

## **Where do they come from? Predictive value of baseline parameters?**

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As far as scalp hair regrowth is concerned, we alluded to 2 recurrent questions about drug efficacy in the manuscript:

- \* where do the re-growing hair come from?
- \* is there a baseline characteristic that might tell us something about the potential for a better future of scalp hair follicles that would be inducible by drug treatments?

### **Preliminary approach and think-tank for future studies**

The test zone on the scalp of 1 MPHL test-subject (on finasteride treatment for several years<sup>[1-2]</sup>) displayed a population of 93 follicular units (FU; numbered in figures 11 and 12 in manuscript). Albeit exogen were removed before imaging as part of our phototrichogram analysis, we decided to trace exclusively growing hair so as to avoid inadvertent inclusion of passively retained hair i.e. trichostasis.

On top of the ongoing oral drug-treatment, the test-subject applied topical minoxidil 5% lotion once/day during 3 months. Within 1 month of combined oral finasteride and topical minoxidil 5% applications the baseline hair counts 123, 39, 16 became 131, 49 and 21 with diameters respectively in the 20-30 $\mu$ m, 40-50 $\mu$ m, 60-70 $\mu$ m categories. Two months later i.e. after complying with the daily treatments for 3 months, there were 45, 72 and 52 hairs in the same categories plus 2 extra hairs with a diameter  $\geq$  80 $\mu$ m.

According to the prevailing theory where drug treatments work by 'reversal of miniaturised hair follicles' i.e. the reduction of thinner hair has been considered as proof for reversal. At first glance, our data seem to support this view but an all too rapid interpretation must be considered with caution in the realm of sound scientific methodology.

From the original work referred to in the manuscript, we know that the number of thicker re-growing hair exceeds by far the minimal reduction of absolute counts of miniaturised hair! We also knew of the arbitrary decision made by the late D. Whiting who counted no more than 3 hair per follicle (personal communication). There is no reason to reject this threshold except for the fact that we knew already the present test-subject had follicles bearing multiple hair.

In a previous longitudinal assay in the same subject, we kept in line with D. Whiting's proposal and monitored follicles bearing  $\leq$  3 hair fibres. The miniaturised follicles did not improve productivity in terms of thickness, duration of growth, lag period between anagen phases. The latter 3 criteria were significantly better when isolated follicles produced intermediate hair fibres during 'no-drug' baseline period. The results of 48 months follow-up are shown in Figure 1S3 (adapted from author's paper<sup>[1]</sup>).

**Figure 1S3. Miniaturised scalp hair follicles resist finasteride induced improvement as opposed to hair follicles able to grow intermediate or terminal hair at baseline.**

