Fagron genomics

TrichoTest™ Scientific validation

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Table of content

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Introduction

The Fagron TrichoTest™ Scientific validation is a document created by Fagron to support healthcare professionals on the complete understanding of the genetic analyzes performed with TrichoTest™.

TrichoTest™ is a genetic test and software solution developed by Fagron Genomics. The test supports prescribers to personalize alopecia treatments, offering individualized solutions in TrichoConcept™ vehicles and other Fagron solutions from more than 175 million therapeutical options, based on the patient's genetic background and lifestyle.

Fagron TrichoTest™ Scientific validation document describes how each analyzed gene in TrichoTest™ is associated with a metabolic pathway involved in alopecia treatment, following a complete and described scientific analysis.

Should you have any questions or remarks, please contact your local sales representative.

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General information

Alopecia is a gradual and multifactorial condition, determined by a combination of both intrinsic and extrinsic factors.

The intrinsic factors are genetic, being related to the genes and their expression, having a higher impact on the development of alopecia. Extrinsic factors such as the patient's lifestyle, alimentation habits and stress, can accelerate the development of alopecia. The combination of genetic characteristics and lifestyle should be addressed as a whole, during alopecia treatment.

TrichoTest™ analyzes 3 polymorphisms within 16 single-nucleotide polymorphisms (SNPs), resulting in 48 (3x16) genetic variations on the most relevant mutations related to the personalized treatment of alopecia. All SNPs analyzed in TrichoTest™ have been scientifically validated from population studies, presenting a significative global incidence.

The genetic variations analyzed with TrichoTest™ are associated with 7 different categories of alopecia treatment options:

- **Prostaglandins metabolism:** GPR44-1; GPR44-2; PTGFR-1; PTGFR-2; PTGFR-3; PTGES2; SULT1A1.
- **Inflammation:** GR-alpha.
- **Androgenic effect:** CYP19; SR5DA1; SR5DA2.
- **Vasodilation and blood circulation:** ACE.
- **Collagen synthesis:** COL1A1.
- **Vitamins and minerals metabolism:** CRABP2; BTD.
- **Insulin metabolism:** IGFR-1.

In the following table, a summary of the analyzed genes in TrichoTest™ and related treatment possibilities are shown.

Gene	Evaluated effect	APIs recommended
GPR44	Activity of PGD2 receptors	Prostaquinon™
PTGFR	Activity of PGF2a receptors	Latanoprost
PTGES2	Activity of PTGES2 enzyme	Minoxidil
SULT1A1	Activity of sulfotransferase	Minoxidil
GR-alpha	Resistance to glucocorticoids therapy	Anti-inflammatory glucocorticoids
CYP19	Activity of aromatase	17α-estradiol
SR5DA	Activity of 5α-reductase type 1 and 2	Finasteride, Dutasteride
ACE	Activity of angiotensin-converting enzyme	Circulation modulators
COL1A1	Synthesis of collagen	Collagen synthesis enhancers
CRABP2	Transport of vitamin A	Tocopherol, Retinol
BTD	Activity of biotinidase	Biotin
IGFR-1	Activity of IGF-1 receptors	IGrantine-F1™

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Prostaglandins metabolism

Prostaglandins (PG) are a class of hormones with direct effect in body functions such as the activation of the inflammatory response, gastric acid regulation, smooth muscle contraction, body temperature regulation and blood pressure control.

PGD, PGF and PGE have been described as having an influence in hair loss through interaction with prostaglandin receptors (GPR44, PTGFR) and regulators (PTGES2 and SULT1A1).

1. GPR44

Prostaquinon™ is a Fagron phytocomplex derived from the essential oils of *Nigella sativa* seeds.

Thymoquinone is one of the principal pharmacological active constituents of *Nigella sativa* [1]. It is known for its anti-inflammatory activity through the inhibition of cyclooxygenase 2 (COX-2) expression and prostaglandin D2 (PGD2) synthesis [2,3].

