

## The Biology and Genetics of Curly Hair

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| Complete List of Authors:     | Westgate, Gillian; University of Bradford, Centre for Skin Sciences<br>Ginger, Rebecca; Unilever R&D, Colworth Science Park<br>Green, Martin; Unilever R&D, Colworth Science Park |
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## The Biology and Genetics of Curly Hair

Gillian E Westgate<sup>1</sup>, Rebecca S Ginger<sup>2</sup> and Martin R Green<sup>2</sup>

<sup>1</sup>Centre for Skin Sciences, University of Bradford, Bradford, West Yorkshire, BD7 1DP, UK and <sup>2</sup>Unilever R&D Colworth Science Park, Sharnbrook, Bedfordshire, MK44 1LQ, UK

Hair fibers show wide diversity across and within all human populations suggesting that hair fiber form and its coloration has been subject to much adaptive pressure over many thousands of years. Human hair fibers typically have the same basic structure in all human hair types. However, the three dimensional shape of the entire fiber varies considerably depending on ethnicity and geography, with examples from very straight hair with no rotational turn about the long axis, to the tightly sprung coils of some African races. This review will introduce the reader to hair follicle formation, the hair growth cycle and basic hair follicle structure and will review the current understanding on how hair fibers are formed by follicles into a non-linear coiled form and which genetic and biological factors are thought to be responsible for hair shape. The creation of the highly complex biomaterials in hair fibre and follicle and how these confer mechanical functions on the fibre so formed is a topic that remains relatively unexplained thus far. We focus here on the links between genetics and protein expression and function in order to understand some of the molecular controls on formation of curly hair.

While it is true that across all mammals, the basic structure of the fiber is the same –a cuticle, cortex and medulla (in some)- how these structures are built by the hair follicle and shaped into the functional hair fiber for both an individual member of a species and the relevant body site, suggests that there is a level of ‘fine control’ on the process of hair fiber formation by the hair follicle.

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3 The distribution of forms of curly hair is shown in Figure 1 and a closer  
4 inspection reveals that curly hair fibers are rarely a true coil but exhibit  
5 heterogeneity in the direction of the curl in all but the mildest cases. Curly hairs  
6 have an elliptical or 'D' shape in cross section. This enables bi-directional  
7 bending stiffness with fibres tending to bend most easily in the direction of the  
8 flattened axis. The relationship of the long and short diameter to the direction of  
9 hair growth also changes (unlike the eyelash where this relationship is  
10 maintained [1]). Therefore, at the level of the follicle, we need to understand how  
11 the arrangement of cells results in a fibre that is elliptical with the orientation of  
12 the ellipse changing with time during hair growth.  
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22 Hair fibres across all races and geographies show degrees of curl that are readily  
23 measurable [2-4]. Previous studies examining the classification of hair  
24 phenotypes reveal potentially important information about the biology of curly  
25 hair formation and the evolutionary and environmental drivers behind curly hair  
26 as a human trait. Both Hrdy and de la Mettrie [2, 5] studied various hair types  
27 sampled from countries and cultures across the world. The degree of curvature  
28 of a fiber in its natural state appears to account for most of the variation (87%  
29 [5]), which is as expected. The presence of a medulla is chiefly correlated with  
30 hair diameter. However 'twist' (as defined by the sudden natural constrictions in  
31 the fibre that produce a discontinuity in curvature and curvature variability);  
32 'crimp' (change in direction of curvature); 'wave' – (the number of  
33 oscillations/coils per unit length) and 'kink' – (a sharp twist or bend) are also  
34 important in differentiating ethnicity and maybe also helpful in defining the  
35 genetic and functional origins of curly hair. Hrdy 1973 [2] showed that kinking  
36 and crimp was not always correlated with curvatures, and irregular curvature  
37 caused by kinking separated a population in the Solomon Islands (Melanesian)  
38 from African. Interestingly, the adaptation of highly curled hair separately in  
39 these two very distant populations achieved the same functional attribute of an  
40 intensely curled hair, suggesting the result of environmental pressures related to  
41 evaporation of sweat and scalp cooling.  
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3 The hair follicle is a self-sufficient and highly organized structure within the skin  
4 that has both proliferating (dividing) and differentiating (functional/specialized)  
5 cell compartments. The hair follicle comprises cells of epithelial, mesenchymal  
6 and neuronal (melanocyte) origin and is intimately connected to the surrounding  
7 dermis through blood and nerve supply and the interchange of individual cells  
8 associated with the follicle, including cells from the immune system such as mast  
9 cells. The hair follicle is an autonomous mini-organ in the skin, thus when  
10 considering how hair shape is controlled by the hair follicle, we must consider  
11 what is know about the first embryonic hair follicles, the diversity of hair shape  
12 within and between individuals and then drill down to investigate how the  
13 component parts of the follicle are arranged in order to make a fiber with low,  
14 moderate or high curl. Because the shapes of cells in the developing hair shaft  
15 are grossly altered during differentiation of the newly forming hair, it is also  
16 necessary to consider some of the biomechanical aspects that govern hair fiber  
17 shape. A global study of hair shape variability and racial classification reported  
18 no gender-based differences [5], suggesting that sex hormone influences are  
19 minimal in hair shape and curl determination.  
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### 35 **Follicle anatomy, structure, size and relationship to hair shape**