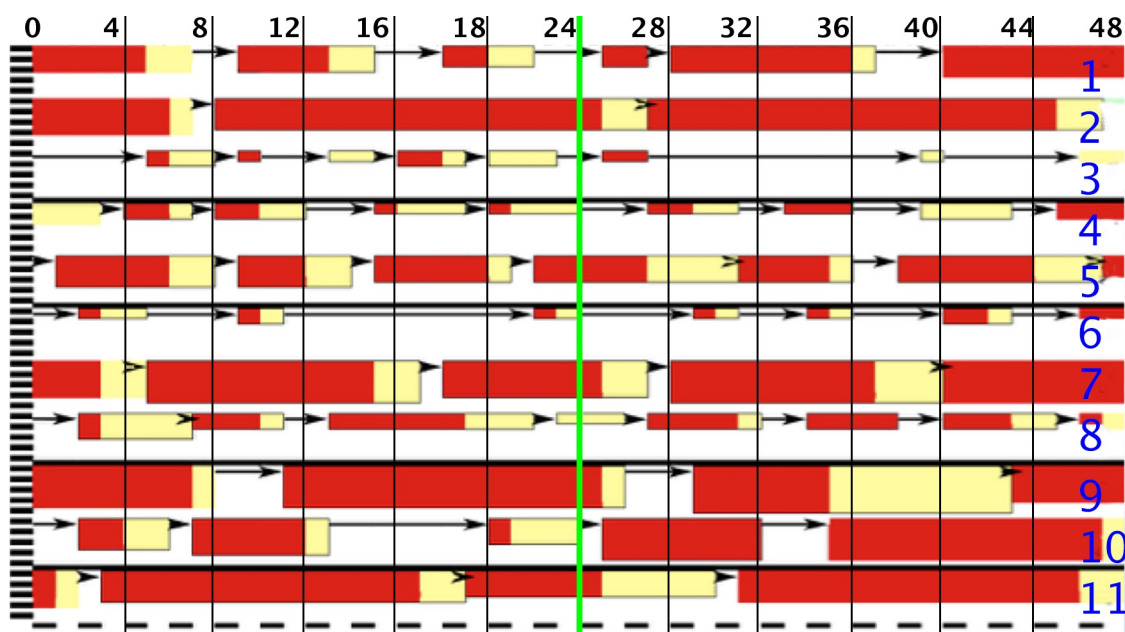


Figure 1S3 displays re-processed results of long-term follow-up of single hair fibres (numbered in blue on the right)<sup>[1]</sup>.

Time is displayed as number of months (on top line) and symbolised as black and white bars at the bottom (1 month each). Thin vertical lines (black) split spaces at 4-months intervals while thicker vertical line (green) splits a 2 year baseline monitoring from the 2 years on drug periods. The black and white bars on the left represent a micrometric scale (10  $\mu\text{m}$  each). It helps tracking maximum thickness reached by individual hair during 1 hair cycle. Colour code is as follows : red for anagen, yellow for telogen, no colour space with arrow points to empty follicle. Daily oral intake of finasteride 1 mg/day was introduced as from month 24 until month 48.

The thicker black horizontal parting lines separate hairs from 5 different follicular units. These units produced respectively hair n° 1, 2 and 3, n°4 and 5, n° 6, 7 and 8, n° 9 and 10 and finally a single hair n° 11.

Each complete hair cycle appears outlined by a rectangle encompassing time spend in anagen and telogen at the exclusion of exogen. The empty phase is shown as a clear space with arrow interconnecting two rectangles. Incomplete cycles beginning before the entry into the study (left margin) or extending beyond 48 months (still ongoing at the right margin), are not outlined.

Hair n° 1, 2, 5, 7, 9, 10 and 11 were considered 'intermediate or terminal'. Thinner hair fibres n° 3, 4 and 6 were in regression during no-drug period and the administration of finasteride did not prevent further regression of the thinner hair. Oral drug intake improved productivity of intermediate hair. For full details the reader is referred to the original report<sup>[1]</sup>.

In the present speculative approach we attempted the opposite point of view as compared with D. Whiting's suggestion. Instead of discarding those FU exceeding 3 hair, we selected from our population follicular units, a subsample bearing at least 3 growing hair per follicular unit. The samples were split into 2 mutually exclusive groups: 10 follicles (sample A) bearing exclusively 3 or more

miniature hairs ( $\leq 30\mu\text{m}$ ) on at least 1 occasion at the 2 baseline phototrichograms and 21 other follicular units containing also exclusively 3 or more hairs but showing a variety of diameters. Statistics are shown in Tables 1S3 and Figures 2S3 and 3S3.

## Results

From the data in Tables 1S3 and Figures 2S3 and 3S3 it is clear that miniaturised hair follicles were much less prone to produce terminal hair as compared with FU capable – during the ‘no-drug’ baseline run-in week – to produce a mixture of thinner, intermediate or terminal hair.

**Table 1S3** Statistics of growing hair counts at baseline in a finasteride-treated MPHL subject and after 1 or 3 months (m1, m3) of daily combination with topical application of minoxidil lotion (1/day).

