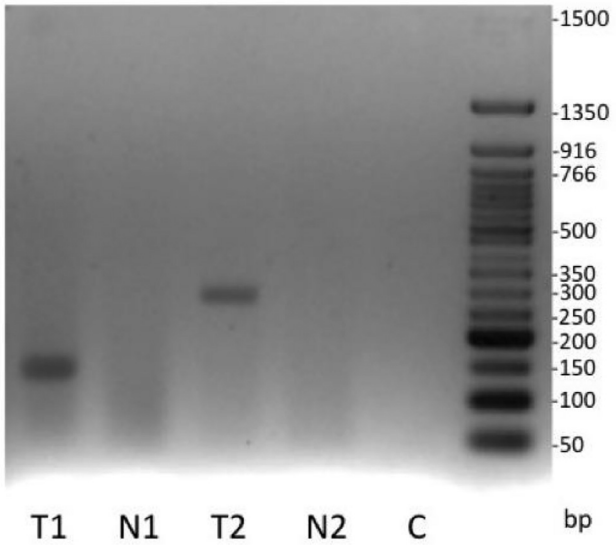


**Fig. 3** Confirmation of *RUFY2-RET* and *KIAA1468-RET* by gel electrophoresis. Electrophoretic separation of PCR products, agarose gel: T1 = tumor of index patient I displaying *RUFY2-RET* fusion (145 bp). N1 = normal tissue (thyroid) without *RUFY2-RET* fusion of index patient I. T2 = tumor of index patient II displaying *KIAA1468-RET* fusion (288 bp). N2 = normal tissue (thymus) without *KIAA1468-RET* fusion of index patient II. C = control, $H_20$ (primer set: *RET*_rv2 and *KIAA1468*_fw2).

fusion *RUFY2-RET* in the tumor tissue, whereas in normal thyroid tissue, it was not present (Fig. 3). Sanger sequencing of the PCR product of tumor tissue confirmed the breakpoint between *RUFY2* and *RET*, as described by "Archer Analysis" software (Fig. 2, Fig. 4).

According to the "UniProt" database research, RUFY2 (RUFY2-213 ENST00000602465.5, AF461266.1, Q8WXA3, 606 amino acids) contains a RUN domain at positions 37–169. Moreover, it harbors a coiled-coil region from positions 210–534 [33]. Whereas the RUN domain is expressed completely in RUFY2-RET, the coiled-coil region is partly represented in the fusion protein (positions 210–274). A zinc finger domain, located at positions 540–589 in RUFY2, is not expressed in RUFY2-RET [33], since the breakpoint to the *RET* proportion follows position 274 of RUFY2.

RUFY2-RET comprises 676 amino acids (Fig. 5). In RUFY2-RET, the aforementioned domains of RUFY2 (RUN domain, coiled-coil domain) are connected to the tyrosine kinase region deriving from *RET* (positions 724–1016 in *RET*, RET-202 ENST00000355710.7, P07949, 1114 amino acids). The transmembrane region of *RET* (positions 636–657) is not expressed in RUFY2-RET.

Positions in RUFY2-RET were exemplarily calculated using the following gene products: RUFY2-213 ENST00000602465.5, AF461266.1, Q8WXA3, 606 amino acids and RET-202 ENST00000355710.7, P07949, 1114 amino acids.

