THYROID TUMORS: HISTOLOGICAL CLASSIFICATION AND GENETIC FACTORS INVOLVED IN THE DEVELOPMENT OF THYROID CANCER

LISKA J.[1, 2], ALTANEROVAV.[3], GALBAVY Š. [4, 5], STVRTINA S.[6], BRTKO J. [2]

[1]Institute of Histology and Embryology, Medical Faculty of Comenius University, 811 08 Bratislava, Slovak Republic:[2]Institute of Experimental Endocrinology, Slovak Academy of Sciences, 833 06 Bratislava, Slovak Republic; [3]Cancer Research Institute, Slovak Academy of Sciences, 833 91 Bratislava, Slovak Republic; [4]Institute of Laboratory Medicine, Medical Faculty of Comenius University, 811 08 Bratislava, Slovak Republic; [5]St. Elisabeth Oncology Institute, 812 50 Bratislava, Slovak Republic; [6]Institute of Pathological Anatomy, Medical Faculty of Comenius University, 811 08 Bratislava, Slovak Republic
E-mail: julius.brtko@savba.sk

Classification of thyroid tumours and their variants is described with special respect to some recent findings on somatic mutations characteristics which are associated with individual types of malignity. Special attention is paid to the interrelations between thyroid nodules and malignity and predictive risk factors are listed.

For details see <www.elis.sk>.

Human thyroid neoplasia represents a variety of lesions starting from well-differentiated benign tumors to anaplastic malignant cancers. In addition tumors can be divided into two groups, non-medullary and medullary thyroid cancer of hereditary or nonhereditary origin. Non-medullary thyroid cancers include papillary cancer, follicular carcinoma, the Hurtle variant of follicular cancer, insular cancer, and anaplastic cancer (MALCHOFF and MALCHOFF 2002). papillary cancer being the most frequent one. Despite that the majority of papillary cancers is sporadic, epidemiological data have revealed that a 4 to 10 fold increase of such cancer exists in the first generation relatives of papillary cancer patients (PAL et al. 2001). This finding indicates the possibility of familial predisposition to papillary cancer which might occur is approximately 5 %. The genetic events responsible for the inheritance which occur in autosomal dominant fashion with partial penetrance have not been identified yet.

Somatic mutation associated with papillary cancer is the activating mutation of *RAS* gene and genes encoding tyrosine kinase receptors RET and TRK. The high prevalence of *BRAF* gene mutations in such cancer was revealed recently (KIMURA et al. 2003). It was no overlap between *RET, RAS* or *BRAF* mutations observed in 66% of papillary cancers analyzed. These mutations were shown to be not present in differentiated follicular neoplasm having the same cell origin like papillary one. The mechanism of this unexpected result is not known yet.

Thyroid cancer appears in less than 5 to 10 % of hypofunctioning thyroid nodules (DEAN and HAY 2000). The prevalence of these nodules being estimated between 5 to more than 20 % in human population. The highest prevalence is found in regions of endemic goitre. Single and multiple nodules arise from thyroid hyperplasia and they may also develop in normal thyroid gland. It has been shown that from 15 to 40 % of thyroid nodules are partly or entirely cystic and the majority of these lesions are benign (SALABE 2001). The most of so-called cystic nodules follows necrosis issues as an imbalance between growth and the precisely regulated process of angiogenesis. SALABE (2001) classified thyroid nodules according their anatomo-clinical image into five types with distinct histological features: Hyperplastic nodule, neoplastic nodule, colloid nodule (or nodular goiter, CHAN 2001), cystic nodule, and thyroiditic nodule (nodular Hashimoto's thyroiditis). Histologically, the neoplastic thyroid nodules correspond to well, moderately, and poorly differentiated carcinomas and they may arise in the tissue

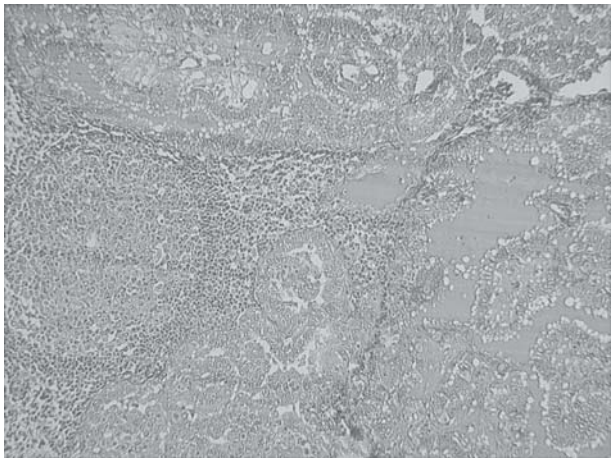

Fig 1 Papillary carcinoma in Hashimoto's thyroiditis: Non-tumoral thyroid gland (left) with distinguishing microscopic features of Hashimoto´s thyroiditis lymphocytic infiltration with germinal center formation) and papillary thyroid carcinoma (right)

of different types of thyroid nodules (Fig. 1). Thyroid cancers are fairly uncommon and include disease types that range from indolent localized papillary cancers to the fulminate and lethal anaplastic disease. The relative infrequency of death from this disease has led to a viewpoint that thyroid cancer is an innocuous tumor (GAGEL et al. 1996). Table 1 summarizes simplified classification of primary thyroid tumours as modified from the World Health Organization (WHO) classification (CHAN 2001).

