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Original Article

Intragenic deletion mutation in the gene desmoglein 4 underlies autosomal recessive hypotrichosis in six consanguineous families

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ملخص

الأهداف: نقص الشعر الجسمي الموضعي المتنحي هو اضطراب فقدان الشعر البشري غير المتلازمة، التي تؤثر على فروة الرأس والحاجبين والرموش، وأجزاء أخرى من الجسم . ستة عوائل مصابين(بحيث كل عائلة فيما بينهم صلة قرابة) بهذا النوع من الاضطراب تساقط الشعر وقد تم التحقيق على المستويين السريرية والجزيئية.

الطريقة: تم اختبار الربط في ستة عوائل مع فرد مصاب واحد من كل عائلة بواسطة التنميط الجيني علامة الميكروستلايت مرتبطة بمواضع نقص الشعر الجسمية المتنحية بما في ذلك نقص الشعر الجسمي الموضعي المتنحي(LAH) ١،٢ و ٣. تم إجراء تحليل تسلسل مواقع الترميز واللصق كاملة من الجينDSG4 للبحث عن الطفرة المسببة للمرض.

النتيجة: ربط إنشاء التنميط الجيني في العوائل على الجين DSG4 في LAH1 الواقع على كروموسوم 18q21.1. الكشف عن تحليل تسلسل طفرة الحذف داخل الجين(EX5_8del)في الأفراد المصابين من كل العائلات الستة.

الخلاصة: تحديد الطفرات المتكررة في ست عائلات باكستانية إضافية يعزز من الأدلة على أن هذا هي طفرة الأجداد التي تنتشر على نطاق واسع بين مختلف الجماعات العرقية الباكستانية.

الكلمات المفتاحية: الجينات DSG4؛ داخل الجين طفرة الحذف؛ باكستان؛ طفرة المتكررة

Abstract

Objectives: Localized autosomal recessive hypotrichosis is a non-syndromic human hair loss disorder, affecting scalp, eyebrows and eyelashes, and other parts of the body. Six consanguineous families with this form of hair loss disorder were investigated at both the clinical and molecular levels.

Methods: Linkage in six families with twenty-one affected members was tested by genotyping microsatellite markers linked to autosomal recessive hypotrichosis loci including localized autosomal recessive hypotrichosis (LAH) 1, 2 and 3. Sequence analysis of the entire coding and splice sites of the gene *DSG4* was performed to search for the disease-causing mutation.

Results: Genotyping established linkage in families to the *DSG4* gene at LAH1 locus on chromosome 18q21.1. Sequence analysis detected an intragenic deletion mutation (Ex5_8 del) in affected members of all six families.

Conclusion: Identification of recurrent mutation in six additional Pakistani families strengthens the body of evidence that this is an ancestral mutation that is widespread among different Pakistani ethnic groups.

Keywords: *DSG4* gene; Intragenic deletion mutation; LAH; Pakistan; Recurrent mutation

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Introduction

Desmosomes are intracellular junctions that play important roles in cell-to-cell adhesion and signal development and differentiation in tissues that sustain mechanical stress, such as the heart, muscle and epidermis. These junctions contain three major protein groups: the desmosomal cadherins that comprise desmogleins (DSG1-4) and desmocollins (DSC1-3), the plakin family member desmoplakin (DSP), and arm (armadillo) proteins plakoglobin (PG) and plakophilins (PKP1-3).[1] Disruption of these junctions leads to a broad spectrum of inherited, infectious and auto-immune diseases. Pathogenic autosomal dominant and recessive mutations have been reported in ten different desmosomal genes, resulting in a spectrum of phenotypes that variably affect the skin, hair and heart. Out of these desmosomal proteins, the functional absence of desmoglein 4 (DSG4), usually expressed in the hair shaft cortex, leads to localized autosomal recessive hypotrichosis (LAH1) in humans and the lanceolate hair phenotype in rodents (lah).[1–3]

Localized autosomal recessive hypotrichosis is a non-syndromic human alopecia affecting scalp, eyebrows and eyelashes, trunk, arms and legs. In males, moustache and beard hair are either sparse or not affected. In addition, a few patients reported developing hyperkeratotic follicular papules, erythema, and pruritus in affected areas.[2,4] Three

