Original Contribution

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# Topical application of the Wnt/β-catenin activator methyl vanillate increases hair count and hair mass index in women with androgenetic alopecia

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## Summary

*Background* Activation of the WNT/β-catenin pathway has emerged as a potential therapeutic target in androgenetic alopecia (AGA). Methyl vanillate (MV) – a safe plant-derived ingredient – has been recently shown to activate the WNT/β-catenin signaling.

*Objectives* Two distinct substudies were conducted. First, we designed a 6-month, uncontrolled, open-label clinical study to investigate whether topically applied MV may increase hair count and hair mass index (HMI) in female AGA. Second, we conducted a molecular study on the effect of MV on WNT10B mRNA expression in scalp biopsies of women with AGA.

*Methods* A total of 20 Caucasian women (age range: 25−57 years) with AGA (Sinclair grade 1−2) were included. The research product was an alcohol-free formulation supplied in the form of a spray containing 0.2% MV as the active ingredient.

*Results* In the clinical study, hair count and HMI were found to increase at 6 months by 6% ($P < 0.01$) and 12% ($P < 0.001$), respectively, compared with baseline. No participant discontinued treatment due to adverse effects, and the overall patient satisfaction was good. At the molecular level, the topical application of the research product resulted in a 32% increase in WNT10B mRNA expression levels in the temporal scalp area ($P < 0.001$).

*Conclusion* Our pilot data suggest that topical MV can increase hair count and HMI by inducing WNT10B expression in the scalp, potentially serving as a novel treatment strategy for female AGA.

*Keywords*: androgenetic alopecia, female pattern hair loss, hair count, hair mass index, WNT/β-catenin, women

## Introduction

Androgenetic alopecia (AGA) is a common condition characterized by the replacement of terminal scalp

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hairs with smaller vellus hairs, ultimately resulting in progressive hair thinning.[1,2] The prevalence of AGA is generally high, with about half of men and women being affected above 40 years of age in the Caucasian population.[3] Increased androgen activity (especially 5-alpha-dihydrotestosterone) and genetic predisposition appear the main pathogenetic drivers of AGA.[1] Despite extensive research efforts, the currently approved therapeutic options for AGA are limited to oral finasteride in men and topical minoxidil in women.[4,5] Consequently, alternative treatment options are actively being pursued.[6,7]

WNT/β-catenin signaling is a widely conserved pathway known to be involved in embryonic development, adult homeostasis, as well as cell growth and proliferation.[8,9] Growing evidence indicates that hair follicle development, initiation of hair follicle formation, and hair growth are dependent on the activation of WNT/β-catenin signals.[10–17] Not only epidermal WNT can act as the initial trigger or hair follicle initiation,[13,15,17] but also WNT/β-catenin-dependent communications between dermal and epidermal cells are required for maintenance and growth of hair follicles.[10] Previous experimental studies have shown that the activation of the WNT/β-catenin pathway – and specifically of WNT10B – can maintain the dermal papilla in an anagen-stimulating milieu.[14,16] Taken together, these findings suggest that activation of the WNT/β-catenin signaling might represent a new potential treatment for AGA.[7]

Methyl vanillate (MV) – a plant-derived ingredient that can be used in cosmetic preparations – has been previously shown to activate the WNT/β-catenin pathway in human cells in a dose-dependent manner.[18] In this report, we intended to prove the concept that stimulation of this pathway by topical MV could serve as a potential strategy for promoting hair growth in AGA. To this aim, two distinct substudies were conducted. First, we designed a 6-month, uncontrolled, open-label clinical study to investigate whether topically applied MV may increase hair count and hair mass index (HMI) in female AGA. Second, we conducted a molecular study on the effect of MV on WNT10B mRNA expression in scalp biopsies of patients with AGA.

## Methods

### Clinical study

This 6-month uncontrolled pilot study had an open-label design. A total of 20 Caucasian female patients (age range: 25–57 years) with AGA (Sinclair grade[19] 1–2) were included. Women were enrolled from the Greater Miami Skin and Laser Center, Miami, FL, USA ($n$ = 10), and European private practices and dermatology clinics ($n$ = 10). Exclusion criteria were as follows: (1) age less than 18 years; (2) congenital or acquired hair shaft abnormalities or cicatricial alopecia; (3) pregnancy or breastfeeding; (4) major physical illness (i.e., cancer, renal impairment, liver failure, positive history of cardiovascular, neurological, or immunological disorders, active infections); (5) history of hair transplantation; (6) current or past (i.e., within 12 months from the beginning of the study) use of topical minoxidil; (7) current or past (i.e., within 12 months from the beginning of the study) systemic treatment for AGA (i.e., finasteride or dutasteride); and (8) current or past (i.e., within 12 months from the beginning of the study) use of topical prostaglandins or prostamide for hair loss. The study participants were not allowed to use other therapies for AGA during the 6-month treatment period.

