Identification of a Novel Mutation in the KITLG Gene in a Chinese Family with Familial Progressive Hyper- and Hypopigmentation

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Research article

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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, which encodes KIT ligand protein, is responsible for FPHH.

Results A novel mutation c.104A > T (p.Asn35Ile) and a recurrent mutation c.101C > T (p.Thr34Ile) in the *KITLG* gene with two Chinese FPHH families were identified. So far, various pathogenic gain-of-function mutations in the *KITLG* gene have been described, which are located in or near the conserved VTNN motif (amino acid 33–37) in exon 2 of the *KITLG* gene. The reported mutations are only involved in 33V, 34T, 36N, 37V but not 35N. As SIFT and Polyphen-2 softwares showed, these two mutations were both predicted to be detrimental variations. Three-dimensional protein structures modeling indicated the mutant KITLG proteins might affect KITLG affinity to its receptor c-KIT. To date, only eight *KITLG* mutations were associated with FPHH and no clear genotype-phenotype correlations had been well established.

Conclusions We have now identified a novel mutation c.104A > T (p.Asn35Ile) of *KITLG* gene, 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 autosomal dominant genodermatosis characterized by diffuse, partly blotchy hyperpigmented lesions intermingled with scattered hypopigmented spots on the skin and mucous membranes. It occurs at birth or early in infancy and the lesions increase both in size and number with age on the face, neck, trunk, and limbs. Lentigines and café-au-lait spots (CALS) are also present [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. 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 [3]. Since then, several mutations (HGMD, http://www.hgmd.cf.ac.uk) in the *KITLG* gene were documented in a few FPHH families [2, 4–6]. However, still many FPHH families without *KITLG* mutation identified indicated that additional locus heterogeneity for this disorder [4, 7, 8].

Here, we reported two Chinese progressive hyper- and hypopigmentation families, 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.

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).

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 “possibly damaging” and “deleterious” with Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/) and SIFT (http://sift.jcvi.org/), respectively. 34Thr and 35Asn of KITLG, which polar, neutral, hydrophilic R-based amino acids had changed in to 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/).

Review Of The Previous Studies Documented Kitlg Gene Mutations

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. [9] 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. [10] 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 [11]. 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 (Supplemental Table S1, 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 VTNN domain of *KITLG* protein (amino acids 33–36), 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 (Supplemental Table S1).

Discussion

KITLG, as KIT LIGAND, is produced locally in human skin by epidermal keratinocytes and endothelial cells. The signaling of *KITLG* and its receptor KIT plays an important role in melanocyte proliferation and pigment production [15, 16]. After KITLG binds the c-KIT receptor, oligomerization is triggered. This initiates signal transduction via the RAS/MAPK pathway to upregulate melanoblast proliferation [15, 16]. The KITLG/C-KIT/RAS/MAPK signalling 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 [17, 18]. Mutant alleles of the *KITLG* gene are lethal in homozygous mice and produce a variable level of coat-color dilution in heterozygous mice [18]. 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. As far as we know, only eight different missense *KITLG* mutations have been reported to cause FPHH (Supplemental Table S1). Notably, seven known mutations were clustered in a highly conserved short amino acid sequence VTNNV (amino acids 33–37) (Fig. 4). It was known VTNN domain of *KITLG* protein (amino acids 33–36), 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 VTNN domain and predicted to be detrimental variations by SIFT and Polyphen-2 tools. Using the Swiss-Model servers [19, 20], 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. 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.

Methods

The characteristics of participants

Family 1, a four-generation Chinese pedigree, had seven individuals affected (three men and four women) by FPHH. 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 (0.2 cm-0.8 cm) progressed successively over her face, neck, trunk and limbs with age. There were also a small number of larger pigmented lesions that were several centimetres 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. The clinical appearances of this sporadic patient was 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 [7]. 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.

Abbreviations

FPHH
Familial progressive hyper- and hypopigmentation.
CALS
café-au-lait spots.
sKITLG
the soluble form of KITLG.
MIM
Mendelian Inheritance in Man.