Thyroid adenoma is the only benign epithelial tumour of thyroid gland. It is encapsulated and differentiates into follicular cells. Microscopically distinguished forms are as follows – normofollicular, macrofollicular (colloid), microfollicular (fetal), trabecular and solid (embryonic). Follicular adenoma occurs mostly in adults aged from 20 to 50 years, and its appearance is more common in females. The characteristic feature that distinguishes a carcinoma from an adenoma is the presence of vascular or capsular invasion in the carcinoma. The adenomas usually lack uptake on iodine scans – "cold" nodules, but rarely may be "hot"(with autonomous production of L-thyroxine) and can cause hyperthyroidism (so-called "toxic adenoma") (CHAN 2001). On radioisotope scanning they generally appear as hot nodules because they concentrate radioiodide,

Table 1
Simplified classification of primary thyroid tumors, modified from the World Health Organization (WHO) classification (CHAN 2001)

Tumours of thyroid follicular or metaplastic epithelium
1. Follicular adenoma (including Hürthle cell adenoma)
2. Follicular carcinoma (including Hürthle cell carcinoma) ➢ minimally invasive ➢ widely invasive
3. Papillary carcinoma
4. Poorly differentiated thyroid carcinoma including insular carcinoma
5. Anaplastic (undifferentiated) and squamous cell carcinoma including so-called carcinosarcoma
6. Columnar cell carcinoma
7. Mucoepidermoid carcinoma
8. Sclerosing mucoepidermoid carcinoma with eosinofilia
9. Mucinous carcinoma
Tumours showing C-cell differentiation
Medullary thyroid carcinoma
Tumours showing both follicular and C-cell differentiation
1. Collision tumor: follicular/papillary and medullary carcinomas
2. Mixed follicular-parafollicular carcinoma (differentiated thyroid carcinoma, intermediate type)
Tumours showing thymic or related branchial pouch
Tumours of lymphoid cells
Intrathyroid parathyroid tumours
Mesenchymal and other tumours

whereas the surrounding tissues concentrate a little amount of the isotope (CORVILAIN 2003). It has been shown that "thyroid hormone receptor-thyroid hormone response element" complex formation and the activity of type I iodothyronine 5'-activity may be altered in thyroid adenoma (BRTKO et al. 2002).

Thyroid cancer accounts (in the U.S.A.) roughly 1 % of all new malignant diseases (FIGGE 1999). The female sex, according to CHAN (2001) is usually associated with slightly better prognosis. Thyroid cancer usually presents as a solitary thyroid nodule. Papillary, medullary, and anaplastic camcers can be diagnosed by cytological examination of a fine-needle aspirate of the nodule. To distinguish between follicular cancer and benign follicular adenoma, histological examination needs to be performed (SHERMAN 2003). The presence of nuclear atypia in a thyroid tumour is not necessarily synonymous with malignancy. Presence of atypical hyperchromatic nuclei in an endocrine organ is more often a reflection of hyperstimulation rather than presence of malignant potential (CHAN 2001). Immunohistochemical analysis does not usually differentiate benign from malignant lesions, although staining for galectin-3 has often been reported in follicular carcinomas but it was found to be rare in adenomas (SHERMAN 2003). Histological examination remains necessary for the evaluation of tumour invasiveness, as well.

Radioisotope scans usually show that malignant lesions are hypofunctioning or cold, however, these findings are in general non-specific. CT and MRI have no role in the routine diagnostic assessment of the most of thyroid nodules (SHERMAN 2003). Recently, KROLL (2002) recommended focusing more on the study of thyroid cancer pathogenesis and the degree to which thyroid tumour morphology correlates with underlying molecular genetic events. The better differentiated tumours generally occur in younger patients, while less differentiated tumours occur in older patients. Age for low-grade, intermediate-grade and high-grade thyroid tumours is ranging from 40 to 60 years, moreover, for the same tumor type, young patients below age of 40 years, generally have better prognosis than older patients. The TNM (T-extent of primary tumor, N-condition of regional lymph nodes, M-presence of distant metastasis) staging system appears to be the most useful for predicting of death from thyroid cancer. Thyroid cancer in children is more aggressive when compared to adults, with more frequent extrathyroidal extension and higher incidence of lymph node or distant metastasis, however, the prognosis is highly favorable (GILLILAND et al. 1997).

Table 2
Classification of thyroid carcinoma (GAGEL et al. 1996)

Thyroid carcinoma derived from the follicular cell
1. Papillary thyroid carcinoma
2. Mixed papillary-follicular thyroid carcinoma
3. Hurtle cell carcinoma
4. Follicular thyroid carcinoma
5. Anaplastic thyroid carcinoma
Thyroid carcinoma derived from the parafollicular or C cells
1. Sporadic medullary thyroid carcinoma
2. Hereditary medullary thyroid carcinoma ➢ Multiple endocrine neoplasia type 2A ➢ Multiple endocrine neoplasia type 2B ➢ Familial medullary thyroid carcinoma

Primary cancers are usually classified as differentiated ones (e.g. papillary and follicular carcinomas), medullary thyroid carcinomas, and undifferentiated or anaplastic cancers (Table 2). Less frequent classifications include Hurtle cell cancers, squamous cell cancers, lymphomas and other hematopoietic lesions, and a variety of unusual cancers and soft tissue sarcomas (GAGEL et al. 1996). Differentiated cancers are diagnosed in women twice as often as in men. These can be inherited as a component of familial adenomatous polyposis, Gardner's syndrome, and Cowden's disease. Potential loci of susceptibility have been identified on chromosomes 1q21, 2q21, and 19p13.2 (MCKAY et al. 2001). CHAN (2001) stated that some studies made no distinction between papillary and follicular cancers and simply lump them under the category of "differentiated thyroid cancers", but there is good evidence that papillary cancer and follicular cancer are clinically, biologically and histopathologically distinct entities.

Papillary thyroid cancer, the most common histological type of thyroid cancer, accounts for approximately 60 % (GAGEL et al. 1996), 70 – 80 % (CHAN 2001) of all thyroid cancers. This type of thyroid cancer appears also in young people. Papillary cancer is defined, according to the WHO classification, as a "malignant epithelial tumour" showing evidence of follicular cell differentiation, typically with papillary and follicular structures as well as characteristic nuclear changes (ground glass, large size, pale, irregular outline with deep grooves and pseudoinclusions)" (Table 3). Tumours designed in the past as "mixed papillary-follicular cancer" should be reclassified as papillary carcinoma (CHAN 2001). This cancer may be also multifocal without dominant mass. Multifocal disease

Table 3
Variants of papillary carcinoma: behavior compared with classical papillary carcinoma (CHAN 2001)

Type	Variants
More aggressive	Diffuse sclerosing variant Diffuse follicular variant Tall cell variant Trabecular variant Dedifferentiated variant
Better prognosis	Encapsulated variant Papillary microcarcinoma
No difference	Follicular variant Solid variant Oxyphil cell variant Variant with exuberant nodular Fascitis-like stroma Warthin tumor-like variant Cribriform-morular variant Variant with lipomatous stroma

