Polymorphisms as Tool for Individual Tailor Customed Estrogen and Testosterone Replacement - Therapy

Kişiye Özgü Uyarlanmış Östrojen Replasman Tedavisi İçin Polimorfizmler Bir Araç Olabilir

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ABSTRACT This presentation displays the new and approach to an endocrinological treatment strategy, which should be 1. individual, 2. custom attracted and 3. secure. However, today we are able to answer four main questions:

- **Cardiovascular diseases**: Who will get the most profit by using hormone replacement therapy?
- **Thrombosis** : Which patient will be reckoned to the risk of clotting and CV- complications .
- **High plasma- levels of estradiol**: How can we avoid supraphysiological hormone plasma levels, which have been recognized to a high risk for breast cancer?
- **Neurological age related diseases as Alzheimer, Parkinson**

Increased burdening of the organism by supraphysiological doses of 17-ß Estradiol increases the proliferation pressure of the mammary glands, increases the radiological density of the mammogram and at least increases the lifetime risk of breast cancer.
Therefore it seems necessary to maintain low Estrogen levels at the systemic level or by the parakrine way.
The breast cancer risk depends on the cumulative risk of lifetime estrogen exposition.
17-β Estradiol effects directly on the genome, bound by estrogen receptor complex. This has impact on the effects of supraphysiological increased Estradiol levels and the cell metabolism, cell division and transcription of the DNA.
Since we know, that the difference between individuals is based on genetic mutations and polymorphism, the HRT managing physician should use
1. personal history dates (weight gain in pregnancy, endometriosis, breast cysts, ovarian cysts),
2. clinical profile, hormone plasma tests (high Estrone, Estradiol levels)
3. images producing methods like bone density scan (high bone density, mammogram (high tissue density) and ultrasound (cysts,
4. high endometrium)and the
5. **polymorphism diagnostics** to get an overview over the estrogen history and the future's risk of HRT of the patient.

Key Words: Hormone replacement therapy, estrogen, polymorphisms

ÖZET Bu bildiri, 1. kişiye uyarlanmış, 2. alım kolaylığı ve 3. güvenilir olması gereken yeni bir endokrinolojik tedavi stratejisi yaklaşımı sunmaktadır. Ancak günümüzde ancak şu 4 ana soruya cevap verebiliyoruz:

- **Kardiyovasküler hastalık:** Hormon replasman tedavisinden en fazla faydayı görecek olanlar kimlerdir?
- **Tromboz:** Hangi hastaların kardiyovasküler hastalık komplikasyonları ve pıhtılaşma riski ile karşı karşıya oldukları hesaplanmalıdır.
- **Östrodiolün yüksek plazma düzeyleri:** Göğüs kanseri için yüksek risk oluşturan suprafizyolojik hormon plazma düzeylerinden nasıl sakınılacaktır?
- **Alzheimer, Parkinson gibi yaşla ilgili nörolojik hastalıklar.**

17-β östrodiolün suprafizyolojik dozları nedeniyle kişide sürekli artan yükleme yapmanın meme bezlerinde proliferasyonu artırıp basınç oluşturduğu üstelik, mamografide radyolojik yoğunluk artışına ve meme kanseri riski artışına yol açtığı bilinmektedir. Bu nedenle, östrojen düzeylerini sistemik seviyede ya da parakrin yolla salınmasını sürdürmek en iyi yöntem olacaktır.
Göğüs kanseri riski, hayat boyu östrojen maruziyetinin kümülatif riskine bağlıdır.
17-β östrodiol, östrojen reseptör kompleksi tarafından bağlanarak doğrudan genomu etkiler. Bu, suprafizyolojik yüksek östrodiol düzeylerinin etkilerinin yanısıra hücre metabolizması, hücre bölünmesi ve DNA transkripsiyonu üzerinde etkilere sahiptir.
Genetik mutasyonlara ve polimorfizme bağlı olarak, bireyler arasındaki farklılıkları öğreninceye kadar, HRT tedavisi veren hekim şunları yapmalıdır:
1. kişisel öykü tarihçesi (gebelikte alınan kilolar, endometriozis, meme kistleri, over kistleri),
2. klinik profil, hormon plazma testleri (yüksek östron, östrodiol düzeyleri),
3. kemik tarama testi, mamografi ve ultrason gibi görüntülü yöntemler,
4. kistler ve yüksek yoğunluktaki dokular, kemik ve endometrium,
5. hastanın HRT'nin olası riskleri ve östrojen öyküsü konusunda genel değerlendirme.

Anahtar Kelimeler: Hormon replasman tedavi, östrojen, polimorfizmler

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This presentation displays the new and approach to an endocrinological treatment strategy, which should be 1. individual, 2. custom attracted and 3. secure. The great advances in medicine in the last years can realize this idea for the near future. Women ask more and more after a custom attracted and individualized HRT. We can observe a change of paradigm in nearly all medical subject areas. The standard of an individualized therapy for our patients requires more and more comprehensive epidemiological investigations, meta analyses and genetically analysis, which is especially supported by the detection of the human genome.

Today we are able to answer four main questions:

- **Cardiovascular diseases**: Who will get the most profit by using hormone replacement therapy?
- **Thrombosis** : Which patient will be reckoned to the risk of clotting and CV- complications .
- **High plasma- levels of estradiol**: How can we avoid supraphysiological hormone plasma levels, which have been recognized to a high risk for breast cancer?
- **Neurological age related diseases as Alzheimer, Parkinson**

The very interesting overview by M. Clemons,and P. Goss published in 2001 in the New England Journal of Medicine[1] and their presented studies substantiate the assumption that the continued increased burdening of the organism by supraphysiological doses of 17-ß Estradiol increases the proliferation pressure of the mammary glands, increases the radiological density of the mammogram and at least increases the lifetime risk of breast cancer.

Therefore it seems necessary to maintain low Estrogen levels at the systemic level or by the parakrine way. This efforts to maintain the estrogen concentrations in the sex – steroid dependent tissues as low as possible is a demand on the evidence based medicine.

The breast cancer risk depends on the cumulative risk of lifetime estrogen exposition.[2-4]

The demand after estrogen replacement bases on the experience, that estrogens have a positive influence on a lot of age – related health – risk developments, especially on the support of bone metabolism, and the improvement of the lipid profile, which can affect the CV – risk.

17 – ß Estradiol effects directly on the genome, bound by estrogen receptor complex. This has impact on the effects of supraphysiological increased Estradiol levels and the cell metabolism, cell division and transcription of the DNA. Therefore should the practised HRT of administering female sex steroids without knowledge of the of the production and breakdown of Estrogens and other sex steroids hormones, especially the genetically dependent influence of the steroid metabolism belong to the past.

Since we know, that the difference between individuals is based on genetic mutations and polymorphism, the HRT managing physician should use

1. personal history dates (weight gain in pregnancy, endometriosis, breast cysts, ovarian cysts),

2. clinical profile, hormone plasma tests (high Estrone, Estradiol levels)

3. images producing methods like bone density scan (high bone density, mammogram (high tissue density) and ultrasound (cysts),

4. high endometrium) and the

5. **polymorphism diagnostics**

to get an overview over the estrogen history and the future's risk of HRT of the patient.

This new possibilities of the molecular genetic diagnostics, especially the gene chip techniques allow us to weigh up the risk of HRT.

So we can estimate the profit of a HRT for men women to reduce the CV- risk. As we know, weight reduction, exercises and no smoking improve the CV- risk. We also know, that the possibility of CV- diseases increases dramatically after menopause, with a 10 years latency compared with men, while the fertile phase of women apparently displays a protection against those diseases.

To know three very important polymorphisms is a necessary condition before starting HRT:

TESTOSTERONE THERAPY, BENEFITS AND RISKS-PROSTATE CANCER-RISK

Hormones as all medicaments and environmental toxins are converted and eliminated by enzymes. The genetically code of them can give us an information about the enzymiatical activity, which can caus accumulation or fast elimination.To undersatnd your results you should know, that each gene consits of two alleles, one from mother, one from father. Depending if the mutation is found in both alleles (homocygoty) or only in one of

them (heterocygoty) is important for the phenotypically expression and at least for your personal risk.

