

TrichoTest™ Scientific validation

21 February 2019





Table of content

Introduction	3
General information	4
Prostaglandins metabolism.....	5
Inflammation	7
Androgenic effect – DHT metabolism.....	7
Vasodilation and blood circulation	8
Collagen synthesis.....	9
Vitamins and minerals metabolism.....	9
Insulin metabolism	10
Non-genetic factors in alopecia	10
References.....	11
Disclaimer	16



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.



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 significant 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; BTB.
- **Insulin metabolism:** IGF-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™
PGTFR	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
BTB	Activity of biotinidase	Biotin
IGF-1	Activity of IGF-1 receptors	IGRantine-F1™



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, PGTFR) 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 miniaturization². 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 cycle³.

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. PGTFR

Latanoprost is an analogue of prostaglandin F2α (PGF2α), commonly used for glaucoma treatment, with a reported side effect of cilia density increase. This observation led to the development of studies that evaluated and support its use in alopecia treatment⁷⁻⁹.

The PGTFR (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 papilla¹⁰⁻¹².



TrichoTest™ analyzes a set of genetic variations of PGF2R 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 PGF2R have an effect on the FP receptor that can lead to different responses when PGF2 α binds to it ¹³. As Latanoprost is an analogue of PGF2 α , 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. GR α

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: GR α and GR β ²⁹. GR α is the classic GR isoform that mediates the actions of glucocorticoids, while GR β is known to enhance resistance to the pharmacological effects of glucocorticoids ^{27, 29}. Increased levels of GR β isoform lead to a dominant negative inhibition of the GR α isoform ²⁷.

The A3669G polymorphism (rs6198) of the GR gene codifies for the GR β isoform due to an A to G mutation, which favors its protein stability and dominant negative function over the GR α 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 GR β . 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 5 α -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 5 α -reductase ³⁵.

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

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



The type I 5 α -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 5 α -reductase and, consequently, higher levels of DHT^{85,86}.

The SRD5A2 gene codifies for the type II 5 α -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 present³⁸⁻⁴².

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 5 α -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 women⁴⁵⁻⁴⁷. 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 aromatase⁴³.

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 vasoconstrictor⁵¹.

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 gene⁵². Patients with allele I typically present reduced ACE activity when in comparison to patients carrying allele D⁵²⁻⁵⁵.



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 expression⁵⁹. It has been reported that COL1A1 is overexpressed in situations of androgenic alopecia⁵⁸.

The rs1800012 polymorphism has been identified as an important SNP in regulation of collagen transcription⁵⁸⁻⁶⁰. This polymorphism affects the DNA-protein interaction and increases the levels of $\alpha 1$ chains of type I collagen, resulting in an imbalance in the normal ratio of $\alpha 1$ to $\alpha 2$ chains⁶⁰. 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 development⁶². 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}. If 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. IGF-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-1 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-1R. 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 from 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.

References

1. Rossi A, et al. *Evaluation of a Therapeutic Alternative for Telogen Effluvium: A Pilot Study*. Journal of Cosmetics, Dermatological Sciences and Applications, Vol. 3, 3A, 2013, pp. 9-16.
2. Garza LA, et al. *Prostaglandin D2 Inhibits Hair Growth and Is Elevated in Bald Scalp of Men with Androgenetic Alopecia*. Science Translational Medicine, Vol. 4, 126, 2012, pp. 126-134.
3. Hill RP, et al. *Human Hair Follicle Dermal Cells and Skin Fibroblasts Show Differential Activation of NF- κ B in Response to Pro-Inflammatory Challenge*. Experimental Dermatology, Vol. 21, 2, 2012, pp. 158-160.
4. Alberto CE, et al. *The single nucleotide polymorphism CRTh2 rs533116 is associated with allergic asthma and increased expression of CRTh2*. Allergy. 2012 Nov;67(11):1357-64.
5. Cornejo-García JA, et al. *Pharmacogenomics of Prostaglandin and Leukotriene Receptors*. Front. Pharmacol. 2016. 7:316.
6. Huang JL, et al. *Sequence variants of the gene encoding chemoattractant receptor expressed on Th2 cells (CRTH2) are associated with asthma and differentially influence mRNA stability*. Hum Mol Genet. 2004 Nov 1;13(21):2691-7.
7. Kelly Y, et al. *Androgenetic Alopecia: An Update of Treatment Options*. Drugs. 2016 Sep;76(14):1349-64.
8. Blume-Peytavi U, et al. *A randomized double-blind placebo-controlled pilot study to assess the efficacy of a 24-week topical treatment by latanoprost 0.1% on hair growth and pigmentation in healthy volunteers with androgenetic alopecia*. J Am Acad Dermatol. 2012 May;66(5):794-800.
9. Coronel-Pérez IM, et al. *Latanoprost in the treatment of eyelash alopecia in alopecia areata universalis*. J Eur Acad Dermatol Venereol 2010; 24: 481–5.
10. Woodward DF, et al. *International Union of Basic and Clinical Pharmacology. LXXXIII: Classification of Prostanoid Receptors, Updating 15 Years of Progress*. Pharmacological Reviews. Sep 2011, 63 (3) 471-538.



