

Fig. S1. Pedigrees of the 14 families with punctate palmoplantar keratoderma (PPKP1). Unaffected family members are indicated by clear symbols and affected members by black symbols. Index patients are marked with an arrow. Asterisks denote individuals genotyped for variants in AAGAB. To maintain confidentiality, the genotypes of unaffected individuals are not shown. Diagonal bars through the symbols denote deceased individuals. Question marks in the symbols denote missing information about the phenotype.

Appendix S1

SUPPLEMENTARY RESULTS

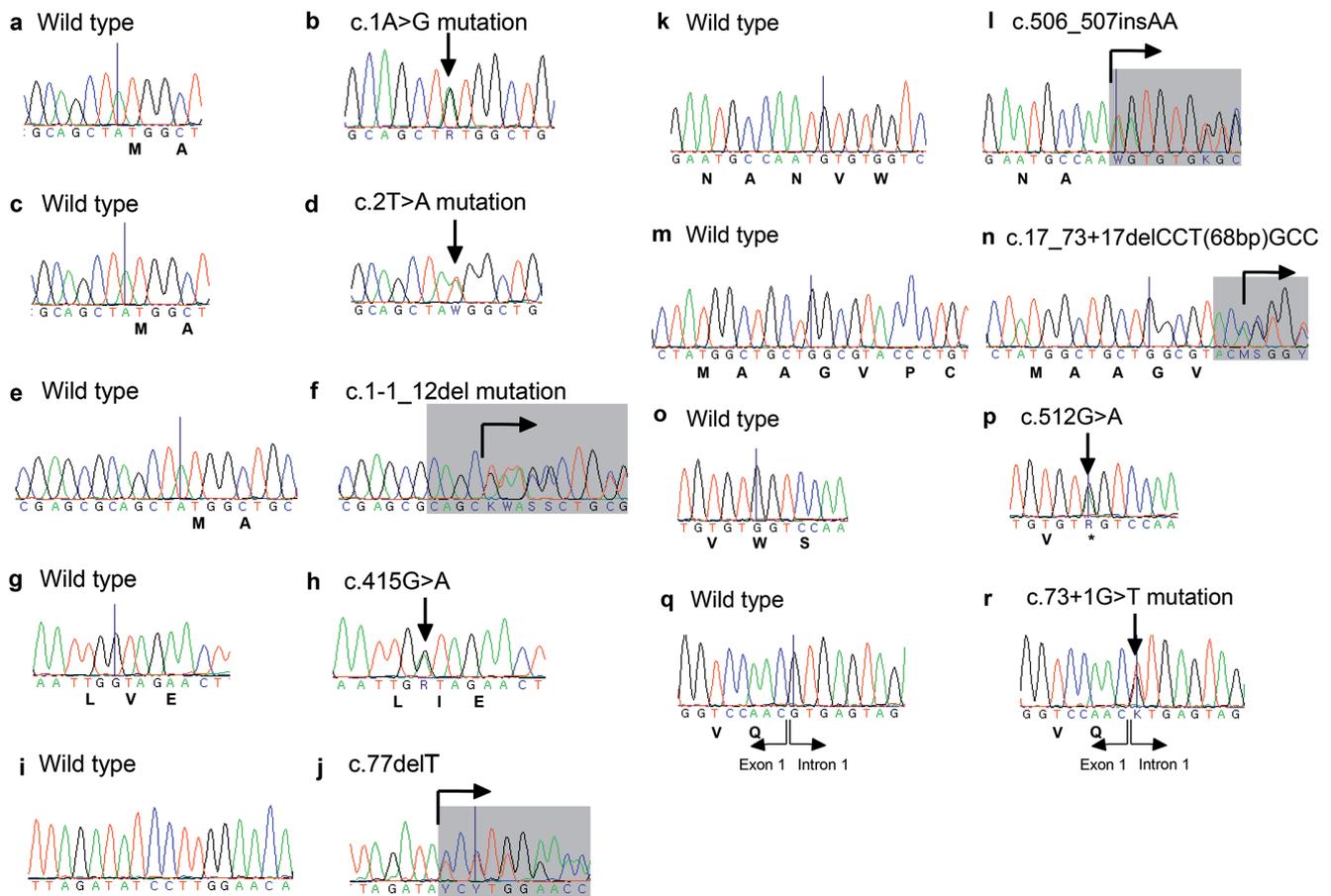
Mutations affecting the start codon

Mutation analysis revealed a c.1A>G substitution in exon 1 that altered the translation initiation site of *AAGAB*. SIFT predicts this variant as deleterious; PolyPhen-2 predicts this variant as probably damaging. Here, the initiating methionine is lost, probably leading to a protein isoform initiated from a start codon downstream of the mutated initiation codon. This mutation (SFig. 1a, b) was seen in a 51-year-old man (66651, family 10) and in an unrelated 44-year-old woman (68077, family 4), both of German origin and with a mild to moderate phenotype. In the affected individual 66651, the age of onset was 40 years, no pain was reported, and there was no known family history of palmoplantar keratoderma. It was not possible to examine other family members for the PPKP1 phenotype.