The conduct of the study was carried out in accordance with the tenets of the Declaration of Helsinki. Written informed consent was obtained from all participants. All assessments were performed at baseline and 6 months thereafter. The primary endpoint of the clinical study was the change in hair count and HMI from baseline to the end of the study. The secondary endpoints were tolerance and overall satisfaction with the topical spray.

The research product was an alcohol-free formulation supplied in the form of a spray containing 0.2% MV as the active ingredient. The study participants were instructed to apply approximately 2 mL of the research product to the vertex area of the scalp (8 pumps per day) every other day for a total of 6 months. Patients were required to apply the product on clean and dry hair, if necessary parting their hair to expose the scalp. The subjects were asked to gently massage the solution throughout the area of hair loss to ensure coverage of the entire area of hair thinning.

Hair count before and after treatment with topically applied MV were measured with TrichoScan (Tricholog GmbH, Freiburg, Germany).[20] The procedure was carried out according to the manufacturer's protocol. The recorded photographs were automatically analyzed using the TrichoScan software, version 3.0, and the results expressed as number of hair detected. The HMI – a measure of hair mass that depends on hair density (number of hair fibers) and hair diameter – was measured using a commercially available trichometer

(HairCheck® System; Divi International Co., Miami, FL, USA).[21] Tolerance was assessed by asking patients about any signs or symptoms of local (burning, itching or stinging sensation) or systemic adverse reactions. The overall satisfaction with the topical spray was rated on 4-point scale, as follows: excellent, good, average, or poor.

### Molecular study

Four-mm$^2$ punch biopsies before and after treatment (paired samples) were removed from a well-defined temporal scalp area in the 10 patients recruited from European private practices and dermatology clinics. All specimens were collected after obtaining written informed consent under Institutional Review Board approval. RNA isolation was performed using the RNeasy Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's protocol. The integrity of RNA was analyzed with agarose gel electrophoresis and its quantity measured by spectrophotometry. A 1 μg amount of RNA was reverse-transcribed using the iScript cDNA Synthesis Kit (Bio-Rad, Hercules, CA, USA) according to the manufacturer's instructions, and the resulting cDNA was stored at −20 °C. All quantitative real-time polymerase chain reactions (qRT-PCRs) were carried out on a Bio-Rad iQ5 Cycler (Bio-Rad). In brief, a 25 μL reaction solution consisted of iQ SYBR Green Supermix (Bio-Rad), forward and reverse primers (final concentration 400 nM each), and cDNA mixture (40 ng). The conditions for qRT-PCR were as follows: preheating at 94 °C for 12 min, followed by 50 cycles of 94 °C for 15 s, 55 °C for 40 s, and 72 °C for 25 s. Analysis of fluorescence data was performed using the Bio-Rad iQ5 Optical System Software Version 2.0. The housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the internal standard. The primers used in this study are as follows: GAPDH, 5′-CGCTCTCTGCTCCTCC TGTT-3′ (forward) and 5′-CCATGGTGTCTGAGCGATG T-3′ (reverse); WNT10B, 5′-GGTCCACGAGTGTCAGCA C-3′ (forward) and 5′-GGAAAAAGCACTTTCTCGGA-3′ (reverse). The mRNA expression levels were expressed in arbitrary units and calculated according to the following formula: $2-\Delta CT$, where ΔCT (sample) was defined as CT (WNT10B) − CT (GAPDH).

### Data analysis

The power was calculated using the program GraphPad StatMate 2.0 (GraphPad Inc., La Jolla, CA, USA). To this aim, the HMI was used as the main endpoint. Our experiment had a 80% power to detect a smallest average difference between 10 pairs of 14.18 for HMI with a significance level (alpha) of 0.05 (two-tailed). Data are expressed using descriptive statistics. Pre- and post-treatment data were compared with paired Student's *t*-tests. Correlations between the study variables were tested with the Spearman's correlation coefficient. All calculations were performed with the Statistical Package for the Social Sciences software, version 17.0 (SPSS Inc., Chicago, IL, USA). A $P$ value <0.05 (two-tailed) was considered statistically significant.