36 Hair follicles have a multi-layered structure with seven layers of specialized  
37 epithelial cells arranged in a concentric pattern (like an onion), with the hair  
38 fiber at the centre, (Figure 2). These concentric layers of epithelial cells all have  
39 unique differentiation pathways and properties. Most cells of the follicle are  
40 epithelial, however, a group of mesenchymal (fibroblast-like) cells lie right inside  
41 the lower follicle bulb called the dermal papilla (DP) and is continuous with the  
42 very outer layer of the follicle, the connective tissue sheath. The dermal papilla  
43 plays an essential role in directing the regulation of hair growth and the hair  
44 cycle.  
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52 In terms of the formation of the hair fiber inside the curly hair follicle, it is useful  
53 to consider activity in two compartments:-  
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3 i). *The mitotic region*, where cells of the lower bulb are undergoing rapid cell  
4 division and generating the 'force' behind hair formation. In curly hair the  
5 mitotic zone can be imagined as being organized in an asymmetrical  
6 arrangement around the dermal papilla. Studies in mice on zigzag hairs reveals a  
7 relationship between an asymmetric location of dermal papilla cells which  
8 results in the change in direction of hair growth generating the zig-zag [6]. More  
9 recent studies show that curved hair follicles emerge from wool follicles with  
10 asymmetric distribution of mitotic cells [7]. In human curly hair follicles there is  
11 some asymmetry in the proliferating pool of cells and this is described later.  
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14 ii). *The zone of differentiation*, where cells in the follicle inner root sheath and  
15 hair fiber become fully keratinized and confer rigidity to these structures. Both  
16 the mitotic 'force' and the subsequent hardening of the fiber and root sheath  
17 cells are considered important factors in establishing ultimate fiber shape, as  
18 described below.  
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21 In order to envision this arrangement of cells, it is helpful to view the hair follicle  
22 from a three dimensional viewpoint (Figure 3), in which the relationship of the  
23 fibre growth axis to the orientation of the dermal papilla is depicted. In Figure 3a  
24 there is full symmetry around the long axis of the follicle in a straight hair. In  
25 Figure 3b the axis is symmetrical through the bisected dermal papilla, however  
26 this does not match the long axis of hair growth and in Figure 3c the curved  
27 nature of the lower hair follicle bulb is shown as out of plane with the long axis of  
28 hair growth. The principle is now established that a curly follicle makes a curly  
29 hair [8] [9] and that some form of asymmetry in the follicle drives the formation  
30 of the coiled/curly hair. It has been shown in several studies that the shape and  
31 size of the follicle determines the shape and size of the hair and that curved/bent  
32 follicles produce curly hair fibers in all ethnicities [8-12] [13]. Thus while it is  
33 perhaps correct to assume that hair shape is defined in the follicle, the  
34 considered question for the hair biologist and biophysicist is how the follicle  
35 shape and associated cell distribution can set up a fixed or variable curl  
36 phenotype and generate features such as crimp (change in curl direction) and  
37 kink (discontinuity in curl) and also why these features may have been usefully  
38 inherited?  
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### Hair follicle development and the hair cycle

Given that hair follicle structure is basically the same in human populations, we can propose that the development of the follicle structure is also very similar, even though this is poorly studied in embryonic tissues for reasons of ethics. The hair follicle forms as an organized involution of the epidermis during the early weeks of gestation in human scalp [14]. Firstly a hair placode forms that responds to the signals derived from the mesenchymal (dermal) cells immediately below the placode, which will eventually become the dermal papilla [15]. The epithelial cells expand in number and the epithelial hair peg extends into the dermis and cells of the newly forming hair matrix and upper hair canal start to differentiate [16]. Melanocytes, which are derived from the neuroectoderm (neuronal tissues of the embryo), arrive in the follicle during embryogenesis to provide hair with a source of melanin and so its colour. It is assumed that this process is identical for the formation of curly hair; however, this has not been studied in any detail. Children of African Negroid descent are born with loose 'silky' curls and that they may not attain the tight curls for another 12 months or so [17]. This suggests that the first hair cycle, or possibly the embryonic 'lanugo' hair has a different shape to subsequent hair cycles. The first hair cycle can be considered as somewhat different to subsequent hair cycles, given that it is governed by embryonic development processes, which are not all required in the post-natal hair cycles. Lanugo hair is normally shed very early in a child's life, much of it inter-uterine. Interestingly, when interrogating the internet for information on this topic, most African American or mixed race babies are reported to have rather straight hair at birth which curls a little more when wet and is replaced by very curly hair over the coming months and early years.

The cyclic behavior of hair follicles as the regulation of the hair cycle has led to an impressive amount of research into the molecular factors responsible for hair growth. Furthermore, because hair shape is reasonably fixed parameter in the adult (save for exogenous influences), the factors that control the re-growth of hair in each hair cycle must also maintain the shape characteristics of each new hair follicle formed during a lifetime. We understand that the hair follicle cycle

