BJD
British Journal of Dermatology

Autosomal recessive hypotrichosis with loose anagen hairs associated with *TKFC* mutations*

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Linked Comment: Brown. *Br J Dermatol* 2021; **184**:800–801.

Summary

Background Loose anagen hair is a rare form of impaired hair anchorage in which anagen hairs that lack inner and outer root sheaths can be gently and painlessly plucked from the scalp. This condition usually occurs in children and is often self-limiting. A genetic basis for the disorder has been suggested but not proven. A better understanding the aetiology of loose anagen hair may improve prevention and treatment strategies.

Objectives To identify a possible genetic basis of loose anagen hair using next-generation DNA sequencing and functional analysis of variants identified.

Methods In this case study, whole-exome sequencing analysis of a pedigree with one affected individual with features of loose anagen hair was performed.

Results The patient was found to be compound heterozygous for two single-nucleotide substitutions in *TKFC* resulting in the following missense mutations: c.574G> C (p.Gly192Arg) and c.682C> T (p.Arg228Trp). Structural analysis of human TKFC showed that both mutations are located near the active site cavity. Kinetic assays of recombinant proteins bearing either of these amino acid substitutions showed almost no dihydroxyacetone kinase or D-glyceraldehyde kinase activity, and FMN cyclase activity reduced to just 10% of wildtype catalytic activity.

Conclusions *TKFC* missense mutations may predispose to the development of loose anagen hairs. Identification of this new biochemical pathobiology expands the metabolic and genetic basis of hypotrichosis.

What is already known about the topic?

- Loose anagen hair syndrome is a rare condition with an estimated incidence of two cases per million per year, mainly observed in female children aged between 2 years and 6 years.
- Loose anagen hairs are loosely anchored and can be easily plucked from the scalp.
- To date, no gene pathology has been associated with loose anagen syndrome.

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Accepted for publication
7 August 2020

Funding sources
This study was supported by the UK National Institute for Health Research comprehensive Biomedical Research Centre award to Guy's and St. Thomas' NHS Foundation Trust, in partnership with King's College London and King's College Hospital NHS Foundation Trust. This work was also funded by the National Children's Research Centre, Dublin. J.C.F.N. is supported by a studentship from the Croucher Foundation, Hong Kong. L.C.S. is supported by a Career Development Award from the Dermatology Foundation. F.F. is supported by the Medical Research Council (MR/L01257X/1). The work in Leiria was financially supported by Associate Laboratory LSRE-LCM - UID/EQU/50020/2019, funded by national funds through FCT/MCTES (PIDDAC). Research in the Grupo de Enzimología in Badajoz is funded by Consejería de Economía e Infraestructuras, Junta de Extremadura, Spain (grant numbers IB16066 and GR18127), cofunded by the European Regional Development Fund.

Conflicts of interest
The authors declare they have no conflicts of interest.

*Plain language summary available online

DOI 10.1111/bjd.19481

What does this study add?

- By undertaking whole-exome sequencing in an individual with loose anagen hairs, and in other unaffected family members, we identified compound heterozygosity for missense mutations in *TKFC*.
- Functional analysis of both amino acid variants showed defective catalytic activity implicating TKFC in hair cycling and structural maintenance.
- The identification of *TKFC* mutations expands the molecular basis of hypotrichosis and provides a new biochemical target for potential therapeutic intervention.

What is the translational message?

- DNA variants in many different genes, including *TKFC*, contribute to hypotrichosis.
- Defining individual gene pathology provides more precise mechanistic insights into the diverse aetiology of inherited forms of hypotrichosis and a basis to develop personalized medicine to improve hair growth.

Inherited forms of hypotrichosis are genetically heterogeneous, with> 30 genes implicated in a spectrum of syndromic and nonsyndromic Mendelian disorders.[1] However, in some forms of hair loss the genetic contribution is more circumspect. For example, loose anagen hair (or syndrome) is a rare condition mainly affecting female children and is characterized by poor anchorage of the anagen hairs that can be plucked easily and painlessly from the scalp. Most cases occur sporadically and are self-limiting, but in other cases a genetic basis has been proposed,[2,3] and variants in the hair follicle keratin *K6HF* (now known as keratin 75 within the companion layer of the hair follicle) have only rarely been identified.[4] Minoxidil 5% lotion has been suggested as a possible treatment for loose anagen hair but most reports are anecdotal and separating therapeutic response from natural resolution of the hair anchorage is difficult.[5,6] The aim of our study was to search for an underlying genetic defect in a patient presenting with hypotrichosis and loose anagen hair using next-generation sequencing and functional characterization of potentially pathogenic variants. We identified biallelic mutations in *TKFC*, whereas recently, different biallelic mutations in *TKFC* have also been reported to cause cataracts and multisystem disease without any hair abnormalities.[7]

