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"Predictors of neuropsychiatric side effects of dopamine-agonist therapy in patients with prolactinomas"

Dissertation

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To my parents and my sister

Contents

1. LIST OF ABBREVIATIONS

- PRL: **Pr**o**l**actin
- kDa: **k**ilo**da**lton
- GH: **G**rowth **H**ormone
- hPL: **H**uman **P**lacental **L**actogen
- REM: **R**apid **E**ye **M**ovement
- GnRH: **G**o**n**adotropin **R**eleasing **H**ormone
- TRH: **T**hyrotropin **R**eleasing **H**ormone
- VIP: **V**asoactive **I**ntestinal **P**eptide
- LH: **L**uteinizing **H**ormone
- MRI: **M**agnetic **R**esonance **I**maging
- DA: **D**opamine **A**gonists
- D2R: **D**opamine **2** **R**eceptor
- PRL-R: **PRL** **R**eceptor
- CSF: **C**erebro**s**pinal **F**luid
- NFPA: **N**on – **F**unctioning **P**ituitary **A**denomas
- PD: **P**arkinson's **D**isease
- ICDs: **I**mpulse **C**ontrol **D**isorders
- BBB: **B**lood-**B**rain **B**arrier
- MDR1: **M**ulti**d**rug **r**esistance **1** gene
- SNPs: **S**ingle **N**ucleotide **P**olymorphisms
- P-gp: **P**-**g**lyco**p**rotein
- RLS: **R**estless **L**egs **S**yndrom

2. INTRODUCTION/BACKGROUND

2.1 Epidemiology of hyperprolactinemia

The prevalence of hyperprolactinemia in women with secondary amenorrhea or oligoamenorrhea is estimated to be 10% to 25%. Hyperprolactinemia is noted in approximately 30% of women with galactorrhea or infertility and in 75% of those with both amenorrhea and galactorrhea (1, 2). Hyperprolactinemia is present in 16% of patients who have erectile dysfunction and in approximately 11% of patients who have oligospermia (3).

2.2 Secretion of Prolactin

Prolactin is a protein (198 amino acids with a molecular mass of 21500 daltons) synthesised in lactotrope cells (~ 20% of anterior pituitary cells). In pregnancy lactotrope cell hyperplasia is induced by estrogen (especially the last two trimesters and in lactation) (4). PRL is secreted, in a pulsatile manner, reaching its peak levels in the morning and has also a circadian fluctuation with higher levels during non–rapid eye movement (REM) sleep (5). It is inhibited by hypothalamic dopamine and transported to the pituitary by portal vessels, mediated through the dopamine type 2 receptors (D2R) and stimulated from thyrotropin releasing hormone (TRH) and vasoactive intestinal peptide (VIP). The normal adult serum PRL levels are below 25 ng/ml and 20 ng/ml in women and men, respectively, as detected with the more commonly used assays (1 ng/ml is equivalent to 21,2 mIU/l, WHO Standard 84/500).

2.3 Role and action of prolactin

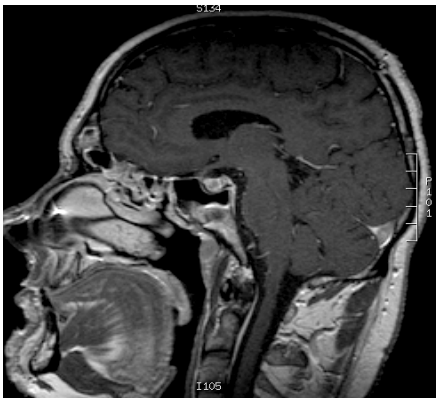
The main role of PRL is to induce and maintain lactation, decrease reproductive function and libido, so that maternal lactation is sustained and not interrupted by a new pregnancy. PRL suppresses hypothalamic GnRH and pituitary gonadotropin secretion and impairs gonadal steroidogenesis in both women and men. In the ovary, PRL directly blocks folliculogenesis leading to hypoestrogenism and anovulation. The luteolytic effect of PRL leads to a shortened or inadequate, luteal phase of the menstrual cycle. In men, attenuated LH secretion leads to low testosterone levels and decreased spermatogenesis (4).

The PRL receptor (PRL-R) is a transmembrane protein, encoded by a single gene on chromosome 5 (6-8) and is mainly present in the mammary gland and the ovary, but also in multiple tissues (pituitary gland, thymus, spleen, liver, pancreas, kidney, adrenal gland, uterus) (9). Interestingly, PRL receptors are also found in several areas of the CNS (10, 11).

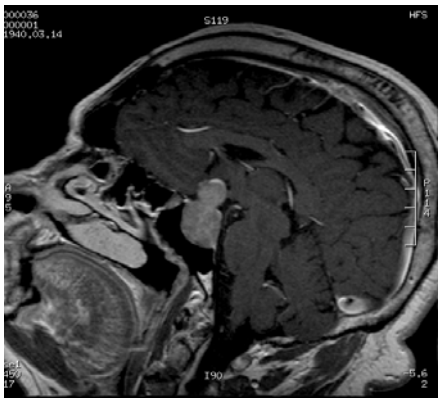
2.4 Definition and classification of prolactinomas

Prolactin hypersecretion is the most common endocrine abnormality due to hypothalamic-pituitary disorders and prolactinomas, the tumours arising from lactotrope cells of the anterior pituitary accounting for about 40% of all pituitary tumours. Microprolactinomas, as for all the pituitary adenomas, are classified as <1 cm in diameter and do not usually invade the parasellar region, whereas macroprolactinomas have a diameter >1 cm and may be locally invasive on adjacent structures.

***Figure 1**: Micro- and macroadenoma, sagittal MRI views (courtesy of Prof. Dr. Schopohl).*



Microadenoma (< 1cm)



Macroadenoma (> 1cm)

Their annual incidence is considered to be 6-10 cases per million population (12). Some more recent data from a study conducted in a tightly defined geographic area in Liege in Belgium, show a much higher prevalence at 55 per 71000 (775 per million) inhabitants (1). Their frequency depends on age and sex, with increased preference in females between 20 and 50 years old, when the ratio between the sexes is estimated to be 10:1. After the fifth decade of life their frequency seems to be equivalent in both sexes (13, 14).

The vast majority of the prolactinomas are benign adenomas. Approximately 50 malignant prolactinomas have been described (15, 16). The presence of metastatic lesions is the most important differential diagnostic tool from the aggressive prolactinomas which are a more frequent finding. The prognosis of malignant prolactinoma is poor, with a 1-year prevalence in less than 50% of patients described in the literature (16).

2.5 Clinical features and differential diagnosis of prolactinomas

The basis of the clinical manifestations of prolactinomas is the hyperprolactinemia and the tumour that can present with neurological signs. Collectively, the above mentioned PRL actions lead to various forms of primary or secondary hypogonadism in both genders.

Women present with the classic amenorrhea-oligomenorrhea with anovulation, galactorrhea, and infertility syndrome but when hyperprolactinemia develops prior to menarche in children or adolescents, results in primary amenorrhea. The galactorrhea is considered abnormal if it persists for longer than 6 months after discontinuation of breastfeeding. It occurs in the majority of women with prolactinomas and is much less common in men. It may come spontaneously or be elicited by nipple pressure, present only transiently or intermittently.
The necessity of measuring PRL levels in patients with unexplained preliminary or secondary amenorrhea in the clinical practise should be emphasised, due to the fact that hyperprolactinemia may be present even in the absence of galactorrhea. Some other symptoms could be weight gain, decreased libido or mild hirsutism (4, 17, 18).

In men, excess PRL presents with hypogonadism, diminished libido, infertility and rarely galactorrhea and gynecomastia. Gonadotropin suppression from PRL leads to reduced testosterone, impotence, and oligospermia. If the disorder is longstanding, decreased beard growth and reduced muscle mass can be present as secondary effects of hypogonadism (4, 17, 18).

In macroprolactinomas, the diagnosis is often made due to local pressure. Neurologic symptoms (headache and visual field defects such as bilateral hemianopsia, initially of superior quadrants) are common in patients who have macroadenomas or giant adenomas and also in men, due to the delayed diagnosis, whereas atypical clinical manifestations (e.g. diplopia, cranial nerve paralysis) are most frequent in aggressive or malignant forms.

Furthermore, some non-specific symptoms of anterior pituitary deficits could be present in cases of macroadenomas.

In cases of pronounced hypoestrogenemia, osteopenia should be considered. This has recently been considered a new indication for early treatment of prolactinoma (19). The mechanism of the negative effect of PRL excess remains rather vague, but some in vitro and in vivo data suggest a predominant role for estrogen deficiency (20).

The prolactin measurement should be considered in one of the following conditions: (18)

- Galactorrhea

- Enlarged sella turcica

- Suspected pituitary tumour

- Hypogonadotropic hypogonadism including unexplained amenorrhea or unexplained male hypogonadism or infertility

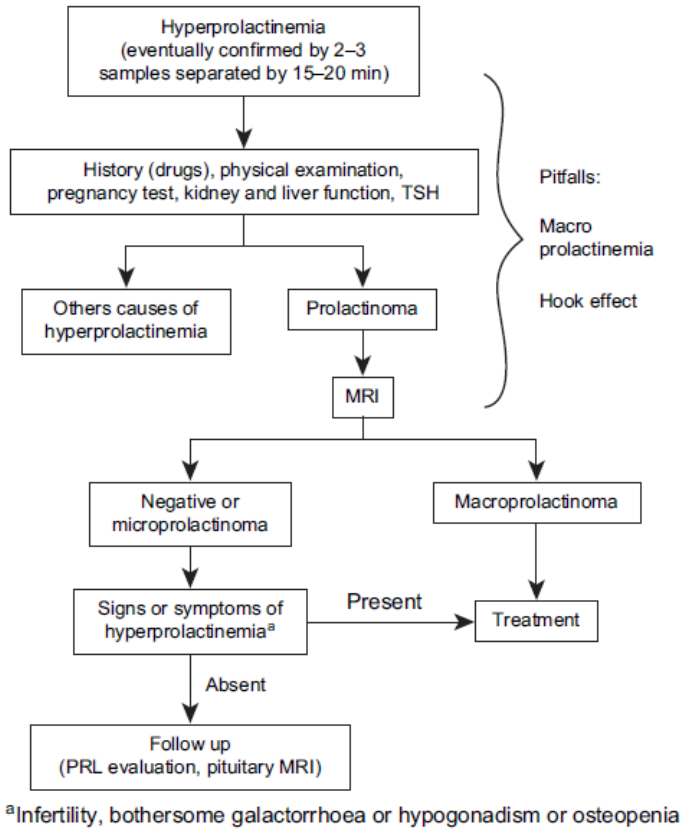
As far as the diagnosis of hyperprolactinemia is established (basal, elevated fasting morning PRL levels or levels on several different occasions), the clinician should carefully exclude secondary causes using the diagnostic tools (careful clinical history, physical examination, pregnancy test, routine biochemical analysis for kidney and liver function and TSH and T4 determination (19). Some of the most common causes of elevated PRL levels that must be differentially diagnosed from the prolactinomas are the following:

Table 1: Causes of hyperprolactinemia (17, 21)

Physiological	**Hypothalamic diseases**	**Pituitary diseases**	**Drugs**
- Pregnancy - Lactation - Breast stimulation - Stress - Sexual intercourse - Exercise	- Tumours - Infiltrative diseases - Cranial irradiation -Vascular abnormalities - Pseudotumour cerebri	- Functioning and non-functioning adenomas - Empty sella syndrome - Lymphocytic hypophysitis - Primitive tumours and metastasis - Infiltrative diseases	- Neuroleptics - Antidepressants - Antihypertensive medications - Gastrointestinal medications - Opiates - Cocaine - Estrogens - Protease inhibitors

In general, serum prolactin levels parallel tumour size. PRL values between the upper limits of normal and 100 ng/ml are usually due to drugs, hormones or functional (idiopathic) causes but can also be caused by microprolactinomas. Most patients with PRL levels over 150 ng/ml will have a prolactinoma. Macroprolactinomas are typically associated with levels of over 250 ng/ml and in some cases over 1000 ng/ml, whereas in recent studies levels greater than 500 ng/ml are referred to as diagnostic (22). Nevertheless, the clinician should be aware that prolactinomas can present with variable elevations in PRL and there may be discordance between tumour mass and PRL value (19).

***Figure 2**: Diagnosis and management of prolactinoma: an algorithm (adopted from Casanueva et al., 2006) (19).*



Two potential pitfalls in the biochemical diagnosis of hyperprolactinemia should be taken into consideration, when PRL is measured: macroprolactin and ‘‘hook effect.’’

Macroprolactin is a complex of PRL with an IgG antibody, with reduced bioactivity and is not detected by all PRL assays. Its reduced clearance is a cause of potential false-positive results (23). For confirmation of macroprolactinemia, polyethylene glycol precipitation and ultrafiltration are the most practical methods (24, 25).

The “hook effect” can be observed in some cases of giant prolactinomas. The extremely high PRL levels cause antibody saturation in the two-site assays, resulting in false low levels. This

artefact can be eliminated by 1:100 dilutions of serum samples. It is recommended to exclude the “hook effect” in all new patients who have a large macroadenoma with unexpectedly normal to mildly elevated PRL levels (26).

After other common causes have been excluded, patients should be investigated for possible structural pathology in the hypothalamo-pituitary region. Gadolinium-enhanced MRI is currently the radiological investigation of choice (27) and increases the detection of microadenomas. CT with intravenous contrast is the second available option in cases where MRI is contra-indicated or inappropriate but remains less effective than MRI in diagnosing small adenomas.

However, it should be noted that microadenomas are present in 10–20% of the normal population, as judged by autopsy studies. On the other hand, a normal MRI scan does not completely exclude a microadenoma <2 mm in diameter or a hyperplasia (28, 29).

The potential problem of differential diagnosis between a large nonsecreting tumour causing modest PRL elevations and a true prolactinoma should be lost by the possible response to the dopamine agonist treatment. Normalisation of PRL levels combined with reduction of adenoma size leads to the diagnosis of prolactinoma.

Many functional tests have been suggested as diagnostic tools in the evaluation of hyperprolactinaemia, including administration of TRH, L-dopa and insulin-induced hypoglycemia but are of no clinical use. Hence, the only reliable diagnostic algorithm of a prolactinoma should be the analysis of basal PRL values, the imaging of the pituitary and the exclusion of other causes as outlined above (29, 30).

The diagnostic algorithm should also include visual-field examination (e.g. computerised Goldman perimetry) and examination for hypopituitarism, mainly for patients with macroadenomas (19).

The diagnosis of idiopathic hyperprolactinemia is made by the exclusion of known causes of hyperprolactinemia in the setting of a normal pituitary MRI. Some of these patients may have small microadenomas below MRI sensitivity (~2 mm).

2.6 Management of prolactinomas

The primary goals of treatment in prolactinomas are:

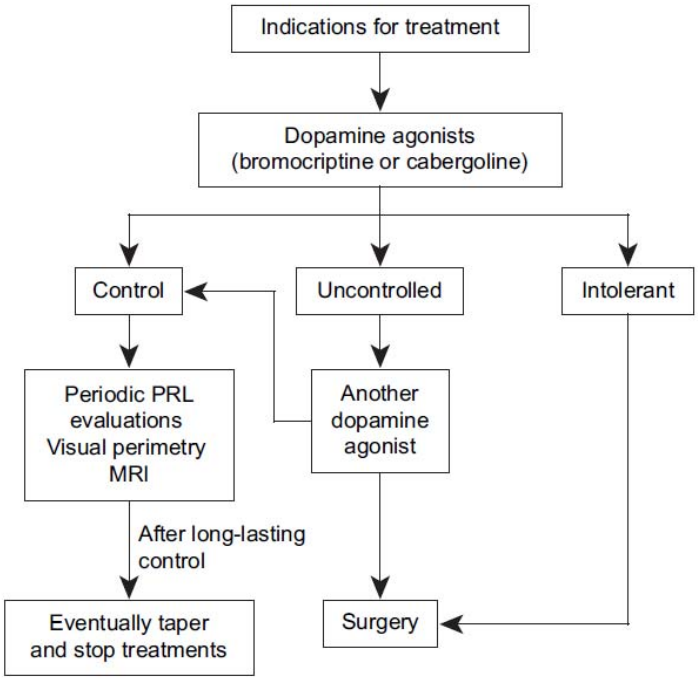
- Satisfactory control of PRL hypersecretion, in order to restore gonadal function and sexual function
- Reduction of the tumour mass

The final decision for treatment should take into consideration that approximately 90% of microprolactinomas remain stable during the follow-up in 4 to 6 years (31-33).
Medical, and sometimes also surgical therapy, is always advisable for all macroadenomas, whereas the indications for treating microadenomas depend on the symptoms. According to current guidelines (34), premenopausal women with normal cycles and tolerable galactorrhea and postmenopausal women with tolerable galactorrhea who have microprolactinomas may be reassured and not treated. These women must be followed clinically with periodic PRL measurements. The increase of PRL levels, or the neurological symptoms of mass development, should be an indication for a MRI study.