Recently, it has been reported an important inhibitory role of PGD2 on hair follicle, related to alopecia. The binding of PGD2 to the prostaglandin D2 receptor (initially termed GPR44 and now known as CRTh2) expressed in the hair follicle seems to induce hair follicle miniaturization [2]. This is probably due to inhibition of the maturation of stem cells into progenitor cells and of vellus hair development into normal terminal hair. Therefore, the inhibition of PGD2 synthesis promoted by thymoquinone can contribute to the regulation of the hair cycle [3].

The GPR44 (or CRTH2) gene codifies for the receptor of PGD2. In TrichoTest™ two variations of GPR44 are analyzed:

- **rs533116** - associated with a higher expression of PGD2 receptors when the AA genotype is present (prevalence of approximately 19% in the global population) [4,5].
- **rs545659 (G1651A)** - associated with an increased GPR44 mRNA stability when the patient presents the GG genotype (prevalence of approximately 34% in the global population) [5,6].

The GPR44 genetic variations analyzed in TrichoTest™ allow for the identification of a potential lower or higher concentration and activity of GPR44 receptors. This information is important to understand the effect of the PGD2 on the patient, as it binds to GPR44 receptors in order to produce an effect. As Prostaquinon™ acts on the inhibition of PGD2 synthesis, the results obtained with TrichoTest™ provide an indication about the dosage and potential efficacy of treatment with Prostaquinon™.

2. PTGFR

Latanoprost is an analogue of prostaglandin F2α (PGF2α), commonly used for glaucoma treatment, with a reported side effect of cilia density increase. This observation lead to the development of studies that evaluated and support its use in alopecia treatment [7-9].

The PTGFR (prostaglandin F receptor) gene mediates the behavior of the main cellular receptor of PGF2α, the FP receptor. It is expressed in different regions of the body, including the dermal papilla [10-12].

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TrichoTest™ analyzes a set of genetic variations of PTGFR highly associated with the probability of obtaining a successful efficacy in treatments with Latanoprost [13]:

- **rs10782665**: the allele variant T is related with a higher efficacy in treatments with Latanoprost [13]. The global prevalence of this allele variation is approximately 38% [14]. If the allele G is present, there is a higher probability of not having a proper response to Latanoprost [13].
- **rs6686438**: the allele variant T is related with a higher efficacy in treatments with Latanoprost [13]. The global prevalence of this specific allele variation is approximately 36% [15]. If the allele G is present, there is a higher probability of not having a proper response to Latanoprost [13].
- **rs1328441**: the allele variant G is related with a higher efficacy in treatments with Latanoprost [13]. The global prevalence of this allele variation is approximately 46% . If the allele A is present, there is a higher probability of not having a proper response to Latanoprost [13].

Genetic variations of PTGFR have an effect on the FP receptor that can lead to different responses when PGF2α binds to it [13]. As Latanoprost is an analogue of PGF2α, differences on FP receptor due to genetic polymorphisms can have an impact on its efficacy in alopecia treatment [7-12]. The results obtained with TrichoTest™ provide an indication about the dosage and potential efficacy of alopecia treatment with Latanoprost.

3. PTGES2

Minoxidil was the first topical drug approved by the FDA for androgenic alopecia treatment [7]. Although the exact mechanism of action of minoxidil on hair growth is still unclear, it has been associated with an increase in prostaglandin E2 (PGE2) levels produced by fibroblasts [16]. A reduction in PGE2 levels is related to the development of alopecia, while an increase in PGE2 levels is related to hair growth enhancement [2, 17, 18].

The PTGES2 gene is related to the synthesis of PGE2, codifying for prostaglandin E synthase 2, the enzyme that converts PGH2 into PGE2 [19-21].

A heterozygous genotype of the rs13283456 variant of PTGES2 gene has been associated with a reduced enzymatic activity of prostaglandin E synthase 2, leading to lower PGE2 levels [20]. TrichoTest™ analyzes the presence of the risk allele T in the rs13283456 variant of PTGES2 (prevalence of approximately 40% in the global population) [22].

Minoxidil acts also on this metabolic pathway, as it can increase PGE2 levels that are lower in a patient due to the presence of a variation on PTGES2 [16].

