INVESTIGATIVE REPORT

Novel and Recurrent \textit{FERMT1} Gene Mutations in Kindler Syndrome

Tanasit TECHANUKUL$^1$, Gomathy SETHURAMAN$^2$, Abraham ZLOTGORSKI$^3$, Liran HOREV$^3$, Michal MACAROV$^4$, Alison TRAINER$^5$, Kenneth FONG$^1$, Marko LENŠ$^1$, Ljiljana MEDENICA$^6$, Venkatesh RAMESH$^7$, John A. McGrATH$^1$ and Joey E. LAI-CHEONG$^1$

$^1$St John’s Institute of Dermatology, King’s College London, London, UK, $^2$Department of Dermatology, All India Institute of Medical Sciences, Delhi, India, $^3$Department of Dermatology and The Center for Genetic Diseases of the Skin and Hair and $^4$Department of Human Genetics and Metabolic Diseases, Hadassah-Hebrew University Medical Center Jerusalem, Israel, $^5$Victorian Clinical Genetics Services, Murdoch Childrens Research Institute, Parkville, Victoria, Australia, $^6$Genetic Epidemiology Unit, King’s College London, London, UK, $^7$Department of Dermatovenerology, School of Medicine, University of Belgrade, Belgrade, Serbia, and $^8$Department of Dermatology, Safdarjung Hospital, New Delhi, India

Kindler syndrome (OMIM 173650) is an autosomal recessive condition characterized by skin blistering, skin atrophy, photosensitivity, colonic inflammation and mucosal stenosis. Fewer than 100 cases have been described in the literature. First reported in 1954, the molecular basis of Kindler syndrome was elucidated in 2003 with the discovery of \textit{FERMT1} (\textit{KIND1}) loss-of-function mutations in affected individuals. The \textit{FERMT1} gene encodes kindlin-1 (also known as fermitin family homologue 1), a 77 kDa protein that localizes to focal adhesions, where it plays an important role in integrin signalling. In the current study, we describe five novel and three recurrent loss-of-function \textit{FERMT1} mutations in eight individuals with Kindler syndrome, and provide an overview of genotype-phenotype correlation in this disorder. 

\textbf{Key words: kindlin-1; epidermolysis bullosa; skin atrophy; poikiloderma; blistering.}

(Accepted November 10, 2010.)


John McGrath, Dermatology Research Laboratories, Floor 9 Tower Wing, Guy’s Hospital, Great Maze Pond, London SE1 9RT, UK. E-mail: john.mcgrath@kcl.ac.uk

Kindler syndrome (KS; OMIM 173650) is a rare autosomal recessive muco-cutaneous disorder. The cutaneous features consist of trauma-induced skin blistering, particularly of acral sites, skin atrophy, poikiloderma (a combination of skin atrophy, hypo- and hyperpigmentation and telangiectases) and varying degrees of photosensitivity (1). The extra-cutaneous features include colonic inflammation, gingivitis, periodontitis and mucosal inflammation affecting the oesophagus, urethra, vagina and anus. There is also an increased risk of muco-cutaneous cancer (1). In 2003, the molecular basis of KS was elucidated with the discovery of loss-of-function \textit{FERMT1} (also known as \textit{KIND1}) gene mutations by genome wide linkage studies and candidate gene sequencing of DNA obtained from large consanguineous pedigrees (2, 3). The \textit{FERMT1} gene encodes kindlin-1 (also known as fermitin family homologue-1), a 77 kDa protein that localizes to focal adhesions, where it mediates actin cytoskeleton-extracellular matrix (ECM) interaction (3). Kindlin-1 participates in integrin activation, a critical process for cell adhesion, differentiation and migration (4, 5). In normal skin, kindlin-1 is present in the basal keratinocyte layer in a polar fashion (3, 6, 7). In KS skin, reduced kindlin-1 expression is noted, associated with severe disruption of the cutaneous basement membrane (3, 6, 7). To date, 40 different disease-associated \textit{FERMT1} mutations in KS have been reported. In this study, we expand the \textit{FERMT1} mutation database to 45 by reporting an additional five novel and three recurrent mutations in eight individuals with KS and provide an overview of genotype-phenotype correlation in this condition.

