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Supplemental Data

Autosomal-Dominant Woolly Hair Resulting from Disruption of Keratin 74 (*KRT74*), a Potential Determinant of Human Hair Texture

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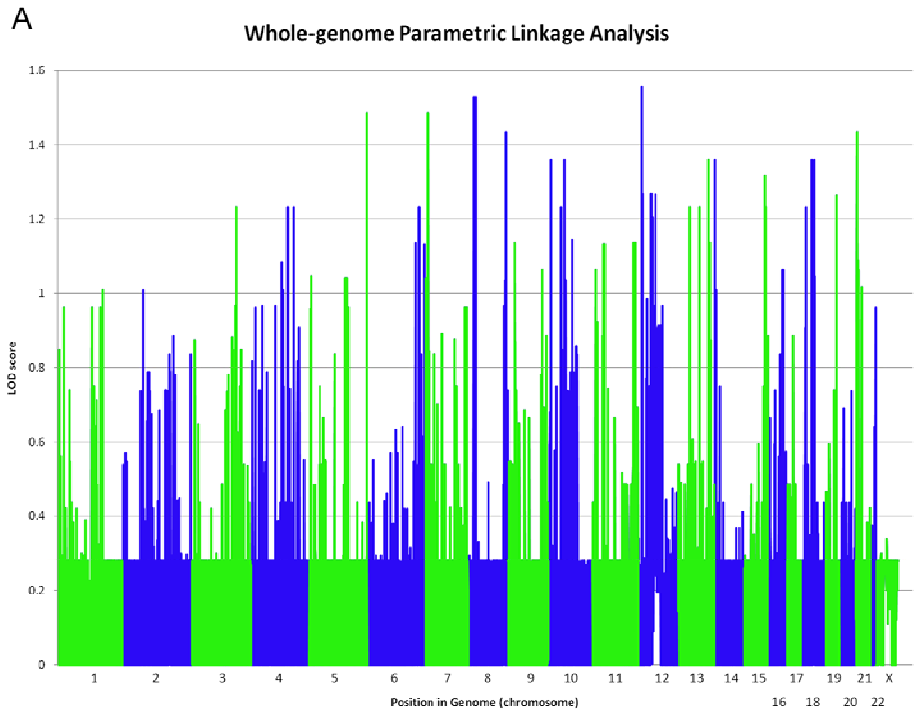
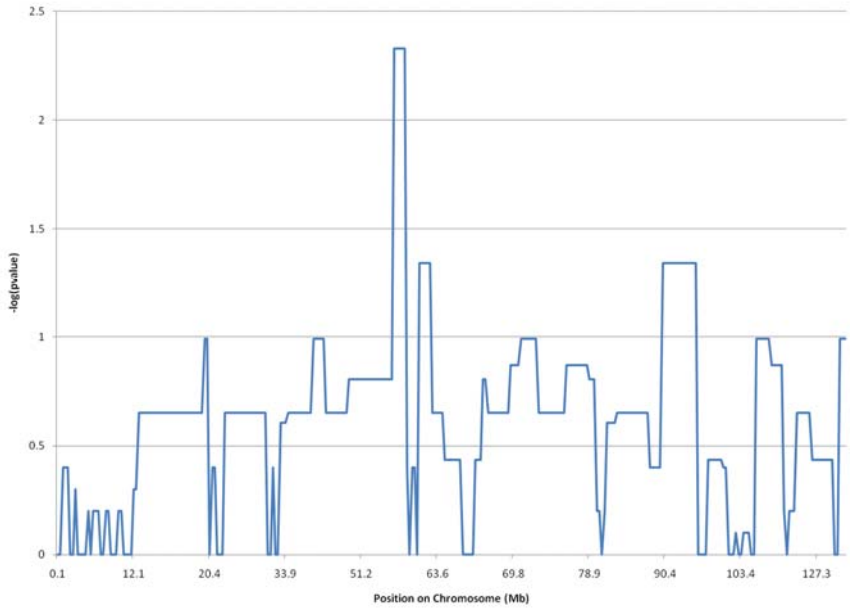
Figure S1. Results of statistical analysis. (**A**) Whole genome linkage analysis was performed on SNP data under the assumption of an autosomal dominant mode of inheritance of a fully penetrant rare allele (frequency of 0.001). A suggestive maximum LOD score of 1.56 was found on chromosome 12. (**B**) The haplotype-based haplotype relative risk (HHRR) analysis was performed on the SNP data across chromosome 12 to more precisely localize the disease locus and implicated 12q13. (**C**) Microsatellite markers were chosen for fine mapping across the putative region and confirmed linkage across the type II keratin gene cluster.