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3 retains an element of developmental dynamics reflected in the interactions  
4 between the mesenchymal and epithelial elements as originally proposed by Sun  
5 et al [18] and Hardy (Hardy 1992) so each time a hair follicle goes through this  
6 're-birth' in the hair cycle, all the factors that govern curl have to be re-  
7 established. The adult hair cycle has a growth phase (anagen) which also  
8 encompasses the very early stages of follicle re-formation, recently termed  
9 'neogen' [19], a regression phase where the lower two thirds of the follicle  
10 undergoes programmed deletion (catagen) [20-22] and the resting and shedding  
11 phases telogen and exogen. [23] [24] [25]. These events occur in all hair-bearing  
12 species and the molecular dynamics of progression through the hair cycle has  
13 been the subject of much study [19, 26-29]. The factors controlling the  
14 progression through the cycle include genetic and epigenetic factors [30], [31]  
15 and the so-called hair follicle clock [32-34]. Anagen is maintained by growth  
16 factors such as VEGF and IGF1[35-37], which help maintain cell proliferation in  
17 the bulb matrix over several years in the case of scalp hair, but only just a few  
18 weeks in the case of eyebrow and eyelash. The signaling pathways that have  
19 been shown to be important in early anagen include Wnt/beta-catenin, bone  
20 morphogenic proteins (BMPs) and Sonic hedgehog (Shh) pathways and are all  
21 involved in hair follicle initiation in embryogenesis [38, 39] [40] and are  
22 governed by Hairless (HR) protein, the product of the hairless gene (*HR*) [41].  
23 This strongly suggests that the program for hair shape is present in the hair  
24 follicle stem/progenitor cells and these may also govern follicle variation in size  
25 and function across the body. It is believed that the size of the hair follicle and  
26 subsequent fiber diameter is determined by the size (number of cells), and  
27 especially the maximum width of the dermal papilla [42] [43]. However the  
28 factors affecting the relationship between follicle size (fiber diameter), anagen  
29 duration (hair length), and curl are not, so far, understood.

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51 We know that the follicle of curly hairs is also curved, but in two directions –  
52 retro-curvature, suggesting curl is set in the follicle [8, 9]. Key questions on  
53 formation of curly hair include 'what factors control the shape of the follicle?'  
54 'what is the driving force for asymmetry?' does the follicle bend because of pre-  
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3 determined strain placed within the fibre owing to asymmetrical fixed protein  
4 structure, or, does the asymmetrical protein distribution arise because the  
5 follicle is curved? The retro-curved nature of the hair follicle in African scalp skin  
6 is shown in Figure 4 where both the bulb and the hair shaft shows curvature in  
7 the skin as indicated by the double bisection of the hair shaft (Figure 4b).

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10 Studies (described in more detail below) suggest five sources of molecular  
11 control in conferring fibre shape:- 1) asymmetric expression of structural  
12 keratins in the pre-cortex; 2) variable cortical cell shape and keratin filament  
13 orientation in relation to the axis of hair growth; 3) asymmetric rates of  
14 proliferation in cells forming the inner and outer root sheaths, 4) polymorphic  
15 variation in the proteins of the IRS which (presumably) alters its ability to form a  
16 specific shape in the keratinizing zone of the hair shaft and 5) the asymmetry of  
17 the dermal papilla within the central 'core' of the hair bulb. The translation of  
18 these molecular 'settings' into curl also requires appreciation of biomechanics as  
19 the curvature of the fibre inside the follicle during growth and then outside when  
20 released, is very different.  
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31 Some of the most instructive studies on molecular factors for curl have come  
32 from examining the expression of a range of proteins in the asymmetric  
33 compartments of the curly hair follicle in relation to the structure and  
34 orientation of cells in the cortex. [1, 9, 12, 44]. These are summarised in Table1.  
35 Notable examples are hair keratin Ha8/K38 which is expressed earlier on the  
36 concave side of the follicle [12], insulin like growth factor binding protein 5  
37 (IGFBP5) which shows elevated expression in the convex side of the outer root  
38 sheath (ORS) in curly hair follicles [44], keratin 71 which is only expressed in the  
39 inner root sheath, but when polymorphic leads to woolly hair syndromes, and  
40 the relationship between the cells of the DP and the bulb matrix. Ki67, which  
41 marks the proliferating compartment, shows asymmetric expression around the  
42 dermal papilla, [1].  
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52 So, how can compartmentalized expression of different proteins and protein  
53 functions influence curl? There are five possible mechanisms that are introduced  
54 below and will be referred to when discussing the genetic and developmental  
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3 origins of variation in hair fibre shape and curl identified through whole genomic  
4 screening studies of the curly hair trait.  
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### 7 1) Asymmetric expression of structural keratins in the pre-cortex.

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9 The cortex of the hair fibre is most likely to structurally support curly hair  
10 characteristics (Figure 2). Thus, we would expect variation in the expression of  
11 the cortical keratins and keratin associate proteins. hHa8, K38 (gene KRT38) is a  
12 Type I acidic member of the hair keratins [45] and the only member of the  
13 complement of cortical hair keratins described as being asymmetrically  
14 distributed in the curly hair follicle [46]. There is no further research on the  
15 regulation of hHa8/K38 expression; this will be needed to help understand why  
16 this particular type I keratin associates with curly hair through its uneven  
17 expression. The cuticle keratin K82 (hHb1), was also shown to be expressed  
18 slightly later on the convex side of the follicle [9]. It is not yet known what  
19 regulates this differential expression pattern.  
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28 2) The cortex comprises three different types of cell (as judged microscopically);  
29 para-, ortho- and meso-cortex. Within the cortical cells, keratin filaments are  
30 formed in dense, almost crystalline arrays. Cortical cells are very long and  
31 aligned with the long axis of the hair. The main variation within these cells is the  
32 orientation of the long axis of the keratin filaments in relation to the long axis of  
33 the hair fibre and the ratio of keratin to keratin-associated-proteins. The  
34 distribution of these different cell types within the hair fibre cortex has been  
35 studied in wool follicles where a distinct microscopical arrangement into ortho-  
36 and para-cortex was originally thought to be associated with crimp [47],  
37 however, more recent studies looking at the orientation of the keratin filaments  
38 in human hair have failed to find such a relationship [48]. In human hair fibers,  
39 three different cortical cell types have been observed and it has been proposed  
40 that the distribution in the different cell types may be related to curl [11, 46].  
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### 51 3) Asymmetric proliferation in cells forming the inner and outer root sheaths.

