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The behavior of hair from different countries

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Synopsis

The properties of curly hair from different countries were assessed using geometrical measurements from a laser scanning micrometer and a new hair shape classification method. In addition, tensile tests, 2-D gel electrophoresis, and differential scanning calorimetry (DSC) were performed. Results concluded that hair characteristics are influenced by the degree of curl. For example, curlier hair tended to be more fragile. Interestingly, the degree of curl in hair also was dependent upon the country of origin. DSC and 2-D gel electrophoresis showed that the thermal behavior and the protein markers in hair differed as a function of hair origin. The variability seen in hair characteristics may potentially influence how the hair responds to treatment and these differences could be caused by regional and cultural diversities in the population from where the hair originated.

INTRODUCTION

Interest in ethnic hair is growing in the cosmetic science field and a number of researchers have reported on the effect of hair type on hair characteristics that pertain to cosmetic attributes such as visual appearance, manageability and response to treatment (1–4). Keis *et al.* studied macroscopic characteristics of hair from different ethnic groups to understand how ethnicity influenced luster (1). They showed that variables such as fiber diameter, cross-sectional shape, and curvature had an impact on luster. Thus, products to modify these characteristics can be considered when enhancing the visual appearance of hair.

Garcia and Diaz have shown that the manageability of African-American hair is more challenging compared to Caucasian hair because the increased degree in curl makes it necessary to use much more force during combing (3). Appropriate surface-enhancements and hair assembly treatments and processes can overcome combing obstacles and thus, manageability can be increased.

Researches have also found that hair type can influence how the hair responds to treatments (4). This is particularly important as it pertains to hair from people of African descent because of its inherent fragility compared to people of other races. In addition, there are hair styles and treatments, such as braids and relaxers, that are common within the Black population. The impact of these styling regimes may be intensified due to inherent properties in hair. As such, there is a need to understand the intrinsic characteristics of Black hair.

Even though contributions in knowledge have been made (1–21), there is still limited information about hair from people of African descent. Among what is available in the literature, most researchers group hair from this population to compare it to other racial groups, such as Asian and Caucasian, or they use low sample numbers because of limited availability of untreated hair. Within the Black population, it is often perceived that hair behaves differently depending on the country of origin. To date, systematic studies have not been performed to understand the characteristics of hair within one racial group with different origins. This study investigates the behavior of curly hair of African descent that originates from different countries to better understand their specific needs.

MATERIALS AND METHODS

HAIR SAMPLES

Curly untreated hair from people of African descent was collected from several countries including Ghana (GA; n=35), Jamaica (JA; n=50), Kenya (KE; n=50); Liberia (LI; n=34), and the United States (US or AA; n=75). Information about the participants' ethnic background was obtained and only participants who were self-identified as a member of the country-specified ethnic group were categorized as such. Thus, the country from where the hair came and its respective ethnic group are used interchangeably throughout this paper.

HAIR PREPARATION

All hair was individually swatched and washed using a 10% ammonium lauryl sulfate solution for 1 minute followed by 1 minute of rinsing in 40°C tap water. The hair was then equilibrated in an environmental chamber at 45% relative for a minimum of 12 hours.

AMINO ACID ANALYSIS

To validate the untreated status of the hair, amino acid analysis on finely chopped hair samples from each subject was performed using acid hydrolysis in sealed tubes with rotary stirring at 120°C. The hair hydrolyzates were then analyzed on an L-8800 Amino Acid Analyzer (Hitachi, Tokyo, JPN) using ion exchange chromatography for amino acid separation. Amino acids were detected colorimetrically with ninhydrin after post-column derivatization. Lanthionine, cysteic acid, and tyrosine were selected as quality markers. The allowable limit of lanthionine was set at less than 0.5 g / 100 g amino acids. Quality limits for cysteic acid and tyrosine were less than 1.0 g / 100 g amino acids and greater than 1.8 g / 100 g amino acids, respectively. Samples were run in duplicate and only hair that met the requirements for quality was used in the study.

HAIR CLASSIFICATION

Two random hair fibers from each of the following 5 different regions of the head were selected: frontal, vertex, occipital, right temporal and left temporal. The fibers were cut to a fully extended length of six centimeters and were individually submerged in deionized water (23°C) that contained two drops of a commercial shampoo. They were immersed for three minutes to allow the fibers to relax and configure to their original shape. After the fibers were blotted dry and equilibrated in air for approximately 5 minutes, the number of waves (*w*), twists (*t*), curl index (*i*) and the smallest curve diameter (CD) were measured for each fiber to evaluate the degree of curl in the hair. Examples of the curl measurements are shown in Figure 1. The mean value of each parameter was used to categorize the hair from each subject into curl groups I–VIII based on a segmentation tree analysis (Figure 2).

