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Novel *transglutaminase-1* mutations and genotype–phenotype investigations of 104 patients with autosomal recessive congenital ichthyosis in the USA

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Abstract

Background—Autosomal recessive congenital ichthyosis (ARCI) is a rare hereditary disorder of cornification. Mutations in the transglutaminase-1 (*TGM1*) gene, which encodes for the epidermal enzyme transglutaminase-1 (TGase-1), are one of the causes of ARCI.

Methods—The *TGM1* mutation spectrum was characterised and genotype–phenotype correlations investigated in 104 patients with ARCI ascertained through the National Registry for Ichthyosis and Related Disorders in the USA.

Methods—Germline mutations in *TGM1* were identified in 55% (57/104) of patients with ARCI. Arginine residues in TGase-1 were mutated in 39% (22/57) of patients overall and 54% (20/37) of those with missense mutations. In total, 55% (12/22) of missense mutations were within CpG dinucleotides and 92% (11/12) of these mutations were C→T or G→A transitions. The genotype–phenotype investigation found that ARCI with *TGM1* mutations was significantly associated with presence of collodion membrane at birth ($p = 0.006$), ectropion ($p = 0.001$), plate-like scales ($p = 0.005$) and alopecia ($p = 0.001$). Patients who had at least one mutation predicted to truncate TGase-1 were more likely to have more severe hypohidrosis ($p = 0.001$) and overheating ($p = 0.0007$) at onset of symptoms than were those with exclusively *TGM1* missense mutations. A logistic model was developed, which predicted that individuals with collodion membrane, alopecia and/or eye problems are about four times more likely to have *TGM1* mutations than patients without these findings.

Conclusion—This is the largest investigation of patients with ARCI to date. It expands the *TGM1* mutation spectrum and confirms that despite genetic and phenotypic heterogeneity in ARCI, *TGM1* is the main causative gene for this disorder. The high frequency of mutated arginine codons in *TGM1* may be due to the deamination of CpG dinucleotides.

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Autosomal recessive congenital ichthyosis (ARCI; OMIM 242100, 242300) classifies a clinically diverse group of autosomal recessive hereditary disorders of cornification characterised by epidermal scaling. ARCI is rare, with estimated incidence rates of 1:200 000–1:300 000 in the USA[1] and as high as 1:91 000 in Norway, owing to a founder effect.[2] Patients with ARCI are often born encased in a very tight, translucent, thick sheath called a collodion membrane. Although the collodion membrane usually desquamates within the first few weeks of life, ectropion, eclabium and digital and joint contractures are some of its more long-lasting sequelae. After the shedding of the membrane, a variable amount of epidermal scaling soon appears. Patients may also have alopecia, hypohidrosis and palmar–plantar hyperkeratosis.

ARCI has traditionally been divided into two major clinical subtypes: lamellar ichthyosis (LI; OMIM 242300 and non-bullous congenital ichthyosiform erythroderma (NBCIE; OMIM 242100.[3] LI, characterised by large, dark, plate-like cutaneous scales with minimal erythema, represents one end of this spectrum and NBCIE, characterised by erythroderma with overlying fine, white scales, represents the other end. However, patients may display cutaneous finding of both subtypes, making it difficult to classify them either as NBCIE or LI, as a patient may lie in the middle of the wide ARCI spectrum.

Russell *et al* mapped the LI locus to the long arm of chromosome 14 (14q11), showed linkage to the *TGM1* gene and identified germline mutations in *TGM1* in patients with classic LI.[4, 5] Since then, > 70 unique *TGM1* germline mutations have been reported.[5–33] Approximately 35–40% of patients with ARCI have germline mutations in *TGM1*.[34] A founder mutation, IVS5-2A→G (also reported as A2526G or +3366A→G), has been identified in 80% of *TGM1* mutated alleles in patients with LI or NBCIE in the Norwegian population.[2] This mutation is also prevalent in families in North America with ARCI, accounting for 9.6% of *TGM1* mutated alleles.[29]

