This introductory chapter will provide background on 1) the diagnosis and treatment options of rare endocrine tumors, and 2) the associated tumor predisposition syndromes included in this thesis: DICER1 syndrome, MEN2a syndrome, *CDC73*-related disorder and *SDHA*-associated paraganglioma

General Introduction

A few years ago I met a then 12-year-old girl, diagnosed with thyroid cancer, at the Department of Clinical Genetics of the Leiden University Medical Center. She and her parents had three important questions: "Why do I have cancer? Are other relatives at risk? And if so, can we prevent cancer?"

These questions, i.e. Why?, Who?, and How?, are the backbone of this thesis, which describes investigations of the genetic background of a wide variety of rare endocrine tumors, including those of the thyroid, parathyroid, adrenal and paraganglia. This introductory chapter will provide background on 1) the diagnosis and treatment options of rare endocrine tumors, and 2) the associated tumor predisposition syndromes included in this thesis: DICER1 syndrome, MEN2a syndrome, *CDC73*-related disorder and *SDHA*-associated paraganglioma (see Figure 1).

In order to provide answers to the questions asked by that 12-year-old girl, patient- and family-centered endocrine cancer care encourages active collaboration between the departments of endocrinology, oncology, surgery, pathology, chemistry, radiology, nuclear medicine and clinical genetics (see Figure 2).

The implementation of high-throughput DNA/RNA sequencing platforms allows novel molecular information to be used to optimize primary endocrine cancer care: firstly, via somatic

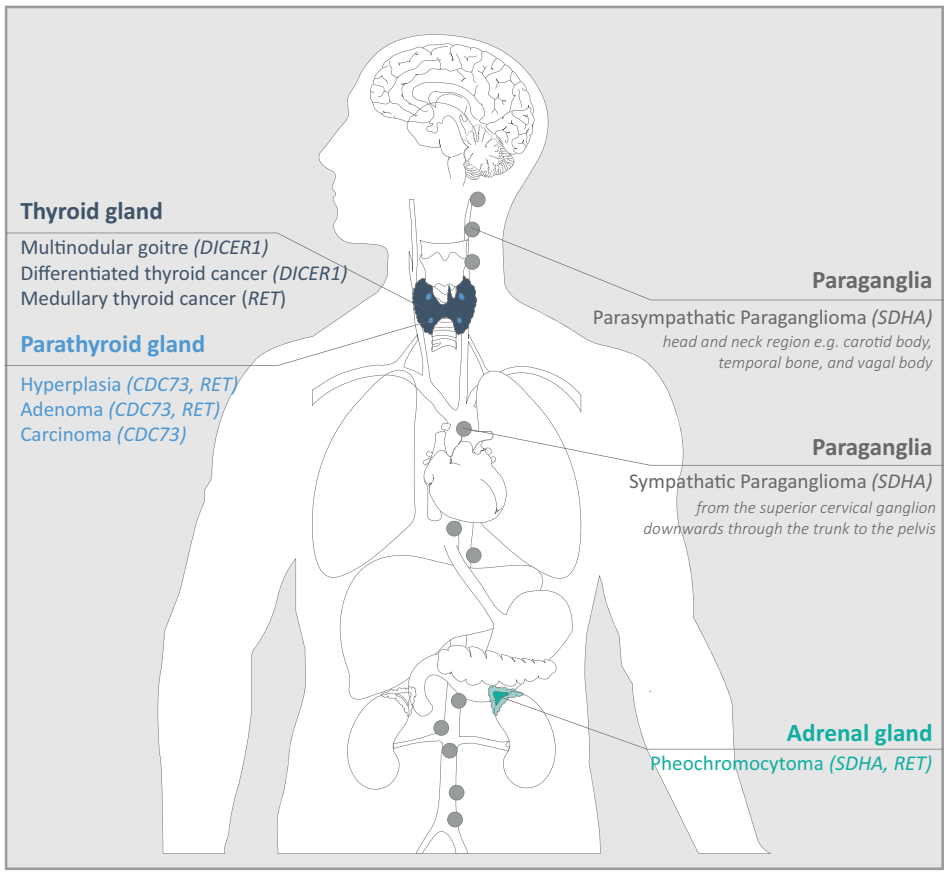


Figure 1. Endocrine tumors and related tumor predisposition syndromes investigated in this thesis.

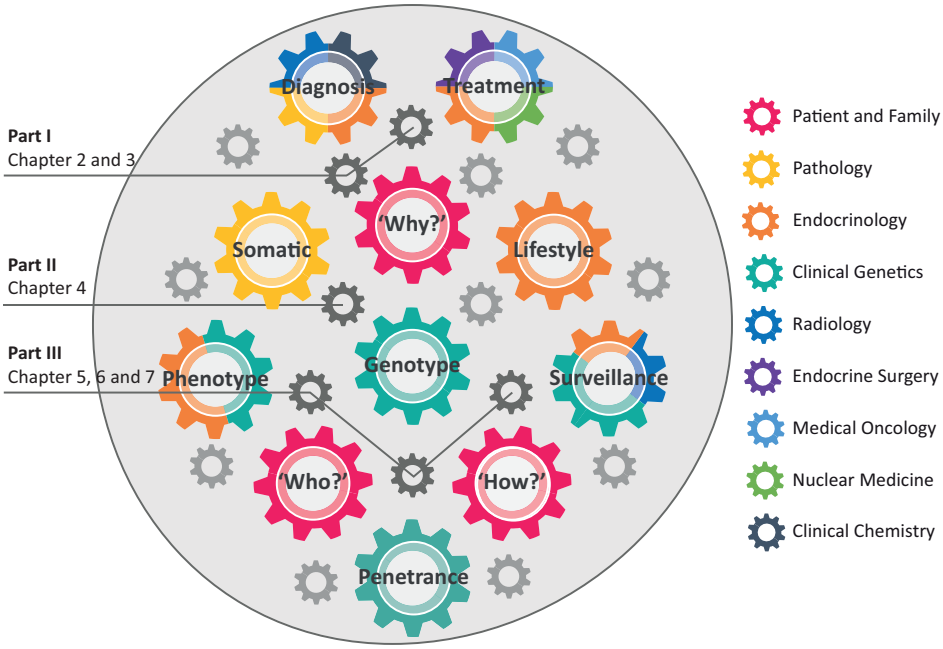


Figure 2. Joining forces in patient- and family-centered endocrine cancer care inspired by the questions: "Why do I have cancer? [Who?], Are relatives at risk? [Who?], And if so, can we prevent cancer? [How?]". Part I. The roll of molecular testing in endocrine cancer diagnostics and treatment decision making. Part II. Identification genetic predisposition in pediatric non-medullary thyroid carcinoma. Part III. Genetic counseling in endocrine tumor predisposition syndromes.

