ANTHROPOLOGICAL NOTEBOOKS

Vol. 27, Issue 2, pp. 33-45, ISSN 2232-3716.
DOI: https://doi.org/10.5281/zenodo.5812129
Research article

Variations in count and width of the human middle phalangeal hair: A microscopic study

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Abstract

The aim of the present pilot study was to analyse human middle phalangeal hair (MPH) at the microscopic level and to study relationships between MPH width and 2D:4D ratio. The relationships between MPH width and 2D:4D ratio have not been studied so far. The pilot sample consisting of 28 volunteers (16 males) was subjected to hand scans, counting their MPH numbers and collecting hair samples. The MPH widths were measured at the microscopic level, and their variations related to sex, body side, finger and the 2D:4D ratio, as measured based on the hand scans, were studied by means of a linear mixed-effects model. In the 3rd and the 4th fingers, we found significant sex differences with higher counts in males, and significant asymmetry in the counts in males with higher counts on the 4th right finger. Males had wider MPH shafts than females. In both sexes, significantly wider hair shafts were found on the 4th finger compared with the 2nd finger. No significant relationship was found between MPH shaft width and the 2D:4D ratio. The present study found that MPH shaft width was significantly different between the sexes and between the 2nd and 4th fingers of both hands.

KEYWORDS: middle phalangeal hair, androgens, microscopy, hair shaft width, 2D: 4D ratio

Introduction

The presence of visible middle phalangeal hair (MPH) on the dorsal side of the middle phalanges of the human fingers has been recognised as a potentially important feature, related to androgen levels, health, and human behaviour (Oinonen, 2009). Variations of MPH related to sex, ethnicity, and age have also been found (Danforth, 1921; Garn, 1951).

Genetic studies of families showed that presence/absence of MPH is related with recessive allele A_0 and alleles with increasing dominance A_1, A_2, A_3 and A_4 (Bernstein & Burks, 1942). Therefore, MPH is a continuously variable feature regarding the numbers and width of the hair (McDonald 2011).

Moreover, the occurrence and detail characteristics of MPH can be affected by environmental factors (McDonald, 2011), endogenous factors including sex steroids and sensitivity of tissues (Blahoš & Zamrazil, 2006). Variations in sex steroids are regarded as proximate factors of the recorded relationships of MPH with sex and age (Oinonen, 2009). In general, hormonal levels have a pronounced effect on body hair. Due to the action of 5α-reductase on testosterone, dihydrotestosterone (DHT) is formed, and it has a strong effect on sensitive/dependent cells (Blahoš & Zamrazil, 2006; Westlund et al., 2015; Winkler & Christiansen, 1993). Also, hormonal effects vary due to different regulation mechanisms in different parts of the body, e.g. DHT has a positive effect on MPH growth, but a negative effect on pubic hair growth in some populations (Winkler & Christiansen, 1993).

MPH is usually investigated as a binary variable, which means that most often, its presence or absence is recorded (Danforth 1921; Garn 1951). Differences in the presence of hair have been also recorded on different fingers. The most numerous MPHs appear on the 4th finger, while they are least frequent on the 2nd finger (Danforth 1921). There is a certain similarity between the variations in the 2nd and the 4th digits (2D:4D ratio).

For the 2D:4D ratio, it applies that the length of the 4th finger is more influenced by levels of prenatal androgens than that of the 2nd finger (Manning, 2002). The values of the 2D:4D ratio within the population overlap between sexes, but in general, males have lower 2D:4D ratios more often than females (Manning et al., 2000). Since more androgen receptors are expressed in the 4th finger during embryonic development (in mice and probably also in man), higher levels of prenatal androgens cause more intensive growth of the 4th finger in males (Zheng & Cohn, 2011).

To our present knowledge, research addressing MPH on the microscopic level and the relationship between MPH shaft width and the 2D:4D ratio for both sexes has not been presented yet. The previous study (Oinonen, 2009), which studied the relationship between MPH and 2D:4D ratio has been performed only on a sample of women and based on MPH counts, not MPH shaft width. For this reason, we decided to research these aspects of the MPHs in the present pilot study.

Materials and methods

The subjects were selected from the Czech Republic population as a convenience sample.

