Gain-of-Function Mutation of KIT Ligand on Melanin Synthesis Causes Familial Progressive Hyperpigmentation

Zhi-Qiang Wang,[1,2,5] Lizhen Si,[1,2,5] Quan Tang,[1,2] Debao Lin,[3,6] Zhangjie Fu,[1,2] Jing Zhang,[1,2] Bin Cui,[1,2] Yufei Zhu,[1,2] Xianghua Kong,[4] Min Deng,[1,2] Yu Xia,[1,2] Heng Xu,[1,2] Weidong Le,[1,2] Landian Hu,[1,2] and Xiangyin Kong[1,2,*]

Familial progressive hyperpigmentation (FPH) is an autosomal-dominantly inherited disorder characterized by hyperpigmented patches in the skin, present in early infancy and increasing in size and number with age. The genetic basis for FPH remains unknown. In this study, a six-generation Chinese family with FPH was subjected to a genome-wide scan for linkage analysis. Two-point linkage analysis mapped the locus for FPH at chromosome 12q21.31-q23.1, with a maximum two-point LOD score of 4.35 ($\emptyset = 0.00$) at *D12S81*. Haplotype analysis confined the locus within an interval of 9.09 cM, flanked by the markers *D12S1667* and *D12S2081*. Mutation profiling of positional candidate genes detected a heterozygous transversion (c. 107A→G) in exon 2 of the KIT ligand (*KITLG*) gene, predicted to result in the substitution of a serine residue for an asparagine residue at codon 36 (p.N→S). This mutant "G" allele cosegregated perfectly with affected, but not with unaffected, members of the FPH family. Function analysis of the soluble form of sKITLG revealed that mutant sKITLGN36S increased the content of the melanin by 109% compared with the wild-type sKITLG in human A375 melanoma cells. Consistent with this result, the tyrosinase activity was significantly increased by mutant sKITLGN36S compared to wild-type control. To our knowledge, these data provided the first genetic evidence that the FPH disease is caused by the KITLGN36S mutation, which has a gain-of-function effect on the melanin synthesis and opens a new avenue for exploration of the genetic mechanism of FPH.

Familial progressive hyperpigmentation (FPH [MIM %145250]), also called "melanosis universalis hereditaria," is a rare form of a congenital genetic disease causing hyperpigmentation. FPH was first reported by Chernosky in a two-generation family consisting of four individuals.[1] The onset of the pigmentation was either at birth or in early infancy, and irregular patches increased with age in size, number, and confluence.[2] This process was rapid during childhood and slower during adolescence, and it resulted in extensive hyperpigmentation of the conjunctive face, neck, trunk, limbs, lips, oral mucosa, palms, and soles.[3] FPH was determined to be an autosomal-dominant inheritable disease, after Rebora's report on an Italian family in 1989 and Lin's report on a Han family from China in 1991.[2,3] The first locus for FPH was reported at chromosome 19p13.1-pter, spanning 45.58 cM between D19S593 and 19pter in 2006.[4] However, no candidate gene has been identified in this region, and the genetic basis and pathologic mechanisms of FPH remain unknown. In an effort to localize the gene for FPH, we undertook an entire genome scan in the six-generation Chinese family with FPH reported by Lin.[3] We found significant evidence for linkage of the FPH-responsible gene to chromosome 12q21.31-q23.1. Subsequently, a missense mutation in the *KITLG* gene (OMIM *184785) was identified as being associated with human FPH.

We investigated a six-generation family, from a rural area of Shandong Province of China, with typical features of FPH. A complete family history was obtained, and 18 affected individuals (nine males and nine females) were identified. Careful physical examination revealed that all of the affected individuals in this family manifested pigmentation disorders without the association of any other system disease (Figure 1). For example, patient IV-1 (Figure 1A) is an affected 53-year-old male with hyperpigmentation on his hands, palms, limbs, conjunctive face, neck, trunk, and soles. None of the affected members in this family was found to have skin cancer. The pedigrees with FPH showed an autosomal-dominant inheritance pattern (Figure 1C).

This research has been approved by the ethical review committees of the appropriate institutions. In total, 25 family members participated in this study, but only 17 are informative for linkage analysis. Samples of peripheral blood were obtained from all available family members after informed consent was obtained, and genomic DNA was extracted with the QIAmp DNA Blood kit (QIAGEN, Germany).

