Lamellar Ichthyosis: further narrowing, physical and expression mapping of the chromosome 2 candidate locus

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Lamellar ichthyosis is an autosomal recessive genodermatosis which has been shown to be both clinically and genetically heterogeneous. Keratinocyte transglutaminase (or transglutaminase 1; TGM1) has been demonstrated to be the disease-causing gene in some families, whilst in others, a second unidentified LI gene was mapped to chromosome 2q33–35 (ICR2B locus). In this study, we present a physical map that encompasses the ICR2B locus, including the mapping of new microsatellite markers. Based on this new map, genotyping additional families highly suggests a reduction in size of the candidate interval. The final interval is covered by a single yeast artificial chromosome (937–H–3) which is 2.2 Mb in length. Fine mapping of potential candidate transcripts was also focused on this region.

Keywords: lamellar ichthyosis; ICR2B; 2q33–35

Introduction

Lamellar ichthyosis (LI) belongs to the complex group of disorders of cornification. Its prevalence is low - 1/200 000 to 300 000 - and its mode of transmission is usually autosomal recessive (MIM 242300). LI can be life-threatening soon after birth, since the neonate skin is covered by a thick collodion-like membrane, exposing the infant to sepsis and dramatic dehydration. Spontaneous shedding of this membrane gives way to a residual and definitive ichthyosis, presenting as an extensive scaling of the entire body surface. The intensity of the ichthyosis ranges from large brown scales to a fine powdery desquamation. It is also variously accompanied by palmoplantar keratoderma, alopecia and erythema. Some controversial classifications separate the erythrodermic form of LI from the ‘pure’ LI, but a lot of patients remain impossible to classify.

Consistent with its variable clinical presentation, LI was demonstrated to be genetically heterogeneous. Transglutaminase 1 (TGM1) was the first gene demonstrated to cause LI. This gene directs the construction of the cornified envelope, a protein structure underneath the plasma membrane of keratinocytes which forms during their late-stage terminal differentiation. However, TGM1 and other known genes involved in cornified envelope construction were firmly excluded in many other LI families (and also in families subclassified as erythrodermic LI). For a set of Moroccan families whose phenotypes were compatible with the ‘pure’ LI definition, we mapped a second...
disease-causing gene to chromosome 2. This locus (referred to as ICR2B in the Genome Database) maps to a 7 cM genomic region which is devoid of any obvious candidate gene. Finally, other LI families were also demonstrated not to be linked to both these loci, suggesting the existence of at least a third disease-causing gene.

In the present study, we attempted to refine the ICR2B locus and to map potential candidate transcripts. In the hope of maximising genetic homogeneity, we focused our work on seven LI families sharing the same geographical origin, similar phenotypes, and presenting a potential linkage to chromosome 2q33–35. The physical order of all polymorphic markers of the region was determined in order to allow reliable haplotype construction. Our results suggested the possibility of a founder effect and, consequently, a substantial refinement of the ICR2B locus. A transcription map of this region was also established and the fibronectin gene was shown to map to the ICR2B locus.

### Table 1

<table>
<thead>
<tr>
<th>Family</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collodion baby at birth</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Generalized ichthyosis</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Severe</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Mild</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Erythema</td>
<td>-</td>
<td>-</td>
<td></td>
<td>mild to null</td>
</tr>
<tr>
<td>Palmoplantar keratoderma</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alopecia</td>
<td>-</td>
<td>-</td>
<td></td>
<td>-</td>
</tr>
</tbody>
</table>

### Patients, Material and Methods

#### Patients

Patients from families A, B, and C were selected as previously reported. Patients from families D and E were examined at CHU IBN R och, Casablanca, Morocco, (HL and CBB). Families C and E originated from the same area of South Morocco. Patients from Algerian families F and G were examined at CHU T Djamerni, Tlemcen, Algeria (OBS). Families C and E originated from the same area of South Morocco. Patients from Algerian families F and G were examined at CHU T Djamerni, Tlemcen, Algeria (OBS). Families C and E originated from the same area of South Morocco.

