THE ROLE OF AIP AND CDKN1B/p27^{Kip1} IN ENDOCRINE NEOPLASIA

Anniina Raitila

Faculty of Medicine
Department of Medical Genetics
Genome-Scale Biology Research Program
Haartman Institute and Biomedicum Helsinki
University of Helsinki
Finland

Academic dissertation

To be publicly discussed, with the permission of the Medical Faculty of the University of Helsinki, in lecture hall 3, Biomedicum Helsinki, Haartmaninkatu 8, on December 21st 2009, at noon

Helsinki 2009

Supervised by

Academy Professor Lauri A. Aaltonen, MD, PhD
Department of Medical Genetics
Genome-scale Biology Research Program
Haartman Institute and Biomedicum Helsinki
University of Helsinki
Finland

Docent Auli Karhu, PhD
Department of Medical Genetics
Genome-scale Biology Research Program
Haartman Institute and Biomedicum Helsinki
University of Helsinki
Finland

Reviewed by

Docent Hannu Haapasalo, MD, PhD
Department of Pathology
Center for Laboratory Medicine
Tampere University Hospital
Finland

Docent Ismo Ulmanen, PhD
Forensic Genetic Paternity Testing Unit
National Institute of Health and Welfare
Helsinki
Finland

Official opponent

Docent Maija Wessman, PhD
Research Program in Molecular Medicine
Folkhälsan Research Center
Helsinki
Finland

ISBN 978-952-92-6471-1 (paperback)
ISBN 978-952-10-5876-9 (PDF)
http://ethesis.helsinki.fi
Helsinki University Print
Helsinki 2009

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original articles referred to in the text by Roman numerals I-IV.

I Georgitsi M*, Raitila A*, Karhu A, Tuppurainen K, Mäkinen MJ,Vierimaa O, Paschke R, Saeger W, van der Luijt RB, Sane T, Robledo M, De Menis E, Weil RJ, Wasik A, Zielinski G, Lucewicz O, Lubinski J, Launonen V, Vahteristo P, Aaltonen LA (2007) Molecular diagnosis of pituitary adenoma predisposition caused by aryl hydrocarbon receptor-interacting protein gene mutations. *Proceedings of the NationalAcademy of Sciences of the United States of America* 104(10): 4101-4105.

II Raitila A, Georgitsi M, Karhu A, Tuppurainen K, Mäkinen MJ, Birkenkamp-Demtröder K, Salmenkivi K, Ørntoft TF, Arola J, Launonen V, Vahteristo P, Aaltonen LA (2007) No evidence of somatic aryl hydrocarbon receptor interacting protein mutations in sporadic endocrine neoplasia. *Endocrine-Related Cancer* 14(3): 901-906.

III Raitila A, Georgitsi M, Bonora E, Vargiolu M, Tuppurainen K, Mäkinen MJ, Vierimaa O, Salmela PI, Launonen V, Vahteristo P, Aaltonen LA, Romeo G, Karhu A (2009) Aryl Hydrocarbon Receptor Interacting Protein (AIP) mutations seem not to associate with familial non-medullary thyroid cancer. *The Journal of Endocrinological Investigation* 32(5): 426-429.

IV Georgitsi M*, Raitila A*, Karhu A, van der Luijt RB, Aalfs CM, Sane T, Vierimaa O, Mäkinen MJ, Tuppurainen K, Paschke R, Gimm O, Koch CA, Gündogdu S, Lucassen A, Tischkowitz M, Izatt L, Aylwin S, Bano G, Hodgson S, De Menis E, Launonen V, Vahteristo P, Aaltonen LA (2007) Germ-line CDKN1B/p27Kip1 mutation in multiple endocrine neoplasia. *The Journal of Clinical Endocrinology & Metabolism* 92(8): 3321–3325.

* Equal contribution

Publication I was included in the thesis of Marianthi Georgitsi (Genetic basis of pituitary adenoma predisposition, Helsinki 2008)

ABBREVIATIONS

A	adenine
aa	amino acid
ACTH	adrenocorticotrophin
AIP	aryl hydrocarbon receptor-interacting protein
AHR	aryl hydrocarbon receptor
ALK	anaplastic lymphoma kinase
ARA9	aryl hydrocarbon receptor-associated protein-9 (or AIP, XAP2)
ARNT	aryl hydrocarbon receptor nuclear translocator
bp	base pair
BIRC5	baculoviral IAP repeat-containing 5 (survivin)
BRCA1	breast and ovarian cancer 1
BRCA2	breast and ovarian cancer 2
C	cytosine
CNC	Carney complex
cAMP	cyclic adenosine monophosphate
CDK4	cyclin-dependent kinase 4
CDKN1B/p27^{Kip1}	cyclin-dependent kinase inhibitor 1B
DNA	deoxyribonucleic acid
EBNA3	Epstein-Barr virus (EBV) nuclear antigen-3
FH	fumarate hydratase
FIPA	familial isolated pituitary adenomas
FKBP	FK506 binding protein
FSH	follicle-stimulating hormone
G	guanine
GADD45γ	growth arrest and DNA-damage-inducible gamma
GH	growth hormone
GNAS	guanine nucleotide-binding protein, alpha stimulating activity polypeptide
Gsα	guanosine triphosphate-binding protein
GTP	guanosine triphosphate
HIF1-α	hypoxia inducible factor 1, alpha subunit
HNPCC	hereditary non-polyposis colorectal cancer
HSP90	heat-shock protein 90
IFS	isolated familial somatotropinoma
IGF-I	insulin-like growth factor 1
IHC	immunohistochemistry
KIT	v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog
LH	luteinizing hormone
LOH	loss of heterozygocity
MAS	McCune-Albright syndrome
MEN1	multiple endocrine neoplasia type 1
MEN4	multiple endocrine neoplasia type 4
MET	met proto-oncogene (hepatocyte growth factor receptor)

MIM	Mendelian Inheritance in Man
MLH1	MutL *E.coli* homologue 1
MSH2	MutS *E.coli* homologue 2
MRI	magnetic resonance imaging
NFPA	non-functioning pituitary adenoma
NMTC	non-medullary thyroid cancer
PAP	pituitary adenoma predisposition
PCR	polymerase chain reaction
PDE2A	phosphodiesterase 2 A
PDE4A5	phosphodiesterase 4 A5
PKA	protein kinase A
PPAR-α	peroxisome proliferation-activated receptor alfa
PPNAD	pigmented nodular adrenocortical disease
PRKAR1A	protein kinase A (PKA) regulatory subunit 1 alfa
PRL	prolactin
PTC	parathyroid cancer
ptg-FGFR4	pituitary tumor-derived fibrolast growth factor receptor 4
RET	rearranged during transfection proto-oncogene
SNP	single nucleotide polymorphism
T	thymine
THRβ1	thyroid receptor beta 1
TOMM20	translocase of the outer membrane of mitochondria 20
TPR	tetratricopeptide repeat
TSH	thyrothophin-releasing hormone
XAP2	hepatitis B virus x-associated protein 2 (or AIP,ARA9)

In addition, standard one-letter codes are used to denote aminoacids.

ABSTRACT

Identification of genes predisposing to tumor syndromes has raised general awareness of tumorigenesis. Genetic testing of tumor susceptibility genes aids the recognition of individuals at increased risk of tumors. Identification of novel predisposing genes enables further studies concerning the classification of potential associated tumors and the definition of target patient group.

Pituitary adenomas are common, benign neoplasms accounting for approximately 15% of all intracranial tumors. Accurate incidence estimation is challenging since a great portion of these adenomas are small and asymptomatic. Clinically relevant adenomas, that cause symptoms due to the expansion of the cell mass or the over-secretion of normally produced hormones, occur in approximately one of 1 000 individuals. Although the majority of pituitary adenomas are sporadic, a minority occur as components of familial syndromes, such as Multiple Endocrine Neoplasia type 1 (MEN1) and Carney complex (CNC). MEN1 syndrome is caused by germ-line mutations in the *MEN1* gene, whereas most of the CNC patients carry the mutated *protein kinase A (PKA) regulatory subunit-1-*α (*PRKAR1A*) gene.

Recently, other conditions predisposing to endocrine tumors have been identified: Pituitary Adenoma Predisposition (PAP) and MEN type 4 (MEN4). PAP was originally identified in a genetically homogeneous Finnish population. In a population based cohort from Northern Finland, *aryl hydrocarbon receptor-interacting protein* (*AIP*) gene mutations were found in 16% of all patients diagnosed with growth hormone (GH) producing pituitary adenoma, and in 40% of the subset of patients who were diagnosed under the age of 35 years.

Since *AIP* mutations were originally described in a defined, homogeneous population from Northern Finland, it was relevant to study whether mutations also occur in more heterogeneous populations. In patient cohorts with different ethnic origins and variable clinical phenotypes, germ-line *AIP* mutations were detectable at low frequencies (range 0.8-7.4%). *AIP* mutation-positive patients were often diagnosed with a GH-producing adenoma at a young age, and usually had no family history of endocrine tumors. The low frequency of *AIP* mutations in randomly selected patients, and the lack of any family history of pituitary adenomas create a challenge for the identification of PAP patients. Our preliminary study suggests that AIP immunohistochemistry may serve as a pre-screening tool to distinguish between the *AIP* mutation-negative and the mutation-positive tumors.

Tumors of various endocrine glands are components of MEN1 and CNC syndromes. Somatic *MEN1* and *PRKAR1A* mutations in sporadic pituitary adenomas are rare, but occur in some of the other tumors related to these syndromes. The role of *AIP* mutations in endocrine neoplasia was studied and

our results indicated that somatic *AIP* mutations are rare or non-existent in sporadic tumors of endocrine glands (0 of 111). Furthermore, germ-line *AIP* mutations in prolactin producing adenomas (2 of 9) confirmed the role of this pituitary tumor type in the PAP phenotype.

Thyroid disorders are common in the general population, and the majority of them are sporadic. Interestingly, it has been suggested that thyroid disorders might be more common in PAP families. For this reason we studied germ-line *AIP* mutations in 93 index cases from familial non-medullary thyroid cancer (NMTC) families. The underlying gene or genes for familial NMTC have not been identified yet. None of the patients had any potentially pathogenic *AIP* mutation. This suggests that AIP is unlikely to play a role in familial NMTCs.

A novel multiple endocrine syndrome was originally described in rats with phenotypic features of human MEN type 1 and 2. Germ-line mutations of *cyclin-dependent kinase inhibitor 1B* (*CDKN1B* also known as $p27^{Kip1}$) gene were reported later in these rats and a germ-line mutation was also identified in one human family with MEN1-like phenotype (later named MEN4). To confirm the importance of this gene's mutations in humans, we performed a mutation screening in MEN-like patients and in patients with pituitary adenoma. Our results indicate that *CDKN1B*/$p27^{Kip1}$ mutations appear in a small portion of MEN1-like patients (one of 36), and that such mutations are rare or non-existent in both familial (0 of 19) and sporadic pituitary adenoma patients (0 of 50).

In conclusion, this work strengthens the tumor susceptibility role of *AIP* and *CDKN1B*/$p27^{Kip1}$ in endocrine neoplasia. Clarifying the PAP phenotype facilitates the identification of potential *AIP* mutation carriers. Genetic counseling can be offered to the relatives and follow-up of the mutation carriers can be organized, hence an earlier diagnosis is feasible.

1. INTRODUCTION

Tumors arise from normal tissue when a cell transforms into a malfunctioning one. The growth advantage of the cell is achieved by accumulation of genetic alterations. Variety of environmental and lifestyle factors can affect tumor formation due to their ability to mutate genes. These factors correlate with the incidence of certain cancers such as exposure to ultraviolet radiation with skin cancer, tobacco smoking with lung cancer, and inadequate diet with stomach cancer (Weinberg 2007).

An individual with an inherited mutation in a crucial gene is predisposed to tumor formation at a higher risk than the general population. However, a single mutated gene alone is seldom sufficient to trigger tumor formation but does contribute to it. For example, it has been estimated that 15 mutations are involved in the initiation, progression, and maintenance of colorectal and breast cancer (Wood *et al.* 2007). Approximately 1.5% of human genome carries the sequence information that encodes the structures of protein and occurrence of somatic mutations in human tissues is estimated to be less than 10^{-8} per base pair (Bielas *et al.* 2006; Weinberg 2007). Thus, sporadic tumor formation is a slow process. Mutation patterns vary between different tumor types but all of them display features uncommon to normal cells. These include self-sufficiency in growth signals, insensitivity to growth-inhibitory signals, evasion of apoptosis, limitless replication potential, sustained angiogenesis, and the capability of invasion and metastasis (Hanahan and Weinberg 2000).

Tumors can be divided into two categories according to their growth pattern. Benign tumors grow locally without invading adjacent tissues, whereas malignant tumors are able to invade and send metastases. The majority of the primary tumors are actually benign and thus cause no symptoms. In rare cases, however, the over-secretion of hormones and/or excessive tumor expansion causing pressure on the adjacent tissues, lead to clinical symptoms. For example growth hormone (GH) producing pituitary adenomas lead to acromegaly. Even though benign tumors only rarely cause deaths, those tumors can be clinically important.

This work focuses on endocrine neoplasia caused by mutations in two genes, *aryl hydrocarbon receptor-interacting protein* (*AIP*) and *cyclin-dependent kinase inhibitor 1B* (*CDKN1B* also known as $p27^{Kip1}$). Both genes predispose to pituitary adenomas and the latter gene also to other endocrine tumors. The contribution of these genes to tumorigenesis in endocrine neoplasia is not completely clarified. This work aims to study the role of *AIP* and $CDKN1B/p27^{Kip1}$ in endocrine neoplasia.

2. REVIEW OF THE LITERATURE

2.1. Tumor genes

As mentioned above, the transformation from a normal cell to a tumor lesion requires several cellular events. These include mutations in the genome leading to loss of function of tumor suppressor genes or to gain of function of oncogenes.

2.1.1. Tumor suppressor genes

Tumor suppressor genes can be divided into three groups according to their role in cellular processes: gatekeepers, landscapers, and caretakers (Kinzler and Vogelstein 1997; Kinzler and Vogelstein 1998). Inhibitors of the cell cycle progression and promoters of the apoptosis of abnormal cells are called gatekeepers. Landscaper genes are involved in the cellular microenvironment (stromal cells). Caretaker genes repair DNA errors and therefore mutations in these genes leads to the accumulation of alterations in the genome (Kinzler and Vogelstein 1997; Kinzler and Vogelstein 1998).

Tumor suppressor genes are mainly inactivated when the activity of both alleles is lost (Knudson's two hit hypothesis) (Knudson 1971). In heritable neoplastic syndromes the "first hit" is in the germ-line and the "second hit" occurs somatically to the wild-type allele. Often the inherited mutation is small, such as a point mutation or a small deletion/insertion, and the somatically occurring one is larger, such as loss of a particular chromosomal area. Loss of the tumor suppressor function may also be due to haploinsufficiency or dominant-negative effect when only a single allele inactivation is enough to promote tumorigenesis (Payne and Kemp 2005).

Predisposing mutations in tumor suppressor genes have been identified in several well known syndromes. These genes include *BRCA1* and *BRCA2* in hereditary breast and ovarian cancer (Futreal *et al.* 1994; Miki *et al.* 1994; Wooster *et al.* 1995), *MEN1* in multiple endocrine neoplasia type 1 (MEN1) (Chandrasekharappa *et al.* 1997), *FH* mutations in hereditary leiomyomatosis and renal cell cancer (Tomlinson *et al.* 2002), and mismatch-repair genes (*e.g. MLH1* and *MSH2*) in hereditary nonpolyposis colorectal cancer (HNPCC) (Bronner *et al.* 1994; Fishel *et al.* 1993; Leach *et al.* 1993; Papadopoulos *et al.* 1994).

2.1.2. Oncogenes

Oncogenes are abnormally activated normal genes, called proto-oncogenes. These normal genes are involved in several cellular processes, such as cell proliferation, differentiation, and apoptosis. Oncogene activation is achieved by activating point mutations in the proto-oncogene, chromosomal translocations, or gene amplifications. This leads to either increased expression of the normal protein or to aberrant activation of the gene in unfamiliar conditions. Activating point mutations and translocations are normally initiating events in the tumor formation or occur during tumor progression, whereas amplifications mainly occur later in tumorigenesis (Croce 2008).

Inherited oncogene mutations are rare but some have been identified such as *RET* in multiple endocrine neoplasia type 2 (Mulligan *et al.* 1993), *MET* in hereditary papillary renal cell carcinoma (Schmidt *et al.* 1997), *KIT* in familial gastrointestinal stromal tumors (Nishida *et al.* 1998), *CDK4* in familial malignant melanoma (Zuo *et al.* 1996), and *ALK* in familial neuroblastoma (Mosse *et al.* 2008).

2.2. Endocrine system

Major endocrine glands consist of pituitary, pineal, thyroid, parathyroids, adrenals, endocrine pancreas, thymus, testes (males), and ovaries (female) (Fig. 1). These tissues release signaling molecules, known as hormones, mainly into the bloodstream to affect the functions of the target tissues such as growth, development, and metabolism. Through the bloodstream the hormones can be circulated to every part of the body. This is called endocrine signaling. Some hormones are secreted straight from the neural cells to the endocrine gland (neuroendocrine signaling), for example the hypothalamus secretes hormones that regulate the function of the anterior pituitary gland. The target of hormones can also be the cells nearby (paracrine signaling), the cell's own receptors in the outer membrane (autocrine signaling) or the cell's own receptors in the nucleus (intracrine signaling) (Valimaki *et al.* 2009).

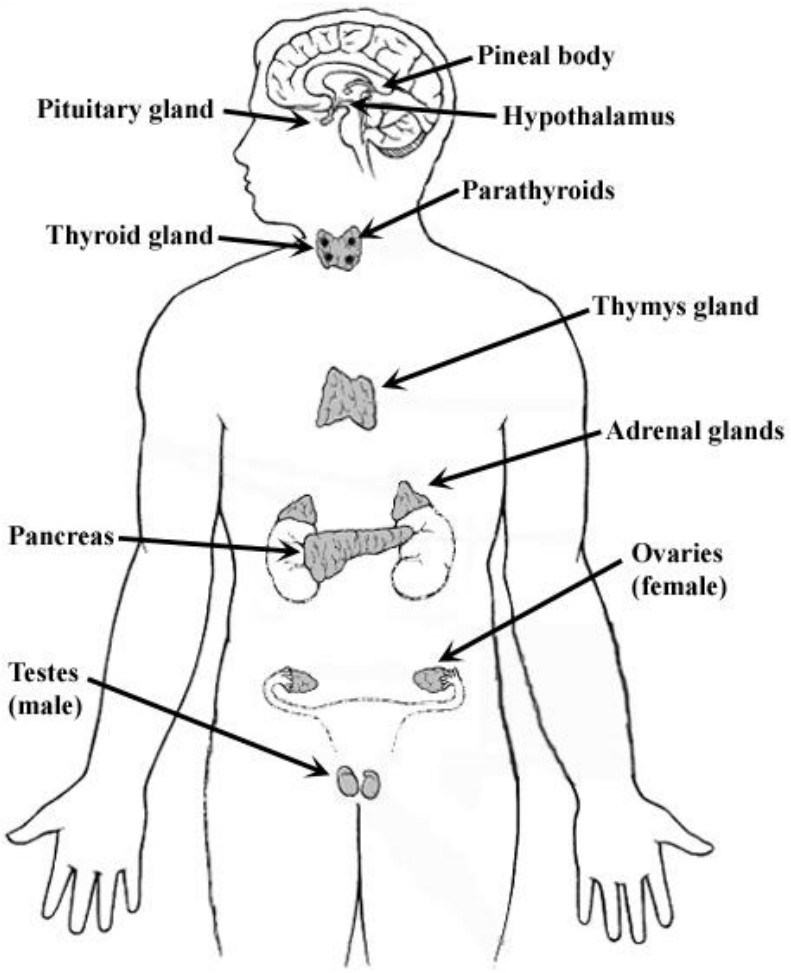

Figure 1. Major endocrine glands (modified from the website www.ama-assn.org).

