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Supplemental Data

Mutations in Three Genes Encoding Proteins Involved in Hair Shaft Formation Cause Uncombable Hair Syndrome

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Supplemental Note: Case Reports

Clinical data for individuals with *PADI3* mutations

The English family has also been described in the manuscript. The female sibling was referred to the Department of Dermatology in Brigthon at the age of 11 years with a lump in her right and left arm. The clinical and later histological diagnosis was of pilomatrixomas. At the same appointment it was noted that she had abnormal hair. This was first noticed by the parents after 8 weeks of hair shedding in early childhood. The hair was then very slow growing, came out easily and painlessly. Her hair was hard to brush or comb and she rarely had to have her hair cut. Teeth and nail development was normal. The girl has recently been diagnosed as diabetic. Her brother was examined at the age of 15 years and had white brittle nails. His hair when long was described as being like sheeps wool which had improved with age (Figure 1J). The hair had a spangly appearance. The features of the hair shaft observed by electron microscopy were diagnostic of uncombable hair with longitudinal running ridges (Figure 1K), some twisting and triangular or heart shaped cross sections (Figure 1L).

A 3-year old Danish girl was referred for evaluation of abnormal hair (Figure 1F). Besides hair shaft anomaly she was otherwise healthy. From birth she had sparse hair until the age of 1.5 years, when the parents noted her dry and unruly hair which could not be properly combed. It was also noted that she could never grow her hair long. Hair microscopy showed hair shaft anomalies with pilli canaliculi et trianguli. When she was 4 years old biotin therapy 5 mg daily was given for 6 months, which apparently improved the hair texture. Today the girl is 8 years old, and overall there has been also a spontaneous improvement of the condition (Figure 1G). This clinical signs of this girl have been described in detail elsewhere.[1]

A German boy was seen at the age of 3 and 8 years with unimproved wiry hair. The blond, lusterless hair was closely cropped and irregularly stuck out from the scalp growing in lots of different directions. Of interest, the hair originating from a congenital melanocytic nevus of 2.5 x 1.5 cm size at the right temporal side of the scalp was not only darker but also appeared structurally normal without any signs of uncombability (Figure 1D).

An 18-month old Swiss boy was referred to the Department of Dermatology in Zurich for the evaluation of abnormal hair. From early infancy the parents noted dry, unruly and slow growing hair which did not maintain its shape after styling. Apart from mild obstipation he was otherwise well. No one else in the family hat any similar hair problems. On examination the boy's entire scalp was observed to be covered with brown, dry, frizzy hair that projected outward and resisted any attempt to flatten it. On hair shaft microscopy longitudinal grooving and triangular cross sections were observed, consistent with pili canaliculi et trianguli. Today the boy is nearly 6 years old, and there is an improvement in the hair phenotype (Figure 1E).

A Spanish girl was seen at the age of 4 years for ‘funny looking’ hair. Clinical examination revealed normal brown light hair without any clear alteration on eyelashes nor eyebrows. Teeth and nails are normal. Under the scanning and optical microscope typical finding of pili canaliculi were seen. This clinical signs of this girl have been described in detail elsewhere.[2]

The scalp hair of a German girl was normal at birth and started to grow curly and badly combable from the age of 3 months. Hair growth was claimed to be decelerated by the mother. On clinical examination at the age of 3.5 years her shoulder-length light hair appeared dry and stood out in all directions. Hair density was not reduced, and no other abnormalities could be identified (no picture available).

Another German girl presented with very fair, uncombable hair at the age of 6 years (Figure 1B). Physical examination was within normal ranges. Until the age of 15 years the structure of the hair remained unchanged.

Another German girl was examined at the age of 4 years because of uncombable hair from infancy on (Figure 1A). Short hairstyles were preferred until spontaneous improvement occurred at the age of 10-11 years, when scalp hair gradually became more flat and easier to manage. Today, at the age of 25 years, few untamable hairs are left over in the frontal area which are straightened with hair gel.

A little German girl presented with normal hair at birth; soon after birth, the regrowing hair was noticeable, and UHS syndrome was diagnosed (Figure 1C). Nowadays, at the age of 10 years, the girl has long pretty hair that is well combable. There are no other abnormalities, a sibling has normal hair. No other individual in this family has abnormal hair structure.

Clinical data for the individual with the *TGM3* mutation

The clinical signs of this young men have recently been described elsewhere.[3]

Clinical data for the individual with the *TCHH* mutation

The German girl with the *TCHH* mutation came to the clinics at the age of 19 years complaining that her hair grows too slowly being otherwise healthy. Since her childhood, she haid brittle, curly hair, which was barely combable. These symptoms improved until her 14th year of life.