TGM1 consists of 15 exons and encodes a 90 kDa, 817-amino-acid protein called transglutaminase-1 (TGase-1).[35] TGase-1 is one of eight catalytic transglutaminases identified in humans and one of three transglutaminases found in the epidermis.[36] TGase-1 is a Ca^{2+}-dependent, membrane-bound enzyme that functions in the formation of the cornified cell envelope (CCE). The CCE, an insoluble 10–20-nm structure composed of proteins cross-linked by N^{ε}-(γ-glutamyl)lysine bonds, is laid down on the inside of the plasma membrane in keratinocytes in the stratum corneum. The CCE acts as a mechanical barrier and protects against water loss and infectious agents. TGase-1 is responsible for catalysing the critical N^{ε}-(γ-glutamyl) lysine cross-linking of ARP preserver proteins such as involucrin and loricrin during the formation of the CCE.[37, 38] Decreased to absent enzyme activity in TGase-1 has been reported in patients with germline mutations in *TGM1*.[6–9, 14, 17, 25, 32, 39, 40] Knockout *TGM1* mice present at birth with ARCI-like features, including erythematous, tight, translucent skin, reminiscent of a collodion membrane. Newborn $TGM1^{-/-}$knockout mice do not feed and consequently develop severe dehydration, dying within 4–5 hours of birth.[41]

ARCI is a genetically heterogenous disease and to date, germline mutations in five other genes besides *TGM1* have been identified in patients with ARCI. *ABCA12* encodes an ATP binding epidermal transporter involved in lipid trafficking.[42, 43] *ABCA12* mutations causing premature termination of the protein lead to harlequin ichthyosis,[44, 45] whereas missense mutations have been reported in patients with LI.[46] Germline mutations in *ALOX12B* and *ALOXE3*, which encode for epidermal lipoxygenases,[47] and in *Ichthyin*[48] and *CYP4F22*,[49] have also been associated with ARCI.

Because of the genetic heterogeneity and the rarity of the disorder, few genotype–phenotype association studies have been conducted. Some studies suggest that patients with ARCI *TGM1* mutations are associated with LI, although only some patients with NBCIE have *TGM1* mutations.[15, 29, 50, 51] In this study, we identified novel *TGM1* mutations and genotype–phenotype correlations in 104 patients with ARCI identified through the National Registry for Ichthyosis and Related Disorders in the US. This is the largest and most comprehensive study of ARCI patients with *TGM1* mutations to date.

METHODS

Patient ascertainment and study design

From 1995 to 2004, subjects with inherited scaling skin disorders were ascertained for enrolment in the National Registry for Ichthyosis and Related Disorders through announcements by the Foundation for Ichthyosis and Related Skin Types (FIRST), in dermatology journals, at national dermatology meetings and through recruitment letters mailed to members of the American Academy of Dermatology. Enrolment in the Registry included a telephone interview, medical record review and blood samples and/or buccal swabs.

Patients were enrolled in the Registry through their local dermatologist. All subjects were enrolled in a protocol approved by the University of Washington's institutional review board. Each patient or their guardian provided written informed consent/assent before participation.

Every participant had a dermatological evaluation and a standard clinical-information form was completed by their dermatologist. Patients or their guardians were mailed a 10-page questionnaire and a 45–60 minute telephone interview, based on the standardised questionnaire, was administered by the Registry coordinator, who was blinded to patients' *TGM1* mutation status. The telephone interview elicited information regarding various aspects of dermatological history, such as presence or absence of scales, erythema, sweating, temperature regulation, skin infections, ectropion, eclabium, alopecia, palm or sole involvement and specified areas of skin involvement. Each of these topics had accompanying questions regarding severity at its onset, currently or at its worst, using a scale of mild, mild/moderate, moderate, moderate/severe and severe. Patients were also asked about their family, personal (including eye problems), birth and treatment medical histories and their quality of life. Eye problems included ectropion, corneal erosions, scarring, impaired vision, glaucoma, cataracts and history of eye surgery.

In total, 610 patients were enrolled in the study, of whom 206 were diagnosed with ARCI. None of the patients had a diagnosis of bathing-suit ichthyosis. All 206 subjects with ARCI were invited to submit buccal swabs or blood for sequencing of *TGM1* and 104 (62 female, 42 male; 94 white, 10 Hispanic; average age at the time of interview 24.4 years, range 2 months to 72 years) provided buccal samples for analysis.

Mutation analysis

DNA was extracted from blood and/or buccal swabs according to standard procedures.[52] DNA from the submitted specimens was amplified by PCR for analysis of exons 2–15 of the *TGM1* gene and their flanking splice-sites. Bidirectional sequence was obtained for analysis and compared with the published gene sequence. DNA from at least 160 unrelated control individuals was examined for each disease-associated sequence variant.