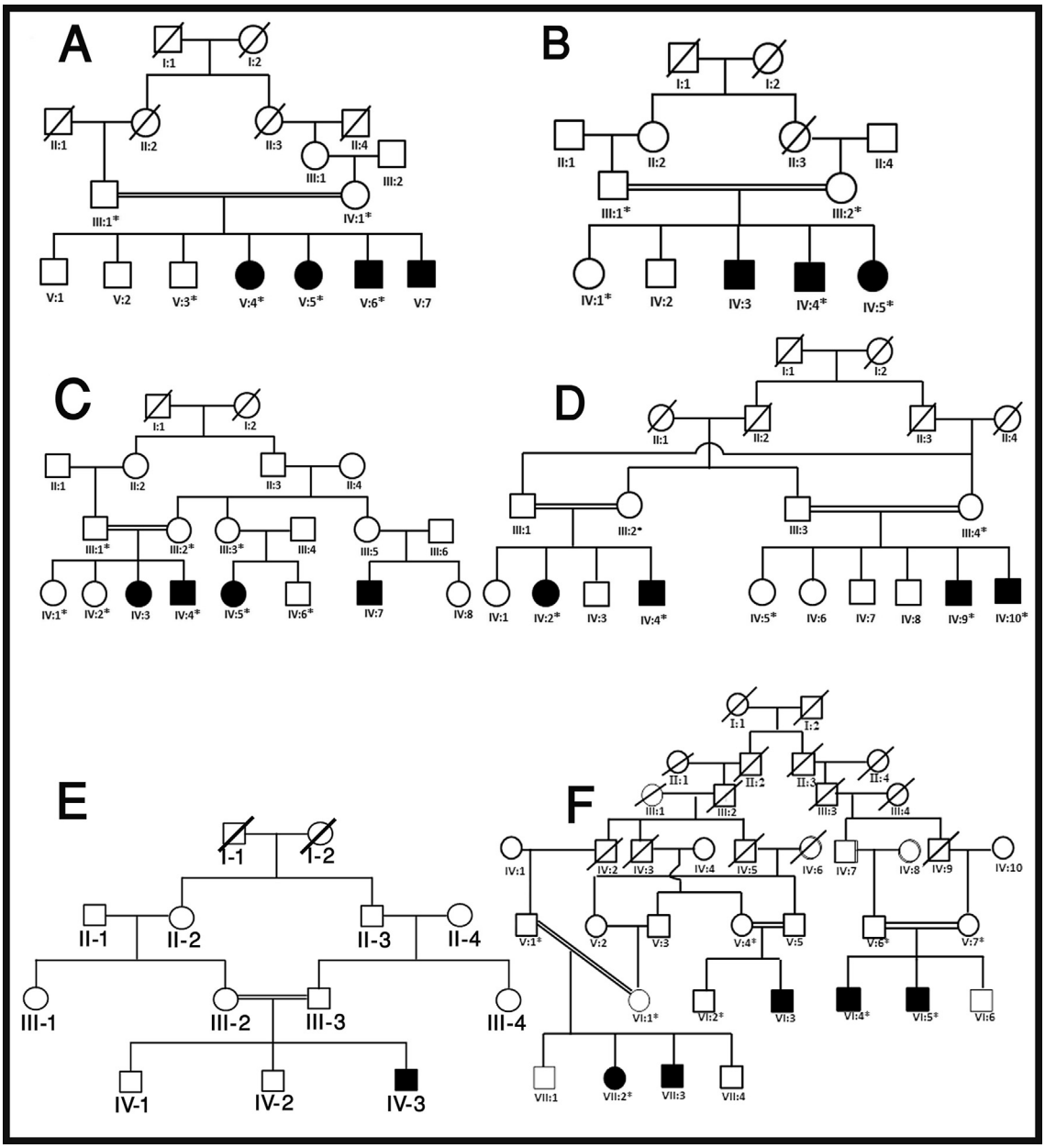

Figure 1: Pedigree drawing of the six families (A, B, C, D, E, F) segregating autosomal recessive localized hypotrichosis. Affected males and females are indicated by filled squares and circles, respectively. Crossed symbols indicate deceased individuals. Double lines between individuals represent consanguineous unions. The individual numbers labelled with asterisks indicate the samples available for this study.

genetically distinct forms of LAH with similarities in clinical features have been mapped to human chromosomes: LAH1 (MIM 607903) to 18q12.1,[2] LAH2 (MIM 609167) to 3q27.3[5] and LAH3 (MIM 611452) to 13q14.11-q21.32[6] caused by mutations in desmoglein 4 (*DSG4*, MIM 607892),[2] lipase-H (*LIPH*, MIM 607365)[4,7–10] and lysophosphatidic acid receptor 6 (LPAR6, MIM 609239),[11–13] respectively. Mutations in *LPAR6* and *LIPH* also result in the autosomal recessive woolly hair (ARWH) phenotype.[14,15] A null mutation in another desmosomal cadherin DSC3 has also been identified to cause hypotrichosis with recurrent skin vesicles in a large family from Afghanistan.[16]

In the present study, we have sequenced the gene *DSG4* in six unrelated Pakistani families with hereditary hypotrichosis, which showed linkage to microsatellite markers linked to the LAH1 locus on chromosome 18q12.1. Sequence analysis of the gene *DSG4* in affected individuals revealed a previously reported in-frame deletion of exons 5–8 (Ex5_8del), predicted to remove amino acids 125–335 in the DSG4 protein.

Materials and Methods

Human subjects

Six unrelated consanguineous families (A, B, C, D, E, F), demonstrating autosomal recessive hypotrichosis were identified from different geographical regions of Pakistan. The pedigrees provided convincing evidence of an autosomal recessive mode of inheritance of the phenotype (Figure 1). Two families, A and E, belonged to the Punjab province while the other four families (B, C, D, F) originated from the Sindh province of Pakistan. Venous blood samples were collected from a total of 39 individuals, including *21* that showed the phenotype hypotrichosis. Genomic DNA was extracted from whole blood using the standard phenol-chloroform procedure. Approval of the study was obtained from the institutional review board (IRB) of Quaid-i-Azam University, Islamabad, Pakistan. Written informed consent was provided by the patients or their guardians to perform the genetic study.

Instrumentation and procedure

Genotyping was performed using microsatellite markers (D18S877, D18S49, D18S36, D18S47, D18S456, D18S1124, D18S1133, D18S57) flanking the gene *DSG4* mapped on chromosome 18q12.1 (Table 2a). The polymerase chain reaction (PCR) was performed as described previously.[17] *Primers were designed using primer3 software* (Table 2b). The PCR amplified products were resolved on 8% nondenaturing polyacrylamide gel, stained with ethidium bromide, and genotypes were assigned by visual inspection.

The gene *DSG4* on chromosome 18q12.1 was sequenced in affected and unaffected individuals of the six families from whom DNA samples were available for the study. Sequencing was performed bidirectionally (forward and reverse strands) with the Big Dye Terminator v3.1 Cycle Sequencing Kit, together with an ABI Prism 310 Genetic Analyzer (Applera, Foster City, CA). Sequence variants were identified via BIOEDIT sequence alignment editor version 6.0.7 (Carlsbad, CA). Sequence analysis of the gene *DSG4* was performed using a control reference obtained from the Ensembl database (Ensembl accession ID ENST00000359747).