and germline molecular information that can be used in the pre- and postoperative phase for primary diagnostics and to select therapy choices. Secondly, in case of cancer recurrence molecular information increasingly stratifies for the effectiveness of targeted drugs (mainly antibody or small molecule drugs) or for the effectiveness of immunotherapeutic drugs. Finally, DNA sequencing aids in the elucidation of genetic factors underlying the increased disease susceptibility in patients and their families.

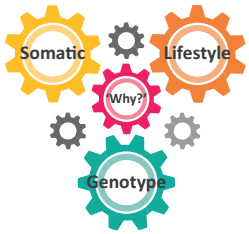
Not unlike multidisciplinary patient care, interdisciplinary efforts are increasingly important to scientific discoveries and translational research efforts. This thesis emphasizes not only local, national and international collaborations between the medical disciplines involved but also the interaction between basic and clinical research, taking research from bench to beside and back again.

Q1: WHY DO I HAVE CANCER?

Tumors evolve from benign to malignant lesions by acquiring a series of non-synonymous variants* (i.e. single nucleotide substitutions, structural variants that alter protein products).[1] This gradual accumulation of gene mutations† is attributable to hereditary, replicative and environmental factors (see textbox 1).[2]

* Genetic terminology is included in the glossary in the appendix (page - 160-164)
† In this thesis, the word *mutation* is used as a synonym for pathogenic (disease causing) variant

Textbox 1. Q1. Why do I have cancer?
A: Due to the accumulation of non-synonymous gene mutations over time, attributable to hereditary, replicative and environmental factors.
- Hereditary factors: Germline mutations that can be transmitted via eggs or sperm cell and are present in every cell of the offspring (also referred to as 'genotype' in Figure 2).
- Replicative factors: Somatic mutations that result from (unavoidable) DNA replication errors (also referred to as 'somatic' in Figure 2).
- Environmental factors: Somatic mutations that result from external mutagens such as smoking, alcohol and UV light (also referred to as 'lifestyle factors' in Figure 2).



Determining the contribution of each factor is challenging and differs among cancer types.[2] In general, the acquisition of de novo somatic variants (due to replicative and environmental factors) accounts for approximately 90% of all new cancer diagnoses. Somatic variants generally occur in cells with high proliferation rates (leading to random mistakes during normal DNA replication) and/or occur in barrier tissue through prolonged exposure to environmental carcinogens (e.g. smoking, alcohol and UV light). As a result, the number of sporadic cancers increases with age. The remaining 10% of cancer diagnosis are related to inherited mutations. In patients with a germline mutation in a tumor suppressor gene or oncogene, the first step in the development of cancer has already been taken in every cell. This differs from patients with sporadic cancers who do not harbor constitutional gene mutations and therefore must acquire multiple somatic gene mutations within a single cell before tumorigenesis can occur.[2] Unsurprisingly, one of the main indicators of a genetic predisposition to cancer is the development of one or more malignancies at an earlier than expected age. The proportion of inherited disease in the context of the total disease population might be an underestimate owing to still unidentified genetic causes or because heredity is not recognized due to an unavailable, incomplete or misdiagnosed family history and/or variable penetrance. Identification of a causative germline mutation may not only have important clinical implications for the index patient (proband), it also facilitates cascade testing and surveillance of relatives in order to prevent, or at least allow early identification of, (pre)malignant conditions.

ENDOCRINE TUMORS

All endocrine tumors are considered rare diseases. To date, a total of six to seven thousand rare diseases have been discovered and new diseases are described regularly in the medical literature. The reported number of rare diseases depends to a large extent on the degree of specificity used when classifying the different entities. Comprehensive genetic analysis, including genomics, transcriptomics and proteomics, for example by The Cancer Genome Atlas (TCGA), has recently led to a large increase in (neuroendocrine) cancer subtypes.[3,4] Interestingly, different cancer subtypes may have a partially comparable genetic background (e.g. solid tumors with *NTRK* gene fusions). This type of molecular reclassification may also extend beyond the current boundaries of organ-specific histologic tumor classification.[5] The primary reason for classification of tumors is to better assign appropriate (targeted) therapy. In 2018, the *NTRK* inhibitor, Larotrectinib, was approved by the American Food and Drug Administration (FDA) for adult and pediatric patients with advanced solid tumors harboring an *NTRK* gene fusion without a known acquired resistance mutation. In contrast to other cancer types (e.g. melanoma and ovarian cancer), molecular profiling in endocrine tumors is mainly used for primary diagnostics (i.e. subtyping and prognostic

forecasting) and has not yet been implemented for tailored treatment in clinical practice.[6] Current treatment options are limited for some endocrine cancer subtypes (e.g. advanced radioactive iodine-refractory thyroid cancer, parathyroid carcinoma and metastatic paraganglioma).

The following paragraphs will give an overview of the diagnostic procedures and treatment options for the endocrine tumors investigated in this thesis, i.e. of the thyroid, parathyroid, adrenal gland and paraganglia.

