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The mouse wellhaarig (*we*) mutations result from defects in epidermal-type transglutaminase 3 (*Tgm3*)

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Abstract

The recessive wellhaarig (*we*) mutations, named for the wavy coat and curly whiskers they generate in homozygotes, have previously been mapped on mouse Chromosome 2. To further limit the possible location of the *we* locus, we crossed hybrid (C57BL/6 x AKR)F_1, we^{4J}/+ females with AKR, we^{4J}/we^{4J} mutant males to create a large backcross family that was typed for various microsatellite markers and single-nucleotide polymorphisms (SNPs) that distinguish strains AKR and B6. This analysis restricted the location of we^{4J} between sites that flank only one gene known to be expressed in skin: epidermal-type transglutaminase 3 (*Tgm3*). To test *Tgm3* as a candidate for the basis of the wellhaarig phenotype we took two approaches. First, we sequenced all *Tgm3* coding regions in mice homozygous for four independent, naturally-occurring wellhaarig alleles (*we*, we^{Bkr}, we^{3J} and we^{4J}) and found distinct defects in three of these mutants. Second, we crossed mice homozygous for an induced mutant allele of *Tgm3* ($Tgm3^{Btlr}$) with mice heterozygous for one of the wellhaarig alleles we possess (we^{4J} or we^{Bkr}) to test for complementation. Because the progeny inheriting both a recessive *we* allele and a recessive $Tgm3^{Btlr}$ allele displayed wavy hair, we conclude that the classic wellhaarig mutations result from defects in *Tgm3*.

Keywords

Positional candidate approach; Complementation testing; Intraspecific backcross mapping; Hair morphology

1. Introduction

The recessive wellhaarig mutations in mice (abbreviated *we*) generate curly vibrissae and a first hair-coat with a striking wavy texture (see Figure 1). The original *we* variant arose as a spontaneous mutation in the stocks of Agnes Bluhm (1862–1943, a physician and researcher at the Kaiser-Wilhelm Institute, Berlin, Germany) but wellhaarig's description and

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assignment to Linkage group V were first reported by Peter Hertwig [1] who obtained the inbred line from Bluhm in 1936. This original mutation has been joined by the "wellhaarig; Bunker" allele (*we*^{Bkr}, named for its discoverer, Helen P. Bunker), which spontaneously arose at The Jackson Laboratory (Bar Harbor, ME) in 1964 and was later shown not to complement *we* [2]. Two more spontaneous remutations were subsequently discovered (also at The Jackson Laboratory), "wellhaarig; wellhaarig 3 Jackson" (*we*^{3J})[3] and "wellhaarig; wellhaarig 4 Jackson" (*we*^{4J})[4], although the *we*^{3J} variant is no longer extant. While *we* was used extensively as a phenotypic marker in early mapping studies of Linkage Group V [5,6], its position on the physical map of Chromosome (Chr) 2 is not well defined and its molecular basis has not been determined.

In order to assign the mutant wellhaarig phenotype to a specific genetic cause, we have fine-mapped the *we*^{4J} mutation with respect to various microsatellite and single-nucleotide polymorphisms on mouse Chr 2. This analysis has identified a small number of co-localizing candidate genes, one of which has been found to harbor distinct mutations in *we*, *we*^{Bkr} and *we*^{4J} mutant mice.