Table 2: Indications for therapy (19, 34, 35)

Absolute indications	**Relative indications**
- Mass effects - Hypopituitarism - Visual field defects - Cranial nerve deficits - Headaches - Effects of hyperprolactinemia - Hypogonadism - Amenorrhea or oligomenorrhea - Infertility - Impotence - Osteoporosis or osteopenia	- Bothersome hirsutism - Bothersome galactorrhea

***Figure 3**: Indications for treatment: an algorithm (Adopted from Casanueva et al., 2006) (19).*



2.6.1 Medical treatment with dopamine agonists

There are five dopamine receptors already described in the literature, all of which are members of the superfamily of G protein-coupled receptors. The regulation of prolactin is mediated by the D2 receptor (encoding gene localised in chromosome 11q). D2R-mRNA seems to be expressed in the substantia nigra, ventral tegment area, hippocampus, caudal putamen, nucleus accumbens and olfractory tubercle but is also expressed in high levels in anterior pituitary (36).

The primary therapy is medical treatment with dopamine agonists (DA) such as bromocriptine and cabergoline. These drugs not only normalise PRL levels but can also significantly reduce the volume of the tumour in most patients and extensive experience has demonstrated their utility in treating prolactinomas of all sizes (19). These agents bind to the dopamine D2 receptors on pituitary lactotrope cells, resulting in a decrease in synthesis and release of PRL (37, 38). However, none of the dopamine agonists are absolutely specific for any dopamine receptor subtype (36).

The most common used dopamine agonists, bromocriptine and cabergoline, bind to the D2 receptors, whereas quinagolide, pergolide and lisuride are not that widely used, and are preferred as an alternative therapy in cases of intolerance/resistance of bromocriptine or cabergoline. While all three lower serum PRL on oral administration and also reduce tumour size, they have different affinity for D2 receptors and plasma half-life. Cabergoline has the highest affinity and greatest selectivity for D2 receptors. The half-lives of cabergoline, quinagolide and bromocriptine are approximately 65 h, 24 h and 8–12 h, respectively (39).

Bromocriptine**,** a short acting ergot alkaloid**,** was developed in the 1970s as the first DA to be introduced for the prolactinomas and there is plenty of data regarding its safety, efficacy and mechanism of action. The useful clinical experience collected over these years shows that this

medication is efficient and normalises PRL levels in 80–90% of patients with microprolactinomas and nearly 70% of those with macroprolactinomas, having also a significant effect on the reduction of the tumour mass (40-42).

Therapy with bromocriptine (tablet of 2,5-5 or 10 mg) is initiated with an oral dose of 0,625–1,25 mg daily and increased by 1,25 mg at weekly intervals until a dose generally of 2,5 mg twice or thrice daily is reached (max. dose 15 mg/day) (19). Starting with a low dose and gradually increasing the dose over days and weeks until the PRL level is suppressed to the normal range, could increase tolerance and diminish possible side effects.

Cabergoline is a long active and more selective ergoline derivative dopamine agonist of D2 receptors. The drug, due to a longer half life, can suppress PRL for longer than 14 days after a single oral dose.

Cabergoline (tablet of 0,5 or 1 mg) should be started at a dosage of 0,25 mg once or twice per week and increased to 0,5 or 1 mg twice weekly. Doses more than 3 mg per week are rarely necessary. The final goal of normoprolactinemia and resumption of normal gonadal function is achieved in approximately 80% of patients with microadenomas; galactorrhea improves or resolves in 90% of patients (4). In the case of macroprolactinomas, the therapy response is lower (~70%). Cabergoline has been proved as efficient and better tolerated in most patients previously intolerant or resistant to bromocriptine (4) and according to the most recent guidelines should be considered the gold standard therapy (34).

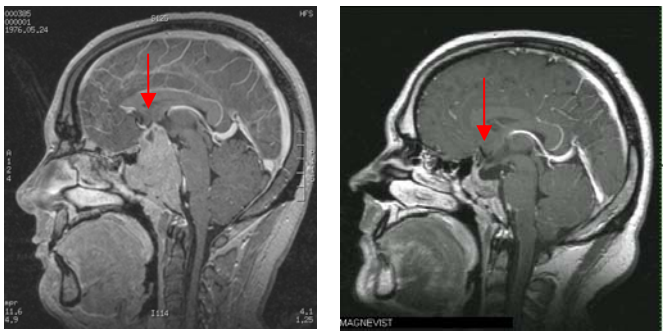


***Figure 4**: Macroprolactinoma after 2 years with DA therapy (courtesy of Prof. Dr. Schopohl).*

Resistance to dopamine agonists, defined as a failure to normalise PRL levels and /or reduce tumour size, is reported in 10-15 % of patients (35). The reasons for this phenomenon remain unclear and possible mechanisms have been speculated such as reduction of dopaminergic binding sites or polymorphisms of the D2 receptor gene (43-45). An increase of cabergoline dose up to 11 mg/week is suggested, under regular echocardiographic control for the potential risk of cardiac valvular regurgitation (34).

2.6.2 Surgical treatment and radiotherapy

The effective medical treatment in restoring normal PRL levels and reducing the tumour size, without the possible complications of a pituitary insufficiency, has limited the indications for surgical resection of prolactinomas only to rare cases (19, 35). The transsphenoidal approach is considered to be the standard surgical treatment (46). Complications from transsphenoidal surgery are quite infrequent, including mortality, cerebrospinal fluid (CSF) rhinorrhea, pituitary insufficiency etc.

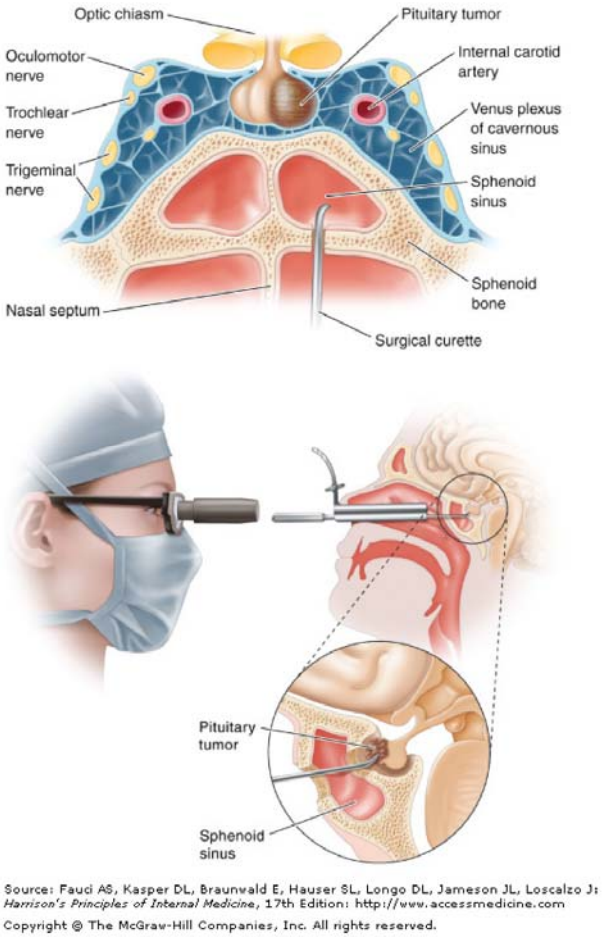


***Figure 5**: Transsphenoidal resection of pituitary mass via the endonasal approach, (adopted from Fahlbusch et al., 1992).*

Radiotherapy is nowadays very rarely used and is associated with significant incidence of major side-effects, including pituitary insufficiency, damage to the optic nerve, neurological dysfunction and increased risks of stroke and secondary brain tumours. It should be reserved for patients in whom medical and surgical therapy have failed (18).

2.6.3 Side effects of DA

The most common side effects are gastrointestinal (nausea, dyspepsia, abdominal pain), postural hypotension, dizziness and headache.

Bromocriptine can cause gastrointestinal (nausea and vomiting), cardiovascular and neurologic side effects. These can be minimised by an incremental dosage schedule and taking tablets with or after the meal at night. The most frequent neurologic adverse effects are headache and drowsiness. Moreover, dyskinesias are well recognised effects of high-dose treatment. Reversible pleuropulmonary changes and retroperitoneal fibrosis have been reported in patients treated with a high dose of bromocriptine for Parkinson's disease; however, because the effects seem to depend on dose, they are unlikely to occur at the low doses used for prolactinoma.

Side effects of cabergoline are similar to those reported for other dopamine agonists but are generally less frequent, less severe, and of shorter duration; in fact, withdrawal of this drug because of side effects is reported in less than 3% of patients. The most common adverse event is nausea or vomiting, followed by headache and dizziness. Recently, several studies have been published describing increased prevalence of cardiac valve regurgitation in patients who were treated with cabergoline. However, regurgitation was only an echocardiographic finding and was not accompanied by symptoms. Echocardiography should be therefore recommended to all patients with hyperprolactinemia planned to be treated with or are under cabergoline therapy (47).

According to the latest consensus statement of February 2011 (34) and a recent meta-analysis of randomised controlled trials (48), cabergoline seems to be more effective than bromocriptine in terms of normalising prolactin levels and menstruation, probably due to its higher affinity for dopamine receptor binding sites. Furthermore, cabergoline seems to be superior and more tolerable in terms of nausea and vomiting, increasing therefore the drug compliance.

2.6.3.1 Neuropsychiatric side effects of DA in different patient groups

The loss of dopaminergic neurons in Parkinson's disease is characterised by motor, cognitive, behavioural and autonomic symptoms. The dopamine replacement therapies with dopamine agonists are very effective in treating the symptoms but have recently been associated with de novo onset of adverse events, which are, amongst others, impulse control disorders (ICDs) such as pathological gambling, compulsive shopping, hypersexuality and binge eating. These symptoms are generally characterised by the maladaptive nature of the preoccupations and the inability to control these urges (49) and they mainly have a de novo onset after the initiation of dopamine replacement therapy. Problems related to pathological gambling have been described in Parkinson patients with a varying frequency from 3,4 % up to 6,1 % (50), significantly increased beyond that of the general population. In nearly all cases, the patient had no gambling history and in some studies the dopamine agonist therapy was adjunctive (51), whereas only rare cases have been associated with carbidopa or levodopa monotherapy (52). Recent data from a multicentre study of 3090 patients with PD in the USA revealed a much higher occurrence of ICDs up to 13,6% (53).

However, symptoms such as pathological gambling, compulsive shopping and hypersexuality tendency have been also described in patients with Restless Legs Syndrome (RLS) who are treated with dopamine agonists, but the prevalence is less established (54, 55).

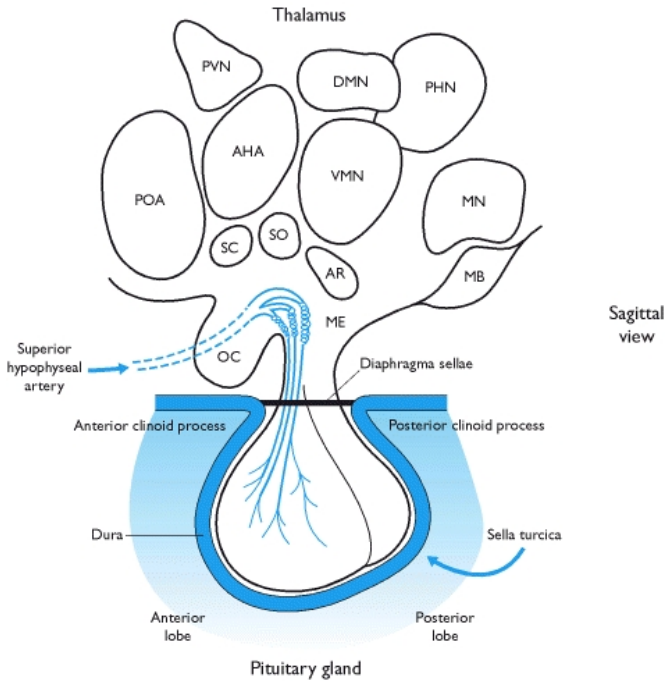
Regarding the patients with prolactinomas, where the medical treatment of choice are also the dopamine agonists, there is a lack of published data. Up to date, there are two case reports that describe cabergoline-induced gambling under a low dose (<1 mg weekly) of cabergoline as treatment for microprolactinoma (56, 57). Both patients had a free psychiatric and gambling history and the symptoms ceased after the withdrawal of the medication. In one of these two

patients, gambling was also combined with excessive libido and hypersexual activities that concluded to divorce proceedings (56).

2.6.4 The role of the MDR1 transporter (coded by the ABCB1 gene) and the action of cabergoline

The pituitary gland maintains its anatomical and functional connections with the brain though sitting outside the blood-brain barrier. Despite the lack of anatomical connection of the anterior pituitary with the hypothalamus, there is a functional connection with this part of the brain via hypothalamic dopaminergic neurons that release dopamine which, via a system of hypophyseal portal vessels, act on the endocrine cells of the anterior lobe to inhibit the synthesis or secretion of prolactin.

***Figure 6**: The pituitary gland and its anatomical and functional connections. Abbreviations: AHA, anterior hypothalamic area; AR, arcuate nucleus; DMN, dorsomedial nucleus; MB, mammillary body; ME, median eminence; MN, medial nucleus; OC, optic chiasm; PHN, posterior hypothalamic nucleus; POA, preoptic area; PVN, paraventricular nucleus; SCN, suprachiasmatic nucleus; SO, supraoptic nucleus; VMN, ventromedial nucleus (adopted from: Endocrinology, An Integrated Approach, Stephen Nussey and Saffron Whitehead. St. George's Hospital Medical School, London, UK).*

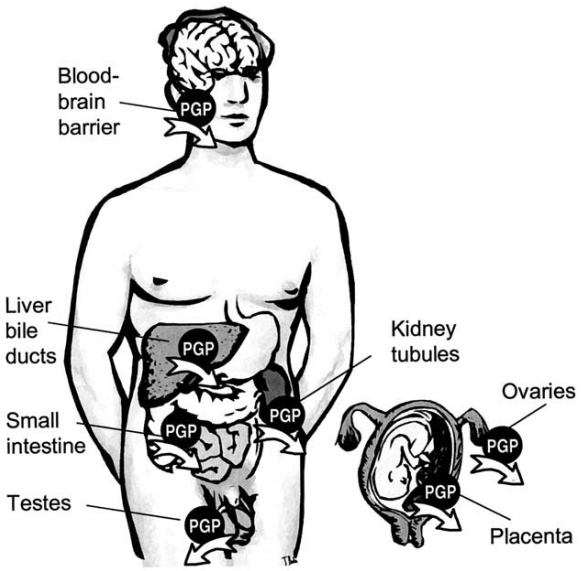


The central side effects of the systematically administered cabergoline and dopamine agonists in general, depend on their ability to pass the blood-brain barrier (BBB). The concentration of the medication into the central nervous system has been found to be actively controlled from transporters that are expressed at the luminal membrane of the endothelial cell-lining that is formed from small blood capillaries. One of these molecules that actively (upon ATP) bind their substrates transporting them out of cells back into the blood circulation against a concentration gradient, acting as a gatekeeper in controlling the passage of substances between the blood and the brain, is a P-glycoprotein (P-gp), encoded by the ABCB1 gene (or

multidrug resistance gene - MDR1) located on chromosome 7. Cabergoline has been found recently to be a substrate of this transporter (Uhr et al., 2008 unpublished data).

However, P-gp has been found to be localised, apart from in the brain, in many different tissues such as gonads, bone marrow, liver and small intestine as well as in the fetus and its function and anatomic localisation suggests it acts as a protective barrier to keep potentially toxic P-gp substrate compounds out of the body limiting tissue exposure (58).

Figure 7 : *P-gp tissue distribution - adopted from Marzolini et al., 2004 (58).*

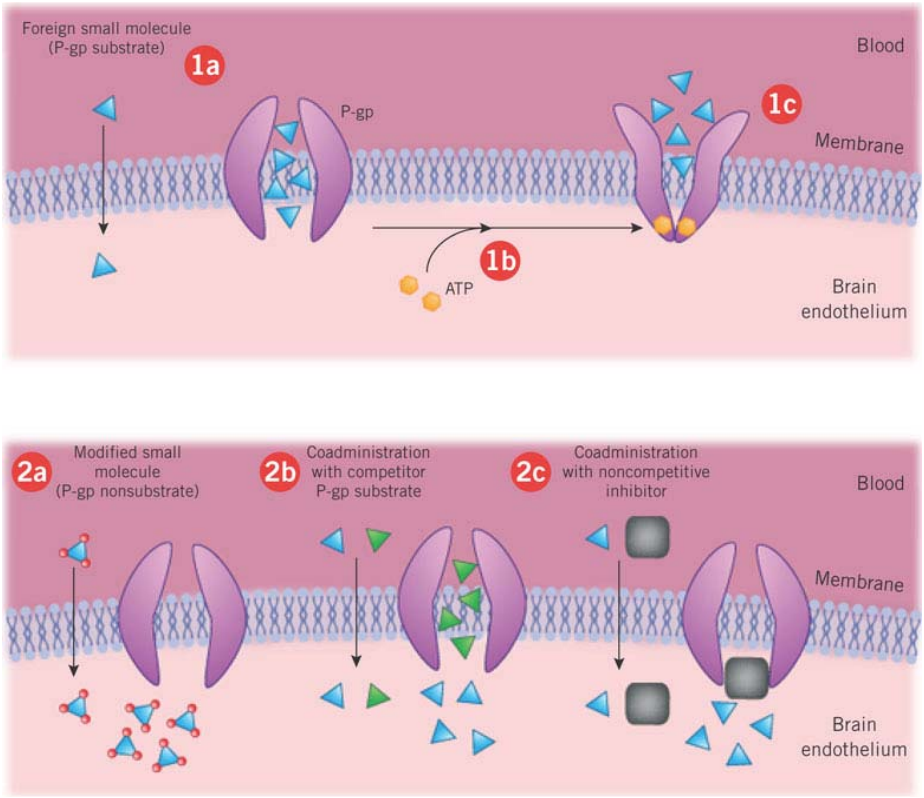


Extended studies have revealed that genetic variants in the ABCB1 transporter P-gp correlate with different intracerebral concentrations of antidepressants and therefore clinical response (59). To date, there are more than 95 SNPs that have been identified. More specifically, single nucleotide polymorphisms (SNPs) have been tested and among others, the following 4 SNPs have been associated with an altered expression and function of the P-gp: rs045642, rs2032582, rs2032583 and 2235015 and were therefore selected for genotyping in our study. In the recent study of Uhr et al. (59) there was a clear difference in the genotype distribution

of rs2032583 and rs2235015 between remitters and non-remitters (for treatment with antidepressants acting as substrate for the P-gp) in favour of C- and T-carriers respectively. The study of Kato et al. (60) led to the conclusion that the C-variant of rs1045642 and the G-variant of rs2032582 were also linked to a higher expression and function of P-gp, resulting in a poor treatment response and decreased remission rates.