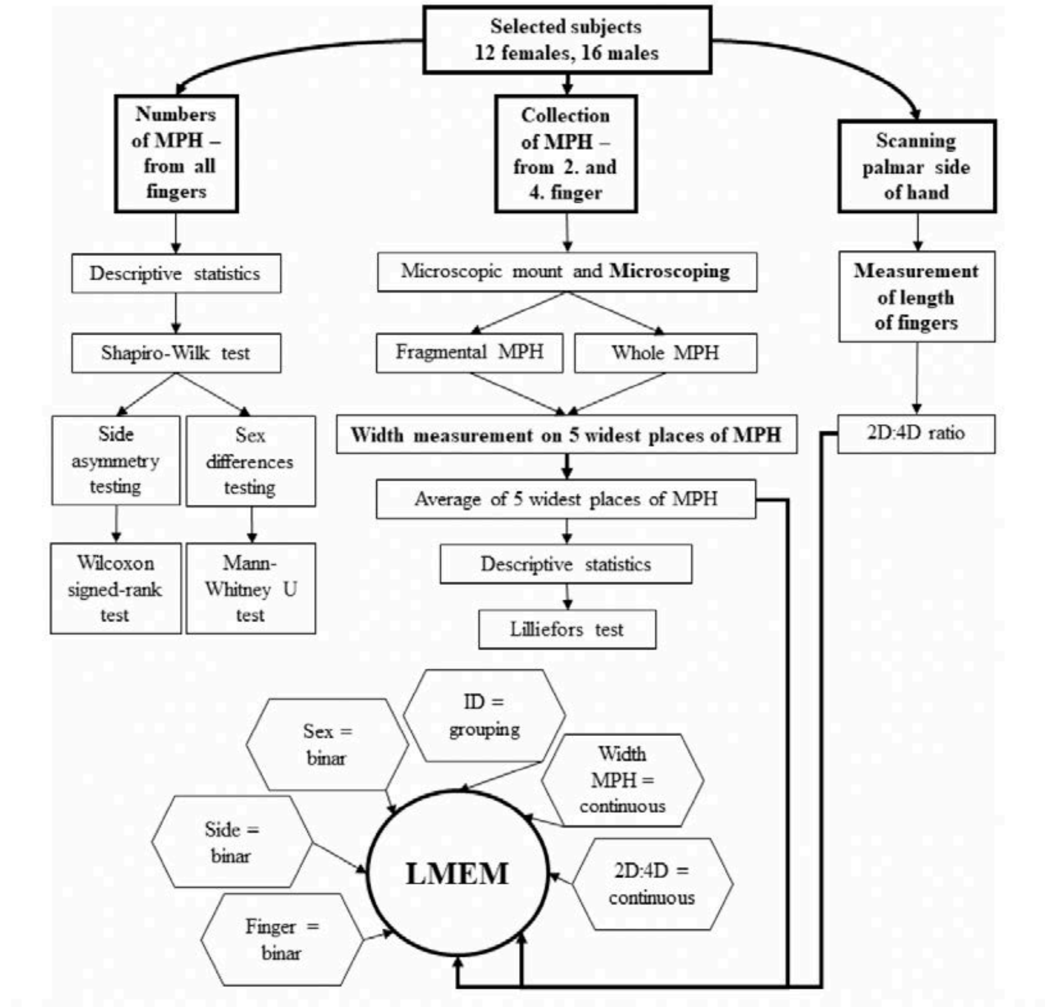

Figure 1: The flowchart of approaches and statistical tests which were applied on a specific subsample; the main statistical output was the linear mixed-effects model (LMEM)

The final sample consisted of mainly university students rather than a cross section of the general population. The subjects participated voluntarily in the study and signed an informed consent form. The final pilot sample was made up of 16 males (57.14 %) with a mean age of 21 ± 1.39 (SD) years, and 12 females (42.86 %) with a mean age of 21 ± 1.62 (SD) years. The minimum age was 19 years, and the maximum age was 25 years for both sexes. Each subject filled out an entrance questionnaire which was designed to exclude those who ingested any form of substance that may affect the growth of hair or the skin (exogenous hormones, alcohol, immunosuppressive and cytostatic drugs, cigarettes). Transsexuals, patients with endocrine disorders, psychiatric and chronic diseases were also excluded. Included subjects were without any previous hair treatment or removal and they were questioned on sex, age, and ethnicity. In this sample, the MPHs were counted, harvested, and subsequent analytical procedures were applied. See Figure 1 for a flowchart of the study.