Statistical analysis

Data collected from patients with ARCI addressed the following specific features: presence of collodion membrane, plate-like scales, erythema, lack of sweating, temperature regulation, skin infections, ectropion, eclabium, alopecia and palm or sole involvement. Analyses comparing two dichotomous parameters were performed using the Fisher exact test. The Mehta modification to this test was used to compare an unordered categorical parameter to a dichotomous parameter.[53] An exact Cochran–Armitage test for trend was used to determine the association between a dichotomous parameter and ordered categorical parameters.[54] Associations between two ordered categorical parameters, such as the number of mutations present and the level of severity of a clinical feature, were determined using an exact Jonckheere–Terpstra test for trend.[55] All p values were two-sided and were not adjusted for multiple comparisons. In view of the exploratory nature of the study and the large number of comparisons performed, only $p < 0.005$ was considered significant.

We performed a logistic regression analysis to determine whether the *TGM1* mutation status of patients with ARCI could be predicted by the presence of a combination of the following clinical variables: collodion membrane, ectropion, other eye problems, alopecia, plate-like scales, use of systemic retinoids and use of topical retinoids. Using the backward selection method, a model was developed.

Literature review

We performed an electronic literature search from 1994 to 2008 designed to capture all reported cases of ARCI with *TGM1* germline mutations. Cases were included in our review even if the method of mutation detection was not included. In total, 36 articles were included.[2, 5–33, 40, 50, 51, 56–58]

RESULTS

TGM1 mutation analysis

Using direct sequencing, we identified *TGM1* mutations in 57 patients (55% *TGM1* mutation detection rate), giving a total of 103 mutated alleles.

Direct sequence analysis of the 14 coding exons and splice-site junctions of *TGM1* showed 44 different *TGM1* germline mutations including 22 missense, 10 nonsense, 8 frameshift and 4 splice-site mutations (Figure 1A). Of these 44 mutations, 22 were novel, including 8 missense (R126H, Y134C, I243S, G291D, N330H, S331P, R396H, Y544C), 7 frameshifts (c.343insT, c.566dupG, c.882_888delCCACGGG, c.932dupC, c.1107dupA, c.1331insA, c.1421insA), 4 nonsense (W193X, Q227X, L235X, L697X) and 3 putative splice-site mutations (IVS5+2T→C (also known as c.876+2T→C), IVS8+5G→C, IVS11+1G→A) (fig 1A). The identified *TGM1* mutations affected all exons, with the exception of exons 1, 13 and 15 (fig 1A). The most 5′ mutation (S42Y) occurred in exon 2 and the most 3′ mutation (L697X) occurred in exon 15.

In total, 65% (37/57) of patients (48 mutated alleles) had at least one missense mutation. Of the 37 patients with missense mutations, 27 (73%) had at least one mutation in the catalytic core, 9 had mutations in the β-sandwich domain only and 1 had a mutation in the anchoring domain. The catalytic core was the most commonly mutated protein domain, accounting for 67% (32/48) of all missense mutated alleles, the β-sandwich was second with 31% (15/48), followed by the anchoring domain with one mutation. No missense mutations were found in either of the β-barrel domains. Of patients who had at least one missense mutation, 54% (20/37) had at least one missense mutation at an arginine residue. Of the six codons that encode arginine, four contained CpG dinucleotides (CGG, CGC, CGT, CGA). We found

that 54% (12/22) of *TGM1* missense mutations in patients with ARCI were within CpG dinucleotides and 92% (11/12) of these mutations were G→A or C→T transitions. Overall, arginine residues were mutated in 39% (22/57) of all patients with ARCI with *TGM1* mutations. Of the 57 patients with *TGM1* mutations, 19% (11/57) had at least one nonsense mutation and 12% (7/57) had at least one frameshift mutation.

Overall, the most common *TGM1* mutation was the splice-site mutation IVS5-2A→G (also referred to as c.877-2A→G and A2526G), which was present in 39% (22/57) of patients (7 homozygous and 22 compound heterozygous). *TGM1* mutations in exon 3 were found in 28% (16/57) of patients (18 mutated alleles). The missense mutation R142H was the most common mutation in exon 3 and the second most common mutation in our cohort overall (5 patients with 7 mutated alleles).