Thyroid gland

Diagnosis

Thyroid nodules are common, as around 5% of adults harbor thyroid nodules by palpation and up to 60% show nodules on ultrasound. Only a small fraction (4.0%-6.5%) of all evaluated thyroid nodules is found to be malignant.[7] Thyroid ultrasound characteristics, such as size, echogenicity, and presence of macrocalcifications and/or irregular margins, have been used to stratify the risk of malignancy in thyroid nodules and aid decision-making regarding whether further investigation is indicated.[8,9] Fine-needle aspiration is typically performed to further stratify thyroid nodules suspect for malignancy. A definitive morphological diagnosis of benign or malignant nodules can be provided by cytology examination in up to 70-75% of cases, whereas the remainder is considered undetermined (Bethesda category III and IV).[10,11] In these cases in particular the increasing use of molecular testing improves diagnosis and clinical management.[12]

Thyroid cancer (TC) is the most common endocrine malignancy and its incidence has increased appreciably over the last few decades, especially in Europe and North America. TC now accounts for 1-3% of all new malignant tumors.[13,14] Of these, the vast majority (>90%) are differentiated thyroid carcinomas (DTC) that derive from the follicular epithelial cells and have an indolent clinical course and low mortality.[15] Trends in TC incidence probably largely reflect incidental detection of asymptomatic disease through the increasing use of medical imaging modalities.[16] The incidence of DTC is about three to four times higher among females than males and shows distinct age-related patterns regarding gender and different histological subtypes.[16]

Histological subtypes can be distinguished based on morphological features and molecular background.[17] Papillary thyroid carcinoma (PTC, 85-90%) is the most common subtype and specific nuclear features are important diagnostic hallmarks. Many morphological variants of PTC have been described.[18] Classic PTCs are associated with somatic *BRAF*^{V600E} variants or gene rearrangements (*RET-PTC*, *NTRK*- and *ALK)*.[19] The follicular variant of PTC (FVPTC) more commonly harbors *RAS* or *BRAF* non-V600E variants.[20] These mutations, leading to activation of the mitogen-activated protein kinase (MAPK) signaling pathway, are almost always mutually exclusive.[21] Follicular thyroid carcinoma (FTC, 5-10%) is diagnosed by minimal or wide follicular cell invasion of the tumor capsule and/or blood vessels and has frequently been linked to somatic mutations (*RAS* or *PTEN)* or *PAX8-PPARγ* gene rearrangements.[19] Hürthle cell carcinomas are characterized by oncocytic cells as a result of mitochondrial abundance and are associated with whole chromosome loss accompanied by endoreduplication or genomic doubling, in the absence of alterations in the abovementioned genes.[22,23] In contrast to PTC, poorly differentiated (PDTC, 2%) and anaplastic thyroid carcinomas (ATC, 2%) are aggressive tumors that have undergone dedifferentiation due to additional or relatively frequent somatic *TERT*, *TP53, CTNNB1* and/or *PIK3CA* mutations.[24] Medullary thyroid cancer (MTC, 2%) originates from the calcitonin-producing parafollicular cells and is associated with (germline and somatic) activating *RET* proto-*oncogene* mutations or somatic *RAS* mutations. [25,26]

Treatment

Treatment decisions are guided by the extent of disease and include lobectomy or total thyroidectomy with or without radioactive iodine (RAI) therapy to treat persistent loco-regional, nodal disease or distant metastases not amenable to surgery.[27] These treatments are highly effective in the majority of DTC patients and the 10-year survival rate ranges between 80 and 95%.[13,14] However, up to 5% of DTC patients become refractory to RAI (RAI-R). The 10-year survival rate in these patients is about 20-40%, due to frequently unresectable metastatic lesions.[15,28] The survival rates for less common TC histological subtypes range from 65% for MTC after 10 years, less than 20% for PDTC at 5 years, and less than 10% for ATC at 6 months after the initial diagnosis.[26,29] A range of targeted treatments have been approved by the FDA for the treatment of advanced RAI-R DTC, ATC and MTC. Several clinical trials are currently investigating the potential of (primarily) alternative kinase inhibitors (e.g. NTRK-, ALK-, BRAF- and RET- inhibitors).[30]

Parathyroid gland

Diagnosis

Hyperparathyroidism (i.e. increased parathyroid hormone levels in blood; HPT) results either from autonomous hyperfunction of the parathyroid glands themselves (primary hyperparathyroidism; pHPT) or secondary/tertiary to an underlying condition (e.g. vitamin D deficiency or kidney failure). HPT is typically characterized by the quartet *stones, bones, groans, and psychiatric overtones* referring to the presence of renal stones, osteoporosis, gastrointestinal symptoms and depression, respectively. Nevertheless, most patients are asymptomatic. pHPT is a relative common endocrine disease, with a prevalence of 1-4 per 1000, a female predominance (3:1) and a peak incidence in the sixth decade of life.[31] Benign, sporadic parathyroid adenomas (PA) are the most common cause of pHPT (~85%). A further 15% of pHPT is attributable to multi-gland disease (including hyperplasia and double adenomas) and less than 1% is due to a parathyroid carcinoma (PC).[32,33] The lack of specific discriminating clinical, biochemical and radiological features makes distinguishing between PA and PC challenging. However, discriminating the two conditions is of the utmost importance as it determines the extent and radical nature of initial surgery, which is in turn the major determinant of prognosis.[34] Pre-operative features that should raise suspicion of PC are: calcium >3mmol/L, PTH >3 times upper limit, parathyroid lesion >3cm and a family history of PC.[35] Intraoperative findings that suggest carcinoma are firm, large grayish to white irregularly-shaped tumors that can be adherent or invade surrounding structures. Even the histological diagnosis remains in some cases difficult and the diagnosis of PC is often made retrospectively, after tumor recurrence or metastasis.[36,37] The criteria to unequivocally diagnose PC include: capsular invasion, vascular invasion, invasion in surrounding tissue and/or distant metastasis.[38] Parathyroid lesions without unequivocal histological signs of PC but with some features of malignancy (e.g. fibrotic bands, questionable capsular invasion, increased mitotic figures) are defined as atypical adenoma and might require closer follow-up. Inactivating *CDC73* mutations are a major driver of PC (~70%) and in one-third of cases the mutations are found in the germline.[39,40] In contrast, these mutations are extremely rare in sporadic PAs.[41] When found, they were typically associated with unusual histologic features, such as cystic appearance.[42] Immunohistochemical staining of the protein product of *CDC73*, parafibromin, and somatic *CDC73* mutation analysis can be useful in the differential diagnosis of PC and may serve as a prognostic factor.[43,44] *MEN1*, *CCND1/cyclin D1* and the *CDKI* genes have been established as primary tumorigenic drivers in PAs.[40]