Knowing polymorphisms of the genes of steroid metabolising enzymes gives us possibilities to estimate the risk of not familiar-dependent breast and prostate cancer.

The combination of personal history, Mammogram (high density), ultrasound (ovarian cysts, high build up Endometrium) and bone density (increased bone density leads to the suspicion of elevated lifelong estradiol levels.

TESTOSTERONE

The physiologic aspects of aging are presented in the myth of Tithonus, the lover of Aurora, goddess of dawn. Aurora loved Tithonus so much that she asked her father, Zeus, to grant him eternal life. Unfortunately she forgot to request eternal youth for her lover, who began to experience the failure of his libido at approximately age 50 years and at age 60 to 70 years was somewhat impotent. By the age of 80 years, Tithonus had lost much of his muscle strength, and by the time he turned 90, he walked around stooped, because his bone was disappearing and he had some kyphosis.

By the time he reached 100 years, he had developed some age-related cognitive dysfunction, which was shown in the myth by the fact that he babbled incessantly.

Many of the changes cited in this myth are associated with declining testosterone production. They include age-related disturbances in memory, muscle mass, and strength. Clearly, loss of libido and impotence are testosterone effects, and osteopenia may be another. There is evidence to suggest that disturbances in balance and declines in maximal oxygen uptake capacity (VO2max) also relate to declines in testosterone levels, although these effects have been understudied. Changes in food intake may also be effects of testosterone loss. Male sexual function declines with age. It bis now clear that the decrease in T levels as a function of age has both a testicular and hypothalamo-pituitary origin, but Leydig cell function decreases not always together with increase of pituitary hormone LH. However, in elderly men, the LH levels are frequently not increased or only modestly increased, a consequence of the alteration of neuroendocrine controle of gonadal function. Moreover the circadian rhytmicity of LH and T secretion is blunted in elderly men and the amplitude of LH pulses decrease. The effects of androgens in men and women are very similar and different. (interest in sexuality, sebum production, hair growth and lost, Epo- production, improvement of lipid profile in men, but not in women. Being a source for Estradiol stimulating bone cells, effecting muscle and fat mass.. Controlling CYP 19 P450 gene (Aromatase) has a great impact of the prevention of supraphysiological Estradiol levels..

Whereas it has long been debated whether plasma testosterone (T) concentration decreases with age in healthy men, the occurrence of an age- associated decrease in bioactive testosterone concentration is no longer disputed. Normal plasma T levels range vary between 11 and 40 nMol/L and reach their maximum at 25 to 30 years.. Testosterone circulates in plasma bound for about 50% to Sex Hormone Binding Globulin (SHBG), a ß-globulin with high affinity but limited binding capacity for T and is bound for about 50% to Albumin (low affinity, high binding capacity). In young healthy males, the plasma FT concentration varies between 0,2 and 0,7 nMol/L. Due to the high binding affinity of SHBG, only the free and part of the albumin bound T is bio available. The significance of SHBG bound testosterone is poorly understood. It has been shown, that some tissues (prostatic cells) carry SHBG receptors, the activation which leads to stimulation of cyclic AMP.

Today scientists are looking for hormonal substances that will rejuvenate human beings and allow them to live longer. Can this be done with testosterone? Probably not, but the full range of its potential may be under appreciated. Twenty years of clinical experience and current research findings provide a convincing argument that testosterone replacement has a role to play in improving the quality of life in older men. Testosterone deficiency in men is manifested typically by symptoms of hypogonadism, including decreases in erectile function and libido. 25% of men over 65 have subnormal T levels. Testosterone also has an important role in the regulation of normal growth, bone metabolism and body composition. Specifically, testosterone deficiency is an important risk factor for osteoporosis and fractures in men. In men older than 65 years of age, the incidence of hip fracture is 4-5/1000 and approximately 30% of all hip fractures occur in men. Men with testosterone deficiency have significant decreases in bone density, particularly in the trabecular bone compartment. Testosterone deficiency has been reported in over half of elderly men with a history of hip fracture. Men with testosterone deficiency also have alterations in body

composition that includes an increase in body fat. Using quantitative CT scans to assess fat distribution, we have shown that testosterone deficiency is associated with an alteration in site-specific adipose deposition with increased deposits in all areas particularly in the subcutaneous and muscle areas. Because truncal fat correlates with glucose intolerance and cardiovascular risk, hypogonadism may have important implications with regard to overall health and mortality. In one study, the alteration in skeletal muscle composition was associated with a decrease in muscle strength. Therefore, testosterone deficiency is associated with an enhanced risk for osteoporosis, altered body composition including increases in truncal fat, and, possibly, decreases in muscle performance.

Administration of adequate testosterone replacement therapy leads to improvements in libido and erectile function. Following testosterone replacement, men note an increase in energy and mood, which may reflect either direct behavioral effects of androgens, and/or, an elevation of hematocrit due to rising testosterone levels. Testosterone therapy also leads to important beneficial effects on the skeleton and lean tissue mass. Testosterone replacement increases bone density in hypogonadal men with the most dramatic effects seen in the trabecular bone compartment. These effects may be seen as early as 6 months following initiation of testosterone therapy. In one recent study of the long term benefits of testosterone therapy, the greatest benefits in trabecular bone were seen in the first several years of therapy. With regard to body composition, testosterone replacement therapy results in a dramatic reduction in adipose content, with the greatest effects seen in the subcutaneous and skeletal muscle areas. Androgen therapy leads to a significant increase in lean skeletal muscle mass and strength. Therefore, there are beneficial effects of testosterone replacement on body composition and bone mineral density in adult hypogonadal men that may serve as indications for therapy in addition to libido and sexual function.

CARDIOVASCULAR RISK

While environmental factors such as diet, physical activity, and alcohol intake play an important role in determining triglyceride levels, results from family studies have all suggested a strong genetic component to triglyceride levels. Although family members tend to share a similar environment, studies on twins reared together have shown that triglyceride levels are highly influenced by genetic variability. However, considerable inter-individual variation is observed in the response of LDL and other lipoproteins to dietary change. The hypothesis that genetic differences contribute to this variability has led to several studies in which associations of dietary lipoprotein responses have been sought with polymorphisms in genes affecting lipoprotein metabolism.[5]

There is increasing awareness of the potential for genetic variation among individuals to influence nutrient requirements and biological responses to nutrient intake. In the case of genes influencing LDL subclass patterns, gene-diet interactions contribute to wide inter-individual differences in the effects of low fat, high carbohydrate diets on risk for coronary heart disease. The degree of the inter-individual differences may be magnified in certain genetic sub-populations. Some of these sub-populations will likely be ethnic minorities. The recognition of such differences in metabolic response is prompting greater appreciation of the concept of intelligent nutrition based upon knowledge of nutritional status, nutritional requirements, and genotype

PROSTATE CANCER-RISK AND SEE BELOW: COMMUNE RISKS MEN AND WOMEN

Hormones as all medicaments and environmental toxins are converted and eliminated by enzymes. The genetically code of them can give us an information about the enzymatic activity, which can cause accumulation or fast elimination. To understand your results you should know, that each gene consists of two alleles, one from mother, one from father. Depending if the mutation is found in both alleles (homocygoty) or only in one of them (heterocygoty) is important for the phenotypically expression and at least for your personal risk.

Knowing polymorphisms of the genes of steroid metabolising enzymes gives us possibilities to estimate the risk of not familiar – dependent breast cancer.

The combination of personal history, Mammogram (high density), ultrasound (ovarian cysts, high build up Endometrium) and bone density (increased bone density leads to the suspicion of elevated lifelong estradiol levels.

The demand for estrogen and testosterone replacement bases on the experience, that estrogens, as well as androgens have a positive influence on a lot of age – related health – risk developments, especially on the support of bone metabolism, and the improvement of the lipid profile, Diabetes profile and have antiinflammatory

and antioxidative effects, which can positively affect the CV – risk, the brain- aging, the joints and ligaments.