Cooperation of the endocrine systems is composed of feedback mechanisms in both hormonal and neural communication networks. Positive and negative feedback control the hormone secretion. For instance, releasing and inhibiting factors secreted by the hypothalamus affect the function of the pituitary gland (Fig. 2). The pituitary hormones induce the hormone secretion of target organs and tissues. These peripheral hormones inhibit the hormone secretion of the hypothalamus and the pituitary gland. Also, para- and autocrine signaling are involved in the feedback system (Fig. 2) (Valimaki *et al.* 2009).

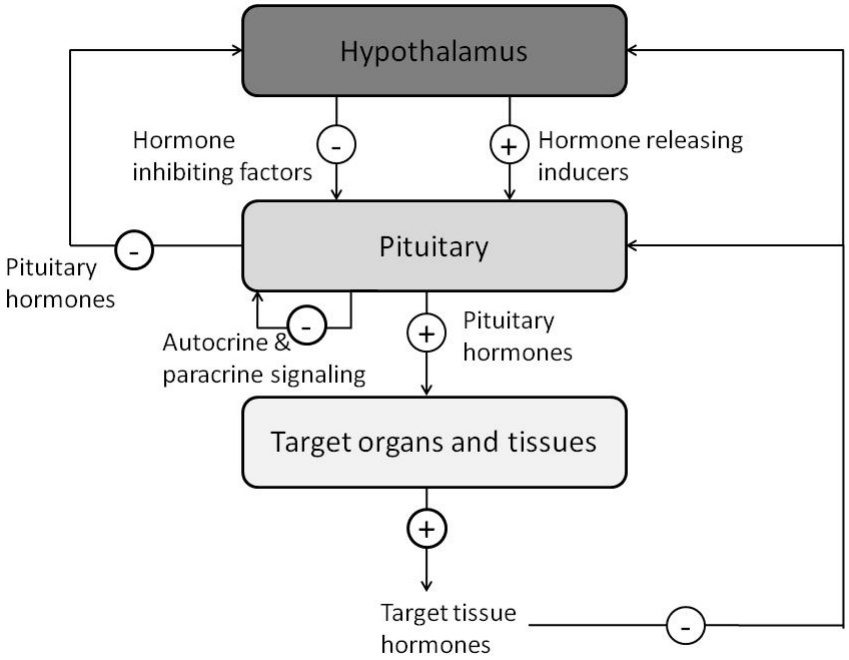

Figure 2. Diagram of the feedback control in the hypothalamic-pituitary-target tissue axis. Secretion of the pituitary hormone-releasing hormone induces the target tissue-stimulating hormone secretion from the pituitary gland. Pituitary hormones stimulate the synthesis and release of the hormones from the target tissue. Inhibition of the hormone secretion is achieved by negative feedback from the target tissues to the pituitary gland and the hypothalamus. Also autocrine and paracrine signaling affect the function of the pituitary gland and the hypothalamus. Stimulation is indicated by a plus sign, whereas inhibition is by a minus sign. The direction of the feedback is indicated by arrows. (Valimaki *et al.* 2009)

In this study, pituitary adenomas relate to both of the studied genes (*AIP* and *CDKN1B/p27^{Kip1}*) and therefore pituitary gland (section 2.2.1.) and its adenomas (section 2.3.1.) are introduced in more details.

2.2.1. The pituitary gland

The pituitary gland is located behind the eyes, at the base of the brain, and on top of the sphenoid bone (Fig. 1). The gland is a bean-shaped tissue and it is divided into the anterior and the posterior lobes (Fig. 3). Hypothalamic antidiuretic hormone (arginine vasopressin) and oxytocin are stored and secreted to the blood-stream from the posterior lobe when needed. Six different pituitary hormones are produced and released from the anterior lobe (Fig. 3, Table 1). These pituitary hormones regulate growth and development in general and as well as the function of three other endocrine glands: the thyroid, the adrenals, and the gonads (Valimaki *et al.* 2009).

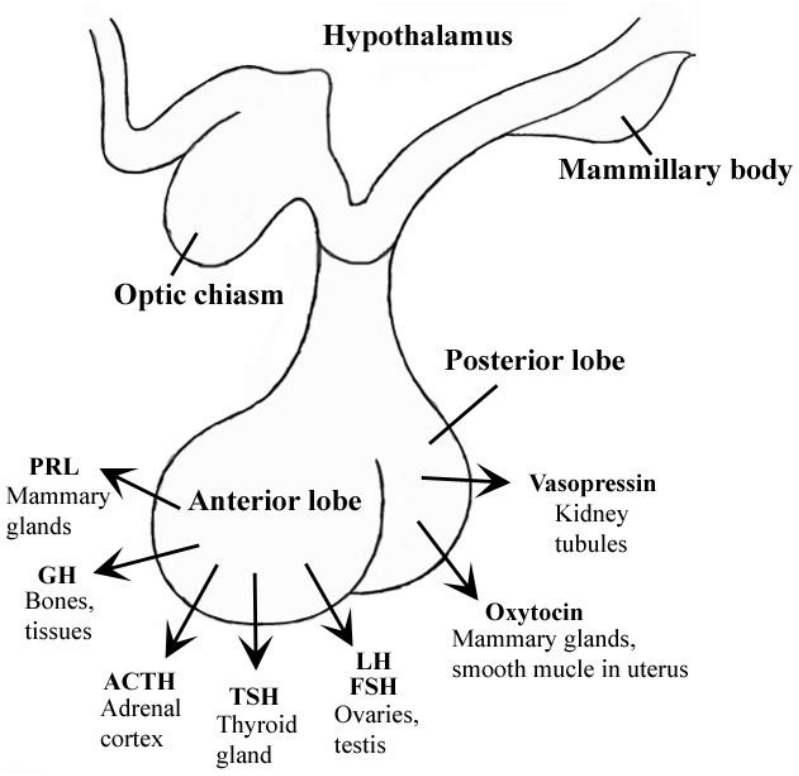

Figure 3. A schematic presentation of the pituitary gland, the secreted hormones, and the target tissues of the hormones.

2.3. Endocrine neoplasia

Neoplasias of the endocrine system are normally benign. Even thought malignancies are rare, thyroid cancers are the most common type, accounting for 1% of all cancers in the developed countries (Steward and Kleihues 2003). Tumors are prevalent in some endocrine tissues such as in the pituitary and thyroid glands. However, adrenal and pancreatic endocrine tumors are rare (DeLellis *et al.* 2004). Most of the endocrine tumors are sporadic but some arise as

components of familial syndromes such as multiple endocrine neoplasias, and Carney complex (introduced in section 2.4. below).

Clinical symptoms can vary in different endocrine disorders according to hormone activity. Decreased or lack of hormone synthesis can be due to a missing endocrine gland, dysfunction of hormone synthesis or destruction of the endocrine gland *e.g.* after infection or radiation. A defective response to hormones can be due to the incorrect structure of the hormone or malfunction of the receptor. Increased hormone synthesis is a consequence of a hyperplasia, a tumor or extra stimulation of the endocrine gland. (Valimaki *et al.* 2009)

Table 1. The pituitary hormones secreted by the anterior lobe. The target tissues and functions are also presented (Valimaki *et al.* 2009).

Pituitary hormone	Target tissue	Function
Prolactin (PRL)	Mammary glands	Initiation and maintenance of milk production
Growth hormone (GH)	Liver and other tissues	Metabolic and anabolic effects, growth stimulation, and induction of particularly hepatic IGF-I production
Adrenocorticotrophin (ACTH)	Adrenal glands (cortex)	Stimulation and maintenance of steroid synthesis
Thyroid stimulating hormone (TSH)	Thyroid gland	Stimulation of thyroid hormone (T_3 and T_4) production
Follicle stimulating hormone (FSH) and luteinizing hormone (LH)	Gonads (ovaries and testis)	Germ cell development and stimulation of sex steroid hormone production

2.3.1. Pituitary adenomas

Tumors, mostly benign, arise mainly from the anterior lobe of the gland. These adenomas are common accounting for ~15% of all intracranial tumors (Karhu and Aaltonen 2007). Hormonally inactive, small tumors with a slow growth rate do not necessarily cause symptoms, and therefore those are not detected until radiographic examination or post-mortem examination. In autopsy studies and in radiological imaging done for other indications, the prevalence of incidentally discovered pituitary adenomas is estimated to be 5-20% (Burrow *et al.* 1981; Hall *et al.* 1994; Molitch and Russell 1990). This was supported recently by a meta-analysis where pituitary adenomas were reported to occur with a frequency of 16.7% (Ezzat *et al.* 2004).

The majority of the clinically relevant adenomas produce hormones excessively, which can affect many organ systems, thus causing severe symptoms or even

death. In addition to the over-secretion of hormones, the expansion of the tumor mass causes symptoms, such as headache, and visual-field disturbance. Furthermore, tumor compression of the normal pituitary tissue can lead to loss of normal hormone production *i.e.* hypopituitarism (Arafah and Nasrallah 2001). These clinically relevant adenomas are rare, but more common than previously estimated, as indicated by recent cross-sectional study conducted in Belgium (1/1064 population) and an international study (average 0.75/1000 population) (Daly *et al.* 2006b; Daly *et al.* 2007a).

Pituitary adenomas appear to have reversible plasticity, varying from hypoplasia to hyperplasia (Melmed 2003). X-chromosome inactivation in human pituitary adenomas suggests that these lesions arise from a single pituitary cell that has acquired growth advantage due to genetic or epigenetic alterations (Alexander *et al.* 1990; Herman *et al.* 1990). According to animal models, long-term pituitary hyperplasia predisposes to tumor progression (Asa *et al.* 1992; Heaney *et al.* 1999). In humans, however, pituitary hyperplasia due to pregnancy or lactation, its enlargement due to estrogen administration, or untreated primary hypothyroidism does not seem to enhance tumor formation (Coogan *et al.* 1995; Ghannam *et al.* 1999; Horvath *et al.* 1999; Kovacs *et al.* 1994).

2.3.1.1. Classification of pituitary adenomas

The World Health Organization's classification of pituitary adenomas is based on clinical and biochemical features. These include imaging, operative findings, histology, immunocytochemistry, and electron microscopy (Kovacs *et al.* 1996). Adenomas can also be classified according to the size of the tumor where microadenomas are equal or less than 10 mm in diameter, macroadenomas greater than 10 mm but less than 4 cm, and tumors over 4 cm in diameter are giant adenomas (DeLellis *et al.* 2004). Classification can also be based on the endocrine activity as detailed in the following paragraphs and summarized in Table 2.

The majority of the pituitary adenomas over-secrete prolactin (PRL; 40-45%; Table 2) (Arafah and Nasrallah 2001). Prevalence of these adenomas, also called prolactinomas, is estimated to be 60-100 cases per million and their incidence is around 6-10 new cases per million per year (Ciccarelli *et al.* 2005; DeLellis *et al.* 2004). Besides symptoms due to the expansion of the tumor mass and variable degrees of hypopituitarism, over-secretion of PRL can lead to menstrual irregularities (amenorrhea or oligomenorrhea) or lactation (galactorrhea) in females, and to sexual impotence or decreased libido in males (DeLellis *et al.* 2004).

The second largest group of pituitary adenomas over-secrete growth hormone (GH; 20%; Table 2) (Arafah and Nasrallah 2001). These adenomas, also called somatotropinomas, can simultaneously over-secrete PRL. The clinical

manifestation depends on the age of occurrence of the adenoma and the time of exposure. GH over-secretion during childhood or adolescence, before epiphyseal fusion is completed, leads to accelerated linear growth termed "gigantism" (Eugster and Pescovitz 1999). Adulthood exposure to high GH-levels causes acromegaly with clinical features such as coarse facial features, broadened nose, enlarged extremities, obesity, organomegaly, sweating, and nausea (Chanson and Salenave 2008; Melmed 2006). Often adenomas causing acromegaly are large and therefore headaches and visual disturbances are common symptoms (Laws *et al.* 1985). Incidence of acromegaly is estimated to be 3-4 new cases per million per year and the prevalence 40-60 cases per million people (Alexander *et al.* 1980; Bengtsson *et al.* 1988; Kauppinen-Makelin *et al.* 2005). Morbidity of acromegaly depends on the exposure time of high GH and IGF-I levels (GH induces IGF-I production which controls linear and organ growth). Furthermore, untreated patients face an increased mortality risk caused by *e.g.* diabetes, cardiovascular or cerebrovascular disease (Colao *et al.* 2004; Erfurth and Hagmar 2005). Due to the slow progression and insidious onset of this disease, the diagnosis may take from four to more than ten years (Chanson and Salenave 2008).

Adrenocorticotrophin (ACTH) producing adenomas, also known as adenocorticotropinomas, account for 10-12% of all pituitary adenomas (Table 2) (Arafah and Nasrallah 2001). These tumors are mostly benign, but they show more invasive features than other pituitary adenomas (Arafah and Nasrallah 2001). ACTH over-secretion causes increased glucocorticoid production *i.e.* hypercortisolism with clinical features of central obesity, hypertension, proximal myopathy, striae, hirsutism, easy bruisability, mood changes, poor wound healing, menstrual irregularity, osteoporosis, hyperglycemia, increased supraclavicular, and dorso-cervical fat pads (Arafah and Nasrallah 2001). This clinical manifestation caused by the excess pituitary secretion of ATCH is called Cushing's disease.

Thyrotropin (TSH) producing adenomas, *i.e.* thyrotropinomas, are rare among pituitary adenomas (1-2%; Table 2) (Arafah and Nasrallah 2001). Diagnosis is often delayed and tumors tend to be macroadenomas with invasive and aggressive nature (DeLellis *et al.* 2004). Patients with these adenomas often present a goiter and hyperthyroidism but co-secretion of GH and/or PRL can occur in a minority of cases leading to acromegaly and/or amenorrhea/galactorrhea (Arafah and Nasrallah 2001; Beck-Peccoz *et al.* 1996; Beckers *et al.* 1991; Sanno *et al.* 2000).

Table 2. Classification of pituitary adenomas according to their hormone activity. (modified from Arafah and Nasrallah 2001; DeLellis *et al.* 2004)

Name	Prevalence	Clinical signs and symptoms
Prolactinomas (PRL-producing)	40-45%	Reproductive and sexual dysfunction
Somatotropinomas (GH-producing)	20%	Pre-pubertal: unrestrained somatic growth *i.e.* gigantism
		Post-pubertal: enlargement of acral parts of body *i.e.* acromegaly
Adenocorticotropinomas (ACTH-producing)	10-12%	Cushing's disease
Gonadotropinomas (NFPA)	10-15%	Mass effects causing headaches, visual disturbance, and hypopituitarism
Null-sell adenomas (NFPA)	5-10%	Mass effects (see gonadotropinomas)
Thyrotropinomas (TSH-producing)	1-2%	Goiter and mild hyperthyroidism

Endocrinically silent adenomas are non-functioning pituitary adenomas (NFPA) including both gonadotropinomas and null-cell adenomas (Table 2). The majority of NFPAs, accounting for 10-15% of all adenomas, are gonadotropinomas producing follicle-stimulating hormone (FSH) and/or luteinizing hormone (LH) (Arafah and Nasrallah 2001). However, the hormone secretion is mainly minimal or inefficient and therefore clinical behavior is due to the tumor mass expansion (Samuels and Ridgway 1995; Snyder 1995). Null-cell adenomas are truly endocrinically non-functioning and those account for 5-10% of all pituitary adenomas (Table 2) (Arafah and Nasrallah 2001). Null-cell adenomas grow slowly and their diagnosis is often made in the 6th decade of life (Kontogeorgos *et al.* 1993; Yamada *et al.* 1988). Because of the late diagnosis, these adenomas are often macroadenomas with cavernous sinus invasion and suprasellar extension occasionally reaching to the hypothalamus (DeLellis *et al.* 2004).

2.4. Disorders of pituitary adenomas

2.4.1. Sporadic pituitary adenomas

The majority of the pituitary adenomas (~95%) are sporadic tumors. Microarray studies have shown that the gene expression patterns vary, not only between pituitary adenomas and normal pituitary, but also between different pituitary adenoma subtypes (Fernandez-Ranvier *et al.* 2008; Moreno *et al.* 2005; Morris *et al.*

2005; Ruebel *et al.* 2006). These studies, among other approaches, have indicated potential factors in pituitary tumorigenesis such as pituitary tumor-derived fibroblast growth factor receptor 4 (*ptg-FGFR4*) (Ezzat *et al.* 2002), growth arrest and DNA damage-inducible gamma (*GADD45*γ) (Zhang *et al.* 2002), and guanine nucleotide-binding protein, alpha stimulating activity polypeptide (*GNAS*) (Vallar *et al.* 1987). However, in many cases it is still unclear which of these alterations (reviewed in Asa and Ezzat 2005) are involved in the initiation of sporadic pituitary adenoma formation, which of them promote the progression of the tumor growth or which are bystanders.

GNAS encodes guanosine triphosphate (GTP)-binding protein Gsα. This protein activates adenylyl cyclase that increases the cellular levels of cyclic adenosine monophosphate (cAMP). In sporadic GH-producing adenomas activating mutations of *GNAS*, also known as *gsp* oncogene, cause constitutive activation of Gsα, overactivity of adenylyl cyclase, and increased cAMP levels (Vallar *et al.* 1987). Approximately 40% of human GH-producing adenomas carry activating mutations in *GNAS* gene (Lyons *et al.* 1990). Furthermore, the expression of Gsα can be high in the GH-producing adenomas without these mutations (Picard *et al.* 2007).

McCune-Albright syndrome (MAS; MIM 174800) is a genetic, but not an inherited, congenital syndrome due to a post-zygotic somatic mutation in the *GNAS* gene (Schwindinger *et al.* 1992; Weinstein *et al.* 1991). These mutations lead to over-secretion of hormones in endocrine cells and also to excessive bone matrix formation. MAS is characterized by polyostotic fibrous dysplasia, café-au-lait spots, endocrine abnormalities such as excess of GH and PRL, and in rare cases by other tumors.

2.4.2. Familial pituitary adenomas

Approximately 5% of pituitary adenomas arise as components of familial syndromes. These include Multiple Endocrine Neoplasia type 1 (MEN1), Carney's Complex (CNC), and recently identified conditions: Pituitary Adenoma Predisposition (PAP) and Multiple Endocrine Neoplasia type 4 (MEN4). These familial syndromes and other conditions involving pituitary adenomas are described below and summarized in Table 3.