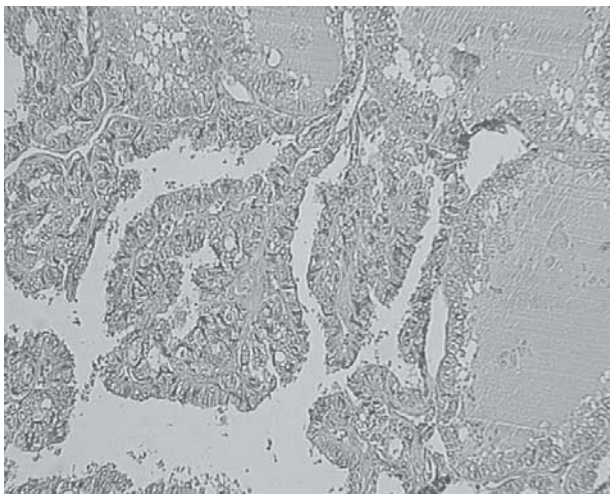

Fig 2 Papillary thyroid carcinoma. Papillary formation with nuclear characteristic of papillary carcinomas. Ground glass appearance and overlaping, several pseudoinclusions and nuclear grooves

is common (~65 %), although, this is traditionally attributed to intraglandular metastasis. Molecular analysis suggests that the individual tumours are usually independent neoplasms (GOULD and SOMMERS 1988). Some familial cases with autosomal dominant inheritance have been also observed, so-called familial non-medullary thyroid cancers. It has been suggested that this form of non-medullary cancer is more aggressive than the sporadic form (CHAN 2001).

Papillary thyroid cancer is recognized by i) epithelium piled up on the surfaces of papillae, ii) empty, ground glass ("Orphan Annie eye") nuclei (Fig. 2), and iii) laminated masses of calcareous material (psammoma bodies) in half of the cases (GOULD and SOMMERS 1988). Calcified colloid materials, which are commonly found in Hürthle cell neoplasms and hyalinizing trabecular adenoma, can be distinguished from psammoma bodies (found in approximately half of the papillary carcinomas) by their exclusive location in the follicular lumina. The keys to diagnosis of papillary cancers are the nuclear characteristics, while invasion, either vascular or capsular, is not prerequisite. However, a feature number and also further characteristics in papillary cancer – nuclear groove formed by deep folding of the nuclear membrane are not entirely patognomic (CHAN 2001) because some benign lesions in thyroid gland can exhibit similar changes in nuclei. Multinucleated histiocytes present in the luminal space of some follicles and papillae are also of diagnostic importance because they are extremely rare in benign lesions and other tumor types. Immunohistochemical markers are not totally specific in the diagnosis of papillary cancer versus other thyroid tumours. Staining may be patchy and weak even in classical papillary cancer (CHAN 2001). Papillary cancers are usually infilrative (tumour cells usually spreads into the adjacent thyroid tissue), but some cells may be circumscribed or even encapsulated. More than 30 % cells may invade the regional lymph nodes, and approximately 10 % of patients develop hematogenous metastasis (GAGEL et al. 1996). It has been suggested that the extent of infiltration is proportional to the rate of lymph

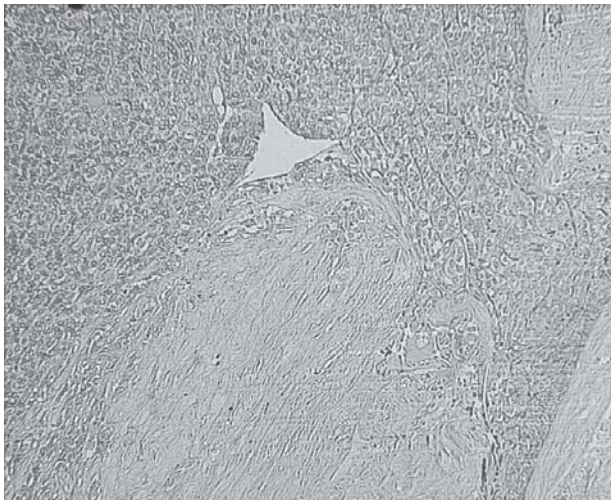

Fig 3 Follicular thyroid invasive carcinoma. Predominant solid and partly trabecular growth pattern transversed by delicate fibrovascular septa. Significant nuclear atypia. The tumour tissue expands in a mushrooms-like fashion on the outside of the nodule

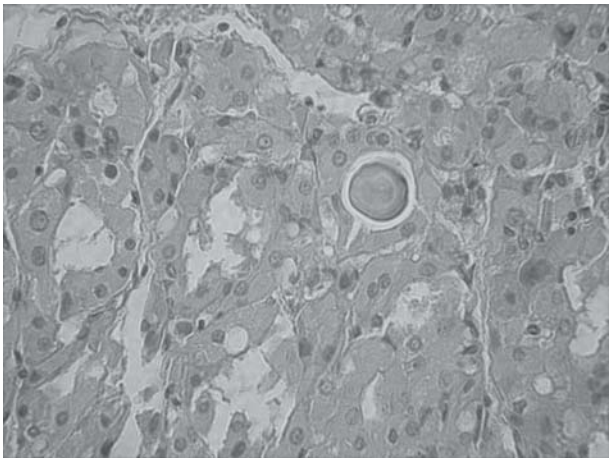

Fig 4 Hürthle cell thyroid carcinoma. Follicular pattern of growth. Oncocytic cells with pleomorphous cells and one psammoma body in center are seen

node metastasis (CHAN 2001). Papillary cancers exhibit a spectrum of histological patterns (classic, solid, and tall cell) that have different biological features. Follicles are frequently present. Very often, there is an intricate blending of the follicles with papillae, resulting in a complex tubulopapillary pattern. The dense hyaline fibrosis of tumour stroma may be useful feature for distinguishing papillary cancer from follicular one. Many variants of papillary thyroid carcinoma have been recognized, but only some are of prognostic significance.