2. Materials and methods

2.1. Mice

All animals were housed and fed according to Federal guidelines, and the Institutional Animal Care and Use Committee at CCSU approved of all procedures involving mice. Mice from the standard inbred strain C57BL/6J (Jax Stock Number 664), inbred AKR/J-*we*^{4J}/J mice (Jax Stock Number 3656, homozygous *we*^{4J}), and B10.129-*we*^{Bkr}/CyJ mice (Jax Stock Number 475, homozygous for *we*^{Bkr}) were obtained from The Jackson Laboratory (Bar Harbor, ME, USA). Inbred C57BL/6J-*Tgm3*^{m2Btlr}/Mmmh mice, carrying the "tortellini" mutation, an EtNU-induced point mutation in the *Tgm3* gene produced by Bruce Beutler and colleagues [7] (herein designated *Tgm3*^{Btlr}), were obtained from the Mutant Mouse Regional Resource Center at the University of Missouri (Columbia, MO USA). The *Tgm3*^{Btlr} mutation is a G to A transition at position 2:130024585 that destroys the splice donor site at the 5' end of Intron 3–4, and is predicted by Won, Moresco and Buetler [7] to cause the splicing of Exon 2 with Exon 4, eliminating the 80 amino acids (61–140) encoded by Exon 3. These wellhaarig and tortelinni mutants were reliably identified by the presence of curly vibrissae that appear shortly after birth, and persist throughout the life span. The first coat of hair in these mutants also grows in with a wavy texture that is most striking at about 10 days to 3 weeks of age (see Figure 1 for a *we*^{4J}/*we*^{4J} mutant), but becomes less marked in subsequent hair coats.

2.2. DNA analysis

Genomic DNA was isolated from 2 mm tail-tip biopsies taken from two to three-week-old mice using Nucleospin® Tissue kits distributed by Clontech Laboratories, Inc. (Mountain View, CA, USA), as directed. DNA samples from standard inbred strains that we do not routinely maintain in our colony—including NX129-10/Ty-*we*^{3J} (homozygous for *we*^{3J}), which are no longer extant—were purchased from The Jackson Laboratory's Mouse DNA Resource before it ceased operations in December 2013.

The polymerase chain reaction (PCR) was performed in 13 ul reactions using the Titanium PCR kit from Clontech Laboratories, as directed. Oligonucleotide primers for PCR were designed and synthesized by Integrated DNA Technologies, Inc. (Coralville, IA, USA), based on sequence information available online [8,9]. To score PCR product sizes for dimorphic microsatellite markers, reactions plus 2 ul loading buffer were electrophoresed through 3.5% NuSieve® agarose (Lonza, Rockland, ME, USA) gels. Gels were stained with ethidium bromide and photographed under ultraviolet light. In addition to nine standard microsatellite markers [10] on Chr 2 (see Supplementary Figure S1), four DNA markers based on single nucleotide polymorphisms previously-reported to differ between strains AKR and C57BL/6J [8,9] were also scored. These markers (herein designated *SNP A–D*) are described in detail in Supplementary Tables S1 and S2. For sequence analysis, about 1.5 μg of individual PCR amplimers were purified and concentrated into a 30 μl volume using QIAquick® PCR Purification kits (Qiagen Sciences, Germantown, MD, USA). Amplimers were shipped to SeqWright DNA Technology Services (Houston, TX, USA) for primer-extension sequence analysis. Carriers of the recessive $Tgm3^{Btlr}$ allele were identified by primer-extension DNA sequence analysis of a 187 bp PCR product directed by a forward primer (5’ AAAGCCAACAGTGGCAATAATC 3’) that anneals within Exon 3, and a reverse primer (5’CCAACCAGATCTAAGCCCATAC 3’) that anneals within Intron 3–4.

2.3. mRNA analysis

Total RNA was isolated from skin samples taken from 1-month-old mutant and wild type mice using the Nucleospin® RNA Midi kit by Macherey-Nagel (Bethlehem, PA, USA). From these total RNA samples poly A^+ mRNA was purified using the NucleoTrap® mRNA kit, also by Macherey-Nagel, and cDNA was generated using the SMARTer® RACE 5’/3’ kit (Clontech Laboratories, Inc.). To detect *Tgm3*- or *Actb*-specific sequences, primer pairs that flanked exon-junction boundaries were used to direct standard PCR amplifications of these cDNAs, and the resulting products were visualized in 3% NuSieve® agarose gels. For primer-extension sequencing, these products were purified and concentrated (as described above) and shipped to SeqWright DNA Technology Services.