The breast cancer risk depends on the cumulative risk of lifetime estrogen exposition.[2-4]

CYTOCHROME P450 17 ALPHA HYDROXYLASE (CYP17)

CYP 17 decodes a key enzyme of the streroid metabolism, the 17 alpha Hydroxylase.. This enzymes act converting Pregnenolon and DHEA to Androstenedione.. Polymorphism in this gene causes increased androgen levels in the tissue and in so far an increased risk for prostate and breast cancer.

The CYP 17 T-34-C mutation in the promotorregion of this gene, correlates with increased androgen levels, and diminished need for androgens in men´s HRT.

Men with this mutation are recommended to control their dose of DHEA, and to keep their testosterone dose rather low, perhaps taking it on alternating days. They should undergo regularly PSA measurements,prostate sonography and urological exam on a regulary time interval.

Men with this mutation displax higher risk of prostate cancer and should keep their tesosterone dose in HRT low. They should undergo regulary urological exam of the prostate and measure their PSA.

STEROID METABOLISM AND PROSTATE CANCER

Cytochrome P450 17 A1	*CYP17*	T>C Pos. -34		

Cytochrome P450 17 A1 (CYP17) mediates androgen biosynthesis via the conversion of pregnenolone to dehydroepiandrosterone (DHEA) or via the conversion of progesterone to androstenedione (Figure 1). A single base-pair change in the 5'-untranslated region (5'-UTR; T>C. Pos.-34) of the CYP17 gene has been associated with increased gene expression resulting in slightly altered hormone levels. The CYP17 polymorphism has been associated with an increased risk of prostate cancer, but its distinct role has been discussed controversially in literature until now.A homozygous genotype of the variant allele (C/C) is present in ~17% of the population. This genotype may alter circulating hormone levels to a moderate extent. A recent meta-analysis[6] suggests that the CYP17 polymorphism is unlikely to increase the risk of sporadic prostate cancer, especially within subjects of European descent. Within African C/C carriers, a moderately increased risk of prostate cancer (OR=1.56) has been reported, although date were limited. Furthermore, there is some evidence, that the CYP17 polymorphism is associated with an increased risk of prostate enlargement.[7] Because of the possible role of the CYP17 C/C polymorphism in increased DHEA production, a long-term and/or high dose DHEA supplementation should be regarded critically

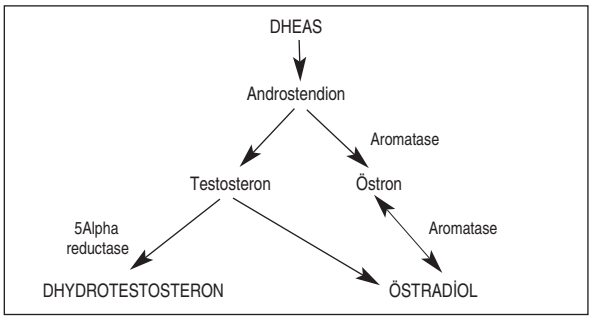

FIGURE 1: Conversion probabilities of steroids.

For men we recommend :

Vitamin E 400 IU

Selen 100 mcg

Lycopene 30 mg

Vit D3 50 IU

Indol 3 carbinol

Soy

Women display higher androgen levels, if they dont have an aromatse polymorphisms. In the case of aromatse snp (CYP 19) DHEA will be converted very fast to androstendione and to estrone. This decreases the need for estrogens in postmenopausal women, but incerases the breast cancer risk. A protection of breast cancer are high androgen levels. Women with a CYP 17 mutation and a joint SRD 5 Alpha mutation convert androstenedion very fast to Dihydro Testosterone (DHT), This would be a protection, especially if the Androgen receptor has a high androgen sensitivity, following the CAG AR-polymorphism (lost of CAG repeats).[8]

P450 CYTOCHROME 19 GENE, AROMATASE

Of great importance is the knowledge of the activity of the aromatase, which is coded by the CYP 19 gene.High activity of this enzyme leads to a faster conversion rate from testosterone to estradiol and from androstenedione to estrone, which at least increase the estrogen tissue- and plasma levels.We know several polymorphisms of

this gene, which all are of great practical importance. The C 1558 T Mutation doubles the risk of breast cancer in women (OR 2.0,95%KI 1,3 – 3,1), another CYP 19 Mutation decreases significantly the risk to get breast cancer over lifetime.(OR o,39, 95% KI- 0,17- 0,89) In men, this snp causes higher E2 levels and the risk of benign prostate hyperplasic growth, gynaecomasty and higher thrombosis risks, but improve the osteoporosis risk. As Alcohol is a potent stimulator of the aromatase, it should be avoided in both genders.

SRD 5A2 GEN POLYMORPHİSM OF THE 5 ALPHA STEROİD REDUCTASE

The Enzyme 5 Alpha reductase type II catalyses the formation of Dihydrotestosteron from Testosteron.. This enzyme consist of 2 variants (Arg49Thr and Val89Leu), which distinguish in their activity. On a genetical level the Arg 49Thr variable enzymes codes for increased activity.. Men display a higher risk for prostatic cancer, women decresae their risk for breast cancer because the amount of newly formated estardiol from tesosterone by aromatase is diminished, and furthermore the Testosterone, DHT -AR complex inhibits DNA replication and transcription. The contrary is the Val89Leu mutation which decreases SRD5A2 activity and is considered to be protective for the prostate.

Steroid 5-alpha-reductase type II	*SRD5A2*	Ala>Thr Codon 49		

Steroid 5-alpha-reductase exists in the 2 isoforms SRD5A1 and SRD5A2, and catalyses the conversion of testosterone into its biologically active form, dihydrotestosterone (DHT). Two polymorphisms within the steroid 5-alpha-reductase type 2 (SRD5A2) gene, Ala>Thr Codon 49 and Val>Leu Codon 89 affect the activity of the SRD5A2 enzyme: the Ala>Thr Codon 49 polymorphism increases the activity of SRD5A2, whereas the Val>Leu Codon 89 polymorphism decreases the activity of SRD5A2. SRD5A2 is the predominant isozyme detectable in the prostate, including benign prostatic hyperplasia and prostate adenocarcinoma tissues. Both polymorphisms have been associated with a predisposition to prostate cancer. In contrast to the isoenzyme SRD5A1, SRD5A2 is relatively sensitive to the enzyme inhibitor finasteride.A homozygous genotype for the variant allele (Thr/Thr) is present in ˜0.3 % of the population and been associated with increased enzyme activity and increased production of DHT. A recent meta-analysis ruled out a >50% increased risk of prostate cancer within Thr-carriers.[9] A small study showed, that Thr-carriers had larger prostates and higher PSA levels,[10] but this effect should be regarded critically and deserves further studies.

The SRD5A2 enzyme is a target of pharmacological treatment of benign prostatic hyperplasia or prostate cancer using specific inhibitors such as finasteride, but it should be noXted that a definite correlation between the response of an SRD5A2 inhibitor therapy and the SRD5A2 Ala>Thr polymorphism has not been studied until now.

Steroid 5-alpha-reductase type II	*SRD5A2*	Val>Leu Codon 89		

The Val>Leu Codon 89 polymorphism decreases the activity of SRD5A2 and has been associated with decreased levels of dihydrotestosterone (DHT). A homozygous genotype for the variant allele (Leu/Leu) is present in ˜10% of the population and is associated with a decreased enzyme activity leading to lower levels of DHT. The homozygous Leu-genotype has been shown to exert a protective effect with respect to prostate cancer. It has been suggested that carriers of the Leu/Leu genotype are on average at a 20% decreased risk of prostate cancer compared to carriers of the Val/Val genotype.[11]

Androgen receptor	*AR*	(CAG)n long>short alleles		

The androgen receptor (AR), alternatively known as the dihydrotestosterone receptor (DHTR), is involved in the transcriptional regulation of hormone-responsive genes. The length of a 3-basepair repeat (CAG) within the AR gene is associated with the transcriptional activity of AR. Shorter CAG repeat lengths (<22) are associated with higher transcriptional activity of AR and furthermore with an increased expression of AR mRNA and protein (Figure 2). Because prostate carcinogenesis is dependent on androgens, men with shorter repeat lengths may be at higher risk for prostate cancer.