Declarations

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Authors’contributions

NHZ, SMZ, XLL, ZLL and ZLY collected and collated the data. JBW, WSL and MS analysed 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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Availability of data and materials

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

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.

Competing interests

The authors declare that they have no competing interests.

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Figures

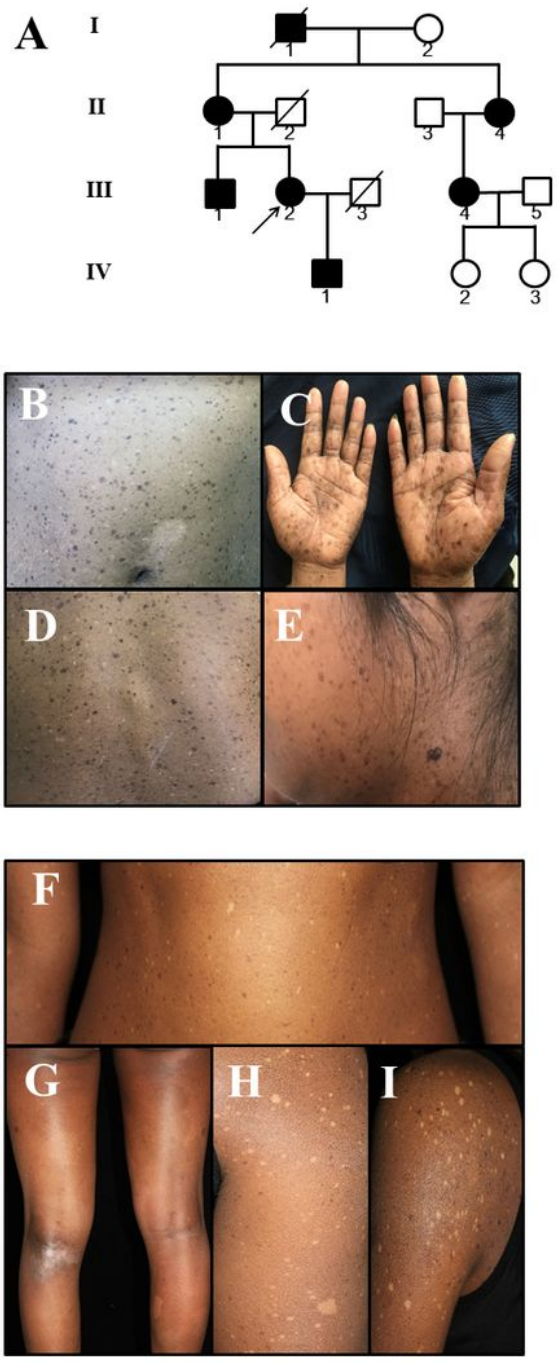

Figure 1

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.