A genome-wide screening was performed in this family for determination of the chromosomal regions linked to FPH, with the use of 382 fluorescent microsatellite markers covering 22 autosomes at a resolution of approximately 10 cM (Linkage Mapping Set Version 2, Perkin Elmer). PCR

[1]State Key Laboratory of Medical Genomics, Ruijin Hospital, Shanghai Jiaotong University School of Medicine, Shanghai 200025, P.R. China; [2]Institute of Health Science, Shanghai Institutes for Biological Sciences of the Chinese Academy of Sciences, Shanghai 200025, P.R. China; [3]Department of Dermatology, Guiyang Medical College, Guiyang, Guizhou 550001, P.R. China; [4]Clinical Laboratory, Affiliated Hospital of Binzhou Medical College, Binzhou, Shandong 256603, P.R. China
[5]These authors contributed equally to this work
[6]Present address: Department of Dermatology, Cangshan Skin Disease Prevention and Control Station, Shandong 277700, P.R. China
*Correspondence: xykong@sibs.ac.cn
DOI 10.1016/j.ajhg.2009.03.019.

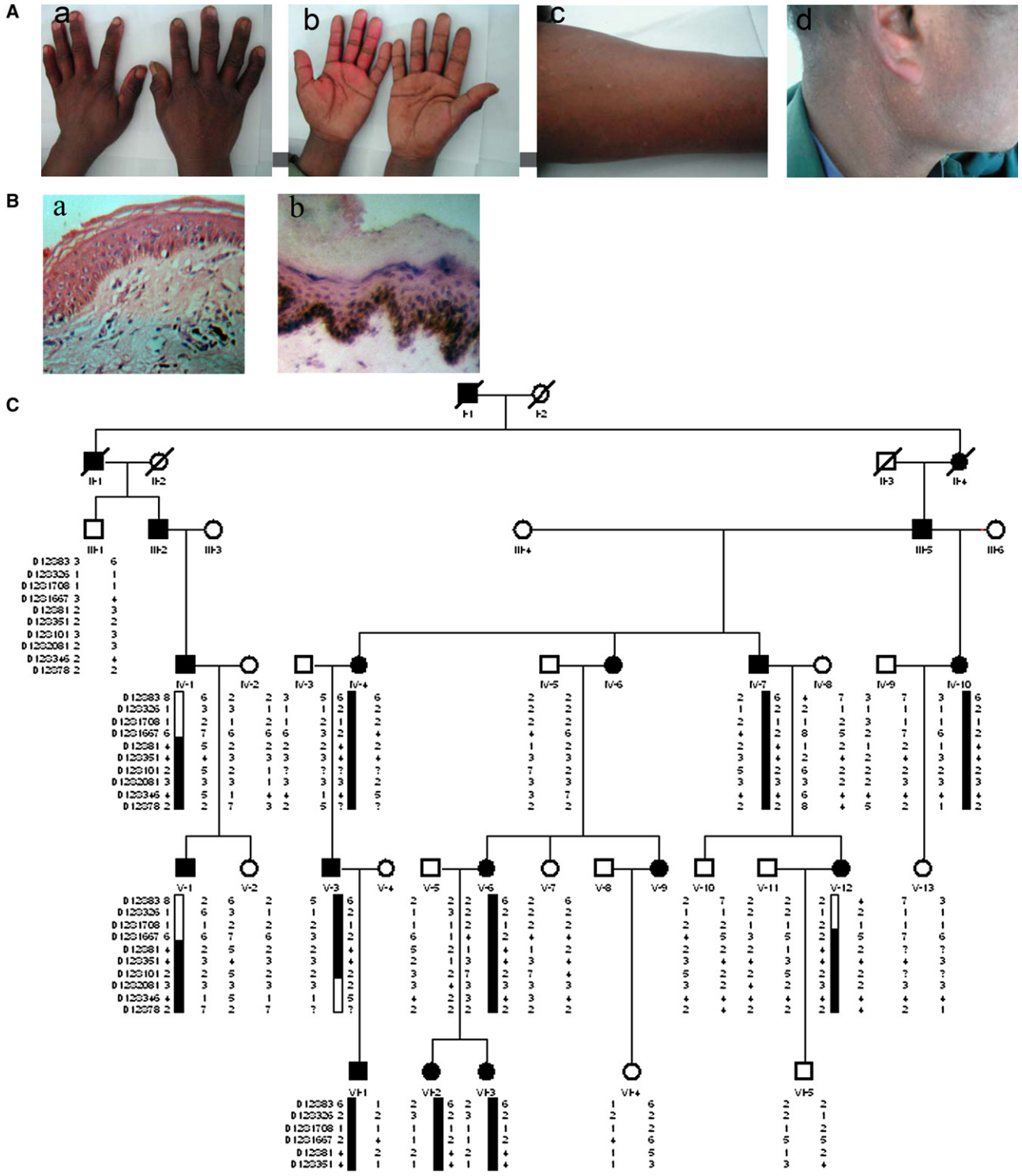

Figure 1. Familial Progressive Hyperpigmentation in a Six-Generation Family

(A) The affected individual, IV-1, manifests a typical clinical feature of FPH. The hyperpigmentation can be observed on his hands (a), palms (b), limbs (c), conjunctive face (d), neck (d), trunk (not shown), and soles (not shown).