2.4.2.1 Multiple Endocrine Neoplasia type 1 (MEN1)

MEN1 (MIM131100; Table 3) is an autosomal dominant syndrome characterized by a predisposition to different combinations of tumors of the endocrine system. These tumors occur most commonly in parathyroids (~95%), pancreatic islet cells (~40%), and the anterior pituitary (~30%) (Lemos and Thakker 2008). Less common tumors include adrenocortical tumors, angiomyolipomas, foregut lipomas, angiofibromas, thyroid adenomas, carcinoids, and spinal cord

ependymomas (Chandrasekharappa *et al.* 1997; Thakker 1998). MEN1 is inherited with a high degree of penetrance; <95% of the patients over 40 year of age develop clinical manifestations of the syndrome (Bassett *et al.* 1998). Clinical symptoms are mainly due to hormone over-secretion, and without treatments MEN1 tumors are mortal (Lemos and Thakker 2008). Furthermore, relatives of MEN1 patients are at 50% risk of developing the disease (Benson *et al.* 1987; Calender *et al.* 1995; Marx *et al.* 1998; Trump *et al.* 1996).

The predisposing gene for MEN1 locates at 11q13 and codes for a protein called "menin" (Chandrasekharappa *et al.* 1997; Larsson *et al.* 1988). *MEN1* gene is comprised of ten exons, and encodes ten transcripts resulting in 555 aa to 615 aa long proteins (Ensembl 54). This protein is mainly nuclear and involved in transcriptional regulation, genome stability, cell division, and cell cycle control (reviewed in Lemos and Thakker 2008). In sporadic pituitary adenomas *MEN1* is rarely somatically mutated (*e.g.* Schmidt *et al.* 1999; Wenbin *et al.* 1999; Zhuang *et al.* 1997a) and also menin expression is rarely altered (*e.g.* McCabe *et al.* 1999; Theodoropoulou *et al.* 2004; Wrocklage *et al.* 2002). However, somatic *MEN1* mutations are found in other lesions related to this syndrome such as in pancreatic islet cell tumors (*e.g.* insulinomas ~15% and glucagonomas ~60%) (Hessman *et al.* 1998; Shan *et al.* 1998; Zhuang *et al.* 1997b), lipomas (~30%) (Pannett and Thakker 2001; Vortmeyer *et al.* 1998), and parathyroid tumors (~10-20%) (*e.g.* Carling *et al.* 1998; Farnebo *et al.* 1998; Heppner *et al.* 1997).

It has been estimated that 5-10% of MEN1 patients do not carry *MEN1* mutations in the coding region or adjacent splice sites (Lemos and Thakker 2008). These patients, however, might carry predisposing mutations in untranslated regions, in the promoter area, or in introns. It is also possible that they may present phenocopies of the disorder or occasionally belong to the multiple endocrine neoplasia type 4 (MEN4) (described in section 2.4.2.6.).

2.4.2.2. Carney Complex (CNC)

CNC (MIM160980; Table 3) is a rare, autosomal dominant, multiple endocrine neoplasia syndrome with spotty skin pigmentation, myxomatosis, schwannomas, and endocrine tumors (Carney *et al.* 1985). Endocrine tumors include *e.g.* testicular neoplasm (33% of male patients), pigmented nodular adrenocortical disease (PPNAD) (26% of patients), GH- or PRL-producing pituitary adenomas (~10%), and thyroid adenoma or carcinoma (5%) (Stratakis *et al.* 2001). The manifestation of CNC can be diverse and occur in different ages. For instance, PPNAD or cardiac myxomas are normally seen in the second or third decade of life but also already in the first couple of years of life (Boikos and Stratakis 2007a). Over half of the disease-specific deaths among CNC patients are due to cardiac myxomas (Boikos and Stratakis 2007a).

More than 60% of the CNC patients that meet diagnostic criteria have mutations in *protein kinase A (PKA) regulatory subunit-1-α* (*PRKAR1A*) gene (Kirschner *et al.* 2000). This gene locates at 17q24 and comprises of 11 exons and three possible transcripts each encoding for identical 381 aa long protein (Ensembl 54). PKA, also known as cAMP-dependent kinase, is involved in transcription, metabolism, cell cycle progression, and apoptosis. Loss of PRKAR1A function leads to enhanced intracellular signaling by PKA, thus causing elevated cAMP levels in CNC tumors (Kirschner *et al.* 2000). Somatic *PRKAR1A* mutations are infrequent in sporadic pituitary adenomas (Kaltsas *et al.* 2002; Sandrini *et al.* 2002a; Yamasaki *et al.* 2003) and in cardiac myxomas (Fogt *et al.* 2002; Mantovani *et al.* 2009). However, somatic mutations are detected in sporadic PPNAD (three out of five) (Groussin *et al.* 2002), adrenocortical tumors (3/44) (Bertherat *et al.* 2003), thyroid carcinomas (2/17) (Sandrini *et al.* 2002b), and odontogenic myxomas (2/21) (Perdigao *et al.* 2005).

Apart from the 17q14 *locus*, linkage to 2p16 has also been reported but a predisposing gene or genes have not been identified (Stratakis *et al.* 1996). Not all the CNC families map to predisposed areas and it is possible that a third so far unidentified region exist.

Table 3. Familial pituitary adenoma syndromes.

Clinical condition	Tissues most often affected by endocrine tumors	Chromosomal *locus*	Predisposing gene
Multiple Endocrine Neoplasia type 1	parathyroid glands, pancreas, pituitary gland	11q13	*MEN1*
Carney Complex	adrenal glands, testicles, pituitary gland, thyroid glands	17q23-4 2p16 other *locus*?	*PRKAR1A* unidentified unidentified
Pituitary Adenoma Predisposition	pituitary gland	11q13	*AIP*
Isolated Familial Somatotropinomas	pituitary gland [a]	11q13 other *locus*?	*AIP* unidentified
Familial Isolated Pituitary Adenomas	pituitary gland	11q13 other *locus*?	*AIP* unidentified
Multiple Endocrine Neoplasia type 4	parathyroid glands, pituitary gland, kidney [b]	12p13	*CDKN1B/p27^{Kip1}*

[a] Only GH-producing adenomas.
[b] One family reported.

2.4.2.3. Pituitary Adenoma Predisposition (PAP)

PAP is a recently characterized condition with familial pituitary adenomas (MIM102200; Table 3) (Vierimaa *et al.* 2006). The occurrence of PAP was suspected when three small clusters of familial pituitary adenomas were detected in Northern Finland. Two of these families were related through common ancestor couple born in the 1700s, which was found by additional genealogical studies (Vierimaa *et al.* 2006). The putative predisposing *locus* at 11q12-13 was identified using whole-genome single nucleotide polymorphism (SNP) genotyping and narrowed down with fine-mapping (Vierimaa *et al.* 2006). Previous studies had already suggested that a gene other than than *MEN1* in 11q13 would predispose to GH-producing pituitary adenomas as allelic loss of the region had indicated (Gadelha *et al.* 1999; Soares *et al.* 2005; Thakker *et al.* 1993; Yamada *et al.* 1997).

With the combined information from linkage data and whole transcriptome expression data the primary candidate predisposing gene *aryl hydrocarbon receptor-interacting protein* (*AIP*) was defined (Vierimaa *et al.* 2006). And indeed, pituitary adenoma clusters detected from Northern Finland harbored a nonsense germ-line mutation c.40C>T (p.Gln14X). This mutation segregated perfectly with GH- and GH/PRL-producing adenomas. *AIP* mutation screening was also performed for 45 acromegaly patients belonging to a population based cohort from Northern Finland (Kauppinen-Makelin *et al.* 2005), and six c.40C>T and one c.469-1G>A mutations were detected (Vierimaa *et al.* 2006). Thus, *AIP* mutations accounted for 16% (7 out of 45) of all acromegaly patients, and 40% of those diagnosed under the age of 35 years (6/15). *AIP* as a novel pituitary adenoma predisposing gene was further confirmed with the detection of a nonsense mutation c.910C>T (p.Arg304X) in two Italian acromegalic siblings (Vierimaa *et al.* 2006).

Loss of the wild-type allele was seen in all studied GH-, PRL, and GH/PRL-producing tumors from *AIP* mutation carriers (Vierimaa *et al.* 2006). Authors concluded that the mutations predispose to at least these tumor types, that the gene is likely a tumor suppressor, and that the penetrance of PAP appears to be low. The tumor suppressor role of *AIP* was further confirmed with functional studies (Heliovaara *et al.* 2009; Leontiou *et al.* 2008). In general, a typical PAP patient (*i.e. AIP* mutation carrier) is diagnosed at a young age and the family history of pituitary adenomas might not be convincing (Vierimaa *et al.* 2006).

AIP gene, also known as *XAP2* or *ARA9*, locates at 11q13 and the six exons code for a 330 aa long protein (Ensembl 54). An FKBP-homology domain is located in the amino-terminus of *AIP* and three tetratricopeptide repeats (TPR), which mediate protein-protein interactions, in the carboxy-terminal region. AIP is known to form a complex with aryl hydrocarbon receptor (AHR), with two 90

kDa heat-shock proteins (HSP90), and with p23 (Carver and Bradfield 1997; Chen and Perdew 1994; Kazlauskas *et al.* 1999). Interactions have been shown at least with PDE4A5, PDE2A, PPAR-α, BIRC5, THRβ1, TOMM20, EBNA3, and RET (Bolger *et al.* 2003; de Oliveira *et al.* 2007; Froidevaux *et al.* 2006; Kang and Altieri 2006; Kashuba *et al.* 2000; Sumanasekera *et al.* 2003; Vargiolu *et al.* 2009; Yano *et al.* 2003). Many of these proteins can be linked to pituitary tumorigenesis such as AHR that is involved in xenobiotic-induced metabolism and in the absence of xenobiotics in *e.g.* cell proliferation, cell adhesion, and migration (Barouki *et al.* 2007). Interestingly, a recent study showed significantly reduced immunoreactivity for AHR translocator (ARNT) and somewhat increased localization of AHR in the nucleus in the *AIP* mutation-positive adenomas compared to the mutation-negative ones (Heliovaara *et al.* 2009). This indicated that these molecules are potentially involved in tumorigenesis of *AIP* mutation-positive pituitary tumors.

Many interaction partners of AIP are known but the mechanism between how the loss of *AIP* tumor suppressor gene leads to the pituitary tumorigenesis remains to be elucidated. Hopefully the ongoing functional studies, both *in vitro* and *in vivo*, will clarify this issue.

2.4.2.4. Isolated Familial Somatotropinomas (IFS)

IFS (Table 3) is defined as follows: at least two individuals of acromegaly or gigantism in a familial setting in the absence of MEN1 and CNC (Gadelha *et al.* 1999). These patients are often diagnosed with macroadenomas at a young age (Soares and Frohman 2004). Efforts to identify the predisposing *locus* have shown loss of heterozygocity (LOH) in chromosome 11q13 but *MEN1*, locating in this area, was not mutated (Gadelha *et al.* 1999; Tanaka *et al.* 1998; Teh *et al.* 1998; Yamada *et al.* 1997). At the same *locus* Vierimaa *et al.* (2006) reported truncating mutations in *AIP* in three families with at least two cases of pituitary adenomas. Two of these families harbored only GH-producing adenomas *e.i.* IFS. Later on, the following mutation screening studies have identified additional IFS cases with *AIP* mutations (*e.g.* Daly *et al.* 2007b; Iwata *et al.* 2007; Leontiou *et al.* 2008; Toledo *et al.* 2007).

2.4.2.5. Familial Isolated Pituitary Adenomas (FIPA)

Compared to IFS, FIPA is a wider clinical entity with familial cases of pituitary adenomas not restricted only to GH-producing adenomas (Table 3). These families can be divided into subcategories according to tumor phenotype between the family members. Homogeneous are those families where only one tumor type exists and heterogeneous those with varying tumor types (Daly *et al.* 2006a). All subtypes of pituitary adenomas have been identified in heterogeneous kindreds but often at least one GH-producing or PRL-producing adenoma is present (Beckers and Daly 2007). The majority of the pituitary adenomas

(approximately 75%) in the FIPA cohort are PRL-producing, GH-producing, or GH/PRL-producing adenomas (Beckers and Daly 2007). Moreover, FIPA patients are often diagnosed at a younger age and with larger pituitary adenomas compared to corresponding sporadic pituitary counterparts (Beckers and Daly 2007).

Pituitary adenomas in the FIPA families were reported to be significantly larger in the *AIP* mutation-positive cases when compared to the *AIP* mutation-negative ones (Daly *et al.* 2007b). Authors concluded that the pituitary adenomas in the *AIP* mutation-positive patients were of an aggressive type. Indeed, preliminary results have indicated a poor response to therapy in the *AIP* mutation-positive patients (Daly *et al.* 2008; Leontiou *et al.* 2008). However, the majority of the FIPA patients do not harbor *AIP* mutations and therefore the underlying genetic component(s) of FIPA remains to be elucidated.

2.4.2.6. Multiple Endocrine Neoplasia type 4 (MEN4)

MEN4 (MIM610755; Table 3) is an autosomal recessive condition with MEN1-related tumors. Identification of this novel syndrome was based on a naturally occurring rat strain with features overlapping with both human MEN type 1 and type 2 (Fritz *et al.* 2002). These included bilateral pheochromocytomas, parathyroid adenomas, medullary thyroid cell neoplasia, parathyroid hyperplasia, pituitary adenomas, and endocrine pancreas hyperplasia (Fritz *et al.* 2002; Pellegata *et al.* 2006). A recent study identified a homozygous germ-line mutation in *Cdkn1b/p27^{Kip1}* gene in these rats (Pellegata *et al.* 2006). In the same study, a germ-line mutation in humans was reported in a family with two individuals diagnosed with MEN1-related tumors: the proband was diagnosed with primary hyperparathyroidism and pituitary adenoma and her sister with renal angiomyolipoma. Although the wild-type allele was retained in the tumor tissue, immunohistochemical staining of the *CDKN1B/p27^{Kip1}* gene protein product, called p27, showed no protein in the tumor tissue (Pellegata *et al.* 2006).

The *CDKN1B/p27^{Kip1}* gene at 12q13, has three protein coding transcripts containing two (205 aa) or three (104 aa and 198 aa) exons (Ensembl 54). Nuclear p27 negatively regulate the cell cycle by inhibiting cyclin and cyclin-dependent kinase complexes. Previous knockout mouse models had already indicated that this gene is involved in multiple endocrine neoplasia (Fero *et al.* 1996; Kiyokawa *et al.* 1996; Nakayama *et al.* 1996). Despite the efforts to screen human pituitary adenomas, neither pathogenic mutations nor loss of heterozygocity was found (*e.g.* Ikeda *et al.* 1997; Tanaka *et al.* 1997). However, lowered or even absent expression of p27 has been reported in most human pituitary adenomas (Bamberger *et al.* 1999; Komatsubara *et al.* 2001; Lidhar *et al.* 1999). Interestingly, the regulation of the expression of p27 includes both menin (Karnik *et al.* 2005; Milne *et al.* 2005; Scacheri *et al.* 2006) as well as AIP through AHR (Kolluri *et al.* 1999).

Additional *CDKN1B/p27^{Kip1}* mutations in humans would strengthen the role of this gene in endocrine neoplasia.

3. AIMS OF THE STUDY

I Clarify the clinical and molecular identification of PAP patients

II Analyze somatic *AIP* mutations in sporadic endocrine neoplasia

III Analyze germ-line *AIP* mutations in familial non-medullary thyroid cancer

IV Study the role of *CDKN1B/p27*^{Kip1} in MEN-like phenotype

4. MATERIALS AND METHODS

4.1. Subjects

This study was approved by the Ministry of Social Affairs and Health, the Ethics Review Committees of the Hospital District of Helsinki and Uusimaa, and the Department of Medical Genetics of the University of Helsinki. The permission to use patient samples was obtained either with appropriate informed consent or with the permission of the National Authority for Medicolegal Affairs. Detailed information of samples can be found from the corresponding publications or from their supplementary material.

4.1.1. Pituitary adenoma patient samples (I, II, IV)

Altogether 460 samples were analyzed in study I. This cohort consisted of heterogeneous pituitary adenoma patients originating from several populations from Europe and the United States. The samples can be divided into the following subcategories: a) *young acromegaly patients* consisting of 27 samples from German patients (<40 years at the time of surgery) and 36 from Finnish patients (<45 years at the time of diagnosis), b) *unselected acromegaly patients* including 71 samples from Italian patients, c) *unselected pituitary adenoma patients* consisting of 113 samples from American patients and 122 from Polish patients, and d) *MEN1 suspected patients without MEN1-gene mutations* including 55 samples from Spanish patients and 36 from Dutch patients.

Fifty pituitary adenoma samples were available to study AIP protein expression (study I). Two patients with PRL-producing and seven with GH-producing adenomas had c.40C>T mutation. Three GH-producing adenomas were from patients with c.280-1G>C, c.824_825insA, or c.469-1G>A mutation. *AIP* mutation-negative tumors accounted for 32 GH-producing and five PRL-producing adenomas, and one GH- and PRL-negative adenoma.

In study II, thirty-two sporadic Finnish pituitary adenoma samples were analyzed: nine were PRL-producing and 23 GH-producing adenomas. Twenty-one of the GH-producing adenomas originated from the population-based cohort of 54 acromegaly patients diagnosed in five Finnish university hospitals during 1980-1999 (Kauppinen-Makelin *et al.* 2005; Vierimaa *et al.* 2006). As previously reported, 20 samples from this cohort of 54 patients are *AIP* mutation-negative (Vierimaa *et al.* 2006).

Thirty-five out of 36 suspected MEN1 patients (also in study I) without *MEN1*- or *AIP*-mutation from Netherlands and one additional suspected MEN1 patient from Germany were included in study IV. Furthermore, sequence analysis consisted of 34 out of 36 young Finnish acromegaly patients (also in study I), 16

AIP-mutation negative sporadic pituitary adenoma patients (Oulu University hospital, also in study I), and 19 familial acromegaly/pituitary adenoma patients originating from various nationalities. All of the samples were *AIP*-mutation negative.

4.1.2. Sporadic endocrine tumors (II)

The tumors analyzed from tissues of the endocrine system other than the pituitary gland, included 61 Finnish and 18 Danish samples. The samples were from the thyroid gland (26 tumors), the adrenal gland (19 tumors), lung, cecum, appendix and small intestine (16 neuroendocrine tumors), and the parathyroid gland (8 tumors). Four paragangliomas, four pancreatic endocrine tumors, and two mixed endocrine-exocrine tumors were also included in study II.

4.1.3. Familial thyroid cancer patient cohort (III)

Up to 7% of all thyroid tumors belong to the clinical entity of familial non-medullary thyroid cancer (NMTC). Eighty percent of a familial NMTC cohort, including 261 families, did not connect to previously identified predisposing *locus* 2q21 and 19p13.2 (Canzian *et al.* 1998; McKay *et al.* 2001; McKay *et al.* 2004). *AIP* was sequenced in 93 index cases of these non-linked families. Eighty-five patients were diagnosed with papillary thyroid cancer (PTC), one with PTC and Hashimoto thyroiditis, one with PTC and hypothyroidism, and one with PTC and colloidal adenoma. Two patients had micropapillary thyroid cancer, two had multinodular goiter, and one had thyroid adenoma.

4.1.4. Healthy control samples (I, II, III, IV)

Control DNA samples were received from 749 unrelated, anonymous, healthy individuals. These samples consisted of 288 Caucasians from UK (Human Random Control DNA panel, Porton Doen, Salisbury, Wiltshire, UK), 110 Caucasians from the Centre d'Étude du Polymorphisme Humain (Fondation Jean Dausset-CEPH, Paris, France), 209 Finnish Red Cross blood donors, 90 Germans (Leipzig University, Germany), and 52 Italians (Treviso General Hospital, Italy). The analyzed control samples in each study are described in corresponding publications.