## Results

### Clinical study

All patients successfully completed the study. Hair count significantly increased after 6 months of treatment with topically applied MV; the mean hair counts were 40.2 ± 6.7 (range, 26–49) at baseline and 42.5 ± 7.8 (range, 24–52) at 6 months. The mean increase in total hair count from the baseline to 6 months was 2.3 (95% confidence interval, 0.7–3.9, $P < 0.01$, paired Student's *t*-test, Fig. 1). The mean HMI significantly increased after 6 months of treatment with topically applied MV; the mean HMI was 65.6 ± 15.2 (range, 38.0–68.0) at baseline and 73.2 ± 18.2 (range, 42.0–115.0) at 6 months. The mean increase in the HMI from the baseline to 6 months was 7.6 (95% confidence interval, 4.5–10.7, $P < 0.001$, paired Student's *t*-test, Fig. 2). The treatment was well tolerated and none of the patients reported burning, itching, or stinging sensation after topical application. No patient discontinued treatment due to adverse local or systemic effects. The overall satisfaction with the topical spray was rated as excellent by seven patients (35%), good by eight patients (40%), average by three patients (15%), and poor by two patients (10%).

### Molecular study

WNT10B expression in the scalp in the 10 women who underwent paired biopsies significantly increased after 6 months of treatment with topically applied MV; the mean WNT10B expression levels were 88.2 ± 11.7 (range, 56–102) at baseline and 116.3 ± 14.6 (range, 72–155) at 6 months. The mean increase in WNT10B expression from the baseline to 6 months was 28.1 (95% confidence interval, 12.4–51.7, $P < 0.001$, paired Student's *t*-test), that is, a 32% increase. Notably, a significant association was found