11. Colombe L, et al. *Prostaglandin metabolism in human hair follicle*. *Experimental Dermatology*. 2007; 16: 762–769.
12. Colombe L, et al. *Prostanoid receptors in anagen human hair follicles*. *Experimental Dermatology*. 2008; 17: 63–72.
13. Ussa F, et al. *Association between SNPs of Metalloproteinases and Prostaglandin F2 α Receptor Genes and Latanoprost Response in Open-Angle Glaucoma*. *Ophthalmology*. 2015 May;122(5):1040-8.e4.
14. U.S. National Library of Medicine. Reference SNP (rs) Report rs10782665. Available in: https://www.ncbi.nlm.nih.gov/snp/rs10782665#frequency_tab (last access on 03/01/2019).
15. U.S. National Library of Medicine. Reference SNP (rs) Report rs6686438. Available in: https://www.ncbi.nlm.nih.gov/snp/rs6686438#frequency_tab (last access on 03/01/2019).
16. Choi N, et al. *Minoxidil Promotes Hair Growth through Stimulation of Growth Factor Release from Adipose-Derived Stem Cells*. *Int. J. Mol. Sci*. 2018, 19, 691.
17. Kvedar JC, et al. *Selective inhibition by minoxidil of prostacyclin production by cells in culture*. *Biochem Pharmacol*. 1988;37(5):867–74.
18. Michelet JF, et al. *Activation of cytoprotective prostaglandin synthase-1 by minoxidil as a possible explanation for its hair growth-stimulating effect*. *J Invest Dermatol*. 1997;108(2):205–9.
19. Park JY, et al. *Prostaglandin E2 synthesis and secretion: the role of PGE2 synthases*. *Clin Immunol*. 2006 Jun;119(3):229-40.
20. Fischer A, et al. *Association analysis between the prostaglandin E synthase 2 R298H polymorphism and body mass index in 8079 participants of the KORA study cohort*. *Genet Test Mol Biomarkers*. 2009 Apr;13(2):223-6.
21. Diamond JM, et al. *Genetic variation in the prostaglandin E2 pathway is associated with primary graft dysfunction*. *Am J Respir Crit Care Med*. 2014 Mar 1;189(5):567-75.
22. U.S. National Library of Medicine. Reference SNP (rs) Report rs13283456. Available in: https://www.ncbi.nlm.nih.gov/snp/rs13283456#frequency_tab (last access on 03/01/2019).
23. Buhl AE, et al. *Minoxidil sulfate is the active metabolite that stimulates hair follicles*. *J Invest Dermatol*. 1990 Nov;95(5):553-7.
24. Goren A, et al. *Novel enzymatic assay predicts minoxidil response in the treatment of androgenetic alopecia*. *Dermatologic Therapy*, Vol. 27, 2014, 171–173.
25. Raftogianis RB, et al. *Phenol sulfotransferase pharmacogenetics in humans: association of common SULT1A1 alleles with TS PST phenotype*. *Biochem Biophys Res Commun*. 1997 Oct 9;239(1):298-304.
26. Grabinski JL, et al. *Genotypic and allelic frequencies of SULT1A1 polymorphisms in women receiving adjuvant tamoxifen therapy*. *Breast Cancer Res Treat*. 2006 Jan;95(1):13-6.
27. Gasic V, et al. *Pharmacogenomic Markers of Glucocorticoid Response in The Initial Phase of Remission Induction Therapy in Childhood Acute Lymphoblastic Leukemia*. *Radiol Oncol*. 2018 Sep; 52(3): 296–306.
28. Rodrigues DM, et al. *Decreased comfort food intake and allostatic load in adolescents carrying the A3669G variant of the glucocorticoid receptor gene*. *Appetite*. 2017 Sep 1;116:21-28.
29. Oakley RH, et al. *The biology of the glucocorticoid receptor: new signaling mechanisms in health and disease*. *J Allergy Clin Immunol*. 2013 Nov;132(5):1033-44.
30. Bunce CM, Campbell MJ. *Nuclear Receptors: Current Concepts and Future Challenges*. 2010. Springer.
31. Varricchio L, et al. *Increased frequency of the glucocorticoid receptor A3669G (rs6198) polymorphism in patients with Diamond-Blackfan anemia*. *Blood*. 2011 Jul 14;118(2):473-4.
32. Hamilton JB. *Patterned loss of hair in man: types and incidence*. *Annals of the New York Academy of Sciences* 1951; 53:708-728.
33. Severi G, et al. *Androgenetic alopecia in men aged 40-69 years: prevalence and risk factors*. *The British journal of dermatology* 2003; 149:1207-1213.



