TO THE EDITOR

Olmsted syndrome (OS; MIM614594) is a hereditary disorder characterized predominantly by palmoplantar keratoderma and periorificial keratosis. Frequent additional features include intolerant itching on palms and/or soles, diffuse alopecia, keratosis pilaris, onychodystrophy, and digital autoamputation (Mevorah et al., 2005). Recently, mutations in transient receptor potential vanilloid type 3 (TRPV3) (Lin et al., 2012) and membrane-bound transcription factor protease site 2 (MBTPS2) (Haghighi et al., 2013; Wang et al., 2014) have been associated with autosomal-dominant and X-linked OS, respectively. In TRPV3-associated OS, we previously demonstrated that certain missense mutations led to overactivation of the TRPV3 channel, as an underlying pathogenesis of OS (Lin et al., 2012). In addition to the autosomal-dominant trait, autosomal-recessive OS caused by homozygous or compound heterozygous mutations in TRPV3 has been described in two families (Duchatelet et al., 2014; Eytan et al., 2014). Herein we report a TRPV3-associated OS family of semidominant inheritance.

The proband was a 6-year-old boy of Chinese Han ethnicity. He was noted to have itchy palmoplantar keratoderma affecting the weight-bearing areas of his soles at the age of 1 year. At 2 years old, mild keratosis developed on both corners of his mouth and perianal areas. He was diagnosed with OS and was started on oral acitretin 0.5 mg/kg/day. One year later, hyperkeratosis relieved greatly and periorificial keratosis disappeared. Acitretin was then ceased for fear of adverse effects of skeletal abnormality. Hyperkeratosis gradually aggravated to involve his palms with mild flexion contraction of fingers (Figure 1b and c), whereas his periorificial areas remained spared. In his fourth year of life, he started to experience paroxysmal burning pain and redness on his distal extremities (Figure 1d). The erythromelalgia was flared by exercise or warmth, relieved after rest or cooling, and became persistent later that required oral carbamazepine to control the symptoms. Four additional family members, including the proband’s father, developed asymptomatic, callus-like focal plantar keratosis only on the weight-bearing points with onset ages ranging from 7 to 10 years (Figure 1a, e and Supplementary Figure S1 online). No mutilation or periorificial keratoderma was found. The proband’s mother and sister were phenotypically normal.

We extracted genomic DNA from peripheral blood after written informed consent was obtained in adherence to the Declaration of Helsinki Principles. This study was approved by the Clinical Research Ethics Committee of the Peking University First Hospital. TRPV3 coding exons and their flanking regions were amplified and directly sequenced. Four additional family members, including the proband’s father, developed asymptomatic, callus-like focal plantar keratosis only on the weight-bearing points with onset ages ranging from 7 to 10 years (Figure 1a, e and Supplementary Figure S1 online). No mutilation or periorificial keratoderma was found. The proband’s mother and sister were phenotypically normal.

We extracted genomic DNA from peripheral blood after written informed consent was obtained in adherence to the Declaration of Helsinki Principles. This study was approved by the Clinical Research Ethics Committee of the Peking University First Hospital. TRPV3 coding exons and their flanking regions were amplified and directly sequenced. Two heterozygous mutations, c.1702G>T and c.643+1G>T, in TRPV3 were detected in the proband (Figure 1f and g), which were absent in the 1000 Genome Project, National Heart, Lung, and Blood Institute Exome Sequencing Project (NHGRI ESP), Exome Aggregation Consortium databases, or 200 ethnically matched normal individuals. Mutation c.643+1G>T was from the mother and c.1702G>T, which led to substitution of glycine by cysteine at position 568 (p.G568C), was of paternal origin and present in all the individuals with plantar keratosis (Figure 1a) and absent in unaffected family members. In silico mRNA splicing prediction of mutation c.643+1G>T showed abolishment of the canonical donor splice site of exon 6, which was confirmed by reverse-transcription PCR using RNA extracted from the dorsal skin of the proband’s feet (Supplementary Figures S2 and S3 online, primers are available in Supplementary Table S1 online). The results showed that a cryptic donor splice site located at position c.643+2953 was used. Such alternative splicing caused insertion of a 2953-bp intronic sequence downstream of the initiation of intron 6 (Figure 1h, i and Supplementary Figure S3), which resulted in a new premature stop codon p.G215Vs*82. The same splicing pattern was detected in the mother’s skin, but not in normal control skin. To assess the consequence of the splicing mutation, we performed real-time quantitative PCR using the patient skin RNA (primers are available in Supplementary Table S2 online). Our data showed a reduction of approximately 50% of total TRPV3 mRNA in the proband, compared with normal control skin (Supplementary Figure S4 online), indicating nonsense-mediated mRNA decay.

