Analysis of Newly Identified and Rare Synonymous Genetic Variants in the RET Gene in Patients with Medullary Thyroid Carcinoma in Polish Population

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Abstract Gain-of-function germline mutations of the RET proto-oncogene are responsible for initiation of carcinogenesis within the thyroid gland and development of hereditary form of medullary thyroid carcinoma and MEN2 syndrome. Genotype-phenotype correlations are established for most RET mutations, but the importance of the synonymous changes in this gene remains debatable. We aimed to analyze RET gene variants in Polish population. Genetic testing for the RET gene variants was performed with standard methods in 585 people aged 1–85, including 448 patients with medullary thyroid carcinoma and 131 of their first- and second-degree relatives, as well as six patients suspected of MTC/MEN2.

Besides the most frequent synonymous changes, p.Leu769Leu, p.Ser836Ser, and p.Ser904Ser, four rare changes—c.1827C>T (p.Cys609Cys), c.2364C>T (p.Ile788Ile), c.2418C>T (p.Tyr806Tyr), and c.2673G>A (p.Ser891Ser)—were found in the RET gene, in the Polish population. Two of the rare changes, p.Cys609Cys and p.Ile788Ile, had not been previously described. The frequency of molecular synonymous variants in the general population was evaluated by testing 400 anonymous blood samples of neonates. Our findings may contribute to a better understanding of the genetic diversity of the RET gene and the involvement of synonymous variants in this diversity.

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Background

Proto-oncogene RET (rearranged during transfection) encodes a single-pass transmembrane receptor of the tyrosine kinase family. The RET gene lies on the chromosome 10q11.2 [1] and comprises 21 exons. RET protein is composed of three domains: an extracellular ligand-binding domain with four cadherin-like repeats and a cysteine-rich region, a transmembrane domain and a cytoplasmic region with the tyrosine kinase domains [2, 3]. Missense, germline gain-of-function mutations in the RET proto-oncogene are associated with type 2 multiple endocrine neoplasia syndromes (MEN2A or MEN2B) and familial medullary thyroid carcinoma (FMTC) [4–7]. Medullary thyroid carcinoma (MTC) is a common component of these syndromes. MTC occurs as a part of an inherited disorder (approx. 20–25% of cases) and as a sporadic tumor (the remaining 75% of cases) [8]. The disease phenotypes and the age of onset are associated with codon-specific RET mutations and their transforming potential. Considering MTC aggressiveness and the co-existing endocrinopathies such as pheochromocytoma (PHEO), hyperparathyroidism (HPT), cutaneous lichen amyloidosis (CLA), and Hirschsprung’s disease (HD), the American Thyroid Association (ATA) Guidelines currently divide germline RET mutations into three risk categories: ATA–HST (the highest risk—patients with MEN2B and the RET codon M918T mutation), ATA–H (the high risk—patients with RET codon C634 mutations and the RET codon A883F mutation), and ATA–MOD (moderate risk—patients with all other mutations in the RET gene) [9].

Besides the changes of the confirmed pathogenic significance, there are polymorphic changes (frequency ≥1%) [10] in the RET gene. In the European population, the most common polymorphic variants (MAF (minor allele frequency) >5%) [11] are the following: c.2307 T>G (p.Leu769Leu) in exon 13 (allele G frequency: 24%); c.2071 G>A (p.Gly691Ser) in exon 11 (allele A frequency: 19%); c.2712 C>G (p.Ser904Ser) in exon 15 (allele G frequency: 19%); and c.2508 C>T (p.Ser836Ser) in exon 14 (allele T frequency: 6%) [12, 13]. Their role in tumorigenesis is still unclear, and there are conflicting data as to whether these changes can modify the risk of developing MTC [14–16]. In addition to these common polymorphic changes in the RET gene, there are also rare synonymous or nonsynonymous allelic variants (MAF < 0.5%) of uncertain significance. Identification of these rare changes in the context of specific symptoms of the disease is extremely significant for a better understanding of the role they potentially play in the RET receptor function.

The role of synonymous genetic variants is a matter of a particular controversy. Such changes, according to the Anfinsen’s principle postulating that the amino acid sequence of the protein alone determines the structure and functions of a protein, were for a long time referred to as “silent” [17]. Recent studies have revealed that the synonymous changes may affect the protein function and cause many diseases.

Several mechanisms have been proposed to explain the pathogenic role of synonymous changes in cancer. Synonymous changes can influence post-transcriptional RNA processing [18–24] and post-transcriptional miRNA-dependent regulation, by altering miRNA binding sites [25–31]. At present, a few miRNAs regulating protein RET expression are known [32, 33], all of which bind the 3’UTR region of RET. Synonymous changes may affect the global mRNA stability [34–37], or the local stability in the start codon region [38–40], or the maintenance of cell homeostasis [41]. Synonymous changes can also influence the speed and accuracy of translation. One way of kinetic control of translation is codon usage. The synonymous mutation can slow down or accelerate the rate of protein synthesis and lead to protein misfolding. SNPs can generate translation pause sites (ribosome stalling) resulting in alternative conformers during co-translational folding [38, 42].