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53 Limited data exists to understand the role of asymmetric proliferation rates but  
54 it is likely that this is linked to the asymmetry in the shape and activity of the DP.  
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3 Ki67, a marker of proliferating cells, is distributed in an asymmetric pattern in  
4 both curly hair follicles and human eyelash follicles [1, 46]. The proliferating  
5 compartment extended higher up in the bulb on the convex side of the hair  
6 follicle. IFGBP5 is involved in the action of the growth factor IGF1 that is known  
7 to be required for hair growth [22, 35, 49] and the increased expression of  
8 IFGBP5 on the convex side of the follicle was also shown to impart asymmetric  
9 hair growth. This suggests that asymmetrical growth rate of cells forming the  
10 hair cortex influence curl degree. It should be considered whether the  
11 proliferating zone itself is mobile about the long axis of the follicle generating  
12 some form of curl force?  
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#### 23 4) Inner Root Sheath links to variation on fibre shape

24 Perhaps the most compelling evidence for control on hair fibre shape supports  
25 the role of the IRS. Genetic studies (further described below) and hair diseases  
26 that give rise to Woolly hair, have revealed polymorphic variation in several  
27 proteins of the IRS and this is presumed to alter its ability to form specific shapes  
28 in the keratinizing zone of the hair shaft. The importance of the IRS was  
29 demonstrated by the namesakes for the two key layered structures; Jacob Henle  
30 and Thomas Huxley [50] and much molecular genetic evidence is emerging to  
31 support this view with several IRS protein polymorphisms associated with  
32 curliness. The inner root sheath in mammals is comprised of three layers; the  
33 cuticle that directly abuts the hair shaft cuticle; Huxley's layer and Henle's layer  
34 (Birbeck and Mercer 1957) Figure 2. Studies on monotremes reveal a simpler  
35 structure without a distinguishable Henle layer which could be the forbear of the  
36 more complex mammalian IRS and give a clue to how hairs arose from reptilian  
37 scales during evolution [51][52]. Henle's layer keratins are the first to fully  
38 keratinise or 'harden' in the follicle to support hair shape. Cells in Huxley's layer  
39 produce keratins that interact with the protein trichohyalin, the latter also  
40 specific to Huxley's layer. The cells of Huxley's layer are fully differentiated more  
41 distal to the bulb than Henle's layer and the interaction between these two layers  
42 forms the bulk of the IRS. Trichohyalin expression is only found where a  
43 hardened keratin structure is needed, such as hair, nail and the filiform papillae  
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3 on the tongue [53-56]. The interaction of trichohyalin with keratin is preceded  
4 by the enzymatic conversion of arginine to citrulline within the trichohyalin by  
5 the enzyme peptidyl arginine deiminase (PAD) that reduces its overall charge so  
6 facilitating stable interaction with the IRS keratins [57-60]. So it was very  
7 interesting to note that mutations in the trichohyalin gene were described for  
8 uncombable hair syndrome, which includes a curly hair phenotype [61]. In  
9 addition to TCHH, the genes for PAD (PADI3) and transglutaminase 3 (TGM3),  
10 both involved in the transformation of the IRS from 'soft' to 'hard' were also  
11 mutated in this rare genetic condition. On the inner side of the IRS, the IRS  
12 cuticle cells form a 'mirror' of the cuticle cells of the hair shaft holding the shaft  
13 very firmly into the follicle. Outside Henle's layer there is the companion layer  
14 (or innermost layer of the outer root sheath). These cells are intimately  
15 connected to the IRS and migrate with the shaft as it grows. Of special mention  
16 are so called flügelzellen, structures in Huxley's layer which project through  
17 Henle's layer to the companion layer. These structures are predicted to  
18 strengthen and stabilise the IRS [62, 63] with the latter already known to be  
19 highly influential on hair curl formation. Flügelzellen may be visualised by  
20 staining with antibodies to K74, directly linking this keratin with Flügelzellen.  
21 Further study of the spatial disposition of Flügelzellen in relation to hair curl is  
22 warranted. Thus, the current thinking is that the IRS is not merely a scaffold  
23 holding the shaft but is able to be programmed to confer properties on the shaft  
24 including shape.

