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This chapter 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.

Discussion and Further Perspectives

This thesis began with the questions of a 12-year-old girl diagnosed with thyroid cancer: *"Why do I have cancer? Are other relatives at risk? And if so, can we prevent cancer?"* In the included studies we have attempted to address these questions, and they contributed to the formulation of the general objectives of this thesis, which were 1) to investigate the role of molecular testing in thyroid cancer (TC) diagnostics and in treatment decision making, 2) to improve knowledge of the genetic background of pediatric non-medullary TC, and 3) to further delineate the genotype and phenotype of DICER1 syndrome, MEN2a syndrome, *CDC73*-related disorder and *SDHA*-associated paraganglioma. The current chapter summarizes the main findings of the studies described in the six previous chapters in the context of the current literature. Moreover, future perspectives for genetic testing will be discussed in a broader context.

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

Over the last decade, the study of genetic alternations contributing to tumorigenesis has improved tumor classification, prognostic forecasting and the development of personalized treatment. Emerging evidence from clinical practice indicated that molecular tumor analysis could guide treatment choice, thus optimizing the selection of effective targeted treatments and reducing side effects and treatment costs.

In **Part I** of this thesis we explored the germline and somatic DNA/RNA variants in TC with the dual aims of improving diagnostics and guiding treatment decisions.

In **chapter 2** we described the genetic characterization of ten DICER1-related young onset cases of differentiated thyroid carcinoma (DTC) and reported on follow-up of the affected persons.[1] The identification of distinct somatic *DICER1* RNase IIIb hotspot variants in separate presumed-malignant and benign nodules sampled from individual patient lesions indicated that the tumors were polyclonal lesions. Furthermore, nine of the ten DICER1-related DTC lacked the well-known oncogenic driver DNA variants (e.g. *BRAF*, *RAS*) and gene rearrangements (e.g. *RET/PTC1-12*, *PPARg-PAX8*, *ALK*-, and *NTRK*-) that are frequently observed in sporadic TC. In addition to these molecular findings, occasional ambiguous histological features and lack of extrathyroidal extension, infiltrative growth, vascular invasion, or lymph node or distant metastasis (at a mean follow-up of 8 years), suggests that most DICER1-related young onset DTCs form a low-risk subgroup within the DTC spectrum. Which is in contrast to children with sporadic DTC whom frequently present with advanced disease (e.g. lymph node involvement at diagnosis, distant metastases, and multifocal disease).[2] Despite the excellent prognosis for pediatric DTC patients (30-year mortality <5%), morbidity caused by the treatment (surgery and radioactive iodine) remains considerable.[3] Based on our findings, DICER1-related thyroid neoplasia might often require hemithyroidectomy or total thyroidectomy due to the extent of nodules, but radioiodine treatment may be unnecessary given the patients' age and the tumors' low propensity for metastases (based on reports published thus far).[4-13]

While TC typically has a good prognosis following standard treatments, advanced cases of radioactive iodine refractory (RAI-R) DTC, anaplastic thyroid carcinoma and medullary thyroid carcinoma (MTC) have a poorer prognosis.[14-17] Management options in these patients include active surveillance, local therapy for metastatic sites (e.g. surgery or external beam radiation), or multi-kinase inhibitor therapy for rapidly progressing, symptomatic, or life-threatening disease.[17,18] Our improved understanding of the molecular alterations underlying TC has allowed researchers to develop targeted drugs and strategies.[19]

Our study, described in **chapter 3**, shows that mitogen-activated protein kinase (MAPK)-related gene fusions are relatively frequently found in recurrent RAI-R TC. For technical reasons, gene fusion analysis in RNA isolated from formalin-fixed tumor tissues has been limited until recently. We now show that extensive gene fusion analysis on formalin-fixed samples is feasible and effective. This is important because patients with TC and other tumor types are often treated in hospitals where tumors are solely processed using formalin fixation and paraffin embedding. The identified gene fusions in RAI-R TC provide a rationale for the incorporation of specific kinase inhibitors in the treatment regimen for these patients, with the intention to restore iodine transport and/or take advantage of a direct effect on tumor cell viability. However, the identification of variants in actionable genes does not necessarily mean that an associated targeted treatment will be effective in clinical practice.

Not unlike RAI-R TC, treatment options for metastatic parathyroid carcinoma (PC), paraganglioma (PGL) and pheochromocytoma (PHEO) are limited. The following paragraphs will discuss the future perspectives for metastatic PC, PGL and PHEO treatment, respectively, in relation to recently published integrated molecular data.