Treatment

Surgery is the most common treatment for pHPT and provides a cure in about 95% of all cases. The extent of surgery (focused vs. bilateral exploration, selective vs. extensive parathyroidectomy) depends on the differential diagnosis and possible underlying hereditary setting. Most patients with PC achieve long-term survival (5-year mortality ~10%) after surgical resection.[33,35] However, following multiple operations, systemic therapy may be required for recurrent or metastatic disease. Radiotherapy and cytotoxic regimes have not been proven to be effective and current treatment focuses on controlling hypercalcemia. Chapter 8 will discuss the future perspectives for metastatic PC treatment, based on recent comprehensive genetic profiling studies.[45-48]

Paraganglia and adrenal medulla

Diagnosis

Paragangliomas (PGLs) are rare neuroendocrine tumors (*i.e.,* 2-5/1.000.000/year) and carry the highest degree of heritability among human neoplasms.[49,50] PGLs are classified according to their anatomical location (intra or extra-adrenal PGL) and whether they are of sympathetic or parasympathetic origin. Head and neck paragangliomas (HNPGL) emerge from the parasympathetic nervous system and are usually benign, slow-growing non-secreting tumors.[51,52] Common sites include the carotid body, the temporal bone, and the vagal body. Pheochromocytoma (PHEO) and sympathetic paraganglioma (SPGL) are catecholamine-secreting tumors, with associated clinical features such as high blood pressure, a rapid heartbeat, flushed skin, sweating, headache and tremors.[53] PHEOs are derived from the chromaffin cells of the adrenal medulla and SPGLs are found in close relationship to the peripheral sympathetic nervous system, from the level of the superior cervical ganglion downwards through the trunk to the pelvis.[54] Diagnostic workup generally includes measurement of metanephrines (i.e. the O-methylated metabolites of catecholamines) levels in blood and/or urine, one or more anatomic or nuclear imaging tests (i.e. CT, MRI, MIBG, and/or PET) for differential diagnosis and to accurately define the location of the lesion, and might also include germline genetic testing.[49,54] Immunohistochemistry for SDHB and SDHA has been shown to be a valuable additional tool in the histopathological analysis of these tumors, and can be considered a surrogate marker for molecular analysis.[55]

Treatment

Treatment of PGL depends on the location and origin of the tumor. For PHEO and SPGL surgical resection is generally the treatment of first choice due to excess production of hormones. For non-producing, slow-growing HNPGL watchful waiting might be more appropriate. Metastases are more often present in SPGL compared to PHEO or HNPGL.[51] Patients with metastatic disease have limited treatment options[56] and a markedly variable prognosis (reported 5-year survival rates range between 24% and 85%).[38] Recently, an integrated analysis identified several molecular markers that were associated with an increased risk of metastatic disease and which may serve as potential drug targets.[4] Chapter 8 will discuss the future perspectives for metastatic PGL treatment.

In summary, (somatic) genetic information has the potential to improve endocrine tumor classification, prognostic forecasting, and the development of personalized treatment. Furthermore, molecular analysis of tumor tissue can be used as a pre-test tool for the identification of patients at high risk for a genetic predisposition syndrome.

Q2: ARE RELATIVES AT RISK?

Identification of the causative gene variant in a cancer patient offers his/her relatives the possibility of pre-symptomatic genetic testing, i.e. at-risk family members can be screened for the presence of the mutation to establish 'who' has inherited an increased cancer risk (mutation carrier vs. non–mutation carrier). Most cancer predisposition syndromes follow an autosomal dominant inheritance pattern in which the patient's first-degree relatives (i.e. parents, children, and siblings) have a 50% risk of carrying the causative mutation. Successful implementation of genetic testing in diagnostics requires accurate estimates of variant pathogenicity classification, phenotype and disease penetrance.

Although an increasing proportion of cases can now be attributed to inherited gene mutations, a substantial fraction of suggestive hereditary cases (i.e. young onset, multiple tumors and/or strong family history) are still genetically unaccounted for. For individuals with clinical features suggestive of a hereditary cancer syndrome, but without a mutation in the known predisposition genes, predictive testing of family members, genetic counseling and preventive medical management are hampered.

ENDOCRINE TUMOR PREDISPOSITION SYNDROMES

Among the first hereditary tumor predisposition syndromes to be recognized were Multiple Endocrine Neoplasia (MEN) type 1-2 and von Hippel-Lindau syndrome.[57,58] Depending on the specific endocrine tumor type, 10-30% of cases are associated with genetic factors, in which up to 15 different genes per tumor type may be implicated.[34,50] The relatively large role of inherited DNA variants in endocrine tumors compared to other cancer types (e.g. 5%-10% in breast cancer) has been suggested to be a counterpart of the relatively low contribution of somatic mutations. The latter is the result of both fewer replicative alterations (due to relatively low proliferation rates[59]) and the limited influence of environmental factors.[2]

While endocrine neoplasia syndromes show many features commonly seen in familial disease (early onset, family history, multifocal neoplasia, multiorgan involvement), some of these syndromes are considered to be phenotypically complex and heterogeneous. Moreover, endocrine predisposition syndromes commonly present with *de novo* mutations. The latter presentation can make them difficult to recognize and classify on purely clinical grounds.

Due to an active international research community, over time the number of endocrine tumor syndromes and associated genes has expanded significantly.[60] Furthermore, new disease patterns have emerged following the identification of non-endocrine tumors and other clinical features as part of hereditary endocrine tumor syndromes, and with the occurrence of endocrine tumors in non-classical endocrine tumor syndromes.[60]

The following paragraphs provide an overview of genetic predisposition for the endocrine tumors and syndromes discussed in this thesis:

- Thyroid cancer, focusing on DICER1 syndrome and MEN2a syndrome (*Figure 3*)
- Parathyroid tumors, focusing on *CDC73*-related disorder (*Figure 4*)
- Paraganglioma, focusing on *SDHA*-associated paraganglioma (*Figure 4*)

Genetic predisposition of thyroid cancer

DTC can manifest as part of a tumor predisposition syndrome, including PTEN hamartoma tumor syndrome, DICER1 syndrome (see below), Carney complex, familiar adenomatous

