A novel homozygous mutation of GJB2—A new variant of keratitis-ichthyosis-deafness syndrome?

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INTRODUCTION
Keratitis-ichthyosis-deafness (KID) syndrome is a rare congenital disorder characterized by skin lesions, hearing loss, and vascularizing keratitis.1 Genetically, it is heterogeneous and may be caused by mutations in the connexin gene GJB2 and/or GJB6 encoding connexin proteins (Cx) 26 and 30, respectively.2 Most of these cases are sporadic,3 but those that appear in families are result of GJB2 mutations in an autosomal dominant pattern.3,4

Mutations in the GJB2 gene, located on chromosome13q12,5 are common in humans and can result in 8 different disease patterns with deafness alone, deafness and cutaneous manifestations, and skin involvement alone (Table I). In humans, there are more than 20 different connexin proteins (named by their molecular mass ranging from 26-62 kilodaltons), which make up gap junctions between the cytoplasm of adjoining cells.6,7 Gap junctions are intercellular channels that permit exchange of ions and small metabolites, nutrients, and signaling molecules between the cytoplasm of adjoining cells. Each gap junction consists of 2 halves, 1 each in adjacent cells, called a hemichannel. A hemichannel comprises 6 connexin proteins.

Virtually all cell types make more than 1 connexin at any given time. For example, keratinocytes express Cx26, Cx30, Cx30.3, Cx31, and Cx43.8,9 The co-expression of multiple connexins within a single cell diversifies the composition of the channels that can be assembled. On the other hand, one connexin protein type is produced by the cells of different tissues. For example, Cx26, Cx30, Cx31, and Cx43 are expressed in the epithelia of the inner ear, cornea, and the epidermis and its appendages. Therefore, genetic mutations of a single connexin gene can influence many types of channels and different tissues, contributing to the diversity of clinical diseases that can result.

PATIENTS AND METHODS
Three siblings from the southern region of Saudi Arabia were referred to our center in 2010 for workup and treatment of chronic cutaneous complaints. They had a history of recurrent abscesses and acneiform lesions since early childhood, scarring alopecia, congenital deafness, short stature, and scoliosis. All 3 were mute. The remaining 4 siblings were normal; their parents were first-degree relatives (first cousins).

Patient 1
A 26-year-old woman presented with history of multiple recurrent skin abscesses since the age of 6 months, mainly affecting the face and scalp resulting in multiple scars. At the age of 1, profound sensorineural hearing loss was diagnosed. She had multiple episodes of otitis media and conjunctivitis. Age of menarche was 18. On examination, there were multiple nodules, abscesses, and atrophic and hypertrophic scars on the face and back with scarring alopecia of the occipito-parietal region (Fig 1, A).
Pubic and axillary hairs were sparse with hypoplastic breasts (Fig 1, B). She had thoracolumbar scoliosis. She was 143 cm tall and weighed only 26.6 kg.

PATIENT 2
A 16-year-old girl presented with recurrent abscesses over her face, scalp, and back since infancy eventuating in scarring alopecia. She was deaf and mute. Age of menarche was 17 (a year after initial presentation). Menses were irregular from the beginning, and she has had amenorrhea for the last 2 years. She had history of recurrent blepharitis bilaterally, and the vision in her right eye deteriorated gradually from keratitis, neovascularization, and finally to corneal opacity (Fig 2, A). On examination, there were hyperkeratotic impetiginized plaques and nodules with scarring over the axillae and face. Axillary and pubic hairs were almost absent, and breasts were hypoplastic (Fig 2, B). She had mild thoracolumbar scoliosis. She was 152 cm tall and weighed only 36 kg.

PATIENT 3
A 14-year-old boy presented with history of congenital deafness, recurrent cutaneous abscesses, severe blepharitis, and scoliosis of spine. On examination he had severe scarring alopecia, bilateral blepharitis (Fig 3 A), thoracolumbar scoliosis, and hyperkeratotic impetiginized plaques over his limbs and axillae (Fig 3, B). His testes were smaller than normal, and there was no axillary or pubic hair. He was 156 cm tall and weighed only 27.2 kg. With time, he had progressive loss of vision in his left eye caused by exposure keratitis and corneal ulcer secondary to severe blepharitis and ectropion.

Based on clinical findings, our provisional diagnosis was KID syndrome with some new undescribed features. Clinical photographs were taken on the first and subsequent visits after obtaining consent from accompanying family members.