Sequence alignments

To evaluate the sequence conservation in TGase-1, we ran a BLAST analysis of the amino acid sequences of human, dog, mouse TGase-1 and found that TGase-1 is highly conserved among species (fig 2).[59] We found that human and mouse TGase-1 had 88% identity and 91% similarity and human and dog TGase-1 had 89% identity and 92% physical and chemical similarity. Our study showed that missense mutations in *TGM1* occurred at evolutionarily conserved residues across species. We identified 22 missense mutations in *TGM1* in 20 different residues in TGase-1. Most (95%; 19/20) missense mutations identified in patients with ARCI in this study occurred at residues identical to those of dog and mouse TGase-1(fig 2).

Genotype–phenotype analyses

Patients with autosomal recessive congenital ichthyosis with *TGM1* mutations *versus* patients without *TGM1* mutations—Patients with ARCI with *TGM1* mutations (n = 57) were significantly associated with increased probability of collodion membrane at birth (p = 0.006), alopecia (p = 0.001), plate-like scales (p = 0.005), ectropion (p = 0.001) and systemic retinoid use (p = 0.002) compared with patients with ARCI without *TGM1* mutations (n = 47) (table 1). We found that subjects without *TGM1* mutations reported only slightly higher frequency of erythema (77%) compared with ARCI subjects with a *TGM1* mutation (67%), but they were not significantly different (p = 0.029) (table 1).

There was a trend towards differences between patients with ARCI with and without *TGM1* mutations in severity of ectropion at its onset (p = 0.019) and its worst (p = 0.034) (table 1). More patients with *TGM1* mutations reported moderate/severe or severe ectropion at its onset and at its worst than did patients without *TGM1* mutations. Of 23 patients reporting moderate/severe or severe ectropion at onset, 83% had *TGM1* mutations compared with 17% without *TGM1* mutations (table 1).

Patients with autosomal recessive congenital ichthyosis who had at least one mutation predicted to truncate transglutaminase *versus* those who had only missense *TGM1* mutations—Patients with ARCI who had only mutations predicted to truncate TGase-1 were more likely to have sweating abnormalities than were patients who had at least one *TGM1* missense mutation (p = 0.041) (table 2). More specifically, when asked about hypohidrosis, all 20 patients with mutations predicted to truncate TGase-1 reported hypohidrosis, compared with 76% of patients who had at least one missense mutation (p = 0.020). Similarly, we found a trend for increasing severity of overheating among patients who had only truncating mutations compared with patients who had at least one *TGM1* missense mutation (p = 0.022).

Patients with autosomal recessive congenital ichthyosis who had only mutations predicted to truncate transglutaminase-1 *versus* patients who had at least one *TGM1* missense mutation—Similar to the findings above, patients with ARCI who had at least one mutation predicted to truncate TGase-1 were significantly more likely to have moderate/severe or severe hypohidrosis at its onset ($p = 0.005$) or at its worst ($p = 0.001$) compared with patients who had only *TGM1* missense mutations (table 3). Of the patients reporting moderate/severe or severe hypohidrosis at its worst, 83% had at least one mutation predicted to truncate TGase-1 and the remaining 17% had only missense mutations. There was also a significant association between severity of overheating at its worst and the presence of at least one truncating TGase-1 mutation ($p = 0.0007$) (table 3). Of patients who reported moderate/severe or severe overheating at worst, 92% had mutations predicted to truncate TGase-1, and the remaining 8% had only missense mutations. Finally, we found that patients who had at least one truncating mutation were less likely to report eclabium than were patients with only missense mutations ($p = 0.007$) (table 3), but this association did not reach statistical significance.

Modelling results

Using a backward selection method and adjusting for the set of parameters which remain in the final model, we found that patients with a collodion membrane were 4.24 times (95% CI 1.58 to 11.39; $p = 0.004$) more likely to have a *TGM1* mutation, those with alopecia were 4.13 times (95% CI 1.49 to 11.5; $p = 0.007$) more likely to have a *TGM1* mutation and patients with eye problems were 3.60 times (95% CI 1.37 to 9.46; $p = 0.009$) more likely to have a *TGM1* mutation. Eye problems included ectropion, corneal erosions, scarring, impaired vision, glaucoma, cataracts and history of eye surgery. The specificity and sensitivity of the backward selection model for predicting who has a mutation, based on presentation of clinical features, were 76% and 69%, respectively.