MPH counting and microscopy

MPH were counted directly on the middle phalanges of fingers of both hands in appropriate lighting using a technical magnifying glass. All present MPHs were collected from the 2nd and 4th fingers of both hands and cosmetic tweezers (made by Heiko Wild, Tuttlingen, Germany) were used to collect them. Then MPHs were immediately placed on microscope slides.

In order to temporarily mount the specimens, microscope slides and cover glasses (made by Paul Marienfeld GmbH & Co. KG, Germany) were used, with paraffin oil as a mounting medium. The sample was studied under the Nikon Eclipse 50i microscope (made by Nikon Corporation, Shinagawa, Tokyo, Japan) with a Nikon DS Fil digital camera. The MPHs were photographed with 100 and/or 200-time magnification, with scales of 500 μm and 200 μm scale respectively. In both magnifications, every hair shaft was photographed from its basal end to the apex and the maximum width of each hair shaft was measured using the ImageJ software (developed by Rasband et al., Maryland, 2007). The widest parts (basal ends) of non-damaged MPHs were measured in five places on the image, approximately equidistant from each other and perpendicular to the axis of the particular hair shaft (Figure 2).

The reliability of the measurement was determined on 30 randomly selected MPHs. The measurement was repeated, and reliability parameters were computed (technical error of measurement = 0.37 μm; relative technical error of measurement = 1.19; reliability coefficient = 0.99). An appropriate level for the subsequent metric analysis was ascertained.

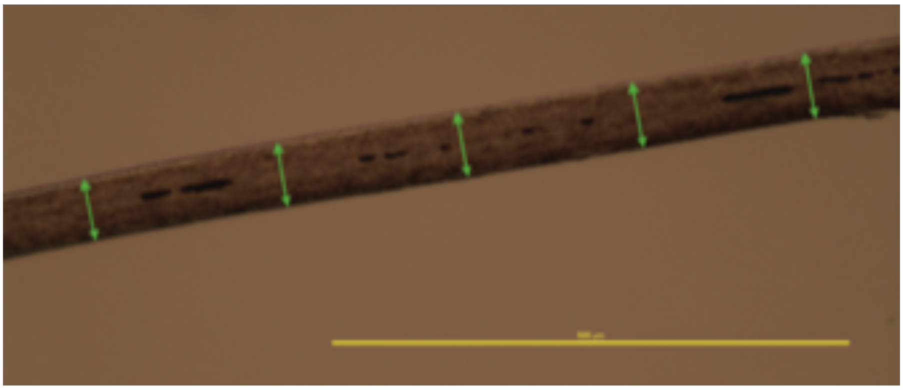

Figure 2 The widest part (basal end) of MPH shaft measured in five places

Hand measurements

The hand measurements followed the method used by Králík et al. 2014. Both hands were scanned using a CanoScan 4200 (made by Canon Inc., Tokyo, Japan) in a standardised position. Four landmarks were recorded: at the centre of the 2 metacarpophalangeal joint creases and at the distal ends of the 2^{nd} and 4^{th} fingers. The fingers lengths were computed, and the 2D:4D ratio was calculated from them.

Statistical analyses

All statistical procedures were processed using the R (developed by R Core Team, Vienna, Austria, 2018) software with some additional packages, namely: nortest: Tests for Normality, for the Lilliefors test (developed by Gross & Liggens, 2015) and nlme: Linear and Nonlinear Mixed Effects Models, for the linear mixed-effects models (developed by Pinheiro et al., 2017) The significance level was set at α 0.05 for all tests.

Middle phalangeal hair counts

When the Shapiro-Wilk test rejected the normal distribution, median tests were performed. Sex differences in hair counts were tested in each finger separately by means of the Mann-Whitney U test. To test the bilateral asymmetry, the Wilcoxon signed-rank test was used.

Statistical analysis of hair shaft width

Due to the hierarchical arrangement of the data (measurement—hair shaft—finger—subject) with complex correlation structure between the levels, a linear mixed-effect model (LMEM) was used to evaluate the data. We presumed a correlation within subject measurements, but no correlation between subjects. We defined the following dependent continuous variable – arithmetic mean of the five widest measurements of the individual MPH shaft width (entered into the LMEM) and 5 explanatory variables: subject ID (individual number of subjects), body side (right, left), finger (2nd, 4th), sex (male, female), 2D:4D ratio (continuous variable). For the model, reference groups were determined: male, 2nd finger, and the right side and the grouping variable (random effect) was the ID. The LMEM without specification of correlation matrix and with specification of covariance matrix was used (enables different variances between levels for the variable sex). For the parameter value search, the restricted maximal likelihood (REML) method was used. For quality test of LMEM, the following were used: the Lilliefors test (testing normal distribution of residues), plots (graphical assessment of standardized residues independence), the compliance of the fitted values with the measured values, rating by the Akaike information criterion and the Bayesian information criterion.