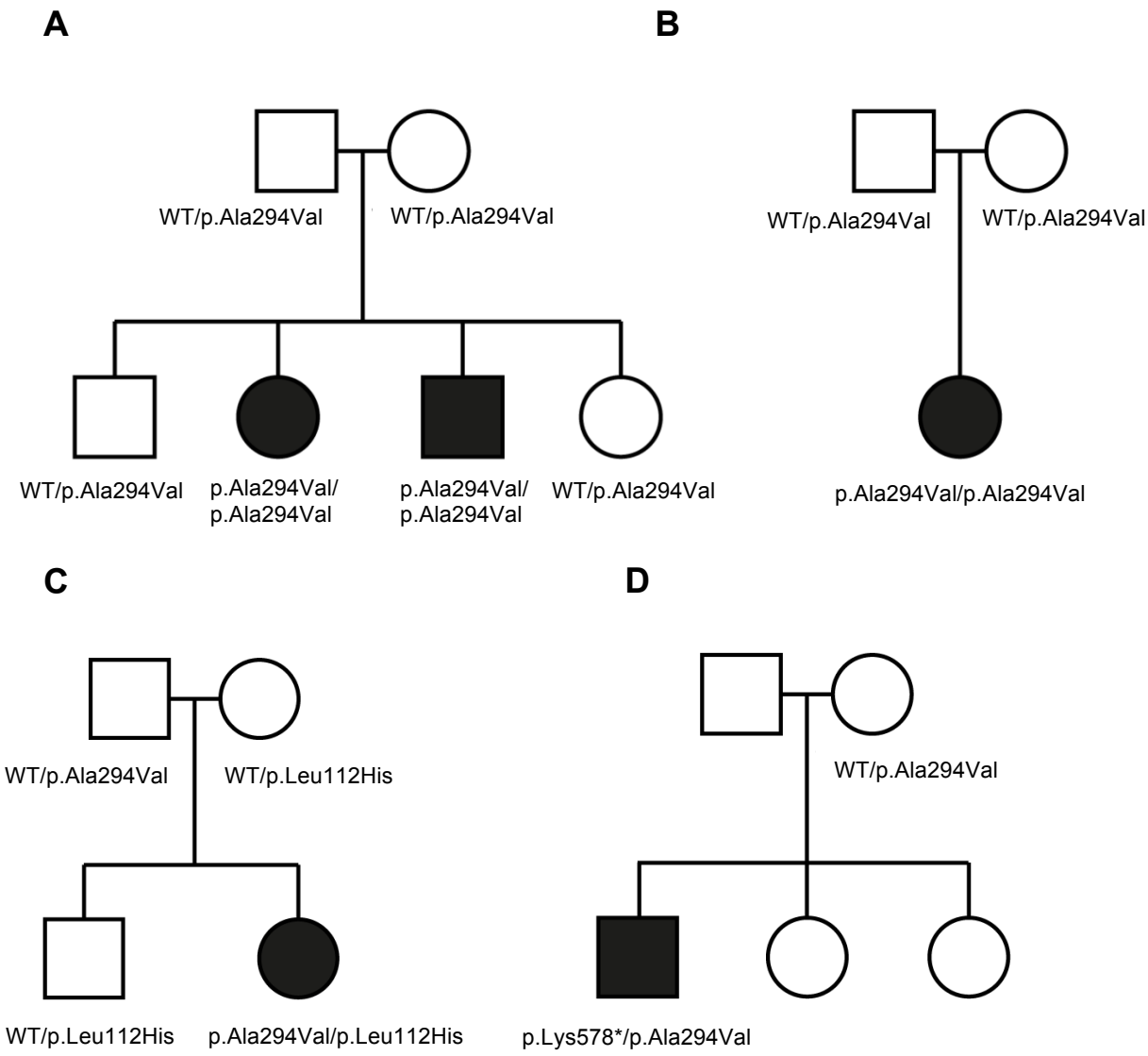


Figure S1

Co-segregation of the disease-causing mutations in pedigrees

(A) Discovery pedigree from the UK with two affected and two unaffected siblings. **(B)** Danish family with an affected daughter. **(C)** Spanish family showing compound heterozygosity for the mutations p.Leu112His and p.Ala294Val in the affected daughter. **(D)** Swiss family showing compound heterozygosity for the mutations p.Ala294Val and p.Lys578* in the affected son. DNA was not available from the healthy father and siblings. WT, wild type

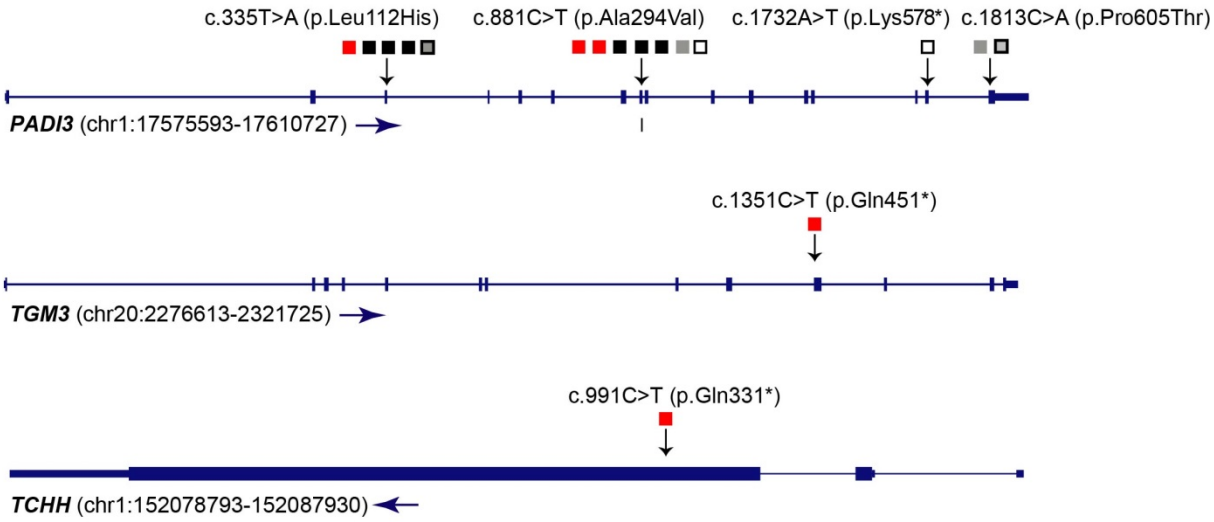
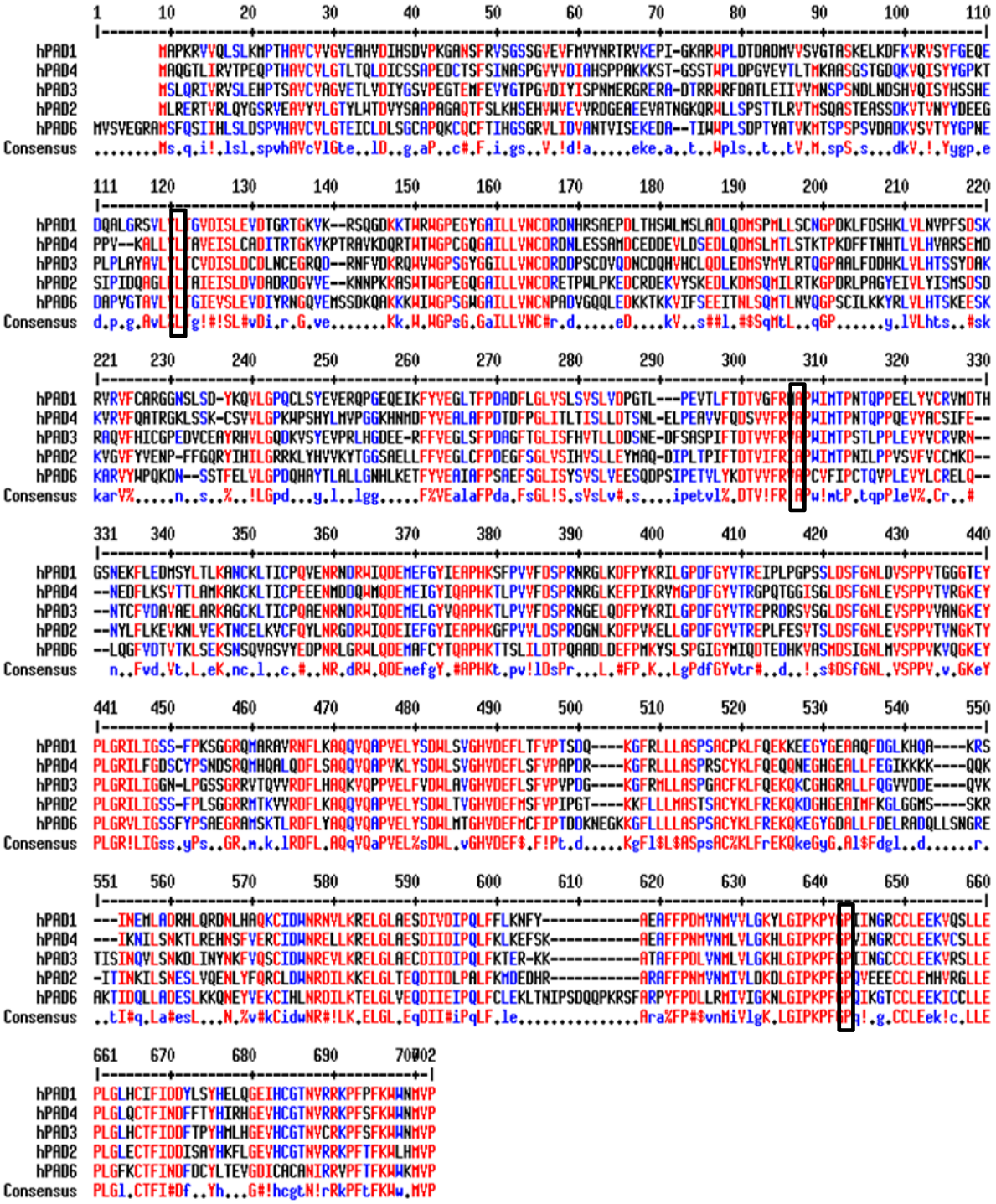


Figure S2

Cartoons depicting the positions of the mutations in *PADI3*, *TGM3* and *TCHH*

The start/end positions of the genes are based on hg19. Direction of transcription is depicted with the arrow heads. Squares indicate individuals carrying the respective mutation. Red squares denote homozygotes. Black squares denote individuals carrying p.Leu112His and p.Ala294Val, grey square denotes the individual carrying p.Ala294Val and p.Pro605Thr, grey square with a black frame denotes the individual carrying p.Leu112His and p.Pro605Thr, and white square denotes the individual carrying p.Ala294Val and p.Lys578*.

