Novel KIND1 Gene Mutation in Kindler Syndrome With Severe Gastrointestinal Tract Involvement

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Background: Kindler syndrome (online Mendelian Inheritance in Man No. 173650) is an autosomal recessive genodermatosis characterized by acral trauma-induced blistering that improves with age and by progressive poikiloderma in later life. Other clinical features include photosensitivity, webbing of the fingers and toes, nail dystrophy, periodontal disease, and mucosal alterations. Aside from esophageal or anal stenosis, gastrointestinal tract involvement seems to be rare in Kindler syndrome. Recently, mutations in the KIND1 gene that encode for the membrane-associated protein kindlin-1 have been identified. Kindlin-1 links the actin cytoskeleton to the extracellular matrix and is supposed to have cell-signaling functions owing to different functional domains. In particular, a domain with high homology to 4.1/ezrin/radixin/moesin (FERM) proteins is closely related to the sequences of talin that mediate integrin binding and therefore may play a role in integrin-dependent processes such as cell growth, differentiation, and apoptosis.

Observation: Complete loss of this multifunctional protein in our patient with Kindler syndrome resulted in severe gastrointestinal tract involvement with hemorrhagic colitis. Mucosa of the descending and sigmoid colon and the rectum showed erosions and ulcers with pseudomembranous alterations of an overall highly vulnerable mucosa. Mutation analysis revealed a homozygous status for the novel mutation 20/21delTT in exon 2 of the KIND1 gene resulting in a preterminal stop codon creating a nonfunctional peptide 17 amino acids in length.

Conclusion: Because of our experience with this and another patient, we propose that gastrointestinal tract involvement should be looked at more frequently in Kindler syndrome.

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Kindler syndrome (online Mendelian Inheritance in Man No. 173650) is an autosomal recessive genodermatosis first described by Kindler in the 1950s. It is characterized in infancy by acral trauma-induced blistering and photosensitivity that improves with age. Later in life, progressive poikiloderma with diffuse cutaneous atrophy, telangiectases, and reticulocyte pigmentation develops. Other features may include nail dystrophy, webbing of the fingers and toes, ectropion formation of the eyelids, chronic inflammation of the oral mucosa, poor dentition with early-onset periodontal disease, and esophageal, anal, vaginal, or urethral stenosis.

Siegel and colleagues demonstrated recently that the molecular basis of this distinct disease is loss of function mutations in the KIND1 gene at chromosome 20p12.3, encoding for the 677–amino acid protein kindlin-1, with a calculated molecular weight of 77.3 kDa. Until now, 18 different mutations were described, and consanguinity of parents was found in about 44% of cases. The kindlin-1 protein consists of 4 functional domains with homologies to other polypeptides. The N-terminal domain with homology to filopodin and the C-terminal domain with homology to talin are considered components in the linkage of the actin cytoskeleton to the extracellular matrix in focal adhesion plaques. A bipartite domain with homology to the band 4.1/ezrin/radixin/moesin (FERM) protein family is thought to be involved in the cytoskeleton attachment to the plasma membrane, and the pleckstrin domain in between is a feature of cytoskeletal-associated and/or cell-signaling molecules. Therefore, the kindlin-1 protein is strongly suggested to have structural functions, but also functions in directing cell migration, normal cell growth, and cell differentiation and in signal transduction. Also, the increased apoptosis and photosensitivity (UV light induces keratinocyte apoptosis in vivo) that we and others have found in Kindler syndrome can be caused by impair-
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grins and bullous pemphigoid antigen-2 (BPAG2 [col-
types IV and VII. Staining with antibodies against inte-
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membrane (BM). However, typically, there is a broad, re-
ation in staining of structural proteins of the basement
ping studies do not show any reduction or major alter-

clusively within the epidermis, particularly in basal kera-
small intestine. In normal skin, kindlin-1 is present ex-
in keratinocytes and in the colon, kidney, and placenta,
intrafibrillary antibody levels were not elevated. Further
cases could be found within the dilated clefts of neighboring basal
may show additional interrupted portions of BM lamina
densae (which in our case have been observed to be as long
as 3-4 basal keratinocytes), sublaminar clefing, and the
an increased amount of fibroblastlike cells beneath the
BM zone, the cellular projections of which sometimes can
be found within the dilated clefts of neighboring basal
keratinocytes.17 In addition, multiple planes of cleavage
within the basal keratinocytes and/or in the lamina lu-
cida can be found, but the hemidesmosomes and anchor-
ing fibrils appear normal.4

Figure 1. Multiple erosions and blisters secondary to minimal trauma on the extremities and the trunk, ie, below the waist on the lower back. Erosion on the hand below the plastic identification band is covered by protective absorbent gauze.