34. Zhang Y, et al. Serum Levels of Androgen-Associated Hormones Are Correlated with Curative Effect in Androgenic Alopecia in Young Men. *Med Sci Monit*. 2018 Oct 30;24:7770-7777.
35. Libecco JF, et al. Finasteride in the treatment of alopecia. *Expert Opin Pharmacother*. 2004 Apr;5(4):933-40.
36. Hirshburg JM, et al. Adverse Effects and Safety of 5-alpha Reductase Inhibitors (Finasteride, Dutasteride): A Systematic Review. *J Clin Aesthet Dermatol*. 2016 Jul;9(7):56-62.
37. Arif T et al. Dutasteride in Androgenetic Alopecia: An Update. *Curr Clin Pharmacol*. 2017;12(1):31-35.
38. Shiota M, et al. SRD5A gene polymorphism in Japanese men predicts prognosis of metastatic prostate cancer with androgen-deprivation therapy. *Eur J Cancer*. 2015 Sep;51(14):1962-9.
39. U.S. National Library of Medicine. Reference SNP (rs) Report rs523349. Available in: https://www.ncbi.nlm.nih.gov/snp/rs523349#frequency_tab (last access on 04/01/2019).
40. Hsing AW, et al. Polymorphic markers in the SRD5A2 gene and prostate cancer risk: a population-based case-control study. *Cancer Epidemiol Biomarkers Prev*. 2001 Oct;10(10):1077-82.
41. Allen NE, et al. The association between polymorphisms in the CYP17 and 5alpha-reductase (SRD5A2) genes and serum androgen concentrations in men. *Cancer Epidemiol Biomarkers Prev*. 2001 Mar;10(3):185-9.
42. van Gils CH, et al. The V89L polymorphism in the 5-alpha-reductase type 2 gene and risk of breast cancer. *Cancer Epidemiol Biomarkers Prev*. 2003 Nov;12(11 Pt 1):1194-9.
43. Zhang XL, et al. SNP rs2470152 in CYP19 is correlated to aromatase activity in Chinese polycystic ovary syndrome patients. *Mol Med Rep*. 2012 Jan;5(1):245-9.
44. Eriksson AL, et al. Genetic Variations in Sex Steroid-Related Genes as Predictors of Serum Estrogen Levels in Men. *J Clin Endocrinol Metab*. 2009 Mar; 94(3): 1033–1041.
45. Olson SH, et al. Variants in estrogen biosynthesis genes, sex steroid hormone levels, and endometrial cancer: a HuGE review. *Am J Epidemiol*. 2007, 165:235–245.
46. Haiman CA, et al. Genetic variation at the CYP19A1 locus predicts circulating estrogen levels but not breast cancer risk in postmenopausal women. *Cancer Res*. 2007, 67:1893–1897.
47. Sowers MR, et al. Endogenous estradiol and its association with estrogen receptor gene polymorphisms. *Am J Med*. 2006, 119:S16–S22.
48. Jiang J, et al. Association of genetic variations in aromatase gene with serum estrogen and estrogen/testosterone ratio in Chinese elderly men. *Clin Chim Acta* 411: 53-58, 2010.
49. Hoffmann R, et al. 17alpha-estradiol induces aromatase activity in intact human anagen hair follicles ex vivo. *Exp Dermatol*. 2002 Aug;11(4):376-80.
50. Adashi EY, et al. Estrogens augment the stimulation of ovarian aromatase activity by follicle-stimulating hormone in cultured rat granulosa cells. *J Biol Chem*. 1982 Jun 10;257(11):6077-83.
51. Namazi MR, et al. Angiotensin converting enzyme activity in alopecia areata. *Enzyme Res*. 2014;2014:694148.
52. Glenn KL, et al. An alternative method for genotyping of the ACE I/D polymorphism. *Mol Biol Rep*. 2009 Jul;36(6):1305-10.
53. Eisenmann JC, et al. ACE I/D genotype, adiposity, and blood pressure in children. *Cardiovasc Diabetol*. 2009 Mar 16;8:14.
54. Montgomery HE, et al. Association of angiotensin-converting enzyme gene I/D polymorphism with change in left ventricular mass in response to physical training. *Circulation*. 1997 Aug 5;96(3):741-7.
55. Costa AM, et al. Association between ACE D allele and elite short distance swimming. *Eur J Appl Physiol*. 2009 Aug;106(6):785-90.
56. U.S. National Library of Medicine. Reference SNP (rs) Report rs4343. Available in: https://www.ncbi.nlm.nih.gov/snp/rs4343#frequency_tab (last access on 07/01/2019).