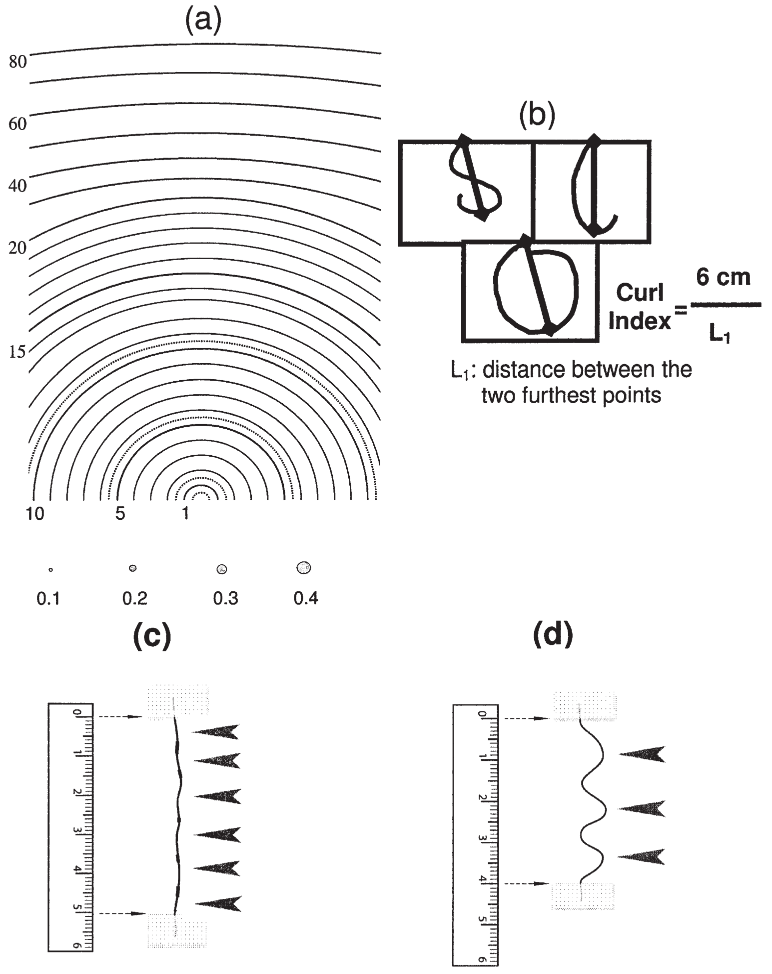

Figure 1. Schematic representations of the curl measurements. (a) CD meter used in the determination of the curve diameter. (b) Curl index, which is the ratio of 6 cm/l_1. (c) The number of natural constrictions along the length of a 5-cm extended fiber. (d) The number of waves present when the end-to-end distance of a 5-cm extended fiber is reduced to 4 cm.

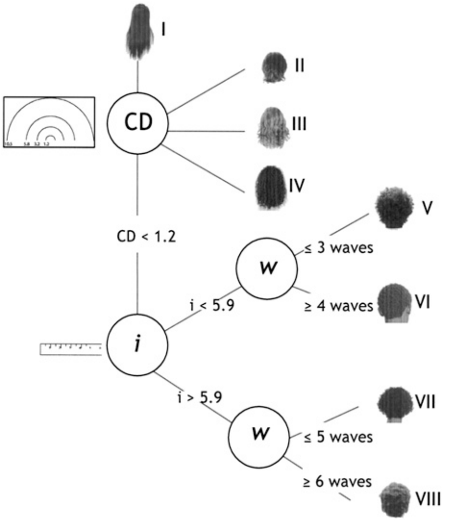

Figure 2. Segmentation tree that partitions the hair into 8 curl classes.

A class value of I was the lowest degree in curl and VIII was the highest. Statistical detail about the classification procedure can be found in earlier publications (5,6).