Table I. Clinical diseases with GJB2 mutations

| Nonsyndromic hearing loss | Syndromic hearing loss | Only skin manifestation |
|---------------------------|------------------------|-------------------------|
| Autosomal dominant - DFNA3 | Bart-Pumphrey syndrome | PEODDN                  |
| Autosomal recessive - DFNB1 | PPK with deafness      | KID syndrome            |
|                           | HID syndrome           |                         |

HID, Hystrix-like ichthyosis with deafness; PEODDN, Porokeratotic eccrine ostial and dermal duct nevus; PPK, palmoplantar keratoderma.

In addition to routine laboratory investigations and skin biopsies, special tests were done for all patients to see immune status. Immunoglobulin levels, leukocyte adhesion test, oxidative burst assay, hormonal assays, and pus for culture and sensitivity were obtained. For patient 2 and 3, cultures were done from the eyes because of severe blepharitis. All were referred to the ophthalmology, audiology and medical genetics departments for further workup. Gene analysis of the entire family was done through exome sequencing 70X.

RESULTS
Exome sequencing found a novel homozygous GJB2 mutation (p.[Ile82Val]) in all 3 patients. The rest of the nonaffected siblings and parents were found to be heterozygous carriers.

Audiologic examination found profound bilateral sensorineural hearing loss in all 3 patients. Leukocyte adhesion tests and oxidative burst assays were normal in all patients.

- All 3 had raised levels of IgG and IgA.
  - Patient 1: IgG, 23.2 g/L (normal range, 7-16) and IgA, 9.04 g/L (normal range, 0.7-4)
  - Patient 2: IgG, 20.3 g/L and IgA, 6.01 g/L
  - Patient 3: IgG, 24.4 g/L and IgA, 6.71 g/L

Cultures from skin were positive for Staphylococcus aureus and Pseudomonas in all patients, whereas eye culture from the third patient was also positive for the same organisms.

A nerve conduction study was normal for patient 1 but showed axonal polyneuropathy for patient 2 and moderate axonal motor neuropathy in the lower limbs of patient 3. Hormonal assays for the female patients were within normal limits, but testosterone level of the male patient was less than 0.1 nmol/L (normal range, 0.98-38.5).

Biopsies specimens were taken from all 3 patients. Biopsies from the scalp found dilated follicular infundibula associated with heavy perifollicular plasma-rich chronic inflammation and dermal fibrosis. Biopsies from the skin had benign epithelial hyperplasia, dermal inflammation, and crusts with gram-positive bacterial colonies.

All patients were treated initially with topical chlorhexidine 2% solution and systemic antibiotics, according to the culture and sensitivity reports. Later, all were shifted to oral isotretinoin. Initially they responded well in the form of reduction in the number and intensity of abscesses but then lost the response in next couple of years. Currently, they are on adalimumab, 40 mg subcutaneously every 2 weeks, with improvement, albeit, suboptimal.
DISCUSSION

Mutations in GJB2 give rise to varied clinical diseases (Table I). Most pathogenic GJB2 mutations causing both hearing loss and skin manifestations cluster at first extracellular loop of Cx26 peptide. To date, more than 100 connexin mutations have been identified, but only 16 of them cause syndromic phenotype. Among all syndromic deafness, KID is the most severe connexin disorder because of involvement of several epithelia of ectodermal origin. Most of the patients with KID have ophthalmic problems such as corneal neovascularization, conjunctivitis, and chronic blepharitis, which may cause reduced vision and, ultimately, blindness. These features were present in our reported cases as well. Patient 1 had history of recurrent conjunctivitis, and all 3 had blepharitis of variable extent. Patients 2 and 3 had keratitis later on, ultimately resulting in blindness of 1 eye.

In addition to the clinical triad, KID syndrome patients are at high risk of infectious complications. In 2004, Montgomery et al reported a young male suffering from KID who had features of severe
follicular occlusion triad since early childhood, which was aggravated with the onset of puberty. It resulted in cyst formation, scarring alopecia, and scarring of the face, axillae, and groin. In our reported cases, features of follicular occlusion triad also worsened with advancing age. Mazereeuw-Hautier et al also described 6 patients who had inflammatory nodules in addition to classical signs of KID syndrome.

