Identification of a novel mutation in the *KITLG* gene in a Chinese family with familial progressive hyper- and hypopigmentation

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Abstract

Background: Familial progressive hyper- and hypopigmentation (FPHH, MIM 145250) is a rare hereditary skin disorder that is predominantly characterized by progressive, diffuse, partly blotchy hyperpigmented lesions intermingled with scattered hypopigmented spots, lentigines and sometimes Cafe-au-lait spots (CALs). Heterozygous mutations of KIT ligand (*KITLG*, MIM 184745) gene is responsible for FPHH. To date, only eight *KITLG* mutations were reported to be associated with FPHH and no clear genotype-phenotype correlations had been well established. This study aimed to identify the causative mutations of the *KITLG* gene in two Chinese FPHH cases.

Methods: Directly sequencing of the coding regions of *KITLG* was performed. The pathogenicity prediction was assessed using bioinformatics tools including SIFT, Polyphen2, and SWISS-MODEL, and further evaluated following the American College of Medical Genetics and Genomics (ACMG) guideline 2015.

Results: A novel mutation c.104A>T (p.Asn35Ile) and a recurrent mutation c.101C>T (p.Thr34Ile) in *KITLG* were identified. As SIFT and Polyphen-2 softwares showed, both mutations identified in this study were predicted to be detrimental variations. Three-dimensional protein structures modeling indicated the mutant KITLG proteins might affect KITLG affinity to its receptor c-KIT. According to the ACMG guideline 2015, the novel mutation c.104A>T was 'Likely Pathogenic'.

Conclusions: So far, most of the *KITLG* mutations are clustered within the conserved VTNNV motif (amino acid 33-37) in exon 2. The known mutations are only involved in 33V, 34T, 36N, 37V but not 35N. We have now identified a novel mutation c.104A>T of *KITLG*, which was first reported in FPHH located within the conserved 35N of the motif. These results strengthen our understanding of FPHH and expand the mutational spectrum of the *KITLG* gene.

Background

Familial progressive hyper- and hypopigmentation (FPHH, MIM 145250) is a rare, genetic, skin pigmentation anomaly disorder characterized by progressive, diffuse, partly blotchy, hyperpigmented lesions that are intermixed with multiple café-au-lait spots, hypopigmented maculae and lentigines, and are located on the face, neck, trunk and limbs, as well as, frequently, the palms, soles and oral mucosa. Dispigmentation pattern can range from well isolated café-au-lait/hypopigmented patches on a background of normal-appearing skin to confetti-like or mottled appearance [1, 2].

FPHH locus was mapped at chromosome 12q21.31-q23.1 by a genome-wide linkage analysis in a six-generation Chinese family. Positional candidate genes screening revealed that a heterozygous transversion (c.107A>G; p.Asn36Ser) in exon 2 of the KIT ligand (*KITLG*, MIM 184745) gene is responsible for this disorder[3].

KITLG gene, also known as stem cell factor (SCF) and mast cell growth factor, encodes the ligand for the KIT receptor tyrosine kinase. By KITLG binding, KIT dimerizes and initiates diverse cellular responses, and plays a crucial role in the development and maintenance of the melanocyte lineage in adult skin. Injection of the soluble form of sKITLG resulted in hyperpigmentation of the grafted skin tissue while injection of the KIT- or KITLG-blocking antibodies into the explanted human skin led to a loss of melanocytes [4]. Function analysis of the soluble form of KITLG (sKITLG) showed that mutant sKITLG (Asn36Ser) increased the content of the melanin by 109% compared with the wild-type sKITLG in human melanoma cells, and a gain-of-function effect of this missense mutation was indicated to possibly trigger hyperpigmentation of skin in patients[3].

Several mutations (HGMD, http://www.hgmd.cf.ac.uk) in the *KITLG* gene were documented in a few FPHH families [2, 5-7]. Most of the FPHH causing mutation in *KITLG* are clustered within the conserved VTNNV motif (amino acid 33-37) in exon 2. and mutated VTNNV domain may increase the affinity of KITLG to the c-Kit receptor, suggesting that the mutation causes a downstream gain of-function effect. However, still many FPHH families without *KITLG* mutation identified indicated that additional locus heterogeneity for this disorder [5, 8, 9].