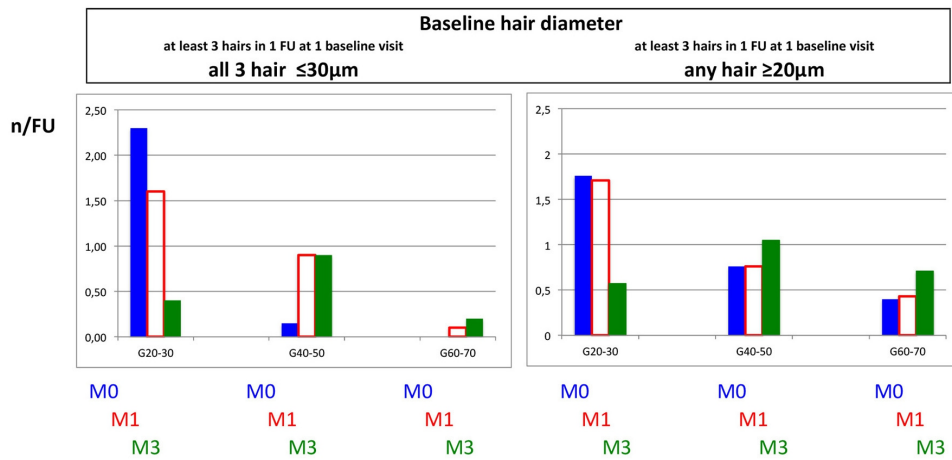
Baseline parameters				Absolute counts of growing hair per FU according to diameter						
Number	Diameter	Sample		G 20-30µm		G 40-50µm		G 60-70µm		Total per FU
m0 (average)	≥3	≤30µm	A (10)	2.30		0.15		0.00		2.45
		≥40µm	B (21)		1.76		0.76		0.40	2.93
m1	≥3	≤30µm	A (10)	1.60		0.90		0.10		2.60
		≥40µm	B (21)		1.71		0.76		0.43	2.90
m3	≥3	≤30µm	A (10)	0.40		0.90		0.20		1.50
		≥40µm	B (21)		0.57		1.05		0.71	2.33
				Average relative counts (% of baseline growing hair per FU according to diameter)						
m1 %	≥3	≤30µm	A (10)	70		600		N.A.		106
		≥40µm	B (21)		97		100		106	99
m3 %	≥3	≤30µm	A (10)	17		600		N.A.		61
		≥40µm	B (21)		32		138		176	80

Table 1S3 details statistics of variations (absolute in 6 top lines, relative in the 4 bottom highlighted lines) in growing hair counts.

The average anagen hair per follicular unit at baseline (m0) in a finasteride-treated MPHL subject are shown comparatively after 1 or 3 months (m1, m3) of daily topical application of minoxidil lotion in 2 samples (A, B).

Follicular units in sample A or sample B were mutually exclusive. The baseline averages were from 2 phototrichograms recorded at 1 week interval i.e., 1 week before and at entry into the topical treatment phase (m0) and growing intermediate hairs were only rarely found in such follicles (average of 40-50µm hairs during 2 baselines : 0.15). FU in A were able to mount a minoxidil response however mainly limited to intermediate hair. The miniaturised hair (G 20-30µm) faded away especially at m3 in all sub-groups (A and B). In terms of total hair counts, there was a highly statistically significant difference at m3 between samples A and B.

**Figure 2S3 : Baseline predicting parameter: number and diameter of growing hair per FU**



Samples growing predominantly miniaturised hair at baseline (left panel ; sample A baseline at least once all hair  $\leq 30\mu\text{m}$ ) produced much less terminal hair per FU after 3 months of combined drug treatment as compared to the therapeutic response of FU bearing a diversity of hair including intermediate and terminal hair at baseline (right panel ; sample B any hair  $\geq 20\mu\text{m}$ ).