***Figure 8**: P-gp: a drug efflux pump in the brain's vascular endothelium.*

(adopted from: http://www.nature.com/scibx/journal/v2/n19/pdf/scibx.2009.773.pdf).



3. AIM OF THE PROJECT

The aim of our project was to investigate the genetic predictors of the side effect profile of treatment with dopamine agonists in prolactinoma patients.

We hypothesised that the neuropsychiatric side effects of DA therapy in particular (not only in prolactinoma, but also in other patient groups) are dependent on the concentration of dopamine agonists in the brain.

The intracerebral concentration of DA (and especially cabergoline) in the brain is controlled from the MDR1 transporter P-gp at the blood-brain-barrier level as shown by our collaborator Manfred Uhr and colleagues in a mouse model (unpublished data). If the function of P-gp is reduced, lower is the amount of cabergoline that is removed from the brain tissue and the higher remains its concentration in the brain.

Genetic variants of the encoding gene ABCB1 (or MDR1 gene), among other SNPs rs045642, rs2032582, rs2032583 and 2235015, control its expression and function, leading to an individual predisposition to develop some neuropsychiatric side effects.

In 9 studies up to now, the role of these genetic variants in the remission rate under antidepressants-substrates of P-gp is being examined. In 6 of them, a significant genotype and remission interaction has been established (61).

Hence, we hypothesised that the genetic variants of the MDR1 transporter, namely the ABCB1 gene variants, will equally predict the occurrence of neuropsychiatric side effects in patients treated with cabergoline and/or DA.

To elucidate this question, we designed a prospective, diagnostic study in prolactinoma patients (and NFPA controls) treated with DA. Patient's neuropsychiatric side effects were investigated on the basis of questionnaires and self rating.

Additionally, we collected blood and performed genetic analyses, to determine the potential associations between the ABCB1 gene variants and the number of neuropsychiatric side effects in this patient group.

4. SUBJECTS AND METHODS

4.1 Type of the study

This study is a case-control study. In a cross-sectional approach, we prospectively enrolled 92 patients during a period of two years between December 2008 and January 2011 (including the planning phase, recruitment of patients, acquisition of the data, the analysis and writing of the results). As a clinical control group, patients with non-functioning pituitary adenomas (n=60) were recruited at the same clinic.

4.2 Patient sample

Ninety two patients with prolactinomas, treated at the Endocrine Outpatient Unit of the Max Planck Institute of Psychiatry (23 male and 69 female, mean age at study time 49,2 ± 13,8 years, mean BMI 25,6 ± 6,9 kg/m²), were enrolled. Patients were identified through the electronic database of the Institute and agreed to participate in this study. Questionnaires were sent to these patients and they were asked to return them in prepaid envelopes. Written informed consent was obtained from all the participants in the study and the study was approved by the Ethics Committee of the Ludwig-Maximilian-University of Munich. Clinical characteristics of the subjects were collected with regard to disease history, tumour characteristics, previous and present therapy and comorbidities as well as present complaints. Comorbidities were diagnosed according to the respective guidelines. In the case of missing data or uncertainty, additional information was obtained by review of patient files.

4.3 Inclusion and exclusion criteria

Inclusion criteria

(i) Patients over 18 years of age.

(ii) Diagnosis of a prolactinoma (micro- or macroprolactinomas).

(iii) Written informed consent.

Exclusion criteria

(i) Patients under 18 years of age.

(ii) Patients unwilling to participate.

(iii) Hyperprolactinemia due to other causes (e.g. medical treatment, hypothalamic disease, other pituitary disease, pregnancy, lactation).

4.4 Questionnaire

The first draft of the questionnaire was developed by identifying areas of interest and after screening and research on potential standardised questionnaires. Additionally, we inserted general questions as published previously (62).

The questionnaire encompassed the following parts (see attached document in the appendices):

- Socioeconomic and baseline characteristics including sex, age, height and weight, occupational and family status, alcohol and tobacco consumption.
- Medical specific history including questions regarding the diagnosis of prolactinoma (date, prolactin value, size of adenoma, visual field evaluation) and medical (type,

dose and duration) or surgical treatment and radiation. All the symptoms as well as possible side effects of the medical treatment were evaluated.

- Gender specific questions including menstruation of female, number of children, medical and clinical history (apart from the prolactinoma), life-time comorbidities and family medical history.

The first version of the questionnaire was distributed to a) a group of 5 experts at the Max-Planck-Instiute, b) a small group of patients of the Endocrine Outpatient Unit of the Max Planck Institute of Psychiatry for improvements and revision.

The final questionnaire was evaluated by the department of epidemiological psychology of the Max Planck Institute of Psychiatry and sent to the patients in May 2009. The response rate was about 30%. A database was created with Microsoft Access (Windows 2000), where all data were transferred for statistical analyses.

4.5 DNA preparation, SNP selection and genotyping of the MDR1 transporter (ABCB1 gene)

Forty millilitres of EDTA blood was drawn from each patient and DNA was extracted from fresh blood using the Puregene whole-blood DNA extraction kit (Gentra Systems, Minneapolis, MN, USA). Alternatively, for the patients that did not attend our Outpatient Unit at study point, Oragene®•DNA Self-Collection Kit (OG-500-tube format and OG-250-disc format) was sent to their home address and they were asked to return them with saliva samples in prepaid envelopes. Saliva samples were collected according to the manufacturer's instructions that were enclosed in the German language. A 500 μL aliquot of an Oragene•DNA saliva sample was used for the DNA extraction according to the manufacturer's instructions. Genotyping was performed with a LightCycler® 480 Genotyping Master (detailed genotyping procedure presented in appendices). Four ABCB1 SNPs were

genotyped (further details are given in Table 3). SNPs were selected from dbSNP (http://www.ncbi.nlm.nih.gov:80/) according to previous published data that revealed altered expression and function of the P-gp. None of the SNPs showed a significant deviation from the Hardy-Weinberg equilibrium and all genotypes could be determined (call rate 100%).

Table 3: Information on genotyped SNPs, HWE, MAF, call rate and genotypes

SNP	Chromosomal position	Genomic localisation	Map_Pos	Alleles	HWE	MAF	Call rate
ABCB1							
rs1045642	7	Exon 26	87138645	C/T	0,65	0,44	1,00
rs2032582	7	Exon 21	87160618	G/T	1,00	0,47	1,00
rs2032583	7	Intron 21	87160561	C/T	0,68	0,16	1,00
rs2235015	7	Intron 4	87199564	G/T	0,49	0,20	1,00

HWE: P-values of the Hardy-Weinberg equilibrium test; MAF: minor allele frequency

Chromosomal positions are given according to the February 2009 (hg19) human reference sequence database of the International Human Genome Sequencing Consortium.

4.6 Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS Version 16) for Windows.

In the frame of the formulated hypothesis of the side effects and symptoms of treatment with cabergoline, percentages and frequencies of these symptoms under hormonal treatment were calculated. We also retrieved data from the disease and personally history and also medical procedures and we calculated means and standard deviations were calculated (SD).

The differences in continuous and categorical variables between 2-groups were analysed by the unpaired t-test (two-tailed) and Chi-square. A two-tailed p-value of 0,05 was considered statistically significant with a 95% confidence interval (CI) after corrections for multiple tests were performed. Differences that were considered statistically significant are marked in ***bold***. Empirical instead of asymptotic p-values are reported: these have been calculated with a permutation-based method using 100000 permutations. SNPs were tested for 3 models of inheritance: allelic, carrier and heterozygote vs. homozygote. To correct for multiple testing, the method proposed by Westfall and Young (1993) was applied (Pwycor).

5. RESULTS

5.1 Description of the prolactinoma patient group

The clinical study included a total of 92 patients consisting of 23 men and 69 women. The mean age of our group at the time of the study was 49,2 ± 13,8 years, whereas the mean Body Mass Index (BMI) was 25,6 ± 6,9 kg/m². Thirty six of the women that were asked had at least one child and 25,8% were already at the menopause (mean age 46 years). Higher was the percent of men having children (81%). Twenty six women reported of any irregular menstrual cycle since menarche, which occurred at a mean age of 13,5 years.

Regarding their occupational status, 58,8% of the patients were employed at study point, whereas 22,4% reported being pensioned and 12,9% to be housewives. About half of the participants had rare or no alcohol consumption and also had never smoked. 68,2% were married and 22,4% single.

Further demographic characteristics are presented in Table 4. Results of variables are presented as mean ± standard deviation (SD).

Table 4: Basic socio-demographic characteristics of 92 prolactinoma patients

	Mean	**SD**
Age	49,2	13,8
BMI	25,6	6,9
Age of menarchy (female patients)	13,5	1,24
Age of menopause (female patients)	46	8
	N	**%**
Sex		
- men	23	25
- women	69	75
Work status		
- employed	50	58,8
- unemployed	2	2,4
- retired	19	22,4
- housewife	11	12,9
- other	3	3,5
School years	3,4	11,4
Legal status		
- single	19	22,4
- married	58	68,2
- divorced/widowed	8	9,4
Smoking		
- no	48	56,5
- yes	24	28,2
- past	13	15,3
Alcohol		
- daily	10	12
- occasionally	10	12
- rarely	16	19,3
- extreme rarely/never	47	56,6
Any irregular menstrual cycles	26	45,6
Women in menopause	16	25,8
Women with children	36	57,1
Men with children	17	81

5.2 Lifetime comorbidities of prolactinoma patients

Twenty four point four percent (24,4%) had the diagnosis of pituitary insufficiency, at least of one axis, that was not surprisingly, strongly correlated with the macroprolactinomas ($p<0,001$). The most affected axis was the gonadotropic axis with 17%, followed by the corticotropic (14,9%), the somatotropic (8,1%) and the thyreotropic axis (8%). Twenty one point two percent (21,2%) presented with hypertension, 12,9% had positive medical history of pulmonary disease, whereas the diagnosis of cancer and diabetes mellitus was lower at 4,7% each. Arrhythmia was present in up to 9,3% of our group. Five point four (5,4%) of patients had a positive medical family history for endocrine diseases (mostly thyroid abnormalities) and 30,4% for psychiatric diseases, including a wide range of psychiatric conditions, such as depression, psychosis, bipolar disorder, attempted suicide or schizophrenia.

Table 5: Lifetime comorbidities of 92 prolactinoma patients

	N	%
Pituitary insufficiency	22	24,4
- corticotropic	13	14,9
- thyreotropic	7	8
- gonadotropic	15	17
- somatotropic	7	8,1
Hypertension	18	21,2
Pulmonary disease	11	12,9
Arrhythmia	8	9,3
Diabetes mellitus	4	4,7
Cancer	4	4,7
Cerebrovascular disease	3	3,5
Coronary disease	1	1,2

5.3 Disease and treatment history of prolactinoma patients

The initial diagnosis was equal to micro- (51,1%, female-to-male ratio 8:1) and macroprolactinomas (48,9%, female-to-male ratio 1,5:1). The mean age at diagnosis was 38 ± 13,8 years, whereas the study was conducted, on average, 11,3 ± 7,1 years after the first diagnosis of the tumour. The median serum prolactin concentration at baseline was 214,5 ng/ml (range: 25-14900 ng/ml, median value for microprolactinomas 98 ng/ml, median value for macroprolactinomas 643 ng/ml). Data on precise tumour size at diagnosis were available only for the half of the adenomas, with a maximal diameter ranging from 2 to 60 mm. Sixteen patients had visual field disturbances at the time of diagnosis. Thirteen patients had undergone surgery (11 transsphenoidal, 2 transcranial) and two of the patients that had been operated, had undergone additional radiotherapy. Regarding the medical treatment, in 79 cases cabergoline was used (mean maximum dose 1 mg/week, range 0,25-7 mg), but 55 patients were treated with at least two different dopamine agonists. At study point, 49 patients were under treatment with cabergoline, six with bromocriptine and two with quinagolide and 62 patients had normalised prolactin values.

Table 6: Disease diagnosis and treatment characteristics of 92 prolactinoma patients

	N	%
Primary prolactinoma type		
- microprolactinoma	47	51,1
- macroprolactinoma	45	48,9
Visual field at diagnosis		
- influenced	16	17,6
- not influenced	56	61,5
- unknown	19	20,9
Surgery	13	14,8
Radiotherapy	2	2,3
Medical treatment		
- Bromocriptine	31	34
- Lisuride	3	3
- Cabergoline	79	86
- Quinagolide	17	18

5.4 Comparison of basic socio-demographic characteristics, lifetime comorbidities and disease characteristics of prolactinoma patients to patients with non-functioning pituitary adenomas (NFPA)

To compare the basic socio-demographic characteristics, the lifetime comorbidities and the disease characteristics of our patients, 60 patients with non-functioning pituitary adenoma as a clinical control group were recruited at the Endocrine Outpatient Unit of the Max Planck Institute of Psychiatry and the Department of Internal Medicine, Ludwig-Maximilian-University of Munich in equal parts. The NFPA group included significantly older (mean age 60,2 ± 10,6 years, $p < 0,001$) and more overweight patients (mean BMI 28,5 ± 5,1 kg/m², $p = 0,006$). In the NFPA group, men were mainly affected (65%, $p < 0,001$) and the group included mostly macroadenomas up to 81,7%. The NFPA tended to be diagnosed later, at a mean age of 47,1 years. The study was conducted approximately 13 years after the diagnosis

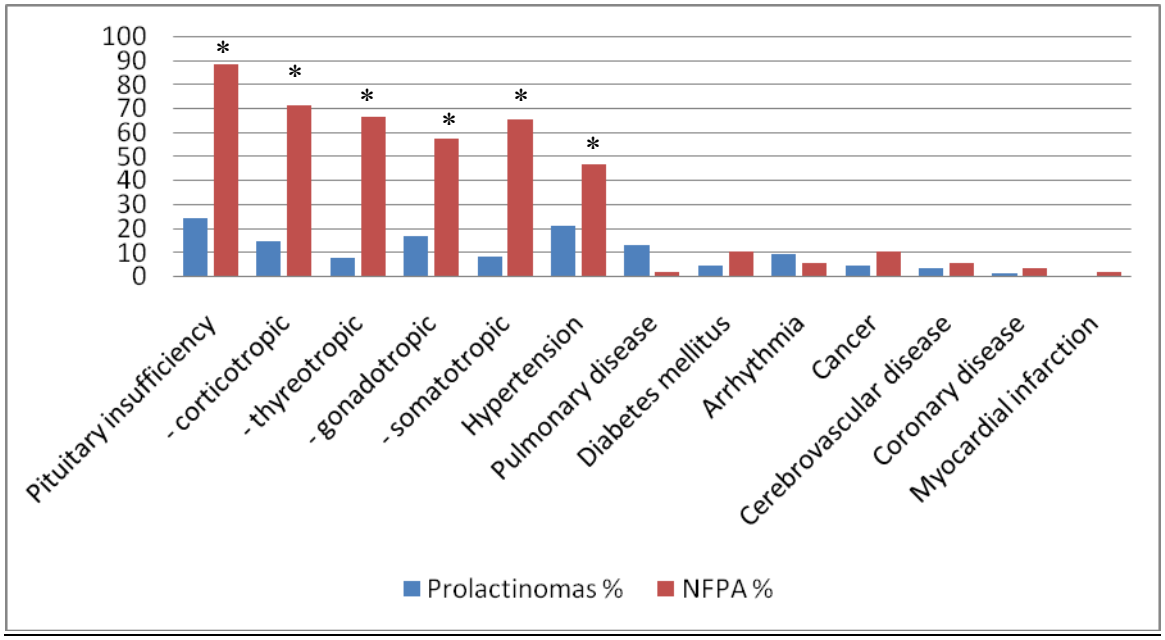
of the NFPA, not significantly different from the time of diagnosis of prolactinomas. Regarding the treatment followed, the NFPA patients underwent significantly more surgery (96,3%) and radiotherapy (28,3%) compared to the prolactinoma patients ($p < 0,001$).