Some synonymous changes are directly related to the pathogenesis of a disease, e.g., in Treacher-Collins’ syndrome, the synonymous variant c.3612A>C in the TCOF1 gene causes exon 22 skipping and mis-splicing and results in defective mRNA [43], and in cystic fibrosis, a structural instability of mRNA, caused by the synonymous polymorphism p.Ile507Ile in the context of ΔF508 CFTR, could be responsible for the reduced translational rate and lower cellular expression level of CFTR protein [44]. Several synonymous mutations have been shown to be associated with carcinogenesis and influence cancer risk by various mechanisms. For example, the specific silent mutations (p.Pro36Pro) in TP53 gene lower the affinity of the TP53 mRNA for the regulatory protein MDM2, and thereby reduce the ability of TP53 to activate apoptosis [45]. The synonymous variant p.Pro72Pro has been associated with an elevated risk of lung cancer [46], and the synonymous changes, rs1061302 and rs709816 in the NBS1 gene, are linked with smoking-related cancers (lung, larynx, liver, and bladder) [47].

The aim of this study was to examine a few rare synonymous allelic variants of the RET gene in MTC patients in Polish population. Some of the variants have not been previously studied in MTC patients.

Patients

Genetic testing for RET mutations was performed in 585 people, aged 1–85 years, including 448 patients with MTC and
131 of their first- and second-degree relatives, and six patients suspected of MTC/MEN2 with other diseases (PTC, PHEO, renal carcinoma, adrenal gland tumor, nodular thyroid disease, and carcinoid of the stomach) (Table 1).

Most patients and their kindred were taken care of at the Outpatient Clinic of Thyroid Diseases and Genetic Counseling Unit Cancer Prevention Center and the Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology in Warsaw between 1998 and 2016. Twenty-nine adolescent patients aged 1–18 had been admitted to other hospitals: the Department of Pediatric Surgery at the Collegium Medicine of Nicolaus Copernicus University in Bydgoszcz, the Department of Oncology at The Children’s Memorial Health Institute in Warsaw, and the Department of Pediatrics and Endocrinology of the Warsaw Medical University. The peripheral blood of adolescent patients was collected for genetic testing in the Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology in Warsaw. All patients were subjected to the standard diagnostic procedures, as published by Paszko et al. [48]. Patients with cytologically or histopathologically confirmed MTC were enrolled for the detailed genetic testing. Six exons (10, 11, 13, 14, 15, and 16) of the RET gene were analyzed in all patients. In 26 patients, including those at risk of inherited MTC (genetic load in the family), those with aggressive MTC disease, or with the specific MEN syndrome symptoms, as well as those with the early age of onset, additional exons (5, 8, 9, 12, 18, and 19) were sequenced, to check for other mutations in the RET gene. The analyzed exons were selected based on the ATA Guidelines Task Force on Medullary Thyroid Carcinoma [49] and ARUP Scientific Resource for Research and Education Mutation Databases [50].

The frequency of molecular synonymous variants in exons 10, 11, 13, 14, and 15 in the general population was evaluated by testing 400 anonymous blood samples of neonates.

### Methods

DNA was extracted from the peripheral blood lymphocytes using a commercial kit Genomic Midi AX (Biotechnology). Germline RET gene mutations were screened in exons 10, 11, 13, 14, 15, and 16 and additionally in exons 5, 8, 9, 12, 18, and 19 (see Supplementary Material for primer sequences). All the tested fragments of the RET gene were amplified using PCR technique. Following purification on Centri-Sep Spin Columns, (Applied Biosystems), PCR products were subjected to electrophoresis in a Perkin Elmer ABI Prism Sequencer using fluorescently labeled terminators BIG DYE v.3.1 (Applied Biosystems). Germline mutations were identified by comparing the sequences of the tested samples with the relevant correct RET sequences: NM 020630.4. Genotype-phenotype correlations and the identified changes-related risk of aggressive MTC were verified by analyzing public databases [32, 49–51].

### Results

Direct sequencing analysis of the RET proto-oncogene in 585 people revealed germline pathogenic mutations in eight exons (10, 11, 13, 14, 15, 16, 18, and 19) in 79 patients (aged 1–75 years) (17.4% of patient group) and their 30 unaffected kindred (aged 1–80 years) (22.9% of asymptomatic group). Three hundred seventy patients (aged 7–85 years) (81.5% of patient group) were diagnosed with sporadic MTC (sMTC) and five patients suspected of MTC/MEN2 with other diseases (1.1% of patient group) (Table 1). No carrier of the pathogenic mutation was found in this group. As revealed by genetic studies of patients and their relatives, individuals of all series presented several synonymous genetic changes in the RET gene. Apart from the most frequent polymorphic variants (p.Leu769Leu, p.Ser836Ser, and p.Ser904Ser) [15], four rare synonymous changes were found, and two new changes were identified, c.1827 C>T (p.Cys609Cys) and c.2364 C>T (p.Ile788Ile) (Table 2).