Here, we reported two Chinese progressive hyper- and hypopigmentation cases, with one is familial and the other is sporadic. A novel mutation c.104A>T (p.Asn35Ile) and a recurrent mutation c.101C>T (p.Thr34Ile) in the *KITLG* gene were identified. Furthermore, we summarized the information on the mutations of the *KITLG* gene associated with the progressive hyper- and hypopigmentation previously reported.

Methods

The characteristics of participants

Family 1, a four-generation Chinese FPHH pedigree with seven affected individuals (three men and four women) (Fig.1a; Supplementary Fig.1). The pedigree presented an autosomal dominant inheritance manner (Fig.1a). The proband (III2) from family 1 was a 37-year-old woman. Generalized hyper- and hypopigmentation with irregular patches was found at birth, and the patches (0.2cm-0.8cm) progressed successively over her face, neck, trunk and limbs with age. There were also a small number of larger pigmented lesions that were several centimeters in diameter on her trunk and limbs (Fig. 1b-e). All the affected individuals in this family had similar lesions, and none of them showed any other skin, nails, hairs, teeth, mucosae or systemic diseases.

The sporadic case was a 26-year-old woman and her parents were unaffected (Supplementary Fig.2). The clinical appearances of this sporadic patient were mostly similar to that seen in the proband of the family 1. However, diffuse hyperpigmentation was found on the entire body and two vast café-au-lait (CAL)-like lesions presented on her legs at birth. One week after birth, it was shown that her diffuse hyperpigmented skin intermixed with some small lentigines/CAL-like lesions, as well as hypopigmented macules and spots on her trunk. With the increase of age, the lesions increased both in size and number, and also

became more noticeable and appeared on her trunk, and limbs (Fig.1f-i). She was born to healthy, non-consanguineous parents. Her nails, hairs, teeth and mucosae are normal.

Mutation screening of KITLG gene by direct sequencing

Genomic DNA was extracted from blood samples of the family 1 and a sporadic case by using QIA-amp® DNA blood mini Kit (Qiagen, Shanghai, China). All exons and their flanking intronic sequences of the *KITLG* gene were amplified by polymerase chain reaction (PCR) as described before [8]. Purified PCR products were sequenced directly using an ABI Prism®3730 automated sequencer (Applied Biosystems, Foster City, CA, USA). DNA sequences were analyzed by comparing to the human *KITLG* reference sequence (NM_000899.5). The mutations were checked with HGMD, Clinvar (https://www.ncbi.nlm.nih.gov/clinvar/) and the 1000 Genome project (http://www.ncbi.nlm.nih.gov/variation/tools/1000genomes/). Furthermore, samples from 100 unrelated normal Chinese Han individuals were also sequenced to exclude polymorphic variants.

KITLG encoding protein function prediction and molecular modeling

Online in silico programs were applied to predict the potential impact of an amino acid substitution on the structure and function of the KITLG protein, with Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/) and SIFT (http://sift.jcvi.org/), respectively. Furthermore, we performed analysis using an online sever, SWISS-MODEL (http://swiss model.expasy.org/), to construct the three-dimensional structure of KITLG. The pathogenicity prediction was evaluated following the American College of Medical Genetics and Genomics (ACMG) guideline 2015.

Results

Identification of *KITLG* gene mutations

All subsequently detected variants were then filtered on the basis of population after filtering all variants, a novel heterozygous missense mutation c.104A>T (p.Asn35Ile) and a recurrent mutation c.101C>T (p.Thr34Ile) in the *KITLG* gene were revealed in the family 1 (Fig.2a) and in the sporadic case respectively (Fig.2b). These two mutations were not detected in the unaffected family members or 100 unrelated population-match controls (Fig.2c). The variation c.102T>A (p.Thr34Thr) in family 1 is a synonymous mutation (Fig.2a).