Results

Number of middle phalangeal hairs

Of the total 28 subjects (16 males with MPHs n = 1 to 34, 12 females with MPHs n = 1 to 25), 23 had MPHs, and 5 (3 males and 2 females) did not. Table 1 shows descriptive statistics of MPH counts for each finger. The Mann-Whitney U test showed a statistically significant difference between counts in males and females. More MPHs were found in males than in females on the 3rd (p = 0.0332) and 4th (p = 0.0482) fingers of the right hand.

Table 1: Descriptive statistics of MPH counts for each finger

Sex	Finger	Side	N of subjects with MPH	N of MPH	Max	Median	Mean ± SD
Males	2nd	R	1	8	8	0.00	0.50 ± 2.00
		L	1	7	7	0.00	0.44 ± 1.75
	3rd	R	12	103	17	4.50	6.44 ± 6.22
		L	12	91	23	5.00	5.69 ± 5.97
	4th	R	13	200	33	11.00	12.50 ± 10.99
		L	13	165	34	7.00	10.44 ± 10.77
	5th	R	6	19	6	0.00	1.19 ± 2.14
		L	6	33	11	0.00	2.06 ± 3.43
Females	2nd	R	2	2	25	0.00	0.17 ± 0.39
		L	2	3	1	0.00	0.25 ± 0.62
	3rd	R	3	42	2	0.00	3.50 ± 6.43
		L	4	35	17	0.00	2.92 ± 4.91
	4th	R	8	63	14	2.00	5.25 ± 6.73
		L	10	77	17	2.50	6.50 ± 8.86
	5th	R	4	18	23	0.00	1.50 ± 3.09
		L	4	21	9	0.00	1.75 ± 3.70

R - right, L - left; minimum MPH counts were 0 in each category; MPH had 13 males and 10 females

In terms of the side asymmetry, significantly more MPHs were found only in males on the 4th finger of the right hand than on the 4th finger of the left hand ($p = 0.0376$). The side asymmetry was not statistically significant in females and on the other male fingers.

2D:4D ratio

On the right hand, the mean 2D:4D ratio (Figure 3) was (mean ± SD) 0.98 ± 0.03 (males), 0.98 ± 0.02 (females) and on the left hand 0.97 ± 0.03 (males), 0.99 ± 0.02 (females). The highest 2D:4D ratio was measured on the right side: 1.06 (females) and 1.02 (males). The lowest ratio was 0.91 (males, left side). For females the lowest 2D:4D ratio was 0.92 (right side).

Width of MPH shaft and LMEM

The descriptive statistics showed that the mean shaft width of the MPHs (Figure 3) in males ranged from (mean ± SD) 20.4 ± 3.8 µm (2nd left finger) to 35.3 ± 13.1 µm (4th left finger).

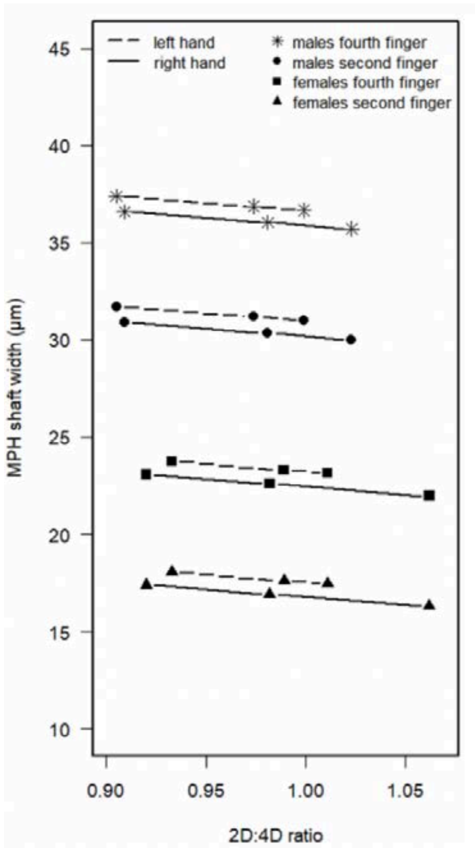

Figure 3: Estimates of mean values of 2D:4D ratio with their minimum, median and maximum values (represented by symbols) with corresponding mean values of MPH width (differences between groups predicted by LMEM)

In females, the mean width ranged from 17.2 ± 1.8µm (2nd left finger) to 24.2 ± 7.5 µm (4th left finger). The highest width was measured on the 4th left finger (males: 75.2µm, females: 48.8µm). The lowest width values were found on the 4th right finger (males: 6.7 µm; females: 7.4 µm).