Table 7: Baseline and disease characteristics - comparison of 92 prolactinoma patients to the 60 patients of NFPA group

	Prolactinomas		**NFPA**		**p-value**
	Mean	**SD**	**Mean**	**SD**	
Age	49,2	13,8	60,2	10,6	***< 0,001***
BMI	25,6	6,9	28,5	5,1	***0,006***
Age at diagnosis	38	13,8	47,1	10,5	***0,002***
Years after diagnosis	11,3	7,1	12,6	7,8	***ns***
	N	**%**	**N**	**%**	
Sex					
- men	23	25	39	65	***< 0,001***
- women	69	75	21	35	
Type of adenoma					
- macroadenoma	45	48,9	49	81,7	***< 0,001***
- microadenoma	47	51,1	4	6,7	
Surgery	13	14,8	52	96,3	***< 0,001***
Radiotherapy	2	2,3	2	28,3	***< 0,001***

In terms of lifetime comorbidities, patients with NFPA suffered hypertension significantly more often ($p=0,002$). Regarding pituitary insufficiency of all axes, patients with prolactinomas had less frequent pituitary deficits, significance that remained statistically significant for each pituitary axis ($p<0,001$). Regarding the other lifetime comorbidities, no statistically significant differences were detected.

***Figure 9**: Lifetime comorbidities of the prolactinoma patients in comparison to the NFPA group (by percentage).*



** Statistically significant differences between prolactinoma and NFPA patients.*

5.5 Side effects of cabergoline in the patient group of prolactinomas treated with cabergoline

Of the 79 patients with prolactinomas treated with cabergoline, the following side effects prevalent under treatment were more prominent: fatigue (n=35), headaches (n=26), depressed mood (n=26), sleep disorders (n=26), dizziness (n=22) and weight loss (n=16). 18 patients reported of decreased and 16 of increased libido. 17 patients had signs of paresthesia equal to aggressiveness and 19 patients reported of anxiety.

We evaluated all the symptoms in terms of presence and change (enhancement vs. reduction or consistency). The patients reported primarily of enhancement of fatigue and increased libido, and secondarily about enhancement of depressed mood. Interestingly, the percentage of those patients who reported weight loss under treatment was higher than those who reported weight gain. In terms of all disorders apart from binge eating, we found statistically significant changes of all symptoms, when compared with the presence of the symptom under treatment.

All the symptoms and their frequencies observed in terms of presence and change under treatment with cabergoline are presented in Table 8.

Table 8: Symptoms and side effects under treatment with cabergoline in the patient group of 79 prolactinoma patients treated with cabergoline

	Presence of symptom under cabergoline		Change of symptom under cabergoline				
			Enhancement		Reduction or consistency		
	N	% (*)	N	% (*)	N	% (*)	p-value[§]
Fatigue	35	44,3	16	20,3	50	63,3	***<0,001***
Headaches	26	32,9	10	12,7	55	69,6	***0,001***
Sleep disorders	26	32,9	11	13,9	53	67,1	***<0,001***
Dizziness	22	27,8	7	8,9	57	72,2	***<0,001***
Weight loss	16	20,3	13	16,5	50	63,3	***<0,001***
Weight gain	13	16,5	10	12,7	51	64,6	***<0,001***
Decreased libido	18	22,8	6	7,6	59	74,7	***<0,001***
Increased libido	16	20,3	16	20,3	46	58,2	***<0,001***
Depressed mood	26	32,9	12	15,2	54	68,4	***<0,001***
Aggressiveness	17	21,5	7	8,9	59	74,7	***<0,001***
Anxiety	19	24,1	7	8,9	57	72,2	***<0,001***
Visual hallucinations	2	2,5	1	1,3	64	81	***0,031***
Gambling	1	1,3	1	1,3	64	81	***0,015***
Compulsive shopping	5	6,3	2	2,5	63	79,7	***0,005***
Binge eating	5	6,3	1	1,3	64	81	0,077
Trichotillomania	3	3,8	0	0	65	82,3	NA

(*) Percentage of the group of patients that answered the question

[§] chi^2 between presence and (enhancement OR reduction and consistency of symptom)

5.6 Association analysis among the seven most common neuropsychiatric side effects of cabergoline and ABCB1 polymorphisms in prolactinoma patients treated with cabergoline

As cabergoline was the only dopamine agonist tested to be a substrate of P-gp, we examined the effects of the ABCB1 SNPs on self reported side effects under treatment with cabergoline (presence and enhancement of symptom). We selected the most common neuropsychiatric side effects according to their frequencies under cabergoline (fatigue, headaches, sleep disorders, dizziness, increased libido, depressed mood and aggressiveness) and evaluated them with permutation analysis. In the carrier model, the carrier of a specific nucleotide was compared with the non-carrier. In the allelic model, each nucleotide was evaluated to have a 2-fold higher effect in the genotype whereas in the last model the heterozygous vs. homozygous genotype of each SNP was compared.

Amongst all 4 SNPs, only SNP rs1045642 and rs2032582 seem to play in role in mainly three neuropsychiatric side effects, fatigue, sleep disorders and dizziness in the carrier and heterozygous genetic model. SNPs rs2032583 and rs2235015 seem not to influence the side effects examined.

The results of the carrier, allelic and heterozygote vs. homozygote models are presented in Tables 9-12.

5.6.1 MDR1 transporter encoding gene ABCB1 SNPs rs1045642 and its association with side effects of cabergoline

We found that the SNP rs1045642 had an influence on two side effects under cabergoline, which were fatigue and sleep disorders. For the other side effects e.g. headaches, increased libido, depressed mood, dizziness and aggressiveness, no association to their occurrence and SNP rs1045642 was found.

More specifically, significant effects were observed for the C-carriers of rs1045642 that presented less frequent fatigue under cabergoline in comparison to non-C-carriers (Pwycor=0,04, OR=0,23).

Additionally, the heterozygous CT-individuals presented less frequent sleep disorders in comparison to homozygous CC or TT (Pwycor=0,02, OR=0,20). There was a marginal statistical significance revealing less frequent occurrence of enhancement of dizziness for the C-carriers both in the carrier model and in the allelic (Pnom=0,02, Pwycor=0,10, OR=0,15, Pnom= 0,08, Pwycor=0,19, OR=0,29). In the heterozygous and allelic model, CT individuals and C-carriers respectively tended to suffer less frequently from fatigue (Pnom=0,06, Pwycor=0,15, OR=0,48 and Pnom=0,07, Pwycor=0,22, OR=0,36). There was no further statistically significant observation regarding this SNP (Table 9).

Table 9: Association of SNP rs1045642 with side effects under cabergoline treatment

(statistically significant effects are marked in ***bold***)

Genotypes CC=18, CT=33, TT=21, missing=7

	Presence of symptom			Enhancement of symptom		
	Pnom	**Pwycor**	**OR**	**Pnom**	**Pwycor**	**OR**
Headaches						
Carrier C	0,49	0,90	1,49	0,12	0,39	0,34
Carrier T	0,73	0,99	0,78	1,00	1,00	0,99
Het./Hom.	0,73	0,99	1,21	0,17	0,44	0,31
Allelic	0,50	0,86	1,31	0,34	0,68	0,59
Increased libido						
Carrier C	0,68	0,98	1,37	0,80	1,00	1,25
Carrier T	1,00	1,00	1,05	0,91	1,00	1,15
Het./Hom.	0,64	0,97	1,37	0,63	0,98	1,33
Allelic	0,79	1,00	1,13	0,92	1,00	1,05
Depressed mood						
Carrier C	0,51	0,91	0,68	0,79	1,00	1,20
Carrier T	0,24	0,63	0,46	0,53	0,86	0,58
Het./Hom.	0,10	0,30	0,39	0,78	0,99	0,78
Allelic	0,80	1,00	1,11	0,57	0,93	1,33
Sleep disorders						
Carrier C	0,07	0,20	0,36	0,36	0,80	0,51
Carrier T	0,24	0,63	0,46	0,24	0,56	0,41
Het./Hom.	***0,01***	***0,02***	***0,20***	0,05	0,16	0,21
Allelic	0,65	0,96	0,83	0,96	1,00	1,03
Fatigue						
Carrier C	***0,02***	***0,04***	***0,23***	0,57	0,96	0,68
Carrier T	0,63	0,99	1,39	0,60	0,99	0,69
Het./Hom.	0,07	0,22	0,36	0,30	0,70	0,52
Allelic	0,06	0,15	0,48	0,90	1,00	0,96
Dizziness						
Carrier C	0,43	0,86	0,61	0,02	0,10	0,15
Carrier T	0,74	0,99	0,80	0,60	0,90	1,85
Het./Hom.	0,31	0,71	0,54	0,10	0,30	0,17
Allelic	0,75	0,99	0,87	0,08	0,19	0,29
Aggressiveness						
Carrier C	0,77	1,00	1,18	0,22	0,64	3,33
Carrier T	0,35	0,68	0,50	0,75	0,95	0,65
Het./Hom.	0,56	0,94	0,69	0,51	0,92	1,74
Allelic	0,46	0,82	1,39	0,30	0,65	1,81

5.6.2 MDR1 transporter encoding gene ABCB1 SNPs rs2032582 and its association with side effects of cabergoline

We found that SNP rs2032582 had an influence only on enhancement of dizziness in favour of G-carriers. For the other side effects headaches, increased libido, depressed mood, sleep disorders, fatigue and aggressiveness, no significant association could be established.

More specifically, in the analysis of SNP rs2032582 G-carriers seemed to be protected from enhancement of dizziness under cabergoline when compared with non-G-carriers (Pwycor=0,05, OR=0,14). There was a trend towards statistical significance in the allelic model in terms of enhancement of dizziness (Pnom=0,03, Pwycor=0,09, OR=0,28) but also in the heterozygous model in terms of enhancement of sleep disorders (Pnom=0,04, Pwycor=0,12, OR=0,20). The G-carriers and the GT-individuals tended to suffer less frequently from enhancement of headaches (Pnom=0,08, Pwycor=0,27, OR=0,27 and Pnom=0,02, Pwycor=0,07, OR=0,11, Table 10).

Table 10: Association of SNP rs2032582 with side effects under cabergoline treatment

(statistically significant effects are marked in ***bold***)

Genotypes GG=21, GT=34, TT=15, missing=9

	Presence of symptom			Enhancement of symptom		
	Pnom	**Pwycor**	**OR**	**Pnom**	**Pwycor**	**OR**
Headaches						
Carrier G	0,86	1,00	1,16	0,08	0,27	0,27
Carrier T	0,39	0,79	0,60	0,37	0,68	0,49
Het./Hom.	0,56	0,94	0,71	0,02	0,07	0,11
Allelic	0,48	0,86	1,32	0,73	0,99	0,82
Increased libido						
Carrier G	1,00	1,00	1,05	0,91	1,00	1,15
Carrier T	0,66	0,99	0,73	0,81	1,00	0,81
Het./Hom.	0,72	0,99	0,78	0,85	1,00	0,91
Allelic	0,75	0,99	1,17	0,76	0,99	1,16
Depressed mood						
Carrier G	1,00	1,00	1,03	0,92	1,00	1,12
Carrier T	1,00	1,00	1,01	1,00	1,00	1,00
Het./Hom.	1,00	1,00	1,02	0,94	1,00	1,08
Allelic	1,00	1,00	1,01	0,96	1,00	1,04
Sleep disorders						
Carrier G	0,60	0,93	0,68	0,23	0,53	0,41
Carrier T	0,62	0,98	0,72	0,25	0,61	0,46
Het./Hom.	0,33	0,74	0,58	0,04	0,12	0,20
Allelic	1,00	1,00	1,01	1,00	1,00	1,01
Fatigue						
Carrier G	0,52	0,91	0,63	0,23	0,49	0,43
Carrier T	0,19	0,35	2,22	0,82	1,00	0,81
Het./Hom.	0,50	0,91	1,42	0,21	0,55	0,44
Allelic	0,20	0,45	0,61	0,62	0,95	0,80
Dizziness						
Carrier G	1,00	1,00	1,06	***0,01***	***0,05***	***0,14***
Carrier T	0,90	1,00	1,08	0,31	0,68	3,03
Het./Hom.	0,87	1,00	1,11	0,35	0,66	0,38
Allelic	0,98	1,00	0,99	0,03	0,09	0,28
Aggressiveness						
Carrier G	0,81	0,99	0,79	0,66	1,00	1,75
Carrier T	0,99	1,00	0,95	0,93	1,00	1,08
Het./Hom.	0,77	1,00	0,81	0,64	0,98	1,49
Allelic	0,91	1,00	0,94	0,86	1,00	1,15

5.6.3 MDR1 transporter encoding gene ABCB1 SNPs rs2032583 and its association with side effects of cabergoline

For SNP rs2032583, our study showed no statistically significant influence to the examined side effects. There were, however, the following marginal effects to be seen:

There was a trend towards statistical significance showing that both C-carriers (in the carrier and allelic model) and CT-individuals tended to suffer more frequently from headaches under the treatment with cabergoline (Pnom=0,05, Pwycor=0,14, OR=3,23, Pnom=0,04, Pwycor=0,14, OR=3,26, Pnom=0,06, Pwycor=0,20, OR=2,59, Table 11). There was no further statistical significance or marginal effect.

Table 11: Association of SNP rs2032583 with side effects under cabergoline treatment

(statistically significant effects are marked in ***bold***)

Genotypes CC=1, CT=22, TT=49, missing=7

	Presence of symptom			Enhancement of symptom		
	Pnom	**Pwycor**	**OR**	**Pnom**	**Pwycor**	**OR**
Headaches						
Carrier C	0,05	0,14	3,23	0,64	0,99	0,60
Carrier T	1,00	1,00	NA	1,00	1,00	NA
Het./Hom.	0,04	0,14	3,26	0,60	0,95	0,61
Allelic	0,06	0,20	2,59	0,67	0,96	0,66
Increased libido						
Carrier C	0,26	0,70	2,08	0,37	0,83	1,89
Carrier T	1,00	1,00	NA	1,00	1,00	NA
Het./Hom.	0,27	0,67	2,06	0,36	0,77	1,88
Allelic	0,28	0,65	1,77	0,40	0,76	1,64
Depressed mood						
Carrier C	0,57	0,96	0,70	0,52	0,96	0,53
Carrier T	1,00	1,00	NA	1,00	1,00	NA
Het./Hom.	0,57	0,95	0,71	0,53	0,91	0,53
Allelic	0,60	0,94	0,75	0,53	0,89	0,58
Sleep disorders						
Carrier C	0,57	0,96	0,70	0,25	0,60	2,17
Carrier T	1,00	1,00	NA	1,00	1,00	NA
Het./Hom.	0,57	0,95	0,71	0,26	0,63	2,18
Allelic	0,60	0,95	0,75	0,28	0,65	1,83
Fatigue						
Carrier C	0,87	1,00	0,86	0,81	1,00	0,81
Carrier T	1,00	1,00	NA	1,00	1,00	NA
Het./Hom.	0,86	1,00	0,86	0,82	1,00	0,81
Allelic	0,88	1,00	0,89	0,78	0,99	0,84
Dizziness						
Carrier C	0,32	0,75	1,85	0,35	0,72	0,33
Carrier T	1,00	1,00	NA	1,00	1,00	NA
Het./Hom.	0,32	0,74	1,87	0,31	0,73	0,33
Allelic	0,34	0,71	1,65	0,40	0,73	0,38
Aggressiveness						
Carrier C	0,59	0,96	0,69	0,96	1,00	0,93
Carrier T	1,00	1,00	NA	1,00	1,00	NA
Het./Hom.	0,60	0,96	0,69	0,93	1,00	0,93
Allelic	0,61	0,95	0,73	0,98	1,00	0,94

5.6.4 MDR1 transporter encoding gene ABCB1 SNPs rs2235015 and its association with side effects of cabergoline

For the SNP rs2235015, no statistically significant association with the examined side effects apart from some marginal effects was found.

There was a trend towards statistical significance showing that T-carriers and heterozygote individuals GT tended to suffer more frequently from headaches under treatment with cabergoline, a difference that turned out to be of no significance when corrected for multiple tests (Pnom=0,04, Pwycor=0,09, OR=3,23, Pnom=0,03, Pwycor=0,08, OR=3,95, Table 12).

Table 12: Association of SNP rs2235015 with side effects under cabergoline treatment

(statistically significant effects are marked in ***bold***)

Genotypes GG=46, GT=22, TT=4, missing=7

	Presence of symptom			Enhancement of symptom		
	Pnom	**Pwycor**	**OR**	**Pnom**	**Pwycor**	**OR**
Headaches						
Carrier G	0,94	1,00	1,43	0,24	0,76	0,33
Carrier T	0,04	0,09	3,23	0,91	1,00	0,89
Het./Hom.	0,03	0,08	3,95	0,60	0,95	0,61
Allelic	0,13	0,27	0,46	0,87	1,00	0,88
Increased libido						
Carrier G	0,78	0,99	0,60	0,82	1,00	0,65
Carrier T	1,00	1,00	1,01	0,92	1,00	0,90
Het./Hom.	0,88	1,00	0,89	0,80	1,00	0,80
Allelic	0,90	1,00	0,92	1,00	1,00	1,00
Depressed mood						
Carrier G	1,00	1,00	1,29	0,72	0,94	0,38
Carrier T	0,25	0,65	0,50	0,33	0,66	0,41
Het./Hom.	0,25	0,63	0,49	0,17	0,42	0,22
Allelic	0,31	0,62	1,72	0,61	0,92	1,51
Sleep disorders						
Carrier G	1,00	1,00	1,29	0,79	0,97	0,44
Carrier T	0,25	0,65	0,50	1,00	1,00	1,01
Het./Hom.	0,25	0,63	0,49	0,79	1,00	0,80
Allelic	0,31	0,62	1,72	0,83	1,00	0,86
Fatigue						
Carrier G	0,60	0,93	2,33	0,77	0,99	0,65
Carrier T	0,28	0,56	0,55	0,47	0,83	0,60
Het./Hom.	0,45	0,87	0,63	0,39	0,79	0,50
Allelic	0,26	0,55	1,73	0,65	0,96	1,34
Dizziness						
Carrier G	1,00	1,00	1,95	0,19	0,62	0,25
Carrier T	0,70	0,99	0,28	0,23	0,51	0,26
Het./Hom.	0,69	0,98	1,32	0,05	0,21	0,00
Allelic	0,75	0,99	0,85	0,61	0,92	1,65
Aggressiveness						
Carrier G	0,78	1,00	0,73	0,17	0,60	0,24
Carrier T	0,33	0,72	1,04	0,77	0,97	0,71
Het./Hom.	0,25	0,63	0,43	0,36	0,80	0,35
Allelic	0,47	0,83	1,53	0,86	1,00	0,91

6. DISCUSSION

6.1 Main and secondary findings

In this study, we investigated potential genetic predictors of neuropsychiatric side effects of cabergoline in patients with prolactinomas under DA therapy.

