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Application of topical gentamicin ointment in the treatment of Nagashima-type palmoplantar keratosis in children with a nonsense mutation

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ABSTRACT

Importance: Nagashima-type palmoplantar keratosis (NPPK) is a hereditary dermatosis mostly caused by a nonsense mutation in *SERPINB7*. Despite the increasing interest in readthrough gentamicin treatment of NPPK, clinical evidence for this treatment is limited.

Objective: This study aimed to provide further evidence for the use of topical gentamicin in the treatment of NPPK in children with nonsense mutations.

Methods: We designed a bilaterally controlled study of topical gentamicin ointment. Children diagnosed with NPPK carrying nonsense mutations were enrolled in this study. A 0.1% gentamicin ointment was applied to one hand and an emollient to the other for 3 months. A bilateral comparison of the visual analog scale scores for clinical manifestations and safety was performed.

Results: Ten children with NPPK were included in this study. In comparison with the emollient side, the topical gentamicin side showed significant improvements in hyperkeratosis, erythema, maceration, and desquamation after 1 and 3 months of treatment ($P < 0.05$). However, hyperhidrosis and odor did not improve significantly. No adverse events were observed during the systemic safety monitoring examinations.

Interpretation: Topical gentamicin ointment showed good safety in the treatment of NPPK with nonsense mutations, indicating that it is a promising therapeutic choice in children with NPPK.

KEYWORDS

Children, Gentamicin, Nagashima-type palmoplantar keratosis, Nonsense mutation, Readthrough treatment

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INTRODUCTION

Palmoplantar keratoderma (PPK) is the term used to refer to a heterogeneous group of inherited disorders characterized by hyperkeratosis of the palms and soles. Nagashima-type palmoplantar keratosis (NPPK; MIM #615598) is a form of diffuse non-epidermolysis PPK that is the most common type of PPK in Asian populations.[1] NPPK is characterized by nonprogressive, mild erythema with hyperkeratosis on palms and soles that extends to the dorsal surfaces, inner wrists, ankles, and the Achilles tendon area, showing a "transgressive" appearance. The accompanying symptoms include maceration (whitish spongy changes after water exposure), desquamation, hyperhidrosis, and odor.[2] NPPK is defined as a rare disease by the World Health Organization definition, with prevalence rates of approximately 1.2 and 3.1 per 10 000 people in Japan and China, respectively.[3] It is an autosomal recessive disorder caused by mutations in *SERPINB7*, which encodes a serine protease inhibitor expressed in granular layer cells.[3] NPPK has mostly been reported in Japanese and Chinese populations and has recently been reported in Finland.[4] Although the cases were distributed across different countries, the nonsense mutation c.796C>T (p.Arg266Ter) of *SERPINB7* was the most frequent and the founder mutation.[2]

Currently, there is no specific curative treatment for NPPK. Previous treatments were symptomatic and helped reduce the thickness of the lesions and the redness to a lesser degree. Topical agents used for the lesions include emollients, calcipotriol (a vitamin D3 analog), salicylic acid, urea, and retinoids, which usually have unsatisfactory and transient effects. Systemic treatments are limited in pediatric patients. Our previous study reviewed new treatment advances for genetic diseases caused by nonsense mutations.[5] Gentamicin, a classical aminoglycoside antibiotic, has the potential to "bypass" the premature termination codons (PTCs) in nonsense mutations, thereby restoring full-length functional proteins and attenuating clinical manifestations. In 2017, Ohguchi et al.[6] first used a 0.1% gentamicin ointment to treat five patients with NPPK and reported good efficacy. Since >90% of patients with NPPK carry the nonsense mutation c.796C>T,[6,7] readthrough therapy is a promising therapeutic choice for NPPK. However, the evidence for this therapy is limited, especially in the pediatric population. In this study, we aimed to verify the efficacy and safety of a 0.1% gentamicin ointment in the treatment of children with NPPK caused by nonsense mutations.

METHODS

Ethical approval

In this open-label, bilaterally controlled study of topical gentamicin ointment, the study protocol was approved by the Independent Ethics Committee of Beijing Children's Hospital ([2022]-E-122-Y) and was registered in the Chinese Clinical Trial Register System (registration number: ChiCTR2300069011). Written informed consent was obtained from the parents for their children's participation in the study and the off-label use of gentamicin.