Follicular thyroid cancer is epithelial thyroid tumor showing follicular cell differentiation, but lacking the diagnostic features of papillary cancer (synonyms include adenoma with invasion, malignant adenoma, adenoma with capsular invasion, angioinvasive adenoma). These synonyms indicate typical feature of follicular carcinoma that is the presence of vascular (and capsular) invasion (Fig. 3) in contrast to follicular adenoma without invasion. Follicular cancers account for about 20 % of all thyroid cancers. It forms a hard thyroid mass that, on section, appears as a granular, pale gray-white, stellate invasive tissue, resembling many other visceral carcinomas. This cancers arise only rarely from benign adenomas as a result of transforming events. However. in the regions of iodine insufficiency, follicular cancer is more common than papillary one (SHERMAN 2003). Thyroid tumours with mixed morphological features of papillary and follicular cancer may be also observed.

The thyroid tumour is classified as **Hürthle cell cancer in such a case**, when more than 75 % of follicular cancer cells exhibit Hürthle cell or oncocytic features (DEAN and HAY 2000). The prognosis of Hürthle cell cancer (Fig. 4) as a malignancy derived from the follicular cells is similar to that of follicular cancer, but somewhat different from that of papillary cancer (GAGEL et al. 1996).

Various studies have not reported consistent influence of the histology pattern on the prognosis in patients with follicular cancer. Actuallz, male patients show far worse prognosis than female ones. The survival is excellent in patients below the age range of30 to 40 years, metastases and age being important prognostic factors. (CHAN 2001). Hematogenous metastases are more common for this type of cancer than for papillary one. Metastases to the lungs, bones, and other sites may occur several years after removal of the thyroid lesion. CAILLOU (1998) stated that the differentiation of diagnosis between encapsulated well differentiated follicular thyroid carcinoma and atypical adenoma is not easy. In spite of that all these tumours show a very good prognosis, it is recommended to fetch these tumors under the same terminology, and actual morphological differences would be then expressed by a histological grading. Histological subtypes of differentiated thyroid cancers that might signal poor prognosis include tall-cell and columnar-cell variants of papillary type cancer, oxyphilic (Hürthle) cells, and poorly differentiated variants of the follicular type (BURMAN et al. 1996). COLLINI et al. (2002) stated that minimally

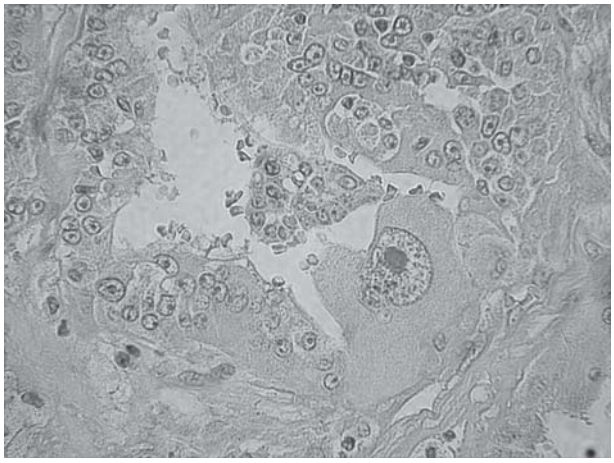

Fig 5 Anaplastic thyroid carcinoma. Anaplastic carcinoma giant cell type. Several giant tumour cells with huge hyperchromatic nuclei

invasive (encapsulated) follicular camcer, widely invasive follicular cancer, and insular cancer are three distinct types of thyroid cancers consisting of follicular cells (insular cancer represents poorly differentiated counterpart). Widely invasive follicular cancer represents a high risk, with an overall survival similar to that of insular one. Widely invasive follicular cancers occur in older age, with a larger size, a widespread growth into the thyroid parenchyma, and presenting with distant metastases (lung, bone, brain, liver). Minimally invasive follicular cancer is the low-risk counterpart of widely invasive follicular thyroid carcinoma, but not of insular cancer which represents a distinct high-risk lesion, showing both follicular- and papillary-related biological behavior characteristics. In insular cancer the criteria of high risk depend upon the degree of differentiation, the low- and high-risk forms of usual follicular cancers being based on their degree of invasiveness irrespective of differentiation grade. THOMPSON et al. (2001) considered the criteria for minimally invasive (low grade) follicular cancer controversial, often resulting in unnecessary treatment.

Insular cancer generally signed as **poorly differentiated thyroid cancer** represents a group of thyroid tumours showing histological and biological features between well differentiated and undifferentiated cancers (CHAN 2001). The name "insular cancer" is connected with insular growth pattern that is often present in this group. Solid nest ("insulae") often contains small follicles. Necrosis, hemorrhage and vascular invasion are common. Only some reported cases of poorly differentiated papillary cancer are, according CHAN (2001), the examples of insular cancer. On the other hand, not all the cases reported as "poorly differentiated cancer" represent currently generally accepted characteristic of this group. The full morphological spectrum remains to be defined, but insular cancer is considered the best characterized group. Approximate frequency of poorly differentiated cancer is ranging from 0.4 to 10 % (CHAN 2001).

Patients with insular cancer are usually middle or old aged and females are generally are more commonly affected than males. The cause of death is usually from metastases rather than from uncontrollable recurrences. Advanced age, large tumor size, extrathyroid extension and lymph node metastasis are correlated with poor prognosis (CHAN 2001).