4.2. Analysis methods

4.2.1. Direct sequencing (I, II, III, IV)

Genomic DNA was extracted from blood or paraffin-embedded tissue according to standard protocols (Kannio *et al.* 1996; Lahiri and Nurnberger 1991; Shibata *et al.* 1988). Primer pairs used for sequencing the coding region of *AIP* and the flanking intronic areas have been previously described (Vierimaa *et al.* 2006).

CDKN1B/p27^{Kip1} primer pairs were designed by using Primer3 (http://frodo.wi.mit.edu/primer3), and are presented in the supplementary material of the original publication (study IV). In both genes, tumor-derived DNA was amplified in shorter fragments compared to normal DNA.

PCR products were analyzed by electrophoresis using 2% agarose gel and the specifically amplified products were purified using ExoSAP-IT purification kit (USB Corporation). Purified PCR-fragments were sequenced using the Big Dye 3.1 Termination chemistry on an ABI3730 DNA sequencer (Applied Biosystems).

LOH analysis was performed on the tumor-derived DNA. From the sequence chromatograms the peak heights/areas between the wild-type and the mutant allele were compared. LOH was detected when the wild-type allele was either completely or nearly completely disappeared, when compared to the mutant allele.

4.2.2. Immunohistochemistry (IHC) (I, III, IV)

Immunoreactions against AIP (1:4000, AIP SP5213P; Acris Antibodies) and p27 (1:500, p27^{Kip1}, clone 57; BD Biosciences) were done according to standard IHC procedures. The AIP IHC is introduced shortly. Five-micrometer-thick sections were cut from the paraffin blocks and pre-warmed from 30 minutes up to one hour at 55°C. After deparaffinization and rehydration in graded alcohol series, antigen retrieval was carried through with either 0.01 M citrate (pH 6.0) buffer in a microwave oven at 800 W for 2 min and at 300 W for 10 min or in 0.01 M Tris-EDTA (pH 6.0) buffer in a microwave oven at 800 W for 2 min and at 300 W for 15 min. The slides were allowed to cool for 20 min and rinsed with PBS. Thereafter, primary antibody was incubated for 30 min at room temperature, followed by rinsing in PBS, after which the bound antibodies were detected using diaminobenzidine (DAKO, Copenhagen, Denmark) with hematoxylin counter stain. The slides were dehydrated in graded alcohol series, embedded in xylene, and covered.

4.2.3. *In silico* analysis (I, III, IV)

Computational programs were used to predict whether the detected intronic, silent, or missense changes affect splicing. The used *in silico* splice site prediction programs were Berkeley Drosophila Genome Project, ESEfinder 2.0, Splice Scan, Alternative Splice Site Predictor, and NetGene2 (Brunak *et al.* 1991; Cartegni *et al.* 2003; Hebsgaard *et al.* 1996; Reese *et al.* 1997; Smith *et al.* 2006; Tchourbanov and Ali 2005; Wang and Marin 2006).

5. RESULTS

5.1. Molecular analysis of PAP (I)

The identification of *AIP* as a predisposing gene was originally described in a homogeneous population (Vierimaa *et al.* 2006). To clarify the clinical features of this condition in a more heterogeneous pituitary adenoma setting, *AIP* was sequenced in 460 pituitary adenoma patients from the European and North American populations. Potentially pathogenic mutations were observed at low frequencies (Table 4). *AIP* mutations were the most common in young acromegaly patients from Germany (2/27 or 7.4%) and Finland (2/36 or 5.5%). Mutations were less common in unselected pituitary adenoma patients from North America (2/113 or 1.8%) and Poland (1/122 or 0.8%). Also, *AIP* mutations were identified in suspected MEN1 patients without *MEN1* mutations from Spain (1/55 or 1.8%) and the Netherlands (1/36 or 2.8%). None of the studied Italian unselected acromegaly patients harbored *AIP* mutations.

Table 4. Detected *AIP* mutations from pituitary adenoma patients from European and North American populations. Only the potentially pathogenic mutations are presented. None of these variations were present in the control samples.

Patient	Detected *AIP* mutation	LOH	Pituitary adenoma type	Age at the diagnosis	Family history of pituitary adenomas
Young acromegaly					
German	c.66_71del	Yes	GH	20 yr	Yes (acromegaly)
German	c.[878_879delinsGT; c.880_891del]	Yes	GH	29 yr [a]	NA
Finnish	c.40C>T	NA	GH	36 yr	No
Finnish	c.40C>T	NA	GH	41 yr	No
Unselected pituitary adenoma					
American	c.280-1G>C	NA	GH	20 yr	No
American	c.824_825insA	Yes	GH	8 yr	No
Polish	c.911G>A	NA	ACTH	26 yr	No
MEN1-negative					
Spanish	c.542del	NA	GH	18 yr	Yes (acromegaly)
Dutch	c.896C>A	NA	GH	16 yr	No

LOH, loss of heterozygocity *e.g.* loss of wild-type allele; NA, not available

[a] Age at the time of operation, the age at the time of diagnosis is unknown.

Other detected *AIP* variations were c.100-18C>T in one German sample, c.47G>A (p.Arg16His) in one Italian, in one American with Polish roots, and in three Polish samples, c.906G>A (p.=) in three American samples, and c.696G>C (p.=) in one Polish sample. These variations were considered as a rare polymorphism according to *in silico* prediction (c.100-18C>T, c.906G>A, c.696G>C), negative LOH analyze (c.47G>A) and/or detection of variations also in the healthy controls (c.100-18C>T, c.47G>A, c.906G>A).

To help in identifying the *AIP* mutation-positive patients, the possibility to use immunohistochemistry as a pre-screening tool was studied. *AIP* mutation-positive tumors were from patients with c.40C>T, c.280-1G>C, c.824_825insA, or c.469-1G>A mutation. Most of *AIP* mutation-positive tumors (9/12 or 75%) showed complete loss of AIP in the cytoplasm and nucleus (Fisher's Exact test, p=0.000004; Fig. 4). Positive AIP immunoreactions in these tumors may be due to *e.g.* unspecific staining or presence of nonfunctional yet immunoreactive AIP protein. Positive immunoreactions of AIP were detected in most of the *AIP* mutation-negative tumors (36/38 or 95%). Therefore, AIP IHC showed 75% sensitivity and 95% specificity for truncating *AIP* germ-line mutations. This indicates the potentiality of AIP IHC as a pre-screening tool.

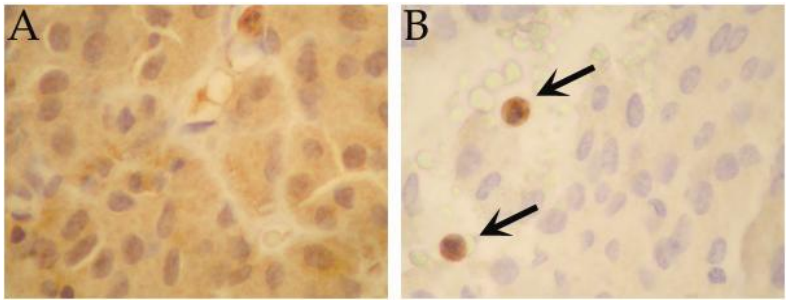

Figure 4. AIP IHC stainings with 40x magnification. (A) *AIP* mutation-negative adenoma shows positive immunoreaction against AIP. (B) Pituitary adenoma from a patient with c.40C>T mutation shows the lack of AIP protein whereas peripheral blood leukocytes, indicated by black arrows, display positive immunoreaction.

5.2. Somatic *AIP* mutation screening in sporadic endocrine neoplasia (II)

To study the possible somatic mutations of *AIP* in sporadic endocrine tumors, thirty-two pituitary adenomas and 79 other tumors of the endocrine system were analyzed. No somatic mutations were identified in the studied tumors. However, two PRL-producing adenoma patients harbored the Finnish founder mutation c.40C>T (p.Gln14X) and one thyroid adenoma patient a potential polymorphism c.69A>G (p.Gly23Glu) in the *AIP* gene.

c.40C>T was detected in two patients with the PRL-producing pituitary adenoma. These patients were diagnosed at the age of 35 years and both of the tumor tissues showed the complete loss of the wild-type allele. One mutation-positive patient was a male with no family history of the endocrine tumors. The mutation was also present in his germ-line. The other mutation carrier was a female whose normal tissue and family history information were not available.

The other detected germ-line variation was c.69A>G in a female patient with thyroid adenoma diagnosed at the age of 60 years. This variation was present in the corresponding normal tissue and wild-type allele was retained in the tumor tissue. Because of this and previous observations of c.69A>G in the healthy controls (five out of 532) and in Finnish colorectal cancer samples (6/373), this variation was considered as a germ-line polymorphism (study I; Georgitsi *et al.* 2007; Vierimaa *et al.* 2006).

5.3. Screening of *AIP* in familial non-medullary thyroid cancer (NMTC) cases (III)

The possible germ-line mutations of *AIP* were studied in index patients from 93 NMTC families. Two previously reported *AIP* variations were detected. One patient, with PTC and colloidal adenoma diagnosed at the age of 44 years, harbored c.47G>A (p.Arg16His). The other patient, with unilateral PTC diagnosed at the age of 36 years, harbored c.36G>A (p.=). These variations did not segregate in the corresponding families. According to *in silico* analysis, neither of the variants had predicted effects on splicing. Tumor tissues were not available for LOH analysis.

In the previous studies, c.47G>A has been identified in fourteen pituitary adenoma patients, two Finnish colorectal cancer patients, and three healthy controls (study I; Buchbinder *et al.* 2008; Cazabat *et al.* 2007; Daly *et al.* 2007b; Georgitsi *et al.* 2007; Yaneva *et al.* 2008). Whereas c.36G>A has been detected in one Finnish prostate cancer patient and one sporadic acromegaly patient, but all of the studied 802 healthy controls have been negative for this change (Cazabat *et al.* 2007; Georgitsi *et al.* 2007). Both variants were considered as rare polymorphisms, although the pathogenic potential of c.36G>A cannot be totally excluded.

In addition, one follicular thyroid adenoma from an *AIP* mutation-positive (c.469-1G>A) patient from an additional cohort was available to the study. No LOH was seen in the tumor tissue and the AIP IHC was positive in the adenoma and the normal cells. This suggested the presence of functional AIP protein in the tumor tissue.

5.4. The analysis of *CDKN1B/p27^{Kip1}* mutations in endocrine neoplasia (IV)

The germ-line mutation of *CDKN1B/p27^{Kip1}* was reported in one patient with multiple endocrine neoplasia (Pellegata *et al.* 2006). To further study the role of *CDKN1B/p27^{Kip1}* in multiple endocrine neoplasia, mutation screening was performed in 36 clinically suspected MEN1 patients without *MEN1* gene mutation. Furthermore, nineteen familial acromegaly or familial pituitary adenoma patients, and 50 sporadic acromegaly patients were included in the study. One suspected MEN1 patient carried 19-bp duplication (c.59_77dup) in the coding region of *CDKN1B/p27^{Kip1}* gene. This mutation causes frame shifting change at Serine-27. It creates a new reading frame ending in a stop codon at 69 residues earlier than the wild-type allele. The sequencing of DNA extracted from the neuroendocrine cervical carcinoma revealed the loss of the wild-type allele, and the IHC staining confirmed the absence of p27 in this tumor. This *CDKN1B/p27^{Kip1}* mutation was the second reported mutation in humans.

The other detected *CDKN1B/p27^{Kip1}* gene variation was a silent change c.426G>A (p.=) in one young sporadic acromegaly patient. According to *in silico* analysis, this change has no predicted effect on splicing and thus it was considered as a rare polymorphism.

6. DISCUSSION

6.1. The PAP phenotype

The Finnish population is overall genetically homogeneous due to the relatively small founder populations, the small genetic drift, and the isolated location resulting from geographical, linguistic, and religious reasons (Peltonen 1997). Thus, rare conditions with low-penetrance can be found as clusters. This facilitates the identification of the predisposing gene(s) as was the case in the identification of the *AIP* mutations in PAP (Vierimaa *et al.* 2006). In the initial study, *AIP* mutations were relatively frequent in a population-based cohort from Northern Finland: 16% in GH-producing pituitary adenomas, and 40% in a subset of patients diagnosed under the age of 35 years (Vierimaa *et al.* 2006). To gain insight into the PAP phenotype and the mutation frequencies it was relevant to study more heterogeneous sample materials as well.

6.1.1. *AIP* mutation frequencies in diverse clinical settings (I)

In study I, the contribution of *AIP* mutations was studied in pituitary adenoma patients from genetically heterogeneous populations from Europe and North America. If taking into account the previously reported two patients belonging to the same Italian cohort of which 71 were analyzed in study I (Vierimaa *et al.* 2006), *AIP* mutations were found from all of the studied clinical subcategories (0.8-7.4%). The highest mutation frequencies were detected in young acromegaly/gigantism patients from Germany (2/27 or 7.4%) and Finland (2/36 or 5.5%). Among all studied *AIP* mutation-positive patients the average age at diagnosis was 23.8 years. Additional studies have detected similar ages in the *AIP* mutation-positive patients (average ~25 years) which are lower than in the mutation-negative ones (average ~38 years) (Daly *et al.* 2007b; Leontiou *et al.* 2008). Besides the young age of the *AIP* mutation-positive patients, the majority of these patients did not have family history of pituitary adenomas. These observations are in concordance with the original study by Vierimaa *et al.* (2006) where the young age of onset and the low penetrance of this condition were reported. In the following studies, even up to 66% penetrance has been suggested (Daly *et al.* 2007b; Iwata *et al.* 2007; Khoo *et al.* 2009; Leontiou *et al.* 2008; Naves *et al.* 2007; Toledo *et al.* 2007). However, this high frequency may reflect a bias caused by the incomplete data from some of the families. More accurate and age-related penetrance estimation requires additional *AIP* mutation-positive families to be studied and extended time period of surveillance.

After the initial study of Vierimaa *et al.* (2006) and followed by study I, *AIP* mutation status has been studied in familial and sporadic pituitary adenoma patients. Daly *et al.* (2007b) performed *AIP* mutation screening in 73 FIPA families. Mutations accounted for 15% of these families and for 50% of those

families with only GH-producing adenomas *e.i.* IFS (Daly *et al.* 2007b). A similar trend was seen in 26 FIPA families where 35% of the families were *AIP* mutation-positive (Leontiou *et al.* 2008). Additional familial cases with *AIP* mutations have also been reported (Georgitsi *et al.* 2008b; Iwata *et al.* 2007; Jennings *et al.* 2009; Khoo *et al.* 2009; Toledo *et al.* 2007). *AIP* mutations seem to account for less than 2% of the sporadic pituitary adenomas (study II; Barlier *et al.* 2007; Buchbinder *et al.* 2008; Cazabat *et al.* 2007; Digiovanni *et al.* 2007; Georgitsi *et al.* 2008a; Igreja *et al.* 2009; Iwata *et al.* 2007; Leontiou *et al.* 2008; Vierimaa *et al.* 2006; Yaneva *et al.* 2008; Yu *et al.* 2006). The majority of the *AIP* mutation-positive patients have been diagnosed with GH-producing adenomas, but cases with PRL-, ACTH-producing adenomas or NFPAs have also been reported (study I; study II; Daly *et al.* 2007b; Georgitsi *et al.* 2008b; Leontiou *et al.* 2008). Recently, male predominance in PAP was suggested: 70% of the reported *AIP* mutation-positive patients were males ($p < 0.001$) (Cazabat *et al.* 2009). This association remains statistically significant in an update of the *AIP* mutation-positive patients (62%, 71 M/44 F, $p \approx 0.014$, Chi-square test). Additional *AIP* mutation-positive patients and careful clinical examinations will show if males are truly at a higher risk of developing pituitary adenomas in PAP.

Approximately 40 different *AIP* mutations, scattered throughout the coding region, have so far been identified (Fig. 5). Interestingly, most of the pathogenic missense mutations occur in a region between codons 241-304 in the two last exons of *AIP*. Two of the TPR motifs, which are critical for protein-protein interactions, are located in this area. In addition, the five last amino acids (codons 325-330) of the protein are required for binding to AHR (Bell and Poland 2000).

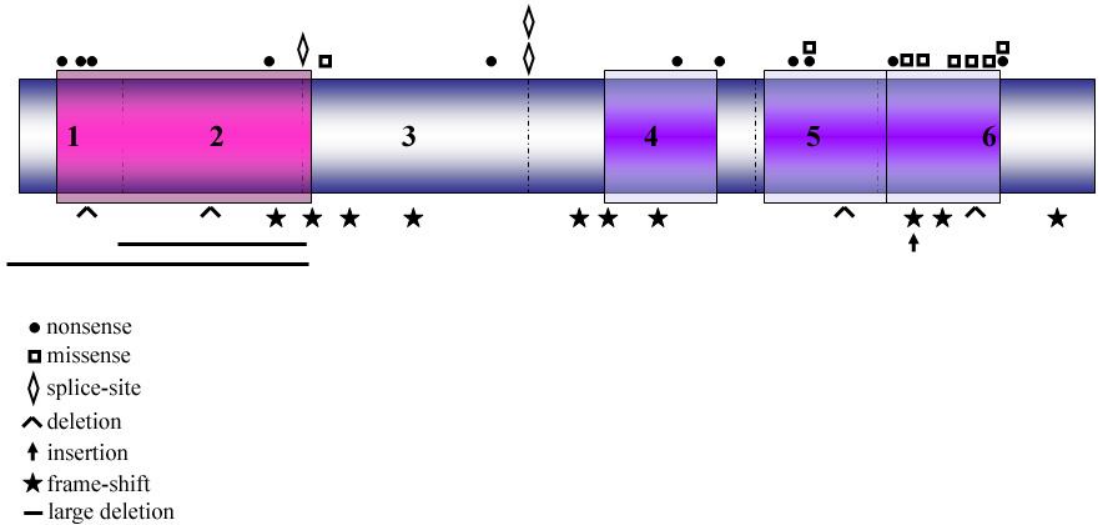

Figure 5. A schematic figure of *AIP* with all pathogenic germ-line mutations reported in the literature by November 2009. The exon boundaries of the *AIP* gene are indicated by dashed lines. The pink box shows the location of FKBP domain and the violet boxes the locations of TPRs.

A large portion of the familial and sporadic pituitary adenoma cases are not explained by the known genes predisposing to pituitary adenomas. LOH of 11q13 is observed in the pituitary adenomas without detectable *MEN1* or *AIP* mutation (*e.g.* Gadelha *et al.* 1999; Tanaka *et al.* 1998; Teh *et al.* 1998; Yamada *et al.* 1997). One explanation might be the limitations of the current sequencing methods, as seen in a recent study where large genomic deletions in the *AIP* area were detected in patient samples previously sequenced to be *AIP* mutation-negative (Georgitsi *et al.* 2008b). However, it is possible that unidentified predisposing gene(s) still lays in the 11q13.

6.1.2. The IHC in identification of PAP (I)

The lack of family history and the low frequency of *AIP* mutations in randomly selected pituitary adenoma patients create a challenge for the identification of the PAP patients. Routine screening of *AIP* in all pituitary adenoma patients seems unreasonable. Since the pituitary adenomas are stained against hormones for diagnostic purpose in many laboratories, we wanted to test the specificity and sensitivity of the AIP IHC.