GEOMETRIC ANALYSIS

Geometrical measurements of hair from each subject were taken using a laser scan micrometer (Mitutoyo, Kanagawa, JPN) at 22 ± 2°C and 45 ± 5% relative humidity (RH). Dimensional values for a single fiber were obtained by taking the average of 10 cross-sectional areas along the length of the fiber that was 3 cm in length. Ellipticity was determined by dividing the major axis by the minor axis. Results from 50 fibers per subject were averaged.

MECHANICAL ANALYSIS

The tensile properties of dry hair from all of the subjects were determined using a Miniature Tensile Tester 675 (Dia-Stron Ltd., Hampshire, UK) using a strain rate of 12 mm/min and a gauge force of 1 g. The environmental temperature and humidity was 22 ± 2°C and 45 ± 5 RH%, respectively. All tensile properties were measured on the first 50 hair fibers that exhibited normal failure profiles. Young's modulus and break stress were normalized using the cross section of each fiber.

THERMAL ANALYSIS

For each country represented, hair from five random subjects that originated from the same country was cut into a fine powder and blended. High-volume DSC pans were charged with 6.5–7.5 mg of this blended hair and 50 μl of deionized water was added. The samples were allowed to equilibrate overnight at 22 ± 2°C. All samples were then thermally characterized using a Q100 DSC (TA Instruments, New Castle, DE). A total of 5 blended samples that represented each country were run, and the denaturation temperature (T_d) and change in enthalpy (ΔH_d) were determined by averaging the 5 values.

CHEMICAL ANALYSIS

Twenty-three to twenty-five individuals from the 5 different countries were used for two-dimensional polyacrylamide gel electrophoresis (2D PAGE) experiments. Three to five fibers were selected from each individual and were delipidated in ethanol (25 ml) and in cyclohexane (25 ml) for 20 minutes, successively. The hair was allowed to air dry at ambient temperature and cut into a powder. The hair powder was placed in an extraction solution which contained 7 molar (M) urea, 2 M thiourea, 50 millimolar (mM) 2-Amino-2-(hydroxymethyl)-1,3-propanediol (trizma), 50 mM dithiothreitol (DTT), and 0.1% Triton ×100 with doubly distilled Millipore water at 40°C for 18 hours. The keratin was post-translationally modified with iodoacetamide in a tris solution prior to dialysis. The protein solution was dialyzed for 3 days in 3500 molecular weight (MW) cassettes suspended in water. The extraction solutions were lyophilized to yield white to off-white keratin.

Protein was rehydrated in a solution containing 7 M urea, 2% 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS), 2% sodium dodecylsulfate (SDS), and 0.2% 3-10 Biolyte ampholytes. The protein solution (125 μl) was loaded onto 7 cm immobilized pH gradient (IPG) 3-11NL support strips and then active rehydration was performed at 50 V overnight. The first dimension was run on a PROTEAN IEF cell (Bio-Rad, Hercules, CA) with a fast gradient voltage of 8000 V at 10000 V-hr. Both current and temperature were kept constant at 50 μA/gel and 20°C, respectively. The second dimension was performed with Novex Sharp Protein Standard MW markers (Invitrogen, Carlsbad, CA) on 10% bis-tris Invitrogen gels in NuPage MES buffer at 200 V using a XCell SureLock system (Invitrogen, Carlsbad, CA).

After each run, the gels were fixed in an aqueous solution containing 10% acetic acid and 40% ethanol prior to staining with Sypro Ruby stain (BioRad, Hercules, CA) using standard techniques. The gels were destained in 7% acetic acid and 10% ethanol with deionized water prior to imaging with the BioRad Molecular Imager Gel Doc XR System (BioRad, Hercules, CA) interfaced to Dell using Quantity One software. Three gels were performed on each sample to assure reproducibility.

STATISTICAL ANALYSIS

The statistical analyses to determine differences in hair from different countires for the geometrical and mechanical data were based on LSD (22), Duncan (23) and Scheffe (24). Group differences were determined by considering the methods that were consistent in their statistical output.

Contrast tests (independent t-tests) (25,26) were used to show ethnic differences in the thermal properties of hair. A Levene test of variance homogeneity (27) showed that ΔH_d

values for virgin hair data had different variances across ethnicity, thus, the p-values are assumed to be of unequal variances.

Both one-way and two-way ANOVA methods (28) were used to determine whether ethnicity or curl type influenced the differences observed in geometrical and mechanical properties for all the ethnic groups within class types VI and VII. In one-way ANOVA, either ethnicity or curl type was considered for the model, whereas, both factors were considered at one time for two-way ANOVA. A Brown-Forsythe test of equality of means (29) was used for one-way ANOVA, which is appropriate for heterogeneous variances and small sample sizes. Significant differences were indicated only if both methods produced consistent results.