Materials and methods

Whole-exome sequencing

Approximately 3 μg of genomic DNA was sheared to a mean fragment size of 150 base pairs (Covaris, Woburn, MA, USA), and the fragments were used for Illumina paired-end DNA library preparation and enrichment for target sequences using the Agilent SureSelect v4 capture kit (Agilent Technologies, Santa Clara, CA, USA). Enriched DNA fragments were sequenced with 100 base-pair paired-end reads on an Illumina HiSeq 2000 platform (Illumina, San Diego, CA, USA; GenePool, Edinburgh, UK). Sequencing reads were aligned to the reference human genome sequence (hg19) using the NovoAlign software (Novocraft Technologies, Selangor, Malaysia). Duplicate and multiple mapping reads were excluded, and the depth and breadth of sequence coverage was calculated with the use of custom scripts and the BedTools package. Single-nucleotide substitutions and small insertions or deletions were identified with SAMtools and in-house software tools and were annotated using the ANNOVAR tool. Variant calling was performed with a previously published in-house pipeline.

Sanger sequencing

TKFC primers were designed with Primer3 software. Polymerase chain reaction products were purified with ExoSAP-IT (GE Healthcare, Chicago, IL, USA) and sequenced with BigDye Terminator v3·1 chemistry (Applied Biosystems, Foster City, CA, USA). Sequences were visualized using the Sequencher software (Gene Codes, Ann Arbor, MI, USA) and variants were detected by direct inspection of chromatograms.

Protein modelling

The conformation of dimeric TKFC having two dihydroxyacetone (DHA) molecules covalently linked to His221 and complexed with two ATP molecules and four Mg^{2+} ions was extracted at time 107·633 ns from the 160-ns molecular dynamics simulation trajectory.[8] This snapshot was taken forward for remodelling of the 535–554 loop, using the Modeller program[9] as follows: firstly, a multiple sequence alignment was generated with (i) the sequence of two identical TKFC chains (chain A + chain B: 2 × 575 = 1150 residues) arranged back-to-back; (ii) a copy of (i), except that residues 540–551 (chain A) and 1115–1126 (i.e. residues 540–551 of chain B) were removed and (iii) two *Escherichia coli* DhaL (PDB 3PNL, chain B) subunits aligned to chains A and B of TKFC, respectively (Figure S1; see Supporting Information). As such, both the TKFC snapshot and the *E. coli* DhaL subunit were used as templates for