A long, >or= 22 (CAG)(n) repeat size have been associated with a reduced risk of prostate cancer [2% decrease in risk of prostate cancer for each additional (CAG) triplet, (12)].A short, < 22 (CAG)(n) repeat is at higher risk for prostate cancer. A recent meta-analysis[12] calculated a 2% increase in risk of prostate cancer for each fewer CAG triplet (OR=1.02). Otherwise, men with short CAG repeats have the highest sperm output within the normal fertile population and the risk of defective spermatogenesis is halved compared to patients with very long (>28) CAG repeats.[13]

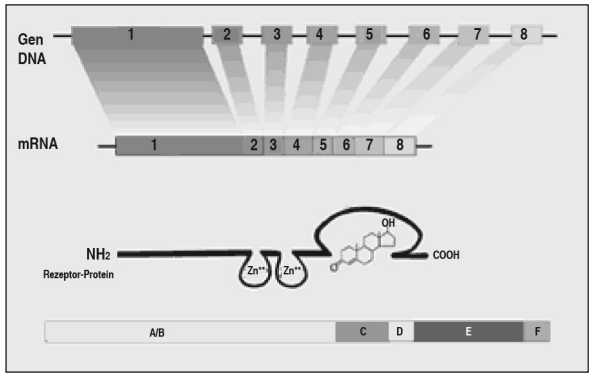

FIGURE 2: Scheme of the androgen receptor (AR) and the mRNA of this gene. The AR gene includes 8 exons of different size.The AR protein = endproduct includes more than 800 amino acids. Regarding its function we have domains A-F.

ElaC homologue 2 / hereditary prostate cancer gene 2	***ELAC2 / HPC2***	**Ala>Thr Codon 541**		

The uncharacterized gene ELAC2 has been proposed to be a prostate cancer susceptibility gene. Two polymorphisms within the ELAC2 gene (Ser>Leu Codon 217, Ala>Thr Codon 541) have been reported to segregate with the disease in multiple-case families and have been associated with a modestly increased risk of prostate cancer.[14] Therefore, some investigators proposed that ELAC2 should be renamed 'hereditary prostate cancer gene 2' (HPC2). Most studies confirm a certain role for the Ala>Thr Codon 541 polymorphism in prostate cancer susceptibility, but not for the Ser>Leu Codon 217 polymorphism.[15,16]

TA heterozygous genotype for the variant allele (Ala/Ser) is present in ~8% of the population. The detected polymorphism has been associated with a modestly increased risk of prostate cancer. While a meta-analysis by Camp and Tavtigian[15] calculated an odds ratio of ~2.4 for Thr541 carriers, another more comprehensive meta-analysis by Severi et al.[16] revealed a moderate effect for the Thr541-allele on prostate cancer risk (OR~1.2). A homozygous genotype for one of the variant (Ala/Ser is present in ~0.1% of the population. The detected polymorphism has been associated with a modestly increased risk of prostate cancer. While a meta-analysis by Camp and Tavtigian[15] calculated an odds ratio of ~2.4 for Thr541 carriers, another more comprehensive meta-analysis by Severi et al.[16] revealed a more moderate effect for the Thr541-allele on prostate cancer risk (OR~1.2).

INFLAMMATION

Interleukin 6	*IL-6*	G>C Pos. -174

Interleukin 6 (IL-6) is a multifunctional cytokine and is involved in both the amplification of and the protection against the inflammation in response to infection and tissue injury. A polymorphism within the promoter region (G>C Pos. –174) has been suggested to modulate IL6-plama levels. Furthermore, IL-6 gene expression is regulated by other cytokines, transcription factors, and several hormones, e.g. estradiol.

No variant allele (G/G) is present in ~38% of the population and is associated with higher IL6-levels,[17] which have been described to be the major predictor of disability and mortality in the elderly. The IL-6 G/G genotype has been found to be underrepresented in centenarians and, therefore, appears to be disadvantageous for longevity. This phenomenon has been observed only among males.[18] The variant allele in form of the heterozygous genotype (G/C) is present in ~49% of the population. C-allele carriers have lower IL-6 serum levels in comparison with homozygous G/G carriers. This genotype has been reported to be advantageous for successful aging.[18]

Interleukin 10	*IL-10*	G>A Pos. -1082		

The cytokine interleukin 10 (IL-10) physiologically limits and down-regulates inflammation. Age related diseases are initiated or worsened by systemic inflammation; conversely, genetic variations determining increased production of anti-inflammatory cytokines have been shown to be associated with successful aging: A polymorphism within the promoter region has been shown to regulate IL-10 levels. An adenine (A) at the site –1082 in the promoter region of the IL-10 gene is associated with low and guanine (G) with high production of IL-10.

No variant allele (G/G. is associated with high IL-10 production. Within male carriers, the G/G genotype has been shown to occur at a higher frequency in centenarians than in younger people.[19] Indeed, IL-10 proved to have several protective features acting against atherosclerotic disease.[20] A homozygous genotype for the variant allele (A/A) is associated with low IL-10 production and is less common within male centenarian carriers.

DETOXIFICATION

Glutathione S-transferase pi	**GSTP1**	**Ile>Val Codon 105**		

Glutathione S-transferase pi (GSTP) plays an important role in detoxification by catalyzing the conjugation of many hydrophobic and electrophilic compounds with glutathione. A polymorphism within the GSTP1-gene (Ile>Val Codon 105) has been shown to have functional effects on the GSTP1 gene product resulting in reduced enzyme activity and to modulate the predisposition to certain cancers, especially to lung cancer within risk groups such as smokers or nonsmokers who are exposed to environmental tobacco smoke. A homozygous genotype for the variant allele (Val/Val) is present in ~8% of the population. This genotype has been associated with an increased risk of lung cancer, especially within individuals exposed to tobacco smoke. A large study reported, that the GSTP1 Val/Val genotype doubles the lung cancer risk among smokers (26 pack-years), compared with the Ile/Ile genotype.[21] This effect increases in combination with the GSTM1 null genotype, which is present in approximately in 50% of the population. Individuals carrying the GSTM1 null genotype and the homozygous GSTP1 Val105 allele were reported to carry a nearly 7-fold increased

Glutathione S-transferase M1	GSTM1	Available > 0 allele		

Glutathione S-transferase M1 (GSTM1) is capable of detoxifying reactive electrophiles which can act as mutagens. GSTM1 is deleted in about half of Caucasians, but the role of the so called GSTM1 null genotype in carcinogenesis has been discussed controversially in the literature and it can be estimated that GSTM1 status has no effect on the risk of lung cancer per se. However, the GSTM1 null genotype can increase the carcinogenic effect of other genetic risk markers like the GSTP1 Val105 polymorphism.If the GSTM1 gene is homozygous deleted (not detectable; GSTM1 null genotype) and although the GSTM1 null genotype does not influence the susceptibility for lung cancer per se, it increases the carcinogenic effect of the GSTP1 Val105 polymorphism: Smokers of the GSTP1 Ile/Val codon 105 heterozygous genotype in combination with the GSTM1 null genotype display an increase in DNA-adduct levels, leading to an increased lung cancer risk.[22] Individuals carrying the GSTM1 null genotype and the homozygous GSTP1 Val105 allele have been reported to carry a nearly nearly 7-fold increased risk of developing lung cancer.[23]

POLYMORPHISMS: RISK EVALUATION BREAST AND PROSTATE

Breast Cancer Risk-General

Transforming growth factor beta receptor type 1	*TGFBR1*	*9A > *6AG>C Pos. -174

Transforming growth factor beta (TGF-ß) is one of the most potent inhibitors of cell growth and acts via transforming growth factor beta receptor (TGFBR). A deletion of three GCG triplets (*6A) within the type 1 TGFBR gene (TGFBR1) results *in vitro* in a less effective response to TGF-ß growth inhibitory signals compared to the 'wild type' TGFBR1 gene (*9A) and therefore, TGFBR1*6A might act as a tumor susceptibility allele. Two recent meta-analyses of 7 and 12 case-control studies showed that TGFBR1*6A carriers have an increased risk of hematological malignancies, breast, ovarian, and colon cancer. Thus, the TGFBR1*6A polymorphism is a useful general marker for various malignancies, among them also gynecologic tumors such as breast and ovarian cancer. Knowledge of the carrier status of TGFBR1*6A might be clinically useful for targeted cancer prevention strategies. The (*9A/*9A); genotype is present in ~83% of the population(Wildtype).Concerning this polymorphism, carriers of the *9A/*9A genotype are not at an increased risk of breast and ovarian cancer.