In study I, the AIP IHC using a polyclonal antibody against mouse recombinant AIP showed high specificity (95%), but weaker sensitivity (75%). The AIP IHC has also been tested in later studies. A monoclonal antibody against the human AIP with amino-terminal epitope (FKBP region) showed decreased immunostaining in a pituitary adenoma sample with early truncating *AIP* mutation (p.Glu174fs) (Naves *et al.* 2007). In another study, the AIP IHC with the same antibody showed weak immunoreactivity in the pituitary adenomas with *AIP* mutation (*e.g.* p.Gln82fs, p.Arg304X, and p.Gln285fs), albeit immunoreactivity was also diminished in the adenomas with probably non-pathogenic variations (*e.g.* c.279+23C>T and c.468+16G>T) (Jaffrain-Rea *et al.* 2009). Moreover, immunostaining with the same AIP antibody in the pituitary adenoma samples with p.Arg304X or p.His274fs mutation showed positive staining of the protein (Leontiou *et al.* 2008). In some of these *AIP* mutation-positive adenomas, the positive immunoreaction may be due to carboxyl-terminal location of the mutation and amino-terminal location of the antibody epitope.

Discrepancies in the sensitivities between the AIP IHC studies may arise from different reasons. For example, the IHC assays may differ between the laboratories. It is also possible that carboxyl-terminal or missense mutation leads to a stable and immunoreactive, yet possibly dysfunctional, protein. Thus, the nature and position of the mutation and the selection of antibody are critical. Improving the AIP IHC requires further optimization with new carboxyl-terminal *AIP* antibodies. Functional AIP IHC could be useful at least as a pre-screening tool to identify the potential PAP patients. For instance, IHC screening

of tumors in HNPCC, also known as Lynch syndrome, has been successfully used even as a diagnostic tool (Hampel *et al.* 2005).

6.2. Somatic *AIP* mutations are rare or non-existent in sporadic endocrine neoplasia (II)

As already mentioned, germ-line *AIP* mutations predispose mainly to GH-producing adenomas and less frequently to PRL-, ACTH-producing adenomas, and NFPAs. Pituitary adenomas also occur in MEN1 and CNC syndromes. Somatic mutations in *MEN1* and *PRKAR1A* are rare in sporadic pituitary adenomas (*e.g.* Wenbin *et al.* 1999; Yamasaki *et al.* 2003). Such mutations are seen in some other tumors related to these syndromes (*e.g.* Farnebo *et al.* 1998; Sandrini *et al.* 2002b). Thus, we studied the occurrence of somatic *AIP* mutations in the pituitary adenomas and also, for the first time, in non-pituitary endocrine tumors.

None of the studied sporadic pituitary adenomas harbored purely somatic mutations. However, the Finnish founder mutation was detected in two PRL-producing adenomas from individuals diagnosed at a young age (two out of nine, 22%). For one of the patients the mutation was confirmed to be in the germ-line. This patient, from whom information was available, had no family history of endocrine tumors. These results supported the previous observations that *AIP* mutations also predispose to PRL-producing adenomas (Daly *et al.* 2007b; Vierimaa *et al.* 2006).

Thus far the number of PRL-producing adenomas, in which somatic *AIP* mutations has been studied, is limited. This may be because of the infrequent surgery of the PRL-producing adenomas; tumors have a slow growth rate and good response to the therapy (Spada *et al.* 2005). Therefore, further studies are required to clarify the possible contribution of somatic *AIP* mutations in the PRL-producing adenomas.

In study II, somatic *AIP* mutations were also examined in the sporadic GH-producing adenomas, but all samples were mutation-negative. This is in concordance with the previous studies which have not identified somatic mutations in that particular tumor type (Barlier *et al.* 2007; Iwata *et al.* 2007). These results indicate that somatic *AIP* mutations do not have a major contribution to the formation of the sporadic GH-producing adenomas. Actually, many tumor susceptibility genes are only rarely somatically mutated in the respective sporadic tumors such as *BRCA1/2* in the sporadic breast tumors (Khoo *et al.* 1999; Yang *et al.* 2002).

In this study, the occurrence of somatic *AIP* mutations were analyzed for the first time in non-pituitary endocrine tumors. No somatic *AIP* mutations were detected. Results suggest that *AIP* mutations do not seem to be strongly involved

in the development of these tumors. In previous studies, LOH of 11q13 has been reported in adrenal carcinomas from two *AIP* mutation-positive patients (Leontiou *et al.* 2008; Toledo *et al.* 2008). However, a larger number of PAP families and additional evidence is needed to clarify whether *AIP* mutations are predisposing to adrenal tumors since LOH of 11q13 is a frequent event in this tumor type (Luccio-Camelo *et al.* 2004).

6.3. *AIP* mutations seem not to be involved in familial non-medullary thyroid cancer (III)

NMTC occurs with a greater frequency than expected in familial syndromes such as CNC (Malchoff and Malchoff 2006). Familial NMTC is a distinct clinical condition characterized by a higher degree of tumor aggressiveness and an increased mortality (Grossman *et al.* 1995). Several susceptibility *loci* for familial NMTC have been suggested (*e.g.* Cavaco *et al.* 2008; McKay *et al.* 2001), but the main genetic components are still unknown. Thyroid disorders have also been identified in FIPA, and PAP families (O Vierimaa and PI Salmela, unpublished observations; Beckers and Daly 2007). Thus, the possible role of *AIP* in familial NMTC was examined in study III. Sequencing of *AIP* did not reveal potentially pathogenic *AIP* mutations indicating that germ-line *AIP* mutations have little or no role in the genesis of familial NMTC.

The possible loss of AIP in thyroid tumors was studied in one tumor sample from a Finnish *AIP* mutation-positive patient. LOH and immunohistochemical analyses suggested that the immunoreactive protein seems to be present in this particular tumor tissue. Interestingly, patients with GH-producing adenomas are at a higher risk of developing non-toxic nodular goiters. Also, these patients have a somewhat elevated risk of thyroid cancer compared to the general population (*e.g.* Herrmann *et al.* 2004; Kurimoto *et al.* 2008). This seems to be linked to the increased plasma levels of IGF-I in patients with GH-producing adenomas (Siegel and Tomer 2005). Thus, at least some of the thyroid disorders seen in the *AIP* mutation-positive patients may be due to the elevated IGF-I levels. On the other hand, thyroid disorders are quite common in the general population (up to 7%) (Roman 2003) and those tumors may occur just by chance in the *AIP* mutation-positive patients.

6.4. AIP in tumorigenesis

So far the number of endocrine tumors screened for *AIP* mutations, other than pituitary adenomas, is limited. Thus, *AIP* mutations may be identified by screening additional non-pituitary endocrine tumors. Besides endocrine tumors, *AIP* mutations have been studied in common cancer types, including colorectal cancer, breast cancer, and prostate tumors, with the negative results (Georgitsi *et al.* 2007). Even though *AIP* is ubiquitously expressed in all human tissues, with current knowledge *AIP* mutations seem to predispose only to pituitary

adenomas. Thus, AIP could be associated to tissue selective tumorigenesis. This was supported in a recent study where the silencing of *Aip* resulted in increased cell proliferation in a rat pituitary cell line (GH3), but not in two other studied cell lines (HEK293 and HeLa) (Heliovaara *et al.* 2009). In the same study, IHC analysis showed that ARNT was less frequently expressed in the *AIP* mutation-positive tumors compared to the mutation-negative ones. Again the tissue-selectivity was indicated since the reduction of Arnt was only seen in the GH3 cell line after *Aip*-silencing (Heliovaara *et al.* 2009). Heliovaara *et al.* (2009) also noticed that the expression of the nuclear AHR was somewhat increased in the *AIP* mutation-positive adenomas compared to the mutation-negative ones. Thus, ARNT and AHR were suggested to be involved in the pituitary tumorigenesis in the *AIP* mutation-positive tumors.

Aberrant cAMP signaling is often detected in pituitary tumorigenesis (Boikos and Stratakis 2007b). This signaling is also possibly involved in the pituitary tumorigenesis of the *AIP* mutation-positive tumors. AIP targets PDE2A to the AHR complex (de Oliveira *et al.* 2007). This binding ensures AHR retention in the cytoplasm by lowering the local cAMP concentrations (de Oliveira *et al.* 2007). Thus, the lack of functional AIP may lead to elevated local cAMP levels and cause the translocation of AHR to the nucleus (Heliovaara *et al.* 2009). Furthermore, the cAMP-mediated translocation of AHR prevents the formation of the AHR/ARNT complex (Oesch-Bartlomowicz *et al.* 2005). Heliovaara *et al.* (2009) suggested that the disturbances in the formation of AHR/ARNT, possibly also HIF1-α/ARNT, complex may unbalance the transcription of target genes of this complex leading to pituitary tumorigenesis.

Aip knock-out mice (*Aip*$^{-/-}$) were reported to show embryonic lethality accompanied by cardiac malformations (Lin *et al.* 2007). The phenotype of these *Aip*$^{-/-}$ mice was different from those of *Ahr*$^{-/-}$ and *Ppar*$\alpha^{-/-}$ mice. Thus, Lin *et al.* (2007) suggested that Aip seems to have a role in another pathway distinct from xenobiotic-induced metabolism. In the same study, heterozygous *Aip*$^{+/-}$ mice appeared phenotypically normal and fertile, but the possibility of endocrine tumor formation was not studied.

In a recent study, a tendency towards a higher risk of pituitary adenomas was seen after massive exposure to dioxin after the Seveso accident, Italy, in 1976 (Pesatori *et al.* 2008). The tumors that occurred did not include GH-producing adenomas, which is the main tumor type in PAP. Moreover, it should be noted that the prevalence of pituitary tumors was not statistically significant (Pesatori *et al.* 2008). Thus, the direct activation of AHR, leading to xenobiotic-induced metabolism, did not cause increased risk of pituitary adenoma development.

Certainly the mechanism how the loss of functional AIP leads to tumor formation requires further studies. Functional studies and work with animal models should help to resolve this issue in the future.

6.5. The role of *CDKN1B/p27^{Kip1}* in multiple endocrine neoplasia (IV)

Recently, a germ-line *CDKN1B/p27^{Kip1}* mutation was reported in one family with multiple endocrine neoplasia (Pellegata *et al.* 2006). In this family, the MEN1-related tumors included GH-producing pituitary adenoma, primary hyperparathyroidism, and angiomyolipoma. To further study the role of *CDKN1B/p27^{Kip1}* gene in endocrine neoplasia, we performed a mutation screening of *CDKN1B/p27^{Kip1}* in suspected MEN1 patients without *MEN1* gene mutation. Also the contribution of *CDKN1B/p27^{Kip1}* mutations in the familial and sporadic pituitary adenomas was studied.

In this study, we were able to confirm the finding of Pellegata *et al.* (2006) by publishing the second germ-line *CDKN1B/p27^{Kip1}* mutation in a clinically suspected MEN1 patient. This patient had three MEN1-related tumors: small-cell neuroendocrine cervical carcinoma, ACTH-producing adenoma, and hyperparathyroidism. The neuroendocrine cervical carcinoma showed a loss of the wild-type allele in tumor-derived DNA. Furthermore, the p27 IHC analysis showed negative immunoreactivity of p27 protein in the particular tumor tissue. These results strengthened the tumor suppressor role of the *CDKN1B/p27^{Kip1}* in endocrine neoplasia (Pellegata *et al.* 2006). The previous knock-out mouse models had suggested the role of *CDKN1B/p27^{Kip1}* in endocrine neoplasia (Fero *et al.* 1996; Kiyokawa *et al.* 1996; Nakayama *et al.* 1996), but our finding confirmed the role of *CDKN1B/p27^{Kip1}* in human endocrine neoplasia.

So far, five mutation-positive index cases have been identified among approximately 340 studied cases (study IV; Agarwal *et al.* 2009; Igreja *et al.* 2009; Owens *et al.* 2009; Ozawa *et al.* 2007). Two of these cases are familial and three apparently sporadic. These results indicate that *CDKN1B/p27^{Kip1}* mutations occur at a low frequency in the suspected MEN1 patients without *MEN1* mutation.

In study IV, none of the familial and sporadic pituitary adenoma patients harbored potentially pathogenic *CDKN1B/p27^{Kip1}* mutations. This is in concordance with the previous negative mutation screenings of *CDKN1B/p27^{Kip1}* in the pituitary adenomas (*e.g.* Ikeda *et al.* 1997; Tanaka *et al.* 1997). Thus, it seems that *CDKN1B/p27^{Kip1}* mutations are rare or non-existent in the familial or sporadic pituitary adenoma patients.

Hyperparathyroidism has been diagnosed in all *CDKN1B/p27^{Kip1}* mutation-positive index cases (study IV; Agarwal *et al.* 2009; Pellegata *et al.* 2006). In a recent study, potentially pathogenic *CDKN1B/p27^{Kip1}* mutations were not detected in presumably familial hyperparathyroidism patients (Vierimaa *et al.* 2009). Furthermore, screening of somatic *CDKN1B/p27^{Kip1}* mutations in sporadic secondary/tertiary hyperparathyroidism was also negative (Lauter and Arnold

2008). These results indicate that *CDKN1B/p27^{Kip1}* does not have a major contribution to familial or sporadic hyperparathyroidism.

The rarity of mutations among the suspected MEN1 patients and the variable phenotype of *CDKN1B/p27^{Kip1}* mutation carriers complicate the identification of these patients. Nonetheless, the clinicians should be aware that at least some of the suspected MEN1 patients without *MEN1* mutation may harbor *CDKN1B/p27^{Kip1}* mutation.

7. CONCLUSIONS AND FUTURE PROSPECTS

This work studied the role of *AIP* and *CDKN1B/p27^{Kip1}* in endocrine neoplasia. Conclusions of this work are summarized as follows:

I) Germ-line *AIP* mutations are found at low frequencies (0.8-7.4%) in diverse settings of the pituitary adenoma patients. Most of the *AIP* mutation-positive patients have GH-producing adenomas. Often these patients are young and they may not display a family history of pituitary adenomas. With further optimization, the AIP IHC could be used as a pre-screening tool for the identification of potential PAP patients.

II) Somatic mutations of *AIP* are rare or do not exist in sporadic endocrine tumors.

III) *AIP* is unlikely to be a predisposing gene for familial NMTC.

IV) The second *CDKN1B/p27^{Kip1}* mutation was identified in suspected MEN1 patients without *MEN1* mutations.

Identification of the predisposing genes for familial syndromes has raised general awareness. The recognition of the mutation-positive families enables regular follow-up of the mutation carriers followed by early diagnosis. With the appropriate treatments, the morbidity of these patients can be diminished. This way the quality of the patient's life can be improved and premature death can even be avoided. In addition, the family members without mutation can be relieved from the unnecessary follow-ups.

So far, mutations in *MEN1*, *PRKAR1A*, *CDKN1B/p27^{Kip1}*, and *AIP* are known to predispose to endocrine neoplasia with pituitary adenomas. However, the majority of the familial, isolated pituitary adenomas do not harbor mutations in these genes and thus new predisposing gene(s) remain to be identified.

The diagnosis of gigantism is unambiguous whereas acromegaly develops insidiously often leading to delayed diagnosis. For this reason, the identification of the *AIP* mutation carriers is important. This is challenging due to the rarity of *AIP* mutations in sporadic cases and often the lack of family history of pituitary adenomas. However, the *AIP* mutation screening should be considered for patients with familial pituitary adenomas, particularly if affected patients have GH-producing adenomas. Furthermore, the contribution of *AIP* mutations should be suspected if a seemingly sporadic patient is diagnosed with GH-producing adenoma at a young age.

For the *AIP* mutation carriers it seems reasonable to offer a non-invasive clinical follow-up, such as a biochemical screening for markers (*e.g.* hormones) from the blood and possibly the pituitary MRI scanning. For instance, in the *MEN1* mutation-positive patients the early marks of neoplasia can be detected biochemically on average 10 years prior to the clinically evident disease (Lairmore *et al.* 2004).

Clinical follow-up of the *CDKN1B/p27^{Kip1}* mutation carriers is recommended. However, the mutation screening of *CDKN1B/p27^{Kip1}* gene seems unreasonable due to the rarity of mutations. Nonetheless, the clinicians should be aware that small portion of suspected MEN1 patients without *MEN1* gene mutations may have *CDKN1B/p27^{Kip1}* gene mutation.

During the last decades, factors contributing to the pituitary tumorigenesis have been identified but the underlying processes are largely unknown. To gain insight into this issue, rigorous *in vitro* and *in vivo* studies are needed. The lack of functional human pituitary cell line requires the use of animal cell lines for these experiments. Currently, there is a great interest towards the possible tumor spectrum of the *Aip$^{+/-}$* mice: is the human PAP phenotype reproduced or not?

8. ACKNOWLEDGEMENTS

This study was carried out at the Department of Medical Genetics, University of Helsinki, during 2006-2009. The present and former heads of the Department of Medical Genetics are thanked for providing the excellent research facilities.

I am most grateful to Lauri Aaltonen for supervising this thesis. It has been privilege to be a member of his excellent group since January 2001! Lauri's enthusiasm and know-how on science combined with his wonderful character make him a great group leader. His genuine concern for the well-being of his group members has encouraged me "in the joy and the sorrow". My other supervisor, Auli Karhu, is deeply thanked for guiding me in my first steps into the world of science. I can honestly say that her guidance has been invaluable during these past years.

I wish to acknowledge Hannu Haapasalo and Ismo Ulmanen for reviewing this thesis and providing the valuable criticism and suggestions to improve the manuscript. I own special thanks to Krista Kauppinen for the excellent and fast editing of this thesis.

I wish to express my deepest gratitude to the patients and families for participating in this study. All collaborators and co-authors are warmly thanked for the fruitful collaboration: Outi Vierimaa, Markus J. Mäkinen, Karoliina Tuppurainen, Pasi Salmela, Kaisa Salmenkivi, Johanna Arola, Timo Sane, Ernesto De Menis, Ralf Paschke, Sadi Gündogdu, Robert J. Weil, Christian A. Koch, Rob van der Luijt, Cora M. Aalfs, Oliver Gimm, Wolfgang Saeger, Mercedes Robledo, Jan Lubinski, Olga Lucewicz, Anna Wasik, Grzegorz Zilienski, Anneke Lucassen, Marc Tischkowitz, Louise Izatt, Simon Aylwin, Gul Bano, Shirley Hodgson, Karin Birkenkamp-Demtröder, Torben F Orntoft, Elena Bonora, Manuela Vargiolu, and Giovanni Romeo.

Past and present members of the Aaltonen lab are thanked for sharing my everyday working life. Special thanks go to post-docs –Virpi, Auli, Pia V, Rainer, Pia A, Heli L, and Sari – for the theoretical and practical advice given. Warm thanks go to Marianna for sharing the moments of success and the moments of frustration in the AIP project. We were a good wet lab team! Rainer, Sini, Iina V, Pia V, Päivi L & co.: it has been pleasure to party with you through the whole night or occasionally even longer ☺ Marianna, Heli S, Silva, and Iina N are thanked for being wonderful and understanding roommates. Good technical assistance is invaluable in research work and huge thanks go to Iina V, Inga-Lill, Maarit, Mairi, Mikko, Päivi H, Sini, and Sirpa.

I would like to thank my dear families – both Leskinen and Raitila – and friends for their support and encouragement during these years. Indeed, you are deeply thanked for non-scientific contribution to this work!

My warmest gratitude goes to Tuomas for always being right there.

This study was financially supported by personal grants from the Paulo Foundation, the Maud Kuistila Memorial Foundation, the Finnish Cancer Organizations, the Biomedicum Helsinki Foundation, the Ida Montin Foundation, and the Research and Science Foundation of Orion-Farmos.

9. REFERENCES

Agarwal SK, Mateo CM, Marx SJ. 2009. Rare germline mutations in cyclin-dependent kinase inhibitor genes in multiple endocrine neoplasia type 1 and related states. The Journal of Clinical Endocrinology and Metabolism 94:1826-1834.