In LMEM (Table 2), statistically significant variables were sex (male or female, $p = 0.0002$) and finger (2nd or 4th, $p = 0.0066$). The average difference between sexes was 13.4 ± 3.0 µm, the mean MPH shaft width was higher in males. In both sexes, the mean MPH shaft width was higher on the 4th finger than on the 2nd finger, with an average difference of 5.7 ± 2.1 µm.

According to the model prediction (Coefficient estimation values β in Table 2), on the right 2nd finger of the male hand, the mean MPH shaft width was (intercept) 37.9 ± 24.2µm, on the 4th right finger, it was of 43.6 µm (Intercept + Finger β). On the right 2nd finger of the female hand, the mean MPH shaft width was 24.5 µm (Intercept – Sex β), and, on the right 4th finger, it was 30.2 µm (Intercept – Sex β + finger β). The side (left or right) and the 2D:4D ratio (continuous) were not statistically significant, but the LMEM showed the following trends in both sexes the numerical value of the MPH shaft width mean was in average higher on the left than on the right side, with an average difference of 0.8 ± 0.8 µm. And if the 2D:4D ratio decreased by 0.01 unit, then the numerical mean value of the MPH shaft width increased by 0.07 µm.

Table 2: Summary of the results of the linear mixed-effects model (LMEM)

	Coefficient estimation values β	**s**	**Test statistic**	**p-value**
Intercept	37.9	24.2	1.57	0.1170
Sex (females)	-13.4	3.0	-4.44	0.0002
Side (left)	0.8	0.8	0.98	0.3274
Finger (4th)	5.7	2.1	2.73	0.0066
2D:4D ratio	-7.7	24.7	-0.31	0.7546

The intercept represents the mean MPH shaft width for reference group: male, 2nd finger, and the right side. Coefficient estimation value β represents predicted difference from the intercept of the mean MPH shaft width for specific variable (sex, side, finger, 2D:4D ratio).

Discussion

The results of our pilot study are in partial congruence with previous knowledge of MPH, but our research would seem to show new interesting findings that require replication.

MPHs counts: sex differences

According to Manning (2002) the 2nd finger is less sensitive to prenatal androgens than the 4th finger, and this statement applied namely to the enchondral primordium of the bony elements of the fingers. It means that the enchondral primordium of the 4th finger possesses more sensitive tissues compared to the 2nd finger. Our results point to a distinct sexual dimorphism, specifically on the 3rd and the 4th fingers of the right hand, where males have statistically significant more MPHs than females. This confirms previous findings in the literature reviewed by Westlund et al. (2015), that women typically have less MPH than men. In contrast to earlier findings of Danforth´s (1921) study, where male subjects without MPHs are less frequent than female subjects, we found the opposite results; however, this may have been due to the fact that in our sample only 5 subjects did not have any MPHs.

MPHs counts: side asymmetry

In our sample, the male subjects had a higher number of MPHs on the right hand compared with the left hands, but in the females, asymmetry in the counts was not demonstrated. According to Manning (2002) and Lutchmaya et al. (2004) the tissues of the right hand are more sensitive to androgens than those of the left hand, which can be also the reason for the higher asymmetry in MPH counts in males, but not in females.

Hair shaft width: differences between fingers

We found statistically significant differences between fingers in MPH shaft width: the shaft width on the 2nd fingers was smaller than on the 4th fingers, and this difference was similar in both sexes. This is in congruence with the premise of the present study. Zheng & Cohn (2011) demonstrated that the most numerous receptors for testosterone are found on the 4th finger, and, on the 2nd finger, they are substantially less plentiful (caveat: these findings were made in mice). However, we must take into account that in our sample, there were only 3 cases of 2nd fingers with MPHs, and 23 cases of 4th fingers with MPHs.

Hair shaft width: sex differences

Different authors (Arenberger, 2002; Garn, 1950) showed that the width of the head hair (*capilli*) differs between the sexes. Males usually have stronger/wider terminal hair. In our LMEM, we found significant sex differences in the MPH shaft width, with higher values in males. The present study is, to our best knowledge, the first study to analyze and report this sex difference.