(B) Histological appearance of the specimen. Normal skin from an age-gender matched control (a) and hyperpigmented skin from a patient (b) are shown. Skin-biopsy specimens were stained with hematoxylin and eosin. Magnification: ×10. In hyperpigmented skin, there is a significant increase of the number of melanocytes and of the melanin content in the basal keratinocytes, as well as a slight increase in the size of melanocytes.

(C) Pedigree structure and haplotype of the family. Markers are listed from top to bottom: centromere-*D12S83-D12S326-D12S1708-D12S1667-D12S81-D12S351-D12S101-D12S2081-D12S346-D12S78*-telomere. Black bars represent disease-carrying haplotypes. Question marks indicate that the genotype is not determined. Squares indicate male family members; circles indicate female family members; blackened symbols indicate affected members; open symbols indicate unaffected members.

Table 1. Two-Point LOD Score Result between the Disease Gene and Ten Markers of Chromosome 12q21.31-q23.1

	Two-Point LOD Score at Ø						
Marker	0.00	0.01	0.05	0.1	0.2	0.3	0.4
D12S83	−∞	−4.69	−2.05	−1.04	−0.26	0.02	0.07
D12S326	−3.95	−0.27	0.27	0.37	0.29	0.15	0.06
D12S1708	2.94	2.88	2.65	2.34	1.73	1.12	0.54
D12S1667	−0.63	2.76	3.10	2.93	2.29	1.51	0.72
D12S81	4.35	4.26	3.92	3.48	2.56	1.62	0.73
D12S351	2.94	2.88	2.62	2.30	1.64	1.0	0.44
D12S101	4.13	4.05	3.73	3.31	2.44	1.55	0.70
D12S2081	−∞	−0.52	0.07	0.24	0.28	0.21	0.11
D12S346	−∞	0.55	1.09	1.17	1.03	0.74	0.38
D12S78	−∞	−0.24	0.42	0.63	0.68	0.54	0.30

amplification was performed in a total volume of 10 μl, with the use of a PTC-225 DNA Engine Tetrad (MJ Research, USA) and standard conditions. PCR products were electrophoresed on a 5% standard denaturing polyacrylamide gel with an Applied Biosystems 377XL DNA Sequencer. The size of the allele was determined on the basis of an internal size standard (GeneScan 400HD ROX, Perkin Elmer) in each lane, and results were analyzed with GeneScan 3.0 and Genotyper 2.1 software (Perkin Elmer).

A two-point linkage analysis was conducted with the use of the LINKAGE V5.10 software package,[5] under an autosomal-dominant genetic model, with a disease-allele frequency of 0.0001, evenly shared allele frequency, full penetrance, and no sex difference. Pedigree drawing and haplotype construction were carried out with the use of the Cyrillic v2.0 software (Cyrillic Software, UK).

An initial genome-wide scan identified a locus for FPH at chromosome 12q. The maximum two-point LOD score obtained was 4.35 with marker *D12S81,* at a recombination frequency of $\emptyset = 0.00$ (Table 1). The analysis of additional markers (*D12S83*, *D12S326*, *D12S1708*, *D12S1667*, *D12S351*, *D12S101*, *D12S2081*, *D12S346*, *D12S78*; Table 1) further confirmed the initial result. Conversely, no significant linkage with markers on other chromosomal regions was found in this FPH family (Table S1, available online).

Pedigree and haplotype analyses were carried out, and three recombinations were identified (Figure 1C). First, an affected male individual (IV-1) had a recombination between *D12S1667* and *D12S81*. Second, an affected female (V-12) had a recombination between *D12S1708* and *D12S1667*. For the affected individual (V-3), recombination took place between *D12S101* and *D12S2081*. All of the combined information indicates that FPH maps to the region of chromosome 12q21.31-q23.1 spanning approximately 9.09 cM and flanked by markers *D12S1667* and *D12S2081*.

The above physical interval contained about 81 defined genes, and *KITLG* (GeneID: 4254) could be a plausible candidate gene on the basis of chromosome position and previous function analysis.[6,7] Sequencing of the *KITLG* gene was initially performed on genomic DNA derived from patient V-5. Upon complete analysis of the coding and the adjacent intron regions of *KITLG,* a heterozygous c.107A → G transversion in exon 2 of *KITLG* was identified in this patient but was not present in unaffected members (Figures 2A and 2B). The transversion caused an asparagine → serine exchange at amino acid 36 of the KITLG protein sequence. This mutant "G" allele cosegregated perfectly with family members affected with FPH, but not with unaffected members. We confirmed that c.107A → G was not listed in the NCBI SNP database (dbSNP). Furthermore, we used sequencing to exclude it as a SNP in a panel of 296 normal, unrelated, healthy Chinese individuals. These results indicated that KITLG N36S was a mutation.