According to the ACMG guideline 2015, the prevalence of the variant in the affected individuals is significantly increased compared with the prevalence in controls, which meet the criterion of pathogenic strong 4 (PS4). These two mutations match the criterion of pathogenic moderate 1 (PM1), since all of them are in the hot spot regions. A novel heterozygous missense mutation c.104A>T (p.Asn35Ile) was cosegregation in each affected family members, indicating that they match the criterion of pathogenic supporting 2 (PP2).

Prediction of the potential impacts of the mutations

The mutation c.104A>T (p.Asn35Ile) and c.101C>T (p.Thr34Ile) was predicted to be 'possible damaging' and 'deleterious' with Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/) and SIFT (http://sift.jcvi.org/), respectively. These predictions indicated that the two variants may have effect on protein function, which meet the criterion of pathogenic supporting 3 (PP3) according to the ACMG guideline 2015.

34Thr and 35Asn of KITLG, which polar, neutral, hydrophilic R-based amino acids had changed into Ile, which non-polar, hydrophobic R-based amino acids, in the family 1 and a sporadic case with SWISS-MODEL (http://swiss model.expasy.org/).

Finally, a novel heterozygous missense mutation c.104A>T (p.Asn35Ile) and a recurrent mutation c.101C>T (p.Thr34Ile) in the *KITLG* gene were revealed in the family 1 (Fig.2a) and in the sporadic case were classified as 'Likely Pathogenic' in accordance with the ACMG guideline 2015. The details of each mutation were shown in the Supplementary Table 1 and the genotype information was in the Supplementary Table 2.

Discussion

FPHH is rare autosomal dominant disorder with variable penetrance caused by mutations in the *KITLG* gene that encodes the C-Kit ligand [5]. Because FPHH is too rare with reduced penetrance, no clear incidence rate of this disease was documented. Patients with FPHH often do not have systemic symptoms. Growth retardation and intellectual disability in some affected members were reported by Westerhof et al. [10]. A 2-year-old Chinese female FPHH patient with frequent seizures, impaired temperature regulation and susceptibility to infection also found mild intellectual disability[11].

There were nine publications in PubMed (https://www.ncbi.nlm.nih.gov/pubmed) is related to pathogenic mutations of *KITLG* gene. Mutations of *KITLG* gene is associated with autosomal dominant nonsyndromic deafness-69 (DFNA69, MIM 616697), Waardenburg syndrome-2 (WS2, MIM 193510), and FPHH. Seco Z et al. [12] reported mutations of *KITLG*, c.286_303delinsT (p.Ser96Ter), c.200_202del (p.His67_Cys68delinsArg), and c.310C>G (p.Leu104Val), cause asymmetric and unilateral hearing loss and Waardenburg Syndrome type 2 (WS2). Ogawa Y et al. [13] reported a patient with WS2 who had the unusual complication of large pigmented macules with homozygous *KITLG* mutation (c.94G>A, p.Arg32Cys). It was speculated that the mechanism of the mutation underlying WS2 leading to membrane incorporation and reducing secretion of KITLG occurs via a gain-of-function or dominant-negative effect. A de novo mosaic *KITLG* variant (NM_000899.3:c.329A>G; p.Asp110Gly) was found with a 6-year-old boy had congenital linear and mottled hyperpigmentation [14]. However, all the phenotypes presented in these three publications with *KITLG* is not defined clearly to FPHH, therefor, we only summarize all the other *KITLG* mutations associated with FPHH here in this study.

Account the novel mutation (c.104A>T, p.Asn35Ile) we reported in this study, to date, eight different missense mutations in the *KITLG* gene responsible for FPHH have been identified (Supplementary Table

3, Fig.4). Seven out of eight mutations were clustered in a short amino acid sequence (VTNNV, amino acid 33-37) in exon2 (Fig.4), except c.337G□A which in exon 4 of the *KITLG* gene. Most pathogenic mutations in FPHH occur within the VTNNV domain of *KITLG* protein (amino acids 33-37), lies within the third b-strand of the protein. Only the p.Val37 change represents the first amino acid of the second a-helix (amino acids 37-46). So far, the reported mutations are only involved in 33V, 34T, 36N, 37V but not 35N. We first report the c.104A>T (p.Asn35Ile) mutation at 35N (Fig.4) with FPHH patients.