As the proband exhibited more severe clinical presentation with earlier onset than the other affected individuals harboring single mutation c.1702G>T, we sought to elucidate the underlying mechanism by assaying the electrophysiological properties of the mutant. We introduced the c.1702G>T mutation at the corresponding site of the human TRPV3 cDNA, and then inserted either wild-type (WT) or mutant cDNA into the pEGFP-N1 plasmid (Clontech, Mountain View, CA). As the TRPV3 channel is a tetramer where four subunits are symmetrically arranged around the ion conduction pore, the subunit composition and functional properties of individual channels vary with TRPV3 allele combinations. We assumed that the proband had only one mutant allele

Abbreviations: 2-APB, 2-aminoethoxydiphenyl borate; OS, Olmsted syndrome; TRPV3, transient receptor potential vanilloid type 3; WT, wild-type

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(G568C) expressing TRPV3 subunit, whereas the other individuals with heterozygous mutation c.1702G>T had equal amount of G568C and WT TRPV3 subunits. To mimic the above situations, we recorded currents from HEK293T cells transiently expressing WT TRPV3 or TRPV3-G568C (G568C) separately, or together (WT plus G568C), via the patch-clamp technique as described previously (Cao et al., 2012) (Figure 2a).

First, we tested the voltage-dependent activation of the TRPV3 channel, which has an important role in TRP channel gating (Voets et al., 2004). When exposed to voltage steps, cells expressing WT showed only small outwardly rectifying currents (Figure 2b), whereas cells expressing WT plus G568C displayed larger outwardly rectifying currents than those expressing WT alone (Figure 2c and e). Remarkably, cells expressing G568C produced the largest outward current with an inactivation-like behavior during depolarization, and had an additional large inwardly rectifying current at negative voltages, implying a constitutively active status at resting membrane potentials (Figure 2d and e). Next, we measured ligand-induced TRPV3 activation by applying 2-aminoethoxydiphenyl borate (2-APB) at concentrations from 1 mM to 1 mM (Figure 2f–i) (Chung et al., 2005). 2-APB activated TRPV3 currents of WT in a concentration-dependent manner with an EC50 value of 65.2 ± 3.4 μM at +80 mV (Figure 2f and i), whereas WT plus G568C showed a 3-fold EC50 shift of 2-APB-dependent activation, with the EC50 being 19.2 ± 4.5 μM (Figure 2g and i). At an even lower concentration (below 100 μM), 2-APB induced measurable currents in cells transfected with G568C alone, with the EC50 value of 5.7 ± 1.6 μM at +80 mV, which was more than 10-fold shift of 2-APB-evoked activation (Figure 2h and i). Previously, it was shown that other TRPV3 mutations such as G573S or G573C were constitutively active and could not be further activated by 2-APB (Lin et al., 2012; Xiao et al., 2008). Although not as robust as in the mutants at position 573, the activation of G568C was desensitized when the 2-APB concentration reached 1 mM (Figure 2h and i). This phenomenon was only observed when the TRPV3 channel was potently activated (Chung et al., 2005; Hu et al., 2009).

Taken together, our results showed that G568C displayed a dramatic gain-of-function property, which could be partially rescued by coassembly with WT subunits.

Semidominant inheritance has been observed in a few genodermatoses, for example, ichthyosis vulgaris (Smith et al., 2006), pachyonychia congenita (Wilson et al., 2010), and epidermolytic ichthyosis (Nousbeck et al., 2013). Herein we report a case of TRPV3-associated OS of semidominant inheritance. We also demonstrated that the clinical severity in the family closely correlated with the electrophysiological properties of the TRPV3 channels. The proband, who is likely
to express G568C homomers, showed relatively severe phenotype, whereas the other affected family members who express WT-G568C heteromers had only mild manifestations, probably due to rescue effects of the WT subunits. This correlation can be supported by significantly more severe, mutilating palmoplantar keratoderma phenotype (flexion, constriction, and autoamputation of digits) caused by TRPV3 G573S or G573C mutations, in which our electrophysiological studies showed a constitutively active channel property with insensitivity to 2-APB (Lin et al., 2012).