The *6A/*9A genotype is associated with an increased risk of breast cancer (OR=1.45) and ovarian cancer (OR=1.53).[24] Carriers also have a 20% increased risk of colon cancer (OR=1.2).[25] A homozygous genotype for the variant allele has been detected (*6A/*6A); this genotype is present in ~1% of the population. The *6A/*6A genotype is associated with an increased risk of breast cancer (at least OR=1.45) and ovarian cancer (OR=3.35).[24] Carriers also have a 20% increased risk of colon cancer (OR=2.02).[25]

Progesterone Receptor	*PGR*	Pos. +331 G>A

The Progesterone receptor (PR) mediates the physiologic effects of progesterone. It exists in two isoforms, PR-A and PR-B. PR-A opposes estrogen or progesterone induced cell proliferation, whereas PR-B contributes to estrogen or progesterone induced cell proliferation. A polymorphism (Pos. 331 G>A) within the promoter of the progesterone receptor gene (PGR) increases the expression of the PR-B isoform and is associated with endometrial cancer and breast cancer. This increased risk has been documented in a prospectively studied cohort within the Nurses' Health

Study.[26,27] The G/G genotype is present in ~90% of the population Concerning this polymorphism, carriers of the G/G genotype are not at an increased risk of endometrial and breast cancer.Carriers of the heterozygous genotype are at an increased risk of endometrial cancer (OR=1.9) and breast cancer (OR=1.4). The risk increases for overweight women (BMI>28kg/m^2) resulting in an OR of 4.7 for endometrial cancer and in an OR of 2.3 for breast cancer. Although HRT is another risk factor for endometrial cancer and breast cancer, no interaction between PGR genotype and HRT has been observed.[26,27] Because too little data for the homozygous genotype are available, the same OD ratios as for the heterozygous genotype apply, although the risk may be in fact higher.

Androgen Receptor	*AR*	short > long alleles

The androgen receptor (AR) is involved in the regulation of hormone-responsive genes. The length of a 3-basepair repeat (CAG) within the AR gene affects the androgen : estrogen balance, mediates trans-activation of cell-cycle regulating genes, and plays a role in breast cancer susceptibility.

If two short (< 22 repeats) alleles or a cumulative (CAG)(n) repeat size of <43 have been detected, we know that shorter alleles [CAG (<22) repeat] of the androgen receptor (AR) are associated with a decreased risk of breast cancer. At least one long allele (>or= 22) has been detected. This genotype of the androgen receptor (AR) is associated with an increased risk of breast cancer. Women with a cumulative (CAG)(n) repeat size of >or= 43 show a modestly increased risk of breast cancer (OR=1.3).[28] The risk increases especially among women with a first-degree family history of breast cancer (OR=1.7). This increased risk has been documented in a prospectively studied cohort within the Nurses' Health Study.[29] If two long alleles >or= 22 have been detected, it is well known, that this genotype of the androgen receptor (AR) is associated with an increased risk of breast cancer. Women with a cumulative (CAG)(n) repeat size of >or= 43 show a modestly increased risk of breast cancer (OR=1.3).[28] The risk increases especially among women with a first-degree family history of breast cancer (OR=1.7). This increased risk has been documented in a prospectively studied cohort within the Nurses' Health Study.[29]

Vitamin D Receptor	VDR	IVS7 +283 G>A (b > B)

The Vitamin D Receptor (VDR) is expressed in breast tissue and moderates the rates of local cell division. A polymorphism (IVS7 +283 G>A / b > B) within the VDR gene has been associated with an increased susceptibility to breast cancer. Furthermore, the VDR polymorphism is associated with lower bone mineral density (BMD) and with a predisposition for osteoporosis.

If no variant allele has been detected (b/b)it is known that Carriers of the b/b-genotype have an increased risk of breast cancer (OD=1.9 for b/b versus B/B); (30) and this genotype is present in ~35% of the population. The b-allele is the most common allele (60%) and is therefore regarded as 'wild type'-allele.). Increases in vitamin D and calcium intakes are associated with decreases in breast densities, suggesting that dietary vitamin D and calcium can increase the detectability of breast cancer possibly through influences on breast tissue morphology.[31] If the variant allele has been detected in form of the heterozygous genotype (b/B),this genotype is present in ~50% of the population and carriers of the b-allele seem to have a moderately increased riskof breast cancer compared to B/B carriers.[30] Because the heterozygous genotype is the most frequent genotype, it should not be regarded as a general risk marker for breast cancer. However, increases in vitamin D and calcium intakes are associated with decreases in breast densities, suggesting that dietary vitamin D and calcium can increase the detectability of breast cancer risk possibly through influences on breast tissue morphology. If there has been detected a homozygous genotype for the variant allele (B/B),this genotype is present in ~15% of the population and carriers of the B/B-genotype have a decreased risk of breast cancer.[30]

Cytochrome P450 17 A1	*CYP17A1*	T>C Pos. -34		

Cytochrome P450 17 A1 (CYP17) mediates androgen biosynthesis via the conversion of pregnenolone to dehydroepiandrosterone (DHEA) and via the conversion of progesterone to androstenedione. A single base-pair change in the 5'-untranslated region (5'-UTR; T>C. Pos.-34) of the CYP17A1 gene has been associated with an increased gene expression resulting in slightly altered hormone levels. The CYP17 polymorphism has been shown to predict use of hormone replacement therapy[32] and is regarded to be a weak modifier of breast cancer risk.[33] The(T/T genotype is present in ~34 % of the population. Regarding breast cancer, the protective effect of a later age at menarche (>/= 13) is limited to women with this genotype.[33] Interestingly, this genotype has been more often observed among women who were current users of HRT, indicating that T/T-women more

often have climacteric symptoms compared to non 'wild type' carriers.[32] If a homozygous genotype of the variant allele has been detected (C/C), this genotype is present in ~17% of the population and alters circulating hormone levels to a moderate extent and is regarded to be a weak modifier of breast cancer risk, but is not a significant independent risk factor.[33] Interestingly, carriers of this genotype have been shown to be about half as likely as carriers of the 'wild-type' genotype T/T to be current HRT users (OR=0.52). It has been speculated that women with the C/C genotype have fewer indications for HRT due to higher levels of endogenous hormones.[32]

Aromatase	*CYP19A1*	C>T Pos. +1558		

The final stage of estrogen synthesis is catalysed by the aromatase enzyme, encoded by the gene *CYP19A1*, which converts the androgens androstenedione and testosterone into the estrogens estrone and estradiol, respectively. A common polymorphism in the CYP19A1gene, 1558 C>T, is associated with an increased enzyme activity leading to increased levels of estrone and estradiol and increased ratios of estrone : androstenedione and estradiol : testosterone. This polymorphism is in strong linkage disequilibrium with anotherpolymorphism, a short tandem repeat [TTTA]n in intron 4, with comparable effects on the estrogen metabolism. C/C homozygous carriers have slightly reduced levels of estrone and estradiol compared to C/T- or T/T carriers.[34] Although circulating levels of estrogens are directly related to the risk of breast cancer, the 1558 C>T polymorphism has given inconclusive results in breast cancer case-control studies, probably due to the relatively minor effect on elevated estrogen levels. Thus, no increased risk of breast cancer can be expected among C/C carriers. On the other hand, lower levels of estrogens are associated with a higher risk of developing osteoporosis or CVD during menopause. Therefore, a HRT may be of special interest.