**Figure 3S3 : Predictive value of baseline parameters for response to topical minoxidil**

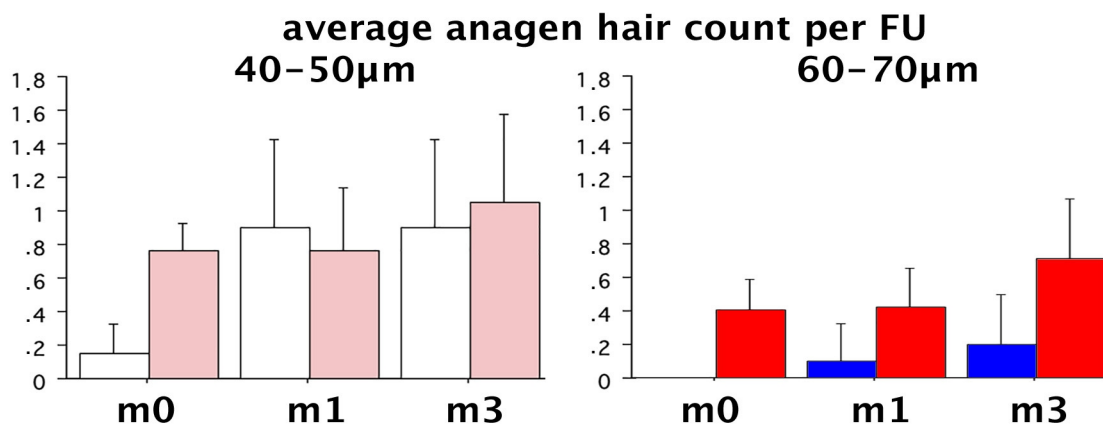


Figure 3S3 illustrates averages (+95% interval bar) of anagen hair growing per FU at baseline (m0) or after stimulation by topical minoxidil (m1, m3).

When hair follicles grew essentially miniaturised hair (baseline panels empty bars, panel on the left; 3 hair  $\leq 30\mu\text{m}$  at least 1 out of the 2 baseline visits), they showed moderate induction of intermediate hair regrowth but in almost equal amounts as follicles bearing a variety of hair ranging from 20 to 70 $\mu\text{m}$  at baseline (pink bars, panel on the left).

However, as shown in the panels on the right, baseline growth of thicker terminal hair (60-70 $\mu\text{m}$ ) was inexistent in the former FU (sample A) at baseline. Induction of growth by minoxidil, the averages were very low (plain blue bars at m1 and m3) as compared with the FU bearing already some terminal hair at baseline visits (pink red bars).

Statistically significant variations established by ANOVA were as follows:

Between A-B n° of growing fibers 40-50  $\mu\text{m}$  (NS), 60-70 $\mu\text{m}$  (P=.0002) and Total (P=.0052) which is obvious by design. Changes over time growing fibers 40-50  $\mu\text{m}$  (P=.007), 60-70 $\mu\text{m}$  (NS) and Total

( $P=.0001$ ) with interactions time\*sample (A or B) growing fibers 40-50  $\mu\text{m}$  ( $P=.0841$ ) with significance between m0-m3 ( $P=.0086$ ; Bonferroni-Dunn). There was also a trend for growing fibers 60-70 $\mu\text{m}$  ( $P=.0220$ ; Bonferroni-Dunn) and a statistically significant change between m0-m3 and m1-m3 for total growing hair counts per FU ( $P=.0008$  and  $.0022$  respectively; Bonferroni-Dunn).

In summary,

- maintenance and/or induction of growth of slightly thicker hair at m1 faded rapidly away in poorest FU in sample A as compared to richer FU in sample B,
- in B the maintenance of a higher number of thicker hair as compared with A indicates a predictable less favourable response when miniaturised hair were predominantly present during the baseline investigations .

We acknowledge that follicles that contained a larger number of miniaturised hair at baseline did occasionally show growth of thicker hair but this was a very short cycling one i.e. a poor result on minoxidil (see also main manuscript for the details on thickest terminal hair).

These findings might serve as an indicator of viability which keeps in line with our earlier conclusions<sup>[5]</sup>.