### Patients Report

#### Genetic Variant in Exon 10: c.1827 C>T (p.Cys609Cys)

A 10-year-old male patient with no family history of MEN syndrome, familial MTC, or sporadic MTC was diagnosed with tumor (16 × 22 × 30 mm) located in the right lobe of the thyroid. The level of serum calcitonin was markedly elevated to 991 pg/mL. The results of abdominal cavity ultrasound and chest x-ray imaging were negative. The patient underwent total thyroidectomy, cervical lymph node dissection, and partial removal of the thymus. The tumor was classified as a monofocal medullary thyroid carcinoma (pT2N1aM0). The postoperative calcitonin level was 5.57 pg/mL. There were no other symptoms of MEN2 syndrome. Two years after the surgery, control studies showed an increase in the level of calcitonin (36.7 pg/mL) and an enlargement of the cervical lymph node. The lymph node was...
removed, and further examinations (USG and PET-CT results, serum calcitonin level of 24 pg/mL, and CEA level of 2 ng/mL) showed no recurrence.

Genetic testing revealed a synonymous variant: c.1827 C>T (p.Cys609Cys) in exon 10 (Fig. 1) (Table 2). No germline mutations in the remaining exons (5, 8, 11, 12, 13, 14, 15, 16, 18, and 19) were found. In exon 13 of the RET gene, only one heterozygous polymorphic change, p.Leu769Leu, was found.

Genetic analysis of the RET gene in patient’s relatives (parents and younger brother) revealed the same synonymous change in exon 10, in the father and the brother (Fig. 2). Due to the family history of cancer, four additional genes, BRCA1, BRCA2, CHEK2, and NBS1, were tested in the father of the patient, and no mutations were found. Neither the father nor the younger brother experienced any symptoms of cancer, as assessed by laboratory tests for serum CEA, calcitonin level, neck, and abdominal ultrasound scanning.

**Genetic Variant in Exon 13: c.2364 C>T (p.Ile788Ile)**

A female carrier of synonymous heterozygous change in exon 13: c.2364 C>T (p.Ile788Ile) (Fig. 3) was diagnosed with sporadic MTC at the age of 46; a tumor of 27 × 36 × 39 mm was located in the left lobe of the thyroid. Serum calcitonin level was elevated to 885 pg/mL. The patient, diagnosed with metastases to the regional cervical lymph nodes, underwent total thyroidectomy, with removal of the central and left lateral lymph node. Apart from the three heterozygous polymorphisms—p.Gly691Ser, p.Leu769Leu, and p.Ser904Ser—no other mutations in the 12 studied exons of the RET gene were found (Table 2).

**Genetic Variant in Exon 14: c.2418 C>T (p.Tyr806Tyr)**

Because of the nodular thyroid and a 13-mm tumor of the left adrenal gland, and a suspicion of MEN2 syndrome, genetic analysis of the RET gene was also performed in 50-year-old female patient with carcinoid of the stomach. There were no pathogenic mutations in the 12 examined exons of the RET gene. Only two heterozygous polymorphic changes in exons 13 and 15, p.Leu769Leu and p.Ser904Ser, and one rare synonymous heterozygous change in exon 14, c.2418 C>T (p.Tyr806Tyr), rs553418132, were found (Table 2).

**Genetic Variant in Exon 15: c.2673G>A (p.Ser891Ser)**

In two MTC patients, a rare synonymous change, c.2673G>A (p.Ser891Ser), rs201612214, was identified (Table 2). One patient, a 37-year-old woman with bilateral tumors of the thyroid gland (10 × 6 × 23 mm in the right lobe and 24.5 × 19.5 × 33.5 in the left lobe), carried an additional pathogenic mutation, p.Cys618Ser in exon 10, and two polymorphic changes, p.Gly691Ser and p.Ser904Ser. It was not
possible to find out whether these genetic variants were cis- or trans-changes. The other, a 53-year-old man with a tumor located in the left lobe of the thyroid gland (7 mm in diameter, pT1a), carried polymorphic variants p.Gly691Ser/p.Ser904Ser of the RET gene.

None of the four rare synonymous changes in exons 10, 13, 14, and 15 were found in the general population group.

Discussion

To assess the significance of rare genetic changes, apart from clinical data, it is necessary to collect information on the carriers of the identified gene changes, their penetration in the family, and their frequency in the general population. We present here several rare genetic variants of the RET gene. We also identified two synonymous variants, p.Cys609Cys and p.Ile788Ile, that have not been identified and described before.

In 10–15% of MEN2A and FMTC cases, codons 609, 611, 618, and 620 are affected [52]. Our previous studies have shown that in patients with MTC in Polish population, pathogenic changes occur most frequently in exon 10 of the RET gene (38.8% of all mutation), while for example, the frequency of mutations in codon 634 was only 26.8% [48]. More than 60% of mutations in cysteine codons of exon 10 occur in FMTC and 10–15% in MEN2A [7,53,54]. All these mutations are associated with a moderate risk of aggressive MTC (ATA-MOD). The most frequent mutations in codon 609 are changes of cysteine into R, G, Y, S, F, and W [50]. However, as different amino acid substitutions of cysteine result in a comparable transforming activity, and it is suggested that the activity depends on the position of the cysteine mutations rather than on the substituting amino acid [14,55]. So far, no synonymous change in codon 609 has been described. Generally, synonymous changes in exon 10 are rare. The germline synonymous genetic variants have been reported in nine codons (588, 591, 594, 601, 608, 619, 620, 621, and 622) of exon 10, so far. The frequencies of these changes are very low [12,13].