KITLG, also known as steel factor, stem cell factor, and mast cell growth factor, binds to and activates KIT and plays a crucial role in the development and maintenance of the melanocyte lineage in adult skin.[7–11] *KITLG* exists in two alternatively spliced transcripts, differing in exon 6. The smaller splice variant of *KITLG*, lacking exon 6, encodes a primarily membrane-bound form of KITLG (mKITLG).[12] In contrast, the larger precursor contains exon 6, which codes for a membrane-proximal extracellularly located protease-cleavage site. This site is readily cleaved by cell-surface proteases for production of a soluble KITLG molecule (sKITLG).[13] The primary translation product of both *KITLG* mRNAs contains a 25 aa signal sequence at the N terminus, not found in the mature form of the proteins.[8]

Many studies have shown that the *KIT* and *KITLG* genes play a critical role in the development of the melanocyte and melanin synthesis in fish, salamanders, avians, and mammals, although the expression pattern of *KIT* and *KITLG* among different species is variable. In addition, the dependence of melanocytes on KIT/KITLG signaling for survival or proliferation shows species-specific differences.[12,14–16] Previous studies revealed that many mutant alleles of *KITLG* are lethal in homozygous mice and produce a variable level of coat-color dilution in heterozygous mice.[17–19] In transgenic mice, specific targeting expression of *KITLG* in the epidermis can rescue the loss of melanocytes in the interadnexal epidermis, resulting in a highly pigmented epidermis.[6,20]

KITLG is produced locally in human skin by epidermal keratinocytes and endothelial cells.[21–23] The signaling of KITLG and its receptor KIT plays an important role in melanocyte proliferation and pigment production.[24–26] Injection of the soluble form of sKITLG into human skin, which was explanted on nude mice, resulted in hyperpigmentation of the grafted skin tissue. On the contrary, injection of the KIT- or KITLG-blocking antibodies into the explanted human skin led to a loss of melanocytes.[7] Similarly, patients receiving KITLG treatment showed an increased number of melanocytes and increased melanin at the site of the KITLG injection, compared with noninjected tissue.[27,28]

Biopsies taken from the hyperpigmented areas of FPH patients displayed strong hyperpigmentation of the epidermia and increased melanin in the basal layer, but no increase in the number of melanocytes within the epidermis.[2–4,29] To determine the effects of the p.N36S

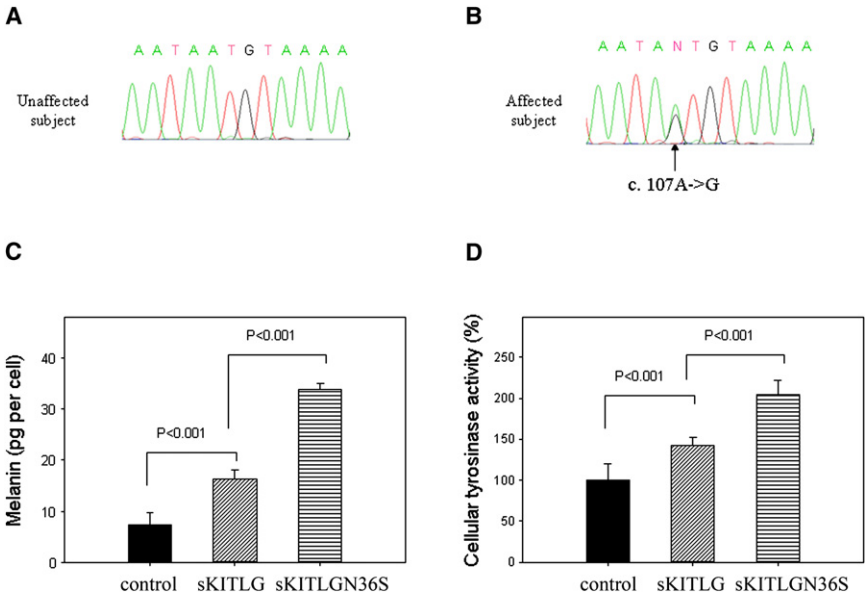

Figure 2. Mutation Analysis of sKITLG in a Family with FPH and Effects of the Mutant sKITLG on Melanin Synthesis

(A) Sequence trace of the WT allele, showing translation of asparagine residue at codon 36 (AAT).