57. Firouzabadi N, et al. Association of angiotensin-converting enzyme (ACE) gene polymorphism with elevated serum ACE activity and major depression in an Iranian population. *Psychiatry Research*. 2012, 200(2-3), 336–342.
58. Michel L, et al. Study of gene expression alteration in male androgenetic alopecia: evidence of predominant molecular signalling pathways. *Br J Dermatol*. 2017 Nov;177(5):1322-1336.
59. Stępien-Słodkowska M, et al. The +1245G/T polymorphisms in the collagen type I alpha 1 (COL1A1) gene in polish skiers with anterior cruciate ligament injury. *Biol Sport*. 2013 Mar; 30(1): 57–60.
60. Zhong B, et al. Association of COL1A1 rs1800012 polymorphism with musculoskeletal degenerative diseases: a meta-analysis. *Oncotarget*. 2017 Sep 26; 8(43): 75488–75499.
61. Reference SNP (rs) Report rs1800012. Available in: <https://www.snpedia.com/index.php/Rs1800012> (last access on 07/01/2019).
62. Zheng WL, et al. "Spatial and temporal patterns of expression of cellular retinol-binding protein and cellular retinoic acid-binding proteins in rat uterus during early pregnancy". *Biology of Reproduction*. Apr 1998, 58 (4): 963–970.
63. Manolescu DC, et al. Newborn serum retinoic acid level is associated with variants of genes in the retinol metabolism pathway. *Pediatr Res*. 2010 Jun;67(6):598-602.
64. Liu RZ, et al. Association between cytoplasmic CRABP2, altered retinoic acid signaling, and poor prognosis in glioblastoma. *Glia*. 2016 Jun;64(6):963-76.
65. Zhang GM, et al. DNA methylation status of CRABP2 promoter down-regulates its expression. *Gene*. 2018 Nov 15;676:243-248.
66. U.S. National Library of Medicine. Reference SNP (rs) Report rs12724719. Available in: https://www.ncbi.nlm.nih.gov/snp/rs12724719#frequency_tab (last access on 07/01/2019).
67. Almohanna HM, et al. The Role of Vitamins and Minerals in Hair Loss: A Review. *Dermatol Ther (Heidelb)*. 2018 Dec 13.
68. Swango KL, et al. Partial biotinidase deficiency is usually due to the D444H mutation in the biotinidase gene. *Hum Genet*. 1998 May;102(5):571-5.
69. U.S. National Library of Medicine. Reference SNP (rs) Report rs13078881. Available in: https://www.ncbi.nlm.nih.gov/snp/rs13078881#frequency_tab (last access on 07/01/2019).
70. Ahn SY, et al. Effect of IGF-I on Hair Growth Is Related to the Anti-Apoptotic Effect of IGF-I and Up-Regulation of PDGF-A and PDGF-B. *Ann Dermatol*. 2012 Feb;24(1):26-31.
71. Gately K, et al. Mutational analysis of the insulin-like growth factor 1 receptor tyrosine kinase domain in non-small cell lung cancer patients. *Mol Clin Oncol*. 2015 Sep; 3(5): 1073–1079.
72. Ben Amitai D et al. I-GF-1 signalling controls the hair growth cycle and the differentiation of hair shafts. *J Invest Dermatol* 2006;126:2135.
73. Bol DK, et al. Overexpression of insulin-like growth factor-1 induces hyperplasia, dermal abnormalities, and spontaneous tumor formation in transgenic mice. *Oncogene* 1997;14:1725-1734.
74. Liu JP, et al. Mice carrying null mutations of the genes encoding insulin-like growth factor I (Igf-1) and type 1 IGF receptor (Igf1r). *Cell* 1993;75:59-72.
75. Itami S, et al. Role of androgen in mesenchymal epithelial interactions in human hair follicle. *J Invest Dermatol Symp Proc* 2005;10:209-211.
76. Tavakkol A, et al. Expression of growth hormone receptor, insulin-like growth factor 1 (IGF-1) and IGF-1 receptor mRNA and proteins in human skin. *J Invest Dermatol* 1992;99:343-349.
77. Bonafè M, et al. Polymorphic variants of insulin-like growth factor I (IGF-I) receptor and phosphoinositide 3-kinase genes affect IGF-I plasma levels and human longevity: Cues for an evolutionarily conserved mechanism of life span control. *J Clin Endocrinol Metab* 88: 3299-3304, 2003.