## Conclusion

We acknowledge that histopathology is mandatory for diagnosis and for the observation of inter-adnexial changes<sup>[3]</sup>. At the same time, we maintain that it is impossible to predict the future from invasive sampling methods like scalp biopsies because of their destructive nature. Dynamic data on follicular performance are missing and destruction also prevents longitudinal studies. This methodological approach appears inappropriate for speculating on reversal i.e., a time-related change of hair follicle performances<sup>[3]</sup>.

In contrast, in previous publications minimal baseline follicular requirements were alluded to in terms of favouring a better therapeutic response<sup>[1,5]</sup> and the findings from our sampling method keep in line with this concept.

It is worth mentioning that we know about the future of the follicles in this particular male with MPHL, as the exact same scalp site was evaluated for several years after the 3 months assay described herein. They maintained growth as miniaturisation was prevented by oral intake of finasteride until interruption of oral drug treatment. After about 18 months without drug, almost all follicles in the probed area engaged into miniaturisation<sup>[4]</sup>.

We hypothesised that after many years on finasteride, the regular cycling and follicular performance became drug dependent. However once follicles engaged in the anagen phase of the hair cycle at the time of drug arrest, growth went on and follicles accomplished their 'programmed' duty. They dropped into massive miniaturisation after completion of the 1st cycle 'off drug'.

The author discussed the matter and received permission to include herein a private communication by A. Messenger. On June 3rd, 2019, he commented our paper on drug dependency and rebound after drug arrest<sup>[5]</sup> as follows:

*'If my understanding is correct -*

- terminal hair growth was maintained and somewhat improved during the finasteride treatment period but there was no change in the vellus population. This was essentially what you and Hugh had reported in your 2016 Exp Derm paper and contradicts the previous claims that finasteride (and minoxidil) 'reverses' miniaturisation. These claims were always based on an increase in mean hair diameters but presumably this was due to a combination of earlier recruitment of resting terminal hairs into anagen and an increase in terminal hair diameter rather than a change in the vellus population. I had long thought this would be the case due to the speed of response to minoxidil, which seemed too quick to be explained by reversal of miniaturisation.*
- the response to finasteride was maintained for at least a year after treatment was stopped suggesting the effect of the drug on hair growth occurs either during telogen or early anagen and is not required once anagen is established. I seem to recall a similar thing has also been proposed for the effect of antiandrogens in hirsutism.*
- once follicles destined to regress go into telogen following finasteride withdrawal there is rapid miniaturisation, presumably in the next cycle. If I understand correctly, this exceeds what one would expect from the predicted natural regression i.e. there is a rebound phenomenon. It also raises the question as to whether miniaturisation is normally a single-cycle event rather than the usually-depicted gradual process over several cycles. I think we had suggested this in our 2001 paper on FPHL as did David Whiting previously though I was never sure how he arrived at this conclusion.*

*One other observation we made years ago but never got round to publishing - we mapped the distribution of major axis diameters in the hair samples we took from 200+ women. The distribution in any individual was rarely normal, there were peaks at various diameters and the distribution of these peaks varied between individuals. We also did the same in a small number of men with vertex thinning, taking samples from the thinning area and from the occipital scalp. The pattern of the peaks was the same in both sites except that the larger diameter peaks were diminished in the vertex samples compared to the occiput. There was no comparable increase in the number of smaller diameter (terminal) hairs. This suggested to me that, in male balding, there is preferential loss of large diameter hairs but no shift of large diameter hairs to smaller i.e. they are either spending longer in a latent state or miniaturise abruptly. We never did enough to confirm the observation and probably never will!'*

Andrew Messenger concluded his email that this type of research requires:

*'...dedication over many years. We can learn much from longitudinal observations but they are few and far between and hard to do.'*

## **References in Supplement 4**

1. Van Neste D. Natural scalp hair regression in preclinical stages of male androgenetic alopecia and its reversal by finasteride. *Skin Pharmacol Physiol* 2006;19:168-76.

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