Diagnosis of Kindler syndrome can be established by the clinical features, course of the disease, and typical findings in immunofluorescence antigen mapping studies and transmission electron microscopy (TEM).17 Antigen mapping studies do not show any reduction or major alteration in staining of structural proteins of the basement membrane (BM). However, typically, there is a broad, reticu-
ticular staining pattern at the dermoeidermal junction with antibodies directed against laminin-5 and collagen types IV and VII. Staining with antibodies against integrins and bullous pemphigoid antigen-2 (BPAG2 [collagen type XVIII]) shows a linear pattern interrupted in a regular fashion.4 In addition, antikeratin-5–positive corp-
cules, corresponding to anti-IgM–positive keratin bodies,
can be found that are generated by apoptotic kerat-
inocyte cell death.16

Transmission electron microscopy of some cases shows excessive reduplications of otherwise normal-appearing lamina densa with branching and folding that can be traced deep below the BM zone.16,19 Other cases may show additional interrupted portions of BM lamina densa (which in our case have been observed to be as long as 3-4 basal keratinocytes), sublaminar clefing, and the
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METHODS

Skin biopsy specimens for TEM and antigen mapping studies were obtained 10 days after birth from clinically normal (inner upper arm) and perilesional (periumbilical) skin.

ANTIGEN MAPPING STUDIES

For diagnostic antigen mapping studies, we used monoclonal antibodies against keratins (keratin 1, 5, 8, 10, and 14), plectin 5B3, bullous pemphigoid antigen-1 and -2, integrins α6 and β4, laminin-5, and collagen types IV and VII.

TRANSMISSION ELECTRON MICROSCOPY

Skin specimens for TEM were analyzed by standard procedures. Briefly, small slices were fixed in phosphate-buffered formaldehyde-glutaraldehyde solution, followed by osmication, dehydration in ascending ethanols, and processing into epoxy resin (Glycidether 100, with hardeners DDSA, MNA, and DMP-30; Serva, Heidelberg, Germany). Semithin (1-μm) and ultra-
thin (approximately 70- to 80-nm) sections were cut using a diamond knife (Diatome Ltd, Biel, Switzerland). Ultrathin sections were stained by a standardized method using tannic acid, uranyl acetate, and lead citrate. Observations and micro-
graphic documentation were performed on an electron micro-
scope at 80 kV (Zeiss EM 109; Carl Zeiss, Oberkochen, Ger-
many), using a 35-mm camera and negative film (Technical Pan Film 2415; Eastman Kodak Company, Rochester, NY). After development, the film was scanned and processed fur-
ther electronically.

GASTROINTESTINAL TRACT ENDOSCOPY

Gastroduodenoscopy and colonoscopy were performed with an ultrathin fiberglass endoscope during uncomplicated general anesthesia. For histological evaluation, biopsy specimens
were taken from the stomach, duodenum, and different parts of the colon and were stained with hematoxylin-eosin by standard procedures.

**MUTATION DETECTION**

We extracted genomic DNA from the patient and both parents from peripheral blood samples by standard procedures (Puregene blood kit; Gentra Systems, Minneapolis, Minn). The KIND1 gene was scanned by polymerase chain reaction (PCR) amplification of individual exons and flanking intronic sequences using specific primers and 100 ng of genomic DNA as a template. The PCR reactions were performed in a final volume of 50 µL containing 2.5 mM magnesium chloride, 10 pmol of each primer, 4% dimethyl sulfoxide, 125 µM deoxyribonucleotide triphosphate, and 2.5 U of Biotherm DNA polymerase (GeneCraft, Muenster, Germany). Cycling conditions were 5 minutes at 94°C, followed by 15 cycles of touchdown PCR starting at 68°C with a temperature decrement of 0.5°C for each cycle, then 40 cycles each for 30 seconds at 94°C, 30 seconds at 61°C, and 30 seconds at 72°C. The PCR fragments were purified using a commercially available PCR and gel purification kit (GFX; Amersham Biosciences, Little Chalfont, England) and forwarded to be sequenced on a capillary electrophoresis system (CEQ8000; Beckman Coulter Inc, Fullerton, Calif). Sequence reactions were performed using a dye terminator cycle sequencing kit (CEQ2000; Beckman Coulter Inc) according to the manufacturer’s instructions. For skin biopsy, gastroduodenal endoscopy, and mutation analysis, written informed consent was obtained from both parents.

