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

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Lamellar ichthyosis (LI) is an autosomal recessive genodermatosis which has been shown to be both clinically and genetically heterogeneous. Keratinocyte transglutaminase (or transglutaminase 1: *TGMI*) 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 2.2Mb 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.¹ Some controversial classifications separate the erythrodermic form of LI from the 'pure' LI,² but a lot of patients remain impossible to classify.³

Consistent with its variable clinical presentation, LI was demonstrated to be genetically heterogeneous.⁴ Transglutaminase 1 (*TGMI*) was the first gene demonstrated to cause LI.^{4–9} 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.¹⁰ However, *TGMI* 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).^{4,11,12} For a set of Moroccan families whose phenotypes were compatible with the 'pure' LI definition, we mapped a second

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Table 1 Summary of the clinical features observed in families D to G. Families A, B and C were reported previously (13)

Family	D	E	F	G
Collodion baby at birth	+	+	+	+
Generalized ichthyosis	+	+	+	+
Severe	+	+	+	+
Mild	-	-	-	-
Erythema	-	-	-	mild to null
Palmoplantar keratoderma	+	+	+	+
		striate	fissurated	fissurated
Alopecia	-	-	-	-

disease-causing gene to chromosome 2.¹³ This locus (referred to as *IRC2B* in the Genome Data Base) maps to a 7cM 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,¹³ suggesting the existence of at least a third disease-causing gene.

In the present study, we attempted to refine the *IRC2B* 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 *IRC2B* locus. A transcription map of this region was also established and the fibronectin gene was shown to map to the *IRC2B* locus.

Patients, Material and Methods

Patients

Patients from families A, B, and C were selected as previously reported.¹³ Patients from families D and E were examined at CHU IBN Rochd, 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 TDjamerni, Tlemcen, Algeria (OBS). Both families were reported to live in the same village of western Algeria. The parents of the patients in families A–F are first cousins; individual III-2 of family G was reported to be related to family F, but the exact genealogical link could not be established. All clinical findings are summarised in Table 1.

Yeast Artificial Chromosome Contig

All yeast artificial chromosomes (YACs) tested were selected from contigs WC2.15 and WC2.16 of the Whitehead Institute

physical map,¹⁴ 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.¹⁵ Sequence-tagged sites (STS) tested were obtained from the Whitehead Institute physical map and completed with additional markers from the last Génethon genetic map.^{14,16} 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 802B12 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(AC) containing clones and subsequent development of STSs were performed as reported.¹⁷ These new markers were mapped on the YAC contig and polymorphism was tested on the LI families.

Genotyping and Lodscore Evaluation

Genotyping was performed using procedures recommended for each marker.^{17,18} For markers D2S3003, D2S3004, D2S3005, D2S3006 and D2S3007, the PCR conditions were as published¹⁷ except that the annealing temperature was 50°C. Haplotype construction was done using the most parsimonious phase.

Multipoint lod scores were calculated using the HOMOZ program,¹⁹ restricting the analysis to the most informative markers when necessary. Linkage disequilibrium was tested using the DISEQ package.²⁰

EST Mapping

Expressed sequence tags (EST) from chromosome 2 were selected from the last version of the human genome transcript map.²¹ The multipoint likelihood evaluation was performed using the rhmaxlik subroutine of the RHMAP package.²² According to the current output of rhmaxlik, the best locus orders were ranked by maximum likelihood. For each EST position, we used the Deltalog10L value,²² 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 D2S143–D2S137 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 and genes) 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

Table 2 Haplotype analysis of the carrier chromosomes in the telomeric region of the candidate interval. For family G, G indicates the paternal chromosome and G' the maternal chromosome. Numbering of alleles is the same as in Figure 2. The common haplotype between family C and the family G paternal chromosome (which extends to D2S157) does not appear in totality. Common haplotypes between Moroccan families C and E and Algerian families F and G are shaded. Common alleles for markers D2S1327 and D2S2394 are also shaded

Families	A	B	D	C	E	F	G	G'
Centro.								
D2S143	4	7	11	6	6	4	6	4
D2S3003	(-12)	(-10)	0	(+2)	(+2)	(-10)	(+2)	(-10)
D2S1345	0	0	0	(-3)	(-3)	(-3)	(-3)	(-3)
D2S1327	(+12)	(+12)	(+12)	(+12)	(+12)	(+12)	(+12)	(+12)
D2S128	10	2	1	4	4	4	4	4
D2S107	0	0	(-2)	(-2)	(-2)	(+2)	(+2)	(+2)
D2S3007	(+16)	(+20)	(+20)	0	0	0	0	0
D2S3004	(+8)	(+12)	(+8)	(+8)	(+8)	(+8)	(+8)	(+8)
D2S2394	1	1	1	1	1	1	1	1
D2S2361	7	4	3	5	7	1	9	1
D2S137	1	7	5	2	2	2	2	2
D2S3005	(-12)	0	(-12)	(-14)	(-16)	(+10)	(-12)	(+10)
Telo.								

only YACs which were positive for at least two STSs. YAC 692C5 was rejected due to ambiguous and non-reproducible results. The YAC clone 961A4 was found positive for a set of markers of the Whitehead Institute contigs WC2.15 and WC2.16. The final order was consistent with recombination events observed in our families. Areas around markers D2S317 and AFMA052YB9 were covered by single YACs only but were consistent with the overall order of the STSs in this region. Markers D2S143, D2S1327, D2S128, D2S1345 and D2S3003 were ordered on the basis of deletions in two YACs (918C8 and 909C10). A deletion in 918C8 was previously reported for D2S1345 from the Whitehead Institute map; no deletion in 909C10 has been reported before.