RESULTS AND DISCUSSION

CURL CLASSIFICATION

The degree of curl in hair is usually described using words such as straight, wavy, curly, kinky, and frizzy. These descriptors are adequate when used to characterize the global appearance of Asian, Caucasian and African hair as straight, wavy, and very curly, respectively. However, these descriptors are poorly defined since they are subjective and depend on a point of reference. They become even more inadequate when distinguishing hair within one ethnic group; thus, to overcome this obscure method of describing the global appearance of hair, de la Mettrie *et al.* (5) developed a classification system on the hair shape measurements that were described in Figure 1.

For the countries studied, curl classification ranged from type IV to VIII (Table I). Type IV was the least populated whereas most of the hair was classified as type VI. The distribution of curl varied according to ethnicity where hair from the United States had a greater number of samples with looser curls and Kenyan hair the highest degree in curl. Hair from other countries varied between two extremes.

INFLUENCE OF CURL

The average cross section and ellipticity values for hair of curl types IV–VIII ranged from 3296–5176 μm^2 1.58–1.73, respectively (Figure 3). These values are consistent with those

Table I
Curl Class Distribution

	I	II	III	IV	V	VI	VII	VIII
AA	0	0	0	3	50	7	15	0
JA	0	0	0	0	10	32	7	1
GA	0	0	0	0	0	22	10	3
LI	0	0	0	1	2	26	4	1
KE	0	0	0	0	2	29	9	7
Total	0	0	0	4	64	116	45	12

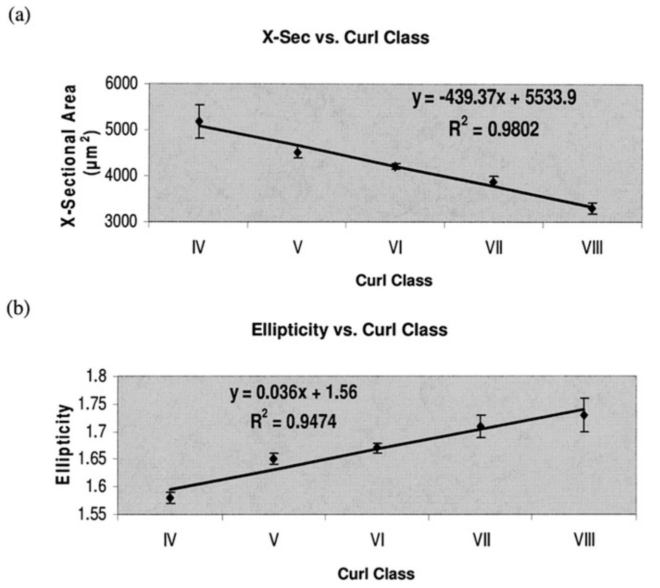

Figure 3. Geometrical properties as a function of curl class; n = 12,050 fibers. (a) Mean x-sectional area ± standard error. (b) Mean ellipticity ± standard error.

reported for black hair in the literature (7–9). A regression analysis between the cross-sectional area and curl type shows that there is a statistically significant relationship between the cross section of hair and the degree of curl. Thicker hair fibers tend to have a looser curl whereas thinner fibers are curlier. A direct correlation between ellipticity and curl classification is shown in Figure 3b. The data shows that, in general, hair that is more elliptical has a higher degree in curl. Earlier studies also revealed inter-ethnic (10) and intra-ethnic (11) correlations between curl and ellipticity. However, Nagase *et al*. did not see a strong correlation with curl radius and ellipticity measurements in Japanese hair (12). Even though there are exceptions where straighter hair is highly elliptical or ellipticity values can vary on one head, this linear relationship seems to be prominent in hair from people of African descent.

Mean Young's modulus and break stress data are shown as a function of curl type in Figure 4. The values range from 2670 to 3147 MPa and 173 to 206 MPa, respectively, and are consistent with those reported by Franbourg *et al*. and Wolfram (13,14). The Young's modulus data shows that less curly hair is more resistant to deformation and break stress shows that it requires higher loads to fracture compared to hair that is curlier. This trend is common knowledge when considering populations based on race since Asian hair, which is straighter, has a higher mechanical integrity compared to Caucasian and Black hair which is curlier (10,13–15). However, this is the first time that a strong correlation is seen in hair samples from one racial group.