In individual 68077, the age of onset was during puberty, and the pain due to the skin lesions was subjectively rated as 5 out of 10. The father (71005) showed the same substitution, but had such a mild phenotype that it was only identified during this study. The sister and mother were clinically unaffected, as confirmed by sequencing analysis.

A c.2T>A substitution in exon 1 which also probably leads to a protein isoform with a different start codon was seen in family 3 (66350, SFig. 1c, d). PolyPhen-2 classifies this variant as probably damaging, while SIFT predicts this variant as deleterious. The affected individual was a 39-year-old woman (66350) of German origin who presented with a mild form of typical PPKP1. She first recognized skin lesions on her hands and feet at age 20 years, but was not experiencing any pain. Her 67-year-old mother (66351) showed similar, but even milder, clinical lesions, which remained unnoticed until her daughter pointed them out.

An additional 13-bp deletion, c.1-1_12del, which includes the start codon of *AAGAB*, was identified (SFig. 1e, f). Due to the loss of the translation initiation site, this mutation probably



SFig. 1. DNA sequencing showing all new identified mutations in *AAGAB*. *AAGAB* exon 1, with the wild-type DNA sequence for codons 1 and 2 (a) and the mutant sequence showing c.1A>G (b) comprising the identified mutation in PPKP 4 and 10. *AAGAB* exon 1, with the wild-type DNA sequence for codons 1 and 2 (c) and the mutant sequence from PPKP 3 (d), showing c.2T>A. *AAGAB* exon 1, with the wild-type DNA sequence for codons 1 and 2 (e) and the mutant sequence from PPKP 2 (f), showing c.1-1_12del. These three mutations (b, d, f) are likely leading to a protein isoform initiated from a downstream start codon. *AAGAB* exon 4, with the wild-type DNA sequence for codons 138–140 (g) and the mutant sequence from PPKP 6 (h), shows a missense mutation, c.415G>A, that leads to an amino acid change, p.Val139Ile. *AAGAB* exon 2, with the wild-type DNA sequence for codons 26–29 (i) and the mutant sequence from PPKP 1 (j), shows a single base pair deletion, c.77delT, resulting in a frame shift and a protein change, p.Ile26Thrfs*11. *AAGAB* exon 5, with the wild-type DNA sequence for codon 167–171 (k) and the mutant sequence from PPKP 12 (l), showing a 2 bp insertion, c.506_507insAA, resulting in a frameshift and protein change, p.Asn169Lysfs*7. *AAGAB* exon 1, with the wild-type DNA sequence for codons 1–7 (m) and the mutant sequence from PPKP 14 (n), showing the mutation c.17_73+17delCCT(68bp)GCC, which leads to a frame shift after the first five amino acids, influencing the splice site of exon 1. *AAGAB* exon 5, with the wild-type DNA sequence for codons 170–172 (o) and the mutant sequence from PPKP 11 (p), showing a nonsense mutation, c.512G>A, which results in a protein change, p.Trp171*. *AAGAB* exon 1, with the wild-type DNA sequence for codons 23 and 24 (q) and the mutant sequence from PPKP 8 (r), which shows a splice site mutation, c.73+1G>T.

also leads to a protein isoform. This mutation was seen in a 65-year-old woman (66352, family 2) with a typical moderate palmoplantar keratoderma since she was approximately 30 years old and reported a pain score of 1. She could not recall having observed palmoplantar keratoderma in her parents who had died early. Her 41-year-old daughter did not show any clinical signs of PPKP1, as confirmed by genetic analysis.

Using the ORF Finder at NCBI, 18 additional open reading frames were predicted, all of which are remarkably shorter than the ORF comprising *AAGAB*.

Missense mutation

One missense mutation in the *AAGAB* gene was identified, c.415G>A in exon 4 (SFig. 1g, h) which leads to the amino acid change p.Val139Ile. The mutation comprises a known single-nucleotide polymorphism (SNP), rs138601241, which has a minor allele frequency of A=0.001/2. PolyPhen-2 classifies c.415G>A as probably damaging, whereas SIFT predicts the known transcripts to be tolerated. The affected individual was a 40-year-old man (69352, family 6) who was diagnosed with a mild clinical phenotype at 10 years of age, but whose condition worsened over time, resulting in a high level of pain. His 2 sisters (71033, 71035), his mother (71034), and a deceased uncle were affected. The disease was also confirmed in his nephew (76427) by mutation analysis, and subsequent clinical examination revealed minimal lesions on both feet. In a segregation analysis, the affected individuals showed the same missense mutation. The phenotype varied within the family from severe (brother, 69352), moderate-severe (sister, 71033), mild (mother, 71034), and very mild (sister, 71035) to almost absent (nephew, 76427).