Results

Clinical features

Six unrelated consanguineous Pakistani families (A–F) affected with localized hypotrichosis were investigated in the present study. Affected members of two families (A and B) had complete hair loss affecting the scalp, eyebrows and

Table 2a: List of microsatellite markers used to test linkage to the DSG4 gene involved in autosomal hair loss disorder.

Phenotype	Gene	Chromosome	Markers	cM*	Mb+
Localized autosomal recessive hypotrichosis (LAH1)	*DSG4*	18q21.1	D18S877	56.26	24.97
			D18S847	57.41	25.95
			D18S47	59.33	28.03
			D18S456	60.41	29.41
			D18S1133	60.90	30.35

Table 1: List of mutations reported in the *DSG4* gene so far.

Mutation	cDNA	Protein	Phenotype	Family origin	Reference
Deletion	Ex5_8 del	Truncated Protein	HT[a]	Pakistan	2, 3, 23, 24, present study
Indel	c.384_385 del GG ins TT	p.A129S	HT	Iraq	29
Missense	c.574T > C	p.S192P	Monilethrix/HT	Japan	19
Insertion	c.2039insT	p.S680FfsX4	Monilethrix/HT	Japan	19
Splicing	c.216 +1G > T	Exon 3 skipping	Monilethrix with HT	Iraq and Iran	18
Missense	c.800C > G	p.P267R	Monilethrix with HT	Iraq and Iran	18
Missense	c.800C > G	p.P267R	Monilethrix	Iraq, Iran, Morocco	20
Nonsense	c.865C > T	p.R289X	Monilethrix	Iraq, Iran, Morocco	20
Splicing	c.216 +1G > T	Exon 3 skipping	Monilethrix	Iraq, Iran, Morocco	20
Deletion	c.763delT	p.C255VfsX6	Monilethrix	Iraq, Iran, Morocco	20
Deletion	c.87delG	p.K30Rfs X54	HT	Pakistan	30
Deletion	c.624delG	p.M208IfsX4	Monilethrix	Japan	31
Nonsense	c.2468G > A	p.W823X	Monilethrix	Japan	31
Deletion	c.85-1_191	Truncated Protein	HT, Monilethrix	Pakistan	21

[a] HT; Hypotrichosis.

Table 2b: Primer sequences used for amplification of the *DSG4* gene.

No	Primer		Sequence		Product	Tm
1.	DSG4-1F	5′	TTCGGAACTGAGAAGACGAG	3′	172 bp	58.1 °C
2.	DSG4-1R	5′	CAGAGACAAGACCTTGAGGC	3′		57.5 °C
3.	DSG4-2F	5′	AAATGTAAATACTTTGGAGGGC	3′	236 bp	56.4 °C
4.	DSG4-2R	5′	ATGCATAGAGATTTGGGAGC	3′		56.8 °C
5.	DSG4-3F	5′	CACTGTTTCTTCTAAATGCACC	3′	362 bp	56.6 °C
6.	DSG4-3R	5′	ATTTGAAAGCTTCCCTGCTC	3′		58.5 °C
7.	DSG4-4F	5′	CCCATTTGGTAAAGAAACCC	3′	372 bp	58.2 °C
8.	DSG4-4R	5′	CTATTTGGGTTCAGTCTGCC	3′		57.2 °C
9.	DSG4-5F	5′	CCCAAATAGAAGACTTGAAGG	3′	626 bp	55.6 °C
10.	DSG4-5R	5′	GCTAAGCACCTGCTAAATCC	3′		56.7 °C
11.	DSG4-6F	5′	GGCCAACCACTCTGTCTTC	3′	532 bp	58.2 °C
12.	DSG4-6R	5′	CAGGTTGTACATACTGTGTTGC	3′		55.8 °C
13.	DSG4-7F	5′	GATCCATGTGTACCCTTACTCC	3′	434 bp	57.4 °C
14.	DSG4-7R	5′	CACATAGGACAGAACCAGGC	3′		58.1 °C
15.	DSG4-8F	5′	TCTCCTGATTGGACTATGGG	3′	479 bp	57.5 °C
16.	DSG4-8R	5′	AGCAGTTACTTAGGACCCTTG	3′		55.3 °C
17.	DSG4-9F	5′	AAACAGCGTATCTCCTGGAC	3′	693 bp	56.8 °C
18.	DSG4-9R	5′	CCAGGGTAGAACAAACTGGC	3′		59.5 °C
19.	DSG4-10F	5′	TAAACCAAGGCAATCATCAC	3′	399 bp	56.1 °C
20.	DSG4-10R	5′	GGGCTTTCCATAAGTCTTGC	3′		58.7 °C
21.	DSG4-11F	5′	ACAAGTTCCATGGCATCATC	3′	425 bp	58.3 °C
22.	DSG4-11R	5′	ATGGCAAGAACTGTGGAAAC	3′		57.6 °C
23.	DSG4-12F	5′	CTAGCCCACCAAGGAATTTC	3′	437 bp	58.6 °C
24.	DSG4-12R	5′	GCTCCATGAACCTAACCATC	3′		57.0 °C
25.	DSG4-13_14F	5′	TCCAGTGACTTCCTAAACCG	3′	560 bp	57.8 °C
26.	DSG4-13_14R	5′	CAAACAGGTCACATTCCCTC	3′		57.9 °C
27.	DSG4-15F	5′	TTTAGCGCCTACGCCTTG	3′	459 bp	60.1 °C
28.	DSG4-15R	5′	AGGTTTGGGATAGGGTTGAG	3′		57.9 °C
29.	DSG4-16-1F	5′	GAGGGATTGCTGTTATTCTTC	3′	514 bp	55.5 °C
30.	DSG4-16-1R	5′	ATTGGGTGCAAGCTGAGG	3′		59.7 °C
31.	DSG4-16-2F	5′	AGAAATGGCAGCATCTGAAC	3′	617 bp	57.8 °C
32.	DSG4-16-2R	5′	GCCTAGCCATATTCACCCTC	3′		58.6 °C