4. SULT1A1

As mentioned previously, the exact mechanism of action of minoxidil in alopecia treatment is not completely defined. However, it has been demonstrated that minoxidil sulfate is the active metabolite of minoxidil responsible for the stimulation of hair follicles [23, 24]. This is due to conversion of minoxidil to minoxidil sulfate by the enzyme minoxidil sulfotransferase (SULT1A1) [24].

SULT1A1 gene codifies for the sulfotransferase that activates minoxidil (SULT1A1). The SULT1A1*2 genotype is associated with a low enzyme activity and low thermal stability of SULT1A1. This polymorphism is due to a C to T substitution (prevalence of approximately 22% in the global population), analyzed in TrichoTest™ [25, 26].

Therefore, knowing the activity and stability of SULT1A1 enzyme allows for a better understanding about the possibility of activation of minoxidil to its active form, in a certain patient.

Inflammation

For cases of alopecia areata, the treatment is usually performed with corticosteroids.

5. GRα

The physiological and pharmacological actions of glucocorticoids are mediated by the glucocorticoid receptor (GR). The GR gene (NR3C1) codifies for GR, which is essential for the effects of glucocorticoids to manifest [27]. Several NR3C1 variants can lead to altered sensibility of GR to glucocorticoids and have been associated with resistance or sensitivity to corticosteroids [27, 28].

There are two isoforms of the glucocorticoid receptor: GRα and GRβ [29]. GRα is the classic GR isoform that mediates the actions of glucocorticoids, while GRβ is known to enhance resistance to the pharmacological effects of glucocorticoids [27, 29]. Increased levels of GRβ isoform lead to a dominant negative inhibition of the GRα isoform [27].

The A3669G polymorphism (rs6198) of the GR gene codifies for the GRβ isoform due to an A to G mutation, which favors its protein stability and dominant negative function over the GRα isoform [27, 28]. Therefore, the A3669G polymorphism has been identified as a contributing factor to glucocorticoids resistance.

TrichoTest™ analyzes the presence of the A3669G polymorphism, a variation with a global prevalence between 20% to 40% in the population [30, 31]. If this polymorphism is observed in the patient the efficacy of glucocorticoids treatment will have a high probability of being reduced, due to the expression of GRβ. In this case, it would be recommended a treatment with anti-inflammatory agents non-glucocorticoids.

Androgenic effect – DHT metabolism

Dihydrotestosterone (DHT) is an androgen synthesized by the 5α-reductase enzyme and the biologically active metabolite of testosterone. High levels of DHT are known to be related to the development of alopecia [32-34].

6. SR5DA

Testosterone is converted to DHT by the action of 5α-reductase [35].

There are two isoenzymes of 5α-reductase described, known as type I and type II 5α-reductase [35, 36]. Type I 5α-reductase is more present in the skin and outer root sheath of the hair follicle, while type II 5α-reductase is more active in the dermal papilla and inner root sheath of the hair follicle [35].

Finasteride and dutasteride, both 5α-reductase inhibitors, are considered first-line treatment for male androgenetic alopecia. While finasteride is known to act only on the type II 5α-reductase, dutasteride inhibits both forms of the enzyme (type I and II) [36, 37].

The type I 5α-reductase is codified by the SRD5A1 gene [38, 39]. The SNP rs248793 (116C>G) of this gene is related to altered activity levels of the enzyme. The presence of the C allele (global prevalence of approximately 40%-45%) is related to an higher activity of type I 5α-reductase and, consequently, higher levels of DHT [85, 86].

The SRD5A2 gene codifies for the type II 5α-reductase [38, 39]. The rs523349 SNP (V89L) of this gene is related to the most active form of the enzyme when the G allele is present (global prevalence of approximately 30%) and to a significant less active form of the enzyme when the C allele is present [38-42].

TrichoTest™ analyzes the presence of V89L and 116C>G polymorphisms, allowing an appropriate API selection and adjustment on finasteride and dutasteride concentrations, based on the expected activity of 5α-reductase type I and II.

