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24. Rat carotid arteries were removed 5 or 20 days after balloon injury and infection with AdBgl or AdΔRb and were embedded in paraffin. Human 293 cells were infected with AdBgl or AdΔRb (20 PFU per cell), and 24 hours later cells were fixed in 4% paraformaldehyde and cell pellets were embedded in paraffin. Paraffin was then removed from 5-μm-thick sections of the carotid arteries or cell pellets and the sections were hydrated and treated with H_2O_2 for 4 min at 42°C to inactivate endogenous peroxidases. Immunohistochemistry was performed with a Ventana ES-320 automated immunostainer (Ventana Medical Systems, Tucson, AZ). Slides were stained with a mAb to von Willebrand factor (Dako, Santa Barbara, CA) or a mAb (12CA5) to HA (Boehringer-Mannheim, Indianapolis, IN) for 32 min at 42°C. Slides were then exposed to biotinylated antibodies to mouse immunoglobulin G (Ventana). Slides stained for von Willebrand factor were finally treated with avidin-conjugated alkaline phosphatase and fast red–naphthol (Ventana), and those stained for HA were exposed to avidin-conjugated horseradish peroxidase and diaminobenzidine plus copper (Ventana) and lightly counterstained with hematoxylin.
25. Carotid arteries of adult rats were injured with a balloon catheter (*18*) and immediately infected with 2 × 10^9 PFU of either AdBgl or AdΔRb, or exposed to vehicle (Hepes-buffered saline) alone. Animals were injected subcutaneously with 5′-bromodeoxyuridine (25 mg per kilogram of body mass) at 12-hour intervals, starting 24 hours after injury, for a total of four doses. Carotid arteries were fixed in situ by intravascular administration of 4% paraformaldehyde, embedded in paraffin, and sectioned. Sections (5 μm thick) from which paraffin had been removed were treated with 3% H_2O_2 in methanol and permeablized by incubation in 0.4% pepsin and 3.3 M HCl. Treated sections were exposed to 1.5% horse serum to block nonspecific sites, and incubated sequentially with a 1:100 dilution of a mAb to 5′-bromodeoxyuridine (Becton-Dickinson, San Jose, CA), a 1:200 dilution of biotinylated horse antibodies to mouse immunoglobulin, and avidin-conjugated horseradish peroxidase (Vectastain Elite ABC kit; Vector Laboratories, Burlingame, CA). Sections were finally treated with diaminobenzidine and counterstained with hematoxylin and eosin. At least 500 nuclei were quantitated in two sections from the balloon-injured and infected arterial segment from each animal. Statistical analyses were performed with Sigmaplot (Jandel Scientific, Corte Madera, CA).
26. Rat carotid arteries were injured with a balloon catheter (*18*) and treated with vehicle alone or infected with 2 × 10^9 PFU of AdBgl or AdΔRb. Twenty days after injury, carotid arteries were removed and tissue sections were stained with hematoxylin and eosin. Neointimal and medial boundaries were determined by digital planimetry of tissue sections with the MOCHA program (Jandel) on a Gateway 486 computer. The neointimal and medial cross-sectional areas were measured from six sections of each artery spanning the 1-cm site of balloon injury and infection, and the mean of these six determinations was used to calculate the neointimal-to-medial area ratio for each animal.
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Molecular Basis of the *cauliflower* Phenotype in *Arabidopsis*

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Genetic studies demonstrate that two *Arabidopsis* genes, *CAULIFLOWER* and *APETALA1*, encode partially redundant activities involved in the formation of floral meristems, the first step in the development of flowers. Isolation of the *CAULIFLOWER* gene from *Arabidopsis* reveals that it is closely related in sequence to *APETALA1*. Like *APETALA1*, *CAULIFLOWER* is expressed in young flower primordia and encodes a MADS-domain, indicating that it may function as a transcription factor. Analysis of the cultivated garden variety of cauliflower (*Brassica oleracea* var. *botrytis*) reveals that its *CAULIFLOWER* gene homolog is not functional, suggesting a molecular basis for one of the oldest recognized flower abnormalities.