Except diffuse hyper- and hypopigmentation, Vast CAL-like lesions had been detected as the most common skin problems present with FPHH patients. Vitiligo was found in one family. Sparse lateral eyebrows and malignancy (pharyngeal cancer, papillary thyroid cancer and melanoma) were found in two families. Short suture was found only with one family and mental retardation was not presented in these FPHH patients (Supplementary Table 3).

KITLG, as KIT LIGAND, is produced locally in human skin by epidermal keratinocytes and endothelial cells, where it induces the migration, development and survival of melanocytes. The signaling of *KITLG* and its receptor KIT plays an important role in melanocyte proliferation and pigment production [15, 16]. The role of the KIT/KITLG system in melanogenesis has been experimentally confirmed using animal models [3, 17]. After KITLG binds the c-KIT receptor, dimerization is triggered. It initiates signal transduction via the RAS/MAPK pathway to upregulate melanoblast proliferation [16, 18]. The KITLG/C-KIT/RAS/MAPK signaling pathways have an important role in the regulation of haematopoiesis, stem cell survival, gametogenesis, and mast cell development, migration and function, as well as skin colour [19, 20]. Mutant alleles of the *KITLG* gene are lethal in homozygous mice and produce a variable level of coat-color dilution in heterozygous mice [20]**.** It is reported that in human studies that variations of the *KITLG* gene is also associated with skin, hair, and eye pigmentation (MIM 611664), autosomal dominant nonsyndromic deafness-69, WS2 and FPHH.

Here we reported a novel c.104A>T (p.Asn35Ile) mutation of *KITLG* in a Chinese FPHH family. According to the ACMG guideline 2015, the mutation was initially identified as a ‘Likely Pathogenic’ mutation[21]. As far as we know, only eight different missense *KITLG* mutations have been reported to cause FPHH (Supplementary Table 3). Notably, seven known mutations were clustered in a highly conserved short amino acid sequence VTNNV (amino acids 33-37) (Fig.4). It was known VTNNV domain of *KITLG* protein (amino acids 33–37), lies within the third b-strand of the protein and is responsible for the binding functions. Both mutations c.104A>T (p.Asn35Ile) and c.101C>T (p.Thr34Ile) found in this study were located in the VTNNV domain and predicted to be detrimental variations by SIFT and Polyphen-2 tools. Using the Swiss-Model servers [22, 23], three-dimensional structures of mutant *KITLG* proteins were found changed as compared with the wild type (Fig.4). Both 35Asn and 34Thr are polar, hydrophilic amino acids, and the mutant became non-polar, hydrophobic Isoleucine, therefore it might change the features of the protein and affect the ligand affinity to its receptor c-Kit, thus may affect migration of melanoblasts, melanosome transfer and melanin synthesis, conferring a phenotype with hyper- and hypopigmentation. The precise mechanisms need to be elucidated by further experiments. Our findings revealed a novel *KITLG* mutation associated with FPHH, and reinforce the evidence that VTNNV was the

hot spot for mutation. However, definitive functional analyses of this mutation are needed to determine the structure-function relationship in patients with FPHH.

Conclusion

In summary, a novel mutation c.104A>T (p.Asn35Ile) of the *KITLG* gene was reported in a Chinese FPHH case. The correlations between genotypes and phenotypes of FPHH were summarized. These results strengthen our understanding of FPHH and expand the mutational spectrum of the *KITLG* gene.

Declarations

Ethics approval and consent to participate

The study was approved by the ethics committee of Henan Provincial People's Hospital and written informed consent from these individuals to participate in our study.