With multiple surgical resections of recurrent or metastatic disease, most patients with PC show long-term survival (5-year mortality ~10%). However, systemic therapy may be required over the long term. Cytotoxic regimes have not proven effective and current treatment focuses on controlling hypercalcemia. Previous studies reported often mutually exclusive somatic genetic alterations in *MEN1* and *CDC73*, which are currently not amenable to targeted therapy. Recent comprehensive genetic profiling studies have reported additional genetic alterations (e.g. *PTEN, PIK3CA, NF1, KDR*), in the presence of *CDC73* mutations, with rationally matched targeted agents (e.g. buparlisib). [20,21] The PI3K/Akt/mTOR pathway was the most frequently altered pathway and clinical trials are currently underway that target the PI3K/Akt/mTOR pathway in solid tumors. Single PC cases have shown clinical benefit from tyrosine kinase inhibitors.[20] As PC is one of the rarest of all human cancers (<0.005% of all cases), patients with unresectable metastatic disease should be enrolled in so called bucket (or basket) trials, i.e. one molecular abnormality targeted across multiple tumor types. Furthermore, immunotherapy that includes immune checkpoint inhibitors (i.e. targeting and disrupting PD-1/PD-L1 and CTLA-4/B7 interactions to boost the immune response against cancer cells) has transformed the treatment approaches for solid tumors in recent years.[22,23] Tumor mutational burden (TMB) serves as one of the biomarkers for response to checkpoint inhibitor treatment. [24] High TMB typically translates into a higher neo-antigen load, and therefore a greater chance that an antigen capable of stimulating an immune reaction will be expressed on the tumor cell surface and recognizable to a cytotoxic T-cell.[24,25] Although the median TMB in PC is relatively low (1.7 mutations per megabase (m/Mb) compared to 3.6m/Mb for all human cancers), about 20% show a high mutational burden (>20m/Mb).[20] These patients might benefit from immunotherapy, including checkpoint inhibitors.

The molecular understanding of PGL and PHEO has recently advanced due to comprehensive characterization of these rare tumors.[26] PGLs/PHEOs are driven by diverse alterations affecting multiple genes and pathways. Several molecular markers (e.g. *MAML3* fusion gene, *SDHB* germline mutations and somatic mutation in *SETD2* or *ATRX*) were associated with increased risk of metastatic disease.[26] Current therapeutic options rely on classic chemotherapy regimens, conventional external beam radiation, radiopharmaceuticals (Iodine-131 metaiodobenzylguanidine, ^{131}I-MIBG) or Peptide Receptor Radionuclide Treatment (PRRT e.g. ^{177}Lu-DOTATATE).[27,28] The described genetic alterations may also serve as potential drug targets in metastatic disease for which treatment options are limited. *SDH*-mutated tumors, associated with high levels of glutamine, might benefit

from the glutaminase inhibitor CB-839, as currently evaluated in a clinical trial (NCT 02071862). PHEOs with an altered Wnt pathway could potentially benefit from downstream inhibitors such as β-catenin and STAT3 antagonists.[29,30] Furthermore, FDA-approved targeted (indirect) therapies are available for specific tumors carrying *VHL, RET, BRAF, EPAS1* or *FGFR1* mutations. Despite the recent progress in this area, none of the therapy options mentioned has been approved for metastatic PGL/PHEO due to the rarity of the disease and lack of prospective studies.

To summarize Part I, recent molecular analysis of advanced endocrine tumors (e.g. RAI-R TC, metastatic PC, PGL and PHEO) has improved both our fundamental understanding of these rare neoplasms and has provided further possibilities for novel targeted therapies. Besides functional analysis of the detected variants, clinical trials are needed to determine the feasibly of these treatments. Furthermore, about half of the advanced endocrine cancer cases do not harbor genetic alterations in known cancer-associated genes. Integration of different 'omics' data (e.g. genomics, epigenomics, transcriptomics, proteomics and metabolomics) will be an important challenge in the near future.[31]

PART II: IDENTIFICATION OF GENETIC PREDISPOSITION IN PEDIATRIC NON-MEDULLARY THYROID CARCINOMA

With the introduction of next-generation sequencing (NGS), the last decades have seen remarkable advances in our understanding of the genetic contribution to disease. A growing list of genes has been associated with hereditary endocrine tumors. Nevertheless, most children who develop DTC are still genetically unexplained. To the best of our knowledge, the frequency of various germline mutations in cancer predisposition genes has not been systematically studied in a large cohort of unselected children with DTC. Earlier studies mainly relied on a candidate-gene approach in selected patients, an approach that is limited by design.