3. Results

3.1. Genetic mapping of we^{4J}

While *we* has been used extensively as a phenotypic marker for genetic mapping on mouse Chr 2 [5,6] , its location has not been well defined with respect to molecular markers except by Zuberi and coworkers [11,12] who found one crossover that placed *we* centromeric to *Itpa* [for inosine triphosphatase (nucleoside triphosphate pyrophosphatase)]. To verify and further refine this location, we produced a 1035-member backcross (N_2) family by crossing (C57BL/6J x AKR/J-we^{4J}/J)F_1 females with AKR/J-we^{4J}/J mutant males. These N_2 mice were typed for their hair phenotype, and DNA isolated from each mouse was characterized for nine PCR-scorable microsatellite markers (see Supplementary Figures S1 and S2) that lie throughout the *we*-critical region identified by Zuberi et al. This analysis located we^{4J} between markers *D2Mit304* and *D2Mit78*, and very near markers *D2Mit135* and *D2Nds3* (from which we^{4J} was never meiotically separated) (Figure 2A).

To more precisely locate *we*^{4J}, the eleven mice recombinant between *D2Mit304* and *D2Mit78* were typed for four single-nucleotide polymorphisms (SNPs) that lie within that interval (and are described in detail in Supplementary Tables S1 and S2). This analysis, summarized in Figure 2B, identified four crossovers that positioned *we*^{4J} telomeric of *SNP B* and three crossovers that placed *we*^{4J} centromeric of *SNP D* (very near *SNPC* which was never separated from *we*^{4J}). This small, 1.1-Mb region contains 21 genes or processed transcripts (see Figure 2C), only one of which, *Tgm3* (for transglutaminase 3, E polypeptide; also known as epidermal-type transglutaminase), is known to be expressed in skin [13].

3.2. Sequence analysis of Tgm3 in four independent wellhaarig alleles

With one obvious candidate, we next sequenced the exons (both coding and untranslated regions) of the *Tgm3* gene in genomic DNA from *we*, *we*^{Bkr}, *we*^{3J} and *we*^{4J} mutants, and from control C57BL/6J and AKR/J mice. DNA defects predicted to cause protein-level alterations were found in three of these four wellhaarig variants: a C to T nonsense mutation in Exon 13 would shorten the *we* mutant's Tgm3 product by 36 amino acids, a C to T missense mutation in Exon 7 would replace a polar serine with a nonpolar leucine in the *we*^{Bkr} mutant, and a 7-base-pair deletion in Exon 10 would terminate translation of the *we*^{4J} mutant's Tgm3 product at R512 (out of the usual 693 amino acids)(as summarized in Figure 2D). The details of these changes are displayed in Supplementary Figure S3. Notably, DNA isolated from two additional strains said to carry the original *we* allele (B6C3Fe *a*/*a*-*we Pax1*^{un} *a*^{t}/J and B6CBACa *A*$^{w\text{-}J}$/*A*-*we a Mafb*^{kr}/J) were shown to encode the same DNA alteration we described for the *we* allele carried by strain B10.UW-*H3*^{b} *we Pax1*^{un} *a*^{t}/SnJ (see Figure 3A). Using additional DNA tests described in Figure 3B and C we also demonstrated that the *Tgm3* defects assigned to *we*^{4J} and *we*^{Bkr} are specific to these variants.

3.3. Complementation analysis

Two other laboratories have engineered recessive, loss-of-function alleles of *Tgm3* [7,14], and these mutants both show abnormal hair morphology similar to the classic wellhaarig phenotype, further supporting *Tgm3* as a candidate for the gene disrupted by the various wellhaarig mutations. We obtained mice homozygous for the recessive *Tgm3*^{Btlr} allele produced by Bruce Beutler and colleagues (UT Southwestern Medical Center, Dallas, TX, USA), and called "tortellini" by these investigators [7] (and see Materials and Methods). Table 1 summarizes the results of crosses we conducted between tortellini mutants and mice heterozygous for either *we*^{4J} or for *we*^{Bkr}. Because the progeny inheriting both a recessive *Tgm3*^{Btlr} allele and a recessive *we*^{4J} (or *we*^{Bkr}) allele showed curly hair (while siblings inheriting only the *Tgm3*^{Btlr} allele did not) we conclude that these mutations "fail to complement". On this basis we suggest that these two wellhaarig mutations (and by extension, all others) result from defects in *Tgm3*.