(B) Sequence trace of the mutant allele, showing the heterozygous c. 107A→G, which is predicted to result in the missense substitution of serine (AGT) for asparagine at codon 36 (p.N36S).

(C) The soluble form of sKITLGN36S significantly increases melanin content in A375 cells, compared with the WT sKITLG. A375 cells were incubated with 100 ng/ml WT sKITLG and sKITLGN36S for 24 hr, respectively. Cell pellets were dissolved in buffer (1N NaOH, 10%DMSO) at 80°C for 2 hr and centrifuged for 10 min at 12,000 × *g*. Absorbance of melanin at 420 nm was measured with TECAN Safire2 (USA). A melanin standard curve was prepared with the use of synthetic melanin (Sigma). Melanin content was normalized to the cell number. Significance was determined according to a two-sided Student's t test performed with Excel software. The results are shown as mean ± SD (n = 6), and similar results were obtained when the experiments and measurements were repeated four times. Error bars indicate ± SD.

(D) The soluble form of sKITLGN36S significantly increases the tyrosinase activity, compared with the WT sKITLG, in A375 cells. The A375 cells were treated as in melanin measurement for tyrosinase-activity analysis. The protein present in the supernatant was estimated by the method of Lowry, with BSA used as the standard, then 40 ug of protein was incubated in1 ml sodium phosphate buffer (pH7.4, containing 0.1% L-dopa) for 2 hr at 37°C. The absorbance was monitored at 475 nm with TECAN Safire2 (USA). Significance was determined according to a two-sided Student's t test performed with Excel software. The results are shown as mean ± SD (n = 10), and similar results were obtained when the experiments and measurements were repeated twice.

substitution in KITLG on the melanin synthesis, we expressed the soluble form of wild-type (WT) sKITLG and mutant sKITLGN36S in Escherica coli and purified the expression products for further analysis (Figure S1). Coomassie brilliant blue staining revealed a single band for each construct (WT and mutant) after SDS-PAGE, and western blot analysis showed that both bands could be recognized by human KITLG antibody (Figure S1).

The A375 human pigmented melanoma cells were treated with WT and mutant sKITLGN36S, then melanin content was measured. Our results indicate that the two types of sKITLG can increase the melanin content of each cell in treated A375 cells; melanin content increased from 16.2 pg per cell (WT sKITLG) to 33.9 pg per cell (mutant sKITLGN36S). Compared with the WT sKITLG, the mutant sKITLGN36S significantly increased the melanin content, up to 209%. This indicated that the mutant sKITLGN36S had a greater melanin-synthesis function than its WT form (Figure 2C). Many reports have shown that tyrosinase is essential in melanin synthase.[30–34] We therefore measured levels of tyrosinase activity in A375 cells treated with either WT sKITLG or sKITLGN36S protein, according to Y.H. Huang et al.[34] Consistent with the improved ability of the mutant sKITLGN36S to induce melanin synthesis, the tyrosinase activity increased significantly in A375 cells after treatment with mutant sKITLGN36S, compared to treatment with the WT sKITLG (Figure 2D). Together, all of these results indicate that the mutant sKITLGN36S protein had the ability to increase melanin synthesis. These results are consistent with the biopsies of FPH patients who displayed increased melanin in the basal layer (Figure 1B).[3] However, there was no proliferation difference found for treated A375 cells between the WT and mutant sKITLG (data not shown).

Although our experimental data show that the soluble form of p.N36S mutant KITLG can increase the melanin synthesis in A375 cells, we cannot exclude the role of the membrane-bound form of mutant KITLG for hyperpigmentation in FPH families. Indeed, reports have indicated that mouse melanocytes require the membrane-bound form of KITLG for survival.[6] Additional work will be required for determination of whether the membrane-bound form of mutant KITLG plays a role in melanin synthesis.

Several human genetic diseases, such as dyschromatosis universalis hereditaria (DUH [MIM %127500]), dyschromatosis symmetrica hereditaria (DSH [MIM #127400]), and xeroderma pigmentation (XP [MIM #278700]), show some overlap with FPH. DUH and DSH are pigmentary dermatoses most commonly seen in Japanese and Korean individuals. DUH is characterized by hyperpigmented and hypopigmented flecks and spots over much of the body.[35] DSH shows a characteristic mixture of hyperpigmented and hypopigmented macules of various sizes,

limited largely to the dorsal aspects of the hands and feet.[36] FPH can be differentiated from DSH and DUH on the basis of the absence of hypopigmented lesions in patients. In addition, the genes for DSH and DUH have previously been mapped to chromosomes 1q21.3, 6q24.2-q25.2, and 12q21-q23.[35,37–39] Thus, the genetic basis of FPH is likely to differ from that of DSH and DUH. With regard to XP, it is an autosomal-recessive syndrome with clinical manifestations of excessive freckling, depigmentation, hyperpigmentation, skin aging, and a very high level of early and multiple skin cancers.[40] Although XP also resembles FPH hyperpigmentation, FPH does not show the atrophy, xerosis, or skin tumors usually observed in XP.