F = forward primer, R = reverse primer, bp = base pairs, Tm = melting temperature, °C = degree centigrade.

eyelashes, axillary and pubic regions. Moustache and beard hairs were also absent in the affected male individuals (Figure 2a and b). Patients in two other families (C and D) had only a few hairs on the scalp at birth that re-grew sparsely after ritual shaving often performed a week after birth. At the time of the study, the degree of hair density on the scalp varied among the affected individuals. They showed sparse to absent hair on the scalp and the rest of the body. The eyebrows and eyelashes were sparse and in affected males the moustache and beard hairs were also sparse (Figure 2c and d). Affected members in the remaining two families (E and F) possessed hair at the time of birth but after ritual shaving sparse, hard, stiff and brittle hairs appeared on the scalp. Eyebrows and eyelashes were sparse too (Figure 2 e and f). Papules were observed on the scalps of affected individuals in five families (A, B, C, E, F) (Figure 2a, b, c, e, f).

Scalp skin biopsies of two affected members IV-3 and VI-4 in family E and F (Figure 2g and h), respectively, revealed the presence of hyperkeratosis, irregular acanthosis (diffuse hyperplasia of the spinous layer of the skin), papillomatosis (small nipple shaped projection), fibroplasias, and paucity of the sebaceous glands with hair fragments lying free in the dermis surrounded by giant cells. The basal cell layer, hair follicle, arrector pili muscles and sweat glands were intact and normal.

Other ectodermal structures such as nails, teeth and sweat glands were found normal in all of the affected individuals of the six families. Obligate heterozygous carrier individuals in each family had normal scalp and body hair and were clinically indistinguishable from genotypically normal individuals.

Haplotype construction and mutation detection

Genotyping highly polymorphic microsatellite markers and haplotype analysis revealed linkage of all 6 families to the gene *DSG4* on chromosome 18q21.1. Comparison of the haplotypes in the six families showed a similar allele pattern (Figure 3).

PCR amplification of *all* exons and splice junction sites of the *DSG4* gene using genomic DNA failed to amplify exons 5–8 in affected individuals of the six families. This showed the presence of a previously reported and very common deletion mutation (Ex5_8del) in all of the affected individuals of the families (Figure 4a). This was further verified by sequencing the PCR product amplified by a set of primers