The aim of **Part II** was to improve knowledge of the genetic background of pediatric DTC by 1) determining the contribution of mutations in known cancer predisposition genes, and 2) identifying novel DTC susceptibility DNA variants.

Chapter 4 describes the first results of our study investigating the Genetic background of Non-medullary Thyroid cancer in Pediatrics (GeNoThyPe) using whole genome sequencing. So far 33 genes are analyzed in 64 out of 100 pediatric thyroid cancer patients. Causative germline mutations were relatively frequent (8%) in a subset of known cancer predisposition genes, including *DICER1* and *APC*. Based on distinct thyroid histology, pathologists may play a crucial role in recognizing features for selecting patients for genetic testing. Future stepwise analysis of whole genome data might result in the identification of novel DTC susceptibility DNA variants. Combining pathway analysis with the use of somatic molecular profiles seems a good strategy. For example, in children with somatic chromosomal alterations such as *RET/PTC*1-12 fusions, we will focus on so-called caretaker genes that are involved in the maintenance of human genome stability (DNA repair pathways). Moreover, the co-occurrence of thyroiditis and DTC is well recognized. However, it remains unclear whether thyroid inflammation is instrumental in causing the cancer or whether inflammation is a result of the cancer. Intrathyroidal lymphocytic infiltration was frequently seen in our series. Focusing on, for example, human leukocyte antigen (HLA) genes and immune response pathways in these cases may result in the detection of susceptibility alleles. Alongside the acquisition of whole genome data, several challenges came to light, including storage, distribution and analysis of large datasets. New bioinformatic strategies in the coming years, may lead to improved interpretation of whole genome data, making these unique data very valuable.

Likewise, in clinical diagnostics traditional exon-by-exon Sanger sequencing of each candidate gene has been (partly) replaced by high throughput NGS that involves the parallel sequencing of millions of short DNA fragments. The advantages and limitations of single gene testing, panel testing and whole exome sequencing are summarized in Table 1.

In general, comprehensive testing (targeted panel and whole exome sequencing) increases in efficiency both in terms of time and costs if more than one gene is related to a certain disease.[32,33] This major improvement has provided the opportunity to incorporate genetic results into treatment decisions, i.e. surgical management and tailored (neo) adjuvant treatment. Whole exome sequencing allows a syndrome to be identified that was not in the initial differential diagnosis (perhaps due to the clarity or lack thereof of a patient's personal or familial characteristics) and therefore would have been missed with single gene/limited gene testing. Why are we therefore not performing whole exome sequencing for each patient? The drawback of testing many genes is the complex interpretation of the results. The probability of 1) 'secondary' findings, i.e. genetic variants not related to the phenotype, and 2) variants of uncertain significant (VUS) increases with the number of tested genes. Clinical geneticists are currently confronted with the question of whether these incidental findings should be shared with patients. The American College of Medical Genetics and Genomics (ACMG) recommends reporting of germline mutations from a specific set of 59 genes when clinical diagnostic sequencing was ordered, even when unrelated to the primary medical reason for testing.[34] Pathogenic variants in these selected genes may require medical intervention aimed at preventing or significantly reducing morbidity and mortality. In contract to the ACMG listed genes, for other conditions such as hereditary neurological diseases, no treatment or prevention is available. Sharing these findings will not influence morbidity or mortality but this knowledge might potentially influence important life decisions and/or reproductive choices. On the other hand this knowledge might represent a psychological burden (e.g. distress and anxiety). Furthermore, identification of a VUS might require additional testing e.g. immunohistochemical staining, somatic mutation analysis and/or functional assays. Pre-test genetic counseling and tiered informed consent is therefore of utmost importance, as shown in recent studies.[35,36] The patient's preference, and tolerance regarding the possibility of ambiguous or secondary findings, plays a crucial role in establishing the preferred sequence modality. Even for highly educated patients this topic can be difficult to understand.[35,37] Furthermore, on some points our present technical capabilities might have outreached our medical knowledge, e.g. specific phenotypes, estimated