A heterozygous or homozygous genotype for the variant genotype is present in~50% of the population. C/T heterozygous carriers have slightly elevated levels of estrone (2%) and estradiol (4%) compared to C/C carriers.[34] Although circulating levels of estrogens are directly related to the risk of breast cancer, the 1558 C>T polymorphism has given inconclusive results in breast cancer case-control studies, probably due to the relatively minor effect on elevated estrogen levels, especially within heterozygous carriers, which should therefore be regarded as 'wild-type' risk carriers.

A homozygous genotype for the variant allele is present in ~28% of the population. T/T homozygous carriers have elevated levels of estrone (10%) and estradiol (13%) compared to C/C carriers.[34] Although circulating levels of estrogens are directly related to the risk of breast cancer, the 1558 C>T polymorphism has given inconclusive results in breast cancer case-control studies, probably due to the relatively minor effect on elevated estrogen levels. Therefore, the 1558 C>T polymorphism has to be considered together with other genetic risk and environmental factors leading to increased levels of estrogens. Alcohol intake promotes aromatization of androgens to estrogens. On the other hand, anti-aromatase chemicals in red wine have been detected, which overcomes the inductive effect of alcohol in wine.[35] A high BMI correlates with an increased risk of breast cancer, and carriers of the T-allele may selectively profit from a reduction in body fat by exercise.[36] Individuals with high levels of estrogens may also profit from a diet rich in cruciferous vegetables (e.g., cabbage and broccoli), which contains indole-3-carbinol (I3C). I3C and its major in vivo product diindolylmethane (DIM) have been reported to modify cytochrome P450 activities involved in the estrogen catabolism, favoring the formation of harmless 2OH estrogen-metabolites.[37] A long term, high dose HRT should be regarded critically.

Estradiol Receptor Alpha Polymorphism

In this case you display an increased sensitivity for estrogens. This causes leiomyoma, Endometriosis and a decreased need for estrogens. There is an incerase of the berast cancer risk for women and may be a higher risk for Cv diseases in men. Also the fat distribution is E2 alpha R dependent. As this receptor is distributed body wide there are many different reactions, e.g. on the gum and bone.

Estrogen Receptor alpha 1	**ESR1**	**IVS1 -401 T>C (p > P)**		

Estrogens have important effects on bone mass and bone remodeling and exert their effects primarily via the estrogen receptor 1 (ESR1), a ligand-activated transcription factor. A common polymorphism within the ESR1 gene (IVS1 -401 T>C / p > P) is associated with bone mineral density (BMD) and fracture risk. This polymorphism also shows a gender specific influence on the occurrence of cardiovascular disease.If a homozygous genotype for the variant allele has been detected (P/P), the P/P genotype is the less common genotype (~20%) and is therefore regarded as variance, although it is associated with higher bone mineral density and an decreased frac-

ture risk compared to carriers of the p-allele.[38] Furthermore, the P/P genotype represents the more hormonesensitive genotype, therefore P/P carriers profit more from the protective effect of HRT than women with the p-allele.[39] Postmenopausal women with this genotype are at a decreased risk of myocardial infarction (MI) and ischemic heart disease (IHD), than carriers with the p-allele.[40] Additionally, P/P carriers have an augmented response (2-fold) of HDL cholesterol to HRT.[41] Therefore, a HRT may be of special interest.

THE BREAK DOWN AND ELIMINATING OF ESTROGENS:

Phase I

CYP 1A1 AND 1B1 GENE POLYMORPHİSM

CYP 1A1

Cytochrome 1A1 mediates the elimination of Eestrogens by C2 hydroxylation and metabolizes environmetal toxins as well as polycyclic hydrocarbons (Figure 3). Polymorphisms in this gene can affect the breast tissue by increased C2- OH products. Two mutations in codon T6235C and 462 are associated with an incerased breast cancer risk. Smoker have an 5 fold risk because of the parallel effects on this enzyme, which eliminates carbon products by hydroxylation as well.

Populations in western countries, who smoke have a 9 fold risk! (OR 9,7;95%-CI 2,0-47,9)

An increased break down of estrogens by CYP 1A1 leads to a high CYP 2OH/CYP 16 OH ratio, a slowed break down to an incerase of C16 OH products, which have a high proliferative and cancerogenic effect on the tissue. You have 2 mutations: stop smoking, no estrogens and inhibit the activity by :increased alimentation of cabagge like broccoli, cauli flower, brussel sprouts, red and yellow garden vegetables, Inbdol-3 Carbinol capsels, flax seed, . It is important, in case of a CYP 1B1 muttaion, to measure the NAT2 and GST activities (2. step) + COMT and Sulftransferase. No smoking!

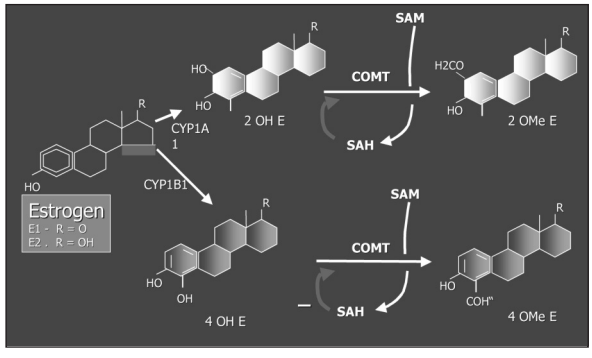

FIGURE 3: Removing Estrogens and Xenoestrogens by CYP 1A1 and CYP 1B1 (step I) and COMT (step II) Stimulation of COMT with folic acid and S-Adenyl –Methionin (methyl donator).

Cytochrome P-450 1 A1	*CYP1A1*	T>C Pos. +3801

Cytochrome P450 1A1 catalyzes the 2-hydroxylation of estrone (E1) and estradiol (E2) into the catecholamines 2-hydroxy estrone (2-OHE1) and 2-hydroxyestradiol (2-OHE2) (42). These 2-hydroxy metabolites show reduced estrogenic effects and behave more like anti-estrogens (in contrast to 4-OH and 16-OH metabolites).[43] Besides that, CYP1A1 also activates procarcinogens, like polycyclic aromatic hydrocarbons (PAH) or heterocyclic aromatic amines (HA), present in tobacco smoke or broiled meat, and play a distinct role in the development of certain cancers (e.g, lung and breast cancer).[44] A polymorphism within the CYP1A1-gene, T>C Pos. 3801, increases CYP1A1 enzyme activity. If no variant allele has been detected (T/T); The T/T genotype is present in ~81% of the population. In this case of Wildtype, the CYP1A1-enzyme shows normal activity. Enzyme activity in the liver may be increased by a diet rich in cruciferous vegetables (e.g., cabbage and broccoli), which contain indole-3-carbinol (I3C). I3C and its major in vivo product diindolylmethane (DIM) are stimulating CYP1A1 activity in the liver, favoring a high ratio of 2OH/16OH metabolites, which is regarded as a protective marker of breast cancer.[45]

If a homozygous or heterozygous genotype has been detected (T/C or CC)than this genotype is present in ~18% or in ~1% of the population. This genotype is associated with an increased enzyme activity. The positive effect of highly active CYP1A1, consisting in a favored formation of 2OH estrogen metabolites, is overcome by its activation of procarcinogens. The CYP1A1 enzyme is also present in the lung and therefore, exposure to tobacco smoke should be avoided. Smokers are showing higher levels of DNA adducts in breast tissue, putting them at an increased risk of breast cancer,[44] while - referring to the CYP1A1 polymorphisms - nonsmokers are not at an increased risk of developing breast cancer.[46] Furthermore, carriers of a polymorphic CYP1A1 allele respond to a lesser degree to an I3C supplementation. Therefore, the 2OH/16OH ratio should be monitored during I3C supplementation.[47]

CYP 1B1

Cytochrome P-450 1 B1	*CYP1B1*	Leu > Val Codon 432

Cytochrome P450 1B1 preferentially catalyzes the

4-hydroxylation of estradiol (E2) into catecholamine 4-hydroxy-estradiol (4-OHE2). 4-OHE2 metabolites may play a functional role in breast carcinogenesis by their high estrogenic potency stimulating estrogen receptor-mediated transcription and increasing local cell proliferation. Furthermore, 4-OHE2 can be metabolized to quinone derivatives, which interact with DNA leading to DNA adducts and DNA damage in breast tissue.[48] CYP1B1 is also involved in the metabolism of polycyclic aromatic hydrocarbons (PAH) or heterocyclic aromatic amines (HA), leading to the bioactivation of these procarcinogens. A polymorphism (Leu > Val Codon 432) within the CY1PB1 gene increases enzyme activity and may be involved in the development of certain cancers (e.g. breast cancer), especially among high risk groups (smokers, women on HRT).