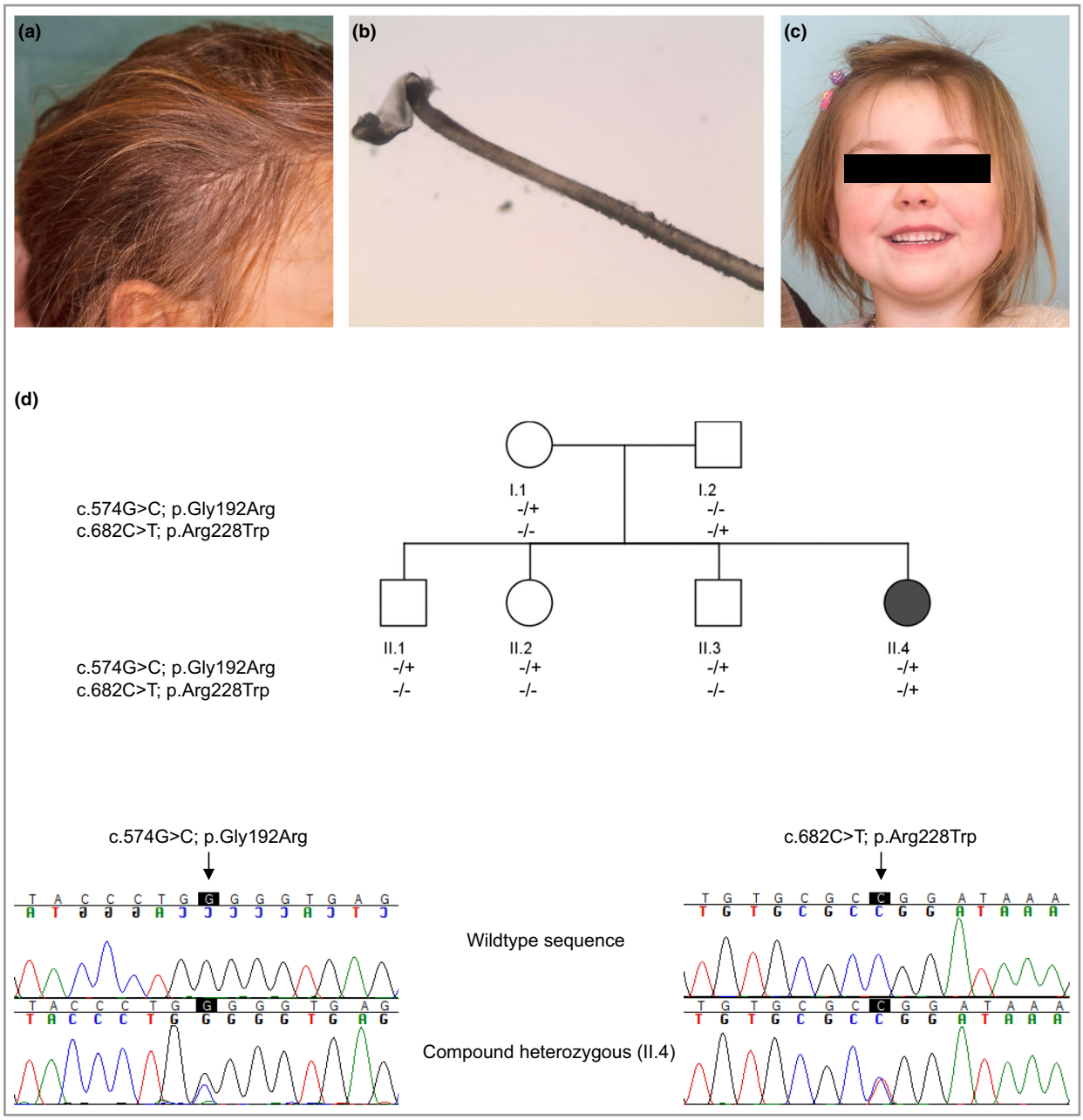

Figure 1 Clinical features and segregation analysis. (a) Hair appearance at 3 years; lank, short hair that had never been cut. (b) Light microscopic analysis of hair pluck samples identified that a preponderance (> 90%) of hairs were anagen hairs with a classical 'hockey-stick' appearance some showing ruffling of hair shaft cuticle. (c) By age 8 years the proband's hair had thickened and generally improved in quality, but lank and short hair remained bitemporally. (d) Segregation analysis and sequence chromatograms of the c.574G> C; p.Gly192Arg and c.682C> T; p.Arg228Trp *TKFC* mutations

modelling. This multiple alignment was used as input to the Modeller program. During the loop refinement process, only residues 535–554 were allowed to move. A total of 100 models were generated and ranked using Discrete Optimized Protein Energy, or DOPE.[10] The best model was then energy-minimized *in vacuo* with Gromacs 2018·6 using the Amber03 forcefield.[11,12] For this process, the coordinates of the best model were processed with AmberTools18 to generate topology and parameters files, which were then converted to Gromacs format with ACPYPE.[13,14] The parameters used for nonstandard species (ATP and histidine with bound DHA) were taken from REDDB (projects F-91 and F-86, respectively).[15]

In vitro protein mutagenesis and recombinant protein expression

Point mutants were constructed as described previously following the QuikChange protocol (Stratagene) using mutagenic

Table 1 Kinetic parameters of the DHA kinase, GA kinase and FMN cyclase activities of TKFC mutants compared with the wildtype