Table 1. Current advantages and limitation of single gene-, panel- and whole exome testing

Characteristics	Single gene testing	Targeted panel	Whole exome sequencing
Comprehensive testing	Low	Moderate	High
Time effective	Low-moderate*	High	Moderate
Phenotypically driven	High	Moderate	Low
Medical management guideline	High	Moderate	Low
Chance of variants of uncertain significance	Low	Moderate	High
Chance of secondary findings	No	Low	Moderate
Costs	Low	Moderate	High

* depending on number of disease-associated genes

cancer risks, lack of surveillance guidelines. Therefore, in many cases, limited phenotype-driven gene testing in clinical diagnostics remains the appropriate testing option.

Given the increasing number of associated genes, in **chapter 5 and 6** we recommend gene panel testing for primary hyperparathyroidism (pHPT)[38] and hereditary PGL, respectively.[39] While NGS technologies are improving rapidly, analysis of some genes with NGS, including *SDHA* and *CDC73*, is still challenging for technical reasons (e.g. presence of pseudogenes, GC-rich regions and/or common deletions/duplication). To obtain comparable sensitivity, additional Sanger sequencing of 'core genes', i.e. genes that are responsible for a significant proportion of the defects, should be considered in cases with a high suspicion of hereditary disease (see below) without detectable mutations following NGS.[40]

To summarize Part II, the introduction of whole exome and whole genome sequencing for diagnostic and research purposes may lead to identification of a syndrome that was not in the initial differential diagnosis. The drawback of testing many genes is the complex interpretation of the results. Pre-test genetic counseling to establish the preferred sequence modality and tiered informed consent is therefore of utmost importance. Genetic testing in a broader context i.e. commercially available DNA tests, is discussed in the end of this chapter.

PART III. GENETIC COUNSELING IN ENDOCRINE TUMOR PREDISPOSITION SYNDROMES

In **Part III** of this thesis we explored the phenotype and penetrance in *CDC73*-related disorder and *SDHA*-related paraganglioma.[38,39] The main objective was to improve identification of individuals with these endocrine tumor predisposition syndromes, thus improving both genetic counseling as well as early detection of tumor development. In addition, we described an unusual case of apparent non-penetrance in MEN2a syndrome.

Chapter 5 demonstrates that germline *CDC73* mutations are frequently (12.4%) found in previously genetically-unexplained pHPT patients. Our finding further suggests that genetic testing should be recommended in four clinical subgroups: 1) individuals with pHPT and HPT-JT syndrome- related features, 2) familiar isolated pHPT, 3) atypical or malignant parathyroid histology, and 4) young individuals with pHPT. The listed clinical subgroups of patients with pHPT are also in line with the 2015 Consensus Report on hereditary hyperparathyroidism of the European Society of Endocrine Surgeons.[41] Clinical use of these criteria could increase mutation carrier detection, thus enabling optimal clinical management of pHPT patients, as well as genetic counseling and surveillance for family members at-risk for developing related disorders. The estimated penetrance of *CDC73*-related disorders in our nationwide study was 83% at age 70 years (95% confidence interval 57-99%) in 43 non-index mutation carriers, comparable with previous studies.[42,43] The relatively low prevalences of ossifying fibroma of the jaw (5/43), renal abnormalities (8/43), and uterine fibroids (n=1/25) in our series could be due to inadequate surveillance and incomplete follow-up data. Alternatively, the high penetrance observed in prior studies (20-60%)[44-47] may be due to ascertainment bias (see below). There are currently no well-established surveillance guidelines for individuals with a *CDC73*-related disorder. Based on our data and literature review we proposed surveillance for *CDC73*-related features as described in Table 2.

Chapter 6 demonstrates that germline *SDHA* mutations are relatively frequent (7.6%) in genetically unexplained PGL patients, even in the absence of familial or clinical indications for inherited PGL. Mutation analysis of *SDHA* should therefore be included in the genetic testing of all patients with PGL. The clinical phenotype in the investigated *SDHA* families is similar to previous studies (i.e. with a few non-PGL tumors such as gastrointestinal stromal tumors, clear cell renal

Table 2. Concept Dutch expert opinion surveillance guidelines for *CDC73*-related disorder