The question whether the discovered substitution of cysteine to cysteine in codon 609 may be involved in the pathogenesis of MTC remains open. The 34-year-old father of the patient with MTC was an unaffected carrier of the same variant (Fig. 3). However, due to a very young age of onset and the lack of other known pathogenic mutations in all the examined exons of the RET gene, the role of this change in the pathogenesis of MTC cannot be ignored.

The other new genetic variant that we found was a synonymous substitution of isoleucine to isoleucine in codon 788 of the RET gene. No genetic variants of this codon have been known so far. The patient had no other known pathogenic mutations in the examined exons of the RET gene. Mutations in exon 13 are thought to lead to a rather mild course of disease. These changes have been assigned to MOD group [9]. According to available databases [12, 13],

![Fig. 1 A synonymous variant c.1827 C>T (p.Cys609Cys) in exon 10 of the RET gene](image1)

![Fig. 2 Pedigree of the patient’s family. The probant is indicated by the black asterisk](image2)

![Fig. 3 A synonymous variant c.2364 C>T (p.Ile788Ile) in exon 13 of the RET gene](image3)
the synonymous changes have been identified in nine codons of exon 13 (763, 766, 768, 769, 774, 777, 786, 790, and 792). Previously, we have suggested a possible association between synonymous variant p.Leu769Leu polymorphism and a risk of sMTC [15].

Another rare mutation identified in this study refers to exon 14. In this exon, synonymous changes occur in 22 codons [12, 13]. A synonymous genetic variant c.2418 C>T (p.Tyr806Tyr) was found in a 50-year-old woman with a stomach carcinoid tumor. This variant has been identified in populations of South Asia, Africa, and Europe, with the total T allele frequency of 0.0001384. In an analysis of the 1000 Genomes Project database, the same variant has been revealed as a somatic change c.2418C>T (p.Tyr806Tyr), COSM1347814 in large intestine tumor. This variant has been identified in populations of South Asia, Africa, and Europe, with the total T allele frequency of 0.002 [12, 13]. In our study, in in patients with gastrointestinal carcinoid tumors and their relatives.

In codon 806 of the RET gene, so far, only one germline change has been described, c.2417A>G (p.Tyr806Cys), rs377767419, co-occurring with the p.Val804Met mutation, in a patient with MEN2B [58].

In exon 15, synonymous changes occur only in seven codons. The clinical significance of the synonymous genetic variant c.2673 G>A (p.Ser891Ser), rs201620214 that we discovered in our population is uncertain. This change has been identified in patients with MEN2. The allele frequency for A in the world population is 0.002 [12, 13, 50]. In our study, in one case, the change appeared as a change accompanying the pathogenic mutation in exon 10, but in the other case, except for polymorphic variants, no other pathogenic changes within the examined exons were found. Apart from synonymous changes in this codon, also a pathogenic change, c.2671T>G (p.Ser891Ala), rs75234356, has been described in patients with FMTC, MEN2A, and MTC [14, 59].

Table 3 Codon usage comparison

| SNP | Codon change | Triplet frequency (H. sapiens) a |
|-----|--------------|---------------------------------|
| Cys690Cys | UGC → UGU | 12.6 → 10.6 |
| Ile788Ile | AUC → AUU | 20.8 → 16.0 |
| Tyr806Tyr | UAC → UAU | 15.3 → 12.2 |
| Ser891Ser | UCG → UCA | 4.4 → 12.2 |

a Values of codon usage (frequency per thousand) in Homo sapiens were taken from the Codon Usage Database [78, 79].

Studies on MTC are ongoing to examine carcinogenic mechanisms other than the pathogenic mutations in the RET gene. So far, a few mechanisms contributing to MTC development have been described. These are the following:

- mutations in the other genes, e.g., in genes encoding the human RET co-receptors GFRA1, GFRA2, GFRA3, GFRA4 [60, 61]; RET ligands ARTN, GDNF, NRTN PSNP [62]; or genes encoding the RET downstream effectors, STAT1, AURKA, BCL2, CDKN2B, CDK6, COMT, and HRAS [63];
- epigenetic modifications, such as CpG DNA methylation or modifications of histones, which may even be inheritable [64, 65];
- changes in the expression level of the various miRNAs that may be the cause and/or a result of the carcinogenesis [66–70];
- changes in degradative pathway of the RET protein [71–73].