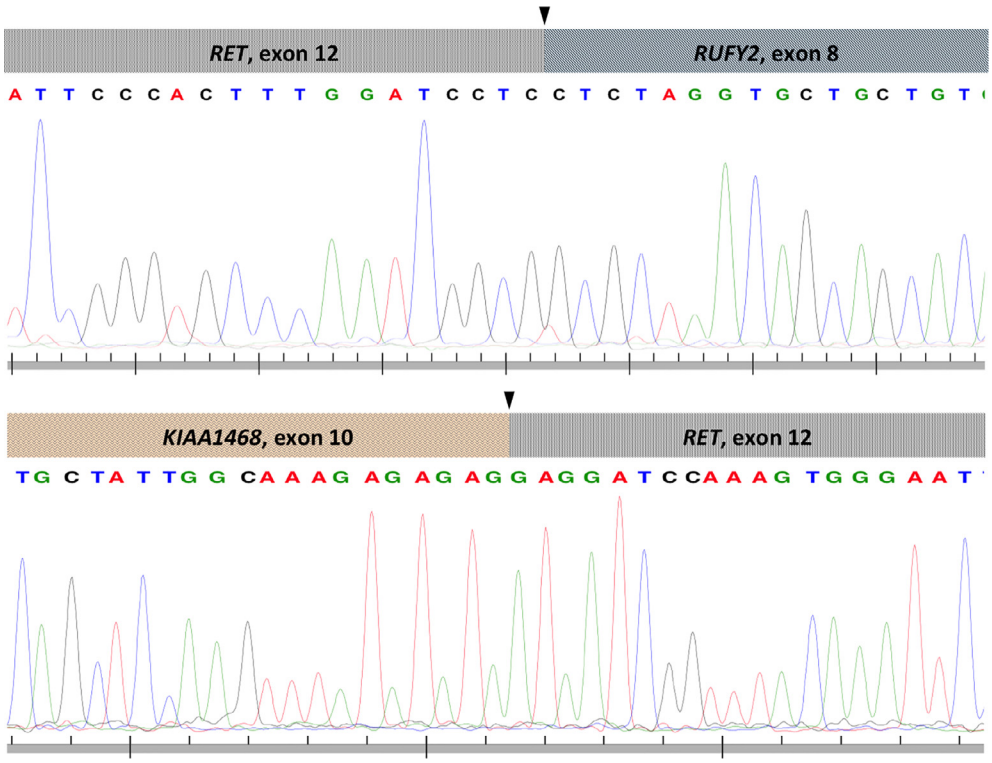


**Fig. 4** Confirmation of *RUFY2-RET* and *KIAA1468-RET* by Sanger sequencing. Sanger sequencing confirmed the in-frame fusion between RUFY-Exon 8 and RET-Exon 12 as well as between KIAA1468-Exon 10 and RET-Exon 12. In both cases the RET sequence is located at the 3'-end of the transcript. Note that Sanger sequencing was only performed in 3' to 5'direction for KIAA1468-RET fusion product, while the reverse complementary strand is shown for RUFY2-RET fusion transcript. Arrows indicate breakpoint.

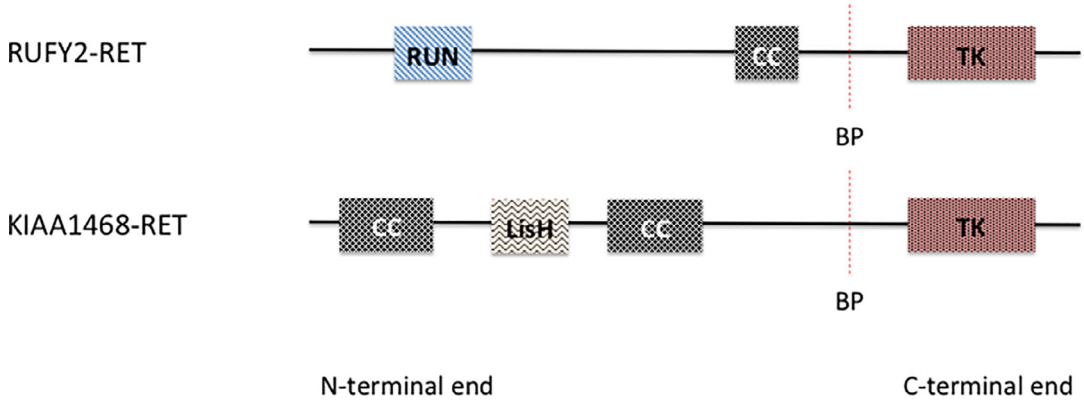


**Fig. 5** Predicted functional domains of RUFY2-RET and KIAA1468-RET proteins. RUFY2-RET (676 amino acids) contains a RUN domain at positions 37–169, and partially a coiled-coil region from positions 210–274, which is connected to the tyrosine kinase region deriving from RET, positions 286–578. KIAA1468-RET (942 amino acids) contains a LisH domain (positions 255–287) and two coiled-coil regions (positions 197–231 and 359–397), which are connected to the tyrosine kinase region at positions 286–578. Positions according to "Uniprot" database analysis. CC = coiled-coil domain, LisH = LisH domain, RUN = RUN domain, TK = tyrosine kinase domain, BP = breakpoint.

## Index patient II: *KIAA1468-RET*

*KIAA1468-RET* was registered with 104 Reads (%Reads: 80). Breakpoint of *RET* on RNA level was at the initial sequence in exon 12 (chr10:43612032). Breakpoint within *KIAA1468* was in exon 10 (chr18:59899660) (Fig. 1).

Fusion gene-specific amplification following RT-PCR confirmed the presence of the gene fusion *KIAA1468-RET* in the tumor tissue. In normal thymus tissue of the index patient, the fusion was not detectable (Fig. 3). Sanger sequencing of the PCR product of tumor tissue confirmed the breakpoint of the novel RET/PTC fusion on RNA level (Figs. 2 and 4).