Anaplastic thyroid cacer (undifferentiated cancer) represents 1 % (SHERMAN 2003) or even 3 % to 5 % (CHAN 2001) of all thyroid cancers and generally derives from the dedifferentiation of differentiated type (SHERMAN 2003). In the areas where goiter is endemic, it may account for10 times higher incidence (CHAN 2001). The fast growing, hard neck mass is composed of dense, granular grayish tissue. These aggressive cancers typically occur in older women. Rapid growth of thyroid cancer mass and regional or distant metastases are typical for this type of cancer which often develops in a preexistent goiter, with or without associated of better differentiated carcinomas. Disease-specific mortality is almost 100 %. Despite vigorous treatment, the most patients die within one year, and the median survival is between three and four months. Histological features are variable. Microscopically, the best known pattern is a bizarre cellular proliferation of spindle and giant cells (Fig. 5), with polyploid nuclei, many mitoses, tumor necrosis, and stromal fibrosis. The diagnostic is spindle and giant cell thyroid carcinoma. Less common types are small cell anaplastic cancer which vaguelly resembles a breast carcinoma, and small cell diffuse carcinoma (GOULD and SOMMERS 1988). Some tumors are predominantly or exclusively sarcomatoid, resembling fibrosarcoma, so-called malignant fibrous histiocytoma, hemangioperycytoma, angiosarcoma (angiomatoid variant) or rhabdomyosarcoma (CHAN 2001). Anaplastic cancer is usually connected with widespread local invasion and a high frequency of distant metastases in lungs, pleura, bone, and brain (SHERMAN 1999). Rapid growth of thyroid cancer mass is a typical feature of this malignant disease. Several classification and staging schemes have been introduced to facilitate identification of important prognostic variables that can guide the clinician (GAGEL et al. 1996). The previously used term "small cell type" of anaplastic cancer has

practically disappeared in the recent literature. Cases so diagnosed are reclassifiable as i) malignant lymphoma (the most of cases); ii) medullary cancer with scanty or no amyloid; iii) metatastatic small cell cancer; iiii) true small cell thyroid cancer which is extremely rare and often exhibits neuroendocrine differentiation. Morphological variants of anaplastic thyroid carcinoma are angiomatoid, osteoclastic, paucicellular variants, carcinosarcoma, lymphoepitelioma-like, adenosquamous and squamous cell cancers. Although the term "cancer" is being used, a convincing proof of epithelial differentiation either at the immunohistochemical or ultrastructural level is not accessible in all cases. However, it is not crucial for practical purposes because tumours conforming to the morphologic features of anaplastic cancer behave in an aggressive fashion (CHAN 2001).

Medullary thyroid carcinoma differs from all other thyroid cancers and derives from the neuroendocrine parafollicular C-cells of the thyroid. It forms bulky, soft, gray tumors, sometimes bilaterally and grows slowly. It is more commonly located in the middle third of the lateral lobe, the region where C-cell density is the highest (WOLFE and DELLELLIS, 1981). Spread and metastases of medullary thyroid carcinoma resemble the pattern of follicular thyroid carcinoma. It readily metastasizes via lymphatic channels to regional lymph nodes, but may invade blood vessels and metastasize to the liver, lings, bones and upper mediastinum (CHAN 2001). The TNM staging system is quite accurate for predicting cause-specific survival in this carcinoma (DEAN and HAY 2000). Medullary thyroid carcinoma differs from normal thyroid tissue, it is composed of pale, cuboidal, and often spindle shaped cells (Fig. 6). Because of the stromal change, the diagnostic term employed is often "medullary cancer with amyloid stroma". Cellular discohesion and interstitial edema are very common (CHAN 2001). Familial medullary cancer is a part of genetic condition associated with pheochromocytoma and parathyroid hyperplasia or adenoma. This combination is designated as multiple endocrine neoplasia type 2A. Type 2B is a condition, which has multiple mucosal neuromas, especially of the lips and tongue, in which parathyroid hyperplasia or adenoma is apparently atypical or absent. Sporadic disease accounts for 80 % of all cases, the remainder of patients have inherited tumour syndromes such as multiple endocrine neoplasia type 2A (medullary thyroid cancer, multigland parathyroid tumours, and unilateral and bilateral phaeochromocytoma), 2B (medullary thyroid cancer, phaeochromocytoma, mucosal neuromas, and marfanoid habitus), or familial medullary cancer (BRANDI et al. 2001). Patients with multiple

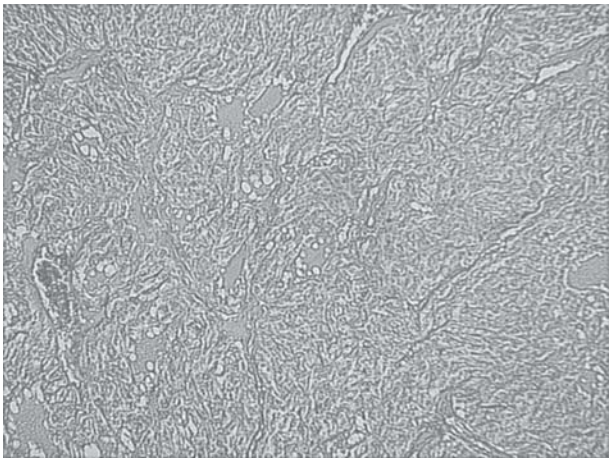

Fig 6 Medullary thyroid carcinoma. Solid pattern of the growth and deposition of amyloid. The tumour cells forms spindle cell fascicles mimicking a mesenchymal tumour

endocrine neoplasia of the type 2B are more likely to have locally aggressive disease than those with either the type 2A or familial medullary thyroid carcinoma (O'RIORDAIN et al. 1994). Patients with inherited disease seem to have a better outlook for survival. Women may be slightly more likely to have the disease than men (Sherman 2003), but the female sex has been associated with better prognosis (SHRODER et al 1988). Experiences with survival rates indicate that this tumor is indolent and not rapidly lethal. Many histological variants of medullary cancer (glandular/follicular, oxyphilic, giant cell, clear cell, spindle cell, pigmented, squamous, papillary, small cell, neuroblastoma-like, hyalinizing trabecular adenoma-like, carcinoid-like, paraganglioma-like, medullary microcarcinoma) have been recognized, but the most of them are of no prognostic importance (CHAN 2001). Metastatic cervical adenopathy is noted in about 50 % of patients with medullary-type cancer at initial presentation. The ability of the tumor to oversecrete measurable quantities of calcitonin, occasionally along with other hormonally active peptides such as adrenocorticotropic hormone or calcitonin-gene related peptide, leads to unexplained diarrhoea, symptoms of Cushing's syndrome, or facial flushing in patients with advanced disease (SHERMAN 2003). Hypocalcemia due to excessive calcitoninemia is extremely rare. Familial non-medullary thyroid cancer is more aggressive than sporadic non-medullary thyroid cancer. It tends to affect younger patients, and the tumours are often multi-focal and bilateral. Histologically, 90 % of these tumours are papillary thyroid cancers and the remaining tumours are Hürthle cell cancers (ALSANEA, 2000).