Table 1. Hereditary syndromes associated with non-medullary thyroid cancer

Syndrome	Gene (locus)	Inheritance	Thyroid phenotype*	Penetrance thyroid phenotype	Syndromic features
PTHS / Cowden	*PTEN* (10q23.31)	AD	FTC > PTC MNG	~10%	e.g. breast- uterine-, colon cancer, hamartomas, macrocephaly
Carney complex	*PRKAR1A* (17q24.2)	AD	MNG PTC, FTC	~60% ~5%	e.g. myxoma, lentigines, endocrine overactivity
DICER1 (Figure 3)	*DICER1* (14q32.13)	AD	MNG PTC, FTC	~35% ~5%	e.g. pleuropulmonary blastoma, cystic nephroma, Sertoli–Leydig cell tumor
FAP	*APC* (5q22.2)	AD	CMV-PTC	~2-10%	e.g. polyposis, colon cancer
Werner	*WRN* (8p12)	AR	PTC, FTC, ATC	~18%	Adult progeria
Pendred	*SLC26A4* (7q21–34)	AR	MNG FTC	~80% ~1%	Congenital deafness

PTHS; *PTEN* hamartoma tumor syndrome, FAP; familiar adenomatous polyposis; AD; autosomal dominant, AR; autosomal recessive, PTC; papillary thyroid carcinoma, FTC; follicular thyroid carcinoma, CMV-; cribriform-morular variant, ATC; anaplastic thyroid carcinoma, MNG; multi nodular goiter, *predominant phenotype.

polyposis, Werner syndrome, and Pendred syndrome (see Table 1).[61,62] However, DTC occurs as a minor component in these syndromes and the majority of apparently hereditary DTC is still genetically unaccounted for. While genome-wide association studies (GWAS) have identified associations with polymorphisms at various loci, additional studies are needed to determine their role in DTC tumorigenesis.[63-65] While the majority of patients with MTC have sporadic disease, 25-30% of cases are diagnosed with MEN2 syndrome (see below) resulting from germline *RET* mutations.[26]

DICER1 syndrome

First reported in 2009, DICER1 syndrome is a rare autosomal dominant inherited disorder that predisposes to a variety of cancerous and noncancerous tumors of mostly pediatric and adolescent onset (see Figure 3).[66] The *DICER1* gene encodes a ribonuclease III enzyme that is crucial for the cleavage of noncoding small RNA precursors to generate mature micro-RNAs (miRNAs), which in turn post-transcriptionally regulate expression of many genes.[67] *DICER1* genetics is consistent with a tumor suppressor two-hit model, whereby a germline inactivating mutation is coupled to a missense "hotspot" mutation within the functional ribonuclease (RNase) IIIb domain in tumor DNA.

Pleuropulmonary blastoma (a rare pediatric lung tumor; PPB), cystic nephroma (CN), ovarian Sertoli-Leydig cell tumor (SLCT) and thyroid neoplasia are the hallmark tumors of DICER1 syndrome.[68] Due to the phenotypic rarity of associated tumors (e.g. PPB, CN and SLCT), the prevalence of DICER1 syndrome was assumed to be low. However, it has recently been estimated that the population incidence of germline *DICER1* mutations could be as high as ~1:2,529 to 1:10,600, based on publicly-available germline whole-exome sequence datasets.[69] The TCGA

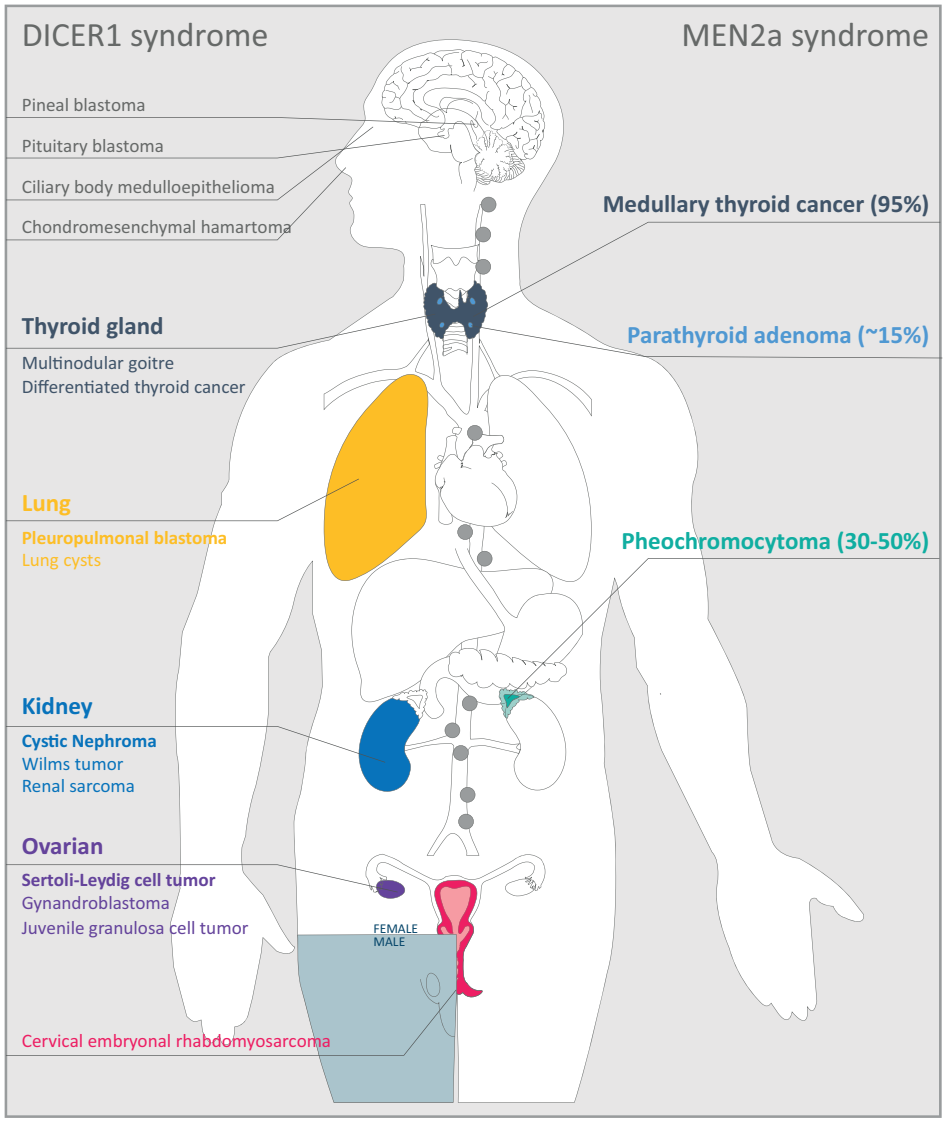


Figure 3. DICER1 syndrome (left) and MEN2a syndrome (right) associated tumors. Clinical hallmarks in bold. Between brackets; estimated MEN2a disease penetrance, DICER1 syndrome disease penetrance is unknown.