**RESULTS**

**ANTIGEN MAPPING STUDIES**

All structural proteins of the BM zone were detectable with normal intensity. Antigen mapping analysis revealed a focal, extremely broadened, reticular staining pattern for laminin-5 and collagen types IV and VII as the typical diagnostic criterion for Kindler syndrome (Figure 2). Staining with antibodies against integrins and bullous pemphigoid antigen-2 showed a linear pattern interrupted in a regular fashion.

**TRANSMISSION ELECTRON MICROSCOPY**

Results of TEM (Figure 3) revealed locally extensive, de-localized remnants of the lamina densa that could be traced as deep as 130 µm below the BM zone. There were also

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**Figure 2.** Antigen mapping results. Extremely broadened reticular staining patterns are seen for collagen types IV (A) and VII (B) and laminin-5 (C) (original magnification ×100). Staining with antibodies against integrin α6 (D) showed a linear pattern interrupted in a regular fashion (original magnification ×400).
areas where the BM was totally absent or interrupted to 15 to 30 µm of length (1-2 basal keratinocytes). Focally, the lamina lucida appeared to be widened; at places, marked clefting between neighboring basal keratinocytes (often filled with projections of fibroblastlike cells) and sub-basal dilatation was observed. Some basal keratinocytes showed marked electron-dense clumping of intracellular tonofilaments and some ultrastructural features of apoptosis. Desmosomes and hemidesmosomes seemed to be ultrastructurally normal.

GASTROINTESTINAL TRACT ENDOSCOPY

Histological findings of biopsy specimens taken during gastroduodenoscopy showed normal esophageal and duodenal mucosa but discrete regenerative alterations of the gastric prepyloric part.

Colonoscopy showed ulcerative colitis with inflammatory longitudinal erosions and ulcers with pseudo-membranous alterations of an overall highly vulnerable mucosa in the descending and sigmoid colon and the rectum. Mucosa of the terminal ileum, cecum, and ascending and transversing colon seemed macroscopically normal. Histological examination of biopsy specimens with intact epithelial layer showed a reduction of goblet cells, an increase of mitotic activity, and an accumulation of plasma cells and eosinophils in the lamina propria. Other biopsy specimens were dominated by unspecific ulcerative lesions with lots of fibrin, detritus, and neutrophils associated with granulation tissue. None of the biopsy specimens showed histological criteria of eosinophilic colitis or other specific pathological pictures such as inclusion bodies, bacteria, parasites, crypt abscesses, granulomata, or absence of ganglia.

MUTATION ANALYSIS

Mutation analysis of the 15 exons and the exon-intron boundaries of the KIND1 gene of the infant revealed the new mutation consisting of a homozygous deletion of TT in codon 7 of exon 2, localized as 20/21delTT (Figure 4). Consequently, the deletion results in a codon frameshift of +2 and a preterminal stop codon of 6 + 11 amino acids downstream of 11 amino acids, creating a nonfunctional minipeptide of 17 amino acids if translated at all. Mutation analysis of the KIND1 gene of the parents uncovered heterozygous status for this deletion at exon 2. No additional mutation could be detected in the father’s gene.

A NEW TURKISH HAPLOTYPE

Sequencing of genomic DNA of affected individuals could identify all 9 single nucleotide polymorphisms, consisting of 1-29T/G, 11+1T/C, 151 + 20C/T, 152-4G/A, 479T/C, 532 + 81T/C, 532 + 34C/T, 533-17A/C, and 722T/C, using the standard numbering system. Intragenic haplotype analysis of these polymorphisms of the affected family revealed a different genetic background from the published Pakistani, white United Kingdom, Omani, and Italian haplotypes and proposed a new Turkish haplotype (Table) associated with the 20/21delTT pathogenic mutation underlying Kindler syndrome.
Gastrointestinal tract involvement in patients with Kindler syndrome is possible but seems to be rare in the literature. To our knowledge, besides esophageal and anal stenosis, no cases with severe gastrointestinal tract involvement have been described. Undoubtedly, the limited accessibility of gastrointestinal tract epithelia and the very low number of patients are reasons for that. In addition, defects that might arise from mutations in structural and functional proteins of the BM zone expressed in internal tissues might not be immediately apparent and may only be detected clinically after further complications have arisen. Besides physical trauma from the movement of gut contents, gastrointestinal tract mucosa is also likely to be subjected to osmotic stress, which causes rapid cell shape changes and reversion.

Figure 4. Sequence part of exon 2. The new mutation, consisting of a homozygous deletion of TT in codon 7 of exon 2 and localized as deletion 20/21delTT, is shown to be homozygous in the child (A) and heterozygous in the parents (B) resulting in a codon frameshift and a preterminal stop codon downstream of 11 amino acids. Met indicates methionine; mut, mutation; and wt, wild type.