79. Rasmussen SK, et al. Studies of the variability of the genes encoding the insulin-like growth factor I receptor and its ligand in relation to type 2 diabetes mellitus. *J Clin Endocrinol Metab* 85: 1606-1610, 2000.
80. Inui S, et al. Acceleration of Hair Growth Rate by Topical Liposomal Cepharranthine in Male Androgenetic Alopecia. *Hair Ther Transplant* 2016, 6:2.
81. Inui S, et al. Induction of insulin-like growth factor-I by cepharanthine from dermal papilla cells: a novel potential pathway for hair growth stimulation. *J Dermatol*. 2013 Dec;40(12):1054-5.
82. Jiang J, et al. The stimulatory effects of cepharanthine on hair growth. *Skin Research* 2005; 4: 99–103.
83. Pazyar N, et al. Cepharanthine: a therapeutic potential candidate for psoriasis. *J Altern Complement Med*. 2012 Jul;18(7):639-40.
84. U.S. National Library of Medicine. Reference SNP (rs) Report rs2229765. Available in: https://www.ncbi.nlm.nih.gov/snp/rs2229765#frequency_tab (last access on 08/01/2019).
85. Graupp M, et al. Association of genetic variants in the two isoforms of 5 α -reductase, SRD5A1 and SRD5A2, in lean patients with polycystic ovary syndrome. *Eur J Obstet Gynecol Reprod Biol*. 2011 Aug;157(2):175-9.
86. Goodarzi MO, et al. Variants in the 5 α -Reductase Type 1 and Type 2 Genes Are Associated with Polycystic Ovary Syndrome and the Severity of Hirsutism in Affected Women. *The Journal of Clinical Endocrinology & Metabolism*. 2006, 91, 10; 4085–4091.
87. Severi G, et al. *Androgenetic alopecia in men aged 40-69 years: prevalence and risk factors*. *Br J Dermatol*. 2003 Dec;149(6):1207-13.
88. Trüeb RM, et al. *The impact of oxidative stress on hair*. *Int J Cosmet Sci*. 2015 Dec;37 Suppl 2:25-30.
89. Fortes C, et al. *The combination of overweight and smoking increases the severity of androgenetic alopecia*. *Int J Dermatol*. 2017 Aug;56(8):862-867.
90. Su LH, et al. *Association of androgenetic alopecia with metabolic syndrome in men: a community-based survey*. *Br J Dermatol*. 2010 Aug;163(2):371-7.
91. Kim BK, et al. *Gender-specific risk factors for androgenetic alopecia in the Korean general population: Associations with medical comorbidities and general health behaviors*. *Int J Dermatol*. 2018 Feb;57(2):183-192.
92. Almohanna HM, et al. The Role of Vitamins and Minerals in Hair Loss: A Review. *Dermatol Ther (Heidelb)*. 2018 Dec 13. doi: 10.1007/s13555-018-0278-6.