We found that:

(I) The comparison of the prolactinoma patients with the clinical control group of NFPA patients revealed a different profile of baseline and disease characteristics and also an individual spectrum of lifetime comorbidities possibly caused by long-term hyperprolactinemia or treatment with dopamine agonists.

(II) In particular, the prolactinoma group showed a high prevalence of neuropsychiatric symptoms under treatment with cabergoline, such as depressed mood, fatigue, sleep disorders, aggressiveness and anxiety.

(III) In regard to our main study hypothesis, we observed that prolactinoma patients with a specific genotypic profile e.g. C-carriers and heterozygous CT-individuals of SNP rs1045642 and G-carriers of SNP rs2032582 presented less frequently fatigue, sleep disorders and also seemed to be protected from enhancement of dizziness respectively under medical treatment with cabergoline. However, SNPs rs2032583 and rs2235015 do not seem to influence the occurrence of the examined side effects under cabergoline. Additionally, for the majority of the neuropsychiatric side effects of cabergoline in prolactinoma patients tested (headaches, increased libido, depressed mood and aggressiveness), the MDR1 transporter encoding ABCB1 gene does not seem to play any predictive role.

Ad (I): The comparison of our prolactinoma patients with 60 patients of a clinical control group of NFPA revealed that our patients were significantly younger. Greenman et al. (63) report an average age of diagnosis between 50 and 55 years for the NFPA, whereas in our group the tumour was earlier diagnosed (mean age at diagnosis 47,1 years). This slight difference could be attributed to the informed group of patients and/or doctors prompting earlier medical consultation when noticing some signs and symptoms that usually tend to be ignored. The patients of the NFPA group included significantly more overweight patients in comparison to the prolactinoma patients but it should be considered that our results are not adjusted for age and gender.

Concurrent to the described sex preference of prolactinomas in the female, our prolactinoma group included more female patients than the NFPA group in a female-to-male ratio 3:1. Gender differences in tumour size are supposed to exist in hyperprolactinemia since microprolactinomas are more commonly found in women and macroprolactinomas in men, results that were also to be seen in our study. Possible reasons for this could be either a delay in diagnosis in men or a true gender difference in tumour pathogenesis (13); however, there is still no exhaustive explanation about this phenomenon. It could be speculated that the increased prevalence of prolactinomas in women could be due to the observation that the symptoms in women are more evident (amenorrhea, galactorrhea) and lead earlier to the diagnosis, whereas the features of hypogonadism (impotence and decreased libido) are less readily evident, not so specific and seem to be ignored. The pathogenesis of the tumour seems also to be under a gender control, as indicated by autopsy studies (64). However, studies comparing the clinical and pathological correlates of growth of these tumours in both sexes are lacking. There is only one study of Delgrange et al. implicating a different pathogenesis and revealing a greater growth potential of macroprolactinomas in men than in women as well

as a male predominance of aggressive forms of the disease (e.g. giant, invasive, and malignant prolactinomas) (65).

We report additionally but not surprisingly, results in consistency with the available literature (63) showing increased prevalence of macroadenomas in the NFPA group.

Due to a lack in sufficient medical treatment of NFPA, surgery remains the main primary therapeutical procedure, whereas in patients with prolactinomas a therapy with dopamine agonist agents is in the vast majority of the cases indicated. The prolactinomas seemed to be diagnosed at a younger age than the NFPA and the reasons seem to be not only the earlier de novo onset of the tumour (63, 66), but also the earlier diagnosis due to the evidence of mostly prominent symptoms such as amenorrhea and galactorrhea, whereas NFPA present often without any symptomatology or with unspecific signs of pituitary deficits and seem to be attributed to other causes both from the physicians and patients.

Regarding the lifetime comorbidities, our patients with prolactinomas reported less frequent hypertension, reflecting most probably their younger age. In terms of pituitary insufficiency, statistically more insufficiency of all axes is being reported in the NFPA group, representing the increased prevalence of macroadenomas and the surgical resection of the tumour mostly followed. We present a 45% prevalence of any axis of pituitary insufficiency in the NFPA group, primarily based on previous pituitary testing, but also on the patient's knowledge of this abnormality. In previous studies, symptoms of hypopituitarism seem to be prevalent in roughly 40-52% of patients (63). However, formal testing of pituitary function always tends to reveal a higher incidence of pituitary hypofunction that can be up to 70% or more.

In the prolactinoma group the gonadal axis was the most commonly affected, followed by the corticotropic. This discrepancy with the available literature data, that reports most frequent

insufficiency of the somatotropic axis (67) reflects most probably a reporting or diagnostic bias, since the somatotropic insufficiency often remains undiagnosed or even untreated and the patients are probably not aware of it.

Ad (II): We present an increased prevalence of neuropsychiatric symptoms under treatment with cabergoline in our prolactinoma group. More specifically, fatigue was present in 44% of our patient group, significantly increasing in 20% of patients. About 33% of patients complained about headaches and more than one third of patients seemed to experience a depressed mood under treatment with cabergoline.

Side effects associated with cabergoline administration are common but seem to be less frequent in comparison with bromocriptine at least in terms of nausea and vomiting (48).

There are many studies examining the different side effects of cabergoline in patients with prolactinoma. However, to our knowledge, the existing studies do not compare the symptoms and signs in terms of changes under treatment with cabergoline and our study is the first in the literature with this approach up to now.

Therefore, we addressed the presence of symptoms under cabergoline and we asked the patients to evaluate them in terms of change, enhancement or not (reduction and consistency). This seems to be important, because some signs such as headaches, fatigue or sleep disorders, when evaluated only under medical treatment, could be frequent in a false positive way because they could be correlated to the medication but also to the state of non-recurrent hyperprolactinemia, the presence of the pituitary mass and secondary pituitary insufficiency. Furthermore, we can assume that the presence of a symptom - when asked to be evaluated in terms of change under treatment - could be in all likelihood attributed to the treatment itself.

More specifically, fatigue seems to be the most common symptom under treatment with cabergoline, significantly increasing in 20% of patients. We observed a higher proportion of patients suffering from fatigue under treatment in comparison to the available literature where data in different studies vary from 13-18% (68). However, our data are rather consistent when evaluated in terms of enhancement of fatigue under treatment, taking into consideration that this percentage is the true attributed side effect to the medication. Additionally, Kars et al. evaluated fatigue with MFI-20 (Multidimensional Fatigue Inventory) and its five different dimensions: a) general fatigue, b) physical fatigue, c) reduced activity, d) reduced motivation and e) mental fatigue and revealed an impaired reduced fatigue profile in the first four traits, when compared to controls. Present use of dopamine agonist (about 60% cabergoline in this patient group) seemed to be a major determinant of reduced activity (c), contributing significantly to the onset of fatigue (69).

Headaches remain one of the main complaints of patients with pituitary lesion of any origin with a frequency up to 40% (70, 71). Approximately one third of our group reported of headaches under cabergoline, with 10% of them complaining of enhancement of the symptom. Webster et al. report of consistent results with approximately 30% of patients suffering from headaches under cabergoline (68). However, headaches also seem to be a common important problem before the initiation of treatment, as Colao et al. (72) states. The physician should keep in mind this important side effect, but should also exclude by persistence of the symptom, other serious causes such as tumour growth or pituitary apoplexy.

Dizziness or vertigo, that occurs in up to 27,8% of our patient collective, appears to be one of the most important reasons of discontinuation of the therapy in consistency with the previous data, reporting of a prevalence of 25% under treatment with cabergoline (68). It seems though to be ameliorated when cabergoline is administered after the meal at night.

In terms of depressed mood, we observed a high proportion of patients (up to 33%) complaining of depressed mood, worsened in 15% under cabergoline. In experimental models (73) and case reports (74) though, cabergoline has been found to exert antidepressant effects but larger studies are needed to fully elucidate this observation. We demonstrated a rather high percentage of depressed mood in our patient collective compared with the available literature. Webster et al. (68), report of 3% of depression under cabergoline, whereas in the studies of Sabuncu et al. and Ono et al., no psychiatric side effects have been described (75, 76).

Our observation could reflect a possible selection bias due to the low response rate, leading to the conclusion that our patient collective included primarily patients of increased age and were more affected from the disease. However, it should also be also taken into consideration, that we did not conducted standardised personal interviews to diagnose depression, but asked the patients' perception of their mood under treatment. A further limitation in the interpretation of our results is also the fact that our Endocrine Outpatient Unit is located in a psychiatric and neurologic clinic (Max Planck Institute of Psychiatry), with patients suffering more often from comorbid mood disorders and prolactinoma being referred to our department more frequently than the patients without these kinds of symptoms.

In the available literature, quality of life seems to be impaired in female patients treated for microprolactinoma, especially due to increased anxiety and depression (69) but the link of this result is poorly defined. In this later study, the authors evaluated the depression and quality of life in 55 female patients with microprolactinoma, where the current use of dopamine agonist or the present prolactin levels were not evaluated in terms of depression. Several points could provide insight to understand the relationship of prolactinoma and emotions, including the central effects of hyperprolactinemia and the patient's knowledge of having a "brain tumour".

Prolactin has been found to act on the central nervous system and may be associated with irreversible changes in neural function, that could probably be translated to alterations in behaviour, emotions, feelings and personality (77). A number of studies have documented an altered psychological profile and emotional difficulties in patients with prolactinomas, mainly characterised by increased anxiety, depression and impaired quality of life (69, 78), changes that occasionally persist even after remission of the hyperprolactinemia (77). The clinician should keep in mind this impaired psychological status of this group of patients when treating them.

Ad (III): In general, our study does not demonstrate any significant correlation of the four examined SNPs of the ABCB1 gene rs1045642, rs2032582, rs2032583 and rs2235015 with the vast majority (headaches, increased libido, depressed mood and aggressiveness) of the most common side effects of cabergoline.

However, we have to point out some interesting results:

We demonstrated that the C-carriers of SNP rs1045642 were rather protected compared to the non-C-carriers, in terms of suffering from sleep disorders and fatigue under cabergoline. Additionally, heterozygous CT-individuals of SNP rs1045642 presented less frequent sleep disorders than homozygous CC and CT. Regarding enhancement of dizziness under cabergoline, non-G-carriers of SNP rs2032582 seemed to experience a 7-fold higher risk to suffer from it than the G-carriers. In terms of further central side effects such as headaches, increased libido, depressed mood or aggressiveness our study failed to demonstrate any statistical significance.

As has been well established from different studies, the P-pg activity depends on the expression of P-gp and also its functionality. Both parameters seem to interfere and secondarily influence intracerebral concentrations of the substrates of P-gp. In terms of expression, MDR1 has been found to be overexpressed in cases of insensitivity of tumour cells toward chemotherapy due to amplifications or other mechanisms (79). Functionality reflects the effectiveness of the MDR1, leading to abnormal increased accumulation and potential adverse events.

To our knowledge, there are few studies addressing the influence of ABCB1 polymorphisms and all of them refer to antidepressants that are substrates of this molecule. In this case, a low ABCB1 activity is desired, in order to achieve higher intracerebral concentrations and increase the remission rate. In the case of cabergoline and dopamine agonist in general though, where the target of treatment is outside the BBB, low intracerebral concentrations do not influence therapy response, protecting at the same time from the occurrence of central side effects.

As indicated from many different studies, the interindividual and genotypic variability of the P-gp in the blood brain barrier could influence secondarily the degree of expression and the functionality of the MDR-1 gene product and therefore directly affect the therapeutic effectiveness of such agents that are substrates of P-gp, with the results reported remaining inconsistent.

Gex-Fabry et al. (80) and Mihaljevic Peles et al. (81) implicated that rs203582 and rs1045642 did not influence the response to paroxetine and both Peters et al. (82) and Laika et al. (83) showed that none of these genetic polymorphisms in the pharmacokinetic genes examined were significantly associated with our response or tolerance.

However, both Hoffmeyer et al. and Kato et al., reported that the C variant of SNP rs1045642 and G variant of SNP rs2032582 were associated with higher P-gp expression and function (60, 84) and therefore poor response to antidepressants, leading to a more severe depressive symptomatology. Conflicting results have also been reported (85), showing an increased remission of depression in the G-carriers SNP rs2032582, presenting with higher CSF concentrations.

These latest results are in accordance with our findings that report both less frequent occurrence of side effects and enhancement of them under cabergoline in the individuals having the "protective" genotype reflecting the lower intracerebral concentrations of the medication.

Nevertheless, our study was focused on the evaluation of some specific genetic predictors of ABCB1. We suppose there are further transporting molecules and genetic pathways that influence the metabolism and action of cabergoline in the brain circulation and further studies need to be conducted to elucidate these mechanisms.

At any case, the study points to the genetically determined investigation of side effects that could have promising results in terms of personalised medicine and could predict, before initiation of treatment, how the individual's genetics affects his or her side effects. This could lead in the future to an adaptation of the treatment to individual patients.

Apart from the three mentioned main findings, we can also point out the following observations:

(i) **Treatment with dopamine agonists and** w**eight loss**

The treatment with cabergoline seems to cause a weight loss in up to 20% of our patients.

Significant interactions seem to exist between hyperprolactinemia and weight control. Hyperprolactinemia in humans is associated with a high prevalence of obesity and insulin resistance (86) and patients with prolactinomas, especially macroprolactinomas tend to have increased body weight in comparison to healthy individuals (87). Human and animal studies have also implicated that dopamine, as a neurotransmitter, modulates rewarding properties of food and plays a significant role in appetite regulation (36) and more recently, different polymorphisms of D2 receptor have been linked to obesity (88), suggesting that individuals may overeat to compensate for hypofunctioning dopaminergic signalling (89, 90).

Normalisation of prolactin levels, after treatment with dopamine agonists, has been associated with weight loss (91-93) but the nature of this link is poorly defined. In most of the available studies a significant weight loss after treatment with bromocriptine was observed (91, 92, 94). More specifically Doknic et al. (94) demonstrated significant weight loss implying that bromocriptine influences body weight by mechanisms in addition to reducing hyperprolactinemia. Adding some controversy, another study of 39 hyperprolactinemic patients treated with different dopamine agonists, failed to demonstrate such correlation (95).

On the other hand, Greenman et al. (91) in a retrospective study of 42 patients with prolactinomas and 36 patients with clinically non-functioning macroadenomas, attributed weight loss not to the therapy with bromocriptine or its pharmacological side effects, but exclusively to the normalisation of prolactin levels.

Regarding cabergoline, there are only two studies available in the literature providing insight to the effect of this commonly used medication on body weight in prolactinoma patients. In the first study of Korner et al. (93), a weight loss effect attributable to cabergoline treatment was noted, whereas in another more recent case-control study of Serri et al. (86), no significant changes in body weight after treating prolactinoma patients with cabergoline occurred.

The animal models are mostly lacking in available weight data, while the weight effect of dopamine agonists in patients with Parkinson's disease is difficult to estimate due to confounding factors affecting this group (dysphagia, anorexia, gastrointestinal dysfunction etc.).

In conclusion, the available data regarding this topic are rather controversial and do not prove causality. To clarify further the relationship between hyperprolactinemia, body weight and dopamine agonist therapy and more specifically the plausible pathophysiological mechanism responsible for this, further studies should be conducted.

(ii) **Impulsive control disorders and dopaminergic signalling**

In our study we managed to show an increased libido under treatment with cabergoline in 20% of patients, whereas small numbers of gambling (1 patient), compulsive shopping (5 patients) and binge eating have been referred (5 patients). The increase of libido could have been anticipated to a certain extent, taken into consideration that hyperprolactinemia and prolactinomas cause hypogonadism and impaired sexual and reproductive function. The normalisation of prolactin levels leads to a restorement of libido and sexual function. To what

extent the increased libido remained in the normal range, or whether it tended to be a pathological hypersexuality, could not be clearly evaluated due to the lacking of a personal interview with the patients. However, the 20% enhancement of increased libido could be implicating a trend to an abnormal extent of this symptom.

In patients with Parkinson's disease under treatment with dopamine agonists, increased prevalence of pathological behaviours characterised by compulsion and impulsivity have been described such as pathological gambling, hypersexuality, compulsive shopping and eating. All these disorders, known as impulsive control disorders may develop, according to the available literature, in up to 30% of people taking higher agonist doses (96) that tend to be 20-fold higher than the mean weekly dose used for prolactinomas and could also persist after the withdrawal of the medication. Additionally, in individuals with Restless Legs Syndrome on dopamine agonist therapy, it was suggested that impulse control disorders can occur over a wide range of dopamine agonist therapy types and dose exposures.