CDC73-related feature	Frequency / starting age	Surveillance modality
pHPT	Annual from age 5-10y old	Clinical, biochemical (Calcium, PTH, vitamin D)*
Jaw	Ones per 5 years from age 18y old	Orthopantomogram
Renal	Ones per 5 years from age 18y old	Ultrasound
Uterine (female)	On indication	Ultrasound

pHPT; primary hyperparathyroidism, y; years
*consider radiology for non-producing parathyroid carcinoma (rare)

cancer and pituitary adenoma).[48,49] Our study confirms the suspected low penetrance of disease in *SDHA* mutation carriers (10% at age 70 years in non-index *SDHA* mutation carriers). This suggests that recommendations for genetic counseling of at-risk relatives and stringency of surveillance for *SDHA* mutation carriers might need to be reassessed. Current Dutch surveillance guidelines for *SDHA*-related PGL starting at age 18 years old are presented in Table 3. Despite very low disease penetrance at young ages, others have proposed surveillance in childhood.[50]

Accurate penetrance estimation is of utmost importance for the development of reliable surveillance guidelines. Current genetic surveillance guidelines and programs are potentially influenced by several forms of bias. The following paragraphs discuss: 1) the different forms of bias, and 2) the phenotypic variability between and within families *(in green)*, as illustrated by the pedigree in Figure 1. These kind of biases, including ascertainment bias (*in blue*), testing bias (*in orange*) and surveillance bias (*in pink*), are often related to a retrospective study design. Likewise, the two nationwide retrospective studies described in **chapters 5 and 6** may have been influenced by these factors.

Ascertainment bias. Disease penetrance can be overestimated due to ascertainment bias *(in blue)*. The clinically ascertained family in Figure 1 was selected due to their compliance with genetic testing and/or referral criteria. Reported families are often severely affected, i.e. an unusually low age at diagnoses (IV-1, 15 years old) and/or many affected family members (e.g. III-1, IV-2). Due to stringent referral criteria for genetic testing (in the past), less severely affected families may not have been referred for genetic testing. Until recently, a step-wise mutation testing protocol was applied in suspected familiar PGL and pHPT. Multiple algorithms were used, including age at presentation, location of tumor, multifocality or metastatic disease, presence of syndromic features and family history.[41,51] This type of testing protocol is relatively expensive,

Table 3. Dutch expert opinion surveillance guidelines for *SDHA*-related paraganglioma

SDHA- related feature	Frequency /starting age	Surveillance modality
HNPGL	Annual control otolaryngology from age 18y old*	Clinical and physical examination MRI head neck 1x/ 3 year
PHEO/SPGL	Annual control endocrinology from age 18y old*	Clinical and physical examination, blood pressure and metanephrines. MRI on indication

HNPGL; head and neck paragangliomas, SPGL; sympathetic paraganglioma, PHEO; pheochromocytoma, y; years
* Consider 5 years before earliest presentation within a family

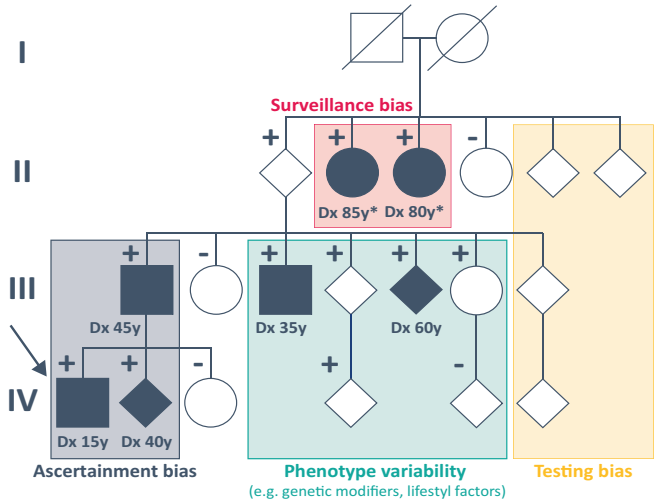

Figure 1. Pedigree illustrating several forms of bias that might occur in retrospective family studies. ircles represent females; squares represent males; diamonds represent undisclosed gender, disease affected (fully shaded), mutation carriers (+) and non-carriers (-), proband indicated with an arrow, Dx; age at diagnose, y; years, *diagnosis after genetic predisposition was confirmed. Patients are in the text referred to their position in the pedigree: generation (I-IV) and number from left to right.