Frameshift mutations

In all 4 affected individuals in family 1 (65325, 65604, 66550, 72382), the same 1 base pair deletion in exon 2 (c.77delT) (SFig. 1i, j) was identified. This frameshift mutation results in a premature stop codon (p.Ile26Thrfs*11). The index patient of this family was an 83-year-old woman (65325) who had a moderate water-sensitive palmoplantar punctate keratoderma (Fig. 1a–c) with no pain since puberty. For many years the patient had been clinically diagnosed with keratosis palmoplantaris maculosa sive papulosa until she was included in our study and the mutation analysis for *AAGAB* was performed. Her daughter (66550), grandmother and great-grandmother, and even her sister (65604), despite taking 10 mg acitretin/day perorally, had a similar phenotype for PPKP1. The phenotype of the nephew (72382) was very mild (Fig. 1d); he played guitar regularly, which lead to callosities at the fingertips of his left hand (Fig. 1e) that were not found on his right hand.

A second frameshift mutation was detected in a family (family 12) of Belgian origin. Patient 76331 showed a 2-bp insertion in exon 5 (c.506_507insAA) (Fig. SFig. 1k, l). This mutation also results in a premature stop codon (p.Asn169Lysfs*7). The index patient of this family was a 57-year-old woman (76331) with a severe phenotype since 50 years of age. She complained of marked pain caused by skin changes on her hands and feet and she had no information about her family history.

A further frameshift mutation occurred in family 14. Individual 78323 showed a 74-bp deletion in exon 1

(c.17_73+17delCCTGTG (62 bases) GGCGCC) (SFig. 1m, n) extending into intron 1, resulting in a frameshift after the first 5 amino acids and influencing the splice site. The index patient was a 55-year-old woman (78323) of German origin with a moderate phenotype since puberty. She experienced pain (score 6) caused by skin changes on her hands and feet. Her mother was reported to have similar skin changes, but she did not participate in the study.

Nonsense mutations

A novel nonsense mutation, c.512G>A, was identified in exon 5 in a Belgian family (73417, family 11) (SFig. 1o, p), resulting in a stop codon at amino acid position 171 (p.Trp171*). The 80-year-old index patient with a moderate-severe phenotype experienced some pain (scale 5). She first noticed skin changes at age 44 years, which worsened as she got older. Her mother and siblings had similar skin lesions.

A second nonsense mutation, c.370 C>T in exon 4, which leads to p.Arg124*, was previously identified in a family of German origin (1). The index patient was a 35-year-old woman (65536, family 9) with a moderate palmoplantar keratoderma since the age of 15 years and a pain score of 6–7. There was no known family history of palmoplantar keratoderma, but close examination of the parents revealed a very mild phenotype in the mother (72608), which was also confirmed by mutation analysis.

A third nonsense mutation, c.481C>T, which leads to p.Arg161*, originally detected in a family of Croatian origin (1), was detected in family 5 and family 13. In family 5, a 41-year-old woman (69783) had a typical moderate phenotype beginning at 20 years of age. Her father (70445) had a mild to moderate phenotype with an onset at 15 years of age. The sister was also affected, but refused to participate in the study.

The index patient (76330) of family 13 was of African origin and 30 years old. He first noticed the skin changes 3 years previously and still had a mild phenotype. He had no knowledge of a family history and examination of other family members was not possible.

Splice site mutations

A novel splice site mutation, c.73+1G>T at the 3' end of exon 1, was identified in another Belgian family (family 8; SFig. 12q, r). CRYP-SKIP predicted a higher probability of exon skipping at this splice site than activation of cryptic splice sites.

The 54-year-old index patient (70562) of this family had a moderate phenotype since the age of 17 years and experienced a lot of pain (score 7–8). Her daughter (73578) had had a mild phenotype since she was 13 years old.

Another splice site mutation, c.451+1G>A at the 3' end of exon 4, was detected in a 49-year-old individual (69354, family 7). This splice site mutation was also recently reported by Eytan et al. 2014 (2), who suggested that exon skipping at this mutated intronic splice site was due to aberrant RNA splicing. The age of onset of the skin changes was 20 years, and the patient reported a high pain score (7–9 over several years). There was a family history of PPKP1 (brother, nephew, and deceased father), but as this part of the family lives in Russia, skin examinations and DNA analyses were not possible.