The relationship between synonymous changes in the RET gene and the increased risk of MTC is still a subject of controversy. Currently, it is widely accepted that despite the lack of a direct influence of the synonymous variants of the amino acid structure of protein, such changes may influence phenotype and may lead to many diseases. Links between synonymous mutations in different genes and different diseases have recently been proven, and the list is still expanding [25, 74]. Five to 10% of human genes are estimated to contain at least one harmful region because of the synonymous mutations [75]. The current release of the database of deleterious synonymous mutation (dbDSM) collects 1936 synonymous mutation disease (SM disease) association entries, including 1289 SMs and 443 human diseases [76]. By employing cancer-related mutation database, Li et al. indicated that, similarly to nonsense and missense pathogenic mutations, synonymous mutations may also change the dynamical parameters of the corresponding proteins in the TNF-α signaling network and cause a significant increase of the critical dose of TNF-α necessary for cell death [77].

It is impossible to assess the mechanism of action and the potential impact of synonymous variants on the protein function, without the precise testing. Based on in silico studies, it can only be concluded that the four described changes may influence protein synthesis rate, by accelerating it (p.Ser891Ser variant) or slightly slowing it down (the other variants) (Table 3).

Conclusions

Rare synonymous changes in the RET gene, c.1827C>T (p.Cys609Cys), c.2364C>T (p.Ile788Ile), and c.2673G>A (p.Ser891Ser), were identified in MTC patients and c.2418C>T (p.Tyr806Tyr) in a patient suspected of MEN2.
Two of the variants, p.Cys609Cys and p.Ile788Ile, had never been previously described. These findings contribute to a better recognition of the whole range of genetic changes of the RET gene and of the involvement of synonymous variants in genetic diversity of this gene.

APUD, amine precursor uptake and decarboxylation; ARTN, artemin; AURKA, aurora kinase A; ATA, American Thyroid Association; ATA-H, RET gene mutations of the high risk; ATA-HST, RET gene mutations of the highest risk; ATA-MOD, RET gene mutations of the moderate risk; BCL2, B cell lymphoma 2; BRCA1/2, breast cancer 1, 2; CAE, carcinomaemobryonic antigen; CDK6, cyclin-dependent kinase 6; CDFK2B, cyclin-dependent kinase inhibitor 2B; CFTR, cystic fibrosis transmembrane conductance regulator; CHEK2, checkpoint kinase 2; CLA, cutaneous lichen amyloidosis; COMT, catechol-O-methyltransferase; FMTC, familial medullary thyroid carcinoma; GDNF, glial cell line-derived neurotrophic factor; GFRA1-4, GDNF family receptor alpha 1–4; HD, Hirschsprung’s disease; HPTH, hyperparathyroidism; HRAS, Harvey rat sarcoma viral oncogene homolog; MEN2A, MEN2B, multiple endocrine neoplasia type 2A, 2B; MTC, medullary thyroid carcinoma; NBS1, Nijmegen breakage syndrome 1; NHLBI, National Heart and Lung Blood Institute; NRTN, neurturin; PHEO, pheochromocytoma; PHEO, pheochromocytoma; SNP, single nucleotide polymorphism; STAT1, signal transducer and activator of transcription 1; TCOF1, treacle ribonoma; sMTC, sporadic medullary thyroid carcinoma; TEL, leukemia/lymphoma; TMEM204, transmembrane protein 204; TOS, Transogine; Wt1, Wilms tumor 1; XPC, Xeroderma pigmentosum complementation group C; XPA, Xeroderma pigmentosum complementation group A; ZEB1, zinc finger E-box-binding homeobox 1.