##### 40 5) Dermal Papilla asymmetry and links to curl

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42 Little or no attention has been paid to whether the size and shape of the papilla  
43 contributes to the shape of the hair fiber and subsequent curliness in human  
44 hair, although the role of the DP generally in contributing to fibre type and shape  
45 was recently reviewed [64] and the links between DP size and fibre size are  
46 known [65]. Differences in DP shape are observed animals in relation to types of  
47 fibre produced [66] with the spiny mouse (*Acomys dimidiatus*) being a good  
48 example of how development of a crescent shaped DP influences the follicle  
49 proliferation and differentiation programs [67] to generate an unusually shaped  
50 fibre. A possible theory has been proposed in which the asymmetrical  
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3 distribution of proliferating cells in the hair follicle bulb matrix leads to a  
4 flattened hair fiber shape [68] and the asymmetrical control on matrix cell  
5 proliferation is assumed to be controlled by the dermal papilla, suggesting that it  
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7 too has an asymmetry in relation to interactions with the surrounding hair  
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9 matrix cells which could lead to the formation of a curly hair. Nissimov 2014  
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11 further hypothesized that the construction of certain features of curly hair had  
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13 explanations within the construction of the hair follicle, proposing multiple  
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15 papillary centres each autonomously influencing growth of adjacent bulb matrix  
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17 cells, so building asymmetry [69].  
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### 20 21 **Curly hair as a genetic trait: Identification of candidate genes and links to** 22 **mechanistic factors involved in curly hair formation** 23

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25 Curly hair traits are straightforward if rather tedious to measure given that hair  
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27 is easily sampled and good methods to quantify curl have been developed [3].  
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29 This has aided genetic studies (so called genome wide association studies  
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31 (GWAS)) to try to identify the causative genes for hair traits and to explain their  
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33 role in hair shape [70, 71].

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35 Factors such as ethnicity and geographic variation must be controlled in these  
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37 studies to minimise false positives. The advantage of GWAS investigations lies in  
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39 the complete survey of the genome without prior hypothesis and the potential  
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41 ability to identify unsuspected, novel genetic links to hair curl and shape. The  
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43 most recent data to emerge from such studies is from the CANDELA cohort, a  
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45 large (6630) admixed South American population with European, Native  
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47 American and African ancestry [71]. In this study, hair shape was scored on a  
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49 fairly simple four-point scale (straight, wavy, curly or frizzy) and was found to be  
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51 associated with polymorphic variation in known curl associated genes (EDAR,  
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53 Trichohyalin) and as yet unknown genes. PRSS53, Protease Serine S1 family  
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55 member 53a is a serine protease expressed in the IRS and was shown by the  
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57 authors to have a variant Q30R substitution causing a change in enzyme activity  
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59 with recent evolutionary selection in East Asian populations. Its expression in  
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the IRS adds weight to the hypothesis that shape of hair fibre is governed by the  
construction of the IRS; mechanism 4 as described above.

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3 In a separate GWAS, designed to examine the curl variation only within South  
4 African populations, Unilever R&D studied 3 separate language (ethnic) groups;  
5 the black African Sotho/Tswana, Xhosa and Zulu peoples, for genetic links to hair  
6 curl variation within what is a largely similar African ancestral population. Prior  
7 observations in South Africa revealed wide variation in curl type and degree,  
8 lending weight to the hypothesis that curl was under polymorphic control; the  
9 key question was which proteins might be variable? The degree of curliness of  
10 hair samples from 2417 volunteers was measured accurately using a flat bed  
11 scanner and image analysis, with the overall curl variation observed shown in  
12 Figure 5. No significant differences in curl variation were seen between language  
13 groups; there was a trend for the Zulu language group to have less curly hair.  
14 DNA from the 25% highest curl and lowest curl subjects was compared using a  
15 DNA pooling strategy and assessing 1.6M single nucleotide polymorphic variants  
16 (SNPs). For general methods used see Stokowski RP et al [72]. A substantial  
17 genetic signal was detected comparing the two hair curl groups but overall there  
18 were no specific associations that passed a strict genome wide statistical test ( $5$   
19  $\times 10^{-8}$  after a Bonferroni multiple testing correction). These data suggest that  
20 black African hair curl variation is 'complex' in that many genes are involved  
21 each having a modest effect on hair curl. Never the less, 3 candidate genes were  
22 selected having suggestive links to curl based on a less strict statistical tests,  
23 follicle location and literature data (Table 2).  
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39 Two of the 3 genes listed in table 2 (KRT74 and TCHH) are located in the IRS,  
40 which again supports the hypothesis that the IRS strongly influences hair shape.  
41 (see also [73], [74], [7, 75], [76], [77] [61]. K74 (keratin 74 the protein product  
42 of KRT74) is found in Huxley's layer (Figure 2) and is also linked to woolly hair  
43 syndromes [76] a disorder manifest by fine curly hair. The role for the IRS in  
44 shaping hair curl is also supported by animal studies, for example Cadieu et al  
45 [78] demonstrated using pure bred dogs, that just three genes control the major  
46 coat attributes of length, curliness and facial hair such as long eyebrows and  
47 beard. In humans polymorphic variation in KRT71 also gives rise to woolly hair  
48 [79]. Thus both KRT71 and KRT74 variants underpin a curly hair phenotype  
49 most likely by altered structural behaviour (e.g. capacity to bend, flex or twist) of  
50 the K71 and K74 proteins.  
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3 Trichohyalin (the protein product of TCHH) is also expressed in Huxley's layer of  
4 the IRS and in the medulla. Trichohyalin is responsible for condensing the  
5 intermediate filaments as they change and harden. Electrostatic links to  
6 intermediate filaments are further stabilised by the action of peptide cross-  
7 linking enzymes called transglutaminases (TGase) [80] and, in particular,  
8 TGase3, appears to be very important in formation of important cross linkages in  
9 the hair fibre [81]; mouse TGase3 gene (TGM3) knock out studies show hair  
10 abnormalities as the major phenotype [82] and TGM3 gene is mutated in  
11 uncombable hair syndrome [61]. In terms of function, it is proposed that  
12 trichohyalin mechanically strengthens the hair follicle inner root sheath to  
13 subsequently contain and permit shape to be set into the hair fibre [59, 83].  
14 Interestingly an independent study in people of western European descent living  
15 in Australia [84] suggests that trichohyalin polymorphisms are linked to the  
16 straightness of hair and therefore that, when combined with the observations  
17 reported here, trichohyalin might influence hair shape across more than one  
18 human population.  
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21 All 3 genes highlighted by the GWAS; KRT71, KRT74 and TCHH, are members of  
22 the so-called epidermal differentiation complex (EDC), a cluster of about 20  
23 genes in chr1q21. A subset of EDC genes is therefore clearly involved in  
24 coordinating hair shape. It is known that the EDC is under epigenetic control in  
25 the epidermis [85, 86] with chromatin organisers key to epidermal  
26 differentiation. It is interesting to speculate that similar factors may also control  
27 genes in the EDC within the IRS, opening up the possibility for epigenetic  
28 regulation of hair shape.  
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31 The third gene listed in table 2 is CUTC (cutC copper transporter) with members  
32 of the family associated with copper homeostasis, namely the uptake, storage,  
33 delivery and efflux of copper. From animal studies copper is known to be  
34 associated with hair conditions including hair curl [87]. For example copper  
35 deficiency in lambs leads to poor quality wool that lacks crimp, an effect linked  
36 to the delayed differentiation of the IRS [7]. Menkes disease, which is associated  
37 with defects in hair traits including hair kinks, [88] is linked to another copper  
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3 transporter ATP7A, further supporting a role for copper homeostasis in affecting  
4 hair curl.  
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### 9 **Curly hair follicle development is under the control of major** 10 **developmental programmes**