In KIAA1468 (KIAA468-202, ENST00000398130.6, Q9P260 1216 amino acids), a LisH domain is located at positions 255–287 [33]. The LisH domain is included in the fusion protein KIAA1468-RET. There are 3 HEAT domains (HEAT1, HEAT2, HEAT3) at from positions 601–639, 640–679 and 1004–1042 in KIAA1468. However, these regions are not expressed in KIAA1468-RET, as the breakpoint to *RET* is located at positions 540. Yet, KIAA1468 contains two coiled-coil regions, encoded at positions 197–231 and 359–397, which are expressed by the fusion protein KIAA1468-RET. The fusion protein KIAA1468-RET (RET-202 ENST00000355710.7, P07949, 1114 amino acids) comprises 942 amino acids and couples the LisH domain and both coiled-coil domains to the *RET* tyrosine kinase domain (Fig. 5). Also KIAA1468-RET does not possess, as already described for RUFY2-RET, a transmembrane region, which is originally present in *RET*.

Positions in KIAA1468-RET were exemplarily calculated using the following gene products: KIAA468-202, ENST00000398130.6, Q9P260 1216 amino acids and RET-202 ENST00000355710.7, P07949, 1114 amino acids.

## Discussion

New next-generation sequencing techniques, based on anchored multiplex PCR, allow for the detection of novel RET/PTC rearrangements. With singularly one fusion partner gene within the panel of analysis, this technique facilitates the detection of genetic rearrangement, and the exact description of the previously unknown fusion partner gene. Sensitivity and specificity of rearrangement analysis with AMP-based next-generation sequencing, compared with reference assays, were shown to reach 100% [34]. Regarding *RET* rearrangements, this technique is more advantageous to the use of fluorescent in situ hybridization or to fusion-specific RT-PCR, as it combines a high sensitivity for detecting rearrangements and a high specificity by denominating the exact gene fusion - qualities, which the aforementioned techniques do not unite.

In the literature, for the two most common rearrangements of the *RET* gene, RET/PTC1 (*CCDC6-RET*) and RET/PRC3 (*NCOA4-RET*), the common breakpoints were described to be located within intron 11 [15,16]. In this RNA based analysis, the breakpoint resulted at the start of exon 12. This implies that on DNA level the common fragile site, intron 11, is involved.

The present findings demonstrate that *RUFY2-RET* and *KIAA1468-RET* are novel RET/PTC rearrangements. The existence of *RUFY2-RET* was already demonstrated in non–small cell lung cancer (NSCLC) using next-generation sequencing [34]. RUFY2 belongs to the RUFY protein family. The *RUFY2* gene encodes for the RUN and FYVE domain containing 2 proteins (also described as RABIP4R or ZFYVE13). It is located on chromosome 10q21.3. Within the RUFY2 protein, the N-terminal RUN domain is connected to a C-terminal FYVE zinc finger by a coiled-coil domain [33,35,36]. The RUN domain was reported to be involved in the regulation of cell polarity and membrane trafficking [37]. An expression of RUFY2 was registered by RNA-seq in normal human tissues primarily in brain, endometrium, ovary and thyroid [38].

The gene fusion KIAA1468-RET, newly discovered in PTC, contains a LisH domain located at positions 255–287 [33]. LisH regions can be involved in protein dimerization [39,40]. KIAA1468 also contains two coiled-coil regions, located at positions 197–231 and 359–397 of KIAA1468

(Fig. 5). KIAA1468-RET was previously observed in invasive mucinous adenocarcinoma of the lung [41].

Physiologically, *RET* activation is regulated by glial cell line-derived neurotrophic factor (GDNF)-family ligands, which, in presence of GDNF-family receptor-$\alpha$ proteins, can bind to the extracellular receptor domain of *RET*, leading to the formation of a dimer complex [18]. From this, an autophosphorylation of selected tyrosine residues at the intracellular kinase region of *RET* results. Depending on the phosphorylation site, different pathways are activated, including RAS-MAPK and PI3K-AKT cascades, which are involved in the regulation of cell survival, proliferation, migration, and differentiation [18,20,21].

Also, coiled-coil domains can facilitate oligo- and dimerization [42,43]. For different RET/PTC fusion proteins, including the most common one *CCDC6-RET* (RET/PTC1), an activation of the tyrosine kinase by protein dimerization via coiled-coil domains was reported in the literature [18,19]. As a coiled-coil domain is partially expressed in the novel gene fusion *RUFY2-RET* (*RUFY2*, positions 210–274), a dimerization might result. For *KIAA1468-RET*, a dimerization mediated by the coiled-coil domains at positions 197–231 and 359–397 and the LisH domain at positions 255–287 of *KIAA1468* is possible. By a resulting constitutive activation of the *RET* tyrosine kinase, a pathological activation of subsequent signaling cascades with oncogenic relevance appears plausible.