INFLUENCE OF COUNTRY

The cross section and ellipticity values of hair from different countries are compared in Figure 5. The trends in cross section decrease as JA > AA > LI > GA > KE and statistical differences are shown in Figure 5a where significance is based on LSD and Duncan methods of analyses where the output gives the same divisions. Ellipticity values show no significant differences across all ethnicities for LSD, Scheffe and Duncan analyses. P-values are shown in the respective graphs.

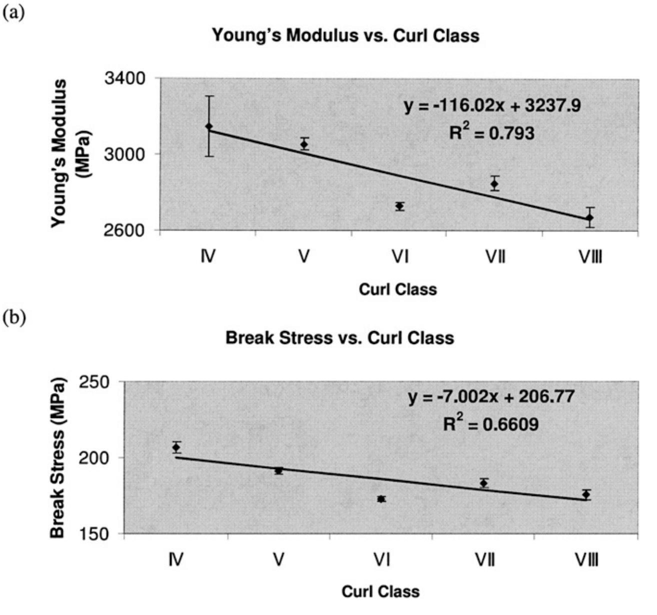

Figure 4. Mechanical properties as a function of curl class; n = 12,050 fibers. (a) Mean Young's modulus ± standard error. (b) Mean break stress ± standard error.

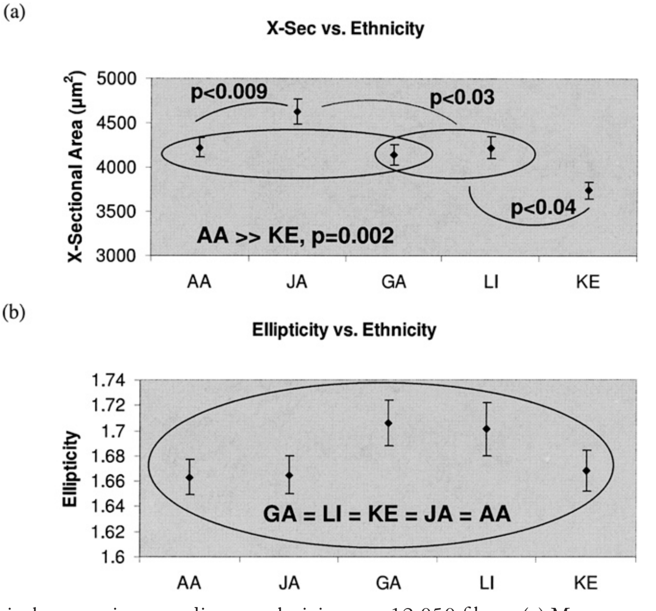

Figure 5. Geometrical properties according to ethnicity; n = 12,050 fibers. (a) Mean x-sectional area ± standard error. (b) Mean ellipticity ± standard error.

Figure 6 shows the mechanical data for the different ethnic groups studied. Young's modulus decreases in the order of AA > JA > KE > LI > GA and break stress decreases from AA > JA > LI > GA > KE. For the statistical groupings, LSD and Duncan methods give an output of the same divisions with differences shown in the graphs. If both Young's modulus and break stress data are considered, the mechanical integrity of African-American hair is shown to be greater compared to the other ethnicities; whereas hair from Kenya is more fragile. To the authors' knowledge, this is the first time that such ethnic comparisons have been made and statistical differences have been observed.