Participants

The inclusion criteria for this study were as follows: (1) diagnosis of NPPK on the basis of both clinical manifestations and genetic confirmation, with nonsense mutations in either one or two alleles, (2) stable vital signs, and (3) provision of informed consent by the patient or the patient's guardian. The exclusion criteria for this study were as follows: (1) allergy to gentamicin sulfate; (2) presence of other chronic concomitant diseases or infectious diseases; (3) impairment of VIII nerves, mutations in the deafness genes, including *GJB2*, *GJB3*, mitochondrial 12S ribosomal RNA (rRNA), *SLC26A4* (PDS), or pre-existing auditory impairment; (4) abnormal electrocardiography, chest radiography, or ultrasound findings; (5) abnormal liver and/or renal function; and (5) other unsuitable conditions or poor compliance.

A 0.1% gentamicin sulfate ointment was prepared by Beijing Children's Hospital Pharmacy. Patients were instructed to apply the 0.1% gentamicin ointment on one hand and emollient alone as a control on the other hand. The side that received each topical application was randomly assigned. The amount of ointment applied complied with the fingertip unit rule.[8] The treatment lasted for three months, with follow-up visits conducted at 1-month intervals.

For the assessment of clinical efficacy, visual analog scale (VAS) scores for six different dimensions were recorded at follow-up visits conducted 1 and 3 months post-treatment. Clinical symptoms in six dimensions, including hyperkeratosis, erythema, maceration, and desquamation, were evaluated by the investigators. Hyperhidrosis and odor were evaluated by the participants or their parents. The VAS scores ranged from 0 to 10 points, with higher scores representing more severe symptoms. To ensure safety, all patients underwent routine examinations, including routine blood, urine, renal, and liver function tests, before and after treatment. Considering potential ototoxicity concerns, hearing tests, including auditory brainstem response (ABR), steady-state auditory evoked potential (ASSR), play audiometry (PA), otoacoustic emission (OAE), and tympanometry, were conducted before and after treatment (ABR and ASSR assessments were conducted in patients aged under 4 years; PA assessments were conducted in patients aged older than 4 years; while OAE and tympanometry assessments were completed in all children).

TABLE 1 General characteristics of the patients with Nagashima-type palmoplantar keratosis

Patients	Age	Sex	Accompanied with AD	Gene	Mutation (allele1/allele2)
1	1 y 1 m	Female	Yes	*SERPINB7*	c.796C>T (homo)
2	15 y	Male	Yes	*SERPINB7*	c.796C>T/c.522dupT
3	1 y 3 m	Female	Yes	*SERPINB7*	c.796C>T (homo)
4	2 y	Male	Yes	*SERPINB7*	c.796C>T (homo)
5	8 y 9 m	Female	No	*SERPINB7*	UK (heterozygous)[†]
6	3 y 2 m	Female	Yes	*SERPINB7*	c.796C>T (homo)
7	5 y 1 m	Male	Yes	*SERPINB7*	c.796C>T (homo)
8	4 y 9 m	Female	Yes	*SERPINB7*	c.796C>T (homo)
9	2 y 2 m	Female	No	*SERPINB7*	c.796C>T (homo)
10	10 m	Female	Yes	*SERPINB7*	c.796C>T/c.827T>C

[†]UK, unknown. This patient had previously undergone genetic screening at another hospital. Her doctor refused to provide details except for a *SERPINB7* heterozygous mutation carrying a classical nonsense mutation.
AD, atopic dermatitis.