A



B

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CLUSTAL O(1.2.1) multiple sequence alignment

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SP|Q9Z184|PADI3_MOUSE  MSLQRIVRVSLEHPTSAVCVAGVETIVDIYGAVPEGTDMFEVYGTPGVDIYVSPSMERSR 60
SP|O02849|PADI3_SHEEP  MSLQRIVRVSLEHPTSAVCVAGVETLVDVYGSVPEGTEMFEVYGTPGVDVYICPSVERDR 60
TR|G3VER6|G3VER6_SARHA MSLQRIVRVSLEHPTSAVCVAGVETLVDIYGSVPEGTETFEVYGTPGVDIYISPNMKASR 60
TR|E2R691|E2R691_CANFA MSPQRIVRVSLEHPTSAVCVAGVETLVDIYGSVPEGTEMFEVYGTPGVDVYISPSMERGR 60
TR|F1NP39|F1NP39_CHICK MAQQRRVQLCTQRPTSTVCVLGTELSLDIQGSAPKDAIAFHVQGTPGVKVYVVHETQSVK 60
                       *: ** *::. ::***:*** *.*  :*: *:.*: :  *.* *****.:*:  . :  :

SP|Q9ULW8|PADI3_HUMAN  ERADTRRWRFDATLEIIVVMNSPSNDLNDSHVQISYHSSHEPLPLAYAVLYLTCV---DI 117
SP|Q9Z184|PADI3_MOUSE  ERADTRRWCFNKGLEIIVIMNSPSNDLNDSHVQIAYHSSREHLPLAYAVLYLTCV---DI 117
SP|O02849|PADI3_SHEEP  ERAVARRWHFDMGSQIVVVMNSPSNVNDTVTFKFPTIPAETPRPLAYAVLYLTCV---DI 117
TR|G3VER6|G3VER6_SARHA ERADTRRWHFNRGLEIIVVMNSPSNDVNDSHVQISYHSRKEPLALAYAVLYLTCVEWTDI 120
TR|E2R691|E2R691_CANFA ERADTRRWHFDNGLEIIVVMNSPSNDLNDSHVQISYHSSHEPLPLAYAVLYLTCV---DI 117
TR|F1NP39|F1NP39_CHICK LPSSVTRWPLATGTEVLLTMEAISKDVGDEKVRISYFGEASGVPVGRAMLYLTCV---DI 117
                         : . ** :    :::: *:: *:      .::          :. *:******   **

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SP|O02849|PADI3_SHEEP  TLDCDLNCEGRQDRDFVDKLQWVWGPSGHGAVLLVNCDRDSMSSN-----------DQEH 166
TR|G3VER6|G3VER6_SARHA SLDTDMNCNGKVERNYVDKRQWVWGPGGHGAVLLVNCDRDDLTCD-----------KRDN 169
TR|E2R691|E2R691_CANFA ALDCDLNCEGRQDRSYVDKRQWVWGPGGYGAVLLVNCDRDDVTCY-----------DQDN 166
TR|F1NP39|F1NP39_CHICK SLDADTGRNGSVSKTLVDKATWTWGPDGHGAILLVNCDCDDPKSRLMGDSSEPQSQLTDN 177
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SP|O02849|PADI3_SHEEP  CDHHVRCLQDLEDMSVMILRTQGPDALFDDHKLVLHTSRADAERARVFHACGPEDSCEAY 226
TR|G3VER6|G3VER6_SARHA CDKCVRCLEDLQDMSLMILRTQGPTALFDDHKLVLHVSSFDAERARVFHACGPEDVCESY 229
TR|E2R691|E2R691_CANFA CDRHVNCLQDLEDMSVMVLRTQGPAALFDDHKLILHTSSYDAKWARVFHACGPEDSCEAY 226
TR|F1NP39|F1NP39_CHICK RDTVVCSYEDLKDMSQMVLRTRGPRTIFTGHGLVLHVNFSDADKVGVFYG-GNSIALEEY 236
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SP|O02849|PADI3_SHEEP  RHVLGQNKVSYEVP-RFHGEEERFFVEGLSFPDAGFSGLVTFHVTLLDDSNEDFSESPIF 285
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TR|E2R691|E2R691_CANFA RHVLGQNKVSYEVP-RFHGDEERFFVEGLSFPDARFTGLVSFHVTLLDDSNEDFSESPIF 285
TR|F1NP39|F1NP39_CHICK KHVLGGEKLAYAVKPSQHQEESIFYVEGLAFPDVSFSGLVAFHVTLLESPEKGLLETPIF 296
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SP|Q9Z184|PADI3_MOUSE  TDTAVFRVAPWIMTPSTLPPLEVYVCRVRNNTCFVEAVEELARKAGCKLTICPQAENRND 345
SP|O02849|PADI3_SHEEP  TDTVVFRVAPWIMTPSTQPPLEVYVCRVRNNTCFVDAVAELARRAGCKLTVCPQAENRND 345
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TR|E2R691|E2R691_CANFA TDTVVFRVAPWIMTPSTLPPLEVYVCRVRNNTCFVDAVAELARKAGCKLTVCPQKENRND 345
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SP|Q9Z184|PADI3_MOUSE  RWIQDEMELGYVQAPHKTLPVVFDSPRNGELQGFPYKRILGLDFGYVTREPKDSSVSGLD 405
SP|O02849|PADI3_SHEEP  RWIQDEMELGYIQAPHKTFPVVFDSPRNGELQNFPYKRILGPDFGYATREPRDNSVSGLD 405
TR|G3VER6|G3VER6_SARHA RWIQDEMELGYIQAPHKTFPVVFDSPRNGELQDFPYKRILGPDFGYVTREPRNKSVSGLD 408
TR|E2R691|E2R691_CANFA RWIQDEMELGYVQAPHKTFPVVFDSPRNGELQDFPYKRILGPDFGYVTREPQDSSVSGLD 405
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TR|F1NP39|F1NP39_CHICK SFGNLEVSPPVTVRSKEYPLGRILIGSSFPRFGGRRMAKAVRNFLAAQRVQAPVELFSDW 475
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TR|F1NP39|F1NP39_CHICK LQVGHVDEFLSFIPAPDRKGFRLLLASPSACYQLLREKQEMGYGEATMFQGLHGV---PK 532
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SP|O02849|PADI3_SHEEP  VSISQVLSNGSLIGYNKFVQSCIDWNREVLKRELGLAERDIVDIPQLFKMER-RKAVAFF 584
TR|G3VER6|G3VER6_SARHA MSINQILSNENLISYNKFVQSCIDWNREVLKRELGLTDRDIIDIPQLFKRER-RKAVAFF 587
TR|E2R691|E2R691_CANFA VSINQVLSNVDLISYNKFVQSCIDWNREVLKRELGLTERDIVDIPQLFKTER-KKAVAFF 584
TR|F1NP39|F1NP39_CHICK PSISEILGNEALRKFNAYAQSCISWNRDILKRELGLAEQDIIDIPQLFQADHQARAVAYF 592
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SP|O02849|PADI3_SHEEP  PDLVNMLVLGKHLGIPKPFGPVINGRCCLEEKVRSLLEPLGLRCTFIDDFTPYHMLHGEV 644
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TR|E2R691|E2R691_CANFA PDLVNMLVLGKHLGIPKPFGPIINGQCCLEEKVRSLLEPLGLHCTFIDDFTPYHMLHGEV 644
TR|F1NP39|F1NP39_CHICK PDMVNMLVLGRHLGIPKPFGPLVDGQCCLEERVRALLQPLGLSCTFINDYFSYHKLAGEV 652
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SP|Q9Z184|PADI3_MOUSE  HCGTNVRREPFAFKWWHMVP 664
SP|O02849|PADI3_SHEEP  HCGTNVRRQPFSFKWWCMEP 664
TR|G3VER6|G3VER6_SARHA HCGTNVRRKPFPFKWWNMIP 667
TR|E2R691|E2R691_CANFA HCGTNVRRQPFSFKWWNMVP 664
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Figure S3