MATERIALS AND METHODS

\textit{Molecular analysis}

This study was approved by the local research ethics committees of the referring hospitals as well as the Guy’s and St Thomas’ Hospitals NHS Foundation Trust and conducted according to the principles of the Declaration of Helsinki. Peripheral blood samples were taken from eight affected individuals and, where possible, from their parents. Polymerase chain reaction (PCR) amplification of genomic DNA was performed using 14 pairs of primers spanning the coding exons and flanking introns of the \textit{FERMT1} gene (RefSeq NM_016213.1) as described previously (3, 8, 9). The amplicons were purified using the QIAquick PCR purification kit (Applied Biosystems, Warrington, UK) and sequenced using an Applied Biosystems 3730 DNA Analyzer. Mutations were confirmed by bi-directional sequencing and excluded in 200 control chromosomes in unrelated control individuals.

RESULTS

\textit{Clinical features of patients with Kindler syndrome}

Eight individuals (subjects 1–8) with KS were studied. Their clinical features and clinical details are illustrated in Fig. 1 and Table I, respectively. The clinical details of subjects 2, 7 and 8 have been reported previously (10–12).
**FERMT1 mutation screening**

Sequencing analysis of DNA from subject 1 showed compound heterozygous FERMT1 mutations, namely p.Trp12X and IVS14+2T>C (Fig. S1A; available at http://www.medicaljournals.se/acta/content/?doi=10.2340/00015555-1063). Sequencing analysis of parental DNA revealed that the mutation, p.Trp12X, was paternally inherited, while the other mutation, IVS14+2T>C, was maternally transmitted. In subject 2, mutation analysis disclosed a homozygous nonsense mutation, p.Trp250X, in the FERMT1 gene (Fig. S1B). Mutation analysis of genomic DNA from subject 3 disclosed two heterozygous mutations; namely, a nonsense mutation, p.Gln49X, and a frameshift mutation, c.676insC (Fig. S1C). Sequencing analysis of genomic DNA from subject 4 showed a homozygous nonsense mutation, p.Tyr403X, in exon 10 of the FERMT1 gene (Fig. S1D). Mutation analysis of subject 5 showed a compound heterozygous FERMT1 mutations, denoted c.384_385+2del4 and IVS13+2T>C (Fig. S1E). In subject 6, a homozygous FERMT1 mutation, denoted c.676insC was identified in exon 5. In subjects 7 and 8, a homozygous c.676insC mutation was present (Fig. S1F). None of these mutations has been identified in screening 200 chromosomes in unrelated control individuals.

**DISCUSSION**

In this study, we report 5 novel and 3 recurrent pathogenic FERMT1 mutations in 8 individuals with KS. The previously unreported FERMT1 mutations were p.Trp12X, IVS14+2T>C, p.Trp250X, p.Gln49X and c.384_385+2del4. The following FERMT1 mutations were recurrent: c.676insC, p.Tyr403X and IVS13+2T>C. When added to the global FERMT1 mutation database in KS, a total of 46 mutations have now been disclosed. The complete FERMT1 mutation database is illustrated in Fig. 2. The c.676insC mutation is a recurrent mutation, previously reported in individuals of Pakistani (8), Brazilian (of Italian descent) (13) and Albanian (14) origins. In this study, we disclose the c.676insC mutation for the first time in an Australian Caucasian individual, an Indian subject and two affected brothers from Serbia. The mutation, p.Tyr403X, has recently been described in a 36-year-old individual with KS, but no further details about his ethnicity or geographical background were available (15). Furthermore, the splice site mutation, IVS13+2T>C, has been reported previously in a 5-day-old neonate who remains, to date, the youngest patient to be accurately diagnosed with KS (16).