3.4. mRNA analysis

The *Tgm3*^{Btlr} mutation is a G to A transition that destroys the splice donor site at the 5' end of Intron 3–4, and is predicted by Won, Moresco and Buetler [7] to cause the splicing of Exon 2 with Exon 4, eliminating the 80 amino acids encoded by Exon 3 (see Supplementary Figure S5A, B). To test this hypothesis and to determine if the *we*^{4J} transcript (with its

predicted early termination of translation in Exon 10) might be subject to nonsense-mediated decay [15], *Tgm3* sequences were amplified between Exons 2 and 5 from cDNA templates based on poly-A^+ mRNA isolated from mutant or wild type skin (see Supplementary Figure S5C). Sequencing of the 586 bp product amplified from wild type, we^{4J}/we^{4J} and we^{Bkr}/we^{Bkr} skin cDNA verified the normal splicing of Exons 2, 3, 4 and 5. Sequencing of the major 346 bp product amplified from tortellini skin demonstrated that the mutant transcript is almost entirely processed to join Exon 2 with Exon 4, as predicted [7]. Furthermore, while the we^{Bkr} and $Tgm3^{Btlr}$ transcripts appear as stable as wild type (as expected, since both of these variants are predicted to be fully translated), the we^{4J} transcript appears consistently underrepresented in both homozygotes and in heterozygotes suggesting that this mutant mRNA is unstable, and is likely subject to nonsense-mediated decay.

4. Discussion

We have taken a positional-candidate approach to assign the classic wellhaarig mutations in mice to defects in the *Tgm3* gene. Finding three different, allele-specific *Tgm3* defects in *we*, we^{Bkr} and we^{4J} mutants (a nonsense mutation; a missense mutation; and a small, frame-disrupting deletion) strongly supports this assignment. In addition, the failure of an induced, recessive allele of *Tgm3* to complement the we^{4J} or we^{Bkr} defects in compound heterozygotes confirms allelism. We therefore recommend that these wellhaarig mutations be formally renamed “transglutaminase 3; wellhaarig” ($Tgm3^{we}$), “transglutaminase 3; wellhaarig 4 Jackson” ($Tgm3^{we\text{-}4J}$) and “transglutaminase 3; wellhaarig Bunker” ($Tgm3^{we\text{-}Bkr}$). No DNA alteration was found in the (mostly) exonic portions of *Tgm3* that we sequenced in DNA isolated from a we^{3J} mutant. While we suspect that the we^{3J} allele likely has a regulatory defect in *Tgm3* outside of the regions we sequenced, we were not able to investigate possible changes in transcript expression, processing or stability because this variant is no longer extant.

The spontaneous wellhaarig mutations join two engineered *Tgm3* alleles [7,14], providing a collection of five phenotypically similar, but mutationally distinct defects. The *we* mutation is predicted to remove just the final 36 amino acids (658–693) of Tgm3, which compose the final third of the 2nd β-barrel (amino acids 595–693; SSF49309 [16]). Because we do not maintain any live *we* mutants in our laboratory, it was not determined if the *we* nonsense defect might destabilize the mRNA (but since this mutant termination codon lies in Exon 13, downstream of the final exon-exon junction in *Tgm3*, nonsense-mediated decay, at least, does not seem likely). The we^{4J} mutation is predicted to more severely truncate the protein (eliminating residues 513–693), deleting all of the second β-barrel and most of the first (amino acids 481–594; SSF49309 [16]), and also appears to result in an unstable transcript. In the we^{Bkr} allele, a missense mutation is predicted to replace Ser299 with a nonpolar leucine residue. While this amino acid lies in the catalytic core of the protein (amino acids 142–461; SSF54001 [16]), this serine is not part of the catalytic triad (Cys273, His331 and Asp354) nor is it near the cleavage site for zymogen activation at Ser465.