References

1. Ishizaka Y, Itoh F, Tahira I, Ikeda I, Sugimura T, Tucker J, Fertitta A, Carrano AV, Nagao M (1989) Human ret proto-oncogene mapped to chromosome 10q11.2. Oncogene 4(12):1519–1521.
2. Takahashi M, Buma Y, Iwamoto T, Inaguma Y, Ikeda H, Hiai H (1988) Cloning and expression of the ret proto-oncogene encoding a tyrosine kinase with two potential transmembrane domains. Oncogene 3(5):571–578.
3. Anders J, Kjaer S, Ibanez CF (2001) Molecular modeling of the extracellular domain of the RET receptor tyrosine kinase reveals multiple cadherin-like domains and a calcium-binding site. J Biol Chem. 276(38):35808–35817. DOI:10.1074/jbc.M104968200
4. Donis-Keller H, Dou S, Chi D, Carlson KM, Toshiba K, Lairmore TC, Howe JR, Moley JF, Goodfellow P, Wells SA Jr (1993) Mutations in the RET proto-oncogene are associated with MEN 2A and FMTC. Hum Mol Genet. 2(7):851–856.
5. Mulligan LM, Kwok JB, Healey CS, Elsdon MJ, Eng C, Gardner E, Love DR, Mole SE, Moore JK, Papi L, Ponder MA, Hakan Telenius H, Tunanclifte A, Ponder BA (1993) Germ-line mutations of the RET proto-oncogene in multiple endocrine neoplasia type 2A. Nature 363(6428):458–460. DOI:10.1038/363458a0
6. Mulligan LM, Eng C, Healey CS, Clayton D, Kwok JB, Gardner E, Ponder MA, Frilling A, Jackson CE, Lehrnt H, Neumann HP, Thibodaux SN, Ponder BA (1994) Specific mutations of the RET proto-oncogene are related to disease phenotype in MEN 2A and FMTC. Nat Genet 6(1):70–74. DOI:10.1038/ng0194-70
7. Eng C, Clayton D, Schuffenecker I, Lenoir G, Cote G, Bagel RE, van Amstel HK, Lips CJ, Nishishio I, Takai SI, Marsh DJ, Robinson BG, Frank-Raue K, Raue F, Xue F, Noll WW, Romei C, Pacini F, Fink M, Niederle B, Zedinen J, Nordenskjold M, Kommippth N, Hendy GN, Mulligan LM, et al. (1996) The relationship between specific RET proto-oncogene mutations and disease phenotype in multiple endocrine neoplasia type 2: International RET mutation consortium analysis. JAMA 20;276(19):1575–9.
8. Schimke RN (1984) Genetic aspects of multiple endocrine neoplasia. Annu Rev Med. 35:25–31. doi:10.1146/annurev.me.35.020184.000325
9. Wells SA Jr, Asa SL, Dralle H, Elisei R, Evans DB, Bagel RE, Lee N, Machens A, Moley JF, Pacini F, Raue F, Frank-Raue K, Robinson B, Rosenthal MS, Santoro M, Schlumberger M, Shah M, Waguespack SG (2015) American Thyroid Association guidelines task force on medullary thyroid carcinoma. Revised American Thyroid Association guidelines for the management of medullary thyroid carcinoma. Thyroid 25(6):567–610. DOI:10.1089/thy.2014.0335
10. Brookes AJ. (1999) The essence of SNPs. Gene 234(2):177–86.
11. Europe PMC Funders Group (2010) Author manuscript; available in PMC 2011 April 01. A map of human genome variation from population-scale sequencing. The 1000 Genomes Project Consortium. Nature 28; 467(7319): 1061–1073. DOI:10.1038/nature09534
12. 1000 Genomes A deep catalog of human genetic variation (Updated 2016) http://browser.1000genomes.org/Homo_sapiens/Gene/Variation_Gene/Table?db=core;g=ENSG00000165731;r=10:4357245–43625799#synonymous_variant_tablePanel
13. The Exome Aggregation Consortium (ExAC) (Updated 2016) http://exac.broadinstitute.org/gene/ENSG00000165731
14. Figlioli G, Landi S, Romei C, Elisei R, Gemignani F (2013) Medullary thyroid carcinoma (MTC) and RET proto-oncogene: mutation spectrum in the familial cases and a meta-analysis of studies on the sporadic form. Mutat Res. 752(1):36–44. DOI:10.1016/j.mrrev.2012.09.002
15. Sromek M, Czetwertyńska M, Skasko E, Zielinska J, Czapczak D, Steffen J (2010) The frequency of selected polymorphic variants of the RET gene in patients with medullary thyroid carcinoma and in...
the general population of central Poland. Endocr Pathol. 21(3):178–185. DOI: 10.1186/s12021-010-9125-8

16. Colombo C, Minna E, Rizzetti MG, Romeo P, Lesic D, Persani L, Mondellini P, Pierotti MA, Greco A, Fugazzola L, Borrello MG (2015) The modifier role of RET-G691S polymorphism in hereditary medullary thyroid carcinoma: functional characterization and expression/penetration studies. Orphanet J Rare Dis. 1;10:25. DOI: 10.1186/s13023-015-0231-z

17. Anfinsen CB (1973) Principles that govern folding of protein chain. Science 20;181(4096):223–230.

18. Cartegni L, Chew SL, Krainer AR (2002) Listening to silence and understanding nonsense: exonic mutations that affect splicing. Nature Rev Genet. 3(4):285–298. DOI: 10.1038/ng775

19. Iida K, Akashi H (2000) A test of translational selection at ‘silent’ sites in the human genome: base composition comparisons in alternatively spliced genes. Gene 30;261(1):93–105. DOI: 10.1016/S0378-1119(00)00482-0

20. Orban TI, Olah E (2001) Purifying selection on silent sites—a constraint from splicing regulation? Trends Genet. 17(5):252–253. doi:10.1016/S0168-9525(01)02281-8

21. Carlini DB, Genut JE (2006) Synonymous SNP provide evidence for selective constraint on human exonic splicing enhancers. J Mol Evol. 62(1):89–98. DOI:10.1007/s00239-005-0055-x

22. Chamary J-V, Parmley JL, Hurst LD (2006) Hearing silence: non-neutral evolution at synonymous sites in mammals. Nat Rev Genet. 7(2):98–108. DOI: 10.1038/nrg1770

23. Pagani F, Raponi M, Baralite FE (2005) Synonymous mutations in CFTR exon 12 affect splicing and are not neutral in evolution. Proc Natl Acad Sci USA 3;102(18):6368–6372. DOI:10.1073/pnas.0502288102

24. Parmley JL, Chamary JV, Hurst LD (2006) Evidence for purifying selection against synonymous mutations in mammalian exonic splicing enhancers. Mol Biol Evol. 23(2):301–309. DOI: 10.1093/molbev/msj035