FPH is also likely to be a heterogenous disease, given that C. Zhang et al. mapped the gene responsible for FPH to chromosome 19p13.1-pter, spanning 45.58 cM between D19S593 and 19pter, using genome screening on a three-generation Chinese family. However, there was no gene reported to be responsible for FPH in this family.[4] In the current work, we have mapped the dominant FPH gene to the region at chromosome 12q21.31-q23.1. Candidate-gene analysis revealed that mutant KITLGN36S was the critical gene for FPH in this family. Protein-function analysis confirmed that the soluble form of mutant sKITLGN36S can increase the melanin content significantly in A375 human pigmented melanoma cells, compared with the WT.

In conclusion, this report identified the p.N36S transversion of KITLG responsible for inherited FPH. The data show that the soluble mutant form, sKITLG, produces a gain-of-function defect in melanin synthesis, possibly triggering hyperpigmentation of skin in patients. These results will provide important clues for understanding of the molecular mechanism of pigmentation in FPH and increase our ability to develop effective intervention strategies.

Supplemental Data

Supplemental Data include one figure and one table and can be found with this article online at http://www.ajhg.org/.

Acknowledgments

We thank all members of the TPT-PS family for participation in this study, and we thank Donghua Chen, Minhua Jin, and Jianqun Wang for their support. This work is supported by the National High Technology Research and Development Program of China (2006AA02Z330, 2006AA02A301), the National Basic Research Program of China (2007CB512202, 2007CB512100, 2004CB518603), the National Natural Science Foundation of China, Key Program (30530450), the Knowledge Innovation Program of the Chinese Academy of Sciences (KSCX1-YW-R-74), and the E-Institutes of Shanghai Municipal Education Commission.

Received: January 10, 2009
Revised: March 6, 2009
Accepted: March 23, 2009
Published online: April 16, 2009

Web Resources

The URLs for data presented herein are as follows:

NCBI dbSNP Database, http://www.ncbi.nlm.nih.gov/projects/SNP/

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

References

1. Chernosky, M.E., Anderson, D.E., Chang, J.P., Shaw, M.W., and Romsdahl, M.M. (1971). Familial progressive hyperpigmentation. Arch. Dermatol. *103*, 581–598.
2. Rebora, A., and Parodi, A. (1989). Universal inherited melanodyschromatosis: A case of melanosis universalis hereditaria? Arch. Dermatol. *125*, 1442–1443.
3. Ling, D.B., and Lo, T. (1991). Familial progressive hyperpigmentation: A family study in China. Br. J. Dermatol. *125*, 607.
4. Zhang, C., Deng, Y., Chen, X., Wu, X., Jin, W., Li, H., Yu, C., Xiong, Y., Zhou, L., and Chen, Y. (2006). Linkage of a locus determining familial progressive hyperpigmentation (FPH) to chromosome 19p13.1-pter in a Chinese family. Eur. J. Dermatol. *16*, 246–250.
5. Lathrop, G.M., and Lalouel, J.M. (1984). Easy calculations of lod scores and genetic risks on small computers. Am. J. Hum. Genet. *36*, 460–465.
6. Kunisada, T., Yoshida, H., Yamazaki, H., Miyamoto, A., Hemmi, H., Nishimura, E., Shultz, L.D., Nishikawa, S., and Hayashi, S. (1998). Transgene expression of steel factor in the basal layer of epidermis promotes survival, proliferation, differentiation and migration of melanocyte precursors. Development *125*, 2915–2923.
7. Grichnik, J.M., Burch, J.A., Burchette, J., and Shea, C.R. (1998). The SCF/KIT pathway plays a critical role in the control of normal human melanocyte homeostasis. J. Invest. Dermatol. *111*, 233–238.
8. Anderson, D.M., Lyman, S.D., Baird, A., Wignall, J.M., Eisenman, J., Rauch, C., March, C.J., Boswell, H.S., Gimpel, S.D., Cosman, D., et al. (1990). Molecular cloning of mast cell growth factor, a hematopoietin that is active in both membrane bound and soluble forms. Cell *63*, 235–243.
9. Copeland, N.G., Gilbert, D.J., Cho, B.C., Donovan, P.J., Jenkins, N.A., Cosman, D., Anderson, D., Lyman, S.D., and Williams, D.E. (1990). Mast cell growth factor maps near the steel locus on mouse chromosome 10 and is deleted in a number of steel alleles. Cell *63*, 175–183.
10. Russell, E.S. (1979). Hereditary anemias of the mouse: A review for geneticists. Adv. Genet. *20*, 357–459.
11. Manova, K., Bachvarova, R.F., Huang, E.J., Sanchez, S., Pronovost, S.M., Velazquez, E., McGuire, B., and Besmer, P. (1992). c-kit receptor and ligand expression in postnatal development of the mouse cerebellum suggests a function for c-kit in inhibitory interneurons. J. Neurosci. *12*, 4663–4676.
12. Huang, E.J., Nocka, K.H., Buck, J., and Besmer, P. (1992). Differential expression and processing of two cell associated forms of the kit-ligand: KL-1 and KL-2. Mol. Biol. Cell *3*, 349–362.
13. Flanagan, J.G., Chan, D.C., and Leder, P. (1991). Transmembrane form of the kit ligand growth factor is determined by alternative splicing and is missing in the Sld mutant. Cell *64*, 1025–1035.