Alexander JM, Biller BM, Bikkal H, Zervas NT, Arnold A, Klibanski A. 1990. Clinically nonfunctioning pituitary tumors are monoclonal in origin. The Journal of Clinical Investigation 86:336-340.

Alexander L, Appleton D, Hall R, Ross WM, Wilkinson R. 1980. Epidemiology of acromegaly in the Newcastle region. Clinical Endocrinology 12:71-79.

Arafah BM and Nasrallah MP. 2001. Pituitary tumors: pathophysiology, clinical manifestations and management. Endocrine-Related Cancer 8:287-305.

Asa SL and Ezzat S. 2005. Genetics and proteomics of pituitary tumors. Endocrine 28:43-47.

Asa SL, Kovacs K, Stefaneanu L, Horvath E, Billestrup N, Gonzalez-Manchon C, Vale W. 1992. Pituitary adenomas in mice transgenic for growth hormone-releasing hormone. Endocrinology 131:2083-2089.

Bamberger CM, Fehn M, Bamberger AM, Ludecke DK, Beil FU, Saeger W, Schulte HM. 1999. Reduced expression levels of the cell-cycle inhibitor p27Kip1 in human pituitary adenomas. European Journal of Endocrinology 140:250-255.

Barlier A, Vanbellinghen JF, Daly AF, Silvy M, Jaffrain-Rea ML, Trouillas J, Tamagno G, Cazabat L, Bours V, Brue T, Enjalbert A, Beckers A. 2007. Mutations in the aryl hydrocarbon receptor interacting protein gene are not highly prevalent among subjects with sporadic pituitary adenomas. The Journal of Clinical Endocrinology and Metabolism 92:1952-1955.

Barouki R, Coumoul X, Fernandez-Salguero PM. 2007. The aryl hydrocarbon receptor, more than a xenobiotic-interacting protein. FEBS Letters 581:3608-3615.

Bassett JH, Forbes SA, Pannett AA, Lloyd SE, Christie PT, Wooding C, Harding B, Besser GM, Edwards CR, Monson JP, Sampson J, Wass JA, Wheeler MH, Thakker RV. 1998. Characterization of mutations in patients with multiple endocrine neoplasia type 1. American Journal of Human Genetics 62:232-244.

Beckers A and Daly AF. 2007. The clinical, pathological, and genetic features of familial isolated pituitary adenomas. European Journal of Endocrinology 157:371-382.

Beckers A, Abs R, Mahler C, Vandalem JL, Pirens G, Hennen G, Stevenaert A. 1991. Thyrotropin-secreting pituitary adenomas: report of seven cases. The Journal of Clinical Endocrinology and Metabolism 72:477-483.

Beck-Peccoz P, Brucker-Davis F, Persani L, Smallridge RC, Weintraub BD. 1996. Thyrotropin-secreting pituitary tumors. Endocrine Reviews 17:610-638.

Bell DR and Poland A. 2000. Binding of aryl hydrocarbon receptor (AhR) to AhR-interacting protein. The role of hsp90. The Journal of Biological Chemistry 275:36407-36414.

Bengtsson BA, Eden S, Ernest I, Oden A, Sjogren B. 1988. Epidemiology and long-term survival in acromegaly. A study of 166 cases diagnosed between 1955 and 1984. Acta Medica Scandinavica 223:327-335.

Benson L, Ljunghall S, Akerstrom G, Oberg K. 1987. Hyperparathyroidism presenting as the first lesion in multiple endocrine neoplasia type 1. The American Journal of Medicine 82:731-737.

Bertherat J, Groussin L, Sandrini F, Matyakhina L, Bei T, Stergiopoulos S, Papageorgiou T, Bourdeau I, Kirschner LS, Vincent-Dejean C, Perlemoine K, Gicquel C, Bertagna X, Stratakis CA. 2003. Molecular and functional analysis of PRKAR1A and its locus (17q22-24) in sporadic adrenocortical tumors: 17q losses, somatic mutations, and protein kinase A expression and activity. Cancer Research 63:5308-5319.

Bielas JH, Loeb KR, Rubin BP, True LD, Loeb LA. 2006. Human cancers express a mutator phenotype. Proceedings of the National Academy of Sciences of the United States of America 103:18238-18242.

Boikos SA and Stratakis CA. 2007a. Carney complex: the first 20 years. Current Opinion in Oncology 19:24-29.

Boikos SA and Stratakis CA. 2007b. Molecular genetics of the cAMP-dependent protein kinase pathway and of sporadic pituitary tumorigenesis. Human Molecular Genetics 16 Spec No 1:R80-7.

Bolger GB, Peden AH, Steele MR, MacKenzie C, McEwan DG, Wallace DA, Huston E, Baillie GS, Houslay MD. 2003. Attenuation of the activity of the cAMP-specific phosphodiesterase PDE4A5 by interaction with the immunophilin XAP2. The Journal of Biological Chemistry 278:33351-33363.

Bronner CE, Baker SM, Morrison PT, Warren G, Smith LG, Lescoe MK, Kane M, Earabino C, Lipford J, Lindblom A. 1994. Mutation in the DNA mismatch repair gene homologue hMLH1 is associated with hereditary non-polyposis colon cancer. Nature 368:258-261.

Brunak S, Engelbrecht J, Knudsen S. 1991. Prediction of human mRNA donor and acceptor sites from the DNA sequence. Journal of Molecular Biology 220:49-65.

Buchbinder S, Bierhaus A, Zorn M, Nawroth PP, Humpert P, Schilling T. 2008. Aryl hydrocarbon receptor interacting protein gene (AIP) mutations are rare in patients with hormone secreting or non-secreting pituitary adenomas. Experimental and Clinical Endocrinology & Diabetes 116:625-628.

Burrow GN, Wortzman G, Rewcastle NB, Holgate RC, Kovacs K. 1981. Microadenomas of the pituitary and abnormal sellar tomograms in an unselected autopsy series. The New England Journal of Medicine 304:156-158.

Calender A, Giraud S, Cougard P, Chanson P, Lenoir G, Murat A, Hamon P, Proye C. 1995. Multiple endocrine neoplasia type 1 in France: clinical and genetic studies. Journal of Internal Medicine 238:263-268.

Canzian F, Amati P, Harach HR, Kraimps JL, Lesueur F, Barbier J, Levillain P, Romeo G, Bonneau D. 1998. A gene predisposing to familial thyroid tumors with cell oxyphilia maps to chromosome 19p13.2. American Journal of Human Genetics 63:1743-1748.

Carling T, Correa P, Hessman O, Hedberg J, Skogseid B, Lindberg D, Rastad J, Westin G, Akerstrom G. 1998. Parathyroid MEN1 gene mutations in relation to clinical characteristics of nonfamilial primary hyperparathyroidism. The Journal of Clinical Endocrinology and Metabolism 83:2960-2963.

Carney JA, Gordon H, Carpenter PC, Shenoy BV, Go VL. 1985. The complex of myxomas, spotty pigmentation, and endocrine overactivity. Medicine 64:270-283.

Cartegni L, Wang J, Zhu Z, Zhang MQ, Krainer AR. 2003. ESEfinder: A web resource to identify exonic splicing enhancers. Nucleic Acids Research 31:3568-3571.

Carver LA and Bradfield CA. 1997. Ligand-dependent interaction of the aryl hydrocarbon receptor with a novel immunophilin homolog in vivo. The Journal of Biological Chemistry 272:11452-11456.

Cavaco BM, Batista PF, Sobrinho LG, Leite V. 2008. Mapping a new familial thyroid epithelial neoplasia susceptibility locus to chromosome 8p23.1-p22 by high-density single-nucleotide polymorphism genome-wide linkage analysis. The Journal of Clinical Endocrinology and Metabolism 93:4426-4430.

Cazabat L, Guillaud-Bataille M, Bertherat J, Raffin-Sanson ML. 2009. Mutations of the gene for the aryl hydrocarbon receptor-interacting protein in pituitary adenomas. Hormone Research 71:132-141.

Cazabat L, Libe R, Perlemoine K, Rene-Corail F, Burnichon N, Gimenez-Roqueplo AP, Dupasquier-Fediaevsky L, Bertagna X, Clauser E, Chanson P, Bertherat J, Raffin-Sanson ML. 2007. Germline inactivating mutations of the aryl hydrocarbon receptor-interacting protein gene in a large cohort of sporadic acromegaly: mutations are found in a subset of young patients with macroadenomas. European Journal of Endocrinology 157:1-8.

Chandrasekharappa SC, Guru SC, Manickam P, Olufemi SE, Collins FS, Emmert-Buck MR, Debelenko LV, Zhuang Z, Lubensky IA, Liotta LA, Crabtree JS, Wang Y, Roe BA, Weisemann J, Boguski MS, Agarwal SK, Kester MB, Kim YS, Heppner C, Dong Q, Spiegel AM, Burns AL, Marx SJ. 1997. Positional cloning of the gene for multiple endocrine neoplasia-type 1. Science 276:404-407.

Chanson P and Salenave S. 2008. Acromegaly. Orphanet Journal of Rare Diseases 3:17.

Chen HS and Perdew GH. 1994. Subunit composition of the heteromeric cytosolic aryl hydrocarbon receptor complex. The Journal of Biological Chemistry 269:27554-27558.

Ciccarelli A, Daly AF, Beckers A. 2005. The epidemiology of prolactinomas. Pituitary 8:3-6.

Colao A, Ferone D, Marzullo P, Lombardi G. 2004. Systemic complications of acromegaly: epidemiology, pathogenesis, and management. Endocrine Reviews 25:102-152.

Coogan PF, Baron JA, Lambe M. 1995. Parity and pituitary adenoma risk. Journal of the National Cancer Institute 87:1410-1411.

Croce CM. 2008. Oncogenes and cancer. The New England Journal of Medicine 358:502-511.

Daly AF, Tichomirowa MA, Ebeling TML, Vierimaa O, Cazabat L, Jaffrain-Rea ML, Naves LA, Eloranta E, Salmela PI, Vanbellinghen JF, Yaneva M, Zacharieva S, Barlier A, Emy P, Murat A, Poplier M, Fajardo Montanana C, Sabate MI, Guitelman M, Ferrandez Longas A, Brue T, Gimenez-Roqueplo AP, Bertherat J, Chanson P, Bours V, De Menis E, Aaltonen LA, Beckers A. 2008. An international, collaborative study of the disease characteristics and response to therapy in 60 pituitary adenoma patients with aryl hydrocarbon receptor-interacting protein (AIP) gene mutations. The Endocrine Society Annual Meeting 2008, San Francisco, California.

Daly AF, Cogne M, Jaffrain-Rea ML, Tabarin A, Murat A, Delemer B, Luger A, Gaillard R, Colao A, Harris AG, Berlocu MC, Petrossians P, Beckers A. 2007a. The epidemiology of pituitary tumors: Result of an international collaborative study. The Endocrine Society Annual Meeting 2007, Toronto, Canada.

Daly AF, Vanbellinghen JF, Khoo SK, Jaffrain-Rea ML, Naves LA, Guitelman MA, Murat A, Emy P, Gimenez-Roqueplo AP, Tamburrano G, Raverot G, Barlier A, De Herder W, Penfornis A, Ciccarelli E, Estour B, Lecomte P, Gatta B, Chabre O, Sabate MI, Bertagna X, Garcia Basavilbaso N, Stalldecker G, Colao A, Ferolla P, Wemeau JL, Caron P, Sadoul JL, Oneto A, Archambeaud F, Calender A, Sinilnikova O, Montanana CF, Cavagnini F, Hana V, Solano A, Delettieres D, Luccio-Camelo DC, Basso A, Rohmer V, Brue T, Bours V, Teh BT, Beckers A. 2007b. Aryl hydrocarbon receptor-interacting protein gene mutations in familial isolated pituitary adenomas: analysis in 73 families. The Journal of Clinical Endocrinology and Metabolism 92:1891-1896.

Daly AF, Jaffrain-Rea ML, Ciccarelli A, Valdes-Socin H, Rohmer V, Tamburrano G, Borson-Chazot C, Estour B, Ciccarelli E, Brue T, Ferolla P, Emy P, Colao A, De Menis E, Lecomte P, Penfornis F, Delemer B, Bertherat J, Wemeau JL, De Herder W, Archambeaud F, Stevenaert A, Calender A, Murat A, Cavagnini F, Beckers A. 2006a. Clinical characterization of familial isolated pituitary adenomas. The Journal of Clinical Endocrinology and Metabolism 91:3316-3323.

Daly AF, Rixhon M, Adam C, Dempegioti A, Tichomirowa MA, Beckers A. 2006b. High prevalence of pituitary adenomas: a cross-sectional study in the province of Liege, Belgium. The Journal of Clinical Endocrinology and Metabolism 91:4769-4775.

de Oliveira SK, Hoffmeister M, Gambaryan S, Muller-Esterl W, Guimaraes JA, Smolenski AP. 2007. Phosphodiesterase 2A forms a complex with the co-chaperone XAP2 and regulates nuclear translocation of the aryl hydrocarbon receptor. The Journal of Biological Chemistry 282:13656-13663.

DeLellis RA, Lloyd RV, Heitz PU, Eng C. 2004. World health organization classification of tumours. Pathology and genetics. Tumours of endocrine organs. Lyon: IARC Press.

Digiovanni R, Serra S, Ezzat S, Asa SL. 2007. AIP Mutations are not Identified in Patients with Sporadic Pituitary Adenomas. Endocrine Pathology 18:76-78.

Erfurth EM and Hagmar L. 2005. Cerebrovascular disease in patients with pituitary tumors. Trends in Endocrinology and Metabolism 16:334-342.

Eugster EA and Pescovitz OH. 1999. Gigantism. The Journal of Clinical Endocrinology and Metabolism 84:4379-4384.

Ezzat S, Asa SL, Couldwell WT, Barr CE, Dodge WE, Vance ML, McCutcheon IE. 2004. The prevalence of pituitary adenomas: a systematic review. Cancer 101:613-619.

Ezzat S, Zheng L, Zhu XF, Wu GE, Asa SL. 2002. Targeted expression of a human pituitary tumor-derived isoform of FGF receptor-4 recapitulates pituitary tumorigenesis. The Journal of Clinical Investigation 109:69-78.

Farnebo F, Teh BT, Kytola S, Svensson A, Phelan C, Sandelin K, Thompson NW, Hoog A, Weber G, Farnebo LO, Larsson C. 1998. Alterations of the MEN1 gene in sporadic parathyroid tumors. The Journal of Clinical Endocrinology and Metabolism 83:2627-2630.

Fernandez-Ranvier GG, Weng J, Yeh RF, Khanafshar E, Suh I, Barker C, Duh QY, Clark OH, Kebebew E. 2008. Identification of biomarkers of adrenocortical carcinoma using genomewide gene expression profiling. Archives of Surgery 143:841-6.

Fero ML, Rivkin M, Tasch M, Porter P, Carow CE, Firpo E, Polyak K, Tsai LH, Broudy V, Perlmutter RM, Kaushansky K, Roberts JM. 1996. A syndrome of multiorgan hyperplasia with features of gigantism, tumorigenesis, and female sterility in p27(Kip1)-deficient mice. Cell 85:733-744.

Fishel R, Lescoe MK, Rao MR, Copeland NG, Jenkins NA, Garber J, Kane M, Kolodner R. 1993. The human mutator gene homolog MSH2 and its association with hereditary nonpolyposis colon cancer. Cell 75:1027-1038.

Fogt F, Zimmerman RL, Hartmann CJ, Brown CA, Narula N. 2002. Genetic alterations of Carney complex are not present in sporadic cardiac myxomas. International Journal of Molecular Medicine 9:59-60.

Fritz A, Walch A, Piotrowska K, Rosemann M, Schaffer E, Weber K, Timper A, Wildner G, Graw J, Hofler H, Atkinson MJ. 2002. Recessive transmission of a multiple endocrine neoplasia syndrome in the rat. Cancer Research 62:3048-3051.

Froidevaux MS, Berg P, Seugnet I, Decherf S, Becker N, Sachs LM, Bilesimo P, Nygard M, Pongratz I, Demeneix BA. 2006. The co-chaperone XAP2 is required for activation of hypothalamic thyrotropin-releasing hormone transcription in vivo. EMBO Reports 7:1035-1039.

Futreal PA, Liu Q, Shattuck-Eidens D, Cochran C, Harshman K, Tavtigian S, Bennett LM, Haugen-Strano A, Swensen J, Miki Y. 1994. BRCA1 mutations in primary breast and ovarian carcinomas. Science 266:120-122.

Gadelha MR, Prezant TR, Une KN, Glick RP, Moskal SF,2nd, Vaisman M, Melmed S, Kineman RD, Frohman LA. 1999. Loss of heterozygosity on chromosome 11q13 in two families with acromegaly/gigantism is independent of mutations of the multiple endocrine neoplasia type I gene. The Journal of Clinical Endocrinology and Metabolism 84:249-256.

Georgitsi M, De Menis E, Cannavo S, Makinen MJ, Tuppurainen K, Pauletto P, Curto L, Weil RJ, Paschke R, Zielinski G, Wasik A, Lubinski J, Vahteristo P, Karhu A, Aaltonen LA. 2008a. Aryl hydrocarbon receptor interacting protein (AIP) gene mutation analysis in children and adolescents with sporadic pituitary adenomas. Clinical Endocrinology 69:621-627.

Georgitsi M, Heliovaara E, Paschke R, Kumar AV, Tischkowitz M, Vierimaa O, Salmela P, Sane T, De Menis E, Cannavo S, Gundogdu S, Lucassen A, Izatt L, Aylwin S, Bano G, Hodgson S, Koch CA, Karhu A, Aaltonen LA. 2008b. Large genomic deletions in AIP in pituitary adenoma predisposition. The Journal of Clinical Endocrinology and Metabolism 93:4146-4151.

Georgitsi M, Karhu A, Winqvist R, Visakorpi T, Waltering K, Vahteristo P, Launonen V, Aaltonen LA. 2007. Mutation analysis of aryl hydrocarbon receptor interacting protein (AIP) gene in colorectal, breast, and prostate cancers. British Journal of Cancer 96:352-356.

Ghannam NN, Hammami MM, Muttair Z, Bakheet SM. 1999. Primary hypothyroidism-associated TSH-secreting pituitary adenoma/hyperplasia presenting as a bleeding nasal mass and extremely elevated TSH level. Journal of Endocrinological Investigation 22:419-423.

Grossman RF, Tu SH, Duh QY, Siperstein AE, Novosolov F, Clark OH. 1995. Familial nonmedullary thyroid cancer. An emerging entity that warrants aggressive treatment. Archives of Surgery 130:892-7; discussion 898-9.

Groussin L, Jullian E, Perlemoine K, Louvel A, Leheup B, Luton JP, Bertagna X, Bertherat J. 2002. Mutations of the PRKAR1A gene in Cushing's syndrome due to sporadic primary pigmented nodular adrenocortical disease. The Journal of Clinical Endocrinology and Metabolism 87:4324-4329.

Hall WA, Luciano MG, Doppman JL, Patronas NJ, Oldfield EH. 1994. Pituitary magnetic resonance imaging in normal human volunteers: occult adenomas in the general population. Annals of Internal Medicine 120:817-820.

Hampel H, Frankel WL, Martin E, Arnold M, Khanduja K, Kuebler P, Nakagawa H, Sotamaa K, Prior TW, Westman J, Panescu J, Fix D, Lockman J, Comeras I, de la Chapelle A. 2005. Screening for the Lynch syndrome (hereditary nonpolyposis colorectal cancer). The New England Journal of Medicine 352:1851-1860.