The sensitivity of male tissues to testosterone, due to the quantity/activity of testosterone receptors, might be the mechanism causing the difference in width, similarly to the sex differences in the hair counts. Moreover, not only the effect of testosterone, but also individual differences in activity of the 5α-reductase (Blahoš & Zamrazil, 2006; Westlund et al., 2015; Winkler & Christiansen, 1993) may be an important factor.

Hair shaft width: side asymmetry

No significant side asymmetry was found in the MPH shaft widths. Nonsignificant prediction from LMEM only was found, where wider MPH shafts were on the left hand than on the right hand. This prediction (statistically nonsignificant) is not consistent with the premise regarding the aforementioned side asymmetry in terms of sensitivity to testosterone (Lutchmaya et al., 2004). There may be several explanations for this contradictory result: a small number of observations result in a reversal effect, or the body side would not play a significant role for the width of MPH itself. But these results need to be interpreted with caution.

Hair shaft width: the relationship with 2D:4D ratio

We tested a possible relationship between the MPHs width and the 2D:4D ratio, which is a putative marker of prenatal testosterone. The question was whether the same prenatal factors are also related to MPH width. If this is the case, we should find a relationship between the variables. Our results showed that the relationship was weak and not statistically significant. This result is consistent with previous results of Oinonen (2009) study. Oinonen (2009) also did not find association between MPH (counts in women) and 2D:4D ratio. The LMEM in our study showed at least a trend that was in congruence with our assumptions: the 2D:4D ratio increased as the width of the MPH shafts decreased. But given that our findings are based on a limited sample size, the results from such analyses should be treated with considerable caution.

Limitations

We are aware that our research may have certain limitations. The first is the pilot character of our sample size. The second is that our sample was assembled from one population. These limitations point out that this pilot study will require replication with a larger sample and in other populations eventually.

Conclusions

In conclusion, analysing the presence/absence, number and width of MPH may be a potentially useful indicator of testosterone levels—for both, prenatal and postnatal levels. The MPH analysis could also indicate more about the biological contexts and hormonal regulation of seemingly unrelated individual parts of the human body. We believe that our research will serve as a base for future studies focusing on: testing of sexual dimorphism in side asymmetry of MPH counts. Next, testing a larger sample of subjects, because LMEM showed some trends (wider MPH on the left hand and increasing 2D:4D ratio with decreasing MPH widths), but these trends were statistically nonsignificant in our pilot sample.

Acknowledgments

The authors would like to thank all volunteers who agreed to be a part of the pilot sample, as well as Ladislav Nejman (University of Sydney, Sydney) and Martin Čuta (Masaryk University, Brno) for reviewing the English version of the manuscript. This work was supported by Masaryk University (Internal grant number MUNI/A/1400/2018).

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Povzetek

Namen te pilotne študije je bil analizirati človeške dlačice srednjih prstnic (DSP) na mikroskopski ravni ten preučiti razmerja med širino DSP in razmerjem 2D:4D. Razmerja med širino DSP in razmerjem 2D:4D doslej niso bila raziskana. Pilotni vzorec, ki ga je sestavljalo 28 prostovoljcev (16 moških), je bil podvržen ročnim skeniranjem, štetjem števila njihovih DSP in zbiranju vzorcev dlačic. Širine DSP smo izmerili na mikroskopski ravni, njihove variacije, povezane s spolom, stranjo telesa, prstom in razmerjem 2D:4D, izmerjenim na podlagi ročnih pregledov, pa smo proučili s pomočjo linearnega modela mešanih učinkov. Pri 3. in 4. prstu smo ugotovili pomembne razlike med spoloma z višjim številom pri moških in pomembno asimetrijo v številu pri moških, z večjim številom na 4. desnem prstu. Moški so imeli širše jamice DSP kot ženske. Pri obeh spolih smo na 4. prstu ugotovlili bistveno širše dlačice v primerjavi z 2. prstom. Med širino telesa DSP in razmerjem 2D:4D nismo ugotovili pomembne povezave. Študija je pokazala, da se širina telesa DSP bistveno razlikuje med spoloma ter med 2. in 4. prstom obeh rok.

KLJUČNE BESEDE: dlačice srednje prstnice, androgenost, mikroskopija, širina telesa dlak, razmerje 2D:4D

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