B


HHRR on Chromosome 12
2.5
2
1.5
1
0.5
0
-log(pvalue)
0.1
12.1
20.4
33.9
51.2
63.6
69.8
78.9
90.4
103.4
127.3
Position on Chromosome (Mb)

C

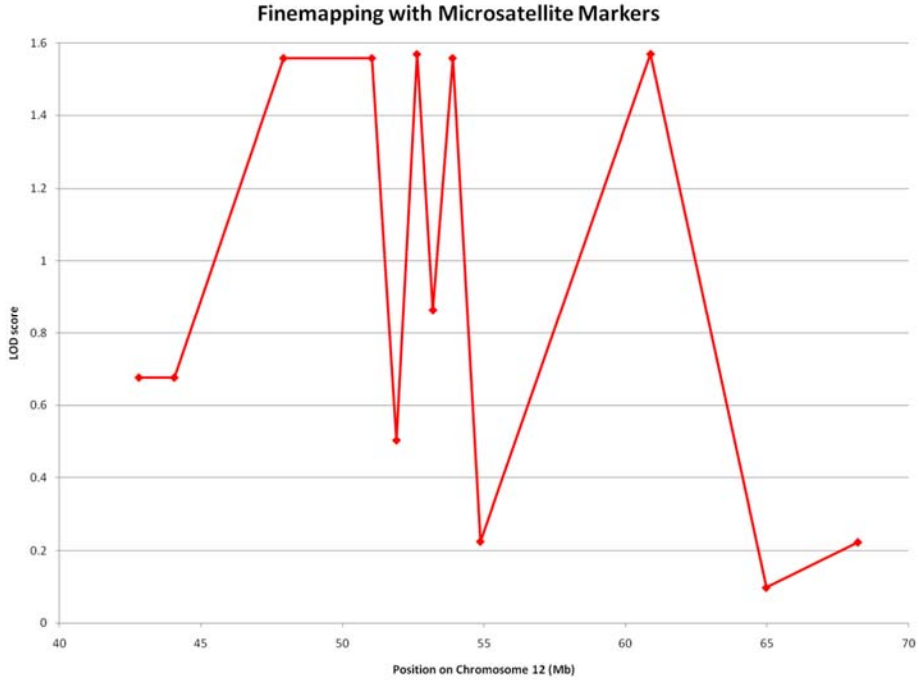

Finemapping with Microsatellite Markers
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
LOD score
40
45
50
55
60
65
70
Position on Chromosome 12 (Mb)

Figure S2. Mutant K74 protein disrupts endogenous KIF formation in MCF-10A cells. (**A-C**) Ectopically expressed wild type (Wt) K74 protein forms a KIF network via heterodimerization with endogenous K14 protein. (**D-F**) The p.Asn148Lys mutant (Mut) K74 protein causes a collapse of the endogenous KIF network around the nucleus. Scale bar: 20 µm.