#### Yeast Artificial Chromosome Contig

A II yeast artificial chromosomes (YACs) tested were selected from contigs WC2.15 and WC2.16 of the Whitehead Institute physical map, between YAC 846F4 and 818G4 (centromeric and telomeric, respectively). Yeast clones were obtained from CEPH; cultures and DNA extractions were performed in microtiter plates using standardised protocols. Sequence-tagged sites (STS) were obtained from the Whitehead Institute physical map and completed with additional markers from the last Généthon genetic map. DNA samples were obtained from multiple different cultures of the same YAC and PCR reactions performed at least twice when results were discordant.

#### Development of New Polymorphic Markers

YACs 914E7 and 802812 were selected since they map to the telomeric portion of the contig. No chimerism was observed by FISH using interALU-PCR products as probes (data not shown). Both YACs were sized by pulse field gel electrophoresis and gel purified. After a total digestion by Sau3A, DNA was cloned in the BamHI site of M13mp19. Identification of poly(A) containing clones and subsequent development of STSs were performed as reported. These new markers were mapped on the YAC contig and polymorphism was tested on the LI families.

#### EST Mapping

Expressed sequence tags (EST) from chromosome 2 were selected from the last version of the human genome transcript map. The multipoint likelihood evaluation was performed using the rhmaxlik subroutine of the RHMAP package. A according to the current output of rhmaxlik, the best loci orders were ranked by maximum likelihood. For each EST position, we used the Deltalog10L value, which expresses the log10 likelihood difference from the best order (ie between the maximum likelihood locus order and the proposed order). ESTs displaying a Deltalog10L value ranging from 0 to 3, that is to say ESTs positioned in the interval D25143–D25137 with likelihoods within 1000 times that of the best order were considered suitable for fine physical mapping on the YAC contig.

#### Results

A unique YAC contig encompassing the previously described LI interval was established (Figure 1). We mapped a total of 68 STSs (including 43 polymorphic microsatellites and 17 ESTs) on 83 YACs. Five of the 43 microsatellites were newly developed for detailed haplotype analysis of the telomeric region (see below). For the establishment of the contig, we retained...
to map between D2S143 and D2S137. Seven ESTs presented the maximum likelihood to map between these last markers, and seven others presented a Deltalog10L value (see Methodology) below 3 for the same interval (Table 3). Finally, seven among them were confirmed to map between D2S143 and D2S137 on the YAC contig (Figure 1). Sequence comparison and UNIGENE cluster21 screening revealed that two of them corresponded to known genes (Table 3, clusters Hs.90280 and Hs.100056). All others failed to show any significant homology with known peptides, and notably those with a transglutaminase catalytic domain.

According to the expression pattern indicated in the UNIGENE database, only the cluster Hs.100056 was found to be expressed in cultured keratinocytes. This cluster corresponds to the fibronectin cDNA, a gene also expressed in a large variety of tissues.

Discussion

Our study was designed to narrow the ICR2B locus, a 7–8cM region of chromosome 2q33–35 that contains a gene responsible for LI. Since LI displays genetic heterogeneity, genetic refinement relies on the study of either large pedigrees or numerous small families with a founder effect. Genotypes of seven North African families, using either public or newly developed polymorphic markers, suggested a potential common founder mutation.

Localisation of ICR2B between D2S154 and D2S2361 is strongly supported by recombination events in families A, B and F, which show individual lod scores above 3. Homozygosity mapping suggests further narrowing of the interval from AFMB399WB5 to D2S137 in nuclear family E. Haplotype analysis in other
pedigrees (F and G) corroborates this result. Moreover, the low prevalence of the disease combined with the very similar geographical origin and clinical presentation of families F and G argues in favour of a common founder event. Since all carrier chromosomes in these two families share the same haplotype between...
D2S1345 and D2S2394, it is tempting to assign ICR2B to this interval. Concordant data are observed in families C and E, both of which originate from South Morocco (Table 2). This would place the LI gene in a 0 cM interval, covered by a single YAC (937h3) which is 2.2 Mb long, (data not shown). Moreover, for all markers but D2S107, haplotypes are identical between the carrier chromosomes of the father in family G and both parents in family C (framed by D2S157 and D2S2394). These data also support a common founder effect between the Algerian and Moroccan families.