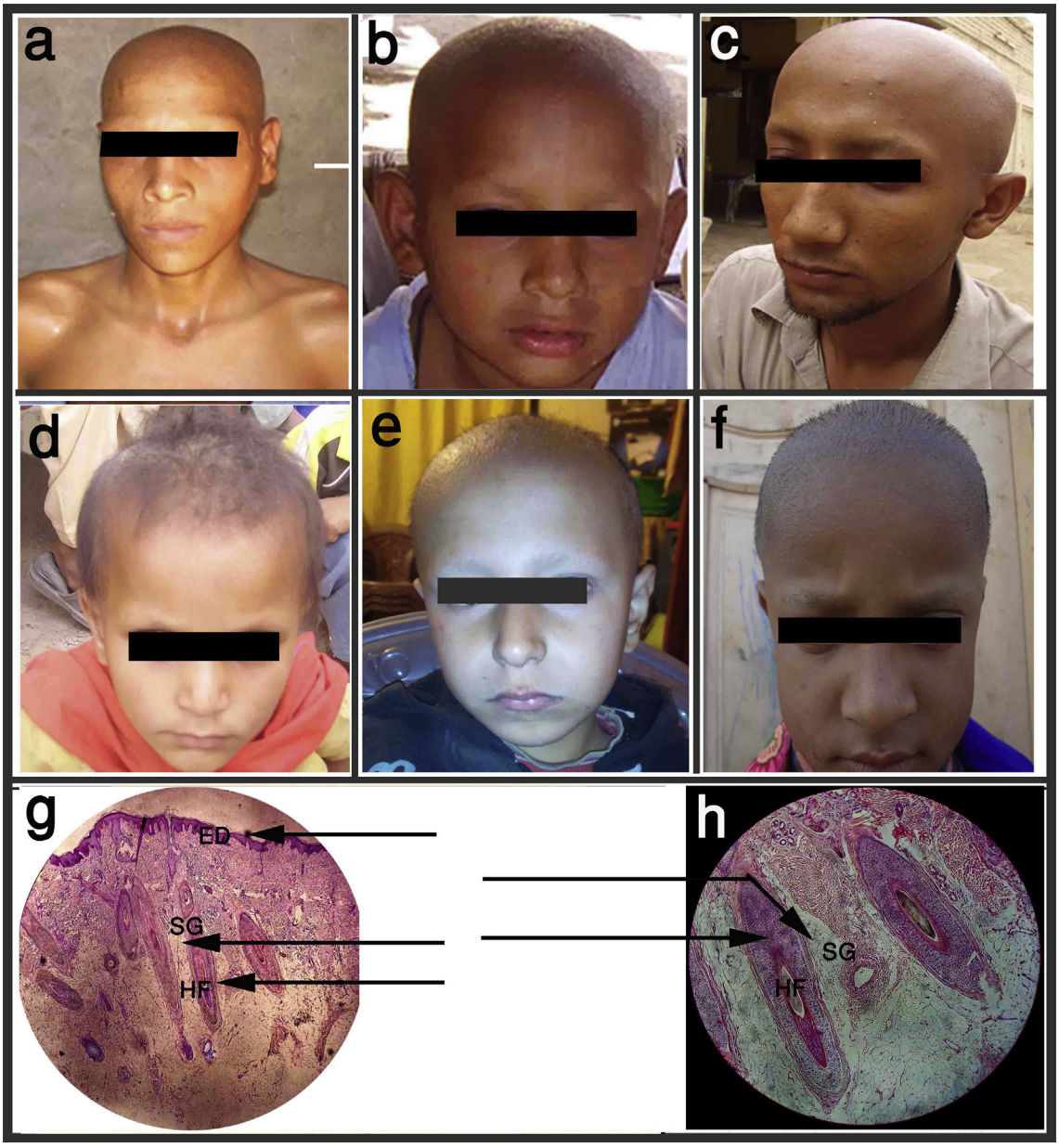

Figure 2: Clinical features of the patients with localized autosomal recessive hypotrichosis (LAH1). Note absence of scalp hair, eyebrows and eyelashes in affected males V-6 (family A) and IV-4 (family B) (a, b). Moustache and beard hair are also missing in patient (V-6) in family A. Eighteen year old male affected individual (IV-4) in family C showing complete absence of scalp hair, sparse eyebrows, and thin sparse facial hair (c). Sparse scalp hair, sparse eyebrows and eyelashes are clearly visible in patient (IV-2) in family D (d). Visible sparse, thick and fragile scalp hair in two patients 1V-3 (family E) (e) and VI-4 (family F) (f). Scalp skin biopsies of the patients IV-3 and VI-4 showing hyperkeratosis, irregular acanthosis and papillomatosis (g, h).

designed from intron-4 and intron-8 of the *DSG4* gene (Figure 4a). The deletion breakpoints of the mutation are present in the non-coding regions of the gene *DSG4* starting 35 bp upstream of exon 5 to 289 bp downstream of exon 8. This is an inframe deletion leading to a truncated protein with 211 amino acids missing in the DSG4 protein.

Discussion

In the present investigation, we identified six consanguineous families with twenty one individuals affected by localized hypotrichosis (LAH1). Affected members in all six families had sparse to absent scalp and body hair. Monilethrix-like scalp hair, as reported in a few cases carrying mutations in the *DSG4* gene,[18–21] was not observed in any affected member of the six families. The phenotypic variations reported due to mutations in the same gene *DSG4* can be attributed to the effect of different modifier genes.[22]

Sequence analysis of the gene *DSG4* detected a previously reported and very common deletion mutation (Ex5_8del)[2,3,23,24] in affected individuals of all six families. Comparing the haplotypes of the six families studied here

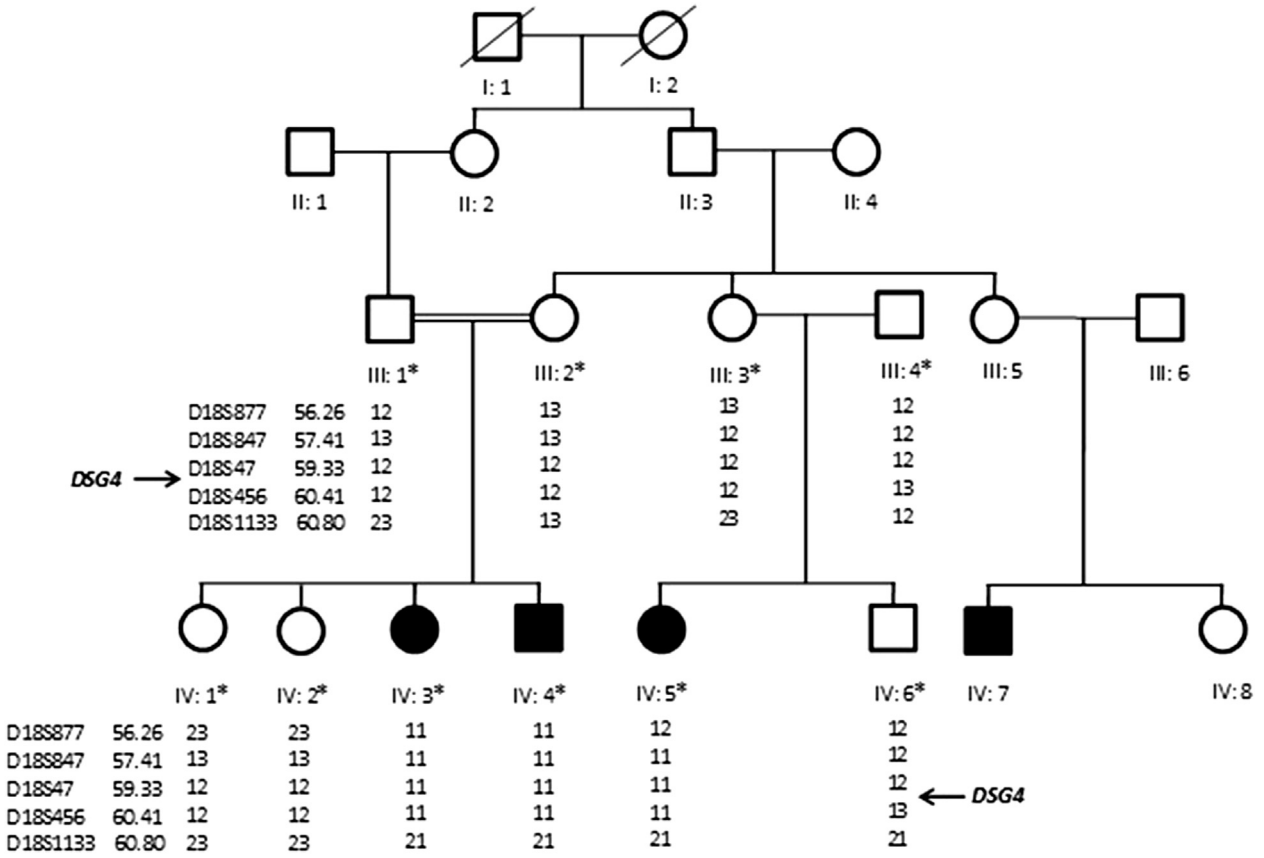

Figure 3: Haplotype of family C segregating autosomal recessive hypotrichosis. For genotyped individuals, haplotypes are shown beneath each symbol revealing that all affected individuals are homozygous for the same haplotypes, whereas normal parents and healthy sibs are heterozygous carriers. Arrows indicate position of the gene DSG4. All affected individuals from other families showed the same haplotype pattern.

with those of families studied earlier who also had the Ex5_8del mutation, revealed that the disease mutation in all of the families appeared on very similar haplotypes, suggesting that the mutation in these families was due to a single mutation event. Moreover, analysis of genomic sequence flanking Ex5_8del did not identify any palindromic sequence, thereby further strengthening the hypothesis that the deletion arose due to an ancestral mutation event.