In *Arabidopsis*, the genes that determine an early event of flower development, the specification of floral meristem identity, include *CAULIFLOWER* (*CAL*), *APETALA1* (*AP1*), and *LEAFY* (*LFY*) (*1*, *2*). In *ap1* single mutants, sepals are replaced by leaf-like organs, and petals generally fail to initiate (*3*). Axillary floral meristems arise at the base of these leaf-like organs, producing secondary flowers that resemble the phenotype of the primary *ap1* mutant flower (Fig. 1B). When the *ap1* mutation is combined with mutations in *CAL*, cells that would normally constitute a floral meristem instead behave as an inflorescence meristem, giving rise to additional meristems in a spiral phyllotaxy (*2*). The resulting *cauliflower* phenotype has an extensive proliferation of meristems at each position that in wild-type

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would give rise to a single flower (Fig. 1C). Plants homozygous for mutations in *CAL* are phenotypically wild-type, indicating that *AP1* is able to compensate for the loss of *CAL*. These genetic data indicate that *CAL* and *AP1* encode partially redundant activities. The *AP1* gene product contains a region, termed the MADS-domain (*4*), that functions as a sequence-specific DNA-binding domain and has a high degree of similarity to transcription factors (*5–7*). We have isolated and characterized the *CAL* genes from *Arabidopsis* and the cultivated garden variety of cauliflower.

Genetic evidence that the CAL and AP1 proteins may be functionally related suggested similar DNA sequences, and DNA blot hybridization experiments revealed that the *Arabidopsis* genome contains a gene that is closely related to *AP1*. This gene was isolated (*8*) and identified as a member of the family of *Arabidopsis* MADS-box genes known as *AGLs* (AG-like), and therefore it was initially termed *AGL10*. *AGL10* encodes a putative 255–amino acid protein (Fig. 2B) of 30.1 kD and a pI (isoelectric point) of 8.78. The AGL10 protein contains a MADS-domain, suggesting that it is a transcription factor. The MADS-domains of AGL10 and AP1 are markedly similar, differing in only 5 of 56 amino acid residues, 4 of which represent conservative replacements. Overall, the putative AGL10 protein is 76% identical to AP1; with allowance for conservative amino acid substitutions, the two proteins are 88% similar. These data suggest that AGL10 and AP1 likely recognize similar target sequences and thus most likely regulate many of the same genes necessary for floral meristems to acquire their identity.

AGL10 was mapped (*9*) to the approximate location of *CAL*, as determined by classical genetic means (*2*). To conclusively determine if *AGL10* corresponds to *CAL*, we used a genomic fragment spanning the *AGL10* gene to transform *cal-1 ap1-1* plants (*10*). Four independent lines transformed with *AGL10* show a complementation of the *cauliflower* phenotype (Fig. 1, D to F), displaying a range of phenotypes similar to those exhibited by *ap1* mutants (*2*). These results demonstrate that *AGL10* corresponds to *CAL*. In addition, they show that *CAL* can function not only to specify floral meristems, but in some instances can completely substitute for *AP1* in specifying petals.

In order to begin to dissect the regions of functional importance in the CAL protein, we analyzed four *cal* alleles. Sequence analysis of the *cal-1* allele, which exists in the wild-type Wassilewskija (WS) ecotype, revealed a cluster of three amino acid differences in the seventh exon, relative to the wild-type gene product from Landsberg *erecta* (*Ler*) (Fig. 2A). It is likely that one or

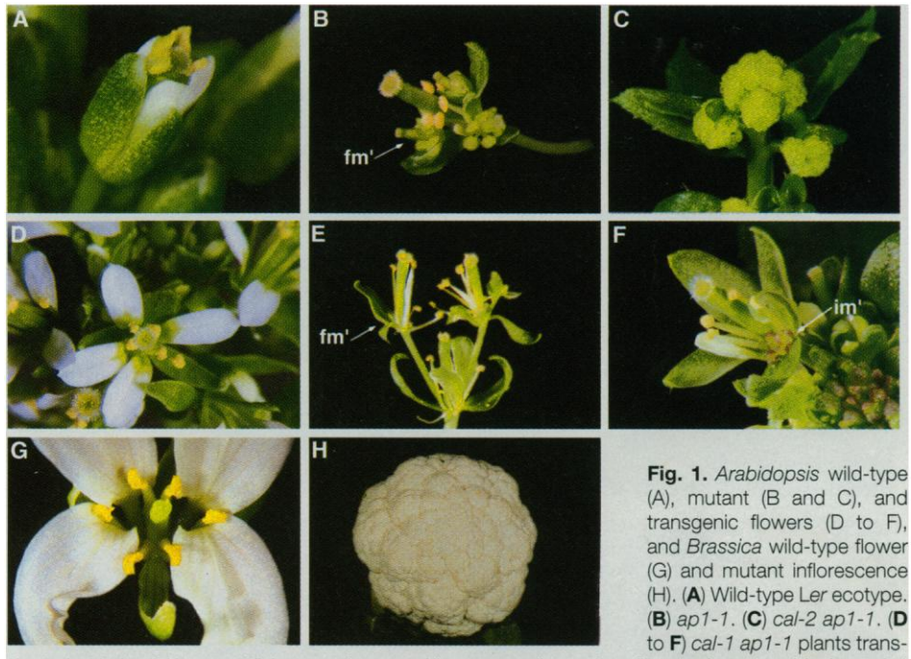
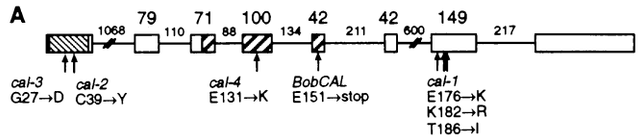