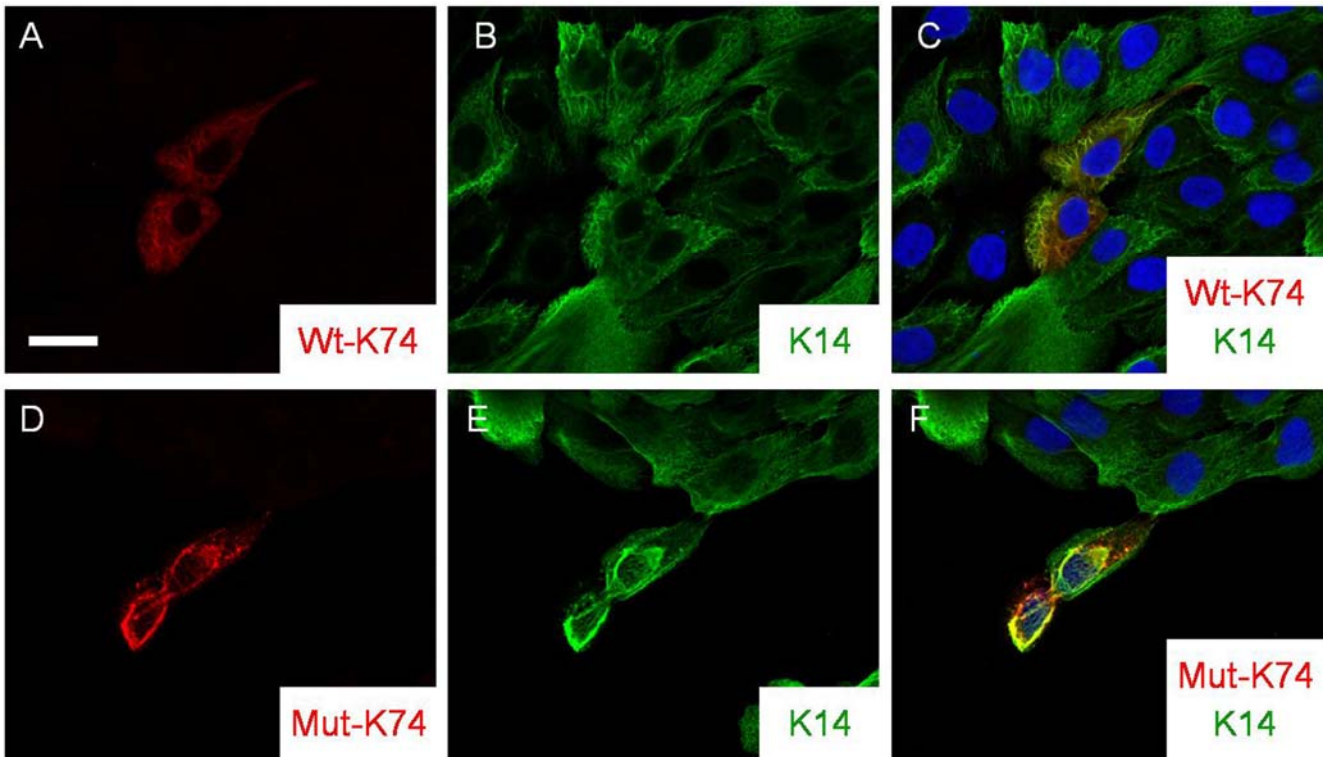


Figure S3. (**A**) Identification of a heterozygous mutation c.1318G>A (p.Glu440Lys) in mouse *Krt71* gene in *Caracul-like 4* (*Cal4*) allele. (**B**) Schematic representation of mouse K71 protein and location of all known *Krt71* mutations. The helix initiation motif (HIM) and helix termination motif (HTM) are colored in yellow and blue, respectively. The mutation p.Glu440Lys in the *Cal4* allele identified in this study is indicated in red. c.172_181del in *Reduced coat 3* (*Rco3*) allele is a recessive mutation, while the others are dominant mutations and occurred in either the HIM or the HTM of the K71 protein.