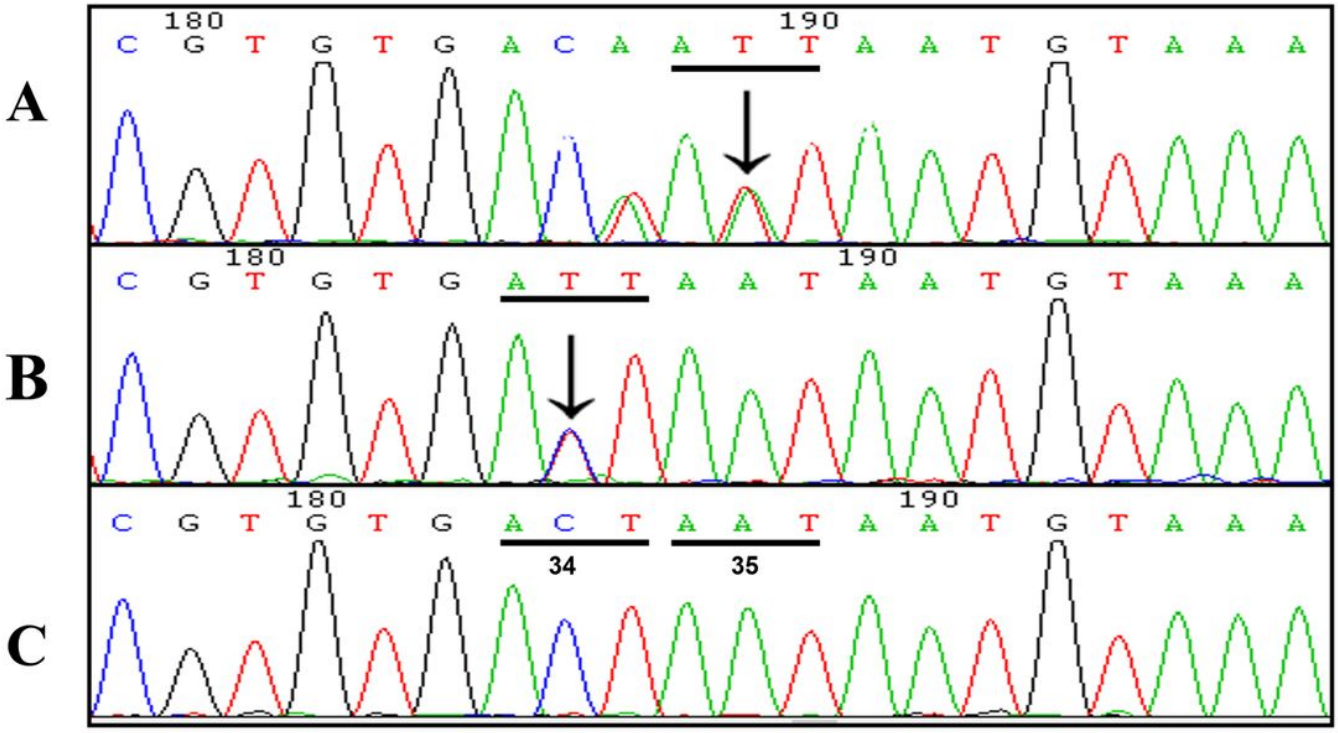

Figure 2

The KITLG gene mutation analysis in these 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).