To our knowledge, there are only 2 case reports in the literature reporting of hypersexuality and gambling in female and male patients, both being treated for a microprolactinoma with a dose of 0,25 mg and 1 mg weekly respectively (56, 57). In both cases, the symptoms were ceased after the withdrawal of cabergoline.

At this point, we have to recall the dopaminergic basis of impulsivity. The chronic exposure to intermittent administration of substances such as dopamine agonists that increase dopaminergic levels, may also affect impulsive choice. A recent study demonstrated that DA use status was associated with a greater choice impulsivity in Parkinson’s patients as compared to PD controls (97). Dopamine neurotransmission, along the mesocorticolimbic pathway, is a potential modulator of risk behaviour. In cases of medical treatment with dopamine agonists, the direct upregulation of the dopaminergic tone could be a plausible

explanation but baseline differences or differences of response to DA should also be addressed. Furthermore, studies with administration of DA in healthy volunteers to evaluate the impulsive behaviour are scarce up to now and therefore only indicative conclusions based on the Parkinson's patient's population can be drawn.

Taking into consideration the aforementioned observations, it remains of clinical importance in the daily practice that these possible complications do not escape doctors' attention remaining underdiagnosed. Such pathological behaviours can frequently lead to considerable marital or occupational conflicts or if severe, become a problem also from ethical and legal points of view. Cabergoline-induced pathological gambling and hypersexuality and in general ICDs are probably under-reported and physicians should consider screening for these in patients treated with dopamine agonists and establish a multidisciplinary approach.

6.2 Strengths and limitations

Of the 308 patients treated at the Endocrine Outpatient Clinic of the Max Planck Institute to whom a questionnaire was sent, 225 were female patients (73%) and the female-to-male ratio was approximately 2,7:1. Of the 92 patients who participated in our study (percentage of participation 30%) the ratio was similar at 3:1. In the initial group of 308 patients, 198 were diagnosed with microprolactinomas and 110 with macroprolactinomas, whereas in our study group we observed an equal prevalence of micro- and macroprolactinomas, reflecting probably the increased health concern of the latest group of patients. Therefore, we have to assume that in terms of tumour art, our group was not reflecting the initial group of patients treated in the Endocrine Outpatient Clinic; however, both types of adenomas were sufficiently represented.

As expected, few patients underwent a surgical approach, reflecting the high rate of remission under medical treatment. 86% of our patients received, in their disease history, cabergoline, which is, according to the latest guidelines of the Endocrine Society (34), the dopamine agonist of choice. As our group included subjects with a long disease history from approximately diagnosis since the 1970s, other dopamine agonists such as bromocriptine, lisuride and quinagolide had been used.

Our patients tended to be overweight at study point. As indicated from Colao (66), patients with hyperprolactinemia often present weight gain, tending also to have an altered body composition with increased fat mass and reduced lean mass, even when non-obese (98). We did not present data regarding weight at the time of diagnosis, which could probably reflect better the effect of hyperprolactinemia on weight gain. Due to the small sample size, we did not conduct further analysis evaluating the BMI of patients in terms of pituitary insufficiency (e.g. of somatotropic axis, knowing to be present with increased fat mass) or prolactin levels, implying that the increased BMI may also reflect insufficient substitution of the pituitary axes or even uncontrolled disease.

The strength of our study is that it is the first to address the important topic of neuropsychiatric side effects of cabergoline in terms of different genetic predictors of ABCB1. The novelty finding of cabergoline, being a substrate of P-gp, presented in our study, will expand the current knowledge on DA-treatment.

An important percentage of patients to whom the questionnaire was sent did not respond (70%). This may be due to the extended and specialised questions that were included regarding medical and disease history or due to their nature of the neuropsychiatric details that the patients may not have wanted to answer, leading to a decreased response rate,

(selection bias). Additionally, due to a relative low response rate we have to hypothesise that the patients that finally participated in our study were probably the older or more affected of the pituitary lesions of our initial group, who due to increased health concerns, dedicated their time to participate in our study.

Another source of bias is additionally the "missing data" that occurs from a false response or misunderstanding of the questions (measurement bias).

The data collected for the study was obtained mainly from the questionnaire and in the case of missing data or uncertainty, additional information was obtained by file review. Therefore, the results consisted partly from information which was based on the patient's own perception. No personal interview was conducted. The reported trends e.g. for depressed mood or aggressiveness, reflected the patient's impression for his symptoms were not validated or diagnosed from a specialised physician according to the ICD-10 (ICD-10: International Statistical Classification of Diseases and Related Health Problems 10th Revision).

Furthermore, due to the small size of our sample we could not evaluate our results controlling for possible confounding factors such as age, sex, BMI, remission of disease or pituitary insufficiency.

7. CONCLUSION

This is the first study demonstrating that polymorphisms of the MDR1 (or P-gp) encoding ABCB1 gene could account for a different occurrence or enhancement of central side effects of the systematically administered cabergoline. As cabergoline was tested and found to be a substrate of MDR1, the individual's genetic polymorphisms account for differences in function and expression of P-gp, controlling the intracerebral concentration of cabergoline.

More specifically in regard to our main study hypothesis, we observed that prolactinoma patients with a specific genotypic profile e.g. C-carriers and heterozygous CT-individuals of SNP rs1045642 and G-carriers of SNP rs2032582, presented less fatigue, less frequent sleep disorders and also seemed to be protected from enhancement of dizziness respectively under medical treatment with cabergoline. SNPs rs2032583 and rs2235015 in our study and for the side effects tested do not seem to influence their occurrence under cabergoline. For the majority of the neuropsychiatric side effects of cabergoline in prolactinoma patients tested (headaches, increased libido, depressed mood and aggressiveness), the MDR1 transporter encoding ABCB1 gene does not seem to play any predictive role.

Secondary, the comparison of the prolactinoma patients with the clinical control group of NFPA patients, revealed a different profile of baseline and disease characteristics and also an individual spectrum of lifetime comorbidities possibly caused by long-term hyperprolactinemia or dopaminagonist therapy.

In terms of prevalence of neuropsychiatric symptoms under cabergoline, our study revealed a significantly higher prevalence of them, such as depressed mood, aggressiveness, anxiety, fatigue and sleep disorders.

8. ABSTRACT/SUMMARY

Background: Prolactinomas are the most frequent pituitary adenomas. The treatment with cabergoline, the most common dopamine agonist used, is associated with side effects such as nausea, vomiting, dizziness, headaches, movement disorders and fatigue. There is some additional evidence from case reports and small studies that some patients report neuropsychiatric side effects such as depression, gambling, hypersexuality and impulsive control disorders.

Objective: In this cross-sectional study we sought to investigate the baseline clinical, demographic and disease characteristics of our patient group as well as life-time comorbidities. Additionally, side effects under treatment with cabergoline (prevalence and enhancement) and whether genetic variants of the ABCB1 gene (coding MDR1 or P-gp) could account for difference in the central neuropsychiatric side effects were investigated.

Methods: Questionnaires evaluating medical history, therapy side effects and further demographic characteristics were sent to all prolactinoma patients currently treated at the Max Planck Institute of Psychiatry in Munich. Additionally, DNA extracted either from blood or saliva samples was genotyped for each patient.

Results: The clinical study included a total of 92 patients (23 male and 69 female, macro-to-microadenoma-ratio 1:1). The mean age of our group at the time of the study was 49,2 ± 13,8 years. Of the 79 patients treated with cabergoline, the following side effects associated with treatment were more prominent: fatigue (n=35), headaches (n=26), depressed mood (n=26), sleep disorders (n=26), dizziness (n=22), aggressiveness (n=17), anxiety (n=19) and weight loss (n=16). 18 patients reported of decreased and 16 of increased libido. Significant effects were observed for the C-carriers and heterozygous CT-individuals of rs1045642 that presented less frequent fatigue and sleep disorders under cabergoline. In the analysis of SNP rs2032582, G-carriers seemed to be protected from enhancement of dizziness under

cabergoline. SNPs rs2235015 and rs2032583 were found to have no association with the examined symptoms.

Conclusion: In our group we described an increased prevalence of symptoms such as fatigue and weight loss under cabergoline, as well as neuropsychiatric side effects such as depressed mood, aggressiveness and anxiety in comparison to the available data of the literature. We demonstrated that polymorphisms of SNPs rs1045642 and rs2032582 of the ABCB1 gene predispose for fatigue, sleep disorders and dizziness under cabergoline. This is the first study demonstrating that individual ABCB1 gene polymorphisms could account for a different occurrence or enhancement of central side effects of this systematically administered medication.

9. ZUSAMENFASSUNG (SUMMARY)

Hintergrund: Prolaktinome sind die häufigste Hypophysenadenome. Medikamentöse Therapie der ersten Wahl sind Dopaminagonisten wie das Ergotderivat Cabergolin. Fallberichte und kleinere Studien weisen darauf hin, dass es bei einigen Patienten unter dieser Therapie zu neuropsychiatrischen Nebenwirkungen wie Spielsucht, Hypersexualität, übermäßigem Einkaufen, Essanfällen oder depressiven Verstimmungen kommt. Cabergolin ist ein Substrat des Transportermoleküls MDR1 (P-Glykoprotein) der Bluthirnschranke. MDR1 wird durch das ABCB1-Gen kodiert und kommt in der Allgemeinbevölkerung in verschiedenen Polymorphismen vor. Das durch das ABCB1 Gen kodierte P-gp führt an den Blut-Hirn-Schranke dazu, dass seine Substrate zurück ins Blut transportiert werden und deren Konzentrationen im Gehirn niedrig bleibt.

Objektive: Es handelt sich um eine nicht-interventionelle diagnostische Querschnittsstudie zur Untersuchung von Nebenwirkungen und genetischer Polymorphismen des ABCB1 Gens für Nebenwirkungen einer dopaminagonistischen Therapie mit Cabergolin bei Patienten mit Prolaktinomen.

Methodik: 308 ambulante Patienten mit der Diagnose Prolaktinom, die in der Endokrinologischen Ambulanz des Max-Planck-Instituts für Psychiatry behandelt werden, wurden angesprochen und 92 haben an der Studie teilgenommen. Entsprechende Fragebögen wurden von den Patienten ausgefüllt und genetische Analysen mittels Extraktion von DNA von Blut- oder Speichelproben wurde durchgeführt.

Ergebnisse: 92 Prolaktinompatienten wurden rekrutiert (23 Männer, 69 Frauen, Mikro-zu-Makroadenom Verhältnis 1:1). Das Durchschnittsalter war 49,2 ± 13,8 Jahre. 79 Patienten wurden mit Cabergolin behandelt. In der Gruppe der Patienten, die mit Cabergolin behandelt sind, wurden vorwiegend die folgenden Nebenwirkungen beobachtet: Müdigkeit (n=35), Kopfschmerzen (n=26), depressive Verstimmung (n=26), Schlafstörungen (n=26), Schwindel (n=22), Aggressivität (n=17), Angst (n=19) und Gewichtsabnahme (n=16). C-carriers und CT

Heterozygoten des SNPs rs1045642 sowie G-carriers des SNPs rs2032582 wiesen weniger Müdigkeit, Schlafstörungen und Verstärkung von Schwindel während der Therapie mit Cabergolin auf. Keine signifikanten Unterschiede für SNPs rs2235015 und rs2032583 konnten nachgewiesen werden.

Zusammenfassung: Wir schließen daraus, dass die Prolaktinompatienten eine erhöhte Zahl von neuropsychiatrischen Nebenwirkungen nachweisen und die SNPs rs1045642 and SNP rs2032582 des ABCB1-Gens eine protektive Rolle im Hiblick auf die Entwicklung von Schlafstörungen, Müdigkeit und Verstärkung von Schwindel spielen. Zukünftig sind klinische Studien wünschenswert, die weitere Aspekte des Phänomens klären können.

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12. APPENDICES

12.1 Questionnaire

Datenerfassungsbogen

Prolaktinomstudie

Name : _____

Vorname : _____

Geschlecht : O weiblich O männlich

Geb. Datum : _____(TT/MM/YYYY)

Gewicht : _____(Kg)

Größe : _____(Meter)

Wir bitten Sie, diesen Fragebogen selbständig auszufüllen und vollständig im frankierten Briefumschlag an unser Institut zurücksenden. Bitte beantworten möglichst Sie alle Fragen (auch wenn eine Frage für Sie nicht zutrifft). Bitte gehen Sie weiter mit den anderen Fragen, wenn sich nicht genau die Antwort erinnern können.

Würden Sie uns bitte einige Angaben zu Ihrer Krankengeschichte machen?

Datum der Erstdiagnose Prolaktinom: _____

Prolaktinwert bei Erstdiagnose:

Datum: _____

Wert: _____

Einheit: _____

Normwerte des Labors: _____

Kommentar: erhöht O unauffällig O nicht bekannt O

_____

MRT bei Erstdiagnose:

Datum: _____

Makroadenom O Kommentar: _____

Mikroadenom O Kommentar: _____

Unbekannt O Kommentar: _____

Gesichtsfeld bei Erstdiagnose:

Datum: _____

eingeschränkt O nicht eingeschränkt O unbekannt O

Letzter Prolaktinwert:

Datum: _____

Wert: _____

Einheit: _____

Normwerte des Labors:_____

Kommentar: erhöht O unauffällig O nicht bekannt O

_____

Letztes MRT:

Datum: _____

Makroadenom O Kommentar: _____

Mikroadenom O Kommentar: _____

Unbekannt O Kommentar: _____

Letztes Gesichtsfeld:

Datum:_____

eingeschränkt O nicht eingeschränkt O unbekannt O

verbessert zum Vorbefund O verschlechtert zum Vorbefund O

Gewicht:

Höchstes Gewicht: kg _____ Datum _____

Niedrigstes Gewicht: kg _____ Datum _____

Hatten Sie zu irgendeinem Zeitpunkt vor Diagnose und Therapie des Prolaktinoms eines dieser Symptome bemerkt oder vom Arzt diagnostiziert bekommen?

Bitte kreuzen Sie Zutreffendes an (ggf. mit Beginn). Bitte schätzen Sie zusätzlich den Grad der Intension des Symptoms auf einer Skala von 1-5 ein:

Intensitätsskala:

1 = überhaupt nicht vorhanden/ausgeprägt

2 = wenig ausgeprägt

3 = mittelgradig ausgeprägt

4 = stark ausgeprägt

5 = sehr stark ausgeprägt

Symptome	**JA**	**NEIN**	**Beginn**	**Intensität** **1-2-3-4-5**
Beispiel	**x**		**2001**	**2**
1. Kopfschmerzen				
2. Milchausfluss **(Galaktorrhoe)**				
3. Schmerzen der Brüste **(Mastodynie)**				
4. Gewichtsabnahme*				
5. Gewichtszunahme*				
6. Reduzierte Libido(= wenig Spaß am Sex, kaum Lust auf Sexualverkehr)				
7. Deutlich gesteigerte Libido(= gesteigerter Spaß am Sex / Lust auf Sexualverkehr)				

Symptome	**JA**	**NEIN**	**Beginn**	**Intensität** **1-2-3-4-5**
8. Verminderte **Knochendichte (Osteopenie)**				
9. Depressive Verstimmung				
10. Schlafstörung				
11. Müdigkeit				
12. Empfindungsstörungen **(Parästhesien)**				
13. Schwindel				
14. Aggressivität				
15. Angst				
16. Visuelle Halluzinationen **(Wahrnehmungen)**				
17. Spielsucht				
18. Übermäßiges Einkaufen				
19. Essanfälle				
20. Ausreißen der Haare				
21. Andere Suchterkrankungen **(ggf. bitte** **eintragen:_____)**				
22. Vermehrter Alkoholkonsum				
23. Verminderter **Alkoholkonsum**				
24. Andere psychiatrische				

Erkrankungen / Symptome (ggf. bitte eintragen:_____)				
Nur für Frauen				
Unregelmäßige Periodenblutungen				
Nur für Männer				
1.Erektile Dysfunktion / Impotenz				
2.Nachlassender Bartwuchs				

*** Bitte Angabe der kg _____ zwischen (Jahr): _____ und (Jahr):_____**

- **Brauchten Sie für eine der o. g. Erkrankungen oder Symptome eine Therapie?**

O Ja O Nein

- **Wenn ja, für welche:_____**
- **Wenn ja, welche Therapie (z.B. Psychotherapie, Antidepressiva etc.):_____**

Erhalten/erhielten Sie eine der folgenden Therapie für Ihre Hyperprolaktinämie/das Prolaktinom?

Bitte füllen Sie die zutreffenden Felder aus.