Complete absence or sparse axillary and pubic hairs in all 3 patients and underdeveloped breasts in the females were additional features in our cases. Similar findings were reported in a case of KID by Van Steensel et al from The Netherlands. Peripheral neuropathy seen in our 2 patients (patients 2 and 3) can be a feature of mutation of gap junction proteins encoding B connexins. However, to the best of our knowledge, the additional clinical features in our cases that have never been described before were scoliosis and short stature.

In 2007 Nyquist et al reported KID syndrome in a mixed Japanese African American. Mutation analysis of the CX26 gene found a homozygous A-to-G nucleotide substitution leading to the replacement of glutamic acid residue at position 114 with glycine; however, the specific homozygous mutation of GJB2 in our patients (Ile82Val) has not been reported before.

It is unclear whether the identified variant is causative for the complete phenotype of our patients because homozygous mutation in any other gene producing these features cannot be ruled out in this consanguineous family, as only targeted exome sequencing was done and not the whole exome sequencing.

A variation in the extent of phenotypes in patients with the same genetic mutations raises the possibility that environmental factors modify the penetrance of the mutation. Moreover, in human skin, Cx 26 is co expressed with other connexins like Cx43 and Cx30, so it is possible that KID mutations change the way Cx26 interacts with other co-expressed connexins.

REFERENCES
1. Mazereeuw-Hautier J, Bitoun E, Chevrant-Breton J, et al. Keratitis-ichthyosis-deafness syndrome: disease expression and spectrum of connexin 26 (GJB2) mutations in 14 patients. Br J Dermatol. 2007;156(5):1015-1019.
2. Srinivas M, Vesselas VK, White TW. Human diseases associated with connexin mutations. Biochem Biophys Acta. 2018;1860(1):192-201.
3. Jonard L, Feldmann D, Parsy C, et al. A familial case of keratitis-ichthyosis-deafness (KID) syndrome with the GJB2 mutation G12R. Eur J Med Genet. 2008;51(1):35-43.
4. Lazic T, Li Q, Frank M, et al. Extending the phenotypic spectrum of Keratitis-ichthyosis-deafness syndrome with the GJB2 mutation G45E. Eur J Med Genet. 2008;51(1):35-43.
5. Iossa S, Maricano E, Franz A. GJB2 gene mutation in syndromic skin diseases with sensorineural hearing loss. Curr Genom. 2011;12:475-485.
6. Wilecke K, Eiberger J, Degen J, et al. Structural and functional diversity of connexin gene in the mouse and human genome. Biol Chem. 2002;383:725-737.
7. Harris AL. Emerging issues of connexin channels, biophysics fills the gap. Q Rev Biophys. 2001;34:325-472.
8. Wisniew L, Limat A, Saurat H, et al. Different expression of connexins during stratification of human keratinocytes. J Invest Dermatol. 2000;115(2):278-285.
9. Kretz M, Euwens C, Hombach D, et al. Altered connexin expression and wound healing in the epidermis of connexin deficient mice. *J Cell Sci*. 2003;116:3443-3452.

10. Montgomery JR, White TW, Martin BL, et al. A novel connexin 26 gene mutation associated with features of the keratitis-ichthyosis-deafness syndrome and follicular occlusion triade. *J Am Acad Dermatol*. 2004;51:377-382.

11. Garcia IE, Maripillan J, Jara O, et al. Keratitis-ichthyosis-deafness syndrome-associated Cx26 mutants produce nonfunctional gap junctions but hyperactive hemichannels when co-expressed with wild type Cx43. *J Invest Dermatol*. 2005; 135(5):1338-1347.

12. Mah H, Liang P, Chen T, Feng P, Lai W. Keratitis-ichthyosis-deafness syndrome accompanied by disseminated cutaneous fungal infection. *J Dermatol*. 2017;44(11):1255-1261.

13. Van Steensel MAM, Van Geel M, Nahuys M, et al. A novel connexin 26 mutation in a patient diagnosed with keratitis-ichthyosis-deafness syndrome. *J Invest Dermatol*. 2002;118:724-727.

14. Robionet R, Gasparini P, Estivill X. Molecular genetics of hearing impairment due to mutations in gap junction genes encoding beta connexins. *Hum Mutat*. 2000;16:190-202.

15. Nyquist GG, Mumm C, Grau R, et al. Malignant proliferating pilar tumors arising in KID syndrome: a report of two patients. *Am J Med Genet*. 2007;143A:734-741.