time-consuming and highly dependent on a comprehensive personal and family history, which might result in a lower diagnostic yield. Nowadays, gene panel testing is fast and cost-effective for germline genetic testing of PGL patients.[32] With more widespread access to genetic testing, an increasing number of apparently sporadic PGLs are now being identified as hereditary, as shown in **Chapter 6**. As expected, following the inclusion of these families in recent studies, *SDH*-related paragangliomas showed lower disease penetrance compared to earlier reports. For example, *SDHB* penetrance estimates have fallen over time from ~55% to ~20% by age 50 years.[52-55] Going one step further, mutation carriers detected as a secondary finding of (large) gene panels or whole exome sequencing for another indication differ phenotypically from patients referred for single gene testing, who generally have typical features. A question worthy of further exploration is whether these patients should be treated and counseled differently. The clinical imaging discussion has focused on balancing the benefits of radiological surveillance versus negative effects in terms of patient anxiety, resource allocation and ionizing radiation. Early removal of tumors may prevent or minimize complications. However, this is counterbalanced by the need for lifelong surveillance starting at an early age and the possible psychological burden of not knowing whether, when, or how (benign or metastatic) tumors will develop. Furthermore, surveillance clearly does not guarantee early detection. Interval carcinomas, i.e. tumors which appear after a negative screening test or examination, may occur for technical or biological reasons (e.g. tumor aggressiveness and surveillance interval).

Testing bias. Penetrance estimates are often influenced by testing bias (*in orange*). Affected family members (III-1, III-3, III-5 and IV-2) were referred for genetic testing, whereas II-2, II-3 and III-4 were not tested for the germline variant, possibly because they didn't show a phenotype. Notably, probands (i.e. in this case the first person in the family with a confirmed germline mutation, indicated with an arrow in Figure 1) are the most obvious example of testing bias and excluding them from penetrance estimations should be considered carefully.

Surveillance bias. As part of the genetic diagnostic process, mutation carriers will undergo surveillance for tumors associated with a germline variant. Which in turn might lead to over diagnosis of small indolent lesions which otherwise would not have been diagnosed (II-7, II-8; surveillance bias, *in pink*).

Nowadays, statistical models are available (e.g. modified segregation analysis) to correct for these types of bias and have been used in large studies of hereditary breast and colon carcinoma.[56,57] However, one disadvantage is possible overcorrection of risk estimates and therefore underestimation of the risk in clinic-based families. Besides using all the available retrospective data, researchers should therefore concentrate on building (international) prospective cohorts to provide more reliable data on gene-stratified disease penetrance. This in turn could lead to gene-specific (personalized) surveillance guidelines with, if applicable, integration of *genotype-phenotype* relationships, *polygenic* risk models and lifestyle factors. Another approach is to use data from publicly available *genomic databases*, such as the Exome Aggregate Consortium (ExAC), to calculate penetrance via Bayesian statistics, i.e. the population frequencies of pathogenic germline variants should be inversely proportional to their penetrance for disease.[58] Using this approach, Maniam *et al*. recently provided support for the etiological role of *SDHA* in PGL formation, while simultaneously suggesting that most germline *SDHA* mutations are associated with very low disease penetrance and that therefore carriers might not benefit from periodic surveillance screening.[59]

Furthermore, additional challenges in the development of tailored surveillance guidelines include the commonly reported phenotypic variability between and within families (*in green*). A range of theories have been proposed to explain this observation: e.g. genotype-phenotype correlations, additional genetic susceptibility loci, parent-of-origin effects and lifestyle factors. A polygenic risk score, i.e. multiple genetic loci with associated weighting, might in the future provide better risk prediction. However, data on endocrine tumor predisposition syndromes are currently very limited.

One genotype-phenotype association (i.e. the association between an individual's genotype and the resulting pattern of clinical findings) that represents an exception is the comprehensively studied *RET* gene. Various consortia have defined the risk profiles of common *RET* mutations associated with Multiple Endocrine Neoplasia (MEN) syndrome type 2, including tailored guidelines for surveillance and prophylactic thyroidectomy.[17,60] Although virtually all patients develop MTC, the age of onset and aggressiveness of MTC, and the incidence of PHEO and pHPT in MEN2 (10-50%), is highly dependent on the specific *RET* mutation.[17] In **chapter 7** we describe an unusual case of apparent non-penetrance in a 93-year-old carrier of an ostensibly *de novo* non-mosaic *RET* germline mutation (codon 620). His affected son (MTC at age 50 and PHEO at age 55) and grandson (MTC at age 19) illustrate that disease penetrance still varies among carriers of an identical *RET* gene mutation. *RET* gene polymorphisms, single nucleotide polymorphisms in other genes,[61,62] mitochondrial DNA mutations,[63] copy number alterations[64] and post transcriptional modifications[65] have been suggested as potential genetic modifiers.