An increased risk of muco-cutaneous squamous cell carcinoma (SCC) has been noted in KS. Specifically, the following mutations have been reported in association with SCC in KS: IVS7-1G>A, c.93_94delGA, IVS9-6T>A, p.Arg110X and g80929_89169del (1). In our study, we report an additional two FERMT1 mutations that were associated with SCC, namely c.676insC and p.Trp250X. To our knowledge, two individuals with KS have died as a result of complications of their SCC. It
FERMT1 mutations in Kindler syndrome

is, however, premature to speculate whether KS-associated SCC is more aggressive than non-KS-associated SCC, given the small number of individuals with KS with SCC. While an upregulation of kindlin-1 has been described in certain cancers (17), the development of SCC in KS provides an intriguing role for kindlin-1 deficiency in the development of cancer. Although there does not seem to be any correlation between the nature of the mutations and the occurrence of cancer in these individuals, it is possible that some mutations may predispose to carcinogenesis. For instance, the splice site mutation, IVS9-6T>A, led to ablation of exons 9, 10 and 11 of the FERMT1 gene. Amplification and sequencing of cDNA from the skin of this individual revealed aberrant splicing with either deletion of exon 10 or deletion of exons 9, 10 and 11, both of which involved loss of the pleckstrin homology domain of kindlin-1 (18).

A possible link has been postulated between intestinal pathology in KS and mutations present within exons 2–7 of the FERMT1 gene. The number of reported cases is, however, small and further work is required to delineate the role of kindlin-1 in colonic pathology. Interestingly, kindlin-1 null mouse, generated by replacing the ATG-containing exon 2 with a neomycin resistance cassette, displayed an ulcerative colitis-like phenotype (5), previously reported in several individuals with KS (14, 19, 20). Although a clear genotype-phenotype correlation is not apparent in KS, the present study expands the global FERMT1 mutation database as well as helps optimize overall mutation detection strategies and highlights specific mutations in patients from particular geographical origins.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Ethnicity/Gender</th>
<th>Age at diagnosis, years</th>
<th>Mutation</th>
<th>Clinical details</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Indian male, 10</td>
<td>Blistering skin at birth</td>
<td>p.Trp12X</td>
<td>Skin atrophy particularly on the dorsal aspects of the hands and feet, poikiloderma affecting sites of previous blistering, anal fissure and severe constipation.</td>
</tr>
<tr>
<td>2</td>
<td>Jewish-Kurdish female, 42</td>
<td>Blistering over pressure points</td>
<td>p.Trp250X</td>
<td>Skin atrophy of acral sites and overlying sites of previous skin blistering, poikiloderma affecting the face, neck, upper back and chest.</td>
</tr>
<tr>
<td>3</td>
<td>Australian female, 7</td>
<td>Blistering on distal limbs</td>
<td>p.Gln49X</td>
<td>Skin atrophy of extremities at 6 years, poikiloderma at 4 months.</td>
</tr>
<tr>
<td>4</td>
<td>Arabic male, 9</td>
<td>Trauma-induced blisters at 1.5 years</td>
<td>p.Tyr403X</td>
<td>Skin atrophy on the dorsal aspects of the hands and feet with palmoplantar hyperkeratosis, photosensitivity beginning at 1.5 years.</td>
</tr>
<tr>
<td>5</td>
<td>Indian female, 11</td>
<td>Trauma-induced blisters at 2 years</td>
<td>c.384_385-2delA</td>
<td>Skin atrophy at 7 days, skin blistering in neonatal period, progressive poikiloderma from the neonatal period.</td>
</tr>
<tr>
<td>6</td>
<td>Indian male, 4</td>
<td>Skin blistering in neonatal period</td>
<td>c.678insC</td>
<td>Skin atrophy and erythema, progressive skin blistering with redness, severe gingivitis, urethral stenosis.</td>
</tr>
<tr>
<td>7 and 8</td>
<td>Serbian brothers, aged 60 and 57, respectively</td>
<td>Trauma-induced skin blistering on acral sites</td>
<td>c.676insC</td>
<td>Progressive skin atrophy, progressive poikiloderma from the neonatal period, dystrophic nails and urethral and oesophageal stenoses, keratoconjunctivitis and strabismus.</td>
</tr>
</tbody>
</table>
ACKNOWLEDGEMENTS
The study was funded by the British Skin Foundation and the British Association of Dermatologists. Support from the Department of Health via the National Institute of Health Research Comprehensive Biomedical Research Centre award to Guy’s and St Thomas’ Hospital NHS Foundation Trust in partnership with King’s College London is gratefully acknowledged.

The authors declare no conflicts of interest.

REFERENCES