14. Hultman, K.A., Bahary, N., Zon, L.I., and Johnson, S.L. (2007). Gene duplication of the zebrafish kit ligand and partitioning of melanocyte development functions to kit ligand a. PLoS Genet *3*, e17.
15. Parichy, D.M., Stigson, M., and Voss, S.R. (1999). Genetic analysis of steel and the PG-M/versican-encoding gene AxPG as candidates for the white (d) pigmentation mutant in the salamander Ambystoma mexicanum. Dev. Genes Evol. *209*, 349–356.
16. Wehrle-Haller, B., Meller, M., and Weston, J.A. (2001). Analysis of melanocyte precursors in Nf1 mutants reveals that MGF/KIT signaling promotes directed cell migration independent of its function in cell survival. Dev. Biol. *232*, 471–483.
17. Rajaraman, S., Davis, W.S., Mahakali-Zama, A., Evans, H.K., Russell, L.B., and Bedell, M.A. (2002). An allelic series of mutations in the Kit ligand gene of mice. II. Effects of ethylnitrosourea-induced Kitl point mutations on survival and peripheral blood cells of Kitl(Steel) mice. Genetics *162*, 341–353.
18. Chui, D.H., Loyer, B.V., and Russell, E.S. (1976). Steel (Sl) mutation in mice: Identification of mutant embryos early in development. Dev. Biol. *49*, 300–303.
19. Poole, T.W., and Silvers, W.K. (1979). Capacity of adult steel (SI/SId) and dominant spotting (W/Wv) mouse skin to support melanogenesis. Dev. Biol. *72*, 398–400.
20. Kunisada, T., Lu, S.Z., Yoshida, H., Nishikawa, S., Nishikawa, S., Mizoguchi, M., Hayashi, S., Tyrrell, L., Williams, D.A., Wang, X., et al. (1998). Murine cutaneous mastocytosis and epidermal melanocytosis induced by keratinocyte expression of transgenic stem cell factor. J. Exp. Med. *187*, 1565–1573.
21. Hamann, K., Haas, N., Grabbe, J., and Czarnetzki, B.M. (1995). Expression of stem cell factor in cutaneous mastocytosis. Br. J. Dermatol. *133*, 203–208.
22. Morita, E., Lee, D.G., Sugiyama, M., and Yamamoto, S. (1994). Expression of c-kit ligand in human keratinocytes. Arch. Dermatol. Res. *286*, 273–277.
23. Miyamoto, T., Sasaguri, Y., Sasaguri, T., Azakami, S., Yasukawa, H., Kato, S., Arima, N., Sugama, K., and Morimatsu, M. (1997). Expression of stem cell factor in human aortic endothelial and smooth muscle cells. Atherosclerosis *129*, 207–213.
24. Halaban, R., Tyrrell, L., Longley, J., Yarden, Y., and Rubin, J. (1993). Pigmentation and proliferation of human melanocytes and the effects of melanocyte-stimulating hormone and ultraviolet B light. Ann. N Y Acad. Sci. *680*, 290–301.
25. Funasaka, Y., Boulton, T., Cobb, M., Yarden, Y., Fan, B., Lyman, S.D., Williams, D.E., Anderson, D.M., Zakut, R., Mishima, Y., et al. (1992). c-Kit-kinase induces a cascade of protein tyrosine phosphorylation in normal human melanocytes in response to mast cell growth factor and stimulates mitogen-activated protein kinase but is down-regulated in melanomas. Mol. Biol. Cell *3*, 197–209.
26. Hemesath, T.J., Price, E.R., Takemoto, C., Badalian, T., and Fisher, D.E. (1998). MAP kinase links the transcription factor Microphthalmia to c-Kit signalling in melanocytes. Nature *391*, 298–301.
27. Grichnik, J.M., Crawford, J., Jimenez, F., Kurtzberg, J., Buchanan, M., Blackwell, S., Clark, R.E., and Hitchcock, M.G. (1995). Human recombinant stem-cell factor induces melanocytic hyperplasia in susceptible patients. J. Am. Acad. Dermatol. *33*, 577–583.