25. Wang Y, Qiu C, Cui Q (2015) A large-scale analysis of the relationship of synonymous SNPs changing microRNA regulation with functionality and disease. Int J Mol Sci. 30;16(10):23545–55. DOI: 10.3390/ijms161023545

26. Brest P, Lapauquette P, Souidi M, Lebrigand K, Cesaro A, Vouret-Craviari V, Mari B, Barby P, Mosnier JF, Hébuterne X, Harel-Bellan A, Mograbi B, Darfeuille-Michaud A, Hofman P (2011) A constitutive exon. Am J Med Genet. 149A(8):1624–1627. DOI: 10.1002/ajmg.a.32834

27. Grey F, Tirabassi R, Meyers H, Wu G, McWeeney S, Hook L, Bellan A, Mograbi B, Darfeuille-Michaud A, Hofman P (2011) A synonymous single nucleotide polymorphism in deltaF508 CFTR alters the secondary structure of the mRNA and the expression of the mutant protein. J Biol Chem. 10;285(37):28741–28749. DOI: 10.1074/jbc.M110.110428

28. Helwak A, Kudlga G, Dudnakova T, Tollervre D (2013) Mapping the human miRNA interaction by CLASH reveals frequent noncanonical microRNA targeting. Mol Cell 44;54(1):1–10. DOI: 10.1016/j.molcel.2013.03.002

29. Huvtagner G, Zamore PD (2002) A microRNA in a multipletumovern RNAi enzyme complex. Science 20;297(5589):2054–60. DOI: 10.1126/science.1073827

30. Loeb GB, Khan AA, Canner D, Hiatt JB, Shendure J, Darnell RB, Leslie CS, Rudensky AY (2012) Transcriptome-wide miR-155 binding map reveals widespread noncanonical microRNA targeting. Mol Cell 14;48(5):760–76. DOI:10.1016/j.molcel.2012.10.002

31. Lynam-Lennon N, Maher SG, Reynolds JV (2009) The role of micro RNA in cancer and apoptosis. Biol Rev. 84(1):55–71. DOI: 10.1111/j.1469-185X.2008.00061.x

32. RET Gene Cards Human Gene Database. http://www.genecards. org/cgi-bin/carddisp.pl?gene=ret&snp=348

33. mirTarBase (Release 6.0 Sept 2015) http://miRtarbase.mbc.nctu.edu.tw
50. ARUP Scientific Resource for Research and Education, The University of Utah (Updated 2016) http://www.arup.utah.edu/database/MEN2/MEN2_display.php

51. The Human Gene Mutation Database. http://www.hgmd.cf.ac.uk/ac/gene.php?gene=RET

52. de Groot JW, Links TP, Pfukker JT, Lips CJ, Hofstra RM. (2006) RET as a diagnostic and therapeutic target in sporadic and hereditary endocrine tumors. Endocrine Reviews 27(5): 535–560. DOI: 10.1210/er.2006-0017

53. Iwashita T, Kato M, Murakami H, Asai N, Ishiguro Y, Ito S, Iwata Y, Kawai K, Asai M, Kurokawa K, Kajita H, Takahashi M (1999) Biological and biochemical properties of Ret with kinase domain mutations identified in multiple endocrine neoplasia type 2B and familial medullary thyroid carcinoma. Oncogene 18:2693-2702. DOI:10.1086/312742

54. Ponder BA, Smith D (1996) The MEN II syndromes and the role of the ret proto-oncogene. Adv Cancer Res. 70:179–222.

55. Carlomagno F, Salvatore G, Cirafici AM, De Vita G, Melillo RM, de Franciscis V, Billaud M, Fusco A, Santoro M (1997) The differential RET-activating capability of mutations of cysteine 620 or cysteine 634 correlates with the multiple endocrine neoplasia type 2B disease phenotype. Cancer Res. 57(3):391–5.

56. Pearse AG (1969) The cytochemistry and ultrastructure of polypeptide hormone-producing cells of the APUD series and the embryologic, physiologic and pathologic implications of the concept. J Histochem Cytochem. 17(5):303–313.

57. Boyd CA (2001) Amine uptake and peptide hormone secretion: APUD cells in a new landscape. J Physiol. 15:531(3): 581. DOI: 10.1111/j.1469-7793.2001.05811.x

58. Miyauchi A, Futami H, Hai N, Yokozawa T, Kuma K, Aoki N, Kosugi S, Sugano K, Yamaguchi K (1999) Two germline missense mutations at codons 804 and 806 of the RET proto-oncogene in the same allele in a patient with multiple endocrine neoplasia type 2B without codon 918 mutation. Jpn J Clin Cancer Res. 90(1):1–5.