Review of published reports of *TGM1* germline mutations

We reviewed cases with published *TGM1* germline mutations and general clinical characteristics associated with ARCI. The number of affected individuals in a family ranged from one to seven. Excluding the present study, 72 unique *TGM1* germline mutations have been previously reported, comprising 49 missense, 13 nonsense, 7 insertion/deletion and 2 putative splice-site mutations (fig 1A,B).[2, 5–16, 19–27, 29–33, 40, 50, 56–58] These previously published *TGM1* mutations affected all translated exons (215). Of the 72 *TGM1* germline mutations reported to date, only 22 mutations were also identified in the present study (fig 1A).[5, 10–17, 19, 20, 23, 29, 33] In combination with previous reports,[5–33] our 22 novel *TGM1* mutations expand the number of unique published *TGM1* mutations to 94, comprising 57 missense, 18 nonsense, 14 insertion/deletion and 5 splice-site mutations.

Excluding the present study, the hotspot mutation IVS5–2A→G was the most common single mutation previously reported to date, being found in 64 individuals from 58 ARCI families.[2, 11, 12, 14–17, 23, 26, 29, 57] Exon 3 was the most common coding sequence site for mutations, with 30 affected individuals from 23 unrelated ARCI families having *TGM1* mutations.[5, 8, 11, 15, 17, 20, 23, 25, 32, 57] The most 5′ and 3′ *TGM1* mutations occurred in exon 2 (c.125C→A (S42Y))[16] and the most 3′ mutation reported occurred in exon 15 (c. 2320C→T (Q774X))[13]

DISCUSSION

We identified three main genotype–phenotype correlations in the largest series to date of 104 patients with ARCI ascertained through the National Registry for Ichthyosis and Related Disorders in the USA. We found that *TGM1* mutations are a major cause of ARCI in

the USA, with 55% of patients with ARCI tested having germline *TGM1* mutations. We characterised 22 novel mutations and in combination with previous reports this study expands the number of identified *TGM1* germline mutations to 94.[2, 5–33, 40, 50, 51, 56–58] Our study showed that missense mutations in *TGM1* occur in highly conserved amino acids, suggesting the functional importance of this enzyme in evolution. Our investigation revealed that 40% of patients affected by *TGM1* mutations had mutations in TGase-1 arginine residues. In combination with the previous reports, this indicates that 41% of all *TGM1* mutations occur at arginine residues.[5–7, 11, 13, 15–17, 19, 20, 22–24, 28–30] Arginine is the most commonly mutated amino acid, accounting for 15% of all mutations in genetic diseases.[60] Arginine residues are very highly mutable due to the deamination of 5′-CpG dinucleotides of arginine codons.[61, 62] Four of the six codons that encode arginine contain CpG dinucleotides, thus partly explaining the tendency of the arginine codon to mutate.[61, 63] The deamination of CpG dinucleotides occurs due to methylation-mediated deamination of 5-methylcystosine in CpG dinucleotides.[61, 62] In phenylketonuria, arginine mutations also have the highest frequency of all *PAH* gene mutations.[63] Furthermore, it has been shown that specific arginine residues in keratin K-14 (K14-R125) and K-10 (K10-R156) are mutated at high frequency in patients with severe epidermal bullosa simplex and epidermolytic hyperkeratosis because both codons contain CpG dinucleotides.[64]

In contrast to most previous studies,[2, 15, 20, 51, 57] our large sample size and detailed clinical data allowed us to conduct a comprehensive genotype–phenotype investigation and identify four main genotype–phenotype associations. We found that patients with ARCI and *TGM1* mutations were significantly more likely to report the presence of (1) a collodion membrane at birth, (2) ectropion, (3) plate-like scales and (4) alopecia compared with patients without *TGM1* mutations. In general, our investigation suggests that patients with *TGM1* mutations were more severely affected than patients without *TGM1* mutations. These findings are consistent with the literature and confirm the previously observation that patients with *TGM1* mutations more commonly have collodion membrane at birth and ectropion compared with patients without *TGM1* mutations.[50] The fact that two independent groups identified similar significant genotype–phenotype correlations in different populations is significantly important.