The Ex5_8del is an inframe deletion leading to a truncated DSG4 protein missing 211 amino acids. The deleted amino acids corresponds to 33 amino acids in cadherin domain 1 (50−157 amino acids), complete cadherin domain 2 (158−269 amino acids) and 66 amino acids in cadherin domain 3 (270−385 amino acids) of the DSG4 protein (Figure 4b, c, d, e). In addition to DSG4, mutations in other cadherins have also been shown to cause skin disorders. Various types of keratodermas are caused by mutations in *DSG1*.[25] A nonsense mutation in another cadherin protein *DSC3* has also been shown to cause hypotrichosis with recurrent vesicles.[16]

To date, only 13 mutations including five deletions, four missense, one nonsense, two insertions, one deletion-insertion and one splice site have been reported in the *DSG4* gene (Table 1). Two of these mutations (p.Arg289*, p.Met208IfsX4,) cause monilethrix-like scalp hair, three (p.Ser192Phe, 216 +1G > T, c.85-1_191del) cause both monilethrix-like scalp hair and hypotrichosis, and the rest of the eight cause only hypotrichosis.

High expression of DSG4 has been reported in the highly differentiated state of the hair shaft especially in the precortex and keratinizing zone of the cortex[26] and is regulated by several transcription factors, such as LEF1, HOXC13 and FOXN1, that control the expression of keratins in differentiating trichocytes.[27] As reported by Kljuic et al.,[2] in some cases aberrant DSG4 proteins may disturb the switch from proliferation to differentiation of trichocytes that cause abnormal and premature keratinization of the hair shaft and result in a beaded hair form as a part of the phenotype.

Conclusion

By identifying the deletion mutation (Ex5_8del) in the six families of the present study, the number of consanguineous Pakistani families carrying the same mutation is increased to fifteen. The frequent occurrence and high incidence of this mutation in the Pakistani population originating from different geographical regions suggests that this is an ancestral mutation. George Grierson's classification (1927)[28] is supports and helps illustrate the hypothesized relationship among these ethnic groups. According to Grierson's classification Punjabi, Sindhi, Hindi-Urdu, Sindhi, Siraiki/Hind-ko, Kurdish, Balochi, Pashto, Kashmiri and Shina languages/groups are descendants of a common ancestor of proto Indo-Aryan origin.