Consent for publication

Written informed consent was obtained from all participants to publish this article.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

NHZ, SMZ, XLL, ZLL and ZLY collected and collated the data. JBW, JLL, WSL and MS analyzed the data. JBW and WSL wrote the manuscript. MS and ML edited the final manuscript. All authors read and approved the final manuscript

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Abbreviations

FPHH: Familial progressive hyper- and hypopigmentation.

CALS: café-au-lait spots.

sKITLG: the soluble form of KITLG.

MIM: Mendelian Inheritance in Man.

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Figures

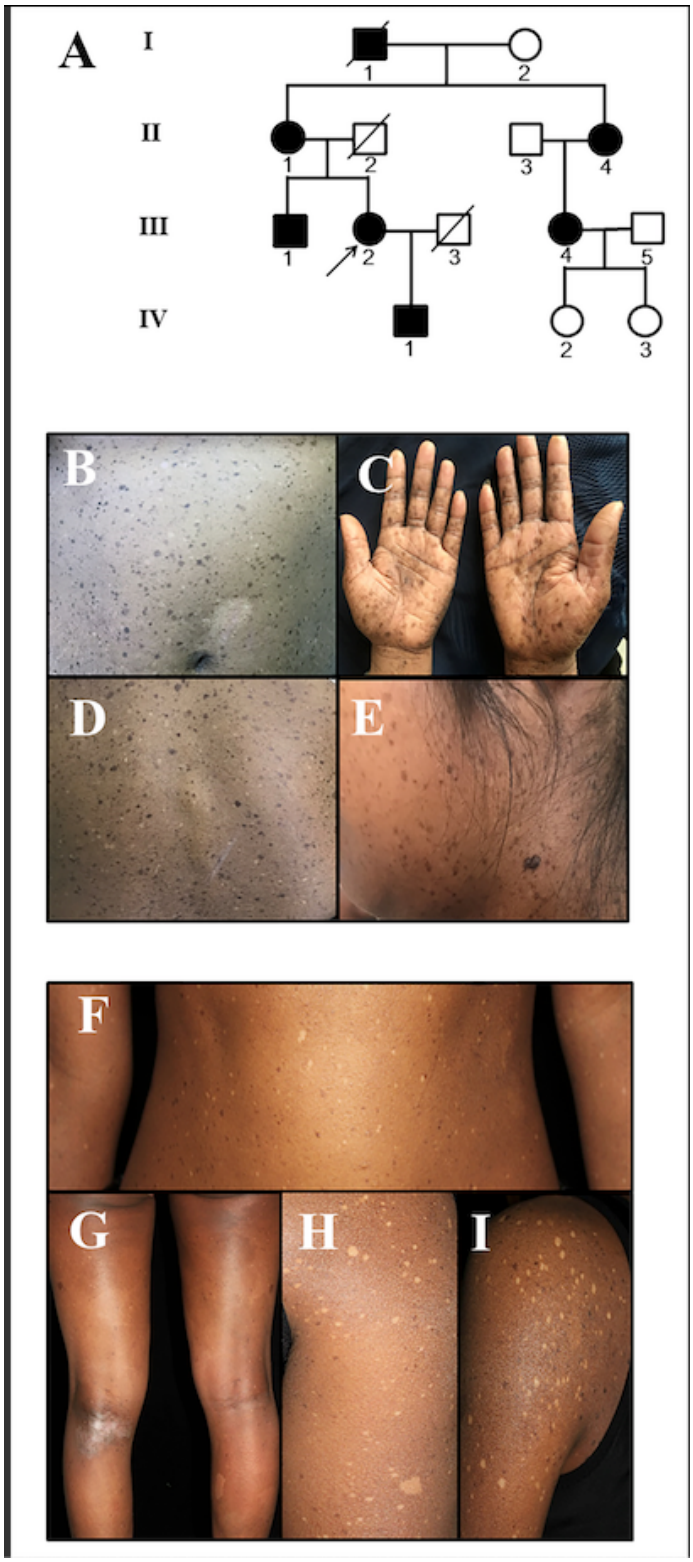

Figure 1

Pedigree and clinical features of the two cases with familial progressive hyper- and hypopigmentation. a. Pedigree of family 1. b-e. Generalized hyper- and hypopigmentation with irregular patches was found at birth, and the patches (0.2cm-0.8cm) progressed successively over her trunk, limbs, face and neck with age. f-i. With age, the lesions increase both in size and number and also became more noticeable and appeared on the trunk and limbs.