TKFC mutation[a]	Varying substrate	Constant substrate	k_{cat}, min^{-1}	K_m, μmol L^{-1}	k_{cat}/K_m, M^{-1} s^{-1}
Wildtype[b]	DHA	5 mmol L^{-1} ATP	300	2	3 210 000
	ATP	0·5 mmol L^{-1} DHA	275	43	105 000
	GA	5 mmol L^{-1} ATP	290	18	275 000
	ATP	0·5 mmol L^{-1} GA	236	62	63 000
	FAD	None	49	7	117 000
G192R	DHA	5 mmol L^{-1} ATP	na[c]	na	na
	ATP	0·5 mmol L^{-1} DHA	na	na	na
	GA	5 mmol L^{-1} ATP	na	na	na
	ATP	0·5 mmol L^{-1} GA	na	na	na
	FAD	None	11	12	15 693
R228W	DHA	5 mmol L^{-1} ATP	13	50	4425
	ATP	0·5 mmol L^{-1} DHA	17	732	384
	GA	5 mmol L^{-1}ATP	na	na	na
	ATP	0·5 mmol L^{-1} GA	na	na	na
	FAD	None	9	17	9426
R227W	DHA	5 mmol L^{-1} ATP	65	0·6	1 700 000
	ATP	0·5 mmol L^{-1} DHA	63	31	33 600
	GA	5 mmol L^{-1} ATP	32	9	60 600
	ATP	0·5 mmol L^{-1} GA	25	29	14 300
	FAD	None	19	4	83 300
R543A	DHA	5 mmol L^{-1} ATP	na	na	na
	ATP	0·5 mmol L^{-1} DHA	na	na	na
	GA	5 mmol L^{-1} ATP	na	na	na
	ATP	0·5 mmol L^{-1} GA	na	na	na
	FAD	None	19	4	74 300

DHA, dihydroxyacetone; FAD, flavin adenine dinucleotide; GA, D-glyceraldehyde. [a]G192R and R228W correspond to the identified mutations in the proband. R227W and R543A correspond to the mutants constructed as controls. Values for the mutants were calculated from the adjustment of the Michaelis–Menten equation to the mean activities obtained in three or four independent experiments (Fig. S3; see Supporting Information). [b]Data for wildtype TKFC are obtained from Rodrigues *et al.*[16] [c]Not assayable (na) owing to activity below detection limit; at 0·5 mmol L^{-1} DHA or GA, and 5 mmol L^{-1} ATP, the molecular activity was < 0·5% of the wildtype under the same conditions.

primers (Table S1; see Supporting Information) and the plasmid pGEX-6P-3-hF2 as template, which encodes a glutathione transferase (GST)-hTKFC fusion protein.[16,17] TKFC, when compared with the reference UniProt protein (Q3LXA3), harbours a p.Ala185Thr substitution, which reflects a common polymorphism (rs2260655) present in almost 99% of the European population, including our patient who is homozygous for this variant. The correctness of all constructs was confirmed by sequencing the two chains of the complete coding sequence (Figure S2; see Supporting Information) (Servicio de Genómica, Instituto de Investigaciones Biomédicas Alberto Sols, Consejo Superior de Investigaciones Científicas-Universidad Autónoma de Madrid, Madrid, Spain). Recombinant proteins were obtained by expression under isopropyl thiogalactoside induction in *E. coli* BL21 cells transformed with each pGEX-6P-3 construct and separated from the GST tag by specific proteolysis with the PreScission protease.[16,17]

Enzymatic assays

TKFC kinetic assays were performed, as described previously.[16] In brief, FMN cyclase activity [flavin adenine dinucleotide (FAD) → cyclic FMN + AMP] was followed discontinuously by high-performance liquid chromatography, evaluating the amount of cyclic FMN accumulated at different timepoints; DHA kinase activity (DHA + ATP → DHA-phosphate + ADP) was coupled to glycerol-3-phosphate dehydrogenase [DHA-phosphate + nicotinamide adenine dinucleotide (NADH) → glycerol-3-phosphate + NAD+]; D-glyceraldehyde (GA) kinase (GA + ATP → GA-3-phosphate + ADP) was coupled to triose-phosphate isomerase (GA-3-phosphate → DHA-phosphate) and glycerol-3-phosphate dehydrogenase. In the kinase assays, the change of A_{340} owing to NADH consumption was recorded continuously. All assays were performed at 37 °C, under conditions of linearity with respect to incubation time and enzyme amount. Controls without TKFC and/or substrate were processed in parallel and used to correct data obtained in full reaction mixtures as required.

Results

Clinical features

A 3-year-old girl born to unaffected parents presented with a history of slow-growing hair that had never been cut. Her scalp hair was sparse, short and lank in character (Figure 1a). Eyebrows, eyelashes and body hair were unaffected. She was otherwise well. Hair pull was painless and revealed almost

100% anagen hairs (Figure 1b). Systematic investigations revealed normal parameters for full blood count and thyroid function. A clinical diagnosis of loose anagen syndrome was made. At review, when the patient was 8 years of age (Figure 1c), hair quality had improved substantially, but she continued to have short and poor-quality hair on both temples, and hair pull still revealed almost 100% anagen hairs.