Sequence alignments for PADI3

When we assessed evolutionary conservation of the three substituted amino acids, we found that all of them were located **(A)** at well-conserved positions across various human PADIs and **(B)** across PADI3 from distinct species, thus suggesting that these mutations may alter the protein function. **(A)** The sequences of human PADI3 and paralogous genes (GenBank accession numbers AB033768, AB03176, AB026831, AB017919 and AY422079) were aligned using MultAlin.[4] Amino acids conserved at 90% and 50% are indicated in red and blue, respectively. The three mutated amino acids of PADI3 are surrounded. **(B)** Primary sequences of human PADI3 and Padi3 from other species (chicken, mouse, sheep, dog (CANFA) and Tasmanian devil (SAHRA) were aligned using Clustal Omega (European Molecular Biology Laboratory-European Bioinformatics Institute). The three mutated amino acids of PADI3 are depicted in red.

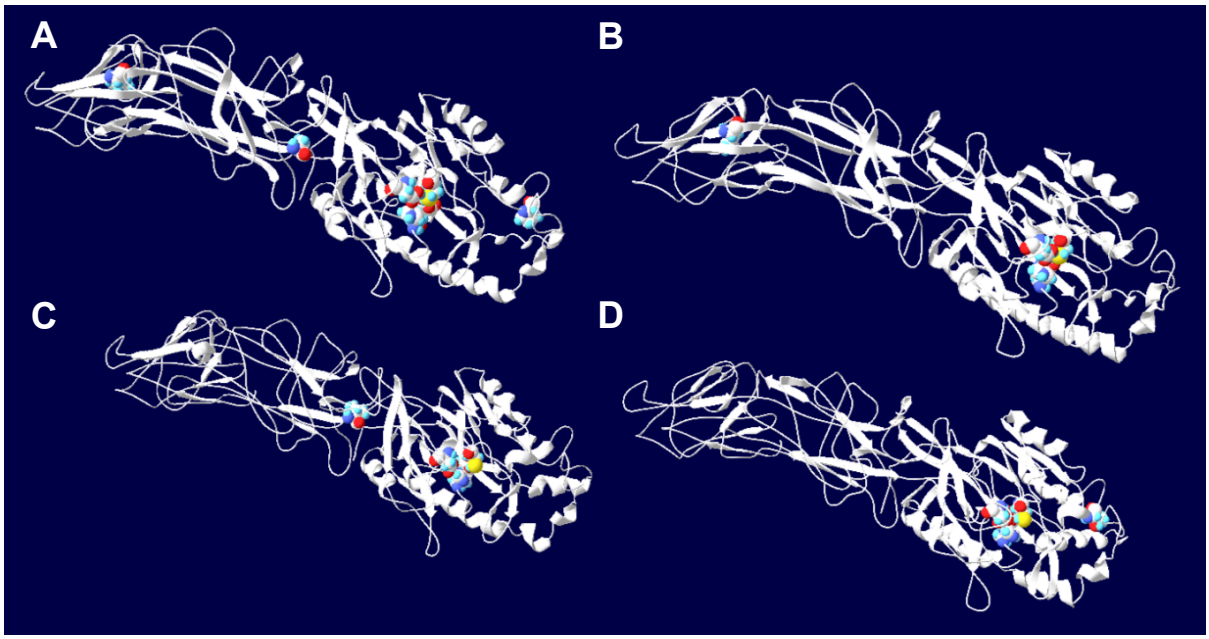


Figure S4

Topological tridimensional models of WT and mutant PADI3: Localization of the 4 major amino acids involved in the catalytic sites and of the 3 amino acids involved in the UHS missense mutations

Overall view of the tridimensional solid ribbon representation of calcium-bound PADI3 models, including WT and three missense mutants. Residues Leu112, Ala294 and Pro605 are reported on the model of the **(A)** WT enzyme, as well as the corresponding substituted amino acids on the structure of the three mutants **(B)** p.Leu112His, **(C)** p.Ala294Val and **(D)** p.Pro605Thr. **(A-D)** The four major amino acids involved in the catalytic site are also shown. According to these models, the **(C)** p.Ala294Val and **(D)** p.Pro605Thr substitutions induce a profound disorganization of the immunoglobulin-likes domains, with clear disappearance of several beta-sheets, and disruption of some alpha-helices in the catalytic domain, as compared to the WT. Effects of the **(B)** p.Leu112His substitution are more discrete.