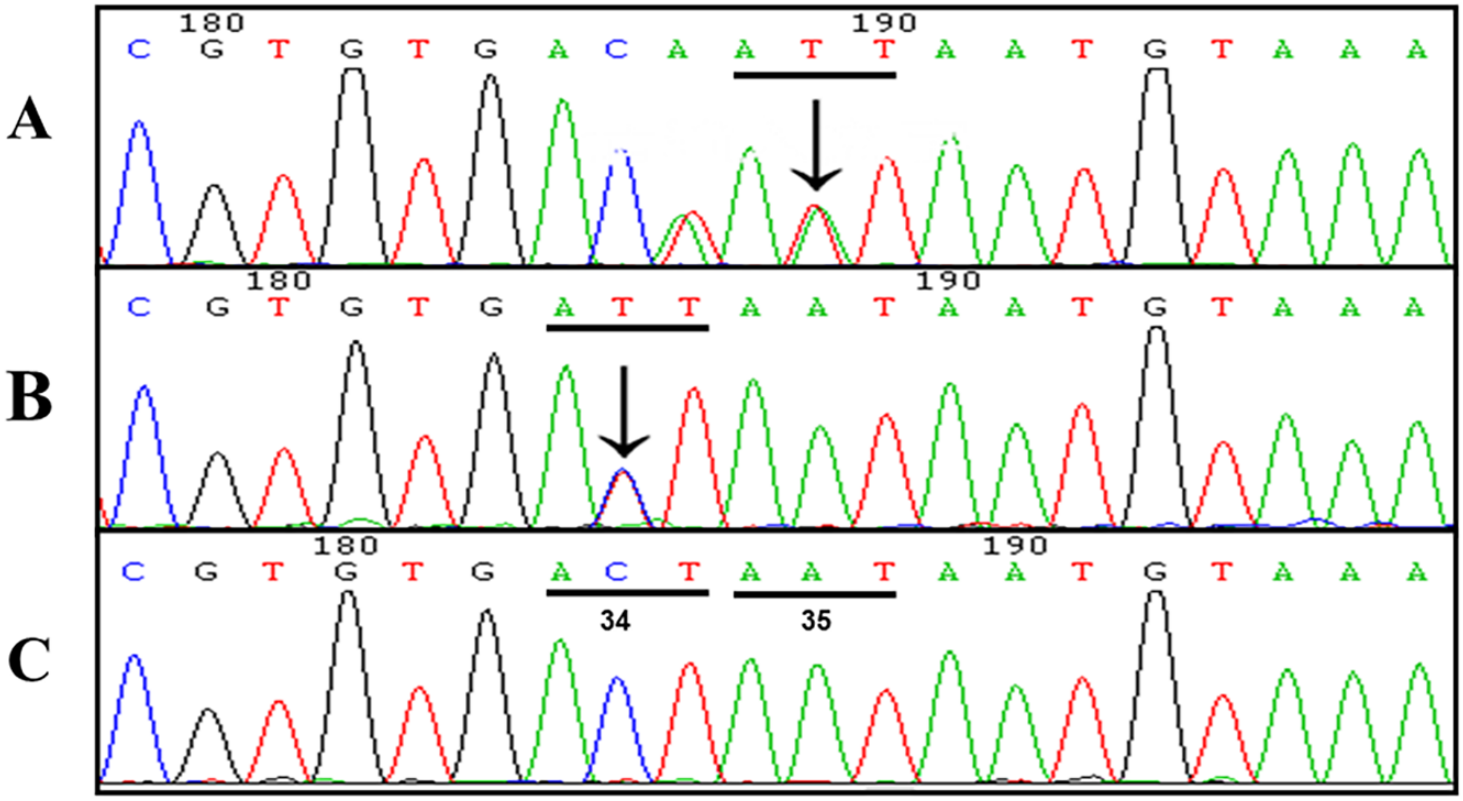

Figure 2

The KITLG gene mutation analysis in two Chinese FPHH cases. a. A novel mutation c.104A>T (p.Asn35Ile) was identified in family 1. The variation c.102T>A (p.Thr34Thr) is a synonymous mutation. b. A recurrent mutation c.101C>T (p.Thr34Ile) was identified in the sporadic case. c. Sequence of the wild type allele, showing translation of Threonine residue at codon 34 (ACT) and Asparagine at codon 35(AAT).