Fig. 1. *Arabidopsis* wild-type (A), mutant (B and C), and transgenic flowers (D to F), and *Brassica* wild-type flower (G) and mutant inflorescence (H). (**A**) Wild-type *Ler* ecotype. (**B**) *ap1-1*. (**C**) *cal-2 ap1-1*. (**D** to **F**) *cal-1 ap1-1* plants transformed with the *CAL* gene. All the complemented lines generally produce flowers that resemble the *ap1-1* single-mutant phenotype (E). Two lines (D) often produce flowers that have three or four normal appearing petals in the second whorl, perhaps as a result of insertion of multiple copies of the *CAL* transgene. Three lines (F) occasionally produce inflorescence meristems from the axils of first whorl organs. (**G**) Wild-type *Brassica oleracea*. (**H**) *Brassica oleracea* var. *botrytis*. fm', secondary flowers; im', axillary inflorescence meristems.



B

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CAL       MGRGRVELKRIENKINRQVTFSKRRTGLLKKAQEISVLCDAEVSLIVFSH   50
BoCAL           M                   A      H    I
BobCAL          M                   A      H    I
AP1             Q                   A      H           A V

CAL       KGKLFEYSSESCMEKVLERYERYSYAERQLIAPDSHVNAQTNWSMEYSRL  100
BoCAL                       H         K  KV             V
BobCAL                                K  K
AP1             TD      I                    E D  --        N

CAL       KAKIELLERNQRHYLGEELEPMSLKDLQNLEQQLETALKHIRSRKNQLMN  150
BoCAL                   D  SI I E         D S              H
BobCAL          W       D  SI I E         D                H
AP1                     D QA  P E         D       T        Y

CAL       ESLNHLQRKEKEIQEENSMLTKQIKERENILKTKQTQCEQLNRSVDDVPQ  200
BoCAL                L     V A   R    S  R H N S  Q    HHVA
BobCAL    *          L       A        S  R H N S  Q    HHVA
AP1         I E  K    A   Q     S       K  RAQ E WD Q QGHNMP -

CAL       PQPFQHPHL---YMIAHQTSPFLNMGGLYQGEDQTAMRRNNLDLTLEPIY  247
BoCAL       QLN YM    -----AS         M     YP  V    R
BobCAL      QLN YM    -----AS         M     YP  V    R
AP1       L P QHQIQHP  LS  P             ED PM      D E      V