In the skin, epidermal transglutaminase is highly expressed in keratinocytes and corneocytes, where it contributes to the formation of the cell envelope by crosslinking substrates such as loricrin and involucrin [17], and in hair follicles, where it catalyzes the

crosslinking of trichohyalin and keratin intermediate filaments to harden the inner root sheath [14]. We propose that mutations of *Tgm3* may result in the wellhaarig phenotype due to asymmetric crosslinking of proteins in the hair cortex. Indeed, genetic variants of trichohyalin [18] and of hair keratins [19,20] have been associated with changes in hair morphology. Defects in *Tgm3* might also, therefore, be expected to influence hair curvature.

In spite of widespread expression of *Tgm3* in skin, its ablation in the mouse does not cause any obvious developmental defects, other than altered hair morphology [14] and contact hypersensitivity to fluorescein isothiocyanate [21]. It is therefore probably not surprising that there are, to our knowledge, no examples of human disorders associated with *Tgm3* defects. It does seem likely that some recessive forms of hair curling or brittleness could be due to changes in *Tgm3*, but their pathology may not be severe enough to draw medical attention. Interestingly, however, epidermal transglutaminase has been identified as the primary autoantigen in the gluten-sensitive disease dermatitis herpetiformis (DH), where IgA directed against Tgm3 in the skin form deposits in the papillary dermis [22]. It might be interesting to learn, for example, if any or all of these murine *Tgm3* variants might be resistant to gluten-induced antibodies, compared with wild type mice.

Other tissues—including the brain; the tongue, esophagus and stomach; and the testes—also display high levels of *Tgm3* expression [23–26] and the role of *Tgm3* in the development, maintenance and function of these tissues certainly deserves further investigation. We anticipate that the series of five distinct, naturally-occurring and engineered variants of *Tgm3* that we have assembled here will help facilitate such studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Appendix A. Supplementary Material

The following supplementary material for this article:

Table S1. Description of SNP markers referred to in the Brennan *et al.* (2015) text;

Table S2. Location of SNP markers referred to in the Brennan et al. (2015) text;

Figure S1. Dimorphic amplimers for each of nine microsatellite DNA markers used in this study;

Figure S2. Segregation of we^{4J} and nine microsatellite markers on mouse Chr 2 among a large backcross family of mice;

Figure S3. Genomic DNA and predicted amino acid sequences at the site of the *we*, $we^{Bkr}$and we^{4J} mutations;

Figure S4. Representative mutant and wild type pups resulting from complementation testing among the recessive we^{Bkr}, we^{4J} and $Tgm3^{Btlr}$ mutations;

Figure S5. Analysis of *Tgm3* mRNA from mutant skin shows that the $Tgm3^{Btlr}$ transcript skips Exon 3, and that the we^{4J} transcript is unstable;

can be found online at http://dx.doi.org/10.1016/ymgme.2015.##.###.

Highlights

- Genetic mapping identifies a small number of candidates for the mouse *we*^{4J} mutation.
- Sequence analysis of four wellhaarig alleles reveals three distinct *Tgm3* mutations.
- Complementation testing shows that two wellhaarig mutations are alleles of *Tgm3*.
- Analysis of skin mRNA indicates that the *we*^{4J} transcript is unstable.
- Analysis of skin mRNA indicates that the *Tgm3*^{Btlr} transcript is aberrantly spliced.

Figure 1.
The classic wellhaarig phenotype. 16-day-old siblings are shown. The mouse on the left is a we^{4J}/we^{4J} mutant, the mouse on the right is a $+/we^{4J}$ heterozygote.