In agreement with Ganemo *et al*,[50] we found that patients with ARCI with *TGM1* mutations reported alopecia more often than those without *TGM1* mutations. However, we found a strong correlation between patients with *TGM1* mutations and alopecia. It is possible that the disparate findings may be related to specific *TGM1* mutations within American, Swedish and Estonian populations. TGase-1 is expressed in the cortex and medulla cells as well as the outer and inner root sheath cells of normal hair follicles.[65] The abundant expression of TGase-1 suggests involvement of this enzyme in hair-follicle formation.[65] Therefore, mutations in *TGM1* may explain the alopecia present in our patients with ARCI.

We found that patients with ARCI who only had mutations predicted to truncate TGase-1 were more likely to report sweating abnormalities, hypohidrosis and overheating than were patients having at least one missense mutation. Similarly, patients who had at least one mutation predicted to truncate TGase-1 were more likely to develop hypohidrosis than were patients having only missense mutations. Mutations predicted to truncate TGase-1 (nonsense, frameshift and splice-site mutations) have been shown to result in drastically reduced to absent TGase-1 mRNA transcripts, protein levels or enzyme activity in the epidermis of affected people.[16, 39, 66] Mutant mRNA transcribed from genomic DNA of individuals with *TGM1* mutations predicted to truncate TGase-1 is likely to be degraded by nonsense-mediated decay (NMD), a surveillance mechanism that eliminates mRNAs with premature termination codons.[67] If NMD effectively eliminates the *TGM1* mutant mRNAs, mutation carriers with truncating mutations may show more phenotypic severity because of

the absence of TGase-1 protein. Hypohidrosis can result in overheating and heat stroke, which can result in a life-threatening situation. The pathophysiology of hypohidrosis in ARCI is not well understood, but obstruction of sweat glands by scale was initially suggested as the causative factor.[68] However, in 1998, Yoneda *et al* showed that normal secretory and intradermal eccrine duct cells contain abundant TGase-1 mRNA, challenging this hypothesis.[65] They hypothesised that hypohidrosis in patients with ARCI is due to dysfunction of TGase-1 in sweat glands and that TGase-1 may contribute to the reinforcement of the characteristic spiral architecture of the acrosyringia.[65] Several case series support our finding of an association between the presence of mutations predicted to truncate TGase-1 and hypohidrosis and/or heat intolerance.[15, 17, 69] Consistent with our findings, Yotsumoto *et al* reported a Japanese patient with ARCI and homozygosity for *TGM1* missense mutations, who had reduced TGase-1 enzyme activity but normal sweating.[32]

Based on our findings we have developed the first model to predict the likelihood a *TGM1* mutation in patients with ARCI with certain clinical features. Our model predicts that individuals born encased in a collodion membrane, with eye problems and/or alopecia are about four times more likely to test positive for a *TGM1* mutation by direct DNA sequencing. Our model may be clinically useful to dermatologists and geneticists in determining which patients with ARCI are more likely to have mutations in *TGM1*. However, this model remains to be validated in a separate population of subjects with ARCI in a future study.

In this study, IVS5-2A→G, the most common *TGM1* mutation, accounted for 28% of the *TGM1* mutated alleles. This mutation allele frequency is almost three times that previously reported (9.6%).[29] IVS5-2A→G affects the canonical splice acceptor site of intron 5, leading to two different consequences on *TGM1* mRNA processing.[2, 17, 29] It has been shown that 90% of transcripts had intron 5 retained and 10% of transcripts had an inserted G nucleotide.[29] This mutation has been reported in ARCI families with diverse ethnic and racial backgrounds.[12, 29, 57, 58] Shevchenko *et al* previously showed that the IVS5-2A→G splice-site mutation is common among North American patients, owing to a founder effect.[29] A common haplotype around *TGM1* was found among North American and Norwegian patients with ARCI with the IVS5-2A→G, supporting a possible common origin for this mutation.[29] In the Norwegian population, where the incidence of ARCI is twice that of the North American population, IVS5-2A→G is also the most common ARCI-causing *TGM1* mutation, again owing to a founder effect.[2] In the middle of the 14th century, a bottleneck effect resulting from the bubonic plague reduced the Norwegian population by half,[29] which could account for the higher incidence of this mutation among Norwegians. Using the Luria–Delbruck method, it was estimated that the IVS5-2A→G mutation originated in Germany and was introduced in the Norwegian population around 1000–1100 AD.[29] It was also hypothesised that German families from Westphalia immigrating to the USA introduced this mutation to the North American population.[29]