CAL       NY-LGCYAA*                                           255
BoCAL      CN  YF
BobCAL     CN  YF
AP1        CN   F
```

Fig. 2. *CAL* gene structure and sequences. (**A**) Exon-intron structure of *Arabidopsis Ler CAL* gene. Exons are shown as boxes and introns as a solid line. Sizes (in base pairs) are indicated above. Locations of changes resulting in mutant alleles are indicated by arrows. MADS- and K-boxes are hatched. (**B**) Deduced amino acid sequences of *CAL* cDNAs isolated from *Arabidopsis thaliana Ler* (CAL), *Brassica oleracea* (BoCAL), and *Brassica oleracea* var. *botrytis* (BobCAL). The complete *Arabidopsis* sequence is displayed. Other sequences are shown directly below the *Arabidopsis* sequence where they differ. The AP1 sequence is shown for comparison. The MADS-domain is indicated in bold. The K-domain is underlined. Asterisk, stop codon. GenBank accession numbers are as follows: *Arabidopsis CAL* (L36925), *BoCAL* (L36926), and *BobCAL* (L36927). Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

more of these amino acid differences is responsible for the *cauliflower* phenotype, because this gene is expressed normally and correctly spliced in the WS background (*11*). We isolated three *cal* alleles, designated *cal-2*, *-3*, and *-4*, which exhibit phenotypes similar to that of the *cal-1* allele (*12*). Sequence analyses revealed a single missense mutation for each (Fig. 2A). It is likely that because mutations in the *cal-2* and *cal-3* alleles lie in the MADS-domain, they affect the ability of CAL to bind DNA and thus to activate its target genes. Because the *cal-4* allele contains a substitution in the K-domain, a motif thought to be involved in protein-protein interactions (*5*), this mutation may affect the ability of CAL to form homodimers or to interact with other proteins such as AP1.

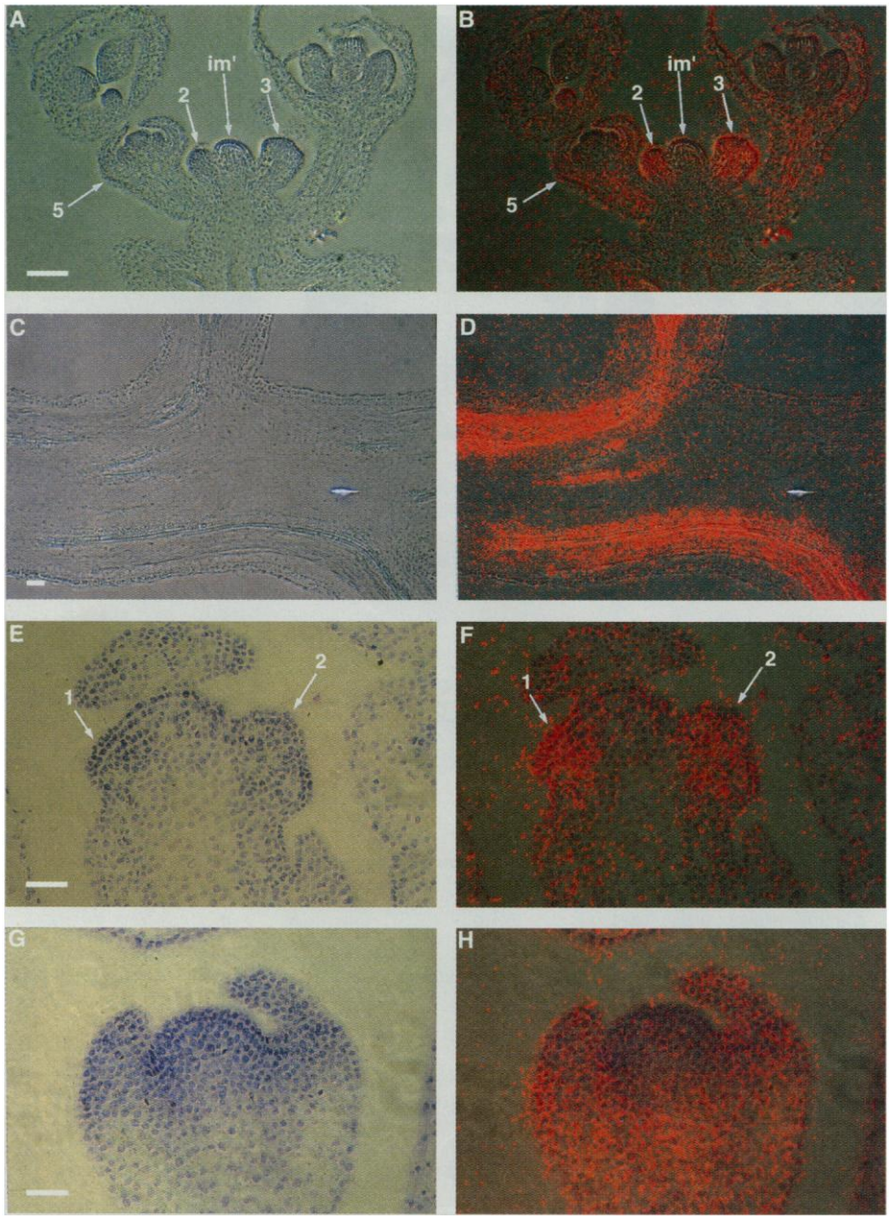

Fig. 3. *CAL* RNA accumulation in wild-type flowers. Longitudinal sections of *Arabidopsis* (A and B) and *B. oleracea* (E to H) wild-type inflorescences, and *Arabidopsis* inflorescence stem tissue (C and D) hybridized with *CAL*-specific antisense mRNA probes. Left column, bright-field photographs; right column, bright- and dark-field (red filter) double exposures. (**A** and **B**) *CAL* RNA is first detected in stage 1 floral primordia, when the floral meristem is first distinguishable from the inflorescence meristem. It is not detected in the inflorescence meristem. *CAL* RNA continues to accumulate uniformly throughout the floral meristem until stage 3, at which time it begins to be excluded from the cells that will give rise to the stamens and carpels. At stage 4 and later stages of development, *CAL* RNA is detected at low levels in cells that will give rise to sepals and petals, and is not detected in the cells that will give rise to stamens and carpels. (**C** and **D**) Unlike *AP1*, *CAL* RNA is detected at high levels in the vascular tissue of the inflorescence stem. (**E** and **F**) As seen in *Arabidopsis*, *BoCAL* RNA accumulates throughout the floral primordia of *Brassica oleracea* early in development and is later (**G** and **H**) excluded from cells that will give rise to stamens and carpels. Numbered arrows indicate the stage of development of the floral primordia (*22*). im, inflorescence meristem. Size bar represents 50 μm.