93. Rushton DH, et al. *Nutritional factors and hair loss*. *Clin Exp Dermatol*. 2002 Jul;27(5):396-404.
94. Upton JH, et al. *Oxidative stress-associated senescence in dermal papilla cells of men with androgenetic alopecia*. *J Invest Dermatol*. 2015 May;135(5):1244-1252.
95. Thompson JM, et al. *The Role of Micronutrients in Alopecia Areata: A Review*. *Am J Clin Dermatol*. 2017 Oct;18(5):663-679.
96. Kaya Erdogan H, et al. *The role of oxidative stress in early-onset androgenetic alopecia*. *J Cosmet Dermatol*. 2017 Dec;16(4):527-530.
97. Nargis T, et al. *Early onset androgenetic alopecia in men and associated risk factors: a hospital based study*. *Int J Res Dermatol*. 2017 Jun;3(2):267-271.
98. Prie BE, et al. *Oxidative stress in androgenetic alopecia*. *J Med Life*. 2016 Jan-Mar;9(1):79-83.
99. Prie BE, et al. *Oxidative stress and alopecia areata*. *J Med Life*. 2015;8 Spec Issue:43-6.
100. Philpott MP, et al. Effects of interleukins, colony-stimulating factor and tumour necrosis factor on human hair follicle growth in vitro: a possible role for interleukin-1 and tumour necrosis factor-alpha in alopecia areata. *Br J Dermatol*. 1996 Dec;135(6):942-8.
101. Naito A, et al. *Lipid peroxides induce early onset of catagen phase in murine hair cycles*. *Int J Mol Med* 2008; 22: 725–729.



102. Trüeb RM, et al. *Association between smoking and hair loss: another opportunity for health education against smoking?*. *Dermatology*. 2003;206(3):189-91.
103. Chalyk NE, et al. Continuous astaxanthin intake reduces oxidative stress and reverses age-related morphological changes of residual skin surface components in middle-aged volunteers. *Nutr Res*. 2017 Dec; 48:40-48.
104. Lance J, et al. *Coenzyme Q10--a therapeutic agent*. *Medsurg Nurs*. 2012 Nov-Dec;21(6):367-71.
105. Fairweather-Tait SJ, et al. *Selenium in human health and disease*. *Antioxid Redox Signal*. 2011 Apr 1;14(7):1337-83.
106. Park SY, et al. *Iron Plays a Certain Role in Patterned Hair Loss*. *J Korean Med Sci*. 2013 Jun; 28(6): 934–938.

Disclaimer

The contents of the TrichoTest™ Scientific validation document have been written with the greatest possible care. However, Fagron cannot guarantee the accuracy or completeness of the information. The content of Fagron's TrichoTest™ Scientific validation document therefore is not legally binding. Fagron accepts no liability which might arise from the content of the TrichoTest™ Scientific validation document