In hereditary PGL, gene-phenotype correlations have been used to guide genetic testing, surveillance, and in some cases, to recommend treatment.[51,66,67] These gene-related phenotypes include tumor location, presence of metastases, biochemical profile and aspects on nuclear imaging.[68,69] Genotype-phenotype correlations in *SDHB*[70] and *SDHD*[53] have been suggested and if confirmed, these findings could be used to stratify tumor surveillance programs according to individual mutation risks.

To summarized Part III, the identification of endocrine predisposition syndromes, i.e. Are other relatives at risk?, cannot be seen separately from the question "Do they need to undergo surveillance?" Our studies contributed to the debate on accurate estimation of disease penetrance. Prospective studies, including genotype-phenotype relationships, genetic modifiers and/or environmental factors, are required to determine the optimal age at which surveillance should be initiated, and the monitoring intervals that best capture the different related manifestations as they develop.

FUTURE GENETICS IN A BROADER CONTEXT

In addition to the questions of the 12-year-old girl with TC, many other questions in which genetics plays a major roll have been and will be asked by our society. The Dutch National Research Agenda for example, driven by the Dutch general public, contains over 150 questions related to DNA. In addition to the research projects described in the previous six chapters, I contributed, in collaboration with others, to different projects that focused on communicating genetics to a broader (non-scientific) audience (e.g. Lowland Science, Science Battle and public lectures - see PhD portfolio). The following paragraphs will give an overview of the fast changing field of genetics and discusses the future of genetics in a broader context, focusing on genetic testing and genome editing.

Genetic testing

History was made in 2003 when the Human Genome Project was completed. Sequencing the first complete human genome took about 13 years and cost more than three billion dollars. Today sequencing takes one to two days and costs less than 1000 dollars. We cannot imagine what the situation will be in 15 years. Is the 100 dollar genome really possible?, as suggested by the largest maker of DNA sequencers (Illumina). Questions worth asking include: In time, will the DNA code of every human be known? Will it become normal to receive the DNA code of your newborn baby on a flash drive directly after birth? And who else will have access to these data? Health insurance companies? Your employer? Facebook or Google? Is it reasonable to 'open' specific parts of the DNA code at different stages of life? Can we subsequently prevent or treat major diseases? Will the potentially expensive medical treatments resulting from this new area of research be covered by health insurance companies?

Currently, most genetic testing goes through the clinical geneticists and/or increasingly through treating physicians. However, a growing number of companies (e.g. 23 and me, My heritage) offer direct-to-consumer genetic tests for a variety of purposes (e.g. heritage, fun DNA facts and health-related features). Direct-to-consumer genetic testing has both benefits and limitations, although they are somewhat different to the genetic tests ordered by a healthcare provider. Customers send the company a saliva (DNA) sample and receive their results directly from a secure website or in a written report without necessarily involving a healthcare provider or health insurance company in the process. Consumers are able to learn about their ancestry and health risks at the cost of just $99 to a few hundred dollars. These companies make DNA testing more widely available, which may lead to increased awareness of genetic disease and might help some people to be more proactive about their health. However, since there is currently little regulation of direct-to-consumer genetic testing services, this genetic information might be inaccurate, incomplete or misunderstood. People may subsequently make important decisions regarding disease treatments or prevention based on incorrect results. Furthermore, genetic

testing for cancer risk can be stressful, provides incomplete information regarding your health and cannot definitively determine whether you will or will not develop cancer. The involvement of other genetic factors, family history and/or environmental factors is discussed during a medical consultation, but is often inadequately addressed in direct-to-consumer tests. People who use direct-to-consumer DNA testing are often apparently healthy, which makes the interpretation of (likely) pathogenic variants, as well as VUS, even more challenging than in patients with a specific disease. Moreover, direct-to-consumer DNA testing raises concerns around the privacy of genetic data. Consumers might not realize that these companies retain data (and DNA samples) and that this information might be accessed by third parties without consumer consent through, for example, sale to other companies, hacking, law enforcement and/or the government. Given the growing fascination with genetic testing due to curiosity, ancestry, or recreational motivations, the government and comparable authorities should facilitate consumer education and the regulation and quality control of providers. However, the combination of internet-based genetic testing, different nations and local authorities remains challenging.