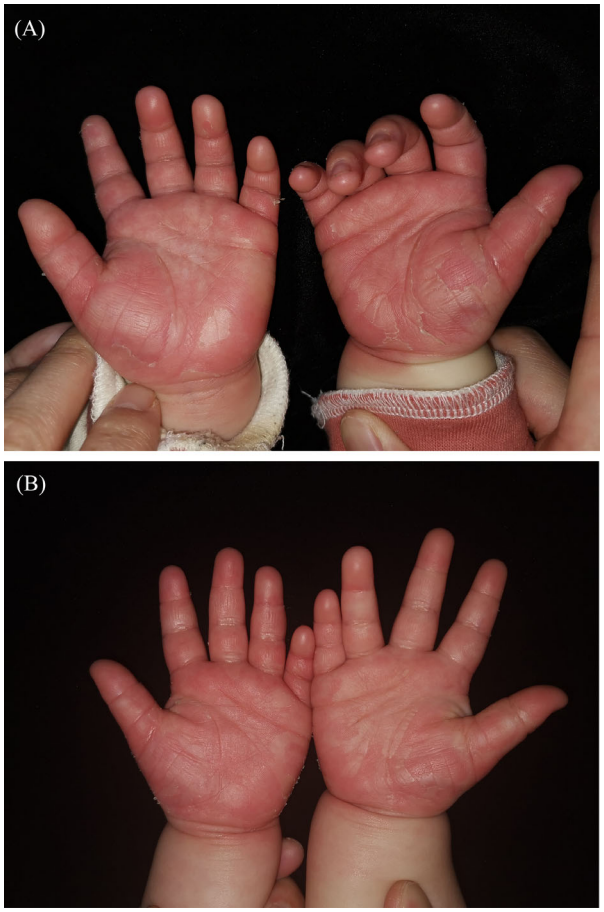

FIGURE 1 Clinical improvement in case 3 with NPPK after topical gentamicin. (A) Pre-treatment; (B) Post-treatment: The figure showed substantial improvements in hyperkeratosis, erythema, maceration, and desquamation after 3-month treatment on the topical gentamicin side (right hand) in comparison with the emollient side (left hand). NPPK, Nagashima-type palmoplantar keratosis.

Treatment-emergent adverse events (TEAEs) were reported throughout the study.

Statistical analysis

Data analyses were performed using the SPSS software (version 22.0; SPSS Inc., Chicago, IL, USA). Non-normally distributed continuous variables were expressed as median (interquartile interval). The Wilcoxon signed-rank test was used for the bilateral comparison of the VAS scores for different aspects. *P*-values less than 0.05 were considered to indicate statistical significance. Figure illustrations were generated using GraphPad Prism 5 software (San Diego, CA, USA).

RESULTS

A total of 10 NPPK patients were enrolled in the outpatient dermatology department of Beijing Children's Hospital between September 2020 and September 2021. General information and clinical characteristics of the patients are collated in detail (Table 1). The study population included seven females and three males aged between 10 months and 15 years, with a median (interquartile range) age of 32.0 (14.5, 72.0) months. A history of atopic dermatitis (AD) or an existing AD lesion was reported in 80% of the patients.

Changes in the VAS scores for each dimension before and after treatment were calculated. The VAS scores indicated significant improvements in four aspects, namely, hyperkeratosis, erythema, maceration, and desquamation, after both 1 and 3 months of treatment ($P < 0.05$). The assessments of hyperhidrosis and odor did not improve significantly after 1 and 3 months of treatment (Figure 1 and Table 2). The degree of improvement varied between 1 and 3 months of treatment. While the 3-month treatment

TABLE 2 Comparison of visual analog scale improvement in different aspects between the treatment groups

	1 m (bilateral)		3 m (bilateral)		1 m *vs*. 3 m (gentamicin)	
Symptoms	**Z-value**	***P*-value**	**Z-value**	***P*-value**	**Z-value**	***P*-value**
Hyperkeratosis	2.251	0.024*	2.739	0.006**	2.070	0.038*
Erythema	2.333	0.020*	2.460	0.014*	2.530	0.011*
Maceration	2.060	0.039*	2.232	0.026*	1.633	0.102
Desquamation	2.271	0.023*	2.271	0.023*	2.000	0.046*
Hyperhidrosis	1.000	0.317	1.890	0.059	1.633	0.102
Odor	1.890	0.059	1.841	0.066	1.342	0.180

*$P < 0.05$, **$P < 0.01$.
Abbreviations: m, month(s).

further improved hyperkeratosis, erythema, and desquamation on the gentamicin-treated side ($P < 0.05$), the therapeutic effects on maceration remained stable from 1 to 3 months ($P < 0.05$).

In the safety monitoring, the results of routine blood tests, renal and liver function assessments, and hearing tests before and after treatment were all within the normal ranges. No TEAEs were reported by parents or recognized by the investigators.