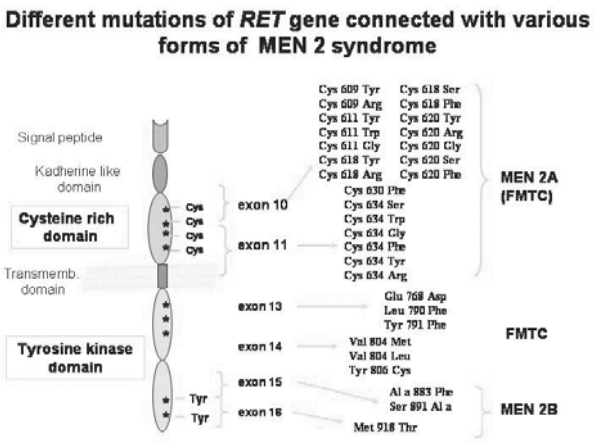

Fig 7 Different mutations of RET gene connected with various forms of MEN 2 syndrome

Medullary thyroid carcinoma a neoplasm of parafollicular C cells secreting calcitonin occurs as a sporadic or hereditary tumour. Missense germ-line mutations in the *RET* gene are responsible for a hereditary tumor syndrome – multiple endocrine neoplasia type 2 (MEN 2) which comprise three subgroups, MEN 2A, MEN 2B and familial medullary thyroid carcinoma (FMTC) depending on the tissue involved. In contrast to other known inherited cancer syndromes connected with loss of function of tumor suppressor genes, MEN 2 specific mutations convert *RET* gene into a dominant transforming gene (Santoro et al. 1995). The mutation is heterozygous, both the wild type and mutated alleles are present in patient's cells. All three syndromes have an autosomal dominant transmission manner, which predict a 50 % risk to inherit the mutation with children of affected parents. Penetrance of the mutated gene is nearly 100 % and is completed at the age of 40 years.

RET gene is a transmembrane tyrosine kinase receptor located on chromosome 10q11.2. It encodes a cell surface protein, which includes a signal peptide, an extracellular domain containing a cadherine like domain and a cysteine rich domain, a transmembrane domain, and an intracellular tyrosine kinase domain (Fig.7).

MEN 2 is characterized by medullary thyroid cancer, phaeochromocytoma (Pheo) and parathyroid hyperplasia (PTH). The site of RET gene mutations determines the clinical phenotype of the syndrome. The common disease for all three subtypes of MEN 2 is medullary thyroid cancer (100 %) and Pheo (50 %). Parathyroid hyperplasia was observed in 15-30 % of MEN 2A families. Medullary thyroid cancer is the only disease, which develop in FMTC members. MEN 2B patients apart from *RET* gene mutation manifest also marfanoid habitus, mucosal gangioneuromas of the lips, tongue, and intestinal tract. MEN 2B syndrome is the most aggressive form with the earlier age of tumor onset and poor prognosis. The most frequent mutation (85%) found in association with MEN 2A is located in codon 634 (exon11) and results in the replacement of TGC to CGC (C634R) followed by TAC (C634Y) and GGC (C634G) (Mulligan et al. 1995). The frequencies of mutations are approximately 50, 26, resp.10 %. Less frequent mutations connected to MEN 2A were found in codons 606, 611 618 and 620 (exon 10). In a limited number of MEN 2A families duplication/insertion mutation (insertion of three or four amino acids) in exon 11 was detected (Hoppner et al. 1997, Hoppner et al. 1998). Dual mutations in *RET* gene which affect both the cysteine rich region and the transmembrane domain were detected in two MEN 2A families (Tessitore et al. 1999, Poturnajova et al. 2005). There was no preferentially mutation in codons 10 and 11 found with FMTC. These mutations are responsible for more then 80 % of FMTC. However the most frequent mutation C634R revealed in MEN 2A was not detected with FMTC (Mulligan et al. 1995, Eng et al. 1996). Some more mutations connected with FMTC include mutations in the tyrosine kinase domain and are affecting exon 13 (E768D or Le790Phe, or Tyr791Phe), or exon 14 (V804L or V804M) and exon15 (S891A) (Berndt et al. 1998, Bolino et al. 1995, Fattoruso et al. 1998, Pasini et al. 1997, Hofstra et al. 1997). Rare mutation in codon 630 and insertion of amino acids, which creates additional cysteine residue in the extracellular cysteine rich domain in family with FMTC was also detected. (Kitamura et al. 1997).

MEN 2B specific *RET* mutation is in 95 % connected with the mutation in codon 918 (exon 16). The result of this mutation is the replacement of ATG to ACG (M918/T). (Carlson et al. 1994). In a minority of MEN 2B cases mutation in codon 883 in exon 15 (A883F) and double mutation V804M and Y806C was reported (Gimm et al. 1997, Myauchi et al. 1999, Smith et al. 1997).

In addition, to the association of the MEN 2 syndromes with individual mutations of the *RET* gene, also tissue specific alterations were detected. Any mutation in codon 634 is strongly correlated with the occurrence of phaeochromocytoma (Eng et al. 1996, Mulligan et al. 1995) while replacement of cysteine to arginine (C634R) is a frequent mutation in connection with parathyroid hyperplasia (Schuffenecker at al. 1998).

The identification of specific mutations in human hereditary diseases and the possible use of a variety of

molecular-genetic approaches to detect these alterations enable the early diagnosis of the disease. Families with hereditary multiple endocrine neoplasia or sporadic tumors of endocrine origins are predictive for genetic screening of the mutated *RET* proto-oncogene. A variety of molecular-genetic methods are available to detect the *RET* gene mutations. These include direct sequencing of the PCR amplified DNA fragment, restriction enzymes analysis (in the cases when gene mutations introduce altered restriction endonuclease site or single strand conformational polymorphism (Fig. 8).

Analysis of the genomic DNA by genetic screening can identify 98 % of the mutated gene carriers and provide secure testing of the members of the affected families. The advantage of genetic screening is the detection of MEN 2 specific *RET* gene mutation in people without any sign of the disease. Individuals with no mutation observed in *RET* gene are not in risk of MEN 2 and are free of further screening. If DNA testing is positive, prophylactic thyroidectomy early in the life is recommended.