database showed germline *DICER1* mutations in ~1:4600 adult cancer cases.[70] The penetrance of each of the *DICER1*-related conditions is not fully understood, but is suggested to be low-to-moderate.[71] Despite reduced disease penetrance, identification of *DICER1* mutation carriers is important, since clinical surveillance is focused on early detection of PPB, and early tumor stages are associated with lower mortality.[72] Large international prospective studies are needed to evaluate and optimize current screening guidelines.[73,74]

Multiple endocrine neoplasia type 2a

MEN2a syndrome is caused by heterozygous germline *RET* mutations, and is characterized by the presence of MTC (>95%), PHEO (40-50%) and/or pHPT (10-20%), see Figure 3.[75] Furthermore, a small number of patients may present with cutaneous lichen amyloidosis or Hirschsprung's disease. Approximately 10% of all cases are caused by *de novo* mutations.[76] Current treatment and surveillance recommendations, from the American Thyroid Association (ATA), are based on the classification of specific *RET* mutations into risk levels according to genotype-phenotype correlations.[77]

Genetic predisposition for parathyroid tumors

A genetic predisposition for pHPT can be found in approximately 10% of pHPT cases and to date, pathogenic variants in at least 11 genes have been associated with hereditary pHPT.[78] The most commonly identified hereditary syndromes associated with pHPT are listed in Table 2, and include MEN type 1, 2a, or 4, *CaSR*-, and *CDC73*-related disorders (see below).[79-81] Disease penetrance and phenotype (predominantly parathyroid hyperplasia, PA or PC) varies among the different syndromes. Therefore, early identification of hereditary pHPT is crucial for optimal clinical and surgical management, e.g. minimal invasive procedure or bilateral neck exploration with (sub) total parathyroidectomy.[34]

CDC73-related syndrome

Inactivation of the *CDC73* tumor suppressor gene (formerly known as *HRPT2* and encoding parafibromin) predisposes heterozygous mutation carriers to pHPT and less frequently, ossifying fibromas of the jaw and/or a variety of benign and malignant renal/uterine lesions (see

Table 2. Hereditary syndromes associated with primary hyperparathyroidism

Syndrome	Gene (locus)	Inheritance	Parathyroid phenotype*	pHPT penetrance	Mean age pHPT	Syndromic features
MEN1	*MEN1* (11q13)	AD	Hyperplasia	95%	20-25y	e.g. pituitary adenoma, pNET, carcinoid
MEN2	*RET* (10q11.21)	AD	Adenoma	20-40%	35–41y	MTC, PHEO
MEN4	*CDKN1B* (12p13.1)	AD	Hyperplasia	High?	36–79y	*Similar to MEN1*
HPT-JT (Figure 4)	*CDC73* (1q31.2)	AD	Adenoma (e.g. cystic, atypical), carcinoma	80-95%	early adulthood	Ossifying fibroma jaw, renal- and uterine lesions
FIHP	*CASR*^ (3q21.1)	AD	Adenoma	High?		None

FIHP; familiar isolation hyperparathyroidism, AD; autosomal dominant, pHPT; primary hyperparathyroidism, y; years; pNET; pancreatic neuro-endocrine tumor (secreting or non-secreting), MTC; medullary thyroid carcinoma; PHEO; pheochromocytoma,^ *CASR* mutations are also associated with other health conditions, *predominant phenotype.

Figure 4).[34,82] pHPT onset is typically in late adolescence or early adulthood and penetrance has been reported to be as high as 80-95%.[34] In contrast to sporadic cases and other hereditary pHPT syndromes, PCs may be found in up to 15-20% of patients with germline *CDC73* mutations.[34] The majority of germline (and somatic) *CDC73* mutations are frameshift and nonsense variants found in exons 1, 2 and 7, although missense variants as well as (small) deletions and insertions