Hanahan D and Weinberg RA. 2000. The hallmarks of cancer. Cell 100:57-70.

Heaney AP, Horwitz GA, Wang Z, Singson R, Melmed S. 1999. Early involvement of estrogen-induced pituitary tumor transforming gene and fibroblast growth factor expression in prolactinoma pathogenesis. Nature Medicine 5:1317-1321.

Hebsgaard SM, Korning PG, Tolstrup N, Engelbrecht J, Rouze P, Brunak S. 1996. Splice site prediction in Arabidopsis thaliana pre-mRNA by combining local and global sequence information. Nucleic Acids Research 24:3439-3452.

Heliovaara E, Raitila A, Launonen V, Paetau A, Arola J, Lehtonen H, Sane T, Weil RJ, Vierimaa O, Salmela P, Tuppurainen K, Makinen M, Aaltonen LA, Karhu A. 2009. The Expression of AIP-Related Molecules in Elucidation of Cellular Pathways in Pituitary Adenomas. The American Journal of Pathology Epub Oct 22.

Heppner C, Kester MB, Agarwal SK, Debelenko LV, Emmert-Buck MR, Guru SC, Manickam P, Olufemi SE, Skarulis MC, Doppman JL, Alexander RH, Kim YS, Saggar SK, Lubensky IA, Zhuang Z, Liotta LA, Chandrasekharappa SC, Collins FS, Spiegel AM, Burns AL, Marx SJ. 1997. Somatic mutation of the MEN1 gene in parathyroid tumours. Nature Genetics 16:375-378.

Herman V, Fagin J, Gonsky R, Kovacs K, Melmed S. 1990. Clonal origin of pituitary adenomas. The Journal of Clinical Endocrinology and Metabolism 71:1427-1433.

Herrmann BL, Baumann H, Janssen OE, Gorges R, Schmid KW, Mann K. 2004. Impact of disease activity on thyroid diseases in patients with acromegaly: basal evaluation and follow-up. Experimental and Clinical Endocrinology & Diabetes 112:225-230.

Hessman O, Lindberg D, Skogseid B, Carling T, Hellman P, Rastad J, Akerstrom G, Westin G. 1998. Mutation of the multiple endocrine neoplasia type 1 gene in nonfamilial, malignant tumors of the endocrine pancreas. Cancer Research 58:377-379.

Horvath E, Kovacs K, Scheithauer BW. 1999. Pituitary hyperplasia. Pituitary 1:169-179.

Igreja S, Chahal HS, Akker SA, Gueorguiev M, Popovic V, Damjanovic S, Burman P, Wass JA, Quinton R, Grossman AB, Korbonits M. 2009. Assessment of p27 (cyclin-dependent kinase inhibitor 1B) and aryl hydrocarbon receptor-interacting protein (AIP) genes in multiple endocrine neoplasia (MEN1) syndrome patients without any detectable MEN1 gene mutations. Clinical Endocrinology 70:259-264.

Ikeda H, Yoshimoto T, Shida N. 1997. Molecular analysis of p21 and p27 genes in human pituitary adenomas. British Journal of Cancer 76:1119-1123.

Iwata T, Yamada S, Mizusawa N, Golam HM, Sano T, Yoshimoto K. 2007. The aryl hydrocarbon receptor-interacting protein gene is rarely mutated in sporadic GH-secreting adenomas. Clinical Endocrinology 66:499-502.

Jaffrain-Rea ML, Angelini M, Gargano D, Tichomirowa MA, Daly AF, Vanbellinghen JF, D'Innocenzo E, Barlier A, Giangaspero F, Esposito V, Ventura L, Arcella A, Theodoropoulou M, Naves LA, Fajardo C, Zacharieva S, Rohmer V, Brue T, Gulino A, Cantore G, Alesse E, Beckers A. 2009. Expression of aryl hydrocarbon receptor (AHR) and AHR-interacting protein in pituitary adenomas: pathological and clinical implications. Endocrine-Related Cancer 16:1029-1043.

Jennings JE, Georgitsi M, Holdaway I, Daly AF, Tichomirowa M, Beckers A, Aaltonen LA, Karhu A, Cameron FJ. 2009. Aggressive pituitary adenomas occurring in young patients in a large Polynesian kindred with a germline R271W mutation in the AIP gene. European Journal of Endocrinology 161:799-804.

Kaltsas GA, Kola B, Borboli N, Morris DG, Gueorguiev M, Swords FM, Czirjak S, Kirschner LS, Stratakis CA, Korbonits M, Grossman AB. 2002. Sequence analysis of the PRKAR1A gene in sporadic somatotroph and other pituitary tumours. Clinical Endocrinology 57:443-448.

Kang BH and Altieri DC. 2006. Regulation of survivin stability by the aryl hydrocarbon receptor-interacting protein. The Journal of Biological Chemistry 281:24721-24727.

Kannio A, Ridanpaa M, Koskinen H, Partanen T, Anttila S, Collan Y, Hietanen E, Vainio H, Husgafvel-Pursiainen K. 1996. A molecular and epidemiological study on bladder cancer: p53 mutations, tobacco smoking, and occupational exposure to asbestos. Cancer Epidemiology, Biomarkers & Prevention 5:33-39.

Karhu A and Aaltonen LA. 2007. Susceptibility to pituitary neoplasia related to MEN-1, CDKN1B and AIP mutations: an update. Human Molecular Genetics 16 Spec No 1:R73-9.

Karnik SK, Hughes CM, Gu X, Rozenblatt-Rosen O, McLean GW, Xiong Y, Meyerson M, Kim SK. 2005. Menin regulates pancreatic islet growth by promoting histone methylation and expression of genes encoding p27Kip1 and p18INK4c. Proceedings of the National Academy of Sciences of the United States of America 102:14659-14664.

Kashuba E, Kashuba V, Pokrovskaja K, Klein G, Szekely L. 2000. Epstein-Barr virus encoded nuclear protein EBNA-3 binds XAP-2, a protein associated with Hepatitis B virus X antigen. Oncogene 19:1801-1806.

Kauppinen-Makelin R, Sane T, Reunanen A, Valimaki MJ, Niskanen L, Markkanen H, Loyttyniemi E, Ebeling T, Jaatinen P, Laine H, Nuutila P, Salmela P, Salmi J, Stenman UH, Viikari J, Voutilainen E. 2005. A nationwide survey of mortality in acromegaly. The Journal of Clinical Endocrinology and Metabolism 90:4081-4086.

Kazlauskas A, Poellinger L, Pongratz I. 1999. Evidence that the co-chaperone p23 regulates ligand responsiveness of the dioxin (Aryl hydrocarbon) receptor. The Journal of Biological Chemistry 274:13519-13524.

Khoo SK, Pendek R, Nickolov R, Luccio-Camelo DC, Newton TL, Massie A, Petillo D, Menon J, Cameron D, Teh BT, Chan SP. 2009. Genome-wide scan identifies novel modifier loci of acromegalic phenotypes for isolated familial somatotropinoma. Endocrine-Related Cancer 16:1057-1063.

Khoo US, Ozcelik H, Cheung AN, Chow LW, Ngan HY, Done SJ, Liang AC, Chan VW, Au GK, Ng WF, Poon CS, Leung YF, Loong F, Ip P, Chan GS, Andrulis IL, Lu J, Ho FC. 1999. Somatic mutations in the BRCA1 gene in Chinese sporadic breast and ovarian cancer. Oncogene 18:4643-4646.

Kinzler KW and Vogelstein B. 1998. Landscaping the cancer terrain. Science 280:1036-1037.

Kinzler KW and Vogelstein B. 1997. Cancer-susceptibility genes. Gatekeepers and caretakers. Nature 386:761, 763.

Kirschner LS, Carney JA, Pack SD, Taymans SE, Giatzakis C, Cho YS, Cho-Chung YS, Stratakis CA. 2000. Mutations of the gene encoding the protein kinase A type I-alpha regulatory subunit in patients with the Carney complex. Nature Genetics 26:89-92.

Kiyokawa H, Kineman RD, Manova-Todorova KO, Soares VC, Hoffman ES, Ono M, Khanam D, Hayday AC, Frohman LA, Koff A. 1996. Enhanced growth of mice lacking the cyclin-dependent kinase inhibitor function of p27(Kip1). Cell 85:721-732.

Knudson AG,Jr. 1971. Mutation and cancer: statistical study of retinoblastoma. Proceedings of the National Academy of Sciences of the United States of America 68:820-823.

Kolluri SK, Weiss C, Koff A, Gottlicher M. 1999. p27(Kip1) induction and inhibition of proliferation by the intracellular Ah receptor in developing thymus and hepatoma cells. Genes & Development 13:1742-1753.

Komatsubara K, Tahara S, Umeoka K, Sanno N, Teramoto A, Osamura RY. 2001. Immunohistochemical analysis of p27 (Kip1) in human pituitary glands and in various types of pituitary adenomas. Endocrine Pathology 12:181-188.

Kontogeorgos G, Kovacs K, Hovart E, Scheithauer BW. 1993. Null cell adenomas, oncocytomas, and gonodotroph adenomas of the human pituitary: An immunocytochemical and ultrastructural analysis of 300 cases. Endocrine Pathology 4:20-27.

Kovacs K, Scheithauer BW, Horvath E, Lloyd RV. 1996. The World Health Organization classification of adenohypophysial neoplasms. A proposed five-tier scheme. Cancer 78:502-510.

Kovacs K, Stefaneanu L, Ezzat S, Smyth HS. 1994. Prolactin-producing pituitary adenoma in a male-to-female transsexual patient with protracted estrogen administration. A morphologic study. Archives of Pathology & Laboratory Medicine 118:562-565.

Kurimoto M, Fukuda I, Hizuka N, Takano K. 2008. The prevalence of benign and malignant tumors in patients with acromegaly at a single institute. Endocrine Journal 55:67-71.

Lahiri DK and Nurnberger JI,Jr. 1991. A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. Nucleic Acids Research 19:5444.

Lairmore TC, Piersall LD, DeBenedetti MK, Dilley WG, Mutch MG, Whelan AJ, Zehnbauer B. 2004. Clinical genetic testing and early surgical intervention in patients with multiple endocrine neoplasia type 1 (MEN 1). Annals of Surgery 239:637-45; discussion 645-7.

Larsson C, Skogseid B, Oberg K, Nakamura Y, Nordenskjold M. 1988. Multiple endocrine neoplasia type 1 gene maps to chromosome 11 and is lost in insulinoma. Nature 332:85-87.

Lauter KB and Arnold A. 2008. Mutational analysis of CDKN1B, a candidate tumor-suppressor gene, in refractory secondary/tertiary hyperparathyroidism. Kidney International 73:1137-1140.

Laws ER,Jr, Scheithauer BW, Carpenter S, Randall RV, Abboud CF. 1985. The pathogenesis of acromegaly. Clinical and immunocytochemical analysis in 75 patients. Journal of Neurosurgery 63:35-38.

Leach FS, Nicolaides NC, Papadopoulos N, Liu B, Jen J, Parsons R, Peltomaki P, Sistonen P, Aaltonen LA, Nystrom-Lahti M. 1993. Mutations of a mutS homolog in hereditary nonpolyposis colorectal cancer. Cell 75:1215-1225.

Lemos MC and Thakker RV. 2008. Multiple endocrine neoplasia type 1 (MEN1): analysis of 1336 mutations reported in the first decade following identification of the gene. Human Mutation 29:22-32.

Leontiou CA, Gueorguiev M, van der Spuy J, Quinton R, Lolli F, Hassan S, Chahal HS, Igreja SC, Jordan S, Rowe J, Stolbrink M, Christian HC, Wray J, Bishop-Bailey D, Berney DM, Wass JA, Popovic V, Ribeiro-Oliveira A,Jr, Gadelha MR, Monson JP, Akker SA, Davis JR, Clayton RN, Yoshimoto K, Iwata T, Matsuno A, Eguchi K, Musat M, Flanagan D, Peters G, Bolger GB, Chapple JP, Frohman LA, Grossman AB, Korbonits M. 2008. The role of the aryl hydrocarbon receptor-interacting protein gene in familial and sporadic pituitary adenomas. The Journal of Clinical Endocrinology and Metabolism 93:2390-2401.

Lidhar K, Korbonits M, Jordan S, Khalimova Z, Kaltsas G, Lu X, Clayton RN, Jenkins PJ, Monson JP, Besser GM, Lowe DG, Grossman AB. 1999. Low expression of the cell cycle inhibitor p27Kip1 in normal corticotroph cells, corticotroph tumors, and malignant pituitary tumors. The Journal of Clinical Endocrinology and Metabolism 84:3823-3830.

Lin BC, Sullivan R, Lee Y, Moran S, Glover E, Bradfield CA. 2007. Deletion of the aryl hydrocarbon receptor-associated protein 9 leads to cardiac malformation and embryonic lethality. The Journal of Biological Chemistry 282:35924-35932.

Luccio-Camelo DC, Une KN, Ferreira RE, Khoo SK, Nickolov R, Bronstein MD, Vaisman M, Teh BT, Frohman LA, Mendonca BB, Gadelha MR. 2004. A meiotic recombination in a new isolated familial somatotropinoma kindred. European Journal of Endocrinology 150:643-648.

Lyons J, Landis CA, Harsh G, Vallar L, Grunewald K, Feichtinger H, Duh QY, Clark OH, Kawasaki E, Bourne HR. 1990. Two G protein oncogenes in human endocrine tumors. Science 249:655-659.

Malchoff CD and Malchoff DM. 2006. Familial nonmedullary thyroid carcinoma. Cancer Control 13:106-110.

Mantovani G, Bondioni S, Corbetta S, Menicanti L, Rubino B, Peverelli E, Labarile P, Dall'Asta C, Ambrosi B, Beck-Peccoz P, Lania AG, Spada A. 2009. Analysis of GNAS1 and PRKAR1A gene mutations in human cardiac myxomas not associated with multiple endocrine disorders. Journal of Endocrinological Investigation 32:501-504.

Marx S, Spiegel AM, Skarulis MC, Doppman JL, Collins FS, Liotta LA. 1998. Multiple endocrine neoplasia type 1: clinical and genetic topics. Annals of Internal Medicine 129:484-494.

McCabe CJ, Gittoes NJ, Sheppard MC, Franklyn JA. 1999. Increased MEN1 mRNA expression in sporadic pituitary tumours. Clinical Endocrinology 50:727-733.

McKay JD, Thompson D, Lesueur F, Stankov K, Pastore A, Watfah C, Strolz S, Riccabona G, Moncayo R, Romeo G, Goldgar DE. 2004. Evidence for interaction between the TCO and

NMTC1 loci in familial non-medullary thyroid cancer. Journal of Medical Genetics 41:407-412.

McKay JD, Lesueur F, Jonard L, Pastore A, Williamson J, Hoffman L, Burgess J, Duffield A, Papotti M, Stark M, Sobol H, Maes B, Murat A, Kaariainen H, Bertholon-Gregoire M, Zini M, Rossing MA, Toubert ME, Bonichon F, Cavarec M, Bernard AM, Boneu A, Leprat F, Haas O, Lasset C, Schlumberger M, Canzian F, Goldgar DE, Romeo G. 2001. Localization of a susceptibility gene for familial nonmedullary thyroid carcinoma to chromosome 2q21. American Journal of Human Genetics 69:440-446.

Melmed S. 2006. Medical progress: Acromegaly. The New England Journal of Medicine 355:2558-2573.

Melmed S. 2003. Mechanisms for pituitary tumorigenesis: the plastic pituitary. The Journal of Clinical Investigation 112:1603-1618.

Miki Y, Swensen J, Shattuck-Eidens D, Futreal PA, Harshman K, Tavtigian S, Liu Q, Cochran C, Bennett LM, Ding W. 1994. A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. Science 266:66-71.

Milne TA, Hughes CM, Lloyd R, Yang Z, Rozenblatt-Rosen O, Dou Y, Schnepp RW, Krankel C, Livolsi VA, Gibbs D, Hua X, Roeder RG, Meyerson M, Hess JL. 2005. Menin and MLL cooperatively regulate expression of cyclin-dependent kinase inhibitors. Proceedings of the National Academy of Sciences of the United States of America 102:749-754.

Molitch ME and Russell EJ. 1990. The pituitary "incidentaloma". Annals of Internal Medicine 112:925-931.

Moreno CS, Evans CO, Zhan X, Okor M, Desiderio DM, Oyesiku NM. 2005. Novel molecular signaling and classification of human clinically nonfunctional pituitary adenomas identified by gene expression profiling and proteomic analyses. Cancer Research 65:10214-10222.

Morris DG, Musat M, Czirjak S, Hanzely Z, Lillington DM, Korbonits M, Grossman AB. 2005. Differential gene expression in pituitary adenomas by oligonucleotide array analysis. European Journal of Endocrinology 153:143-151.

Mosse YP, Laudenslager M, Longo L, Cole KA, Wood A, Attiyeh EF, Laquaglia MJ, Sennett R, Lynch JE, Perri P, Laureys G, Speleman F, Kim C, Hou C, Hakonarson H, Torkamani A, Schork NJ, Brodeur GM, Tonini GP, Rappaport E, Devoto M, Maris JM. 2008. Identification of ALK as a major familial neuroblastoma predisposition gene. Nature 455:930-935.

Mulligan LM, Kwok JB, Healey CS, Elsdon MJ, Eng C, Gardner E, Love DR, Mole SE, Moore JK, Papi L. 1993. Germ-line mutations of the RET proto-oncogene in multiple endocrine neoplasia type 2A. Nature 363:458-460.

Nakayama K, Ishida N, Shirane M, Inomata A, Inoue T, Shishido N, Horii I, Loh DY, Nakayama K. 1996. Mice lacking p27(Kip1) display increased body size, multiple organ hyperplasia, retinal dysplasia, and pituitary tumors. Cell 85:707-720.

Naves LA, Daly AF, Vanbellinghen JF, Casulari LA, Spilioti C, Magalhaes AV, Azevedo MF, Giacomini LA, Nascimento PP, Nunes RO, Rosa JW, Jaffrain-Rea ML, Bours V, Beckers A. 2007. Variable pathological and clinical features of a large Brazilian family harboring a mutation in the aryl hydrocarbon receptor-interacting protein gene. European Journal of Endocrinology 157:383-391.

Nishida T, Hirota S, Taniguchi M, Hashimoto K, Isozaki K, Nakamura H, Kanakura Y, Tanaka T, Takabayashi A, Matsuda H, Kitamura Y. 1998. Familial gastrointestinal stromal tumours with germline mutation of the KIT gene. Nature Genetics 19:323-324.

Oesch-Bartlomowicz B, Huelster A, Wiss O, Antoniou-Lipfert P, Dietrich C, Arand M, Weiss C, Bockamp E, Oesch F. 2005. Aryl hydrocarbon receptor activation by cAMP vs. dioxin: divergent signaling pathways. Proceedings of the National Academy of Sciences of the United States of America 102:9218-9223.

Owens M, Stals K, Ellard S, Vaidya B. 2009. Germline mutations in the CDKN1B gene encoding p27 Kip1 are a rare cause of multiple endocrine neoplasia type 1. Clinical Endocrinology 70:499-500.

