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SHORT REPORT

Refined mapping of the gene encoding the p127 kDa UV-damaged DNA-binding protein (DDB1) within 11q12-q13.1 and its exclusion in Best's vitelliform macular dystrophy

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Best's vitelliform macular dystrophy (Best's disease) is an autosomal dominant disorder of unknown causes and is typically characterised by an accumulation of lipofuscin-like material in the subretinal space of the macula. The disease gene has been localised to chromosome 11q12–13.1 within a 1.4 Mbp interval flanked by markers at D11S1765 and uteroglobin (UGB). Here we report the refined mapping of the gene encoding the p127 kDa subunit (DDB1) of a UV damage-specific DNA binding protein within the D11S1765–UGB region. Northern blot analysis demonstrates an abundant expression of the DDB1 transcript in the retina suggesting a functional role for DDB1 in this tissue. These considerations together with the chromosomal localisation have led us to evaluate the possible involvement of DDB1 in the pathogenesis of Best's disease.

Keywords: Macular degeneration; Best's vitelliform macular dystrophy; chromosome 11; UV-damaged DNA-binding protein; candidate gene approach

Introduction

Best's vitelliform macular dystrophy (Best's disease; OMIM 153700) is an autosomal dominant disorder that appears to be both clinically and genetically homogenous.[1–3] During the life of one patient there is progression of the disorder from the subclinical (reduced electro-oculogram, EOG), to the clinical (typical vitelliform lesion in the macular area) and, finally, to the atrophic stage.[4,5] The underlying biochemical defect in Best's disease is still unknown.

As an initial step towards the identification of the Best's disease gene, its locus was mapped to chromosome 11 by genetic linkage analysis[2,3] and was subsequently refined to a 1–2 cM interval in 11q12–13.1 flanked by loci D11S1765 and uteroglobin (UGB).[6,7] More recently, a high coverage P1-derived artificial chromosome (PAC) contig encompassing the entire Best's disease locus was constructed[8] greatly facilitating further gene cloning efforts.

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By fluorescent *in situ* hybridisation the gene encoding the p127 subunit (DDB1) of a heterodimeric damage-specific DNA binding protein, DDB, was previously assigned to chromosome 11q12–q13.[9] As part of our effort to construct a comprehensive gene map of the Best's disease region we have now mapped the gene within the minimal candidate interval and have determined its expression profile with particular emphasis on retina and retinal pigment epithelium, those tissues which are of primary interest in the pathogenesis of Best's disease. Moreover, we have analysed the entire coding region of the gene in six Best's disease patients unrelated by genealogy.

Materials and Methods

Physical Mapping of the DDB1 Transcript and Northern Blot Analysis

The construction and assembly of the PAC clone contig used to refine the localisation of the *DDB*1 gene has been reported elsewhere.[8] Primers 3'F and 3'R (Table 1) and overlapping PACs dJ398K24, dJ71C4 and dJ506K16 were used to amplify a 287 bp fragment derived from the 3'-UTR of the *DDB*1 gene (Figure 1a,b,c), whilst the location of the 5'-end of the gene was identified by PCR amplification of a 62 bp fragment using primers D1 and D2 (Figure 1c).

For Northern blot analysis, total RNA (12 μg) isolated from frozen lung, cerebellum, RPE and retinal tissue and the RPE cell line, ARPE-19[10] was electrophoretically separated in 1.2% agarose gels containing 0.6 M formaldehyde and vacuum-blotted onto nylon membrane. The filter was hybridised with radiolabelled DDB1 cDNA fragment 6 (Table 1, Figure 1c) or a human β-actin cDNA probe at 65°C. The final wash was in 0.2 × SSC/0.1% SDS at 65°C.

Patients and Families

Diagnosis of patients affected with Best's disease was based on mode of inheritance, funduscopic abnormalities and reduced EOGs. Of six unrelated German patients with a known family history of the disease, we were able to recruit additional affected and unaffected family members in three cases, namely in families R, H and W, for pedigree analysis. Microsatellite markers were selected based on their close vicinity to the Best's disease gene[7,11,12] and included D11S1765, D11S4076 and UGB. Haplotypes were established under the assumption that possible recombination events would be minimised.