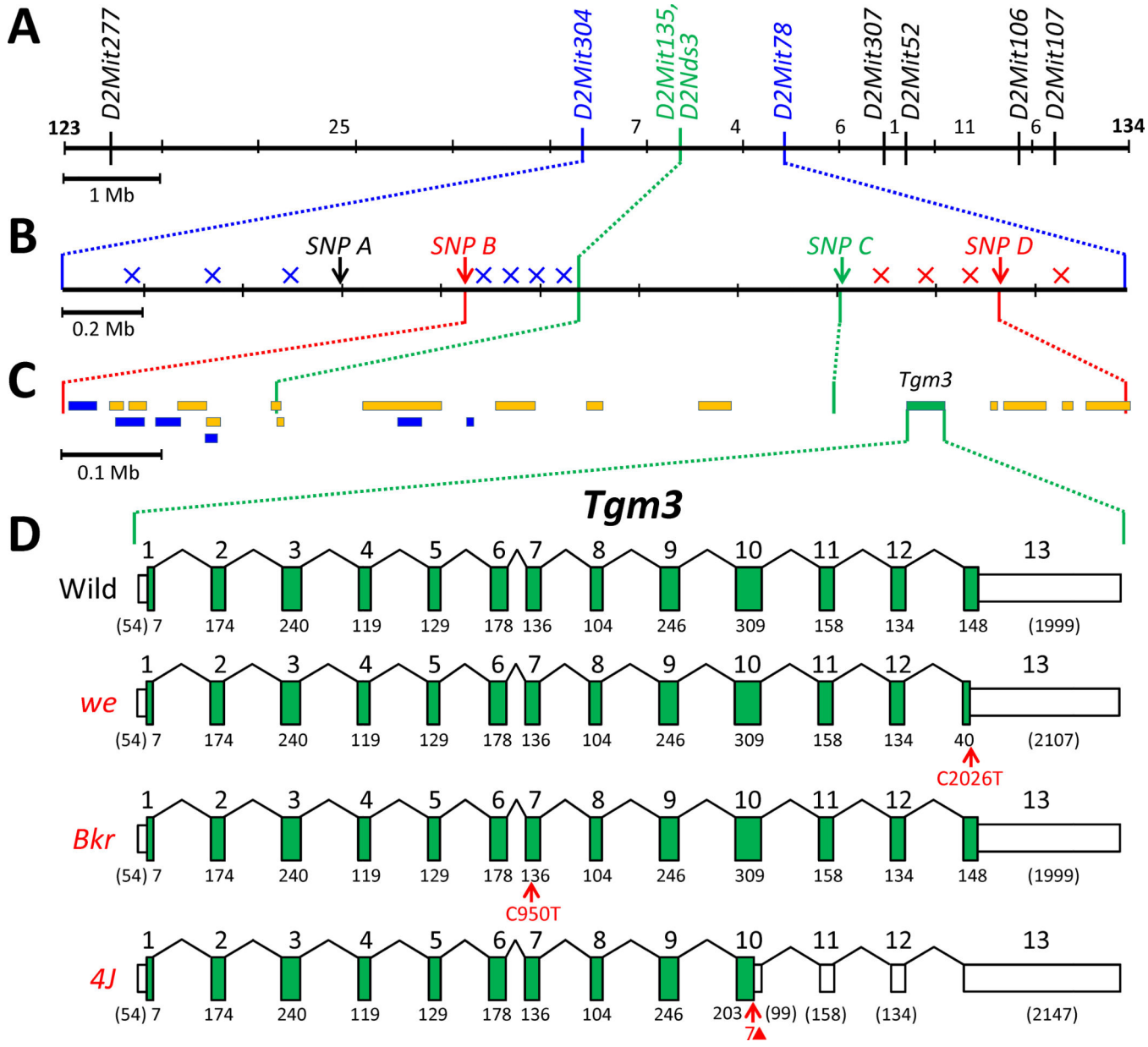

Figure 2.