To characterize the temporal and spatial pattern of *CAL* RNA accumulation, and to see if this pattern revealed insights into the differences in CAL and AP1 activities, we performed RNA in situ hybridizations with a *CAL*-specific probe (Fig. 3, A to D) (*13*). As with *AP1* (*6*, *14*), *CAL* RNA accumulates in young flower primordia, consistent with the ability of *CAL* to substitute for *AP1* in specifying floral meristems. In contrast to *AP1*, whose RNA accumulates at high levels throughout sepal and petal development, *CAL* RNA is detected only at very low levels in these organs. This suggests that *CAL* is unable to substitute for *AP1* in specifying sepals and petals, at least in part as a result of the relatively low levels of *CAL* RNA in these developing organs.

Studies of two distantly related dicot plant species, *Arabidopsis* and *Antirrhinum*, have demonstrated that the genes controlling flower development are highly conserved. The *cauliflower* phenotype in *Arabidopsis* is similar to the inflorescence structure that develops in the closely related species *Brassica oleracea* var. *botrytis* (*15*), the cultivated garden variety of cauliflower, indicating that the *CAL* gene may contribute to the *cauliflower* phenotype of this agriculturally important species. To investigate this possibility, we have isolated the *CAL* gene homologs from a *Brassica oleracea* line that produces wild-type flowers, *BoCAL* (Fig. 1G), and from *Brassica oleracea* var. *botrytis*, *BobCAL* (Fig. 1H) (*16*).

To determine if the *BoCAL* gene from *Brassica oleracea* is expressed like its *Arabidopsis* counterpart, we performed RNA in situ hybridizations (*13*). As seen in *Arabidopsis*, *BoCAL* RNA accumulates uniformly in early floral primordia and is later excluded from the cells that will give rise to stamens and carpels (Fig. 3, E to H). DNA sequence analyses revealed that the open reading frame of the *BoCAL* gene is intact, whereas that of the *BobCAL* gene is interrupted by a stop codon in exon 5 (Fig. 2). The resulting BobCAL protein product is truncated after only 150 of the wild-type 255 amino acids. Because similar stop codon mutations in the fifth exon of the *Arabidopsis AP1* gene result in plants having a severe *ap1* phenotype (*2*, *17*), it is likely that the BobCAL protein is largely nonfunctional. These molecular data suggest that, as in *Arabidopsis*, the molecular basis for the *cauliflower* phenotype in *Brassica oleracea* var. *botrytis* is in part due to a mutation in the *BobCAL* gene. It will be

interesting to determine whether the *Brassica oleracea* var. *botrytis AP1* gene is also nonfunctional. Additional experiments, such as complementation of the *cauliflower* phenotype in *botrytis*, will further define the role of *BobCAL* in this crop plant.

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9. Restriction fragment length polymorphism mapping filters were scored and the results analyzed with the Macintosh version of the Mapmaker program as described (*18*), placing *CAL* on the upper arm of chromosome 1 near marker λ235.
10. A 5850–base pair (bp) Bam HI fragment containing the entire coding region of the *Arabidopsis CAL* gene as well as 1860 bp upstream of the putative translational start site was inserted into the pBIN19 plant transformation vector (Clontech) and used for transformation of root tissue from *cal-1 ap1-1* plants as described (*19*). Seeds were harvested from primary transformants, and all phenotypic analyses were performed in subsequent generations.
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12. Seeds homozygous for the *ap1-1* allele in L*er* were mutagenized with 0.1% or 0.05% ethylmethane sulfonate (EMS) for 16 hours. Putative new *cal* alleles were crossed to *cal-1 ap1-1 chlorina* plants to verify allelism. Two sets of oligonucleotides were used to amplify and clone new alleles: oligos AGL10-1 and AGL10-2 (5′-GATGGAGACCATTAAACAT-3′) for the 5′ portion and oligos AGL10-3 (5′-GGAGAAGGTACTAGAACG-3′) and AGL10-4 (5′-GCCCTCTTCCATAGATCC-3′) for the 3′ portion of the gene. All coding regions and intron-exon boundaries of the mutant alleles were sequenced.