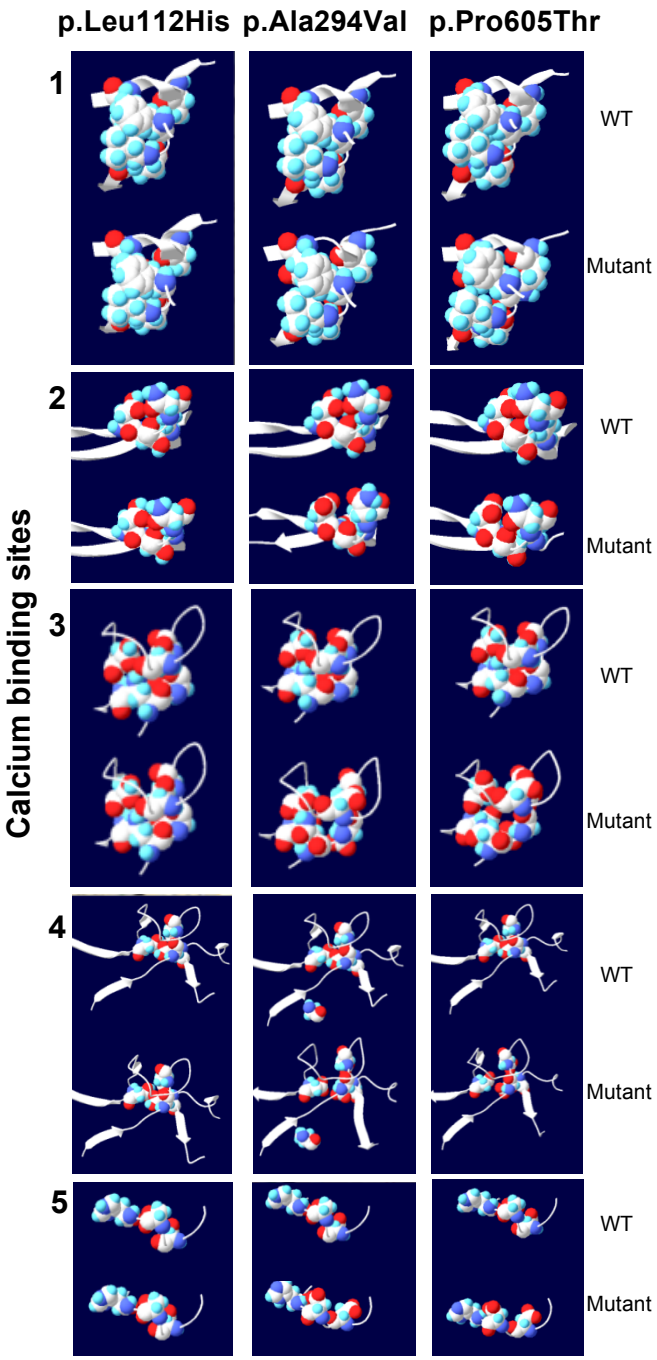


Figure S5

Topological tridimensional models of WT and mutant PADI3: Predicted calcium binding sites

Zoom on the amino acids involved in the five calcium-binding sites (1-5). As previously published,[5] these amino acids (Table S2) were defined by analogy, after a multiple alignment, to the residues of the five calcium binding sites of PADI4 [MIM 605347].[6] None of the substituted amino acids are directly involved in calcium binding. Nevertheless, the predicted models of p.Ala294Val and p.Pro605Thr mutants show clear spatial modifications of, at least, the calcium-binding sites 2-5.

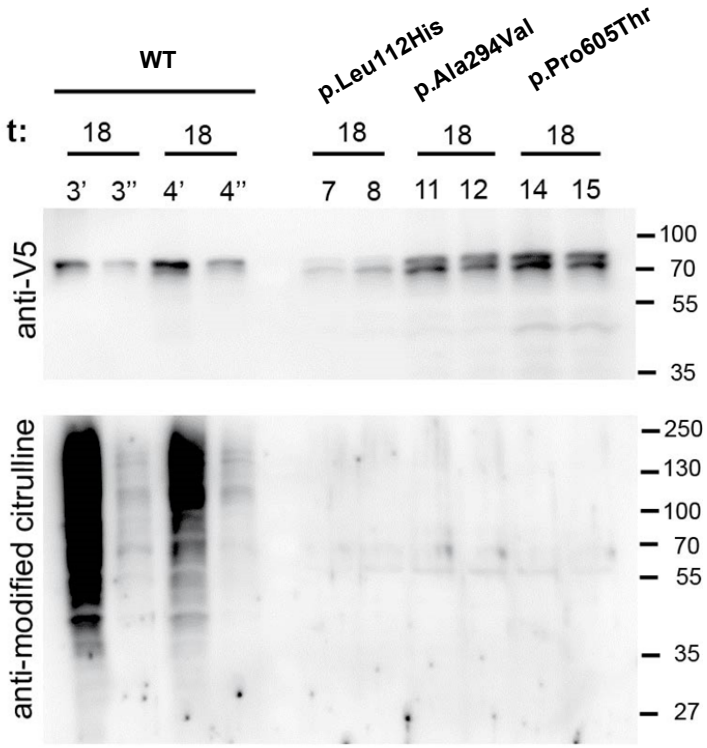


Figure S6

Absence of activity of mutant PADI3 produced in bacteria

Extracts of *E. coli* producing WT PADI3 were diluted either four (3' and 4') or eight times (3" and 4") in order to adjust the amounts of PADI3, as compared to undiluted extracts containing the mutant enzymes, p.Leu112His (clones 7 and 8), p.Ala294Val (clones 11 and 12) and p.Pro605Thr (clones 14 and 15). The extracts were then incubated for 18 hours with calcium, as indicated. After incubations, proteins were immunodetected with either the anti-V5 antibody or anti-modified citrulline antibodies. While citrullinated proteins were detected in the WT-containing extracts, no citrullinated proteins were detected in the mutant-containing extracts. Molecular mass markers are indicated on the right in kDa.