Genome editing

In late 2018 the world was shocked by the news that the first genetically modified twins were born. Jiankui He, a Chinese researcher, claimed via YouTube that he had successfully used CRISPR/Cas9 to disable the *CCR5* gene so that the twin girls might be resistant to potential infection with HIV. Although DNA experts knew that this development - introducing alterations into the human germline that can be passed to offspring - was inevitable, it had been considered off-limits. CRISPR/Cas9 is a technique that permits the highly specific and rapid modification of DNA in any genome. Briefly, the Cas9 protein cuts both DNA strands at the place where a single-guide RNA binds. The double strand break will be repaired by non-homologous end joining which often results in mutation of the target gene, or 'correction' when a replacement DNA segment is supplied. Human-related applications might include curing inherited genetic disorders, treating infectious diseases, and advancing cancer treatment.

There is no doubt that CRISPR will be important in the coming years but this technology nowadays faces two major issues. The first issue is one of safety. While CRISPR is a relatively simple and powerful technique, it isn't flawless. A major concern is that the CRISPR technique might introduce (unpredictable) off-target mutations into the genome.[71] The second issue, particularly when using CRIPR to alter 'germline' cells, is a medical ethical dilemma. Most scientists agree that gene editing should be restricted to medical conditions. However, where does one draw the line between treating serious disease and 'enhancing' humans beyond what the society considers 'normal'? And who should determine these boundaries? Medical doctors/scientists? The government? Health incurrence companies? A dedicated committee? Furthermore, genome editing might need to be restricted to conditions where no alternative is available. In many cases, pre-implantation genetic diagnosis (PGD) i.e. embryo selection, might be an appropriate alternative.[72]

The rapidly expanding possibilities of DNA testing and the concept of editing the human genome both raise questions that science alone cannot answer. While physicians and scientist might can determine what will be possible in DNA testing and editing in near future, the broader public should decide if everything that is possible, retains also desirable. "Just because we could, does not mean we should". The relationship between science and the media has in the past been

characterized by terms such as *distance, gap* and *barrier,* this is the moment for the scientific community to break out of their 'ivory tower' and to discuss these topics with a broader public. In other words, while accurately presenting the facts, scientists could actively initiate a public debate about the science and about societal consequences and implications that may arise from potential new applications.

CONCLUDING REMARKS

Within the broad theme of endocrine cancer genetics, there are a few aspects we would like to highlight:

Clinical implications

- Gene fusion analysis in selected patients is effective and feasible for TC classification and stratification for targeted treatment.
- TC patients with DICER1-syndrome may form an low risk subgroup and should be treated in a center of expertise.
- Mutation analysis of *SDHA* should be included in the genetic testing strategy of all patients with PGL, preferably using gene panels.
- Germline *CDC73* mutation detection using clinical testing criteria enables optimal clinical management of pHPT as well as genetic counselling and surveillance

Implications for further studies and clinical practice

- International prospective studies in DICER1 syndrome, *CDC73*-related disorder and *SDHA*-related paraganglioma are needed to determine the optimal age at which surveillance should be initiated and the best monitoring intervals to capture the different related manifestations as they develop. With, if applicable, integration of genotype-phenotype relationships, polygenic risk models and lifestyle factors.
- Patients with advanced endocrine cancers (e.g. RAI-R TC, PC and PGL) should be enrolled in bucket or basket trials to investigated the potential of targeted treatment and/or immunotherapy.
- Exploring the possibility of (partly) mainstreaming genetic testing i.e. gene testing via attending physicians in patients with a high suspicion for an endocrine tumor predisposition syndrome.

Implications for future genetics in a broader context

- Improve information and regulation of direct-to-consumer genetic testing services.
- Determine CRISPR/Cas9 safety and discuss related medical ethical dilemmas.
- Strengthen the responsibility of the scientific community in the public debate about genetic testing and genome editing.

In order to provide answers to the questions Why?, Who? and How? for every patient, endocrine cancer care encourages active collaboration between among others the departments of endocrinology, oncology, surgery, pathology, chemistry, radiology, nuclear medicine and clinical genetics. Moreover, local- national- and international collaborations between basic- and clinical researchers should take research from bench to beside and back again.

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