Ozawa A, Agarwal SK, Mateo CM, Burns AL, Rice TS, Kennedy PA, Quigley CM, Simonds WF, Weinstein LS, Chandrasekharappa SC, Collins FS, Spiegel AM, Marx SJ. 2007. The

parathyroid/pituitary variant of multiple endocrine neoplasia type 1 usually has causes other than p27Kip1 mutations. The Journal of Clinical Endocrinology and Metabolism 92:1948-1951.

Pannett AA and Thakker RV. 2001. Somatic mutations in MEN type 1 tumors, consistent with the Knudson "two-hit" hypothesis. The Journal of Clinical Endocrinology and Metabolism 86:4371-4374.

Papadopoulos N, Nicolaides NC, Wei YF, Ruben SM, Carter KC, Rosen CA, Haseltine WA, Fleischmann RD, Fraser CM, Adams MD. 1994. Mutation of a mutL homolog in hereditary colon cancer. Science 263:1625-1629.

Payne SR and Kemp CJ. 2005. Tumor suppressor genetics. Carcinogenesis 26:2031-2045.

Pellegata NS, Quintanilla-Martinez L, Siggelkow H, Samson E, Bink K, Hofler H, Fend F, Graw J, Atkinson MJ. 2006. Germ-line mutations in p27Kip1 cause a multiple endocrine neoplasia syndrome in rats and humans. Proceedings of the National Academy of Sciences of the United States of America 103:15558-15563.

Peltonen L. 1997. Molecular background of the Finnish disease heritage. Annals of Medicine 29:553-556.

Perdigao PF, Stergiopoulos SG, De Marco L, Matyakhina L, Boikos SA, Gomez RS, Pimenta FJ, Stratakis CA. 2005. Molecular and immunohistochemical investigation of protein kinase a regulatory subunit type 1A (PRKAR1A) in odontogenic myxomas. Genes, Chromosomes & Cancer 44:204-211.

Pesatori AC, Baccarelli A, Consonni D, Lania A, Beck-Peccoz P, Bertazzi PA, Spada A. 2008. Aryl hydrocarbon receptor-interacting protein and pituitary adenomas: a population-based study on subjects exposed to dioxin after the Seveso, Italy, accident. European Journal of Endocrinology 159:699-703.

Picard C, Silvy M, Gerard C, Buffat C, Lavaque E, Figarella-Branger D, Dufour H, Gabert J, Beckers A, Brue T, Enjalbert A, Barlier A. 2007. Gs alpha overexpression and loss of Gs alpha imprinting in human somatotroph adenomas: association with tumor size and response to pharmacologic treatment. International Journal of Cancer 121:1245-1252.

Reese MG, Eeckman FH, Kulp D, Haussler D. 1997. Improved splice site detection in Genie. Journal of Computational Biology 4:311-323.

Roman SA. 2003. Endocrine tumors: evaluation of the thyroid nodule. Current Opinion in Oncology 15:66-70.

Ruebel KH, Leontovich AA, Jin L, Stilling GA, Zhang H, Qian X, Nakamura N, Scheithauer BW, Kovacs K, Lloyd RV. 2006. Patterns of gene expression in pituitary carcinomas and adenomas analyzed by high-density oligonucleotide arrays, reverse transcriptase-quantitative PCR, and protein expression. Endocrine 29:435-444.

Samuels MH and Ridgway EC. 1995. Glycoprotein-secreting pituitary adenomas. Bailliere's Clinical Endocrinology and Metabolism 9:337-358.

Sandrini F, Kirschner LS, Bei T, Farmakidis C, Yasufuku-Takano J, Takano K, Prezant TR, Marx SJ, Farrell WE, Clayton RN, Groussin L, Bertherat J, Stratakis CA. 2002a. PRKAR1A, one of the Carney complex genes, and its locus (17q22-24) are rarely altered in pituitary tumours outside the Carney complex. Journal of Medical Genetics 39:e78.

Sandrini F, Matyakhina L, Sarlis NJ, Kirschner LS, Farmakidis C, Gimm O, Stratakis CA. 2002b. Regulatory subunit type I-alpha of protein kinase A (PRKAR1A): a tumor-suppressor gene for sporadic thyroid cancer. Genes, Chromosomes & Cancer 35:182-192.

Sanno N, Teramoto A, Osamura RY. 2000. Long-term surgical outcome in 16 patients with thyrotropin pituitary adenoma. Journal of Neurosurgery 93:194-200.

Scacheri PC, Davis S, Odom DT, Crawford GE, Perkins S, Halawi MJ, Agarwal SK, Marx SJ, Spiegel AM, Meltzer PS, Collins FS. 2006. Genome-wide analysis of menin binding provides insights into MEN1 tumorigenesis. PLoS Genetics 2:e51.

Schmidt L, Duh FM, Chen F, Kishida T, Glenn G, Choyke P, Scherer SW, Zhuang Z, Lubensky I, Dean M, Allikmets R, Chidambaram A, Bergerheim UR, Feltis JT, Casadevall C, Zamarron A, Bernues M, Richard S, Lips CJ, Walther MM, Tsui LC, Geil L, Orcutt ML, Stackhouse T, Lipan J, Slife L, Brauch H, Decker J, Niehans G, Hughson MD, Moch H, Storkel S, Lerman MI,

Linehan WM, Zbar B. 1997. Germline and somatic mutations in the tyrosine kinase domain of the MET proto-oncogene in papillary renal carcinomas. Nature Genetics 16:68-73.

Schmidt MC, Henke RT, Stangl AP, Meyer-Puttlitz B, Stoffel-Wagner B, Schramm J, von Deimling A. 1999. Analysis of the MEN1 gene in sporadic pituitary adenomas. The Journal of Pathology 188:168-173.

Schwindinger WF, Francomano CA, Levine MA. 1992. Identification of a mutation in the gene encoding the alpha subunit of the stimulatory G protein of adenylyl cyclase in McCune-Albright syndrome. Proceedings of the National Academy of Sciences of the United States of America 89:5152-5156.

Shan L, Nakamura Y, Nakamura M, Yokoi T, Tsujimoto M, Arima R, Kameya T, Kakudo K. 1998. Somatic mutations of multiple endocrine neoplasia type 1 gene in the sporadic endocrine tumors. Laboratory Investigation 78:471-475.

Shibata DK, Arnheim N, Martin WJ. 1988. Detection of human papilloma virus in paraffin-embedded tissue using the polymerase chain reaction. The Journal of Experimental Medicine 167:225-230.

Siegel G and Tomer Y. 2005. Is there an association between acromegaly and thyroid carcinoma? A critical review of the literature. Endocrine Research 31:51-58.

Smith PJ, Zhang C, Wang J, Chew SL, Zhang MQ, Krainer AR. 2006. An increased specificity score matrix for the prediction of SF2/ASF-specific exonic splicing enhancers. Human Molecular Genetics 15:2490-2508.

Snyder PJ. 1995. Extensive personal experience: gonadotroph adenomas. The Journal of Clinical Endocrinology and Metabolism 80:1059-1061.

Soares BS, Eguchi K, Frohman LA. 2005. Tumor deletion mapping on chromosome 11q13 in eight families with isolated familial somatotropinoma and in 15 sporadic somatotropinomas. The Journal of Clinical Endocrinology and Metabolism 90:6580-6587.

Soares BS and Frohman LA. 2004. Isolated familial somatotropinoma. Pituitary 7:95-101.

Spada A, Mantovani G, Lania A. 2005. Pathogenesis of prolactinomas. Pituitary 8:7-15.

Steward BW,Kleihues P. World cancer report, 2003.

Stratakis CA, Kirschner LS, Carney JA. 2001. Clinical and molecular features of the Carney complex: diagnostic criteria and recommendations for patient evaluation. The Journal of Clinical Endocrinology and Metabolism 86:4041-4046.

Stratakis CA, Carney JA, Lin JP, Papanicolaou DA, Karl M, Kastner DL, Pras E, Chrousos GP. 1996. Carney complex, a familial multiple neoplasia and lentiginosis syndrome. Analysis of 11 kindreds and linkage to the short arm of chromosome 2. The Journal of Clinical Investigation 97:699-705.

Sumanasekera WK, Tien ES, Turpey R, Vanden Heuvel JP, Perdew GH. 2003. Evidence that peroxisome proliferator-activated receptor alpha is complexed with the 90-kDa heat shock protein and the hepatitis virus B X-associated protein 2. The Journal of Biological Chemistry 278:4467-4473.

Tanaka C, Yoshimoto K, Yamada S, Nishioka H, Ii S, Moritani M, Yamaoka T, Itakura M. 1998. Absence of germ-line mutations of the multiple endocrine neoplasia type 1 (MEN1) gene in familial pituitary adenoma in contrast to MEN1 in Japanese. The Journal of Clinical Endocrinology and Metabolism 83:960-965.

Tanaka C, Yoshimoto K, Yang P, Kimura T, Yamada S, Moritani M, Sano T, Itakura M. 1997. Infrequent mutations of p27Kip1 gene and trisomy 12 in a subset of human pituitary adenomas. The Journal of Clinical Endocrinology and Metabolism 82:3141-3147.

Tchourbanov A and Ali HH. 2005. Combinatorial Method of Splice Sites Prediction, 2005 IEEE Computational Systems Bioinformatics Conference (CSB'05) :189-190.

Teh BT, Kytola S, Farnebo F, Bergman L, Wong FK, Weber G, Hayward N, Larsson C, Skogseid B, Beckers A, Phelan C, Edwards M, Epstein M, Alford F, Hurley D, Grimmond S, Silins G, Walters M, Stewart C, Cardinal J, Khodaei S, Parente F, Tranebjaerg L, Jorde R, Salmela P. 1998. Mutation analysis of the MEN1 gene in multiple endocrine neoplasia type 1, familial acromegaly and familial isolated hyperparathyroidism. The Journal of Clinical Endocrinology and Metabolism 83:2621-2626.

Thakker RV. 1998. Multiple endocrine neoplasia--syndromes of the twentieth century. The Journal of Clinical Endocrinology and Metabolism 83:2617-2620.

Thakker RV, Pook MA, Wooding C, Boscaro M, Scanarini M, Clayton RN. 1993. Association of somatotrophinomas with loss of alleles on chromosome 11 and with gsp mutations. The Journal of Clinical Investigation 91:2815-2821.

Theodoropoulou M, Cavallari I, Barzon L, D'Agostino DM, Ferro T, Arzberger T, Grubler Y, Schaaf L, Losa M, Fallo F, Ciminale V, Stalla GK, Pagotto U. 2004. Differential expression of menin in sporadic pituitary adenomas. Endocrine-Related Cancer 11:333-344.

Toledo RA, Mendonca BB, Fragoso CM, Longuini VC, Lourenco DM,Jr, Moyses CB, Soares CI, Jallad SR, Bronstein MD, Toledo SPA. 2008. Germline mutation, loss-of-heterozygosity and low immunohistochemical detection of AIP, which attenuates activity of the cAMP-specific PDE4A5, in adrenocortical carcinoma. 11th International Workshop on Multiple Endocrine Neoplasia, Delphi, Greece.

Toledo RA, Lourenco DM,Jr, Liberman B, Cunha-Neto MB, Cavalcanti MG, Moyses CB, Toledo SP, Dahia PL. 2007. Germline mutation in the aryl hydrocarbon receptor interacting protein gene in familial somatotropinoma. The Journal of Clinical Endocrinology and Metabolism 92:1934-1937.

Tomlinson IP, Alam NA, Rowan AJ, Barclay E, Jaeger EE, Kelsell D, Leigh I, Gorman P, Lamlum H, Rahman S, Roylance RR, Olpin S, Bevan S, Barker K, Hearle N, Houlston RS, Kiuru M, Lehtonen R, Karhu A, Vilkki S, Laiho P, Eklund C, Vierimaa O, Aittomaki K, Hietala M, Sistonen P, Paetau A, Salovaara R, Herva R, Launonen V, Aaltonen LA, Multiple Leiomyoma Consortium. 2002. Germline mutations in FH predispose to dominantly inherited uterine fibroids, skin leiomyomata and papillary renal cell cancer. Nature Genetics 30:406-410.

Trump D, Farren B, Wooding C, Pang JT, Besser GM, Buchanan KD, Edwards CR, Heath DA, Jackson CE, Jansen S, Lips K, Monson JP, O'Halloran D, Sampson J, Shalet SM, Wheeler MH, Zink A, Thakker RV. 1996. Clinical studies of multiple endocrine neoplasia type 1 (MEN1). QJM : monthly journal of the Association of Physicians 89:653-669.

Valimaki MJ, Sane T, Dunkel L. 2009. Endokrinologia. 2nd edn. Kustannut Oy Duodecim, Helsinki.

Vallar L, Spada A, Giannattasio G. 1987. Altered Gs and adenylate cyclase activity in human GH-secreting pituitary adenomas. Nature 330:566-568.

Vargiolu M, Fusco D, Kurelac I, Dirnberger D, Baumeister R, Morra I, Melcarne A, Rimondini R, Romeo G, Bonora E. 2009. The tyrosine kinase receptor RET interacts in vivo with aryl hydrocarbon receptor-interacting protein to alter survivin availability. The Journal of Clinical Endocrinology and Metabolism 94:2571-2578.

Vierimaa O, Villablanca A, Alimov A, Georgitsi M, Raitila A, Vahteristo P, Larsson C, Ruokonen A, Eloranta E, Ebeling TM, Ignatius J, Aaltonen LA, Leisti J, Salmela PI. 2009. Mutation analysis of MEN1, HRPT2, CASR, CDKN1B and AIP genes in primary hyperparathyroidism patients with features of genetic predisposition. Journal of Endocrinological Investigation Epub Mar 26.

Vierimaa O, Georgitsi M, Lehtonen R, Vahteristo P, Kokko A, Raitila A, Tuppurainen K, Ebeling TM, Salmela PI, Paschke R, Gundogdu S, De Menis E, Makinen MJ, Launonen V, Karhu A, Aaltonen LA. 2006. Pituitary adenoma predisposition caused by germline mutations in the AIP gene. Science 312:1228-1230.

Vortmeyer AO, Boni R, Pak E, Pack S, Zhuang Z. 1998. Multiple endocrine neoplasia 1 gene alterations in MEN1-associated and sporadic lipomas. Journal of the National Cancer Institute 90:398-399.

Wang M and Marin A. 2006. Characterization and prediction of alternative splice sites. Gene 366:219-227.

Weinberg RA. 2007. The biology of cancer. 1st edn. Garland Science,Taylor & Francis Group, LLC, New York.

Weinstein LS, Shenker A, Gejman PV, Merino MJ, Friedman E, Spiegel AM. 1991. Activating mutations of the stimulatory G protein in the McCune-Albright syndrome. The New England Journal of Medicine 325:1688-1695.

Wenbin C, Asai A, Teramoto A, Sanno N, Kirino T. 1999. Mutations of the MEN1 tumor suppressor gene in sporadic pituitary tumors. Cancer Letters 142:43-47.

Wood LD, Parsons DW, Jones S, Lin J, Sjoblom T, Leary RJ, Shen D, Boca SM, Barber T, Ptak J, Silliman N, Szabo S, Dezso Z, Ustyanksky V, Nikolskaya T, Nikolsky Y, Karchin R, Wilson PA, Kaminker JS, Zhang Z, Croshaw R, Willis J, Dawson D, Shipitsin M, Willson JK, Sukumar S, Polyak K, Park BH, Pethiyagoda CL, Pant PV, Ballinger DG, Sparks AB, Hartigan J, Smith DR, Suh E, Papadopoulos N, Buckhaults P, Markowitz SD, Parmigiani G, Kinzler KW, Velculescu VE, Vogelstein B. 2007. The genomic landscapes of human breast and colorectal cancers. Science 318:1108-1113.

Wooster R, Bignell G, Lancaster J, Swift S, Seal S, Mangion J, Collins N, Gregory S, Gumbs C, Micklem G. 1995. Identification of the breast cancer susceptibility gene BRCA2. Nature 378:789-792.

Wrocklage C, Gold H, Hackl W, Buchfelder M, Fahlbusch R, Paulus W. 2002. Increased menin expression in sporadic pituitary adenomas. Clinical Endocrinology 56:589-594.

Yamada S, Yoshimoto K, Sano T, Takada K, Itakura M, Usui M, Teramoto A. 1997. Inactivation of the tumor suppressor gene on 11q13 in brothers with familial acrogigantism without multiple endocrine neoplasia type 1. The Journal of Clinical Endocrinology and Metabolism 82:239-242.

Yamada S, Asa SL, Kovacs K. 1988. Oncocytomas and null cell adenomas of the human pituitary: morphometric and in vitro functional comparison. Virchows Archiv 413:333-339.

Yamasaki H, Mizusawa N, Nagahiro S, Yamada S, Sano T, Itakura M, Yoshimoto K. 2003. GH-secreting pituitary adenomas infrequently contain inactivating mutations of PRKAR1A and LOH of 17q23-24. Clinical Endocrinology 58:464-470.

Yaneva M, Daly AF, Tichomirowa M, Vanbellinghen JF, Hagelstein M, Bours V, Zacharieva S, Beckers A. 2008. Aryl hydrocarbon receptor interacting protein gene mutations in Bulgarian FIPA and young sporadic pituitary adenoma patients. The Endocrine Society Annual Meeting 2008, San Francisco, California.

Yang Q, Yoshimura G, Nakamura M, Nakamura Y, Suzuma T, Umemura T, Mori I, Sakurai T, Kakudo K. 2002. BRCA1 in non-inherited breast carcinomas (Review). Oncology Reports 9:1329-1333.

Yano M, Terada K, Mori M. 2003. AIP is a mitochondrial import mediator that binds to both import receptor Tom20 and preproteins. The Journal of Cell Biology 163:45-56.

Yu R, Bonert V, Saporta I, Raffel LJ, Melmed S. 2006. Aryl hydrocarbon receptor interacting protein variants in sporadic pituitary adenomas. The Journal of Clinical Endocrinology and Metabolism 91:5126-5129.

Zhang X, Sun H, Danila DC, Johnson SR, Zhou Y, Swearingen B, Klibanski A. 2002. Loss of expression of GADD45 gamma, a growth inhibitory gene, in human pituitary adenomas: implications for tumorigenesis. The Journal of Clinical Endocrinology and Metabolism 87:1262-1267.

Zhuang Z, Ezzat SZ, Vortmeyer AO, Weil R, Oldfield EH, Park WS, Pack S, Huang S, Agarwal SK, Guru SC, Manickam P, Debelenko LV, Kester MB, Olufemi SE, Heppner C, Crabtree JS, Burns AL, Spiegel AM, Marx SJ, Chandrasekharappa SC, Collins FS, Emmert-Buck MR, Liotta LA, Asa SL, Lubensky IA. 1997a. Mutations of the MEN1 tumor suppressor gene in pituitary tumors. Cancer Research 57:5446-5451.

Zhuang Z, Vortmeyer AO, Pack S, Huang S, Pham TA, Wang C, Park WS, Agarwal SK, Debelenko LV, Kester M, Guru SC, Manickam P, Olufemi SE, Yu F, Heppner C, Crabtree JS, Skarulis MC, Venzon DJ, Emmert-Buck MR, Spiegel AM, Chandrasekharappa SC, Collins FS, Burns AL, Marx SJ, Lubensky IA. 1997b. Somatic mutations of the MEN1 tumor suppressor gene in sporadic gastrinomas and insulinomas. Cancer Research 57:4682-4686.

Zuo L, Weger J, Yang Q, Goldstein AM, Tucker MA, Walker GJ, Hayward N, Dracopoli NC. 1996. Germline mutations in the p16INK4a binding domain of CDK4 in familial melanoma. Nature Genetics 12:97-99.