- **Hypophysenoperation** O ja O nein

Datum: _____

Anzahl: _____

Art: O transphenoidal O transkraniell O unbekannt

- **Bestrahlung der Hypophyse** O ja O nein

Datum: _____

Art: O stereotaktisch fraktionär O Gammaknife O Cyberknife

- **Medikamentöse Therapie**

1) Bromocriptin (Handelsnamen: Bromocrel, Bromocriptin-rathiop., Kirim, Parlodel, Pravidel)

O ja O nein

2) Lisurid (Handelsname: Dopergin)

O ja O nein

3) Cabergolin (Handelsnamen: Dostinex – Cabaseril)

O ja O nein

4) Quinagolide (Handelsname: Norprolac)

O ja O nein

5) Andere medikamentöse Therapie (Handelsname _____)

O ja O nein

Wenn Sie eine ***Hypophysenoperation* erhielten, ist nach der Operation eines der folgenden Symptome neu aufgetreten oder hat sich verändert?**

(Bitte folgende Seite nur ausfüllen, falls Sie diese Therapie erhalten haben)

Bitte kreuzen Sie Zutreffendes an (ggf. mit Beginn). Bitte schätzen Sie zusätzlich den Grad der Intension des Symptoms auf einer Skala von 1-5 ein:

Intensitätsskala:

1 = überhaupt nicht vorhanden/ausgeprägt

2 = wenig ausgeprägt

3 = mittelgradig ausgeprägt

4 = stark ausgeprägt

5 = sehr stark ausgeprägt

Symptome	**Neu**	**Verstärkt**	**Vermindert**	**Unverändert**	**Skala 1-5**
Beispiel	**Nein**	**Ja**			**2**
1. Kopfschmerzen					
2. Milchausfluss (Galaktorrhoe)					
3. Schmerzen der Brüste (Mastodynie)					
4. Gewichtsabnahme*					
5. Gewichtszunahme*					

6. Reduzierte Libido(= wenig Spaß am Sex, kaum Lust auf Sexualverkehr)					
7. Deutlich gesteigerte Libido (= gesteigerter Spaß am Sex / Lust auf Sexualverkehr)					
8. Verminderte Knochendichte (Osteopenie)					
9. Depressive Verstimmung					
10. Schlafstörung					
11. Müdigkeit					
12. Empfindungsstörungen (Parästhesien)					
13. Schwindel					
14. Aggressivität					
15. Angst					
16. Visuelle Halluzinationen (Wahrnehmungen)					
17. Spielsucht					
18. Übermäßiges Einkaufen					
19. Essanfälle					
20. Ausreißen der Haare (Trichotillomanie)					
21. Andere Suchterkrankungen (ggf. bitte					

eintragen:_____)					
22. Vermehrter Alkoholkonsum					
23. Verminderter Alkoholkonsum					
24. Andere psychiatrische Erkrankungen / Symptome (ggf. bitte eintragen:_____)					
Nur für Frauen					
Unregelmäßige Periodenblutungen					
Nur für Männer					
1.Erektile Dysfunktion / Impotenz					
2.Nachlassender Bartwuchs					

*** Bitte Angabe der kg _____ zwischen (Jahr): _____ und (Jahr):_____**

Wenn Sie eine ***Bestrahlung* erhielten, ist unter der Bestrahlung eines der folgenden Symptome neu aufgetreten oder hat sich verändert?**

(Bitte folgende Seite nur ausfüllen, falls Sie diese Therapie erhalten haben)

Bitte kreuzen Sie Zutreffendes an (ggf. mit Beginn). Bitte schätzen Sie zusätzlich den Grad der Intension des Symptoms auf einer Skala von 1-5 ein:

Intensitätsskala:

1 = überhaupt nicht vorhanden/ausgeprägt

2 = wenig ausgeprägt

3 = mittelgradig ausgeprägt

4 = stark ausgeprägt

5 = sehr stark ausgeprägt

Symptome	**Neu**	**Verstärkt**	**Vermindert**	**Unverändert**	**Skala 1-5**
Beispiel	**Nein**	**Ja**			**2**
1. Kopfschmerzen					
2. Milchausfluss (Galaktorrhoe)					
3. Schmerzen der Brüste (Mastodynie)					
4. Gewichtsabnahme*					
5. Gewichtszunahme*					
6. Reduzierte Libido(= wenig					

Spaß am Sex, kaum Lust auf Sexualverkehr)					
7. Deutlich gesteigerte Libido (= gesteigerter Spaß am Sex / Lust auf Sexualverkehr)					
8. Verminderte Knochendichte (Osteopenie)					
9. Depressive Verstimmung					
10. Schlafstörung					
11. Müdigkeit					
12. Empfindungsstörungen (Parästhesien)					
13. Schwindel					
14. Aggressivität					
15. Angst					
16. Visuelle Halluzinationen (Wahrnehmungen)					
17. Spielsucht					
18. Übermäßiges Einkaufen					
19. Essanfälle					
20. Ausreißen der Haare (Trichotillomanie)					
21. Andere Suchterkrankungen (ggf. bitte eintragen:_____)					

22. Vermehrter Alkoholkonsum					
23. Verminderter Alkoholkonsum					
24. Andere psychiatrische Erkrankungen / Symptome (ggf. bitte eintragen:_____)					
Nur für Frauen					
Unregelmäßige Periodenblutungen					
Nur für Männer					
1.Erektile Dysfunktion / Impotenz					
2.Nachlassender Bartwuchs					

*** Bitte Angabe der kg _____ zwischen (Jahr): _____ und (Jahr):_____**

Medikamentöse Therapie

1) Wenn Sie diese medikamentöse Therapie *(Bromocriptin)* erhielten, ist unter der Therapie eines der folgenden Symptome neu aufgetreten oder hat sich verändert?

(Bitte folgende Seite nur ausfüllen, falls Sie diese Therapie erhalten haben)

Bromocriptin (Handelsnamen: Bromocrel, Bromocriptin-rathiop., Kirim, Parlodel, Pravidel) O ja O nein

Beginn: _____

Höchste Dosis: _____

Dauer: _____

Aktuell: O ja O nein

- Therapie senkt/senkte Prolaktin erfolgreich O ja O nein O teilweise

Prolaktinwert vor _____und nach Therapie: _____

- Therapie führte zu einer Tumorschrumpfung O ja O nein O teilweise

Tumordurchmesser vor _____und nach Therapie: _____

Bitte kreuzen Sie Zutreffendes an (ggf. mit Beginn). Bitte schätzen Sie zusätzlich den Grad der Intension des Symptoms auf einer Skala von 1-5 ein:

Intensitätsskala:

1 = überhaupt nicht vorhanden/ausgeprägt

2 = wenig ausgeprägt

3 = mittelgradig ausgeprägt

4 = stark ausgeprägt

5 = sehr stark ausgeprägt

Symptome	**Neu**	**Verstärkt**	**Vermindert**	**Unverändert**	**Skala 1-5**
Beispiel	**Nein**	**Ja**			**2**
1. Kopfschmerzen					
2. Milchausfluss (Galaktorrhoe)					
3. Schmerzen der Brüste (Mastodynie)					
4. Gewichtsabnahme*					
5. Gewichtszunahme*					
6. Reduzierte Libido(= wenig Spaß am Sex, kaum Lust auf Sexualverkehr)					
7. Deutlich gesteigerte Libido (= gesteigerter Spaß am Sex / Lust auf Sexualverkehr)					
8. Verminderte Knochendichte (Osteopenie)					
9. Depressive Verstimmung					
10. Schlafstörung					
11. Müdigkeit					
12. Empfindungsstörungen (Parästhesien)					
13. Schwindel					
14. Aggressivität					

15. Angst					
16. Visuelle Halluzinationen (Wahrnehmungen)					
17. Spielsucht					
18. Übermäßiges Einkaufen					
19. Essanfälle					
20. Ausreißen der Haare (Trichotillomanie)					
21. Andere Suchterkrankungen (ggf. bitte eintragen:_____)					
22. Vermehrter Alkoholkonsum					
23. Verminderter Alkoholkonsum					
24. Andere psychiatrische Erkrankungen / Symptome (ggf. bitte eintragen:_____)					
Nur für Frauen					
Unregelmäßige Periodenblutungen					
Nur für Männer					
1.Erektile Dysfunktion /					

Impotenz					
2.Nachlassender Bartwuchs					

*** Bitte Angabe der kg _____ zwischen (Jahr): _____ und (Jahr):_____**

2) Wenn Sie diese medikamentöse Therapie *(Lisurid)* erhielten, ist unter der Therapie eines der folgenden Symptome neu aufgetreten oder hat sich verändert?

(Bitte folgende Seite nur ausfüllen, falls Sie diese Therapie erhalten haben)

Lisurid (Handelsname: Dopergin) O ja O nein

Beginn: _____

Höchste Dosis: _____

Dauer: _____

Aktuell: O ja O nein

- Therapie senkt/senkte Prolaktin erfolgreich O ja O nein O teilweise

Prolaktinwert vor _____und nach Therapie: _____

- Therapie führte zu einer Tumorschrumpfung O ja O nein O teilweise

Tumordurchmesser vor _____und nach Therapie: _____

Bitte kreuzen Sie Zutreffendes an (ggf. mit Beginn). Bitte schätzen Sie zusätzlich den Grad der Intension des Symptoms auf einer Skala von 1-5 ein:

Intensitätsskala:

1 = überhaupt nicht vorhanden/ausgeprägt

2 = wenig ausgeprägt

3 = mittelgradig ausgeprägt

4 = stark ausgeprägt

5 = sehr stark ausgeprägt

Symptome	**Neu**	**Verstärkt**	**Vermindert**	**Unverändert**	**Skala 1-5**
Beispiel	**Nein**	**Ja**			**2**
1. Kopfschmerzen					
2. Milchausfluss (Galaktorrhoe)					
3. Schmerzen der Brüste (Mastodynie)					
4. Gewichtsabnahme*					
5. Gewichtszunahme*					
6. Reduzierte Libido(= wenig Spaß am Sex, kaum Lust auf Sexualverkehr)					
7. Deutlich gesteigerte Libido (= gesteigerter Spaß am Sex / Lust auf Sexualverkehr)					
8. Verminderte					

Knochendichte (Osteopenie)					
9. Depressive Verstimmung					
10. Schlafstörung					
11. Müdigkeit					
12. Empfindungsstörungen (Parästhesien)					
13. Schwindel					
14. Aggressivität					
15. Angst					
16. Visuelle Halluzinationen (Wahrnehmungen)					
17. Spielsucht					
18. Übermäßiges Einkaufen					
19. Essanfälle					
20. Ausreißen der Haare (Trichotillomanie)					
21. Andere Suchterkrankungen (ggf. bitte eintragen:_____)					
22. Vermehrter Alkoholkonsum					
23. Verminderter Alkoholkonsum					
24. Andere psychiatrische Erkrankungen / Symptome (ggf. bitte					

eintragen:_____)					
Nur für Frauen					
Unregelmäßige Periodenblutungen					
Nur für Männer					
1.Erektile Dysfunktion / Impotenz					
2.Nachlassender Bartwuchs					

*** Bitte Angabe der kg _____ zwischen (Jahr): _____ und (Jahr):_____**

3) Wenn Sie diese medikamentöse Therapie *(Cabergolin)* erhielten, ist unter der Therapie eines der folgenden Symptome neu aufgetreten oder hat sich verändert?

(Bitte folgende Seite nur ausfüllen, falls Sie diese Therapie erhalten haben)

Cabergolin (Handelsnamen: Dostinex – Cabaseril) O ja O nein

Beginn: _____

Höchste Dosis: _____

Dauer: _____

Aktuell: O ja O nein

- Therapie senkt/senkte Prolaktin erfolgreich O ja O nein O teilweise

Prolaktinwert vor _____und nach Therapie: _____

- Therapie führte zu einer Tumorschrumpfung O ja O nein O teilweise

Tumordurchmesser vor _____und nach Therapie: _____

Bitte kreuzen Sie Zutreffendes an (ggf. mit Beginn). Bitte schätzen Sie zusätzlich den Grad der Intension des Symptoms auf einer Skala von 1-5 ein:

Intensitätsskala:

1 = überhaupt nicht vorhanden/ausgeprägt

2 = wenig ausgeprägt

3 = mittelgradig ausgeprägt

4 = stark ausgeprägt

5 = sehr stark ausgeprägt

Symptome	**Neu**	**Verstärkt**	**Vermindert**	**Unverändert**	**Skala 1-5**
Beispiel	**Nein**	**Ja**			**2**
1. Kopfschmerzen					
2. Milchausfluss (Galaktorrhoe)					
3. Schmerzen der Brüste (Mastodynie)					
4. Gewichtsabnahme*					
5. Gewichtszunahme*					

6. Reduzierte Libido(= wenig Spaß am Sex, kaum Lust auf Sexualverkehr)					
7. Deutlich gesteigerte Libido (= gesteigerter Spaß am Sex / Lust auf Sexualverkehr)					
8. Verminderte Knochendichte (Osteopenie)					
9. Depressive Verstimmung					
10. Schlafstörung					
11. Müdigkeit					
12. Empfindungsstörungen (Parästhesien)					
13. Schwindel					
14. Aggressivität					
15. Angst					
16. Visuelle Halluzinationen (Wahrnehmungen)					
17. Spielsucht					
18. Übermäßiges Einkaufen					
19. Essanfälle					
20. Ausreißen der Haare (Trichotillomanie)					
21. Andere Suchterkrankungen (ggf. bitte					

eintragen:_____)					
22. Vermehrter Alkoholkonsum					
23. Verminderter Alkoholkonsum					
24. Andere psychiatrische Erkrankungen / Symptome (ggf. bitte eintragen:_____)					
Nur für Frauen					
Unregelmäßige Periodenblutungen					
Nur für Männer					
1.Erektile Dysfunktion / Impotenz					
2.Nachlassender Bartwuchs					

*** Bitte Angabe der kg _____ zwischen (Jahr): _____ und (Jahr):_____**

4) Wenn Sie diese medikamentöse Therapie *(Quinagolide)* erhielten, ist unter der Therapie eines der folgenden Symptome neu aufgetreten oder hat sich verändert?

(Bitte Seite nur ausfüllen, falls Sie diese Therapie erhalten haben)

Quinagolide (Handelsname: Norprolac) O ja O nein

Beginn: _____

Höchste Dosis: _____

Dauer: _____

Aktuell: O ja O nein

- Therapie senkt/senkte Prolaktin erfolgreich O ja O nein O teilweise

Prolaktinwert vor _____und nach Therapie: _____

- Therapie führte zu einer Tumorschrumpfung O ja O nein O teilweise

Tumordurchmesser vor _____und nach Therapie: _____

Bitte kreuzen Sie Zutreffendes an (ggf. mit Beginn). Bitte schätzen Sie zusätzlich den Grad der Intension des Symptoms auf einer Skala von 1-5 ein:

Intensitätsskala:

1 = überhaupt nicht vorhanden/ausgeprägt

2 = wenig ausgeprägt

3 = mittelgradig ausgeprägt

4 = stark ausgeprägt

5 = sehr stark ausgeprägt

Symptome	**Neu**	**Verstärkt**	**Vermindert**	**Unverändert**	**Skala 1-5**
Beispiel	**Nein**	**Ja**			**2**
1. Kopfschmerzen					
2. Milchausfluss (Galaktorrhoe)					
3. Schmerzen der Brüste (Mastodynie)					
4. Gewichtsabnahme*					
5. Gewichtszunahme*					
6. Reduzierte Libido(= wenig Spaß am Sex, kaum Lust auf Sexualverkehr)					
7. Deutlich gesteigerte Libido (= gesteigerter Spaß am Sex / Lust auf Sexualverkehr)					
8. Verminderte Knochendichte (Osteopenie)					
9. Depressive Verstimmung					
10. Schlafstörung					
11. Müdigkeit					
12. Empfindungsstörungen (Parästhesien)					
13. Schwindel					
14. Aggressivität					

15. Angst					
16. Visuelle Halluzinationen (Wahrnehmungen)					
17. Spielsucht					
18. Übermäßiges Einkaufen					
19. Essanfälle					
20. Ausreißen der Haare (Trichotillomanie)					
21. Andere Suchterkrankungen (ggf. bitte eintragen:_____)					
22. Vermehrter Alkoholkonsum					
23. Verminderter Alkoholkonsum					
24. Andere psychiatrische Erkrankungen / Symptome (ggf. bitte eintragen:_____)					
Nur für Frauen					
Unregelmäßige Periodenblutungen					
Nur für Männer					
1.Erektile Dysfunktion /					

Impotenz					
2.Nachlassender Bartwuchs					

*** Bitte Angabe der kg _____ zwischen (Jahr): _____ und (Jahr):_____**

5) Wenn Sie eine andere medikamentöse Therapie erhielten, ist unter der Therapie eine der folgenden Symptome neu aufgetreten oder hat sich verändert?