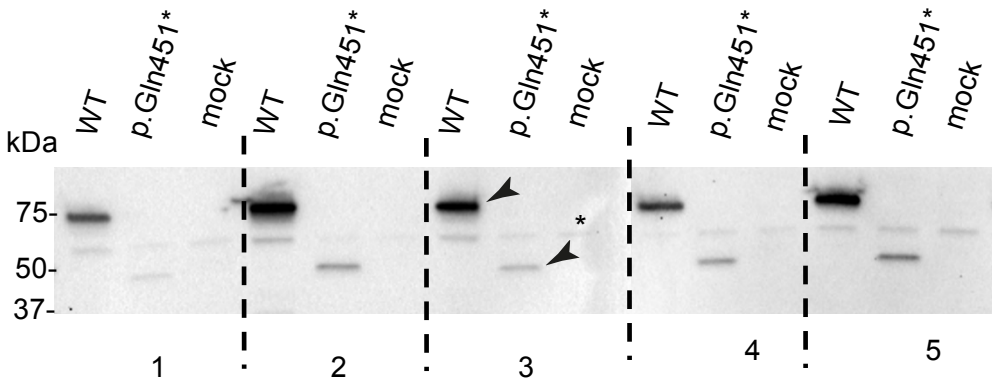
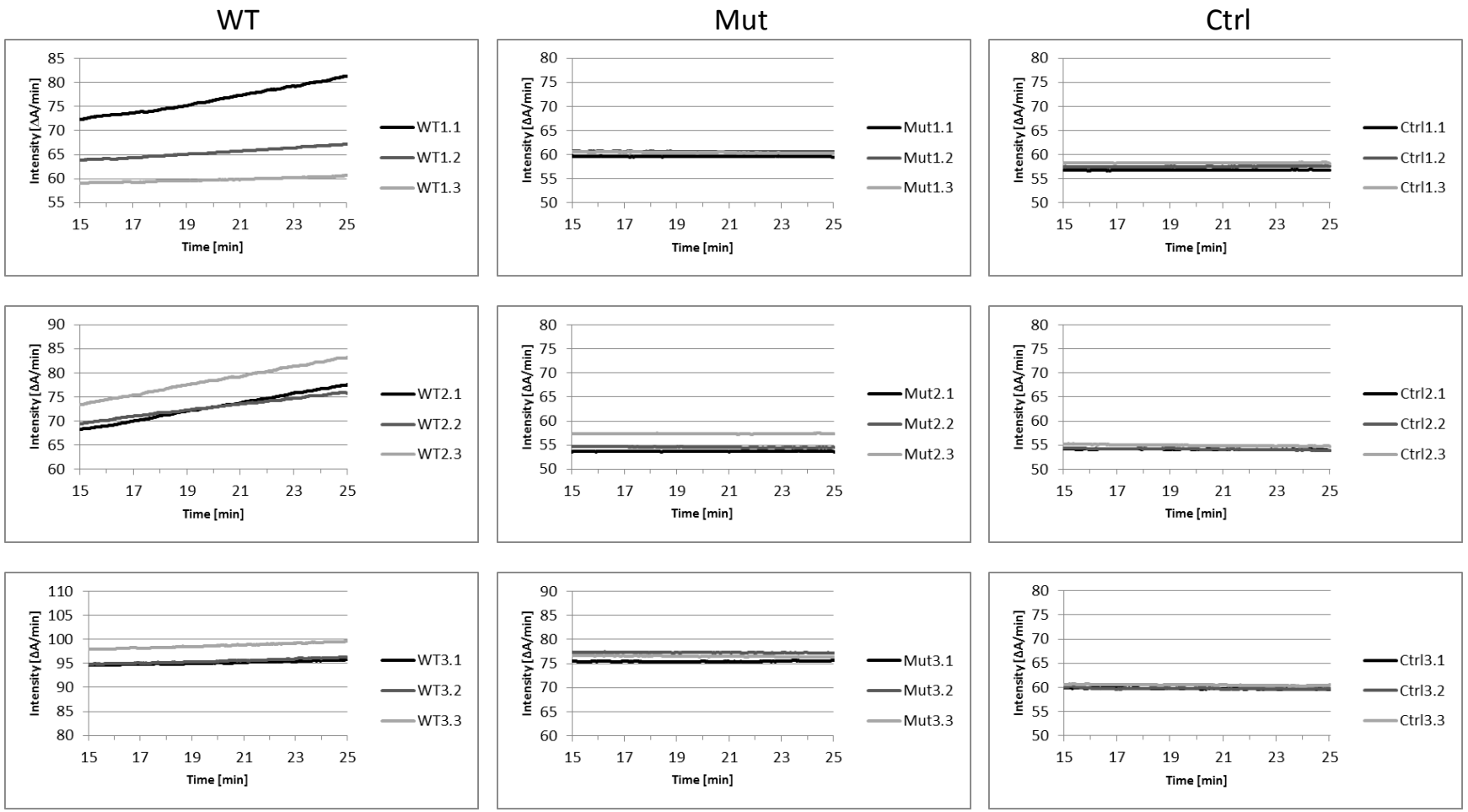


Figure S7

Immunoblotting of HEK293T extracts producing WT and mutant TGM3

Immunoblotting analysis shows the detection of WT and truncated TGM3 in transiently transfected HEK293T cells, which were collected 48 h post-transfection. Cell extracts from independent transfections (1-5), were concurrently immunoblotted (1-3, 4-5) with an anti-Flag antibody. Antibody-specific bands showing the WT (~70 kDa) and truncated TGM3 (~40 kDa) are indicated with an arrow and a non-specific cross-reactive band around 55 kDa is indicated with an asterisk. A persistent lower detection level was observed for the mutated protein in comparison to the WT TGM3. Relative protein quantification was performed using Stain-Free technology that is based on normalization by the total lane protein content (Bio-Rad Laboratories).


WT
Mut
Ctrl
Intensity [ΔA/min]
Time [min]
WT1.1
WT1.2
WT1.3
Mut1.1
Mut1.2
Mut1.3
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Ctrl1.2
Ctrl1.3
WT2.1
WT2.2
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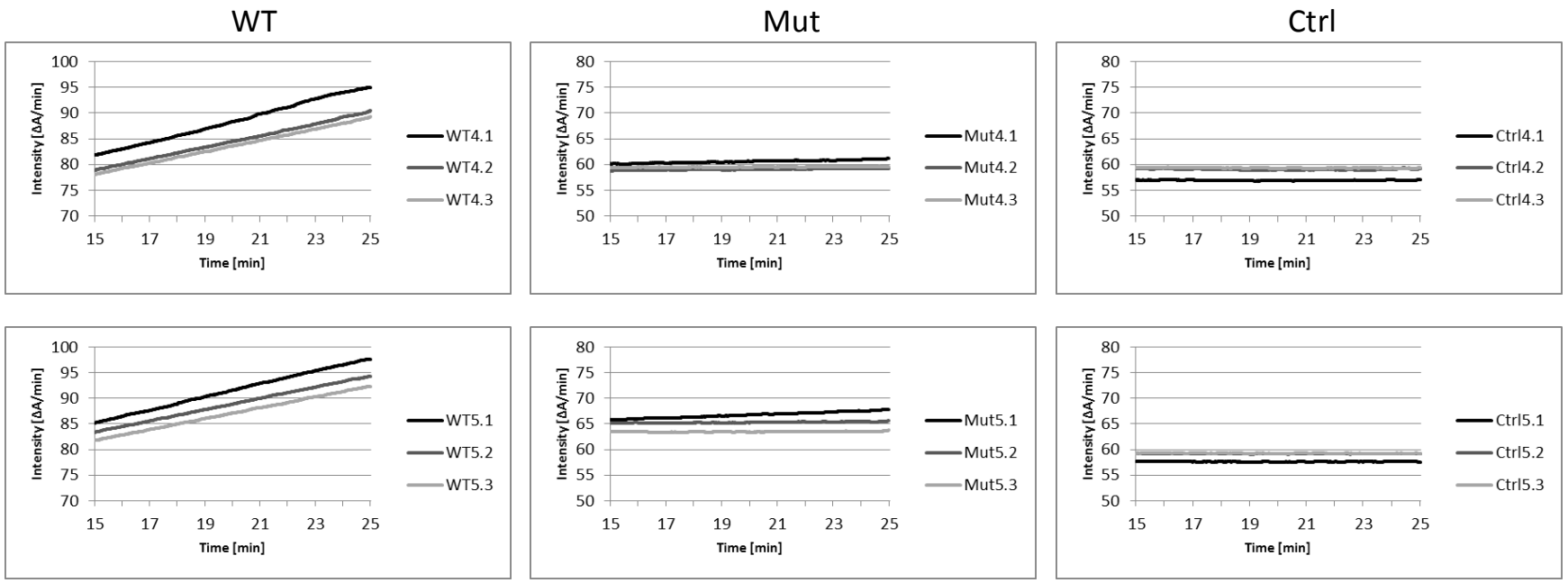

Figure S8

Transglutaminase activity of TGM3 produced in HEK293T cells

Fluorescence intensity augmentation by incorporation of monodansylcadaverine into casein. Measurements from five independent transfections are presented in rows. Three technical replicates for each sample are depicted with color-coded lines. Linear slopes of the measurements represent the transglutaminase activity.