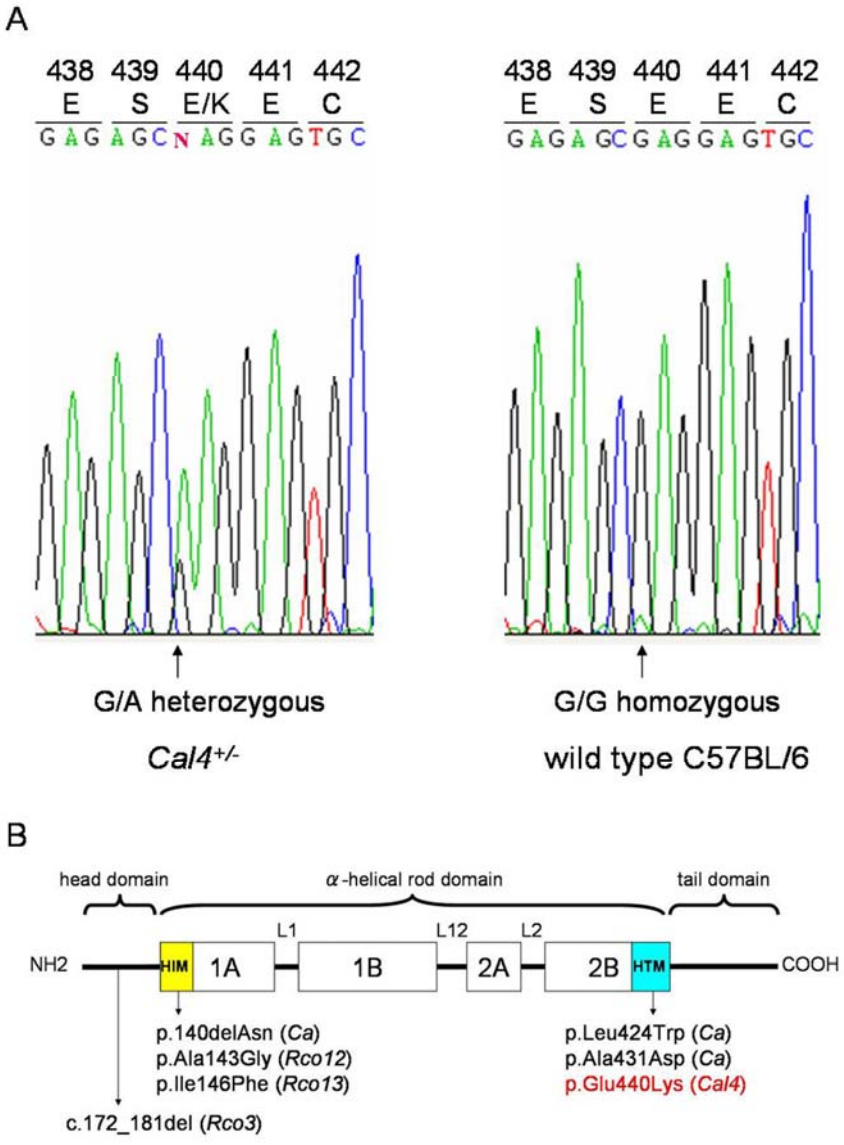


Figure S4. Genetic differentiation between African and European populations at the *KRT71-74* gene cluster. Allele frequencies for all SNPs in each of four *KRT* genes located on chromosome 12q13 were downloaded for an African population (YRI) and a population of European ancestry (CEU) from HapMap. The differentiation between the two populations for each SNP was calculated as $F_{ST} = \frac{\delta^2}{\theta(2-\theta)}$ where δ is the difference in reference allele frequencies between populations and θ denotes the sum of the reference allele frequencies. Pairwise F_{ST} is plotted as a function of physical position for all four *KRT* genes examined in the top panel. In the bottom panel, the location of the SNPs is plotted relative to the *KRT71* gene. Coding SNPs are indicated by red diamonds and non-coding by black diamonds. The red arrow indicates the position of rs10783518, a coding SNP that results in a non-synonymous change in amino acids, p.Gly464Val. Pairwise F_{ST} values were calculated for all SNPs on chromosome 12 and an F_{ST} of 0.3 corresponded to the 97th percentile of scores (data not shown).