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12 Genome-wide searches have also uncovered evidence that developmental genes  
13 are involved in shaping hair curl. The ectodysplasin receptor family (EDAR) are  
14 cell surface receptors of the tumour necrosis factor family (TNF) expressed in  
15 skin and hair follicles during hair follicle development, again at puberty and  
16 during the hair cycle [89]. Recently EDAR has been implicated in the control of  
17 hair shape and fibre thickness [90] [91]. Positive selection of a polymorphism in  
18 east Asian and native American populations about 10,000 years ago is believed  
19 to have affected both follicle size and fibre thickness as well as shovel-shaped  
20 incisor teeth and increased secretions of sebum and meibian lipids in the eye  
21 and saliva [92] [93]. As indicated earlier, the shape of the hair follicle is set  
22 through embryogenesis and then curl manifests during childhood. Thus it is not  
23 surprising that factors involved in embryogenesis affect hair shape. The  
24 interesting question is why East Asians developed straight hair? One  
25 explanation is that the glandular changes may have been the driving force behind  
26 the penetrance of the new gene variant in East Asians, with straighter hair being  
27 a non-selective consequence of advantageous changes in tooth shape and gland  
28 activity. The hair phenotype maybe linked to higher Edar function which,  
29 through signalling via sonic hedgehog [94] may have led to greater symmetry in  
30 growth rates in the follicle bulb with straighter hair arising as a result.  
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45 A second developmental gene associated with hair morphology is suspected to  
46 be WNT10A (wingless-type MMTV integration site family, member 10A; [70].  
47 WNT10A is upregulated at the beginning of the hair growth cycle and mutations  
48 in this gene are known to cause misformed hair [95] and appendage  
49 abnormalities in Hypohidrotic Ectodermal Dysplasia patients [96]. However, a  
50 known mutation in Wnt10A (rs7349332) in combination with mutations in  
51 TCHH (rs11803731 and FRAS 1 (rs1268789) form a potential signature for  
52 straight hair of potential use in forensics [97].  
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3 In summary, the shape, type and colour of hair are determined not just during  
4 embryogenesis but also repeatedly in each hair growth cycle. Aside from pattern  
5 baldness, characteristic hair types are maintained in bodily patterns throughout  
6 life. Natural population variance in hair curl appears to have a largely genetic  
7 basis and environmental pressure selecting for specialised hair morphology may  
8 well have arisen when humans migrated out of Africa. There is evidence that  
9 trichohyalin (TCHH) may affect hair curl in most/all world populations and that  
10 other genes such as EDAR, WNT10A only affect specific populations. Hair curl  
11 variation in native Africans is very likely a complex trait with multiple genes  
12 influencing curl. The strongest evidence for the control of shape comes from the  
13 evidence of the role of the inner root sheath which appears to structurally mould  
14 hair fibre shape, including curl - but we are still a very long way from  
15 understanding the complete biological/biophysical mechanisms that produce  
16 such a wide range of curled, coiled, kinked and wavy hair fibres.  
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31 MR Green and RS Ginger are employed by Unilever R&D.  
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### 36 Conflicts of Interest

37 The authors state no conflicts of interest.  
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## Figures and Tables

Figure 1. Variation in degree of curl in human hair fibres. Hair fibres were sampled from populations in South Africa (a) showing a range from high curl (left) to low curl (right). Magnified images of fibre samples from low (b) and high (c) curl reveal that both degree of curliness (tightness of the curl) and the change in direction of the curl contribute to overall curliness.

Figure 2. Diagrammatic representation of the lower hair follicle bulb. This figure shows the distribution of the 7 layers that comprise the fundamental hair follicle structure as well as the dermal papilla and connective tissue sheath. This diagram has been kindly provided by Dr. Claire Higgins.