Identification of *TKFC* mutations

Following written informed consent and institutional ethics committee approval, whole-exome sequencing (WES) was undertaken in DNA from peripheral blood from this pedigree including the proband (Figure 1d) (Tables S1–S3; see Supporting Information). More than 6·6 Gb of sequence was generated per sample, with> 91% of the target exome at> 20-fold coverage. Filtering of the generated exome variant profiles was performed following a model of either *de novo* dominant or rare recessive inheritance by retaining homozygous or compound heterozygous predicted protein-altering substitutions and indels with a minor allele frequency of less than 0·5% in each of the 1000 Genomes Project, Exome Aggregation Consortium, National Heart, Lung, and Blood Institute Exome Variant Server and our in-house database of in excess of 6000 exomes. Our filtering approach revealed *TKFC* (HGNC:24552; previously known as *DAK*) as the only gene harbouring compound heterozygous variation (c.574G> C; p.Gly192Arg and c.682C> T; p.Arg228Trp) that met our criteria, with predicted Combined Annotation Dependent Depletion scores of 32 and 35, respectively (Table S3; see Supporting Information). Segregation analysis by means of Sanger sequencing in all available members of the family confirmed recessive inheritance of the hair loss (Figure 1d and Table S4; see Supporting Information). Mining of the Genome Aggregation Database (https://gnomad.broadinstitute.org/) did not identify any homozygotes for either variant. We also did not identify further *TKFC* mutations in 14 other pedigrees known to us with loose anagen hair, suggesting that variants in this gene are a rare cause of this type of hypotrichosis. The presenting hair phenotype in those other pedigrees was similar to our patient, although 10 of the 14 other pedigrees had atopy, which was not present in our case, and spontaneous clinical improvement was more rapid in our patient.

TKFC protein modelling

TKFC encodes triokinase/FMN cyclase, which phosphorylates both DHA and D-GA, and splits ribonucleoside diphosphate-X compounds among which FAD is the best physiological substrate.[16] The enzyme is a homodimer of subunits, each containing two domains, K and L (Figure 2a), which form two active sites between K- and L-domains of different subunits (Figure 2b). There is no crystal structure of human TKFC, although models for the protein have been proposed,[8,16] which we have refined further with the *E. coli* homolog as a template to remodel the loop formed by residues 535–554 (Figure S1; see Supporting Information). Both mutations are near the active site, possibly affecting triokinase activity (Figure 2a). The p.Gly192Arg substitution may affect triose binding to His221, or triose hydroxymethyl orientation towards the γ-phosphorus of ATP. The p.Arg228Trp substitution may affect the alternation of the active site between open and closed conformations.

TKFC mutations result in reduced catalytic activity

To evaluate the biochemical significance of both mutations, recombinant proteins G192R-hTKFC and R228W-hTKFC were constructed. We also took into consideration that approximately 92% of the population are carriers of a common polymorphism (rs2260655) in *TKFC*, giving rise to a p.Ala185Thr substitution, with our patient being homozygous for this variant. Two additional mutations were used as controls, i.e. R227W (adjacent to Arg228) and R543A, as Arg543 is conserved in the *E. coli* homolog, where it is needed for kinase activity.[18] For each mutant, DHA kinase, GA kinase and FMN cyclase activities were assayed (Table 1 and Figure S3; see Supporting Information). The assays showed that G192R-hTKFC and R228W-hTKFC were essentially devoid of kinase activities and conserved approximately 10% of FMN cyclase catalytic efficiency. In contrast, R227W-hTKFC conserved most activities, highlighting the specificity of the R228W mutation. The strong effect of the R543A mutation indicates that Arg543 is close to the kinase substrates, as in the remodelled structure of TKFC (Figure 2a).