(Bitte Seite nur ausfüllen, falls Sie diese Therapie erhalten haben)

Andere medikamentöse Therapie des Prolaktinoms O ja O nein

(Handelsnamen: _____)

Beginn: _____

Höchste Dosis: _____

Dauer: _____

Aktuell: O ja O nein

- Therapie senkt/senkte Prolaktin erfolgreich O ja O nein O teilweise

Prolaktinwert vor _____und nach Therapie: _____

- Therapie führte zu einer Tumorschrumpfung O ja O nein O teilweise

Tumordurchmesser vor _____und nach Therapie: _____

Bitte kreuzen Sie Zutreffendes an (ggf. mit Beginn). Bitte schätzen Sie zusätzlich den Grad der Intension des Symptoms auf einer Skala von 1-5 ein:

Intensitätsskala:

1 = überhaupt nicht vorhanden/ausgeprägt

2 = wenig ausgeprägt

3 = mittelgradig ausgeprägt

4 = stark ausgeprägt

5 = sehr stark ausgeprägt

Symptome	**Neu**	**Verstärkt**	**Vermindert**	**Unverändert**	**Skala 1-5**
Beispiel	**Nein**	**Ja**			**2**
1. Kopfschmerzen					
2. Milchausfluss (Galaktorrhoe)					
3. Schmerzen der Brüste (Mastodynie)					
4. Gewichtsabnahme*					
5. Gewichtszunahme*					
6. Reduzierte Libido(= wenig Spaß am Sex, kaum Lust auf Sexualverkehr)					
7. Deutlich gesteigerte Libido (= gesteigerter Spaß am Sex / Lust auf Sexualverkehr)					
8. Verminderte					

Knochendichte (Osteopenie)					
9. Depressive Verstimmung					
10. Schlafstörung					
11. Müdigkeit					
12. Empfindungsstörungen (Parästhesien)					
13. Schwindel					
14. Aggressivität					
15. Angst					
16. Visuelle Halluzinationen (Wahrnehmungen)					
17. Spielsucht					
18. Übermäßiges Einkaufen					
19. Essanfälle					
20. Ausreißen der Haare (Trichotillomanie)					
21. Andere Suchterkrankungen (ggf. bitte eintragen:_____)					
22. Vermehrter Alkoholkonsum					
23. Verminderter Alkoholkonsum					
24. Andere psychiatrische Erkrankungen / Symptome (ggf. bitte					

eintragen:_____)					
Nur für Frauen					
Unregelmäßige Periodenblutungen					
Nur für Männer					
1.Erektile Dysfunktion / Impotenz					
2.Nachlassender Bartwuchs					

*** Bitte Angabe der kg _____ zwischen (Jahr): _____ und (Jahr):_____**

Noch einige allgemeine Angabe:

<u>Nur für Frauen</u>

- **Sind Sie derzeit in den Wechseljahren?** O ja O nein
- **Wenn Sie das Klimakterium bereits hinter sich haben, wann war Ihre letzte Regelblutung? (Menopause) : mit _____ Jahren**
- **Wann war Ihre erste Regelblutung? (Menarche) : mit _____ Jahren**
- **Gibt/ gab es Besonderheiten der Regel?** O ja O nein
- **Haben Sie eigene Kinder?** O ja O nein **Wie viele? ____**
- **Aborte _____. wann _____(Jahr)**

Nur für Männer

- **Haben Sie eigene Kinder?** O ja O nein Wie viele? ____

Nehmen oder nahmen Sie jemals regelmäßig andere Medikamente ein?

Liste aller regelmäßig eingenommenen Medikamente (unabhängig von Prolaktinombehandlung) mit Zeitraum und Diagnose

(Für Frauen und Männer)

1.) Beispiel: L-Thyroxin (2004-2008) wg. Schilddrüsenunterfunktion

2.) _____

3.) _____

4.) _____

5.) _____

6.) _____

7.) _____

Krankheitsanamnese

(Für Frauen und Männer)

Leiden oder litten Sie an folgenden Erkrankungen?

1) **Kardiovaskuläre Erkrankungen (Herzkreislauferkrankungen)**

1a) Arrhythmien (Herzrhythmusstörungen) O Ja O Nein

Dauer: _____

Derzeit: O Ja O Nein

Therapie: O Ja O Nein

Therapie: _____

1b) Kardiomyopathie (Herzrmuskelerkrankung) O Ja O Nein

Dauer: _____

Derzeit: O Ja O Nein

Therapie: O Ja O Nein

Therapie: _____

1c) Cerebrovaskuläre Erkrankungen (Erkrankungen der Hirngefäße)

O Ja O Nein

Dauer: _____

Derzeit: O Ja O Nein

Therapie: O Ja O Nein

Therapie: _____

1d) Hypertonus (Bluthochdruck) O Ja O Nein

Dauer: _____

Derzeit: O Ja O Nein

Therapie: O Ja O Nein

Therapie: _____

1e) KHK (Koronare Herzerkrankung) O Ja O Nein

Dauer: _____

Derzeit: O Ja O Nein

Therapie: O Ja O Nein

Therapie: _____

1f) Myokardinfarkt (Herzinfarkt) O Ja O Nein

Dauer: _____

Derzeit: O Ja O Nein

Therapie: O Ja O Nein

Therapie: _____

2) Erkrankungen des Bewegungsapparats

2a) Arthralgie (Gelenkschmerzen) O Ja O Nein

Dauer: _____

Derzeit: O Ja O Nein

Therapie: O Ja O Nein

Therapie: _____

2b) Arthropathie (Gelenkerkrankungen) O Ja O Nein

Dauer: _____

Derzeit: O Ja O Nein

Therapie: O Ja O Nein

Therapie: _____

2c) Karpaltunnelssyndrom Arthropathie (Gelenkerkrankungen)

O Ja O Nein

Dauer: _____

Derzeit: O Ja O Nein

Therapie: O Ja O Nein

Therapie: _____

3) Metabolische Erkrankung

3a) Diabetes mellitus (Zuckerkrankheit) O Ja O Nein

Dauer: _____

Derzeit: O Ja O Nein

Therapie: O Ja O Nein

Therapie: _____

3b) Hypophyseninsuffizienz O Ja O Nein

Insuffizienz der:

-corticotrope Achse (Substitution mit Hydrocortison) O Ja O Nein

Dauer: _____

Derzeit: O Ja O Nein

Therapie: O Ja O Nein

Therapie: _____

-thyreotrope Achse (Substitution mit Schilddrüsenhormon) O Ja O Nein

Dauer: _____

Derzeit: O Ja O Nein

Therapie: O Ja O Nein

Therapie: _____

-gonadotrope Achse (Substitution mit Sexualhormonen) O Ja O Nein

Dauer: _____

Derzeit: O Ja O Nein

Therapie: O Ja O Nein

Therapie: _____

-somatotrope Achse (Substitution mit Wachstumshormon) O Ja O Nein

Dauer: _____

Derzeit: O Ja O Nein

Therapie: O Ja O Nein

Therapie: _____

4) Respiratorische Erkrankungen (Erkrankungen der Atemwege)

4a) Schlafapnoesyndrom O Ja O Nein

Dauer: _____

Derzeit: O Ja O Nein

Therapie: O Ja O Nein

Therapie: _____

4b) Andere Lungenerkrankungen O Ja O Nein

Dauer: _____

Derzeit: O Ja O Nein

Therapie: O Ja O Nein

Therapie: _____

5) Krebserkrankungen

Krebserkrankungen O Ja O Nein

Wenn ja, welche:_____

Dauer: _____

Derzeit: O Ja O Nein

Therapie: O Ja O Nein

Therapie: _____

Spezifikationen: _____

6) Psychiatrische Erkrankungen O Ja O Nein

Wenn ja, welche:_____

Dauer: _____

Derzeit: O Ja O Nein

Therapie: O Ja O Nein

Therapie: _____

Spezifikationen: _____

7) Sonstige Erkrankungen O Ja O Nein

Wenn ja, welche:_____

Dauer: _____

Derzeit: O Ja O Nein

Therapie: O Ja O Nein

Therapie: _____

Spezifikationen: _____

_____

_____

Familienanamnese

(Für Frauen und Männer)

- **Gibt es hormonelle Erkrankungen in Ihrer Familie? (z. B Hypophysenadenome):**

_____

_____

_____

- **Gibt es psychiatrische Erkrankungen in Ihrer Familie? (z.B. Depression, Psychosen, Suchterkrankungen):**

_____

_____

_____

Zur Person

(Für Frauen und Männer)

- **Sind Sie derzeit:**

  O berufstätig O arbeitslos O sonstiges _____

  O Rentner/Rentnerin O Hausfrau

- **Ihr Familienstand ist:** O ledig O verheiratet O geschieden/verwitwet
- **Wie viele Jahre sind Sie zu Schule gegangen?** _____ Jahre
- **Rauchen Sie zur Zeit täglich Zigaretten oder haben Sie früher täglich Zigaretten geraucht?**

  O Nein, ich habe noch nie Zigaretten geraucht

  O Ja, ich rauche seit ____ Jahren täglich etwa ____ Zigaretten

  O Ja, ich habe früher täglich geraucht. Insgesamt ____ Jahren und ca. ____ Zigaretten pro Tag.

- **Wie oft trinken Sie Alkoholika (Bier, Wein, Likör, Spirituosen)?**

  O täglich oder fast täglich (5-7 mal pro Woche)

  O gelegentlich (3-4 mal pro Woche)

O selten (1-2 mal pro Woche)

O sehr selten (1-2 mal pro Monat)

12.2 DNA preparation and genotyping

Primer F	rs1045642MP_F	GCTGAGAACATTGCCTATGGA
Primer R	rs1045642MP_R	CTCTTCACTTCTGGGAAACC
Sonde sensor	rs1045642MP_FL	GATCGTGAGGGCAGCAAAGGAG-Fluorescein
Sonde anchor	rs1045642MP_LC	LC Red 610-CAACATACATGCCTTCATCGAGTCACTGCC-Phosphate
Primer F	rs2032582MP_F	TGTTACTCTTAGCAATTGTACCCATC
Primer R	rs2032582MP_R	AAACACATTCTTAGAGCATAGTAAGC
Sonde sensor	rs2032582MP_FL	CCCAGCACCTTCTAGTTCTTTCTTATCTTTCAGTG-Fluorescein
Sonde anchor	rs2032582MP_LC	LC Red 640-TGTCCAGACAACATTTTCATTTCAACAACTCCTGC-Phosphate
Primer F	rs2235015MP_F	ACACAATTAAAACTGAGTCAGTTCG
Primer R	rs2235015MP_R	ACAATAGTAAGGAGAATGTCTAATTACCTG
Sonde sensor	rs2235015MP_LC	LC Red 610-AACAAACATACCATTTATGTCTCTTTAGTCTCCAT-Phosphate
Sonde anchor	rs2235015MP_FL	AACCCTGTATCATTGATATCACCTAGACCACCAC-Fluorescein
Primer F	rs2032583MP_F	AGAAAGAACTAGAAGGTTCTGGG
Primer R	rs2032583MP_R	TTGAGTCCAAGAACTGGCT
Sonde sensor	rs2032583MP_FL	AGTAGAGTAAAGTATTCCAATCAGTGTTATTTTGT-Fluorescein
Sonde anchor	rs2032583MP_LC	LC Red 640-CTCCCTACTGCTTACTATGCTCTAAGAATGTGTT-Phosphate

Light Cycler 480 Multiplex: Genotyping Master

Number of probes	**1**

Mix:

Component	Concentration Stock	Capillary concentration	µl / Reaction	µl for probes
Water			4,00	4,40
MgCl2	25000	0,0	0,00	0,00
Primer forward A	10	0,5	0,50	0,55
Primer reverse A	10	0,5	0,50	0,55
Primer forward B	10	0,5	0,50	0,55
Primer reverse B	10	0,5	0,50	0,55
Fluos HybProbe A	4	0,2	0,50	0,55
Light Cycler Red 610 HybProbeA	4	0,2	0,50	0,55
Fluos HybProbe B	4	0,2	0,50	0,55
Light Cycler Red 640 HybProbeB	4	0,2	0,50	0,55
Enzyme Mix (Genotyping Master)	5	1	2,00	2,20
		Total:	10,00	

µl Mix	9
µl cDNA	1

LightCycler® 480 Genotyping Master

Ready-to-use hot start reaction mix for real-time PCR and probe melting-curve based genotyping using the LightCycler® 480 Instrument.

Artikelnr.: 04707524001 → 4 x 384 µl 5x conc. approx. 384 reactions of 20 µl reaction volume each.

Application:

The LightCycler® 480 Genotyping Master is designed to easily perform real-time PCR followed by genotyping via melting curves on the LightCycler® 480 Instrument. It can be used to genotype single nucleotide

polymorphisms (SNPs) and to perform mutation analysis, and is especially recommended for multiplex approaches. The LightCycler® 480 Genotyping Master can be used for the amplification and detection of every DNA or cDNA target. Only template DNA, primers, and suitable sequence-specific probes (HybProbe probes or SimpleProbe probes) have to be added.

Product description:

The LightCycler® 480 Genotyping Master is a hot start reaction mix for PCR. The supplied enzyme contains a 5'-3'-exo-minus, N-terminal deletion of thermostable recombinant Taq DNA polymerase that is inactive at room temperature due to a chemical modification, and becomes activated during a 10-minute incubation at 95°C. HybProbe probes or SimpleProbe probes are used as detection format during PCR and subsequent melting curve analysis.

Since the mix is provided as a one-component, easy-to-use reagent, reaction setup only requires the addition of template DNA and primers. The mix can be used with different types of DNA (*e.g.*, genomic, cDNA), and is ideally suited for high-throughput applications in 96- or 384-well plates.

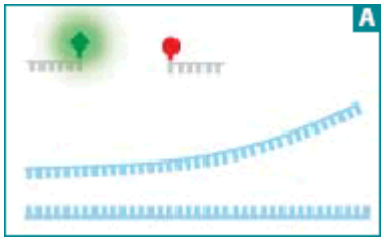
Hybridisation Probes (Hybprobes): Monitor PCR with the LightCycler® HybProbe Format

The unique LightCycler® HybProbe format is based on fluorescence resonance energy transfer (FRET). Two sequence-specific oligonucleotide probes are labeled with different dyes (donor and acceptor), and are added to the reaction mix along with the PCR primers. During the annealing phase, HybProbe probes hybridize to the target sequences on the amplified DNA fragment in a head-to-tail arrangement, thereby bringing the two dyes close to each other. The donor dye (fluorescein) is excited by the blue LED. As long as the two dyes are close to each other (within 15 nucleotides), the energy emitted by the donor dye excites the acceptor dye on the second HybProbe probe, which then emits fluorescent light at a different wavelength. This fluorescence is directly proportional to the amount of target DNA generated during PCR. HybProbe probes are displaced during the elongation and denaturation steps.

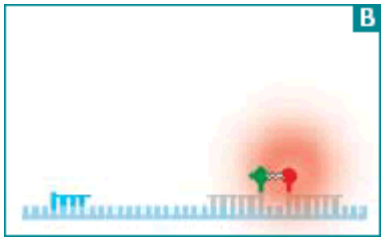
Advantages of the HybProbe Format

- Only the presence of a specific amplification product causes an increase in fluorescence
- Increased specificity, because two sequence-specific probes hybridize to the target
- Primer-dimers do not interfere, because the sequence-specific probes do not recognize them

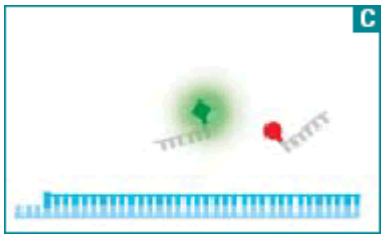
- Probe sequences are not altered by PCR, so they can still be used in a subsequent assay, *e.g.,* for mutation detection or SNP analysis



A The donor-dye probe is labelled with fluorescein at the 3´ end and the acceptor-dye probe is labelled with LightCycler® Red at the 5´ end. Hybridization does not take place during the denaturation phase of PCR and, thus, the distance between the dyes is too large to allow energy transfer to occur.



B During the annealing phase, the probes hybridize to the amplified DNA fragment in a close head-to-tail arrangement. When fluorescein is excited by the light from the LED, it emits green fluorescent light, transferring the energy to LightCycler® Red, which then emits red fluorescent light. This red fluorescence is measured at the end of each annealing step, when the fluorescence intensity is highest.



C After annealing, the temperature is raised and the HybProbe probe is displaced during elongation. At the end of this step, the PCR product is double-stranded and the displaced HybProbe probes are again too far apart to allow FRET to occur.

DNA Purification Protocol for 2ml Saliva Samples

Cell Lysis

1. Incubate Oragene•DNA/Saliva samples at 50ºC in a water incubator for a minimum of 1 hour or in an air incubator for a minimum of 2 hours.

2. Transfer 4 ml lysate sample (2 ml saliva plus 2 ml Oragene•DNA-preserving solution) to a 15 or 50 ml centrifuge tube.

3. Add 1 ml Cell Lysis Solution and 25 µl Gentra RNase A Solution (4 mg/ml). Vortex on high speed for 10 seconds to mix sample and incubate 10 minutes at room temperature.

Protein Precipitation

4. Add 1.67 ml Protein Precipitation Solution to the cell lysate.

5. Vortex vigorously at high speed for 20 seconds to mix the Protein Precipitation Solution uniformly with the cell lysate.

6. Centrifuge at 2,000 x g for 5 minutes. The precipitated proteins will form a tight dark brown pellet. If the protein pellet is not tight, repeat Step 4 followed by incubation on ice for 5 minutes and then repeat Step 5.

DNA Precipitation

7. Pour the supernatant containing the DNA (leaving behind the precipitated protein pellet) into a 15 or 50 ml tube containing 5 ml 100% Isopropanol (2-propanol) and 40 µl of Gentra Glycogen Solution (20 mg/ml).

8. Mix the sample by inverting gently 50 times.

9. Centrifuge at 2,000 x g for 3 minutes; the DNA will be visible as a small white pellet.

10. Pour off supernatant and drain tube briefly on clean absorbent paper. Add 5 ml 70% Ethanol and invert tube several times to wash the DNA pellet.

11. Centrifuge at 2,000 x g for 1 minute. Carefully pour off the ethanol. Pellet may be loose so pour slowly and watch pellet.

12. Invert and drain the tube on clean absorbent paper and allow sample to air dry 5-10 minutes.

DNA Hydration

13. Add 400 µl DNA Hydration Solution (400 µl will give a concentration of 200 µg/ml if the total yield is 80 µg DNA).

14. Rehydrate DNA by incubating at 65°C for 1 hour and overnight at room temperature. If possible, tap tube periodically to aid in dispersing the DNA.

15. For storage, sample may be centrifuged briefly and then transferred to a 1.5 ml tube. Store DNA at 4°C. For long-term storage, store at -20°C or -80°C.