We did not find a common haplotype in seven families, except for identical alleles for markers D2S1327 and D2S2394 (Table 2). Based on these results, it could be hypothesised that a founder haplotype has become unrecognizable due to recombination or mutation events but could still remain in a smaller interval. One could also speculate that the LI gene maps close to D2S1327 or D2S2394, but either their insufficient informativeness or the small number of chromosomes...
tested does not permit observation of a statistically significant linkage disequilibrium. Further confirmation requires the comparative analysis of larger populations of both carrier and non-carrier chromosomes. In an attempt to identify candidate genes in the D2S1345–D2S2394 critical interval, the human gene map was used. Among the seven ESTs for which assignment to the interval was confirmed...
1) none was found to have any homology with a transglutaminase catalytic domain; 
2) Hs.90280 corresponds to AICAR, a housekeeping gene involved in purine metabolism; and 
3) only Hs.100056, which is identical to fibronectin, is known to be expressed in keratinocytes. 

Fibronectin is also expressed in other tissues, and is involved in several physiological processes, some of them involving cell adhesion mechanisms. There are several arguments both for and against fibronectin as a candidate gene for LI. On the other hand, since some LI patients present deleterious mutations in TGM1, one could speculate that a second LI-causing gene product could directly or indirectly interact with this enzyme. There is as yet no evidence that fibronectin could be a substrate for TGM1, or that both proteins could be implicated together in the cornification process. Moreover, homozygous fibronectin null mutants are non-viable in the mouse and the embryo displays dramatic disruption of heart and segment development. This makes the hypothesis of a nonsense mutation of the gene unlikely. On the other hand, it has been shown that fibronectin directs the regulation of keratinocyte differentiation. It also plays a role in inter-keratinocyte adhesion, a process that depends on several specific well characterised regions of the protein. Further screening for mutations in the coding sequence of the gene should therefore focus on these regions, with particular attention to potential missense mutations. In any case, the present study should facilitate the subsequent positional cloning efforts in the identification of the ICR2B locus gene.

Acknowledgements

We wish to acknowledge the essential participation of all LI families in the study. We thank D Samson for her support in computer analysis and A Bullot for her contribution in the family collection. We also gratefully acknowledge the stimulating discussions with J Beckman and the constant support
Figure 2f-g
Table 3  Radiation hybrid mapping of the ESTs from the proposed refined candidate interval. ESTs are identified by their GenBank accession numbers (first column) and their UNIGENE cluster names (second column). For each interval, the Deltalog 10L value is indicated (see Methods) and is shaded when the position of the EST was confirmed between D2S143 and D2S137 on the physical map.

<table>
<thead>
<tr>
<th>GENBANK</th>
<th>UNIGENE</th>
<th>D2S355/D2S317</th>
<th>D2S317/D2S143</th>
<th>D2S143/D2S137</th>
<th>D2S137/D2S164</th>
<th>D2S164/D2S163</th>
<th>Known gene</th>
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<tr>
<td>R02572</td>
<td>Hs100056</td>
<td>4.54</td>
<td>2.27</td>
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<td>3.78</td>
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<tr>
<td>A A 127063</td>
<td>Hs71063</td>
<td>6.45</td>
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<tr>
<td>A A 127924</td>
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<td>T66825</td>
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<td>T56668</td>
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<tr>
<td>A A 172020</td>
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<tr>
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<td>1.86</td>
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<tr>
<td>R 00076</td>
<td>Hs18836</td>
<td>7.82</td>
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<td>0</td>
<td>1.81</td>
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<tr>
<td>T 49027</td>
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<td>9.25</td>
<td>2.58</td>
<td>0</td>
<td>1.98</td>
<td></td>
<td>ATP-dependent DNA helicase II, 86 KD subunit</td>
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</tbody>
</table>
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References