Discussion

We found *TKFC* mutations in one individual with loose anagen hairs but failed to identify mutations in this gene in 14 other pedigrees. It is plausible that these other cases might result from regulatory or deep intronic variants in *TKFC* that we failed to detect, although genetic heterogeneity is perhaps more likely. Moreover, different mutations in *TKFC* have already been implicated in a different phenotype that does not involve hair abnormalities.[7] Those reported *TKFC* mutations impact differently on the protein and its functional domains, which may account for the phenotypic differences. It is therefore important to be cautious in claiming the new genotype–phenotype data we propose. In addition, expression of *TKFC* mainly occurs in the liver, small intestine and adrenal glands (medulla, where catecholamines but not corticosteroids are produced), rather than in the skin or its appendages, based on the GTEx (https://www.gtexportal.org) and BioGPS (http://biogps.org) portals. Nevertheless, our functional studies do support a role for TKFC pathology in the pathobiology of loose anagen hairs, at least in this one individual. Therefore, the key questions from our study are how could *TKFC* mutations contribute to loose anagen hairs (Figure 3) and whether there might be any implications for therapy?

Our functional data highlight loss of kinase activity. The most plausible explanation for the hair changes might implicate GA kinase inactivation leading to the accumulation of GA

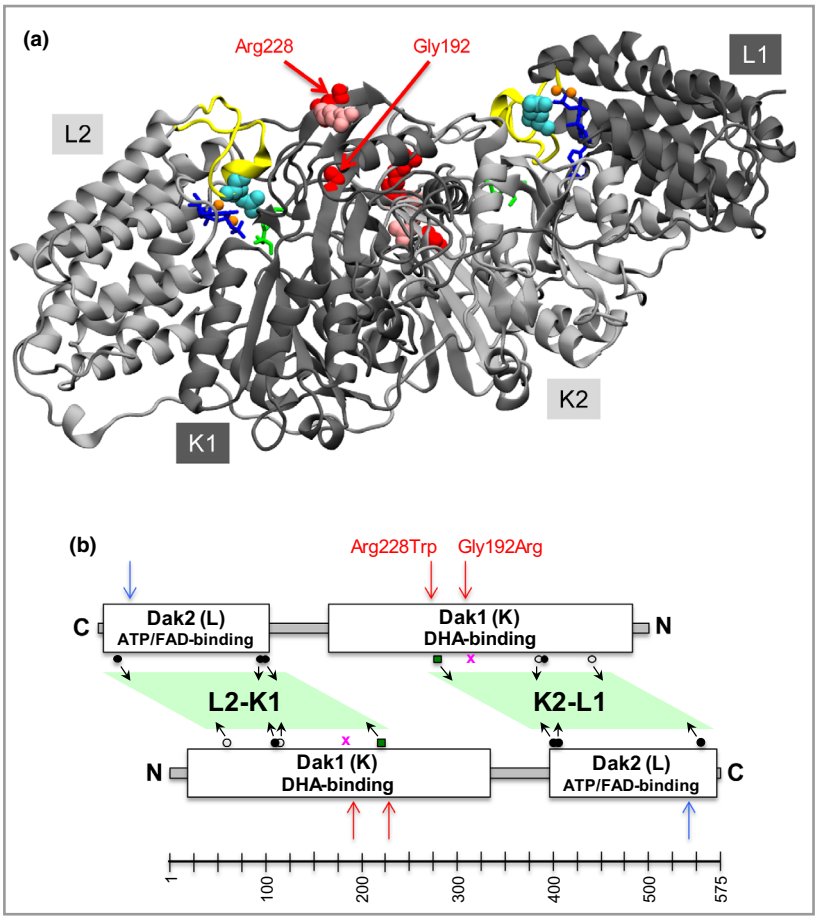

Figure 2 TKFC structural analysis. (a) TKFC model showing the position of mutated residues in the proband. TKFC is a dimer formed by two identical subunits, i.e. chain 1 (dark grey) and chain 2 (light grey), with each containing two domains (K and L) that are connected by a 20-residue linker with an extended conformation. The two active sites formed in the interlocked structure are displayed with ligands [ATP (blue), Mg ions (orange) and dihydroxyacetone (DHA) covalently bound to His221 (green)]. The following residues are highlighted: the 535–554 loop (yellow), which includes Arg543 (cyan); Gly192 and Arg228 (red), which are the residues mutated in the patient (G192R and R228W) and Arg227 (pink). (b) Schematic representation of protein domains. Domain names and boundaries are according to Pfam database [Dak1/K-domain (N-terminal; ID PF02733; residues 19–335), and Dak2/L-domain (C-terminal; ID PF02734; residues 398–571)]. Coloured arrows point to the residues mutated in the patient (red arrows) or mutated to validate the protein model (cyan arrow). Two active centres (light green areas) are formed in the interface between the K- and L-domains of each protein chain [i.e. chain 1 (lower) and chain 2 (upper)], named as L2-K1 and K2-L1 active centres. Circles indicate residues that have been proposed to be involved in substrate binding and were either tested by mutagenesis (solid) or were not tested (clear). The green square marks His221, which covalently binds DHA, and is required for DHA kinase, but not for FMN cyclase activity.[16,18] All these residues help to conform the active centres, and for this reason are connected to the active centres by arrows. The magenta 'x' marks position 185, which is Ala in the reference UniProt sequence (Q3LXA3), but Thr in the recombinant TKFC proteins (Table 1),[16] and in the proband, who is homozygous for the common Ala185Thr variant (rs2260655)