Table SI. AAGAB mutations identified in the current study

	Nucleotide change	Protein change	Computational analysis	Exon	Protein position	Family (patient)	References
Affecting start codon	c.1A>G c.2T>A	p.0? p.0?	Probably damaging deleterious Probably damaging deleterious	1 1	N-terminal N-terminal	PPKP 10 (66651), PPKP 4 (68077, 71005) PPKP 3 (66350, 66351)	Current study Current study
Missense	c.1-1_12del c.415G>A	p.0? p.Val139Ile	Probably damaging deleterious Probably damaging tolerated	-1/1 4	N-terminal Between Rab-like GTPase and adaptin-binding domain	PPKP 2 (66352) PPKP 6 (69352, 71033, 71034, 71035, 76427)	Current study rs138601241 MAF: A=0.001/2
Frameshift	c.77delT c.506_507insAA c.17_73+17delCCT(68bp)GCC	p.Ile26Thrfs*11 p.Asn169Lysis*7 p.?		2 5 1/+1	N-terminal Adaptin-binding N-terminal	PPKP 1 (65325, 65604, 66550, 72382) PPKP 12 (76331) PPKP 14 (78323)	Current study Current study Current study
Nonsense	c.512G>A c.370C>T c.481C>T	p.Trp171* p.Arg124* p.Arg161*	Exon skipping	5 4 5	Adaptin-binding Rab-like GTPase Adaptin-binding	PPKP 11 (73417) PPKP 9 (65536, 72608) PPKP 5 (69783, 70445)	Current study Giehl et al. 2012 (1) Giehl et al. 2012 (1)
Splice-site	c.73+1G>T c.451+1G>A	p.? p.?	Exon skipping Exon skipping	+1 +4	N-terminal Between Rab-like GTPase and adaptin-binding domain	PPKP 13 (76330) PPKP 8 (70562, 73578) PPKP 7 (69354)	Pohler et al. 2012 (12) Current study Eytan et al. 2014

PPKP: punctate palmoplantar keratoderma.

Table SII. Clinical information about patients with AAGAB mutations

	Patient	Sex/age, years	Severity of clinical phenotype (hands/feet)	Pain score 1–10	Age of onset	Family history	Ancestry
Affecting start codon	PPKP 10: 66651	M/51	Mild–moderate	0	40	Not known	German
	PPKP 4: 68077	F/44	Moderate	5	16	Yes	German
	PPKP 4: 71005	M/71	Very mild	0	Not recognized	Yes	German
	PPKP 3: 66350	F/39	Mild–moderate	0	20	Yes	German
	PPKP 3: 66351	F/67	Very mild	0	Not recognized	Yes	German
Missense	PPKP 2: 66352	F/65	Moderate	1	30	Not known	Romanian
	PPKP 6: 69352	M/40	Severe	8–9	10	Yes	German
	PPKP 6: 71033	F/34	Moderate–severe	7–9	6	Yes	German
	PPKP 6: 71034	F/70	Moderate	6–7	6	Yes	German
	PPKP 6: 71035	F/45	Mild	8	40	Yes	German
Frameshift	PPKP 6: 76427	M/18	No skin changes	0	Not recognized	Yes	German
	PPKP 1: 65325	F/83	Moderate, water sensitive	0	16	Yes	German
	PPKP 1: 65604	F/74	Moderate, water sensitive	0–5	30	Yes	German
	PPKP 1: 66550	F/55	Moderate, water sensitive	0	16	Yes	German
	PPKP 1: 72382	M/35	Mild	0–2	16	Yes	German
Nonsense	PPKP 12: 76331	M/57	Severe	9	50	Not known	Belgian
	PPKP 14: 78323	F/55	Moderate	6	15	Yes	German
	PPKP 11: 73417	F/80	Moderate–severe	5	44	Yes	Belgian
	PPKP 9: 65536	F/35	Moderate	6–7	15	Yes	German
	PPKP 9: 72608	F/53	Very mild	0	Not recognized	Yes	German
Splice-site	PPKP 5: 69783	F/41	Moderate	0	20	Yes	German
	PPKP 5: 70445	M/75	Mild–moderate	0	15	Yes	German
	PPKP 13: 76330	M/30	Mild	6	27	Not known	African
	PPKP 8: 70562	F/54	Moderate	7–8	17	Yes	Belgian
	PPKP 8: 73578	F/27	Mild	Not known	13	Yes	Belgian
	PPKP 7: 69354	M/49	Moderate	7–9	20	Yes	Russian

PPKP: punctate palmoplantar keratoderma.