Physical maps of the "*we*-critical" region of mouse Chr 2. (**A**) The relative positions of the nine microsatellite markers typed within the backcross panel are shown, with a 1 Mb scale bar. The number of crossovers found in each marker-defined interval is shown. *D2Mit135* and *D2Nds3* (shown in green) were never meiotically separated from we^{4J} in this backcross panel. (**B**) The relative positions of four SNP markers are shown for the 2.1 Mb region that is flanked by *D2Mit304* and *D2Mit78* (a 0.2 Mb scale bar is shown). Crossovers that fell to the left of (centromeric to) we^{4J} are depicted by blue x's (drawn arbitrarily within the SNP-defined interval where they were mapped); crossovers that fell to the right of (telomeric to) we^{4J} are shown in red. Therefore, we^{4J} must lie between *SNP B* and *SNP D* (shown in red). *SNP C* (shown in green) was never separated from we^{4J} in this backcross panel. (**C**) The

interval from *SNP B* to *SNP D* is expanded (a 0.1 Mb scale bar is shown), and the extent of known genes (in yellow) and processed transcripts (in blue) are depicted by colored rectangles. Of these potential candidates, only *Tgm3* (in green) is known to be expressed in skin. (**D**) The *Tgm3* gene is expanded to show the 13 exons it comprises. Tall green boxes represent coding regions and shorter white boxes indicate the 5' and 3' untranslated regions. The number below each coding segment is its length in base pairs; the base-pair lengths of noncoding segments are in parentheses. The mutant *we* transcript shows a nonsense mutation in Exon 13 that is predicted to truncate the mutant protein (also see Supplementary Figure S3, Panel A). The mutant we^{Bkr} transcript is drawn to show the position of a missense mutation in Exon 7 that is predicted to substitute a highly-conserved, polar serine with a non-polar leucine residue (also see Supplementary Figure S3, Panel B). The we^{4J} transcript is drawn to show the position of the 7-bp deletion we define here (shown as a red triangle) that is predicted to result in an early translational stop (also see Supplementary Figure S3, Panel C)

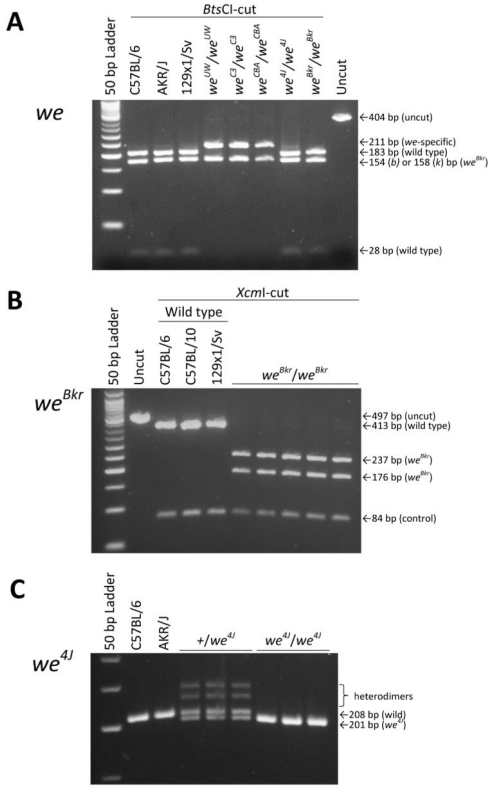

Figure 3.