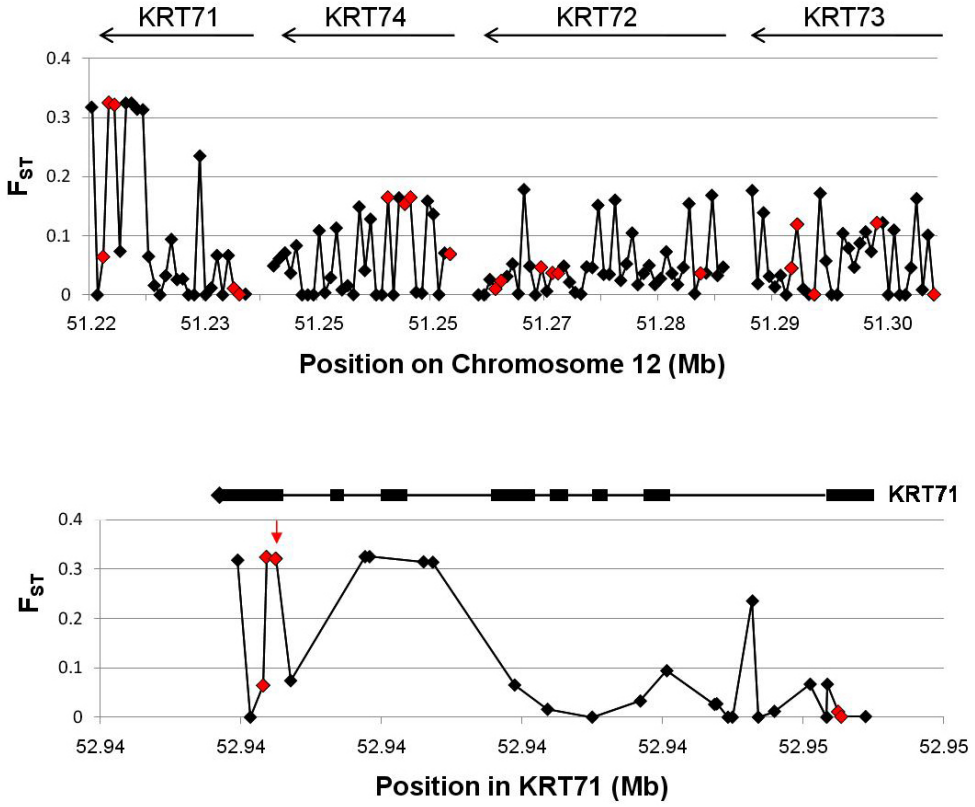


Table S1. Primers used in this study

Primer name	Forward primer (5' to 3')	Reverse primer (5' to 3')	product size (bp)
Primers used in sequencing analysis of human *KRT71-74* genes			
KRT71-ex 1	AAGGCACCTGCCAGTCCTCA	GTCACCCTGTTGATGGGATGTA	682
KRT71-ex 2	CTGTGAGAGACACGTGTGACT	CATTTAAGCTGGGGTGACTGC	360
KRT71-ex 3	CAGCACCTGTATCTTCTGATG	CACCTTGGCAGGCTCTGTTC	239
KRT71-ex 4	CTCCTTTCCCAAGGCAAAGT	AACTGAGGGGTCTCACTGAG	333
KRT71-ex 5	TGGGTTCCAGCCTCCAAGAT	GCACGATCTGTCTCCATCTG	343
KRT71-ex 6	CAGATGGAGACAGATCGTGC	CCATGTTCTCAGCAGCTCATC	327
KRT71-ex 7	CTCTGCCCCACTCTAAGGAC	CTGGATTGAGATGTGTTAGGC	393
KRT71-ex 8	GCTGATGGTGGCAGTAGCT	GCACACAGAGGGTGTCACTA	191
KRT71-ex 9	TGCACCTCCCCACTCAGCT	CAGGTGTATGGGAGCAGGAC	438
KRT72-ex 1	CAAGAGGCCTCAAGGGATCT	GGCTCGGAGAGGTTATGACC	694
KRT72-ex 2	GAGGCAACAACATGGGGACT	GTTTGGAGAACCGGTGTAGG	434
KRT72-ex 3	AGGGGTATGGGAATCCCAGT	GGGAAGTTAGAGTGCCAGTC	315
KRT72-ex 4	GTAGCCATTGCACTGCACGT	CAGTAGGGCCTTGGTGAATG	417
KRT72-ex 5	GAGGGACAGTGAGGAATTTGC	GAGGTCTTGACTGAGCCCAA	384
KRT72-ex 6	ACTCTGCCAAGGCTCTCATG	ACCTACAGTGTGTAGGCTGC	436
KRT72-ex 7	CTCCAAGCAGTTGCCATCAGT	TTCAGTAGGACCTAAACAGGAC	424
KRT72-ex 8	CATTCTGCGTGGAGAGGCTT	CATCCACTGCTTCCCCTTAG	297
KRT72-ex 9	ATCTCAGCAGGAAGCCACTG	TGACTGGACTCCTTGCATCG	486
KRT73-ex 1	ATCACAGGCTGGTGAACTGC	CAGCACCTGGGGAGCTTTCA	677
KRT73-ex 2	CCTGTCCAGGAAGGGAATTC	CTTGCTGGGACAGATGAACC	396
KRT73-ex 3	CTGGTGTGTTGGGATGGACT	CTTCAGCACTTTGGCTCCAG	324
KRT73-ex 4	AAGTCCTGGGCAGCTAGCTA	GAGGTGCCCTGGACTGCTTA	306
KRT73-ex 5	GGAAGTGCAGCTTCCATCTG	GGCAGACAAGACAGAGTCTG	353
KRT73-ex 6	CACAGTCTCTGTACCAGGCT	GGATGAGAGCAGGACCTCCA	330
KRT73-ex 7	GGCTCAACCATTGAGAGCATG	TGGGAAACTTTAAGGTGGAGAC	358
KRT73-ex 8	CAGCCAGTGCCATCTGGCAA	AGAGCCAAGCTCTTCCTCAG	275
KRT73-ex 9	TTGGGAGTCTTGCAGGACTG	AAGAGTCCGGAGCAGTCTGC	513
KRT74-ex 1	ACTCTGGGTGCCCCATCAGT	CTTTCCAGCCACAGTGTGCA	643
KRT74-ex 2	GGGTGGAGAGTCAAGACATG	AAGGGATGTGAGCTCCTGAC	471
KRT74-ex 3	CTCCTGTCACTAGCCTCTTAC	GCTGAAGTCCTGAGCAGAGT	269
KRT74-ex 4	CTCCAGGCATAACAGAGCTG	TTCCATGGTCTCCTGGTGCA	437
KRT74-ex 5	CGTGGATCCTAGCAGCCTAT	CCTCACATTCACTGTGCAGGT	349
KRT74-ex 6	TTACAGGACCCTTCCCCAGT	CCAGCTCTGATGGTAGGGTA	358
KRT74-ex 7	CCCATCTGCAAAATGAGGGCAT	CATGTTGTTCTCCAGGTGGC	397
KRT74-ex 8	GCGATAGTGTCAGTTTCGTAAG	CTTCTTGGGAGGTGAGACAG	261
KRT74-ex 9	TTGGGGACAGCCGTTACCAT	CGTTAGTCCACATGGGACCTA	486
Primers used in sequencing analysis of mouse *Krt71-74* genes			
Krt71-ex 1	AGGCACCTGCTGGTCCTCA	CCAGCATCATGTACAGTAACTG	648
Krt71-ex 7	GGGTGTTTGCTCCTGGATC	CTAGGGCTTCGCACATGCTA	410
Krt72-ex 1	GGACTATTTAAGGGGCGAGCT	TCCCCTTCACTGTGTGAATGC	636
Krt72-ex 7	CTCCAGTTCTGTCCAACCTG	GGGTGGCATTGATGCTTTGC	541
Krt73-ex 1	ATCACAAGCGGGGAACAGTC	GGGTAGCATCATCCTATCTCC	607
Krt73-ex 7	AAGGCCCATCCGAGATTAGC	GTGGTTCAACTGGAGAGCATC	404
Krt74-ex 1	GAACTTCAGGTCATGCAGTGAC	CACAGGAAACAGGAGAACTGC	501
Krt74-ex 7	CTCATAGGCACAACCGGAGC	TGCCAGCAAGGCTGTGCTCA	451
Primers used in segregation analysis and screening assays for the mutation c.444C>G in human *KRT74* gene			
KRT74-AcuI	AGGCCTGGCTCTGGGTATGGA	CTTTCCAGCCACAGTGTGCA	338
Primers used in cloning of human *KRT74*-cDNA into the mammalian expression vector pCXN2.1			
KRT74-rtpcr	AAAAGAATTCAACCTTTCCCACCATGAGTC	AAAACTCGAGTCACCTCTTCTTCCAAGTGC	1665