7. CYP19

The enzyme complex aromatase is responsible for the conversion of androgens into estrogens. This enzyme complex is composed of cytochrome P450 aromatase and NADPH cytochrome P450 reductase [43, 44].
Several reports relate polymorphisms in sex steroid-related genes with a possible impact on serum estradiol levels in women [45 - 47]. Promising findings have been made in the CYP19 gene, the gene that codifies for aromatase [43, 46].

The SNP rs2470152 from CYP19 gene has been reported to be associated with serum estrogen levels and the estradiol/testosterone ratio [43, 44, 48]. TrichoTest™ analyzes the rs2470152 polymorphism, focusing on the 15658C>T variation. The presence of the T allele (global incidence of approximately 47% and 50%) is associated with a decreased activity of aromatase [43].

A decreased activity of aromatase will lead to a decreased conversion of testosterone in estrogens, leading to a higher possibility of conversion into DHT, a metabolite related with the development of alopecia [43, 44]. 17-α estradiol has proven to be a strong aromatase activity stimulator, being used topically on androgenic alopecia treatment .

The results obtained with TrichoTest™ provide information about the need to stimulate the aromatase with 17-α estradiol, allowing also for a concentration adjustment based on the information obtained [49, 50].

Vasodilation and blood circulation

Vasoconstriction is a significant cause of alopecia, as proper transportation of nutrients and APIs is not fully achieved. Information about the genetic tendency to high blood pressure helps in selecting APIs that increase vasodilation and blood circulation.

8. ACE

Angiotensin I converting enzyme (ACE) plays a key role in blood pressure regulation. ACE converts the inactive form angiotensin I into angiotensin II (active form), a well-known vasoconstrictor [51].

There are two forms of ACE that are produced from changes in the genetic code of the ACE gene - ACE I and ACE D - corresponding to an insertion (I) or deletion (D) of a short region of DNA within the gene [52]. Patients with allele I typically present reduced ACE activity when in comparison to patients carrying allele D [52-55].

In TrichoTest™, the genetic variation G2328A (SNP rs4343) of ACE is analyzed. The risk G allele (prevalence of approximately 46% in the population) acts as a marker for patients carrying the allele D (deletion) [53, 54, 56]. Levels of ACE are increased in patients carrying the G allele G2328A variation, which leads to increased conversion of angiotensin I to its active form angiotensin II, therefore increasing vasoconstriction [52-55, 57].

The vasoconstriction levels induced by angiotensin II are analyzed with TrichoTest™ and can be managed in alopecia with APIs that increase blood circulation or vasodilation, such as minoxidil, ginkgo biloba and caffeine.

Collagen synthesis

Collagen is an important component of the hair and deficiencies in collagen levels can enhance hair damage.

9. COL1A1

COL1A1 gene codifies for the largest compound of type I collagen, the most abundant type in the human body [58, 59].

SNPs in COL1A1 gene have been shown to be associated with several complex connective tissue disorders. The G to T substitution in COL1A1 rs1800012 variation (1546G>T) results in increased gene expression [59]. It has been reported that COL1A1 is overexpressed in situations of androgenic alopecia [58].

The rs1800012 polymorphism has been identified as an important SNP in regulation of collagen transcription [58-60]. This polymorphism affects the DNA-protein interaction and increases the levels of α1 chains of type I collagen, resulting in an imbalance in the normal ratio of α1 to α2 chains[60]. This imbalance is associated with instability of the collagen molecule.

TrichoTest™ analyses the presence of the rs1800012 variation (global prevalence of approximately 20%) [61]. If the mutation is observed, oral supplementation with cysteine, silicon or adenosine, for example, will be suggested.

Vitamins and minerals metabolism

Vitamin A contributes to the retinoids production, which stimulate the hair follicle and new hair production. Vitamin B nourishes the hair follicles and group B vitamins help to decrease stress, one of the main causes of hair loss.

10. CRABP2

Retinoic acid (RA) is a metabolite of vitamin A (retinol) that mediates functions required for growth and development [62]. Treatment with minoxidil is sometimes complemented with retinoic acid to increase its effectiveness.

Cellular retinoic acid-binding protein 2 (CRABP2) is a cytoplasmic binding protein, codified by the CRABP2 gene, responsible for the transport of RA to intracellular RA receptors (RARs) [62-65].