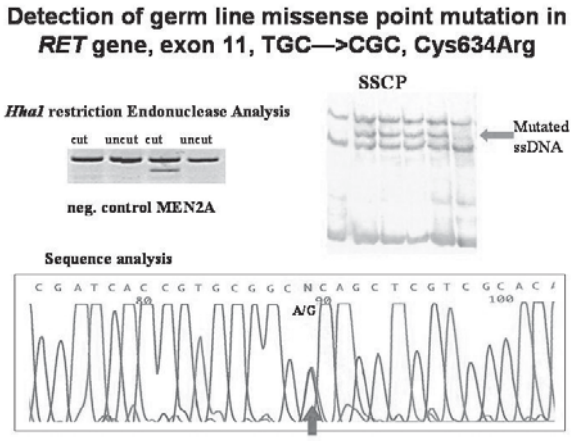

Fig 8 Detection of germ line missense point mutation in RET gene, exon 11, TGC CGC, Cys634Arg

Risk factors influencing thyroid cancer recurrence and cancer death is listed in TABLE 4 as modified from the NCCN guidelines for the diagnosis and treatment of thyroid cancer (MAZZAFERRI and KLOOS 2001).

Table 4
Factors predictive of thyroid cancer risk

Factors predictive of high risk

Patient variables	Age less than 15 and/or more than 45 years Male sex Family history of thyroid cancer
Tumour variables	More than 4 cm in diameter Bilateral disease Extrathyroidal extension Vascular invasion (both papillary and follicular thyroid cancer) Cervical or mediastinal lymph node metastases Certain tumour subtypes: Hürthle cell, tall cell, columnar cell, diffuse sclerosis, insular variants Marked nuclear atypia, tumor necrosis, and vascular invasion (i.e. histology grade) Tumours and metastases that concentrate radioiodine poorly or not at all Distant metastases

Factors predictive of moderate-to-low risk

Patient variables	Age from 15 to 45 years Female sex No family history of thyroid cancer
Tumour variables	Less than 4 cm in diameter Unilateral disease No extrathyroidal extension Absence of vascular invasion No lymph node metastases Encapsulated papillary thyroid carcinoma, papillary microcarcinoma, cystic papillary thyroid carcinoma Absence of nuclear atypia, tumor necrosis, and vascular invasion Tumors or metastases that concentrate radioiodine well No distant metastases

References

ALSANEA O.: Familial nonmedullary thyroid cancer. Curr Treat Options Oncol **1**, 345-351, 2000

BERNDT I, REUTER M, SALLER B et al: A new hot spot for mutations in the ret protooncogene causing familial medullary thyroid carcinoma and multiple endocrine neoplasia type 2A. J Clin Endocrinol Metab **83**, 770-774, 1998

BOLINO A, SCHUFFENECKER I, LUO Y et al: RET mutations in exons 13 and 14 of FMTC patients. Oncogene **15**, 2415-2419, 1995

BRANDI ML, GAGEL RF, ANGELI A et al: Guidelines for diagnosis and therapy of MEN type 1 and 2. J Clin Endocrinol Metab **86**, 5658-5671, 2001

BRTKO J, BOBALOVA J, PODOBA J, SCHMUTZLER C, KOHRLE J: Thyroid hormone receptors and type I iodothyronine 5'-deiodinase activity of human toxic adenomas and benigncold nodules, Exp Clin Endocrinol Diabetes **110,** 166-170, 2002

BURMAN KD, RINGEL VD, WARTOFSKY L: Unusual types of thyroid neoplasms. Endocrinol. Metab Clin North Am **25**, 49-68, 1996

CAILLOU B: Follicular carcinoma. Arch Anat Cytol Pathol **46**, 39-44, 1998

CHAN JKC: Tumors of the thyroid and parathyroid glands in: Diagnostic histopathology of tumors, Vol.2. FLETCHER CDM, Churchil Livingstone, London 959 – 1038, 2001

CARLSON KM, DOU S, CHI D et al: Single missense mutation in the tyrosine kinase catalytic domain of the RET protooncogene is associated with multiple endocrine neoplasia type 2B. Proc Natl Acad Sci U.S.A.; **91**, 1579-1583, 1994

COLLINI P, SAMPIETRO G, ROSAI J, PILOTTI S: Minimally invasive (encapsulated) follicular carcinoma of the thyroid gland is the low-risk counterpart of widely invasive follicular carcinoma but not of insular carcinoma. Virchows Arch **442**, 71-76, 2002.

CORMACK DH: The Endocrine System. In: Essential histology. JB Lippincot Company, Philadelphia 339-356, 1993

CORVILAIN B: The natural history of thyroid autonomy and hot nodules. Ann Endocrinol **64**, 17-22, 2003

DEAN DS, HAY DH: Prognostic indicators in differentiated thyroid carcinoma. Cancer Control **7**, 229-239, 2000

ENG C, CLAYTON D, SCHUFFENECKER I et al: The relationship between specific RET proto-oncogene mutations and disease phenotype in multiple endocrine neoplasia type 2. International RET mutation consortium analysis. JAMA **276**, 1575-1579, 1996

FATTORUSO O, QUADRO L, LIBROIA A et al: A GTG to ATG novel point mutation at codon 804 in exon 14 of the RET proto-oncogene in two families affected by familial medullary thyroid carcinoma. Hum Mutat, Suppl **1**, 167-171, 1998

FIGGE J: Epidemiology of thyroid cancer. In: WARTOFSKY L ed. Thyroid Cancer: a comprehensive guide to clinical management. Totowa: Humana Press 77-83, 1999

GAGEL RF, GOEPFERD H, CALLENDER DL: Changing concepts in the pathogenesis and management of thyroid gland carcinoma. Cancer J Clin **46**, 261-283, 1996

GILLILAND FD, HUNT WC, MORRIS DM, KEY RC: Prognostic factors for thyroid carcinoma: A population-based study of 15,698 cases from the Surveillance, Epidemiology and End Results (SEER) program 1973-1991. Cancer **79**, 564-573, 1997