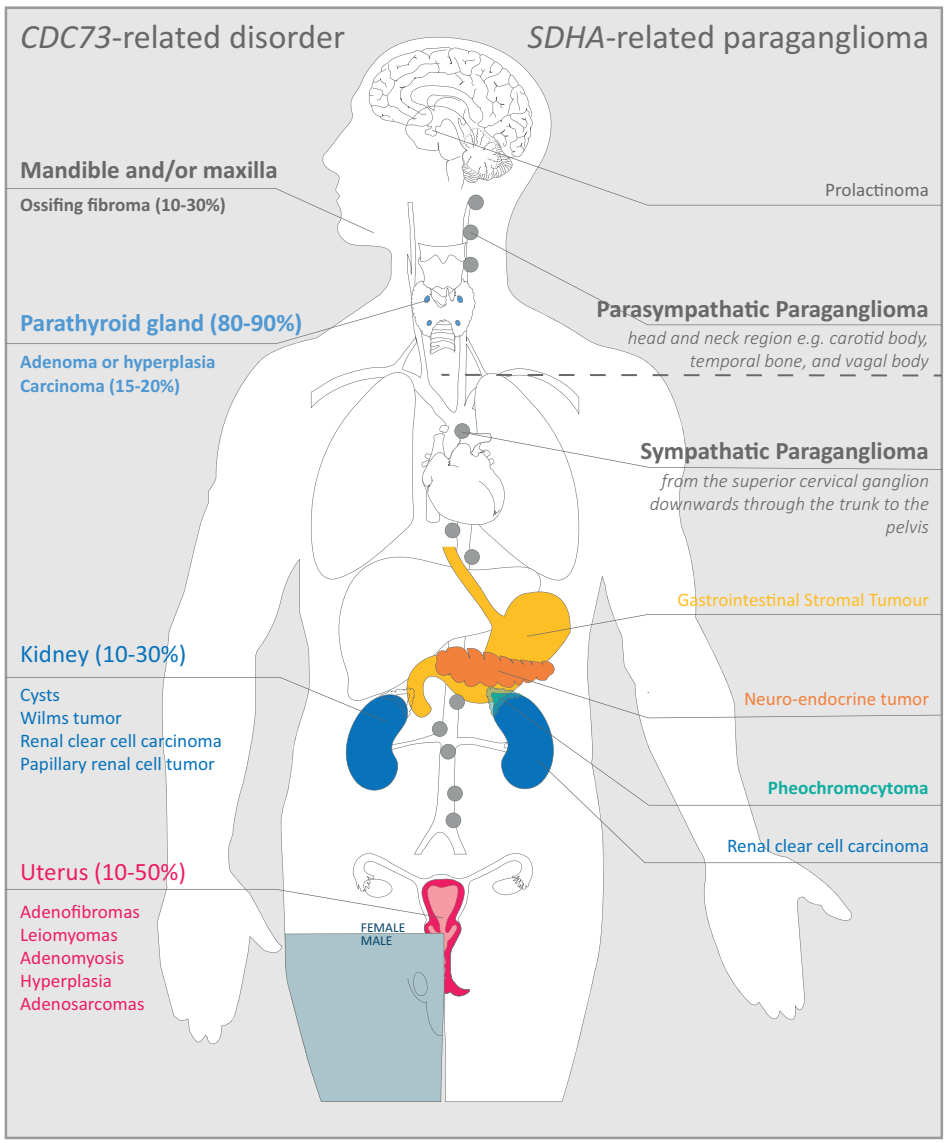


Figure 4. *CDC73*-related disorder (left) and *SDHA*-related paraganglioma (right) associated tumors. Clinical hallmarks in bold. Between brackets; estimated *CDC73*-related disorder disease penetrance. *SDHA*-related disease penetrance unknown.

have been reported.[83-85] No clear phenotype-genotype relationship has been identified in the approximately 120 index *CDC73* mutation carriers described to date.[34]

Genetic predisposition to paraganglioma

About one third of the PGL patients reportedly carry germline mutations in a growing list of susceptibility genes.[86] The best described genes, summarized in Table 3, are: *NF1, RET, VHL, SDHD, SDHC, SDHB, SDHAF2, SDHA (see below), TMEM127* and *MAX*. Germline mutations in the succinate dehydrogenase (*SDH*) genes are the most common genetic cause of PGLs, occurring in up to 15% of all PGL patients and half of all familiar cases.[50,87] In the last decade at least 12 additional genes have been associated with PGL, mostly in case reports (*BAP1, DNMT3A, EGLN1, KIF1Bβ, IHD, FH, MITF, MEN1, MDH2, PHD1, PHD2/EPAS1, and SLC25A11*) and it is likely that further rare and/or low-penetrant genes will be identified.

Table 3. Hereditary syndromes associated with paraganglioma and pheochromocytoma

Syndrome	Gene (locus)	Year report^	Inheritance	Mutation yield	PGL vs PHEO*	Multiple	Metastatic risk	Syndromic features
PGL1	*SDHD* (11q23)	2000	Paternal	8-9%	HNPGL	~50%	Low	Gastro intestinal stromal tumor, prolactinoma, RCC, pNET
PGL2	*SDHAF2* (11q13)	2009	Paternal	<0.1%	HNPGL	~90%	Low	
PGL3	*SDHB* (1q21)	2001	AD	10-25%	PGL	~20%	~50%	
PGL4	*SDHC* (1p35-36)	2000	AD	2-8%	PGL	~20%	Low	
PGL5 (Figure 4)	*SDHA* (5p15)	2010	AD	0.6-3%	HNPGL	Rare	Low	
	MAX (14q23)	2011	Paternal	~1%	PHEO	~60%	~25%	None
	TMEM127 (2q11)	2010	AD	~2%	PHEO	~25%	Low	None
NF1	*NF1* (17q11.2)	1990	AD *(cave de novo)*	<5%	PHEO	~15%	Low	Neurofibromas, café au lait macules, freckling
VHL	*VHL* (3p25-26)	1993	AD *(cave de novo)*	2-11%	PHEO	~40%	<5%	Hemangioblastomas, RCC, pNET
MEN2 (Figure 3)	*RET* (10q133.1)	1994	AD *(cave de novo)*	<5%	PHEO	~60%	<5%	MEN2a: MTC, pHPT MEN2b: neuromas, marfanoid habitus

AD; autosomal dominant, PGL; paraganglioma, HNPGL; head and neck paraganglioma; PHEO; pheochromocytoma, pNET; pancreatic neuro-endocrine tumor, RCC; renal cell carcinoma, MTC; medullary thyroid carcinoma; pHPT; primary hyperparathyroidism, ^direct association between gene and disease, *predominant phenotype.

SDHA-associated paraganglioma

In 2010, a direct association between germline *SDHA* mutations and PGL was reported.[88] The clinical phenotype seems to be comparable with the other SDH genes; e.g. predominately characterized by PGLs, with an additional risk of developing other tumor types such as clear cell renal cancer (RCC), gastrointestinal stromal tumors (GIST) and more rarely, neuroendocrine tumors (NET) and pituitary adenomas (see Figure 4)[89-91] Moreover, germline *SDHA* variants were recently identified in children and adults with various cancers, although a direct association has not been proven.[92] *SDHA* variants are also observed at an unexpectedly high frequency in the general population (Genome Aggregation Database cohort, public available genomic database), with ~1% and ~0.1% harboring a rare missense or loss of function variant, respectively.[93] To date, 39 unique (likely) pathogenic *SDHA* variants have been reported in about 100 index PGL patients, most of which were nonsense or frameshift variants, with the remainder made up of splice site and missense variants.[94-96] Of the index cases, half presented with HNPGL, whereas the remainder manifested either with PHEO or SPGL. The mean age at diagnosis was 40 years (range 15-81), with an equal gender distribution. Germline *SDHA* mutations have been associated with an increased risk of metastatic disease.[95] Notably, few patients reported a positive family history for (possibly) *SDHA*-associated disease, suggesting that the overall penetrance is substantially lower compared to the other SDH genes. The latter conclusion is supported by the high *SDHA* variant frequency in the general population.[93]

Q3: HOW CAN WE PREVENT CANCER?