13. ^{35}S-labeled antisense *CAL* and *BoCAL* mRNAs were synthesized from Sca I–digested cDNA templates and hybridized to 8-μm sections of *Arabidopsis* L*er* or *B. oleracea* inflorescences. The probes do not contain any MADS-box sequences in order to avoid cross-hybridization with other MADS-box genes. Hybridization conditions were the same as previously described (*20*).
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Mutations of Keratinocyte Transglutaminase in Lamellar Ichthyosis

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Lamellar ichthyosis is a severe congenital skin disorder characterized by generalized large scales and variable redness. Affected individuals in three families exhibited drastically reduced keratinocyte transglutaminase (TGK) activity. In two of these families, expression of *TGK* transcripts was diminished or abnormal and no TGK protein was detected. Homozygous or compound heterozygous mutations of the *TGK* gene were identified in all families. These data suggest that defects in TGK cause lamellar ichthyosis and that intact cross-linkage of cornified cell envelopes is required for epidermal tissue homeostasis.

Autosomal recessive lamellar ichthyosis (LI) is a congenital disorder of keratinization [MIM (Mendelian Inheritance in Man) 242100, estimated incidence 1:250,000]. Neonates are often born encased in a tough and inelastic film-like membrane that fissures easily, resulting in a high risk of sepsis and dehydration. Within 2 weeks the membrane sheds, revealing a lifelong disfiguring disease characterized by generalized large scales and variable redness of the skin (*1*). The renewal rate of proliferative basal keratinocytes is strongly enhanced in affected individuals, and this is manifested as thickened epidermis and increased nail and hair growth (*2*). The genetic origin of LI is unknown, and the absence of biological or molecular markers has contributed to disagreements on classification of the disease (*1*).

Transglutaminases (TGs) are a superfamily of enzymes that catalyze transamidation of glutamine residues, a reaction associated with a wide variety of physiological processes such as blood clotting, cytoplasmic coagulation in apoptosis, keratinization, hair follicle formation, fertilization, and dimerization of interleukin-2 in nerves (*3*). The keratinocyte form of TG (TGK) mediates N^{ε}-(γ-glutamyl)lysine cross-linkage (*4*) during formation of the cornified cell envelope (CE), a distinct and highly insoluble structure of 15-nm thickness that replaces the plasma membrane (PM) in terminally differentiating keratinocytes (*5*). This process involves the sequential cross-linking of CE precursor proteins such as involucrin; small, proline-rich proteins; and loricrin on the inner side of the PM (*5*). Simultaneously, the PM is replaced by Ω-hydroxyacyl-sphingosine lipids covalently bound to the outer surface of the protein CE (*5*). TGK is mostly expressed on the PM in upper spinous and granular cell layers of stratified squamous epithelia (*4*). About 5 to 10% of TGK activity is found in the cytoplasmic fraction and may account for the final steps of CE cross-linkage (*4*). TGK activity requires a catalytic thiol center in the enzyme and is sensitive to Ca^{2+} (*3*). The human *TGK* gene has at least two sequence variants, contains 15 exons, and is located on chromosome 14q11 (*TGM1* locus) (*6*).

In previous work, we observed that affected individuals in two families with LI

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