The rs12724719 variation in CRABP2 gene is associated with a higher concentration of RA in the blood, due to a decreased rate of intracellular transport, therefore reducing the efficacy of treatments with retinoic acid [63]. TrichoTest™ analyzes the presence of homozygotic allele A (prevalence of approximately less than 20% in the population), related with a lower efficacy on CRABP2 retinoic acid transportation [63, 66]. It this allele is homozygotic in a patient, the standard treatment with retinoic acid might not have the desired effect. Therefore, it would be suggested an increase in the concentration or treatment with an alternative API.

11. BTD

Biotin is an essential water-soluble B vitamin, having biotinidase has the enzyme responsible for cleaving it from biocytin and from dietary sources. Deficit of biotinidase leads to low biotin levels, which can cause hair loss, skin rashes and brittle nails [67].

The BTD gene codifies for biotinidase and the SNP rs13078881 of the BTD gene is analyzed in TrichoTest™ [68]. The presence of the allele C is related to biotinidase deficiency, having a global prevalence in the population of less than 5% [68, 69]. The presence of this genetic variation allows for an understanding of a possible need for biotin supplementation.

Insulin metabolism

It has been reported that patients with higher resistance to insulin (mutation on the insulin receptor) are more likely to develop alopecia.

12. IGFR-1

Insulin-like growth factor-1 (IGF-1) shares a high degree of structural and functional homology with insulin [70, 71]. IGF-1, being produced by dermal papilla cells, is critically involved in hair growth by regulating cellular proliferation and migration during the development of hair follicles [72-79]. To induce its biological effects, IGF-I must bind to a specific cell-surface receptor known as IGF-1 receptor (IGF-1R) [70, 71].

IGrantine-F1™ is a Fagron innovative phytocomplex with cepharanthine. Cepharanthine is known for stimulating hair growth by increasing the production of IGF-1 [80-83].

TrichoTest™ analyzes the rs2229765 SNP presence in the IGF1R gene, the gene that codifies for IGF-IR. It has been identified that patients carrying at least one A allele in this variation (global prevalence of approximately 39%) have lower free plasma IGF-1 levels [71, 78]. As IGrantine-F1™ increases the production of IGF-1, the results obtained with TrichoTest™ can identify if there is a need to use IGrantine-F1™ in alopecia treatment.

Non-genetic factors in alopecia

As alopecia is a multifactorial condition, TrichoTest™ analyzes not only the genetic profile but also non-genetic factors associated with alopecia, in each patient.

13. Antioxidants

Different factors, such as nutritional habits, metabolic syndrome, exposure to UV, smoking or drinking alcohol have been associated with the development and progress of alopecia [87-96]. A common consequence of all these

factors is the increase on cellular oxidative stress, which is being linked to an important role in alopecia development [88, 94, 96-99].

It has been demonstrated that oxidative stress alters significantly dermal papilla cells morphology, migration, proliferation, senescence and TGF-β signaling, suggesting an important role for the development of androgenic alopecia [94]. Naito et al, observed the effect of lipid peroxides in hair follicle, demonstrating that lipid peroxides, which can produce free radicals, induce the apoptosis of hair follicle cells, followed by early onset of the catagen phase [101]. Most specifically, smoking-induced oxidative stress and a disequilibrium of the anti-oxidant systems may lead to the release of pro-inflammatory cytokines form follicular keratinocytes, which have been shown to inhibit the growth of isolated hair follicles in culture [88, 100].

Besides oxidative stress, other mechanisms by which smoking is related to accelerated hair loss include effects on microcirculation, direct genotoxic effect, imbalance in the follicular protease/anti-protease systems involved in tissue remodeling in the course of the hair follicle cycle, inhibition of aromatase and hydroxylation of estradiol [88, 102].

TrichoTest™ has in consideration the lifestyle factors than can promote an increase in oxidative stress, when suggesting personalized formulations for each patient. Therefore, different DCIs known to be powerful antioxidants can be suggested [103 - 106]. These include astaxanthin, coenzyme Q10, selenium yeast and sulfate iron, among others.

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