Figure 3. Representation of the curly hair follicle in three dimensions. Figure 3a shows the line of symmetry around the long axis of the hair in a straight hair follicle. Figure 3b the long axis is shown as symmetrical through the dermal papilla of a curly hair follicle. Note that the hair shaft is no-longer in plane with the dermal papilla, Figure 3c shows the section through the bulb depicting the curved shape of the lower follicle.

Figure 4. A series of images showing the retrocurvature of the hair follicle when sectioning through a sample of scalp skin from an individual with very curly hair. The curly hair follicle is curved in two directions – left to right and fore and back. a) the upper bulb is out of the image, yet the shaft is sectioned through. b) the follicle curves twice (arrows). c) the bulb is sectioned through the mid dermal papilla d) the dermal papilla is almost out of section and e) the bend in the upper follicle reveals connective tissue sheath (arrow).

Figure 5. Population distribution of South African hair curl covering the main language groups (Sotho/Tswana, Xhosa and Zulu). Average Mean Curvature

(1/radius, AMC) was mathematically averaged over each fibre separately, before the mean value for 20 hairs from each volunteer was calculated. The range of hair AMC sampled was from 0.14 to 1.545. As illustrated in the figure, more curly hair has a higher AMC.

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Table 1  
Biomarkers associated with curl in the hair follicle

| Biomarker    | Localisation | Asymmetry   | Reference |
|--------------|--------------|---|-----------|
| K38          | Cortex       | Earlier expression on concave side                            | [46]      |
| K82          | Cuticle      | Later expression on convex side                               | [9]       |
| Ki67         | Bulb matrix  | Proliferation is above the line of 'Auber' on the convex side | [9] [1]   |
| K14          | ORS          | ORS is thicker on the concave side                            | [1, 9]    |
| IGFBP-5      | ORS          | Greater expression on convex side                             | [44]      |
| K74          | IRS          | Mutations give rise to woolly hair                            | [76]      |
| K71          | IRS          | Mutations give rise to woolly hair and curly hair in dogs     | [78, 79]  |
| Trichohyalin | IRS          | SNP associates with straighter hair in caucasians             | [84]      |
| Fibronectin  | CTS          | CTS is thicker on the concave side                            | [9]       |

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Table 2  
Candidate genetic associations influencing black African hair curl

| Gene region | Possible function   | SNPs                               | p-value   |
|-------------|---|------------------------------------|---|
| KRT74       | IRS keratin linked to woolly hair syndrome. Adjacent to KRT71 which strongly affects hair curl in dogs            | rs3912631                          | $<3 \times 10^{-05}$  |
| TCHH        | Hair follicle specific protein also found in the IRS. Linked to hair curve in peoples of western European descent | Afd_1108920*                       | $<1 \times 10^{-6}$   |
| CUTC        | Copper transport homologue. Copper changes linked to wool crimp in sheep and 'kinky hair' in Menkes disease       | rs4919394<br>rs978554<br>rs7078602 | $<5 \times 10^{-7}$<br>$<9 \times 10^{-7}$<br>$<1 \times 10^{-6}$ |

p-values include a genome wide Bonferroni correction

\* Afd\_1108920 is a SNP used on the Perlegen genotyping platform, 7kb from rs11803731 identified by Medland et al [84]. The rs11803731 alternate allele is found only in populations of European descent and therefore is not informative for black African hair.

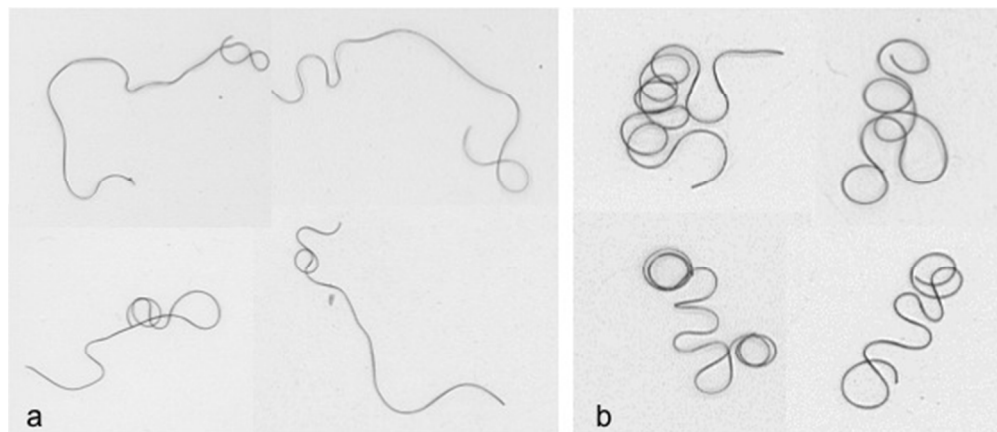


Figure 1. Variation in degree of curl in human hair fibres. Hair fibres were sampled from populations in South Africa (a) showing a range from high curl (left) to low curl (right). Magnified images of fibre samples from low (b) and high (c) curl reveal that both degree of curliness (tightness of the curl) and the change in direction of the curl contribute to overall curliness.