derived from fructose metabolism. The low K_M value of TKFC for GA makes this the major route for GA disposal.[16,19] Moreover, GA (and DHA) generates advanced glycation end products (AGEs).[20] Of note, AGEs have been reported to inhibit mesenchymal–epidermal interaction by upregulating proinflammatory cytokines in hair follicles.[21] Therefore, increased levels of GA, produced in the liver or elsewhere, could lead to GA-dependent AGE in hair follicles and contribute to the loose hair phenotype.

An alternative or perhaps additional explanation for the hair changes might involve DHA kinase and FMN cyclase loss of activity impacting on riboflavin metabolism. Riboflavin contributes to the activation of vitamin B6, which is essential for normal hair growth; riboflavin also helps produce the antioxidant glutathione, which prevents free radical damage, including damage to hair follicles.[22]

A further possibility might involve defective TKFC interactions with the antiviral-response mediator MDA5.[23] However, the lack of any clinical evidence for immune dysregulation in the patient makes this unlikely, even though innate and adaptive immune response abnormalities have been implicated in other hair loss disorders such as alopecia areata.[24]

It is noteworthy that our mutations (p.Gly192Arg and p.Arg228Trp) map to the Dak1/K-domain in the N-terminal

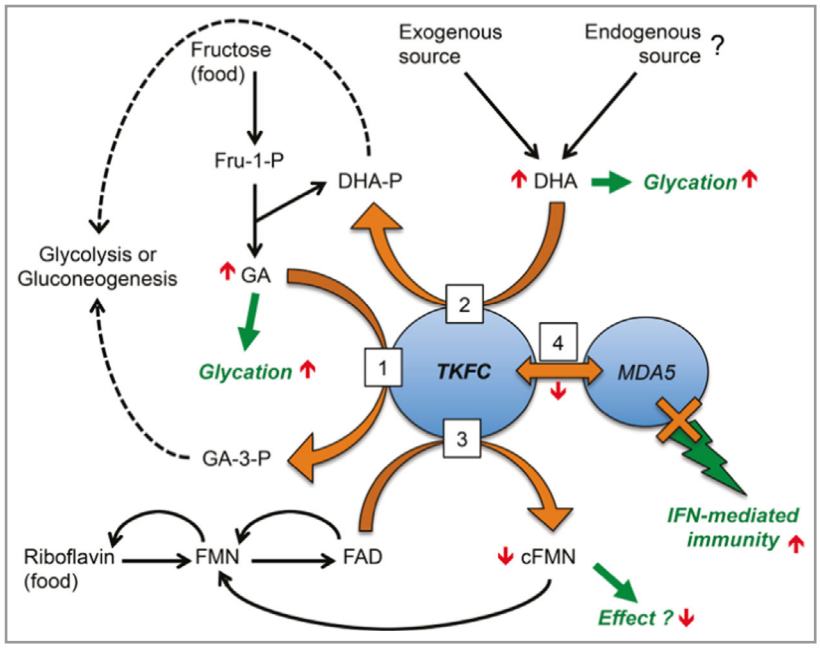

Figure 3 TKFC activities and potential consequences of its inactivation. Schematic showing the four known activities of TKFC (thick orange arrows and 'x'). Green arrows and text show effects of TKFC kinase substrates, cyclase product or protein–protein interactions. Red arrows indicate potential consequences of mutations abolishing the following TKFC activities: (i) D-glyceraldehyde (GA) kinase (GA + ATP → GA-3-P + ADP) represents the third step of fructose (Fru) metabolism, acting after fructokinase (Fru + ATP → Fru-1-P + ADP) and fructose 1-phosphate aldolase [Fru-1-P → dihydroxyacetone (DHA)-P + GA].[28] The absence of GA kinase in the patient may interfere with the use of fructose and elicit some accumulation of GA, a protein-glycating agent that can elicit the formation of advanced glycation end products (AGEs).[9,29] (ii) DHA kinase (DHA + ATP → DHA-P + ADP) is needed for DHA disposal.[30–32] Although there is no clear endogenous source of DHA in mammals,[33] it is approved for use as part of tanning formulations (https://www.fda.gov/cosmetics/cosmetic-products/sunless-tanners-bronzers),[34,35] and there are a few reports of its use in nutritional supplements.[36] The lack of DHA kinase may enhance the (toxic) effects of DHA, which is also a protein-glycating agent.[31,37,38] (iii) FMN cyclase [flavin adenine dinucleotide (FAD) → cFMN + AMP] and its specific flavin product cFMN (cyclic FMN) are of unknown physiological significance. This is shown in the context of the known reactions of flavin metabolism. The occurrence of the FMN cyclase reaction *in vivo* is compounded by the *in vitro* requirement of high concentrations of Mn^{2+} or Co^{2+}, which are not expected in mammals, and by the strong cyclase inhibition by ATP and ADP.[16,39,40] Of note, low nanomolar concentrations of cFMN have been reported in liver extracts.[41] It is noteworthy that cFMN can be hydrolysed by a phosphodiesterase (cFMN + H_2O → FMN) identified in rat liver, which is neither studied in detail nor molecularly identified.[39] A diminished FMN cyclase activity would lead to a reduction of cFMN concentration with unknown consequences. (iv) TKFC binding to MDA5, a cytoplasmic viral RNA sensor, leading to inhibition of MDA5-mediated innate immunity.[23,42] This interaction occurs via the N-terminal domain of TKFC, where residues Gly192 and Arg228 (mutated in the patient) are located, potentially impeding protein–protein interactions. IFN, interferon