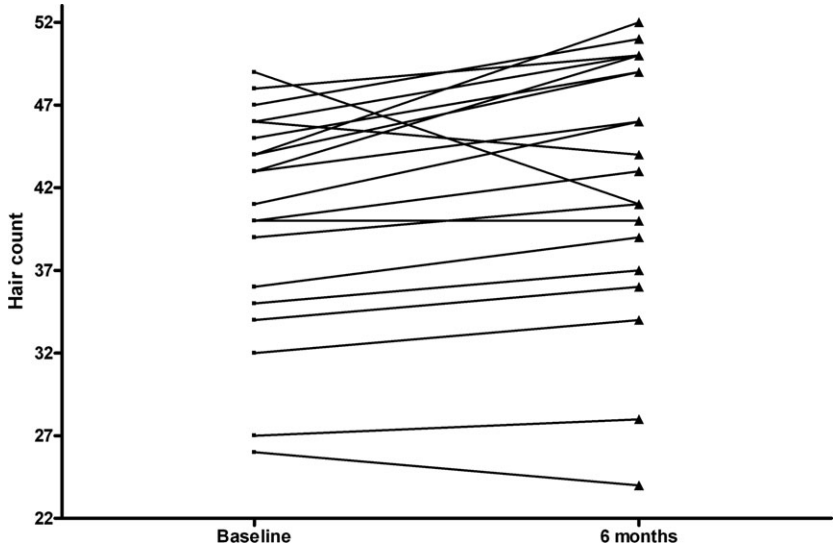


**Figure 1** Changes in hair count from baseline to 6 months in the study participants ($P < 0.01$).

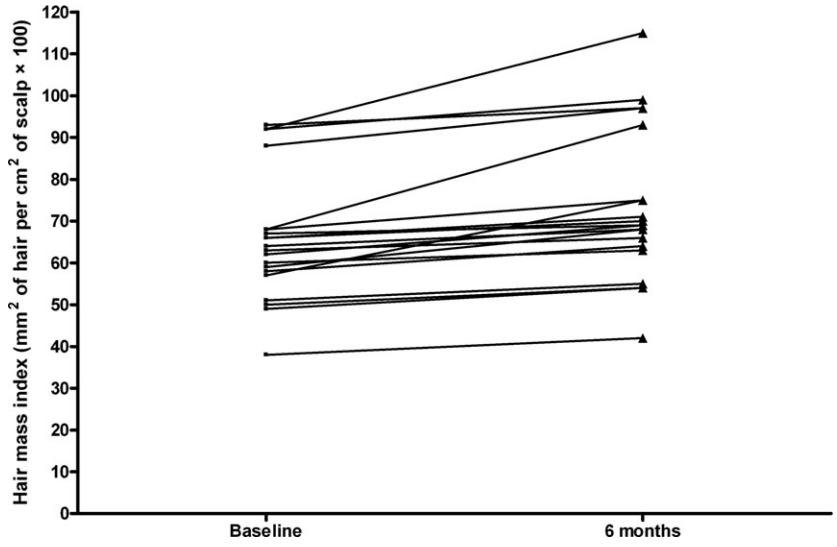


**Figure 2** Changes in hair mass index from baseline to 6 months in the study participants ($P < 0.001$).

between changes in WNT10B expression in scalp biopsies and both hair count (Spearman's rho = 0.52, $n = 10$, $P < 0.05$) and HMI (Spearman's rho = 0.44, $n = 10$, $P < 0.05$). The greater the increase in WNT10B expression, the higher the increase in both hair count and HMI.

## Discussion

The results of our clinical and molecular studies indicate that topically applied MV – a safe plant-derived ingredient[18] – may increase both hair count and HMI by promoting WNT10B mRNA expression in the treated scalp of women with AGA. We also identified significant positive correlations between the increase of WNT10B expression in the scalp and the magnitude of increase in both hair density and HMI. Our results suggest that the clinical effects of MV on the hair may be related to its capacity to promote WNT10B expression. Notably, the treatment approach was safe and associated with good satisfaction levels.

The WNT/β-catenin pathway has recently received attention as a therapeutic target in AGA because androgens have been shown to inhibit its expression.[7] Topical

valproate – a known activator of WNT signals – has been shown to promote hair regeneration and induce the expression of dermal papilla markers.[22,23] In an organ culture model, valproate increased the viability of human dermal papilla cells, promoted elongation of the hair shaft, and reduced catagen transition of human hair follicles.[23] Interestingly, a 24-week clinical feasibility study has recently shown that 8.3% topical valproate can increase total hair count of AGA patients.[24] MV is a natural activator of the WNT/β-catenin signaling pathway. In this study, topical MV was found to increase by 32% the scalp expression of WNT10B, a component of the WNT/β-catenin pathway that promotes the biological switch of hair follicles from the telogenic phase to the anagenic phase.[25] We thus speculate that the observed effects of MV on hair count and HMI may be mediated by an increased expression of WNT10B in the scalp. Although the current results are preliminary and need to be confirmed by large placebo-controlled trials, our studies can be considered as a pilot exploratory proof-of-concept analysis of the WNT/β-catenin pathway as therapeutic target in AGA. Independent replication is necessary to confirm and expand the main findings.

Several caveats of our study merit comment. The lack of a placebo arm, the exclusive focus on Caucasian women, and the fact that scalp biopsies were performed only in a subset on participants ($n$ = 10) are the main limitations of the present research. Currently, our results should not be considered as a basis for treatment recommendations. AGA patients should be treated on an individual basis according to each patient's characteristics based on the results of well-designed clinical trials. Further research including men will be necessary to perform a sex-based analysis of the effects of topically applied MV on hair growth. Our study was also limited by the fact that we did not compare the effect of topically applied MV with that of topical valproate, another molecule known to activate the WNT pathway and potentially useful in AGA.[24] Another significant limitation is the lack of measurement of androgen receptor (AR) expression – a well-known antagonist of WNT/β-catenin pathway signaling in epidermal stem cells[26] – in scalp biopsies. Moreover, we did not specifically assess the expression of dickkopf-1, a powerful suppressor of the WNT/β-catenin signaling pathway whose tissue levels have been reported to be significantly higher in patients with AGA than in controls.[27]

## Conclusion

The results of our clinical and molecular studies indicate that topically applied MV significantly increased hair count and HMI compared with baseline after 6 months of topical treatment. This suggests that the WNT/β-catenin pathway may serve as a therapeutic target in AGA. More research is necessary to determine the optimal dose and treatment duration with MV or other candidate topical WNT activators. The question as to whether systemic agents specifically activating the WNT/β-catenin pathway may have therapeutic implications for AGA remains open. Because these compounds have only recently entered clinical trials,[28] it will be important to investigate whether the artificial modulation of the WNT/β-catenin pathway may be useful for promoting hair growth.

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