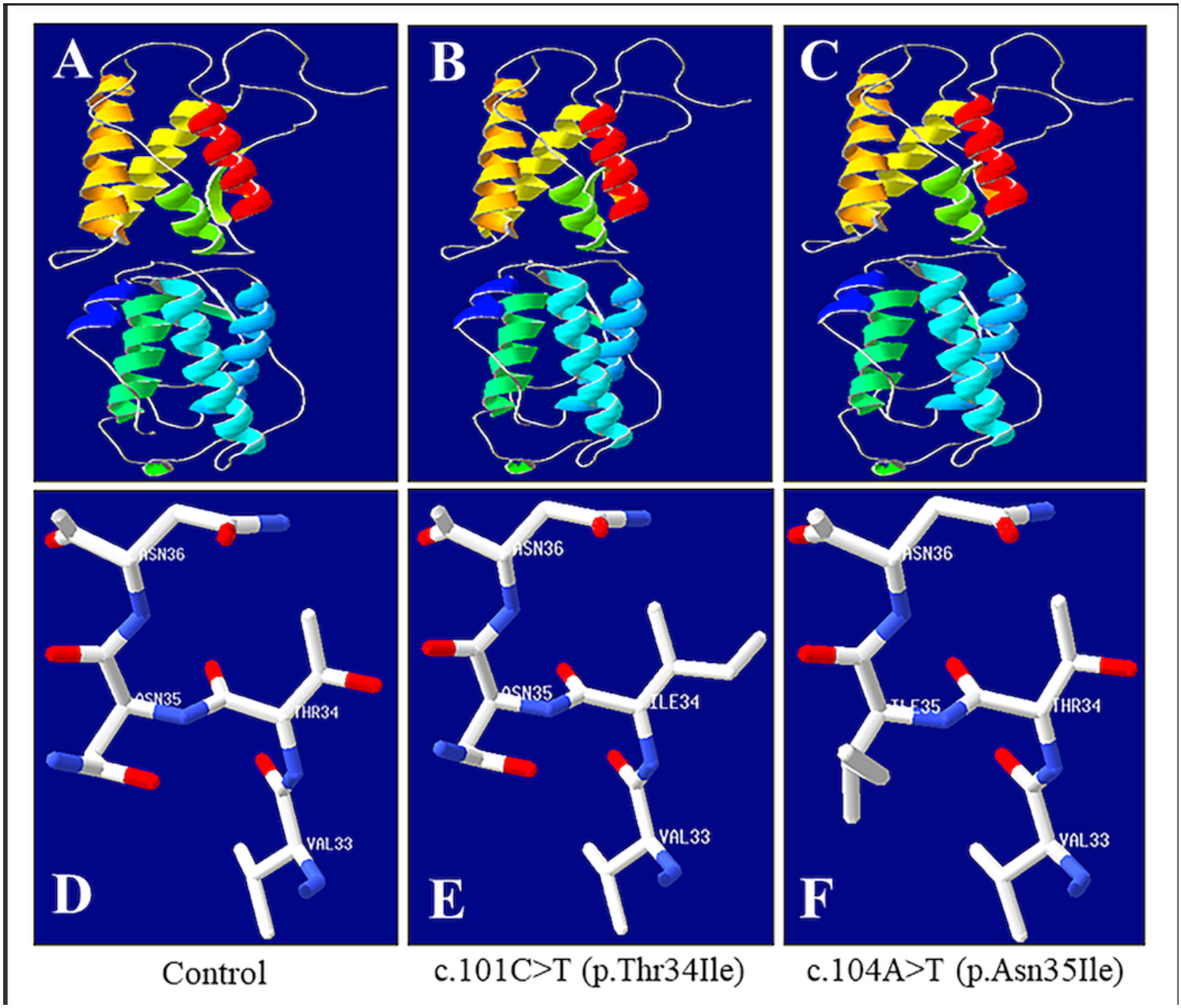

Figure 3

Three-dimensional structure of KITLG. a-c. Protein three-dimensional structure overall picture. d-f. Partial map of protein three-dimensional structure. e. Thr and Asn, as polar, neutral R-based amino acids, became Ile, non-polar, hydrophobic R-based amino acids. When Thr changed to Ile at position 34, the side chain of Ile 34 changed. f. When the Asn changed to Ile at position 35, the side chain of Ile 35 changed.

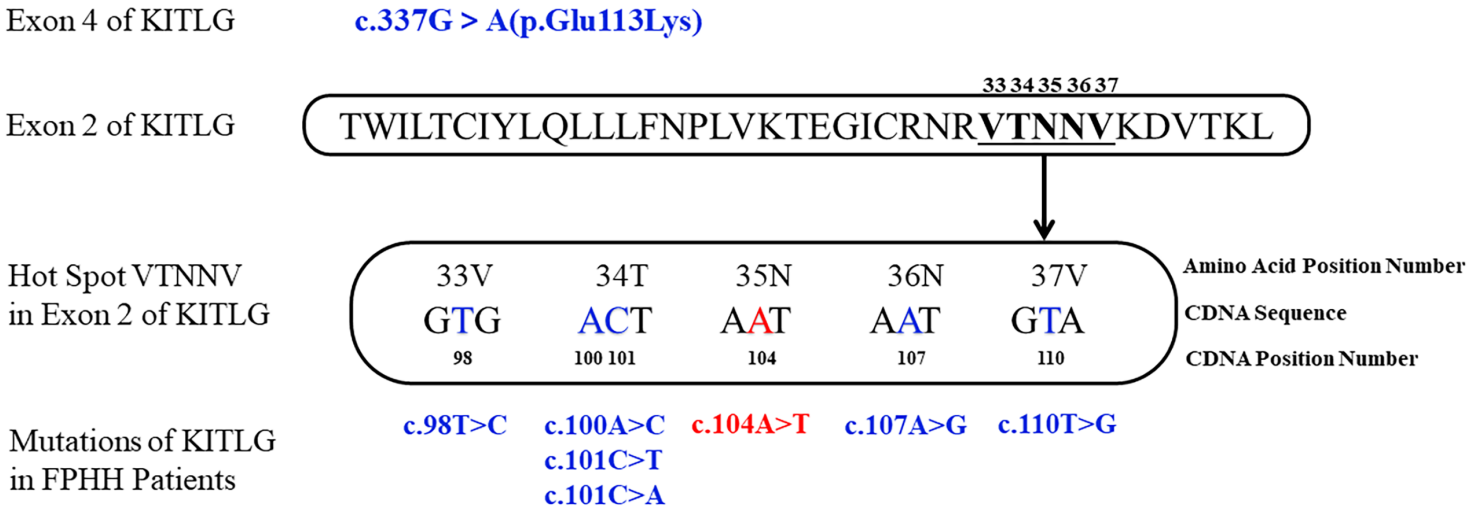

Figure 4

Summary of KITLG gene mutations associated with FPHH. All the mutations associated with FPHH is located within the VTNNV region in exon 2, except c.337G>A that in exon 4 of the KITLG gene. The mutations reported previously are in BLUE, while the novel mutation (c.104A>T) at 35N reported in this study is in RED.

Supplementary Files

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- SupTable1.docx
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- SupTable3.docx
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