of TKFC, whereas the mutations reported by Wortmann *et al.* (p.Gly445Ser and p.Arg543Ile) map to the Dak2/L-domain in the C-terminal of the protein, which may be responsible for the diverse phenotypes.[7] This phenomenon has also been documented for the *LSS* gene, which encodes for lanosterol synthase, an enzyme involved in cholesterol biosynthesis.[25] In fact, mutations that map in the N-terminal of LSS give rise to hypotrichosis, whereas mutations that map to the C-terminal lead to cataracts.[25,26] Moreover, biallelic mutations in LSS have been shown to cause a neuroectodermal syndrome characterized by alopecia and intellectual disability.[27]

Regarding potential treatment, the variable natural history of loose anagen hairs, allied to the complexity and multiplicity of the biochemical pathways impacted by the *TKFC* mutations, poses a challenge to physically improving the loose anagen hair anchorage. Hypothetically, dietary restriction of fructose to diminish GA accumulation might ameliorate the hair phenotype. In addition, treatment with cFMN or its precursors FAD, FMN or riboflavin, to overcome the reduction of FMN cyclase activity, might also have therapeutic value. However, such dietary interventions remain speculative for now. Nevertheless, future functional studies to investigate the role of TKFC, GA and cFMN in hair follicle biology are warranted.

In conclusion, we have identified a single family in which there is evidence that biallelic loss-of-function mutations in *TKFC* are implicated in the pathobiology of hypotrichosis with loose anagen hair. Identification of further patients with loose anagen hair and the search for *TKFC* variants will determine the broader relevance of our study. Our findings expand the genetic causes of hypotrichosis, provide a more accurate model of TKFC structure and highlight its importance for hair anchorage, which may facilitate the development of novel therapeutic approaches, potentially involving dietary manipulation or supplementation.

Acknowledgments

We would like to thank the patient and her family members who kindly contributed samples and granted permission to publish this work. We acknowledge assistance with hair microscopy from Michael McDermott and from Dong Li for WES data mining.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Table S1 Exome sequencing coverage and mapping statistics.

Table S2 Variant calling for exome-sequenced individuals.

Table S3 Summary of whole-exome filtering process.

Table S4 Primer sequences used for co-segregation analysis.

Table S5 Primer sequences used for site-directed mutagenesis.

Figure S1 Comparison of previous and current TKFC models in complex with dihydroxyacetone (DHA), ATP and Mg^{2+}.

Figure S2 Sanger sequencing data of *TKFC* mutations constructed *in vitro*.

Figure S3 Saturation curves of mutant TKFC proteins.

Powerpoint S1 Journal Club Slide Set.

Video S1 Author video.