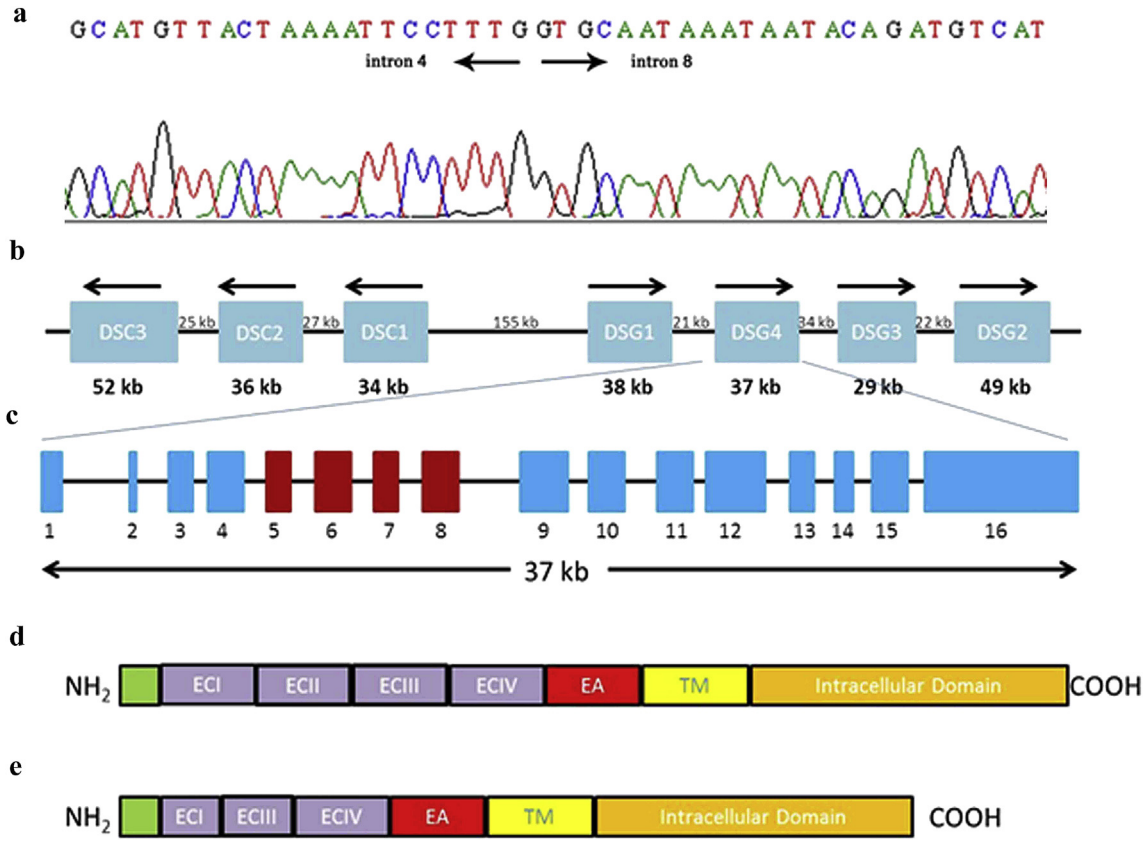

Figure 4: Mutation analysis of *DSG4* gene in all six families (A–F). a: DNA sequence analysis revealed a homozygous deletion mutation (Ex5_8del) in the *DSG4* gene in affected individuals. Arrows indicate deletion breakpoints between intron 4 and 8. b: Genomic organization of human desmosomal cadherin gene cluster on chromosome 18q12.1. Approximate genomic size of the genes and intergenic regions are given according to the UCSC genomes browser. c: Structure of 37 kb *DSG4* gene. Exons deleted in affected individuals in six families (A–F) are shown in red. d: Schematic representation of the organization of wild type DSG4 protein domains. e: Mutant DSG4 protein. EC1-ECIV: extracellular cadherin repeat domains, EA: extracellular anchoring domain, TM: transmembrane domain.

Identification of previously known mutations in the gene involved in human hereditary hair loss disorder in an increasing number of families indicates that inherited hair loss phenotypes are not as rare as earlier predicted. The identification of the causative mutation for a Mendelian disease enables molecular diagnosis and carrier testing in the patient and his or her family. This is of great importance for patient management and family counselling and serves as a starting point for therapeutic interventions.

Conflict of interest

The authors have no conflict of interest to declare.

Authors' contributions

DM, SIR, FA, NAC, MA and SB recruited families, DM, BK, SIR, FA, NAC and SB performed experiments including DNA extraction, amplification and Sequencing, MA, WA, SB analyzed the data. WA provided laboratory space and funds for reagents. WA and SB wrote the manuscript. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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Web resources

GenBank Accession Numbers: http://www.ensembl.org/Homo-sapiens.

Online Mendelian Inheritance in Man (OMIM): http://www.ncbi.nlm.nih.gov/omim/.

USCS Genome Bioinformatics website, March 2006: http://genome.ucsc.edu/cgibin/hgGateway.

References

1. Bazzi H, Christiano AM. Broken hearts, woolly hair, and tattered skin: when desmosomal adhesion goes awry. **Curr Opin Cell Biol 2007**; 19: 515–520.
2. Kljuic A, Bazzi H, Sundberg JP, Martinez-Mir A, O'Shaughnessy R, Mahoney MG, Levy M, Montagutelli X, Ahmad W, Aita VM, Gordon D, Uitto J, Whiting D, Ott J, Fischer S, Gilliam TC, Jahoda CA, Morris RJ, Panteleyev AA,