In addition to this, coiled-coil domains were reported to act as molecular spacers influencing kinase function [44]. By regulating the spatial relation between enzyme and substrate, for Rho kinases, a reformative way of kinase activation was demonstrated to be achieved by coiled-coil domains [45]. A potential regulation for the *RET* tyrosine kinase is possible in case of the fusion protein RUFY2-RET and KIAA1468-RET. Following this principle, in KIAA1468-RET, different processes than in RUFY2-RET could be propelled. For further investigation, larger cohorts of patient samples harboring the specific rearrangements, or artificially created thyrocyte cell lines, are possible models for elucidation.

Thus, the yet large variety of RET/PTC rearrangements ($>$ 20 different fusion genes) should be analyzed with regard to differential activation of subsequent signaling cascades. Whereas a basic dimerization should consequently lead to a similar kinase activation pattern, and similar courses of disease, a specific condition, e.g., by differential kinase regulation potentially caused by alteration of the spatial relation between enzyme and substrate, might influence distinct pathways. Variable courses of disease would be the consequence. In the present case, the 28-year-old patient harboring KIAA1468-RET initially presented with lymph node metastases (pN1b), and an early recurrence after an extensive operative resection and RAI therapy was registered. The patient harboring RUFY2-RET was already 40 years of age at diagnosis. She also harbored lymph node metastases (pN1b). Following an adequate surgical resection and RAI therapy, no recurrence was registered in this case. In the patient harboring *RUFY2-RET*, the follicular variant of PTC was diagnosed (Fig. 1). For *RET* rearrangements, a potential role favoring the development of lymph node metastases was discussed in the literature [30,46,47]. A large-scale analysis of the clinical parameters as the development of lymph node metastases, recurrence, survival and histological subtype of PTC – in correlation with results from next-generation sequencing – could contribute to the understanding, whether different RET/PTC rearrangements are associated with more or less aggressive features. Moreover, the group of *RET* rearrangement positive tumors should be compared to different PTC subgroups, as defined by e.g., *BRAF* mutation. For the invasive form of follicular variant of PTC, BRAF mutations were described to be present with a relatively high frequency compared to the encapsulated variant, in which RAS mutations prevail [48]. Even though analyses of larger cohorts support that RAS mutations are primarily present in the encapsulated, and therefore less aggressive form of the follicular variant of PTC [48,49], there are yet cases of particularly aggressive disease reported, as an isolated NRAS q61K mutation [50]. In the present sample, neither a BRAF mutation, nor any other genetic variant other than *RUFY2-RET*, was present. To prove clinical significance of the finding of RUFY2-RET in the follicular variant of PTC, an analysis of a larger cohort would be required.

The analysis of normal tissue (thyroid/thymus) of the index patients harboring *RUFY2-RET* and *KIAA1468-RET* did not show the specific rearrangement discovered in the tumor, which underlines the oncogenic relevance of the gene fusions. Moreover, the gene fusions *RUFY2-RET* and *KIAA1468-RET* were previously described in NSCLC [34,41]. Rearrangements involving the *RET* gene are observed in 1–2% of NSCLC. A notably higher prevalence in pre-selected cohorts without *EGFR, ALK* or *KRAS* mutations, the prevalence was reported to reach 16% [51–54]. The existence of *RET* rearrangements in NSCLC was found to be associated with more poorly differentiated tumors, and an earlier onset than in *EGFR*-mutant tumors [55].

In lung cancer harboring *RET* rearrangement, targeting the tyrosine kinase by inhibitors, as cabozantinib, vandetanib and sunitinib, is a possible treatment option, which in multicenter analysis led to complete or partial responses in up to 37% [56].

Currently, for advanced, RAI-refractory differentiated thyroid carcinoma, treatment with tyrosine kinase inhibitors sorafenib and levatinib presents an option [57,58]. In addition to surgery and RAI therapy, also possible treatment approaches for NSCLC using additional tyrosine kinase inhibitors might be beneficial in patients suffering from PTC.

This research gives further evidence that multiple, different *RET* rearrangements are present in different subtypes of papillary thyroid carcinoma (classical papillary thyroid carcinoma, follicular variant of papillary thyroid carcinoma). To prove epidemiological significance of the findings, further research in larger cohorts are required. With the results as a basis, correlation with clinical course and treatment response to radioiodine therapy and tyrosine kinase inhibitors can further elucidate the issue, and give an important structure to the existing therapy algorithms.

## Funding

This work was supported by the University Medical Center of the Johannes Gutenberg University Mainz.

## Declarations of interest

None

## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cancergen.2018.11.002.

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