CYP1B1 variants are showing a 2.4 – 3.4 fold higher catalytic efficiencies in expression studies,[49] leading to an increased production of 4-OHE2 metabolites. Nevertheless, *in vivo* data showed only marginal associations between CYP1B1 variants and serum hormone concentrations in pre- and postmenopausal women.[50,51] The CYP1B1 gene polymorphism does not affect the overall breast cancer risk but may modify the risk after long-term menopausal hormone use, compared with long-term users without this genotype (OD=2.0),[52] Therefore, a long term, high dose HRT should be regarded critically. Because of its role in the activation of procarcinogens such as PAHs, exposure to tobacco smoke should be avoided. Smokers with the Val codon 432 allele have been shown to have a higher risk of breast cancer compared to never smokers with the Leu/Leu genotype (OR=2.3).[53] The Val codon 432 allele also increases the susceptibility to breast cancer in women exposed to environmental pollutants like agricultural pesticides or exhaust gases.[54] Thus, affected individuals should avoid exposure.

A slow hydroxylation of estrogens by a CYP 1B1 Polymorphism causes a high 2/ 16 ratio, as CYP 1B1 hydroxylates estrogens in C 16, which leads to osteoporosis and estrogen deficiency over the sexual life span. Increased CYP 16 activity increases the 16 OH estrogens which are high proliferate and cancerogenous. CYP 1B1 eliminates all cyclic hydrocarbons,, Benzpyren, aflotoxins, dioxins, from paintings, gas, petrol, smoking, BBQ products, . Avoid the exposition to these toxins. Inhibit the activity by increased alimentation of cabbage like broccoli, cauliflower, Brussels sprouts, red and yellow garden vegetables, Inbdol-3 Carbinol capsules, flax seed, . It is workfolk, in case of a CYP 1B1 mutation, to measure the NAT2 and GST activities (2. step) + COMT and Sulfotransferase. A ratio from 1.5-3.0 is said to be fine for you. The higher the ratio, the higher the risk for osteoporosis, cardiovascular disease, depressive and menopausal syndromes.

The lower or inversed this ratio is, the higher the risk for breast cancer, uterus cancer, Lupus Erythematodes and chronic polyarthritis. Due to the toxic environmental industry products, tobacco smoke, BBQ products, charcoal products, petrochemical products, cycled hydrocarbons, Benzpyren, stilbene, aromates and estrogens from drinking water (excreted hormones of anti-baby pill) you have a very dangerous risk for the accumulation of this stuff in the fatty tissue depots and liver. Your eliminating activity is very low and slow. To avoid chemical induced cancer we urgently recommend avoiding these products, no hormone replacement therapy, stimulating the enzyme activity by nutrition: add to your normal diet cabbage as broccoli, cauliflower, green and yellow vegetables. Capsules with Indol-3-Carbinol, flaxseed, Omega 3 fatty acids.

Phase II

CATECHOL-ORTHO-METHYL-TRANSFERASE (COMT) AND CYP 1A1, CYP 1B1

The COMT is responsible for the breakdown of catechol- estrogens by hydroxylation, which are converted from Estradiol by P450 oxidases (Cytochrom P450 1A1 (CYP 1A1) and (P450 CYP 1B1). This intermediary produced catechol -estrogens display a high cancerogenous potency.

Polymorphisms of CYP 1A1 – gene leads to increased levels of estrogens and catechol – estrogens, which are themselves a great risk for the development of breast cancer.

Mutation of the COMT gene leads to a slower breakdown rate of catechol-estrogen so that this increased amount of cancer stimulating intermediary estrogen products is available in the breast cells.

Moreover this enzyme is involved in the break down of neurotransmitters as there are nor epinephrine and dopamine, causing different diseases and disorders like anxiety syndrome, panic attacks, obsessive disorders and schizophrenia. As it works estrogens dependent, the amount of this particular hormone in the brain is important due to the competition between E2, E1 and neurotransmitters.pQCT analyses showed that

COMT genotype was an independent predictor of trabecular vBMD of the tibia, radius, and fibula. Trabecular vBMD of the radius and fibula in COMT(LL) was 5.3% and 7.4% lower, respectively, than that of the combined COMT(HL/HH) group. COMT genotype was associated with cortical vBMD but not with cortical cross-sectional area in the tibia. These findings show that the COMT polymorphism is associated with BMD in young adult men

Catechol-O-methyltransferase	*COMT*	Val>Met Codon 158		

Catechol-O-methyltransferase (COMT) converts catechol estrogens (e.g. 4-hydroxy-estradiol, 4OH-E2) into inactive methoxy derivatives (e.g. 4-MeOE2). COMT is also involved in the degradation of other catecholamines, like neurotransmitters and exogenous catechol substances. A common polymorphism (Val>Met codon 158) within the COMT gene is associated with a decreased enzymatic activity and therefore a decelerated catabolism of catecholamines. A homozygous genotype of the variant (Met/Met) is present in ~25% of the population. It leads to a decreased enzyme activity and to increased levels of 4-hydroxy-estradiol, which are associated with an increased risk of breast cancer. However, until now the influence of the Met/Met genotype on the predisposition to breast cancer has been discussed controversially and should therefore *not* be regarded as a general risk marker of breast cancer. Otherwise, within carriers of this genotype with concomitantly low folate / high homocysteine levels, breast cancer cases can be more often observed.[55] Therefore, a dietary supplementation with folic acid can be of special interest. Interestingly, carriers of the Met-allele seem to profit form the protective effect of tea (green or black), due to the less rapid degradation of the catechol-containing tea polyphenols.[56] Data form the Nurses' Health Study demonstrate that the elevated risk of breast cancer among women with a history of regular alcohol use can be reduced by folate supplementation.[57]

HORMONE REPLACEMENT THERAPY RISKS AND BENEFITS

COLLAGEN TYPE I ALPHA 1

Encoded by the COL1A1 gene, is the major protein of the bone. A polymorphism within the COL1A1 gene (Pos. 1546 G>T / S > s) is involved in the regulation of collagen transcription and is regarded as a marker for the predisposition to osteoporosis and an increased fracture risk.