Nguyen VT, Christiano AM. Desmoglein 4 in hair follicle differentiation and epidermal adhesion: evidence from inherited hypotrichosis and acquired pemphigus vulgaris. **Cell 2003**; 113: 249–260.
3. Rafiq MA, Ansar M, Mahmood S, Haque S, Faiyaz-ul-Haque M, Leal SM, Ahmad W. A recurrent intragenic deletion mutation in DSG4 gene in three Pakistani families with autosomal recessive hypotrichosis. **J Invest Dermatol 2004**; 123: 24724–24728.
4. Ali G, Chishti MS, Raza SI, John P, Ahmad W. A mutation in the lipase H (LIPH) gene underlies autosomal recessive hypotrichosis. **Hum Genet 2007**; 121: 319–325.
5. Aslam M, Chahrour MH, Razzaq A, Haque S, Yan K, Leal SM, Ahmad W. A novel locus for autosomal recessive form of hypotrichosis maps to chromosome 3q26.33-q27.3. **J Med Genet 2004**; 41: 849–852.
6. Wali A, Chishti MS, Ayub M, Yasinzai M, Kafaitullah Ali G, John P, Ahmad W. Localization of a novel autosomal recessive hypotrichosis locus (LAH3) to chromosome 13q14.11-q21.32. **Clin Genet 2007**; 72: 23–29.
7. Kazantseva A, Goltsov A, Zinchenko R, Grigorenko AP, Abrukova AV, Moliaka YK, Kirillov AG, Guo Z, Lyle S, Ginter EK, Rogaev EI. Human hair growth deficiency is linked to a genetic defect in the phospholipase gene LIPH. **Science 2006**; 314: 982–985.
8. Jelani M, Wasif N, Ali G, Chishti M, Ahmad W. A novel deletion mutation in LIPH gene causes autosomal recessive hypotrichosis (LAH2). **Clin Genet 2008**; 74: 184–188.
9. Naz G, Khan B, Ali G, Azeem Z, Wali A, Ansar M, Ahmad W. Novel missense mutations in lipase H (LIPH) gene causing autosomal recessive hypotrichosis (LAH2). **J Dermatol Sci 2009**; 54: 12–16.
10. Kalsoom UE, Habib R, Khan B, Ali G, Ali N, Ansar M, Ahmad W. Mutations in lipase H gene underlies autosomal recessive hypotrichosis in five Pakistani families. **Acta Derm-Venereol 2010**; 90: 93–94.
11. Pasternack SM, von Kügelgen I, Al Aboud K, Lee YA, Rüschendorf F, Voss K, Hillmer AM, Molderings GJ, Franz T, Ramirez A, Nürnberg P, Nöthen MM, Betz RC. G protein-coupled receptor P2Y5 and its ligand LPA are involved in maintenance of human hair growth. **Nat Genet 2008**; 40: 329–334.
12. Azeem Z, Jelani M, Naz G, Tariq M, Wasif N, Kamran-Ul-Hassan Naqvi S, Ayub M, Yasinzai M, Amin-Ud-Din M, Wali A, Ali G, Chishti MS, Ahmad W. Novel mutations in G protein-coupled receptor gene in families with autosomal recessive hypotrichosis (LAH3). **Hum Genet 2008**; 123: 515–519.
13. Tariq M, Ayub M, Jelani M, Basit S, Naz G, Wasif N, Raza SI, Naveed AK, ullah Khan S, Azeem Z, Yasinzai M, Wali A, Ali G, Chishti MS, Ahmad W. Mutations in the P2RY5gene underlie autosomal recessive hypotrichosis in 13 Pakistani families. **Br J Dermatol 2009**; 160: 1006–1010.
14. Shimomura Y, Wajid M, Petukhova L, Shapiro L, Christiano AM. Mutations in the lipase H gene underlie autosomal recessive woolly hair/hypotrichosis. **J Invest Dermatol 2009**; 129: 622–628.
15. Khan S, Habib R, Mir H, Umm-e-Kalsoom Naz G, Ayub M, Shafique S, Yamin T, Ali N, Basit S, Wasif N, Kamran-Ul-Hassan Naqvi S, Ali G, Wali A, Ansar M, Ahmad W. Mutations in the LPAR6 and LIPH genes underlie autosomal recessive hypotrichosis/woolly hair in 17 consanguineous families from Pakistan. **Clin Exp Dermatol 2011**; 36: 652–654.
16. Ayub M, Basit S, Jelani M, Ur Rehman F, Iqbal M, Yasinzai M, Ahmad W. A homozygous nonsense mutation in the human desmocollin-3 (DSC3) gene underlies hereditary hypotrichosis and recurrent skin vesicles. **Am J Hum Genet 2009**; 85: 515–520.
17. Khan B, Basit S, Touseef M, Tariq M, Khan MN, Ahmad W. A novel chondroectodermal dysplasia mapped to chromosome 2q24.1-q31.1. **Eur J Med Genet 2012**; 55: 455–460.
18. Schaffer JV, Bazzi H, Vitebsky A, Witkiewicz A, Kovich OI, Kamino H, Shapiro LS, Amin SP, Orlow SJ, Christiano AM. Mutations in the desmoglein 4 gene underlie localized autosomal recessive hypotrichosis with monilethrix hairs and congenital scalp erosions. **J Invest Dermatol 2006**; 126: 1286–1291.
19. Shimomura Y, Sakamoto F, Kariya N, Matsunaga K, Ito M. Mutations in the desmoglein 4 gene are associated with monilethrix-like congenital hypotrichosis. **J Invest Dermatol 2006**; 126: 1281–1285.
20. Zlotogorski A, Marek D, Horev L, Abu A, Ben-Amitai D, Gerad L, Ingber A, Frydman M, Reznik-Wolf H, Vardy DA, Pras E. An autosomal recessive form of monilethrix is caused by mutations in DSG4: clinical overlap with localized autosomal recessive hypotrichosis. **J Invest Dermatol 2006**; 126: 1292–1296.
21. Ullah A, Raza SI, Ali RH, Naveed AK, Jan A, Rizvi SAD, Satti R, Ahmad W. A novel deletion mutation in the DSG4 gene underlies autosomal recessive hypotrichosis with variable phenotype in two unrelated. **Clin Exp Dermatol 2015**; 40: 78–84.
22. El-Amraoui A, Petit C. Cadherins as targets for genetic diseases. **Cold Spring Harbor Perspect Biol 2010**; 2: a003095.
23. Moss C, Martinez-Mir A, Lam H, Tadin-Strapps M, Kljuic A, Christiano AM. A recurrent intragenic deletion in the desmoglein 4 gene underlies localized autosomal recessive hypotrichosis. **J Invest Dermatol 2004**; 123: 607–610.
24. John P, Tariq M, Arshad Rafiq M, Amin-Ud-Din M, Muhammad D, Waheed I, Ansar M, Ahmad W. Recurrent intragenic deletion mutation in desmoglein 4 gene underlies autosomal recessive hypotrichosis in two Pakistani families of Balochi and Sindhi origins. **Arch Dermatol Res 2006**; 298: 135–137.
25. McGrath JA, Wessagowit V. Human hair abnormalities resulting from inherited desmosome gene mutations. **Keio J Med 2005**; 54: 72–79.
26. Bazzi H, Getz A, Mahoney MG, Ishida-Yamamoto A, Langbein L, Wahl JK, Christiano AM. Desmoglein 4 is expressed in highly differentiated keratinocytes and trichocytes in human epidermis and hair follicle. **Differentiation 2006**; 74: 129–140.
27. Bazzi H, Demehri S, Potter CS, Barber AG, Awgulewitsch A, Kopan R, Christiano AM. Desmoglein 4is regulated by transcription factors implicated in hair shaft differentiation. **Differentiation 2009**; 78: 292–300.
28. Grieson G. Part. 1: Motilal Banarsidas Delhi. **Linguistic survey of India. Istedn.**, Vol. 1; 1927. pp. 121–126.
29. Messenger AG, Bazzi H, Parslew R, Shapiro L, Christiano AM. A missense mutation in the cadherin interaction site of the desmoglein 4 gene underlies localized autosomal recessive hypotrichosis. **J Invest Dermatol 2005**; 125: 1077–1079.
30. Wajid M, Bazzi H, Rockey J, Lubetkin J, Zlotogorski A, Christiano AM. Localized autosomal recessive hypotrichosis due to a frameshift mutation in the desmoglein 4 gene exhibits extensive phenotypic variability within a Pakistani family. **J Invest Dermatol 2007**; 127: 1779–1782.
31. Farooq M, Ito M, Naito M, Shimomura Y. A case of monilethrix caused by novel compound heterozygous mutations in the desmoglein 4 (DSG4) gene. **Br J Dermatol 2011**; 165: 425–431.

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