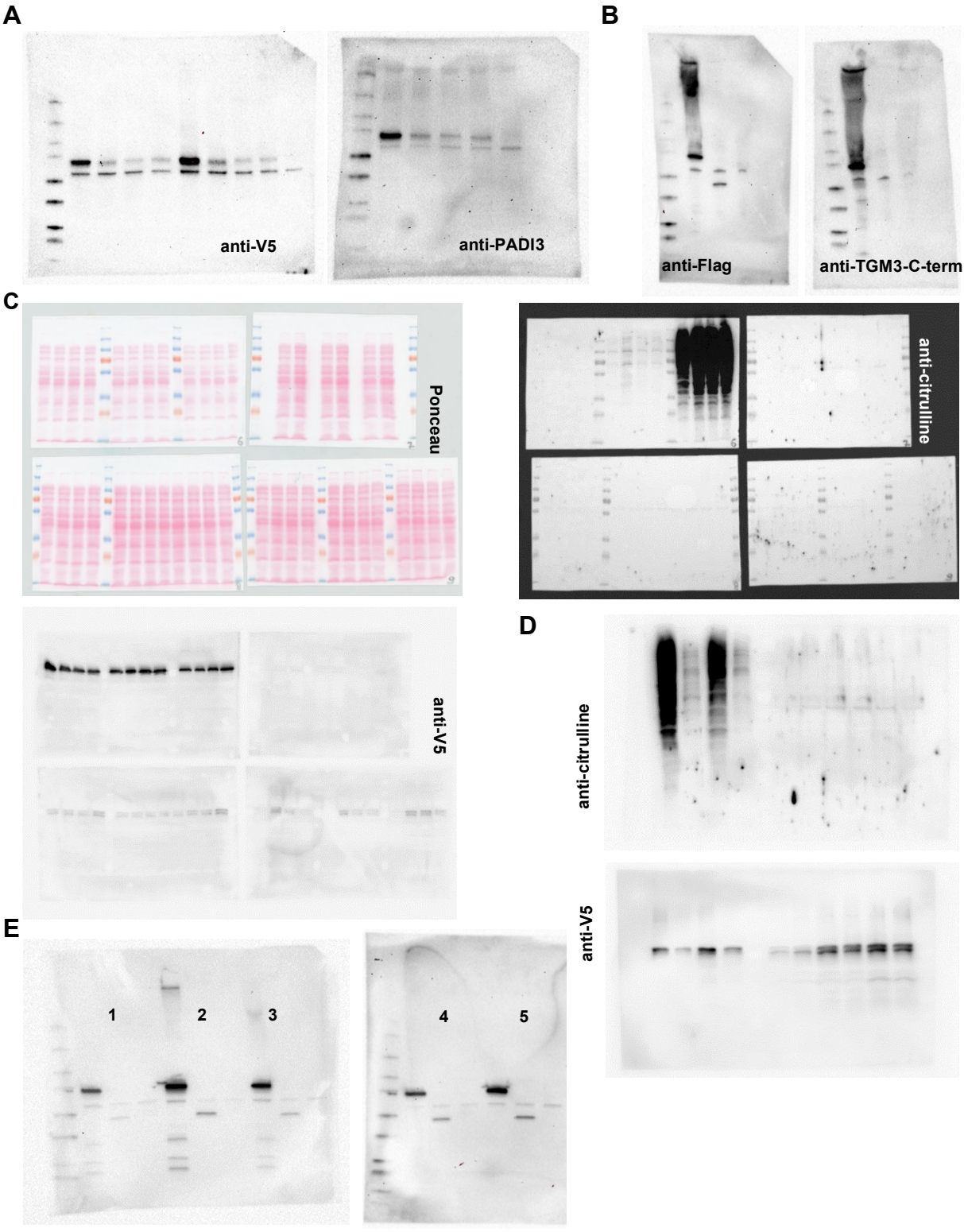

Figure S9

Full-length gel and blot images from the main figures.

Full-length images of the blots/gels in **(A)** Figure 4A**, (B)** Figure 7A, **(C)** Figure 5B**, (D)** Figure S6 and **(E)** Figure S7

Supplemental Tables

Table S1. Primers used for sequencing of *PADI3*

Amplicon	**Forward (5′-3′)**	**Reverse (5′-3′)**
PADI3_Ex1	CTGAGCTCTCAGCTCTGGGA	TCTTCTACCTGGCTCAGCC
PADI3_Ex 2	GCTTGACTCGCAGGAGCTTA	TCTGTAACTTGCAGAGCTGG
PADI3_Ex 3	GGCACAGAGGAGGTTAAAGAA	TCCATTTGTGGAGGCTTAGC
PADI3_Ex 4	CCTCTTGGATGGTGTCTCCTT	AGTCCAGAAGGTCTTGATCCC
PADI3_Ex 5	GCCATTGACATGTCTTGAGAA	AGCATCGAGGTGTGTCTGG
PADI3_Ex 6	CAGCTGGCTATGCCACACT	TTCTGACTGTTCTTACTGCG
PADI3_Ex 7	CCACTGTGTATTACCTGTCCT	TGATCATGGCTCACTGCAAC *TAGGTGTACACCACTATACC**
PADI3_Ex 8/9	GGTTCATTTCCATCTTGCAGA	TGTTGAATCCAGGATCAGC
PADI3_Ex 10	CTGACCTGGGCACATTTATG	TTTAGGGCTGCCAGATTCAG
PADI3_Ex 11	CTCAGGCTCCATGTCCGAT	AATGATCTCTTAGGTCTCTGC
PADI3_Ex 12/13	AGATTTTCCTGGATGGTGGG *GCAGGAATGCTAAACTCTTGT**	AGTCCATGTCCACCTTCTATC
PADI3_Ex 14/15	GGCTGCTGACTCGGCAAGA	TCCCAGCTGATGCCATGTGC
PADI3_Ex 16	CCAGAGTGAGTTCTGCGGAT	AAGTCTGAGAACCACATGGG

Primer pairs are used both for amplicon generation and Sanger sequencing reactions. *Additional primers were used for the cycle sequencing reaction in order to cover the whole region.

Table S2. Primers used for sequencing of *TGM3*

Amplicon	Forward (5'-3')	Reverse (5'-3')
TGM3_Ex 1	AGGCAATCCTTGGCAGCCTG	GATGTCCAGCTGCACTGAACA *AAGGCAGGCAGCTGTCCTGG**
TGM3_Ex 2	AGGATGCACAGAGGTTCAGC	AGAGATGGACAGCAACTTGC
TGM3_Ex 3	GTTGTATTGGAACCTGGTCT	TGCTTAAGTGTCAGAGCTCC *TAGGCCAGGGCTGAGAGTGTG**
TGM3_Ex 4	AAGCAGCTGTCTGAGTGTGG	ACACTAAGGAAGTGTCATCGC
TGM3_Ex 5	TCAGTAGCTCTCAGTTCCAG	TACTCACTGTGTGCCTCAGG
TGM3_Ex 6/7	ACTGTGACAGCAGTGATAGCC	AGACTAGCAGACCGCAGAGC *GTGAGAGCGAGAAGCCACTCA**
TGM3_Ex 8	ACTCACTCGATGCATGTTGTC	AGGCTCTGTGCAGCAACAGTG
TGM3_Ex 9	TTGCAGTGGTCCTGGAAGGC	AGGCAGAACTGGCTGCCAGTG
TGM3_Ex 10	TCCGGTTAAGACAGGCGAGC	TGTGCCATAGCTATGAACTGC
TGM3_Ex 11	TGGCCCAAGGAGGGCTCAGTC	TGGGAGAGCTGTGGCTCACAG
TGM3_Ex 12	AGCACAGGATAATGTCCTGG	AGATTCTAGAGTTCCAAGACC
TGM3_Ex 13	AACAGGACAGGAGGTCACAG	TCCATGGTGAGCTCTCCCTG

Primer pairs are used both for amplicon generation and Sanger sequencing reactions. *Additional primers were used for the cycle sequencing reaction in order to cover the whole region.