Table S2. Pathogenic mutations in the Asn residue at position 9 in the HIM of human type II keratins. Asn to Lys substitutions at amino acid position 9 in the HIM are indicated in bold letters.

Keratins (previous nomenclature)	Mutations	diseases	references
K1	p.Asn188Ser	bullous congenital ichthyosiform erythroderma	18, 19
K1	p.Asn188Thr	bullous congenital ichthyosiform erythroderma	20
K1	**p.Asn188Lys**	bullous congenital ichthyosiform erythroderma	21
K2 (K2e)	p.Asn186Tyr	ichthyosis bullosa of Siemens	22
K2 (K2e)	**p.Asn186Lys**	ichthyosis bullosa of Siemens	23
K5	p.Asn176Ser	epidermolysis bullosa simplex	24
K6a	p.Asn171Asp	pachyonychia congenita type I	25
K6a	p.Asn171Tyr	pachyonychia congenita type I	25
K6a	p.Asn171Ser	pachyonychia congenita type I	26
K6a	**p.Asn171Lys**	pachyonychia congenita type I	27, 28
K74 (K6irs4)	**p.Asn148Lys**	autosomal dominant woolly hair	This study
K86 (Hb6)	p.Asn114Asp	monilethrix	29
K86 (Hb6)	p.Asn114His	monilethrix	30