<table>
<thead>
<tr>
<th>Exon/Intron Position</th>
<th>KIND1 SNP</th>
<th>20/21delTT (Present Family)</th>
<th>676insC (Pakistani)</th>
<th>E304X (White UK)</th>
<th>W616X (Omani)</th>
<th>958-1G-A (Italian)</th>
</tr>
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<tr>
<td>5' Region</td>
<td></td>
<td>G</td>
<td>G</td>
<td>T</td>
<td>G</td>
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<tr>
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<td>T</td>
<td>C</td>
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<tr>
<td>Intron 2</td>
<td>151 + 20C/T</td>
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<td>C</td>
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<tr>
<td>Intron 2</td>
<td>152 – 46/A</td>
<td>A</td>
<td>G</td>
<td>G</td>
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<tr>
<td>Exon 4</td>
<td>479T/C</td>
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<tr>
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<td>T</td>
<td>C</td>
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<tr>
<td>Intron 4</td>
<td>532 + 34T/C</td>
<td>C</td>
<td>C</td>
<td>T</td>
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<tr>
<td>Intron 4</td>
<td>533 – 17A/C</td>
<td>A</td>
<td>C</td>
<td>A</td>
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<tr>
<td>Exon 5</td>
<td>722T/C</td>
<td>T</td>
<td>T</td>
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Abbreviations: KIND1, the gene that encodes for the membrane-associated protein kindlin-1; SNP, single nucleotide polymorphism; UK, United Kingdom; 20/21delTT, a homozygous deletion of TT in codon 7 of exon 2.

“Data regarding 676insC, E304X, W616X, and 958-1G-A are from Ashton et al.”
Our patient is the second with Kindler syndrome observed in our department. The other patient, a 49-year-old man, also showed typical clinical signs and course of Kindler syndrome and characteristic alterations in the antigen mapping and TEM findings. Because of stenosis of the small bowel, which was first thought to be caused by Crohn disease, he underwent partial resection of the small bowel. It remains unclear whether this gastrointestinal tract involvement was caused by Crohn disease or Kindler syndrome, which was diagnosed 4 years after the resection. We believe that this observed stenosis of the small bowel was caused by Kindler syndrome because of the lack of typical complications, recurrences, and extraintestinal manifestations of Crohn disease. Owing to the high expression of kindlin-1 in the colon, it is feasible that the gastrointestinal tract alterations in Kindler syndrome could be observed more frequently than described. Similar to dermatisis herpetiformis or Duhring disease, an autoimmune bullous disease, histological signs of gastrointestinal tract involvement in Kindler syndrome could be a rather frequent but often asymptomatic event.

Our patient's father reported a history of mild blister formation during childhood that improved with age, but whether it was caused by the heterozygous status of the novel deletion mutation 20/21delTT in exon 2 is unclear. This mutation results in a frameshift and premature termination codon and consequently a short polypeptide (consisting of only 17 amino acids), if translated at all. Alternatively, nonsense-mediated RNA decay may result in complete loss of the respective messenger RNA and therefore in a nontranslatable substrate. Maybe this nearly complete loss of all amino acids (98.9%) and complete loss of function could also induce the reported history of blister formation in the patient's father, although he was heterozygous for this mutation, suggesting somatic reversion transmission. Because he refused further examination in our clinic, other blistering skin diseases cannot be excluded.

Finally, more studies about the function and binding partners of kindlin-1 and more examinations of gastrointestinal tract mucosa are obviously needed to confirm the suspicion of frequent gastrointestinal tract alterations and genetic heterogeneity and to enlighten the pathophysiological background of clinical signs and symptoms in patients with Kindler syndrome.

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Author Contributions: Dr Sadler had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Sadler, Laimer, Lanschuetzer, and Bauer. Acquisition of data: Sadler, Klausseger, Muss, Deinsberger, Pohlga-Gubo, Bauer, and Hintner. Analysis and interpretation of data: Klausseger, Pohlga-Gubo, Bauer, and Hintner. Drafting of the manuscript: Sadler, Muss, Deinsberger, Pohlga-Gubo, Laimer, Lanschuetzer, and Bauer. Critical revision of the manuscript for important intellectual content: Klausseger, Muss, Laimer, Lanschuetzer, Bauer, and Hintner. Administrative, technical, and material support: Sadler, Klausseger, Muss, Pohlga-Gubo, Lanschuetzer, and Bauer. Study supervision: Klausseger, Bauer, and Hintner.

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REFERENCES


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