To gain insight on possible differences in the structure of hair from the populations studied, DSC was performed to evaluate the thermal characteristics. Denaturation temperature (T_d) values give information about the cross-link density of the matrix in which the intermediate filaments are imbedded while the change in enthalpy during denaturation (ΔH_d) gives the energy required to denature α-helical content within the intermediate filaments. Wortmann *et al*. and Marsh *et al*. reported differences in T_d and ΔH_d as a function of treatment (30,31); however, values did not significantly differ when Wortmann and Deutz compared the thermal properties of hair from people of different races (20). Figure 7 shows the thermal behavior of hair from the different countries in the present study. T_d and ΔH_d values range from 151.7 to 153.1°C and 17.2 to 19.1 J/g, respectively, and are consistent with reported values (20,21). T_d values decreased from AA > LI > GA > KE > JA and ΔH_d decreased from AA > GA > KE > LI > JA. T_d values showed more statistical differences between ethnic groups compared to ΔH_d (Figure 7). Thus, variations in the cross-link density between ethnic groups were more readily detected compared to differences in the α-helical content. Overall, African-American hair had the highest thermal integrity and Jamaican hair had the lowest, which is not consistent with data from the mechanical studies. One might expect that hair with higher mechanical integrity would also be more structurally robust in regards

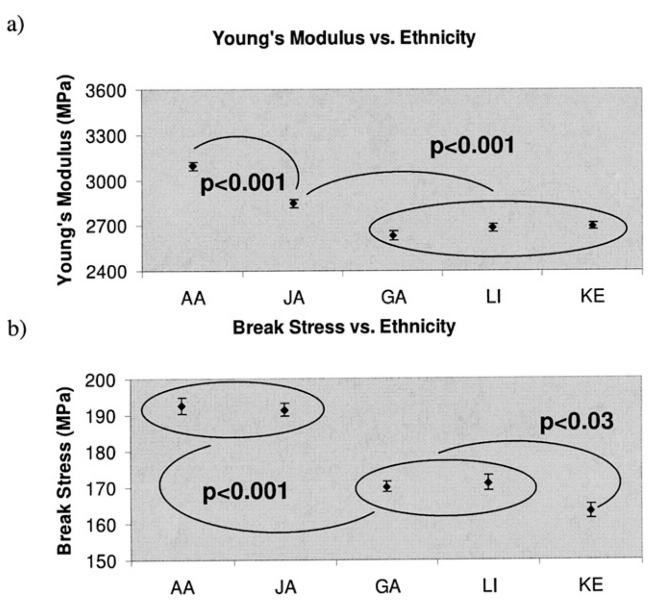

Figure 6. Mechanical properties according to ethnicity; n = 12,050 fibers. (a) Mean Young's modulus ± standard error. (b) Mean break stress ± standard error.

to its matrix cross-link density and α-helical content. In fact, this is what is observed with African-American hair; however, Jamaican hair, which had the second highest mechanical integrity, has the lowest thermal values. Additional studies are needed to assess chemical and structural differences that may be responsible for these results.

Protein variations as a function of ethnic group were explored using 2D-gel electrophoresis and the results are shown in Figure 8. In all cases, minor variations in staining intensity occur when the IF proteins have MWs between 50–75 kDa. Similar results were reported by Nagai *et al*. and Khawar *et al*. (32,33) and Nappe and Kermici (34). In our study, most

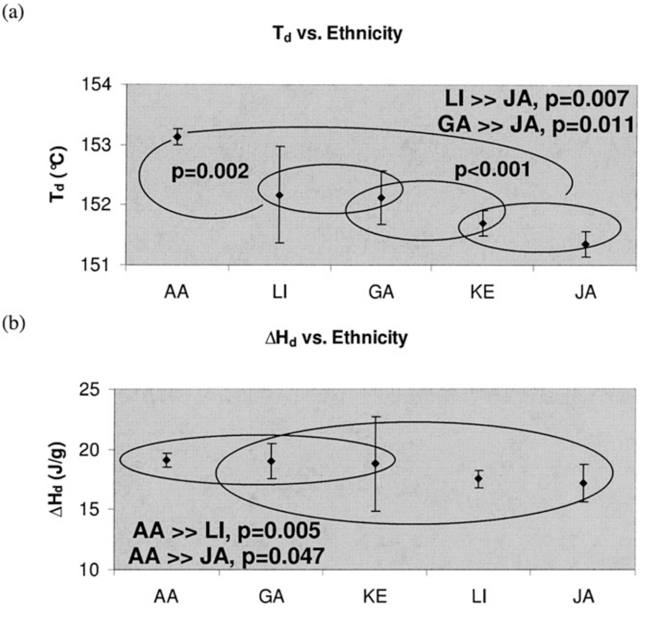

Figure 7. Thermal properties according to ethnicity; n = 5 hair samples / ethnic group. (a) Mean denaturation temperature ± standard error. (b) The mean change in denaturation enthalpy ± standard error.