GIMM O, MARSH DJ, ANDREW SD et al.: Germline dinucleotide mutation in codon 883 of the RET proto-oncogene in multiple endocrine neoplasia type 2B without codon 918 mutation. J Clin Endocrinol Metab; **82**, 3902-3904, 1997

GOULD VE, SOMMERS SC: Endocrine system. In: Pathology, pp. 1118-1177. JB Lippincot, Philadelphia 1988

HOPPNER W, RITTER MM: A duplication of 12 bp in the critical cysteine rich domain of the RET proto-oncogene results in a distinct phenotype of multiple endocrine neoplasia type 2A. Hum Mol Genet; **6**, 587-590 1997

HOFSTRA RM, FATTORUSO O, QUADRO L et al: A novel point mutation in the intracellular domain of the ret protooncogene in the family with medullary thyroid carcinoma. J Clin Endocrinol Metab **82**, 4176-4178, 1997

HOPPNER W, DRALLE H, BRABANT G: Duplication of 9 base pairs in the critical cysteine-rich domain of the RET proto-oncogene causes multiple endocrine neoplasia type 2A. Hum Mutat Suppl **1**, 128-130, 1998

KIMURA ET, NIKIFOROVA MN, ZHU Z et al: High prevalence of BRAF mutations in thyroid cancer: genetic evidence for constitutive activation of the RET/PTC-RAS-BRAF signaling pathway in papillary thyroid carcinoma. Cancer Res. **63**, 1454-1457, 2003

KITAMURA Y, GOODFELLOW PJ, SHIMIZU K et al: Novel germline RET proto-oncogene mutations associated with medullary thyroid carcinoma (MTC): mutation analysis in Japanese patients with MTC. Oncogene **14**, 3103-3106, 1997

KROLL TG: Molecular rearrangement and morphology in thyroid cancer. Am J Path **160**, 1941-1944, 2002.

LIVOLSI VA: Thyroid. In: Histology for Pathologists, Ed: STERNBERG SS, Raven Press, Ltd New York 301-310, 1992

MALCHOFF CD, MALCHOFF DM: The genetics of hereditary nonmedullary thyroid carcinoma. J Clin Endocrinol Metab **87**, 2455-2459, 2002

MAZZAFERRI EL, KLOOS RT: Current approaches to primary therapy for papillary and follicular thyroid cancer. J Clin Endocrinol Metab **86**, 1447-1463, 2001

MCKAY JD, LESUEUR F, JONARD L et al: Localization of a susceptibility gene for familial nonmedullary thyroid carcinoma to chromosome 2q21. Am J Hum Genet **69**, 440-446, 2001

MIYAUCHI A, FUTAMI H, HAI N et al: 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 Cancer Res **90**, 1-5, 1999

MULLIGAN LM,,MARSH DJ, ROBINSON BG et al: Genotype-phenotype correlation in multiple endocrine neoplasia type 2: report of the International RET Mutation Consortium. J Intern Med **238**, 343-346, 1995

O'RIORDAIN DS, O'BRIEN T, WEAVER AL et al: Medullary thyroid carcinoma in multiple endocrine neoplasia types 2A and 2B. Surgery **116**, 1017-1023, 1994

PAL T, VOGL FD, CHAPPUIS PO et al: Increased risk for nonmedullary thyroid cancer in the first degree relatives of prevalent cases of nonmedullary thyroid cancer: a hospital-based study. J Clin Endocrinol Metab. **86**, 5307-5312, 2001

PASINI A, GENESTE O, LEGRAND P et al: Oncogenic activation by two distinct FMTC mutations affecting the tyrosine kinase domain. Oncogene **15**, 393-402, 1997

POTURNAJOVA M, ALTANEROVA V, KOSTALOVA L et al: Novel germline mutation in the transmembrane region of RET gene close to Cys634Ser mutation associated with MEN 2A syndrome. J Mol Med **83**, 287-295, 2005

SALABE GB: Pathogenesis of thyroid nodules: histological classification? Biomedicine and Pharmacotherapy **55**, 39-53, 2001

SANTORO M, CARLOMAGNO F, ROMANO A et al: Activation of RET as a dominant transforming gene by germline mutations of MEN2A and MEN2B. Science **267**, 381-383, 1995

SCHUFFENECKER I, VIRALLY-MONOD M, BROHET R et al: Risk and penetrance of primary hyperparathyroidism in multiple endocrine neoplasia type 2A families with mutations at codon 634 of the RET proto-oncogene. J Clin Endocrinol Metab **83**, 487-491, 1998

SHERMAN SI: Thyroid carcinoma. Lancet **361**, 501-511, 2003

SHERMAN SI: Anaplastic carcinoma: clinical aspects. In: WARTOFSKY L ed. Thyroid Cancer: a comprehensive guide to clinical management. Totowa: Humana Press 319-325, 1999

SHRODER S, BOCKER W, BAISCH H et al: Prognostic factors in medullary thyroid carcinomas. Survival in relation to age, sex, stage, histology, immunocytochemistry, and DNA content. Cancer **61**, 806-816, 1988

SMITH DP, HOUGHTON C, PONDER BA: Germline mutation of RET codon 883 in two cases of de novo MEN 2B. Oncogene **15**, 1213-1217, 1997

TESSITORE A, SINISI AA, PASQUALI D et al: A novel case of multiple endocrine neoplasia type 2A associated with two de novo mutations of the RET protooncogene. J Clin Endocrinol Metab. **84**, 3522-3527, 1999

THOMPSON LD, WIENEKE JA, PAAL E et al: A clinicopathological study of minimally invasive follicular carcinoma of the thyroid gland with a review of the English literature. Cancer **91**, 505-524, 2001

WOLFE HJ, DELELLIS RA: Familial medullary thyroid carcinoma and C cell hyperplasia. Clin Endocrinol Metab **10**, 351-365, 1981

Corresponding author: Julius Brtko, PhD., DrSc
Laboratory of Functional Neuromorphology
Institute of Experimental Endocrinology
Slovak Academy of Sciences
Vlarska str. 3, 833 06 Bratislava, Slovakia
Phone: (42102)54772800
Fax.: (42102)54774247
E-mail address: julius.brtko@savba.sk