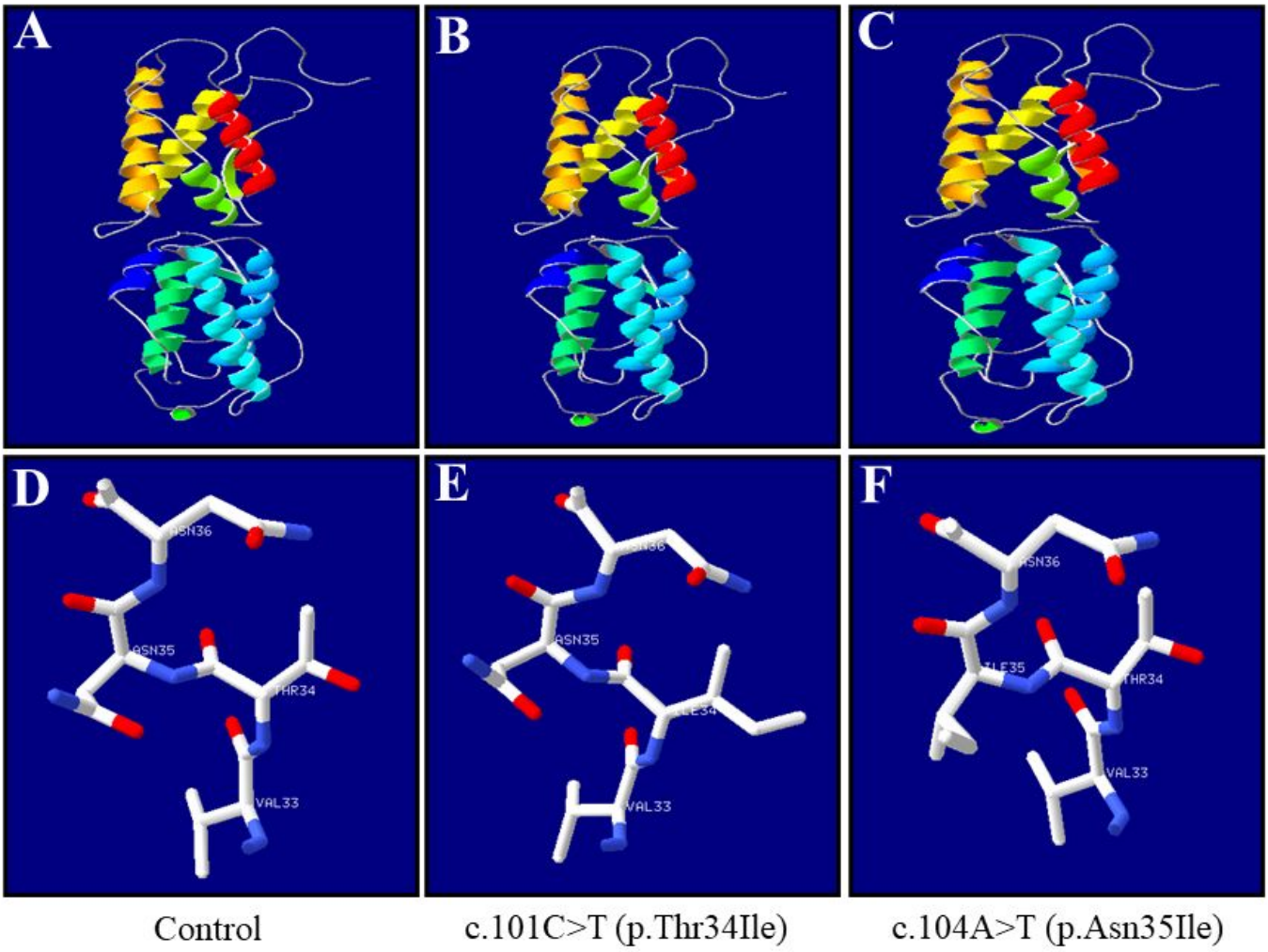

Figure 3

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.

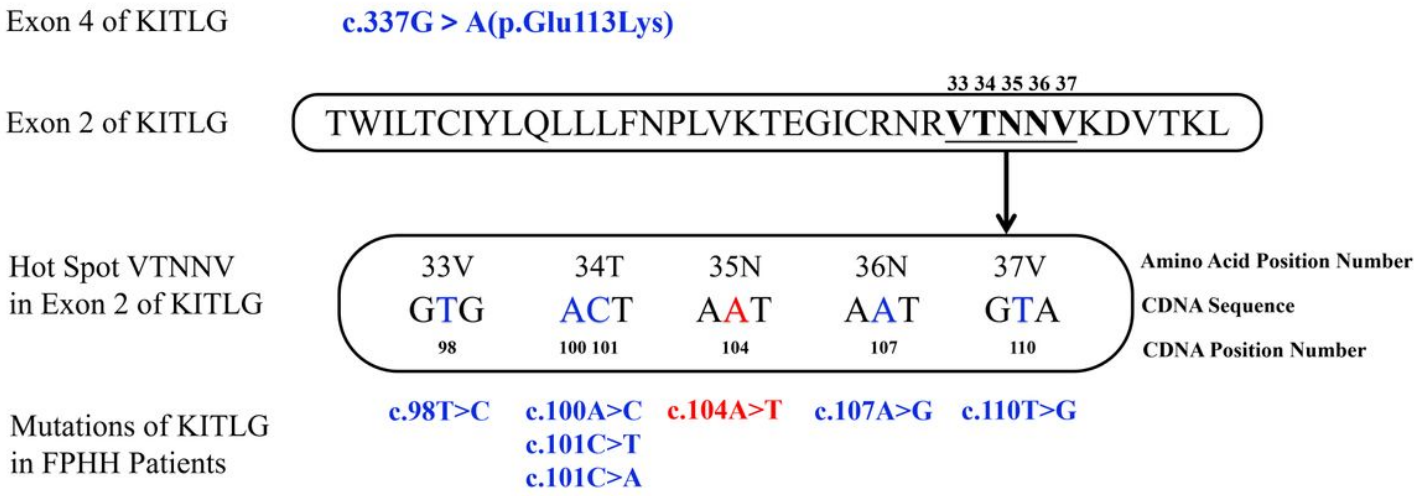

Figure 4

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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