A recent report of a French family with TRPV3-related OS showed that a heterozygous carrier of G568C mutation was clinically normal (Duchatelet et al., 2014a), in contrast to mild, late-onset, focal plantar keratosis in our study. This phenotypic discrepancy indicated that factors other than TRPV3 mutations might also play a role in OS pathogenesis. Our case also developed erythromelalgia, a condition that has been reported elsewhere (Duchatelet et al., 2014a, 2014b). Interestingly, one of two previous cases was compound heterozygous for a splice site mutation and the G568C mutation identified in our patient. Our results further confirmed that certain mutations in TRPV3 could lead to erythromelalgia in patients with OS. Future studies are required to address the association between OS and erythromelalgia in the context of TRPV3 mutations.

CONFLICT OF INTEREST
The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL
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Figure 2. Electrophysiological studies on transient receptor potential vanilloid type 3 (TRPV3) channels of wild-type (WT), G568C mutant, and WT plus G568C. (a) Model of the TRPV3 channel containing WT subunit (blank), G568C mutant (hatched), and WT plus G568C. (b–d) Representative inside-out recordings from cells transfected with WT, G568C mutant, and WT plus G568C. Patches were held at 0 mV, steps from −160 to 240 mV in an increment of 20 mV for 300 ms, and then back to −100 for 100 ms. (e) Respective current density-voltage plots of steady-state currents from b–d. (f–h) Representative current traces at −80 mV were recorded from inside-out patches facing solutions at various concentrations of 2-aminoethoxydiphenyl borate (2-APB). (i) 2-APB dose-response curves of WT, G568C mutant, and WT plus G568C. (j) Comparison of EC50 values of WT, G568C mutant, and WT plus G568C. n = 3–5. Statistical analyses were performed using Student’s t-test. *P < 0.05; **P < 0.01.
Genetic Reversion via Mitotic Recombination in Ichthyosis with Confetti due to a KRT10 Polyalanine Frameshift Mutation

TO THE EDITOR

Ichthyosis with confetti (IWC) is an autosomal dominant disorder of keratinization that is exceedingly rare, with approximately 40 cases reported. Although patients generally demonstrate ichthyosiform erythroderma at birth, the disorder is defined by the hundreds of confetti-like white spots that appear in childhood and grow in size and number over time (Choate and Milstone, 2015; Guerra et al., 2015). The histopathology of the red skin surrounding the macules shows perinuclear vacuolization, loss of the granular layer, and parakeratotic hyperkeratosis, whereas the white, revertant macules are histologically normal (Choate and Millstone, 2015).

The mutations identified in IWC to date arise de novo and cause frameshift deletions affecting the carboxyl tail of keratin 10 (K10) or keratin 1 (K1), causing type I IWC (IWC-I) and type II IWC (IWC-II), respectively (Choate et al., 2010, 2015). Prior investigation of seven independent IWC-I probands identified distinct mutations causing entry into the same aberrant reading frame invariably replacing the endogenous glycine-rich tail of K10 with a polyarginine sequence (Choate et al., 2010; Guerra et al., 2015). In IWC-II, the K1 glycine tail is maintained, but the final 22 residues of the end domain are replaced with a novel 30 amino acid, non-repeating sequence (Choate et al., 2015).

Immunohistochemistry of K10 in IWC-I shows mislocalized K10 in aggregates within the nucleolus, with the corresponding decrease in cytosolic intensity; K1 in IWC-II mislocalizes to the nucleus, along with perinuclear collapse of the cytokeratin network (Choate et al., 2010, 2015). In contrast, the white macules in both subtypes not only demonstrate correct cytosolic localization of their respective keratins, but also represent independent copy-neutral loss of heterozygosity events, in which the heterozygous mutant haplotype is lost without aberrations in chromosomal copy number. Via single nucleotide polymorphism (SNP) genotyping, the loss of heterozygosity track was found to span the proximal q arm of chromosome 17 (IWC-I) or chromosome 12 (IWC-II) to the telomere, consistent with genetic reversion via mitotic recombination, a DNA break-induced event that is otherwise rare on a per cell basis (Choate et al., 2010, 2015; O’Keefe et al., 2010).

Given that nucleolar K10 is unique to IWC and all patients with IWC-I invariably express polyarginine K10, it was hypothesized that the mislocalization results from gain of the arginine-rich motif, as many RNA-binding proteins utilize their arginine-rich motif to interact with the phosphate backbone of RNA; mutant K10 would similarly associate with and aggregate within the ribosomal RNA-rich environment of the nucleolus (Choate et al., 2010; Draper, 1999). However, direct interaction between mutant K10 and ribosome

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Polyalanine Keratin 10 in Ichthyosis with Confetti

REFERENCES


Duchatelet S, Guibbal L, de Veer S, Fraitag S. Michelin star arise de novo and cause frameshift (Choate and Milstone, 2015).