59. Hofstra RM, Fattoruso O, Quadro L, Wu Y, Libroia A, Verga U, Hofstra RM. (2006) CYR61 deletion contributes to the pathogenesis of multiple endocrine neoplasia type 2A. J Pathol. 206:198–206. DOI:10.1002/path.2006

60. Gimm O, Dziema H, Brown J, Hoang-Vu C, Xi L, Raffeld M, Moley J, Chermock RD (2013) Overexpression of miR-10a and miR-375 and downregulation of YAP1 in medullary thyroid carcinoma. Exp Mol Pathol. 95(1):62–67. DOI:10.1016/j.yexmp.2013.05.001

61. Mian C, Pennelli G, Fassan M, Balistreri M, Barollo S, Cavedon E, Galuppi F, Pizzi M, Vianello F, Pelizzo MR, Girelli ME, Rugge M, Opocher G (2012) MicroRNA profiles in familial and sporadic medullary thyroid carcinoma: preliminary relationships with RET status and outcome. Thyroid 22(9):890–896. DOI:10.1089/thy.2012.0045

62. Nikiforova MN, Tseng GC, Steward D, Dikiorov YE (2008) MicroRNA expression profiling of thyroid tumors: biological significance and diagnostic utility. J Clin Endocrinol Metabol. 93(5):1600–1608. DOI:10.1210/jc.2007-2696

63. Citri A, Alroy I, Lavi S, Rubin C, Xu W, Grammatikakis N, Pattarson C, Neckers L, Fry DW, Yarden Y (2002) Drug-induced ubiquitylation and degradation of ErbB receptor tyrosine kinases: implications for cancer therapy. EMBO J 21(10):2407–17. DOI: 10.1093/emboj/c20.10.2407

64. Carniti C, Perego C, Mondellini P, Pierotti MA, Bongarzone I (2003) PP1 inhibitor induces degradation of RET MEN2A and RET MEN2B oncoproteins through proteosomal targeting. Cancer Res. 63(9):2234–43.

65. Scott RP, Eketjall S, Aineskog H, Ibanez CF (2005) Distinct turnover of alternatively spliced isoforms of the RET kinase receptor mediated by differential recruitment of the Cbl ubiquitin ligase. J Biol Chem. 280(14):13442–9. DOI:10.1074/jbc.M500507200

66. Sauna Z, Kimchi-Sarfaty C (2011) Understanding the contribution of synonymous mutations to human disease. Nature Reviews Genetics 31;12(10):683–91. DOI:10.1038/nrg3051

67. Charnley JV, Hurst LD (2009) The price of silent mutations. Sci Am. 300(6):46–53.

68. Chen W, Xiao P, Xia J (2016) dbDSM: a manually curated database of deleterious synonymous mutations. Bioinformatics 32:12(12): 1914–6. DOI:10.1093/bioinformatics/btw086

69. Li X, Chen Y, Qi H, Liu L, Shuai J (2016) Synonymous mutations at codons 804 and 806 of the RET proto-oncogene in a family with medullary thyroid carcinoma: preliminary relationships with RET status and outcome. Thyroid 22(9):890–896. DOI:10.1089/thy.2012.0045

70. Nikiforova MN, Tseng GC, Steward D, Dikiorov YE (2008) MicroRNA expression profiling of thyroid tumors: biological significance and diagnostic utility. J Clin Endocrinol Metabol. 93(5):1600–1608. DOI:10.1210/jc.2007-2696

71. Citri A, Alroy I, Lavi S, Rubin C, Xu W, Grammatikakis N, Pattarson C, Neckers L, Fry DW, Yarden Y (2002) Drug-induced ubiquitylation and degradation of ErbB receptor tyrosine kinases: implications for cancer therapy. EMBO J 21(10):2407–17. DOI: 10.1093/emboj/c20.10.2407

72. Carniti C, Perego C, Mondellini P, Pierotti MA, Bongarzone I (2003) PP1 inhibitor induces degradation of RET MEN2A and RET MEN2B oncoproteins through proteosomal targeting. Cancer Res. 63(9):2234–43.

73. Scott RP, Eketjall S, Aineskog H, Ibanez CF (2005) Distinct turnover of alternatively spliced isoforms of the RET kinase receptor mediated by differential recruitment of the Cbl ubiquitin ligase. J Biol Chem. 280(14):13442–9. DOI:10.1074/jbc.M500507200

74. Sauna Z, Kimchi-Sarfaty C (2011) Understanding the contribution of synonymous mutations to human disease. Nature Reviews Genetics 31;12(10):683–91. DOI:10.1038/nrg3051

75. Charnley JV, Hurst LD (2009) The price of silent mutations. Sci Am. 300(6):46–53.

76. Chen W, Xiao P, Xia J (2016) dbDSM: a manually curated database of deleterious synonymous mutations. Bioinformatics 32:12(12): 1914–6. DOI:10.1093/bioinformatics/btw086

77. Li X, Chen Y, Qi H, Liu L, Shuai J (2016) Synonymous mutations at codons 804 and 806 of the RET proto-oncogene in a family with medullary thyroid carcinoma: preliminary relationships with RET status and outcome. Thyroid 22(9):890–896. DOI:10.1089/thy.2012.0045

78. Codon usage database. 2007. http://www.kazusa.or.jp/codon/

79. Nakamura Y, Gojobori T, Ikemura T. Codon usage tabulated from international DNA sequence databases: status for the year 2000. Nucleic Acids Research. (updated in 2007); 26 (1): 334–334.