28. Bellet, J.S., Obadiah, J.M., Frothingham, B.M., Kurtzberg, J., and Grichnik, J.M. (2003). A patient with extensive stem cell factor-induced hyperpigmentation. Cutis *71*, 149–152.
29. Zanardo, L., Stolz, W., Schmitz, G., Kaminski, W., Vikkula, M., Landthaler, M., and Vogt, T. (2004). Progressive hyperpigmentation and generalized lentiginosis without associated systemic symptoms: A rare hereditary pigmentation disorder in south-east Germany. Acta Derm. Venereol. *84*, 57–60.
30. Luo, D., Chen, H., Searles, G., and Jimbow, K. (1995). Coordinated mRNA expression of c-Kit with tyrosinase and TRP-1 in melanin pigmentation of normal and malignant human melanocytes and transient activation of tyrosinase by Kit/SCF-R. Melanoma Res. *5*, 303–309.
31. Sriwiriyanont, P., Ohuchi, A., Hachiya, A., Visscher, M.O., and Boissy, R.E. (2006). Interaction between stem cell factor and endothelin-1: Effects on melanogenesis in human skin xenografts. Lab. Invest. *86*, 1115–1125.
32. Cook, A.L., Chen, W., Thurber, A.E., Smit, D.J., Smith, A.G., Bladen, T.G., Brown, D.L., Duffy, D.L., Pastorino, L., Bianchi-Scarra, G., et al. (2009). Analysis of cultured human melanocytes based on polymorphisms within the SLC45A2/MATP, SLC24A5/NCKX5, and OCA2/P loci. J. Invest. Dermatol. *129*, 392–405.
33. Tsuchiya, T., Yamada, K., Minoura, K., Miyamoto, K., Usami, Y., Kobayashi, T., Hamada-Sato, N., Imada, C., and Tsujibo, H. (2008). Purification and determination of the chemical structure of the tyrosinase inhibitor produced by Trichoderma viride strain H1–7 from a marine environment. Biol. Pharm. Bull. *31*, 1618–1620.
34. Huang, Y.H., Lee, T.H., Chan, K.J., Hsu, F.L., Wu, Y.C., and Lee, M.H. (2008). Anemonin is a natural bioactive compound that can regulate tyrosinase-related proteins and mRNA in human melanocytes. J. Dermatol. Sci. *49*, 115–123.
35. Stuhrmann, M., Hennies, H.C., Bukhari, I.A., Brakensiek, K., Nurnberg, G., Becker, C., Huebener, J., Miranda, M.C., Frye-Boukhriss, H., Knothe, S., et al. (2008). Dyschromatosis universalis hereditaria: Evidence for autosomal recessive inheritance and identification of a new locus on chromosome 12q21-q23. Clin. Genet. *73*, 566–572.
36. Suenaga, M. (1952). Genetical studies on skin diseases. VII. Dyschromatosis universalis hereditaria in 5 generations. Tohoku J. Exp. Med. *55*, 373–376.
37. Xing, Q.H., Wang, M.T., Chen, X.D., Feng, G.Y., Ji, H.Y., Yang, J.D., Gao, J.J., Qin, W., Qian, X.Q., Wu, S.N., et al. (2003). A gene locus responsible for dyschromatosis symmetrica hereditaria (DSH) maps to chromosome 6q24.2-q25.2. Am. J. Hum. Genet. *73*, 377–382.
38. Zhang, X.J., Gao, M., Li, M., Li, M., Li, C.R., Cui, Y., He, P.P., Xu, S.J., Xiong, X.Y., Wang, Z.X., et al. (2003). Identification of a locus for dyschromatosis symmetrica hereditaria at chromosome 1q11–1q21. J. Invest. Dermatol. *120*, 776–780.
39. Miyamura, Y., Suzuki, T., Kono, M., Inagaki, K., Ito, S., Suzuki, N., and Tomita, Y. (2003). Mutations of the RNA-specific adenosine deaminase gene (DSRAD) are involved in dyschromatosis symmetrica hereditaria. Am. J. Hum. Genet. *73*, 693–699.
40. Stary, A., and Sarasin, A. (2002). The genetics of the hereditary xeroderma pigmentosum syndrome. Biochimie *84*, 49–60.