Genotypes of the five Moroccan families with the polymorphic markers are shown in Figure 2 (pedigrees A to E) and include previous results.¹³ Telomeric and centromeric recombinants are defined by D2S137 (family A) and D2S325/D2S2321 (family B). A region of homozygosity, highly suggestive of linkage with *ICR2B* was observed in families D and E although their individual lod scores remain below the threshold of 3. In the proband of family E, homozygosity encompasses the region telomeric to AFMB299WB5. A common haplotype from D2S143 to D2S2394 is observed in carrier chromosomes of families C and E (Table 2).

Genotyping of two new Algerian families provided results in agreement with those for families A to E (Figure 1, pedigrees F and G). For family F, linkage to

the *ICR2B* locus is supported by a significant lod score (Lod Score = 4.2, theta = 0). Two centromeric recombinants (individuals IV-1 and IV-3) exclude D2S2208 and D2S2242. Visual haplotype analysis of both carrier chromosomes in family G indicated that:

- 1) the carrier chromosomes transmitted either by III-2 in family G or by both parents in family F share the same haplotype for markers telomeric to D2S153 (and also for additional markers telomeric to D2S2382, data not shown);
- 2) a region of homozygosity was observed between markers D2S1345 to D2S2394;
- 3) the haplotype for the paternal contribution was identical with that found in the Moroccan family E, from D2S1345 to D2S2394 (excepting D2S107).

Finally, a common allele was observed in carrier chromosomes of all seven families for both markers D2S1327 and D2S2394 (Table 2) but no significant linkage disequilibrium was found (P value = 0.015 and 0.122 for D2S1327 and D2S2394, respectively). Linkage with the *TGM1* locus on chromosome 14 was excluded in all new families (data not shown).

Since genotyping results strongly suggested that the LI gene maps in the telomeric region (see Discussion), we selected ESTs of chromosome 2 from the human genome transcript map and calculated their likelihood

Figure 1 YAC contig encompassing the LI interval on chromosome 2. The YAC names are from the CEPH YAC library database. Microsatellites are named either by their D-number or their AFM number, and are indicated by an asterisk. ESTs and genes are underlined; they are identified by their GenBank accession numbers or names (*CRYGA* for crystallin gamma A, *CPS1* for carbamyl phosphate synthetase 1, *MYL1* for human alcali myosin light chain 1). Co-localised markers are indicated by square brackets.

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 7–8 cM 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 AFMB299WB5 to D2S137 in nuclear family E. Haplotype analysis in other

Figure 2 Genotypes of families A to G with the set of markers of chromosome 2. Markers are indicated by their D-numbers (except for five AFM markers obtained from the Whitehead Institute physical map). Relative positions are consistent with the YAC contig, see Figure 1. Regions of identity by descent in consanguineous families are outlined. Numbering of the alleles of the different markers were consistent with that used in the CEPH database, when available. Otherwise, they indicate the relative difference in base pairs from the lowest allele of CEPH reference individual 134702. The centromeric part of the ICR2B locus is not detailed in families D and E.

pedigrees (F and G) corroborates this result. Moreover, the low prevalence of the disease combined with the very similar geographical origin and clinical presenta-

tion 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

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 unrecognisable 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

Figure 2c

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.²¹ Among the seven ESTs for which assignment to the interval was confirmed

Figure 2d-e

- 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.²¹

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.²³ This makes the hypothesis of a non-sense mutation of the gene unlikely. On the other hand, it has been shown that fibronectin directs the regulation of keratinocyte differentiation.^{24,25} It also plays a role in inter-keratinocyte adhesion, a process that depends on several specific well characterised regions of the protein.^{26,27} 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.

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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

GENBANK	UNIGENE	D2S355/D2S317	D2S317/D2S143	D2S143/D2S137	D2S137/D2S164	D2S164/D2S163	Known gene
R02572	Hs.100056	4.54	2.27	0	3.78		Fibronectin
AA127063	Hs.71063	6.45	2.27	0	3.63		
AA127924	Hs.71034		6.18	0	0.58	5.86	
T72644	Hs.13960	6.99	3.09	0	2.68		
F09068	no		3.45	0	1.53	3.93	
W90364	Hs.38894		3.13	0	0.79	4.71	Phosphoribosyl-amino-imidazole-carboxamide formyltransferase
R36451	no	4.81	2.23	0	2.38		
T66825	Hs.12950		5.22	0.04	0	3.50	
T56668	Hs.90280		2.82	0.27	0	0.96	
AA172020	no			1.56	0	2.64	
R76848	Hs.79741		6.42	1.86	0	3.36	Insulin-like growth factor binding protein 5
R73554	Hs.83021			2.39	0	0.55	
R00076	Hs.18836		7.82	2.45	0	1.81	
T49027	Hs.84981		9.25	2.58	0	1.98	ATP-dependent DNA helicase II, 86 KD subunit

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