This study is the largest genetic investigation of *TGM1* mutations in patients with ARCI to date, providing us with statistical power to detect genotype–phenotype associations. Owing to the rarity of ARCI, previous studies on ARCI have been limited to case reports or small case series. The large sample size allowed us to detect significant differences between clinical phenotype and type or location of *TGM1* mutation.[50] Our study was registry-based, which avoids the ascertainment and assessment biases associated with hospital and clinical series. Other strengths of our study are the comprehensive clinical and genetic data that were available from our large cohort of subjects with a rare condition.

Our study has some limitations. Despite the large size of our cohort of patients with ARCI, the sample size did not have sufficient power to detect effects by stratification on analyses. Because some participants were referred by their dermatologist, our study population could be biased towards patients who sought medical care or had symptoms. Recall bias is a limitation in studies requiring patients or their parents to remember specific dates and symptoms at birth, infancy or in the past. As this was a cross-sectional study, young *TGM1* mutation carriers may develop additional symptoms in the future. Prospective follow-up of patients with ARCI with *TGM1* mutations will be required to evaluate this issue further and determine its effect on the results.

In conclusion, our study expands the *TGM1* mutation spectrum and shows that, despite genetic and phenotypic heterogeneity in ARCI, *TGM1* is the main susceptibility gene for ARCI. Our comprehensive investigation identified various genotype–phenotype associations and validated some of the findings of a previous ARCI study.[50] The high frequency of mutated TGase-1 arginine residues in patients with ARCI identified in our study may be due to the deamination of 5′-CpG dinucleotides. Based on our results, we have developed the first model to predict the likelihood that patients with ARCI with specific clinical features have a *TGM1* mutation. These findings could provide guidance for the clinical evaluation and practical utility in genetic testing of patients with ARCI.

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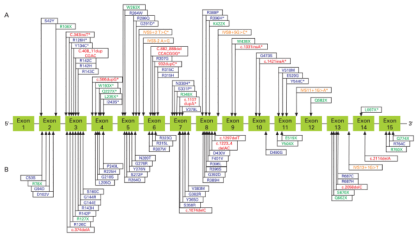

Figure 1.

TGM1 mutations reported in this investigation and previous studies. (A) The 44 germline *TGM1* mutations identified. Asterisks denote the 22 novel mutations identified; mutations identified in both this and previous studies are not marked with asterisks. (B) The 50 previously reported germline *TGM1* mutations that were not identified in the present study. Blue, missense; green, nonsense; red, frameshifts, orange, putative splice site.

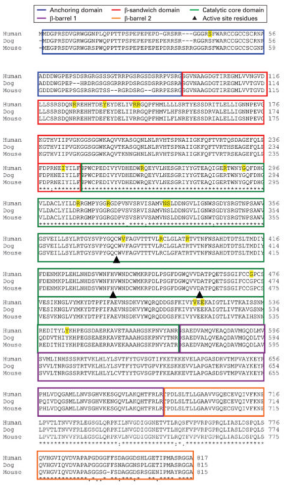

Figure 2.

TGase-1 alignments showing amino acid sequence comparisons among human, dog and mouse. Boxes indicate amino acid residues within the TGase-1 domains. Yellow characters indicate the amino acid positions where missense mutations were identified. Triangles represent active site residues (Cys^{377}, His^{436}, Asp^{459}). The 22 *TGM1* missense mutations identified in our cohort are marked.

