7. Abstract

Medullary thyroid carcinoma (MTC) is a rare neuroendocrine neoplasm originating from parafollicular thyroid cells (C cells). In most patients with a family history of thyroid cancer but also in some apparently sporadic cases, activating mutations in the *RET* gene are detected. They are most frequently located in exons 10 – 11 and 13 – 16. However, mutations in other exons of the gene, including tandem mutations, which can be associated with an aggressive progression of the disease, have also been described.

The clinical course of the hereditary MTC indicates its phenotypic heterogeneity. Moreover, the course of the disease varies considerably between members of the same family with an identical mutation in the *RET* gene. They are diagnosed with MTC at different ages, and have different intensity and localisation of associated symptoms. This suggests an involvement of additional factors, both environmental and genetic, that modulate the disease. However, there have been contradictory reports on the association between single nucleotide polymorphisms (SNPs) within the *RET* gene and an increased risk of MTC.

In this dissertation, the prevalence and distribution of germinal mutations in the *RET* gene as well as their correlation with the course of the disease have been determined in a group of 142 MTC patients from the Wielkopolska region in Poland. To this end, screening tests based on high-resolution melting analysis (HRMA) of DNA fragments as well as pyrosequencing protocols to identify the most frequent *RET* mutations have been designed and optimised. The study has identified mutations in 51 subjects. A higher frequency of mutation of codon 634 in exon 11 (70.6%) were noticed, while common in other reports mutations in exon 10 were rare (2%). In 21% of patients mutations in exon 13 were present. DNA analysis in relatives have allowed identification of mutant *RET* allele carriers who were then included in a specialised healthcare programme and qualified for a prophylactic thyroidectomy. Furthermore, in 125 family members the presence of the mutant allele of the *RET* gene were excluded.

Seven polymorphic variants of the *RET* gene have also been analysed in study subjects and their potential effect on the risk of medullary thyroid carcinoma and the disease course has been determined. Comparing patients with sporadic and hereditary medullary thyroid cancer and a population, statistically significant differences in a distribution of genotypes in four *loci*: IVS1 + 9277C> T, p.A45A, p.A432A and p.L769L were observed.

The results presented in this dissertation show that the IVS1+9277C>T variant, associated with a decreased activity of the MCS+9.7 enhancer located in intron 1 of the

RET gene, may influence the MTC clinical picture – considering the incidence of primary hyperparathyroidism in patients with familial MTC the protective effect of allele c.73+9277T was noticed (OR=0.09, C.I.=[0,01-0,71], p=0.005).

Identification and interpretation of mutations in the *RET* gene is indispensable to implement an appropriate healthcare protocol in MTC patients and, in the future, it may be the basis for developing and implementing a targeted therapy. While further improvement of DNA analysis techniques remains necessary, the results presented in this dissertation greatly contribute to achieving this important goal.