Table S3. Primers used for sequencing of *TCHH*

Primer Name	Sequence (5'-3')
TCHH_EX 2F	**GGTGGAGAGCTGGAAGAAAGACA**
TCHH_EX 2R	**TGGGGGATGTAGTGTAGACCTGTT**
TCHH_EX 3F	**TGAGCTCTTCATGGGACATTACCACA**
TCHH_EX 3R	**TGCACTTTCCACAAGATGGGTCA**
TCHH_SF1	*GCTCTGAATGTCTCTTGAATGTCA*
TCHH_SF2	*CAAAGGCAAGAATGGCAAGAA*
TCHH_SF5	*CTAGCTGAGGAGGAGCAGGAACA*
TCHH_SF6	*GTGGCAACTAGAAGAAGAAAGGA*
TCHH_SF8	*CAAAGGCAGAGAGAATGAACAGTT*
TCHH_SF16	*CAGCAGCGGGAACAACGGTTTCT*
TCHH_SF20	*CGAACAGGAACTGCGCAGTCAGGA*
TCHH_SF23	*GAGCAGCTGCTGAGAGAGGAACA*
TCHH_SEQ1F	*TGGAGCGGCAAGAGCTGAG*
TCHH_SEQ3F	*TCGGAAGGATAAGAAGCTG*
TCHH_SEQ5F	*AGAGTCGTCGTGAGGAACAAG*
TCHH_ EX3.2F	*CGCAGGCAGAAGAGGCAGGAA*
TCHH_ EX3.3F	*GCGGTTGAGGAGCGAGCAAC*
TCHH_ EX3.4F	*CCAGCAGCGGGAACAACGGT*
TCHH_ EX3.5F	*GCGGGAGAGGCAGTATCGGG*
TCHH_ EX3.6F	*CAGCGCGACAGGCATTTCC*
TCHH_ EX3.7F	*CAACAGCTGCGTCACGACCG*
TCHH_ EX3.1R	*CTGTCTTGCCGCTCTCGCCT*
TCHH_ EX3.3R	*CTTGGCGTACAGCGTGTGGC*
TCHH_ EX3.4R	*TGTCGCGCAGCTGGGAATCT*
TCHH_SR2	*TCCTTTCTTCTTCTAGTTGCCAC*
TCHH_SR3	*CAGCTTCTTATCCTTCCGA*
TCHH_SR4	*AACTGTTCATTCTCTCTGCCTTTG*
TCHH_SR5	*CTTGTTCCTCACGACGACTCT*
TCHH_SR9	*GACGGAGCTGCTCTTCCTCTAGGAT*
TCHH_SR10	*CCAGCGATACTTTCCGTCACGCTGTT*
TCHH_SR11	*GAGGAAGAACAGCTGGAGCGAGA*

Primer pairs used for amplicon generation are given in bold and primers used for cycle sequencing reactions are given in italic. Presence of *TCHH* mutations in the coding sequence in screened individuals could not be entirely excluded as overlapping and/or individual regions could not be sequenced in different individuals due to technical limitations arising from the repetitive regions.

Table S4. Primers used for cloning and mutagenesis

Construct	Primers
PADI3 WT	PADI3-WT-F: 5' accATGTCGCTGCAGAGAATCGTG 3' PADI3-WT-R: 5' GGGCACCATGTTCCACCAC 3'
PADI3 p.Leu112His	PADI3-Mut-p.L112H-F: 5' CCTATGCGGTGCTCTACC**A**CACCTGTGTTGACATCTC 3' PADI3-Mut-p.L112H-R: 5' GAGATGTCAACACAGGTG**T**GGTAGAGCACCGCATAGG 3'
PADI3 p.Ala294Val	PADI3-Mut-p.A294V-F: 5' GTGGTGTTCCGAGTGG**T**ACCCTGGATCATGACG 3' PADI3-Mut-p.A294V-R: 5' CGTCATGATCCAGGGT**A**CCACTCGGAACACCAC 3'
PADI3 p.Pro605Thr	PADI3-Mut-p.P605T-F: 5' CCCCAAGCCCTTTGGG**A**CCATCATCAATGGCTG 3' PADI3-Mut-p.P605T-R: 5' CAGCCATTGATGATGG**T**CCCAAAGGGCTTGGGG 3'
TGM3 WT	TGM3-WT-F: 5' accatg*gattacaaggatgacgacgataag*ccaggaccaATGGCTGCTCTAGGAGTCC 3'' TGM3-WT-R: 5' TCATTCGGCTACATCGATG 3'
TGM3 p.Gln451*	TGM3-Mut-p.Q451*-F: 5' GCTCTGACCAGGAAAGA**T**AAGTGTTCCAAAAGGCT 3' TGM3-Mut-p.Q451*-R: 5' AGCCTTTTGGAACACTT**A**TCTTTCCTGGTCAGAGC 3'

The N-terminal flag tag sequence fused to *TGM3* is given in italic. The locations of the mutations are given in bold in the respective mutagenesis primers. WT; wild type; Mut, mutant; F; forward; R; reverse

Table S5. *PADI3*, *TGM3* and *TCHH* mutations in ExAC database

Variant[a]	Gene	Consequence	Allele count	Total allele	Homozygous individuals	Allele frequency
1:17588689 T / A (rs142129409)	*PADI3*	p.Leu112His	459	111360	0	0.004122
1:17597423 C / T (rs144080386)	*PADI3*	p.Ala294Val	809	121318	5	0.006668
1:17609392 C / A (rs144944758)	*PADI3*	p.Pro605Thr	51	113490	0	0.0004494
20:2312665 C / T (rs779702016)	*TGM3**	p.Gln451*	1	114212	0	0.000008756
1:152084702 G / A (rs201930497)	*TCHH**	p.Gln331*	43	112892	0	0.0003809

[a]Variants are annotated by genomic location based on hg19, nucleotide substitution and their dbSNP IDs.* None of the sequenced individuals in ExAC database carry a loss of function mutation in homozygous state in these genes.

Table S6. Amino acid residues and positions[#] involved in the 5 calcium binding sites and the catalytic site of PADI3

Calcium binding sites	Residue-position
1	Gln-349 / Glu-353 / Phe-407 / Leu-410 / Glu-411
2	Glu-351 / Asp-369 / Ser-370 / Asn-373
3	Asn-153 / Asp-155 / Asp-157 / Asp-165 / Asp-176 / Asp-179
4	Asp-155 / Asp-157 / Asp-179 / Asp-388
5	Asp-165 / Asp-168 / His-170
Catalytic site*	Asp-350 / His-470 / Asp-472 / Cys-646

[#]Positions of calcium-coordinating and catalytic site residues are reported according to the PADI3 primary sequence (GenBank accession number AB026831) after a multiple alignment, as previously described.[7] They have been defined by analogy to the analysis of PADI4 crystal structure analysis.[6] *The 4 major amino acids of the catalytic site are mentioned.

Supplemental References

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