Table 1

Analyses of clinical features comparing patients with and without at least one *TGM1* mutation

	Patients, % (n/total n)		
Clinical features or treatment	**Without *TGM1* mutations (n = 47)**	**With at least one *TGM1* mutation (n = 57)**	**p Value**
Collodion membrane	44 (18/41)	73 (38/52)	0.006*
Alopecia	21 (10/47)	53 (30/57)	**0.001***
Plate-like scale	64 (30/47)	88 (50/57)	**0.005***
Ectropion	60 (28/47)	88 (50/57)	**0.001***
Severity at onset‡			
1 or 2 (n = 28)	50 (14)	50 (14)	0.019‡
3 (n = 23)	39 (9)	61 (14)	
4 or 5 (n = 23)	17 (4)	83 (19)	
Severity at worst‡			
1 or 2 (n = 26)	50 (13)	50 (13)	0.034‡
3 (n = 20)	40 (8)	60 (12)	
4 or 5 (n = 30)	23 (7)	77 (23)	
Eye problems	40 (19/47)	65 (37/57)	0.018*
Erythema	77 (36/47)	67 (38/57)	0.29*
Fine scales	96 (45/47)	89 (51/57)	0.29*
Eclabium	15 (7/47)	23 (13/57)	0.33*
Temperature regulation	96 (45/47)	91 (52/57)	0.45*
Hearing problems	51 (24/47)	58 (33/57)	0.55*
Hypohidrosis	89 (42/47)	84 (48/57)	0.57*
Soles	94 (44/47)	89 (51/57)	0.51*
Palms	89 (42/47)	84 (48/57)	0.57*
Skin infections	51 (24/47)	51 (29/57)	1.0*
Treatment			
Systemic retinoids	13 (6/46)	42 (24/57)	**0.002***
Topical retinoids	25 (11/44)	51 (27/53)	0.012*
Systemic steroids	7 (3/44)	0 (0/53)	0.090*

*Fisher's exact test.

‡Cochran–Armitage exact trend test.

‡Severity scale.

Severity: 1 or 2, mild or mild/moderate; 3, moderate; 4 or 5, moderate/severe or severe.

Bold p values indicate significance (p<0.005).

Table 2

Analyses of clinical features comparing patients who had only mutations predicted to truncated TGase-1 versus patients who had at least one *TGM1* missense mutation

	Patients, % (n/total n)		
Clinical features	**With mutations predicted only to truncate TGase-1 (n = 20)**	**With at least one *TGM1* missense mutation (n = 37)**	**p Value**
Sweating abnormality	100 (20/20)	78 (29/37)	0.041*
Hypohidrosis	100 (20/20)	76 (28/37)	0.020*
Overheating			
Severity at worst‡			
1 or 2 (n = 11)	9 (1)	91 (10)	0.022†
3 (n = 16)	37 (6)	63 (10)	
4 or 5 (n = 24)	50 (12)	50 (12)	

*Fisher's exact test.

†Cochran–Armitage exact trend test.

‡Severity: 1 or 2, mild or mild/moderate; 3, moderate; 4 or 5, moderate/severe or severe.

Table 3

Analyses of clinical features comparing patients who had at least one mutation predicted to truncate TGase-1 versus patients who had only *TGM1* missense mutations

	Patients, % (n/total n)		
Clinical features	**With at least one mutation predicted to truncate TGase-1 (n = 37)**	**With *TGM1* missense mutations only (n = 20)**	**p Value**
Hypohidrosis	92 (34/37)	70 (14/20)	0.054*
Severity at onset			
1 or 2 (n = 2)	0	100 (2)	0.005†
3 (n = 8)	50 (4)	50 (4)	
4 or 5 (n = 34)	82 (28)	18 (6)	
Severity currently			
1 or 2 (n = 2)	0	100 (2)	0.027†
3 (n = 12)	67 (8)	33 (4)	
4 or 5 (n = 30)	80 (24)	20 (6)	
Severity at worst			
1 or 2 (n = 2)	0	100 (2)	0.001†
3 (n = 8)	37 (3)	63 (5)	
4 or 5 (n = 35)	83 (28)	17 (6)	
Overheating	97 (36/37)	80 (16/20)	0.047*
Severity at onset‡			
1 or 2 (n = 12)	42 (5)	58 (7)	0.039†
3 (n = 28)	79 (22)	21 (6)	
4 or 5 (n = 9)	78 (7)	22 (2)	
Severity at worst‡			
1 or 2 (n = 11)	36 (4)	64 (7)	0.0007†
3 (n = 16)	63 (10)	37 (6)	
4 or 5 (n = 24)	92 (22)	8 (2)	

*Fisher's exact test.

†Cochran–Armitage exact trend test.

‡Severity: 1 or 2, mild or mild/moderate; 3, moderate; 4 or 5, moderate/severe or severe.

Bolded p values indicate significance (p<0.005).