Ideally, mutation carriers should be enrolled in specific surveillance programs that have been designed to improve their prognosis. In addition, genetic risk factors can be addressed in clinical practice by educating families and their treating physicians about early signs of disease. Collaboration between among others the departments of endocrinology, oncology, surgery, pathology, chemistry, radiology, nuclear medicine and clinical genetics is of the utmost importance. However, the advantages of early tumor detection should be weighed against the disadvantages of tumor screening, e.g. false positive and negative results, potential risk due to the screening modality itself (e.g. radiation), anxiety, negative emotional impact and healthcare costs.

In summary, with the implementation of high-throughput DNA/RNA sequencing platforms, somatic and germline genetic information may provide answers to the question *"Why do I have cancer?"*. Furthermore this molecular information has the potential to improve endocrine tumor classification, prognostic forecasting, the development of personalized treatment and the identification of patients at high risk for tumor development. Identification of endocrine predisposition syndromes, i.e. *Are other relatives at risk?,* cannot be seen separately from the question *"Do these relatives need to undergo surveillance?"*. Current challenges in known tumor predisposition syndromes include accurate estimates of variant pathogenicity, disease penetrance, genotype-phenotype relationships and the variable phenotypes within families, and from there to tailored treatment and surveillance guidelines. Clinical information on the rare endocrine tumor syndromes studied in this thesis, e.g. *DICER1*-related TC, *CDC73*-related disorder, and *SDHA*-associated PGL, has so far been limited to small case series.

OBJECTIVES AND OUTLINE OF THIS THESIS

The main objectives of this thesis were:

1. To investigate the role of molecular testing in TC diagnostics and treatment decision making.
2. To improve knowledge of the genetic background of pediatric non-medullary TC by:
   - determining the contribution of mutations in known cancer predisposition genes, and
   - identifying novel TC susceptibility genes.
3. To further delineate the genotype and phenotype of known endocrine tumor predisposition syndromes, i.e**.** DICER1 syndrome, MEN2a syndrome, *CDC73*-related disorder and *SDHA*-associated PGL.

Thesis outline:

Part I. The role of molecular testing in endocrine cancer diagnostics and treatment decision making

In **Chapter 2** we perform genetic characterization of 10 *DICER1*-related TC and report on follow-up of affected individuals. In **Chapter 3** we determine the contribution of somatic gene fusions in RAI-R TC, with the intention to stratify for targeted therapy.

Part II. Identification of genetic predisposition in pediatric non-medullary thyroid carcinoma

Chapter 4 describes the first results of a whole genome study investigating the contribution of mutations in known cancer predisposition genes and novel TC susceptibility genes in pediatric patients with non-medullary TC.

Part III. Genetic counseling in endocrine tumor predisposition syndromes

In **Chapter 5** we describe the clinical manifestations and penetrance in *CDC73*-related disorders and formulate recommendations to improve case detection in pHPT. In **Chapter 6** we estimate the contribution of germline *SDHA* mutation in PGL patients, assess the clinical manifestations and determine the age-related penetrance. **Chapter 7** describes an unusual case of apparent non-penetrance in a family with MEN2a.

Part IV: General discussion

Chapter 8 summarizes the main findings this thesis in the context of the current literature. Moreover, future perspectives for genetic testing will be discussed in a broader context.

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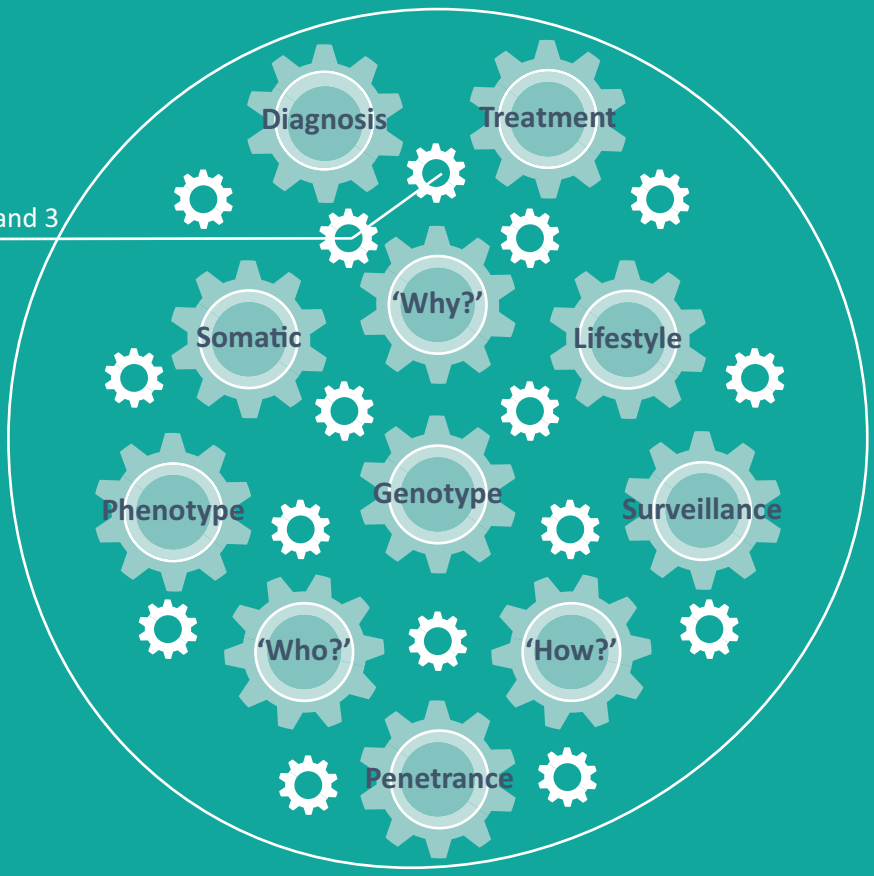
Part I
Chapter 2 and 3



PART I

THE ROLE OF MOLECULAR TESTING IN ENDOCRINE CANCER DIAGNOSTICS AND TREATMENT DECISION MAKING