DNA tests that distinguish three wellhaarig-associated mutations in *Tgm3* from the wild-type sequence. (**A**) 404 or 408 bp amplimers (from C57BL/6-derived $Tgm3^{b}$ templates or from AKR-derived $Tgm3^{k}$ templates, respectively) directed by primers shown in Supplementary Figure S3, Panel A, were cut with restriction endonuclease *Bts*CI. Amplimers based on wild type templates cut twice, while templates from three inbred mouse strains (B10.UW-$H3^{b}$ *we* $Pax1^{un}$ a^{t}/SnJ, B6C3Fe *a*/*a*-*we* $Pax1^{un}$ a^{t}/J and B6CBACa $A^{w\text{-}J}$/*A*-*we a* $Mafb^{kr}$/J) that are homozygous for the original *we* mutant allele (distinguished in the figure as we^{UW}, we^{C3} and we^{CBA}, respectively) cut only once, yielding a 211 bp mutant-specific fragment. Distinct wellhaarig alleles with defects in other regions of the *Tgm3* gene (including we^{4J} and we^{Bkr}, shown here) exhibit the wild-type cutting pattern. (**B**) 495 bp wild-type amplimers directed by primers that flank *Tgm3*, Exon 6 (see Supplementary Figure S3, Panel B) were cut once with endonuclease *Xcm*I, while amplimers based on mutant we^{Bkr} templates were cut twice. Digestions were limited to 45 minutes to avoid star activity. (**C**) Primers located within *Tgm3*, Exon 10 (see Supplementary Figure S3, Panel C) were used in a standard PCR to amplify a 201 bp product from we^{4J} templates and a 208 bp product from wild-type templates. These products were readily distinguished by electrophoresis through 3.5 % NuSieve agarose gels. Two (fainter) slower-moving bands were generated only from heterozygous templates and presumably result from heterodimer PCR products with retarded migration rates.

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Table 1

Complementation testing among the recessive *we*^{Bkr}, *we*^{4J} and *Tgm3*^{Btlr} mutations confirms allelism.

	Wild Type		Mutant			
	Female	**Male**	**Female**	**Male**	χ^2	*P*
Cross A.						
we^{4J}/*we*^{4J} X +/*we*^{Bkr}	6	5	4	2	2.94	0.09
+/*we*^{Bkr} X *we*^{4J}/*we*^{4J}	5	6	2	4		
Cross B.						
we^{Bkr}/*we*^{Bkr} X +/*we*^{4J}	6	2	5	2	1.13	0.29
+/*we*^{4J} X *we*^{Bkr}/*we*^{Bkr}	6	5	2	4		
Cross C.						
Tgm3^{Btlr}/*Tgm3*^{Btlr} X +/*we*^{4J}	3	3	6	1	0.86	0.35
+/*we*^{4J} X *Tgm3*^{Btlr}/*Tgm3*^{Btlr}	3	3	4	6		
Cross D.						
Tgm3^{Btlr}/*Tgm3*^{Btlr} X +/*we*^{Bkr}	2	1	4	5	1.64	0.20
+/*we*^{Bkr} X *Tgm3*^{Btlr}/*Tgm3*^{Btlr}	4	1	3	2		

Combined counts from reciprocal crosses were tested for goodness-of-fit with the 1 wild type:1 mutant ratio expected for non-complementation using the χ^2 test. DNA isolated from the progeny of crosses *A* and *D* were typed using the test shown in Figure 3B to confirm that all mutant progeny received the *we*^{Bkr} allele, and that all the wild type progeny did not. DNA isolated from the progeny of crosses *B* and *C* were typed using the test shown in Figure 3C to confirm that all mutant progeny received the *we*^{4J} allele, and that all the wild type progeny did not. One mutant and one wild type pup from each cross (*A*, *B*, *C* and *D*) are shown in Supplementary Figure S4, Panels A, B, C and D, respectively. Mutant phenotypes resulting from crosses *A* and *B* formally confirm that *we*^{Bkr} and *we*^{4J} are alleles of each other, as well as being alleles of the original *we* mutation, which was previously demonstrated by Graff et al. for *we*^{Bkr} [2] and by Samples et al. for *we*^{4J} [4]. Mutant phenotypes resulting from crosses *C* and *D* demonstrate that these recessive wellhaarig mutations fail to complement *Tgm3*^{Btlr}, and may therefore be considered alleles of *Tgm3*.