The (S/S) genotype (Wildtype) is present in ~64% of the population. With respect to this polymorphism, no increased risk of osteoporosis and fracture has been detected. Furthermore, S/S carriers especially benefit from a biphosphonate (e.g. etidronate) therapy by an increase in femoral neck (FN) bone mineral density (BMD).[58]

The variant allele in form of the heterozygous genotype (S/s), is present in ~33% of the population and carriers of the S/s genotype have an increased risk of osteoporosis. In a meta-analysis of 26 studies, bone mineral density (BMD) values at the lumbar spine (LS) and the femoral neck (FN) were shown to be significantly lower in the S/s genotype group compared to S/S homozygotes. Heterozygous carriers of this polymorphism have a significantly elevated risk of vertebral fractures (OR=1.26).[59] Furthermore, s-allele carriers will profit less from a biphosphonate (etidronate) therapy regarding femoral neck BMD values.[58] S/s carriers are at a significantly increased risk of osteoporotic fractures. However, MacDonald et al. found no significant difference in baseline BMD values at the lumbar spine (LS) or femoral neck (FN) between COL1A1 genotypes or in the rates of bone loss between COL1A1 genotypes in HRT users.[60] Therefore, a HRT may be of special interest for identifying women at increased risk of fracture despite normal baseline BMD values. If there is a homozygous genotype of the variant allele has been detected (s/s) and this genotype is present in ~3% of the population, than carriers of the s/s genotype show an increased risk of osteoporosis. Bone mineral density (BMD) values at the femoral neck (FN) were shown to be significantly lower in the s/s genotype group when compared with S/S homozygotes. Homozygous carriers of this polymorphism have a significantly elevated risk of vertebral fractures (OR=1.26).[61] Furthermore s-allele carriers will less profit from a biphosphonate (etidronate) therapy regarding femoral neck BMD values.[58] s/s carriers are at a significantly increased risk of osteoporotic fracture. However, MacDonald et al. found no significant difference in baseline BMD values at the lumbar spine (LS) or femoral neck (FN) between COL1A1 genotypes or in the rates of bone loss between COL1A1 genotypes in HRT users.[60] Therefore, a HRT may be of special interest for identifying women at increased risk of fracture despite normal

Apolipoprotein E	***APOE***	**Cys>Arg Codon 112**
Apolipoprotein E	***APOE***	**Arg>Cys Codon 158**

baseline BMD values.

Apolipoprotein E (apoE) is a protein component of several plasma lipoproteins, including triglyceride-rich chylomicrons and very-low-density lipoprotein (VLDL) particles. As a ligand for the low-density lipoprotein (LDL) receptor, apoE plays a major role in plasma lipoprotein metabolism. Two polymorhisms within the human *APOE* gene form 6 common genotypes, leading to 3 major isoforms of apoE: apoE2, -E3, and -E4. Carriers of the APOE4 allele have higher levels of total and LDL cholesterol and therefore have a higher risk of cardiovascular disease. Furthermore, the APOE4 allele has been related to dementias like Late Onset Alzheimer's disease. Interestingly, many effects of APOE-polymorphisms are gender specific.

Apolipoprotein A1	***APOA1***	**G>A Pos. -75 Promoter**		

The human apolipoprotein AI (apoAI) constitutes the major protein component of high-density lipoprotein (HDL, the so-called "good" cholesterol). Because apoAI plays an important role in the reverse transport of cholesterol, low ApoAI/HDL serum levels constitute a well-known risk factor of coronary artery disease (CAD). In the human *APOA1* gene, a relatively frequent promoter polymorphism modulates the expression of apoA-I. Important interactions, at least for women, between this polymorphism, dietary habits, and HDL levels are known. Carriers of the variant allele can increase their serum HDL in response to a dietary uptake of polyunsaturated fatty acids.[62,63]

Matrix metalloproteinase 3 (Stromelysin 1)	*MMP3*	5A>6A Pos.-1171 Promoter

Matrix metalloproteinases (MMPs) have important roles in vascular remodeling and in aging dependent stiffening of the arteries. MMP3, a member of the MMP family, has been associated with the dangerous, smooth and lipid-rich structures of arteriosclerotic plaques. A functional, repeat-length polymorphism within the promoter region of the human *MMP3* gene is known to fine-tune the enzymatic activity of MMP3. The 5A allele causes higher activity and has been associated with higher risk for myocardial infarction, while the 6A allele is known to cause lower activity and has been reported as risk marker for artery stenosis and for arteriosclerosis. In case of this polymorphism, experts in the field have suggested that the "optimal" genotype would be the heterozygous. A homozygous genotype for the variant allele 6A/6A genotype has been identified reported as risk marker for internal carotid artery stenosis and for rapid progression of arteriosclerosis. The risk of arteriosclerosis is not modulated by smoking habits. It further has been identified as weak genetic risk factor for myocardial infarction in Japanese women. If applicable, this variant indicates preventive reduction of conventional risk factors for cardiovascular disease.

Paraoxonase 1	*PON1*	Gln>Arg Codon 192

The antioxidant enzyme paraoxonase, an high-density lipoprotein bound serum enzyme, prevents low-density lipoprotein oxidation and is therefore believed to affect the risk of coronary artery disease. There is a common genetic variation in the paraoxonase 1 gene, *PON1*, which is associated with the severity of coronary artery disease. Due to a meta-analysis from 43 studies, the Arg192 variant The variant allele form of the homozygous genotype is weakly associated with coronary heart disease, the estimated per-allele excess risk is +12%. Female carriers of the 192Arg/Arg genotype have increased risk of more severe coronary heart disease. Because there is no robust evidence that this polymorphism is associated with coronary heart disease risk, the clinical interpretation must carefully evaluate other risk factors. Interestingly, there is a modest association between the 192Arg/Arg genotype and longevity in Italy and Irish populations.

Angiotensin converting enzyme	***ACE***	**Ins>Del Intron 16**

Angiotensin converting enzyme (ACE) plays an important role in blood pressure regulation and electrolyte balance by hydrolyzing angiotensin I into angiotensin II. Angiotensin II is a potent vasopressor, and aldosterone-stimulating peptide, maintaining cardiovascular homeostasis. The human *ACE* gene harbors an insertion (I) / deletion (D) polymorphism. The D/D genotype is associated with high plasma levels of ACE leading to increased angiotensin II concentrations. This is reflected in higher genetic predisposition for hypertension. This genotype is further regarded as weak genetic risk marker

for myocardial infarction. A homozygous genotype for the variant allele D/D has been associated with significantly increased systolic blood pressure and risk of hypertension in smokers. This genotype is also a weak genetic marker for ~1.1-fold increased risk of myocardial infarction. If applicable, this genotype would indicate to give up smoking and to set preventive measures against hypertension and cardiovascular disease. Accor-

Angiotensinogen	*AGT*	Met>Thr Codon 235

ding to a meta-analysis from 28 studies the D/D genotype is further associated with a ~2-fold higher risk of left ventricular hypertrophy in untreated hypertensive patients. If applicable, an (earlier) *pharmacological intervention in patients with hypertension should be evaluated.*

Angiotensinogen (AGT), the precursor of Angiotensin I, is another key player within the renin-angiotensin-system in cardiovascular homeostasis. Renin converts AGT to angiotensin I, which is further altered by angiotensin converting enzyme to form angiotensin II, a potent vasopressor. The human *AGT* gene harbors a structural variant, which alters Met235 to Thr. Because this variant allele has been associated with elevated plasma levels of AGT, this polymorphism is suspected to be a genetic risk marker in hypertension. A homozygous genotype for the variant allele,Thr/Thr235 genotype is associated with a moderate ~11% increase of plasma AGT, corresponding to a ~1.2-fold increased risk of hypertension. Importantly, this genotype also confers genetic susceptibility to pregnancy-induced hypertension (preeclampsia). If other risk factors for hypertension are known, this genotype would indicate early prevention.

BETA1-ADRENERGİC RECEPTOR GLY > ARG CODON 389

Beta1-adrenergic receptors (B_1-ARs) are the predominant cardiac receptors for norepinephrine and epinephrine, representing the major mechanism by which cardiac output is increased *via* the sympathetic nervous system. The human B_1-adrenergic receptor is encoded by the *ADRB1* gene, which contains a functional polymorphism, resulting in a Gly to Arg change at codon 389. Although Gly389 is the minor allele, it is referred to as the "reference" receptor, whereas the more common "variant", Arg389, has been shown to be associated with enhanced receptor function. The human Arg389 variant has been suggested to predispose to heart failure by hyperactive signaling and to influence the therapeutic response to Beta blocker treatment. This variant is also reported to be associated with hypertension .A homozygous genotype

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