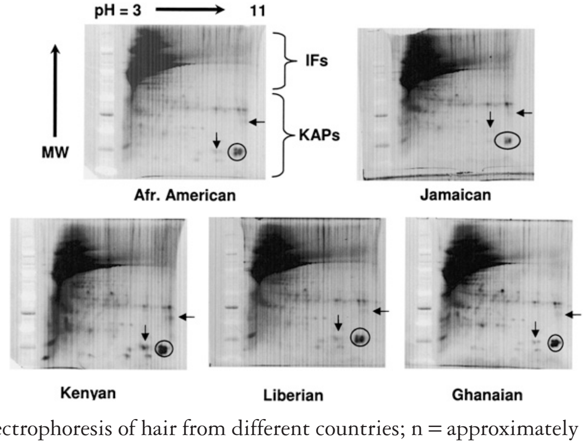

Figure 8. 2-D gel electrophoresis of hair from different countries; n = approximately four fibers from 23–25 subjects / ethnic group. The presence and the intensity of spots give insight about specific proteins.

variations were found with keratin associated proteins (KAPs) at basic isoelectric points > pH 9. Particular to our observations, more KAPs spots are present in the circled portion of the gel for hair from African countries compared to Jamaican and African-American hair. Additionally, the spots indicated by arrows vary with ethnic group indicating different protein concentrations. These differences may be attributed to possible ethnic or origin-related variations.

From the above studies it can be seen that hair characteristics change according to degree in curl and ethnicity. However, since some ethnic groups have curlier hair compared to others, it is difficult to assess whether the results are related to ethnicity or to type of curl or to both. So, answer this question, statistics using one-way and two-way ANOVA were conducted on hair from types VI and VII where there was a larger number of hair samples representative of each ethnic group. The results are shown in Table II. It was found that ethnicity was the sole driver for values in cross-sectional area and Young's modulus, whereas curl class did not solely influence any of the parameters discussed in this paper. Both ethnicity and curl type affected variations in break stress but neither of them affected ellipticity. It seems that these statistics do not agree with our earlier results of them affected ellipticity. It seems that these statistics do not agree with our earlier results since Figure 3 shows a statistically significant correlation between ellipticity and degree of curl. However, when the reduced range in ellipticity values for the latter statistical method is considered (1.67–1.71), it is clear that the reduced range is responsible for the inconsistency. In order to better understand the influence of curl type using this method, more data is needed where more curl types, and hence, ellipticity values, are represented for the different ethnic groups being studied. Nevertheless, the amount of samples represented within both ethnic groups, particularly curl type VI, statistically demonstrates the effect of ethnicity.

The influence of geometry can easily explain why the mechanical properties in hair differ. The geometrical twists and kinks that are more prevalent in curlier hair can be local points of weakness that act as stress concentrators and result in higher fragility. However, when hair from different global regions that has the same curl is compared, as was done in the latter statistical method, the impact of geometry is significantly reduced and one has to consider other possible factors for the observed differences. Regional and cultural diversities, such as diet, environment and grooming practices, may contribute to these differences; however, more research is needed to understand the effect of ethnicity.

CONCLUSIONS

This study has shown that hair fibers with a higher degree in curl are typically thinner, more elliptical and weaker than hair that has looser curls. Based on curl classification, in

Table II
Two-Way ANOVA p-Values

Property	Ethnicity	Curl class
Cross-sectional area	0.038*	0.162
Ellipticity	0.212	0.082
Young's modulus	<0.001*	0.165
Break stress	<0.001*	0.001*

*Significant when one-and two-way ANOVA are consistent and $p < 0.05$.

general, African-American hair is looser in curl and curl degree increases in the order of Jamaican < Ghanaian < Liberian < Kenyan. The cross-sectional area and Young's modulus values are influenced by ethnicity, while break stress is influenced by both ethnicity and the degree of curl in hair. DSC and 2-D gel experiments suggest that structural and chemical composition of curly hair from different ethnic groups may also vary. Collectively, these results show that both curl type and ethnicity are important factors to consider when evaluating the behavior of hair, which may potentially impact the hair's response to treatments and processes.

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