Figure 1  
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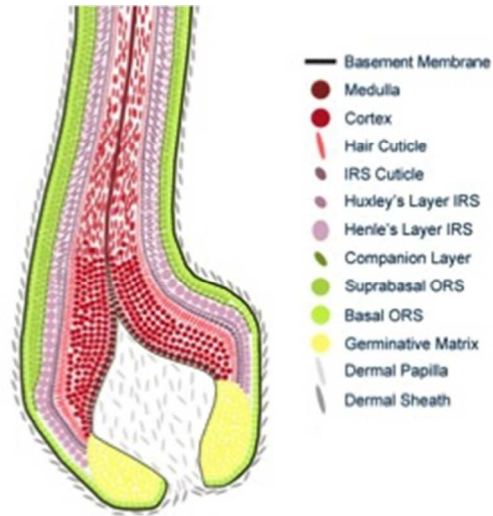


Figure 2. Diagrammatic representation of the lower hair follicle bulb. This figure shows the distribution of the 7 layers that comprise the fundamental hair follicle structure as well as the dermal papilla and connective tissue sheath. This diagram has been kindly provided by Dr. Claire Higgins.

Figure 2  
103x97mm (72 x 72 DPI)

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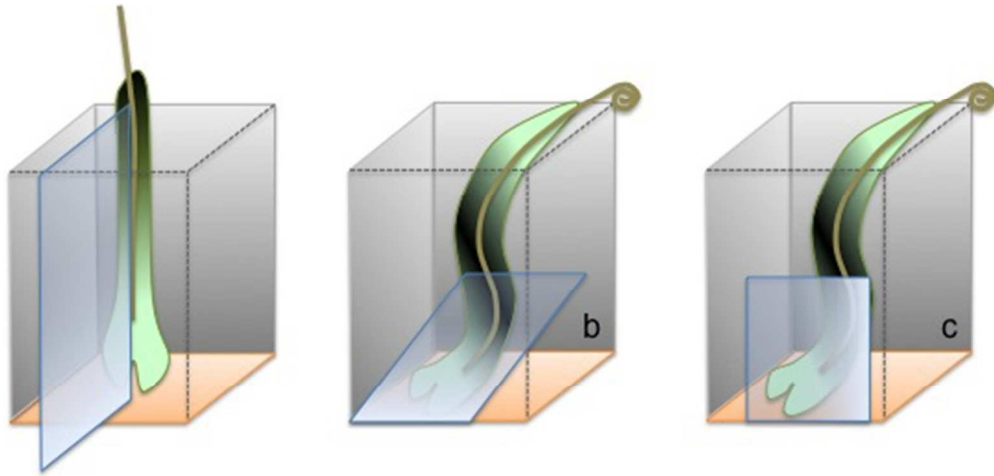


Figure 3. Representation of the curly hair follicle in three dimensions. Figure 3a shows the line of symmetry around the long axis of the hair in a straight hair follicle. Figure 3b the long axis is shown as symmetrical through the dermal papilla of a curly hair follicle. Note that the hair shaft is no-longer in plane with the dermal papilla, Figure 3c shows the section through the bulb depicting the curved shape of the lower follicle.

Figure 3  
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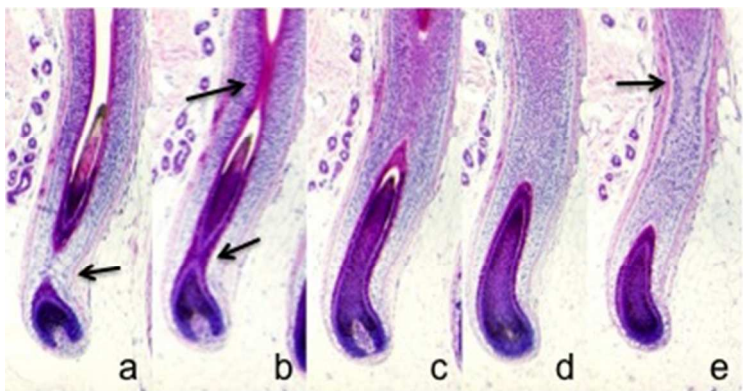
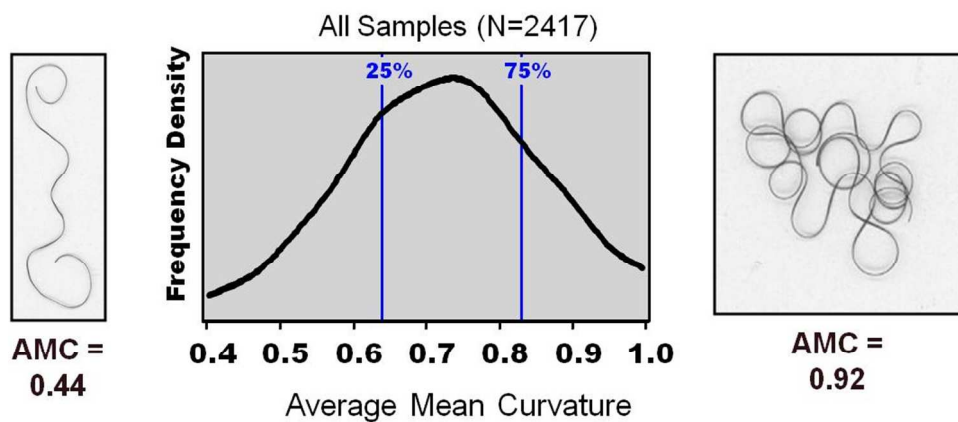


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Figure 4  
130x68mm (72 x 72 DPI)

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23 Figure 5. Population distribution of South African hair curl covering the main language groups  
24 (Sotho/Tswana, Xhosa and Zulu). Average Mean Curvature (1/radius, AMC) was mathematically averaged  
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27 AMC.

28 Figure 5  
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