DISCUSSION

NPPK was first described in 1977, and studies on gentamicin readthrough treatment were initiated in 2017.[6] Over the four years since then, only two clinical studies on this treatment have been reported in the literature. The first was an investigator-blinded, randomized, bilaterally controlled clinical study conducted by Ohguchi et al.[6] in Japanese patients. In their study, topical 0.1% gentamicin in five patients with NPPK harboring c.796C>T mutations significantly improved hyperkeratosis, reduced scaling, and smoothened palmar skin surfaces on the gentamicin-treated side in comparison with the control side, although it did not improve erythema. The second study was conducted by Li et al.[9] in a Chinese population using two gentamicin ointment concentrations (0.1% and 0.3%). Their prospective, randomized, double-blinded, contralateral, and vehicle-controlled clinical trial reported a significant improvement in the symptoms of hyperkeratosis and foul smell, with a significant increase in quality of life, but without a difference in the effect on erythema.[9] Our study is the first to focus on the treatment efficacy of topical gentamicin for NPPK in a pediatric population. We conducted a comprehensive evaluation of the efficacy from six dimensions (hyperkeratosis, erythema, maceration, desquamation, hyperhidrosis, and odor), including both investigator- and patient-oriented outcomes. We found significant improvements in hyperkeratosis, erythema, maceration, and desquamation after 1 month of treatment, while no obvious improvement was observed in hyperhidrosis or odor. This result is partially consistent with the results reported by Ohguchi et al.[6] and Li et al.,[9] both of which reported attenuation of hyperkeratosis and desquamation. Although the degree of erythema did not improve in the previous studies, it was shown to improve in the present study. The foul odor was not significantly alleviated, which was inconsistent with the results reported by Li et al.[9] The aspects of maceration and hyperhidrosis improvement were first illustrated in our study and have not been evaluated elsewhere. Topical 0.1% gentamicin was well tolerated by these children, as they showed no clinical signs of adverse reactions or systemic toxicity in the blood, urine, and auditory tests. The consistency of the findings in these studies demonstrates the efficacy and safety of topical gentamicin in the management of NPPK with nonsense mutations. However, clinical trials with larger sample sizes are required to further explain the inconsistent results.

Gentamicin is a broad-spectrum aminoglycoside antibiotic. Both *in vitro* and *in vivo* studies have shown that aminoglycosides can enhance PTC readthrough by binding to rRNA and impairing codon-anticodon recognition at the aminoacyl-transfer RNA (tRNA) acceptor A site.[6,10] This could lead to mismatch of tRNA, reduce the fidelity of the normal translation process, and insert random amino acids instead, thus bypassing the PTC and restoring full-length functional proteins.[5] In recent years, topical gentamicin has been successfully used to treat many genodermatoses, including epidermolysis bullosa,[11,12] Hailey-Hailey disease,[13] hereditary hypotrichosis simplex of the scalp,[14] and xeroderma pigmentosum group C.[15] Nevertheless, the clinical use of aminoglycosides for genodermatosis remains limited, probably because of the rarity of this disorder and concerns regarding the systemic adverse effects of aminoglycosides. Our study provides evidence for the efficacy and safety of gentamicin in the treatment of NPPK.

Another interesting finding in our study was that 80% of the patients had coexisting AD. This phenomenon is consistent with our previous report in which *SERPINB7*

mutations were shown to lead to AD-associated NPPK.[16] Therefore, we believe that patients with *SERPINB7* mutations are more prone to developing AD. SERPINB7 may function as a skin barrier and as a susceptibility gene for AD. However, this hypothesis needs to be confirmed through further investigation.

The limitations of this study include the insufficient number of participants and the limited follow-up period. Moreover, the assessment of clinical manifestations was not blinded, which may have introduced a subjective bias. Thus, more blinded clinical studies with larger sample sizes and longer follow-up periods are required to provide further evidence of the efficacy of gentamicin readthrough treatment.

In conclusion, this study revealed the efficacy and safety of topical gentamicin for the treatment of NPPK in a pediatric population. Bilateral comparisons showed that gentamicin ointment significantly improved hyperkeratosis, erythema, maceration, and desquamation, but not hyperhidrosis and odor, indicating that gentamicin ointment is a promising therapeutic option for children with NPPK.

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CONFLICT OF INTEREST

None.

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