SSCA and Sequencing Analysis

For single-stranded conformational analysis (SSCA) and sequencing, total RNA was either prepared from Epstein Barr virus (EBV) transformed lymphocytes derived from affected patients or from peripheral blood lymphocytes using the RNA-Clean-LS system (Angewandte Gentechnologie Systeme). RNAs were reverse transcribed using the SUPERSCRIPT preamplification system (Gibco, BRL). The resulting

Table 1 Oligonucleotide primers used for DDB1 analysis

Fragment	*Primer name*	*Primer sequence 5′→3′*	*Annealing temp. (°C)*	*$MgCl_2$ (mM)*	*Restriction enzyme*	*Fragment sizes (bp)*
–	DF DR	(–74)CCACCTGTCTTTTCGCTTG(–56) (+3459)TTGGGGAGGGTCAGAAAG(+3441)	55	1.0	–	3533
1	DF D1	(–74)CCACCTGTCTTTTCGCTTG(–56) (+502)TGACATCAATGACATGCAGC(+483)	56	1.0	AvaII	296 175 and 105
2	D2 D4	(+441)CGATAATAAAGAACTCAAGGC(+461) (+967)ACACAACACCATTATCAAGG(+948)	54	1.0	NcoI	317 210
3	D3 D6	(+925)TCTATTGCTGAGTGCTTGAC(+944) (+1438)AGACCAACCTCACCGATGC(+1420)	56	1.5	PvuII	316 198
4	D5 D8	(+1399)CAGCTTATCCAGATCACTTC(+1418) (+2020)TGAGGTTGACATTTGAGAAG(+2001)	54	1.0	PstI/HhaI	271 190 and 161
5	D7 D10	(+1955)GTTCTGACCGCCCCACTG(+1972) (+2598)CACTTCCTTTTCAGCCACAG(+2579)	59	1.5	HhaI	332 190 and 122
6	D9 D12	(+2548)GTCTTTCAGTATTCGGATGG(+2568) (+3159)GTCCAGCAGGAGGTTGTAC(+3141)	56	1.5	XhoI	322 290
7	D11 DR	(+3105)CATGATAGGGCTGGTGACC(+3123) (+3459)TTGGGGAGGGTCAGAAAGG(+3441)	59	1.5	BstNI	188 167
3′UTR	3′F 3′R	(+3780)TGTATCTCACACTCATGC(+3797) (+4067)CTAACAATTCACATCCTC(+4050)	56	1.5	–	287

cDNAs served as a template for subsequent PCR reactions. Oligonucleotide primers were designed from the full length cDNA sequence of the published *DDB*1 gene (GenBank Acc. No. U18299; Table 1). DDB1 cDNA containing the entire coding sequence was amplified using primer pair DF/DR. For SSCA, nested primers were used to amplify seven overlapping cDNA fragments (Figure 1c) by adding 0.1 µl [α-^{32}P]-dCTP (3000 Ci/mmol) followed by incubation of a 5 µl aliquot with selected restriction enzymes (Gibco, BRL) to obtain appropriate fragment sizes (Table 1). Digested PCR products were mixed 1:1 with stop buffer (95% formamide, 5 mM NaOH, 0.1% bromo-phenolblue, 0.1% xylene cyanol). Prior to loading on 6% non-denaturing polyacrylamide gels with and without 5% glycerol, samples were heated at 80°C for 5 min. After electrophoresis at 35 W for 4–9 h at 4°C, gels were dried and exposed for 12–48 h for autoradiography. Sequencing of forward and reverse strands of fragments 1, 2, 6 and 7 (Figure 1c) were carried out using the Thermo Sequenase radiolabelled terminator cycle sequencing kit (Amersham).

Results

Physical Mapping of DDB*1*

Using oligonucleotide primers 3'F and 3'R a 287 bp fragment corresponding to the 3'-untranslated region (UTR) of the human *DDB*1 gene was specifically PCR amplified in PAC DNA dJ398K24, dJ71C4 (Figure 1a,b). This positions the *DDB*1 gene approximately 100 kb distal to PGA and approximately 400 kb proximal to FEN1 (Figure 1a). In addition, PCR amplification of a 62 bp fragment derived from the 5'-end of the gene (corresponding to nucleotides + 441– + 502 with A = + 1 of the ATG start codon) in PAC clone dJ71C4, but not in dJ398K24 or dJ506K16 (Figure 1b), defines the orientation of transcription of the *DDB*1 gene from telomere to centromere (Figure 1a).

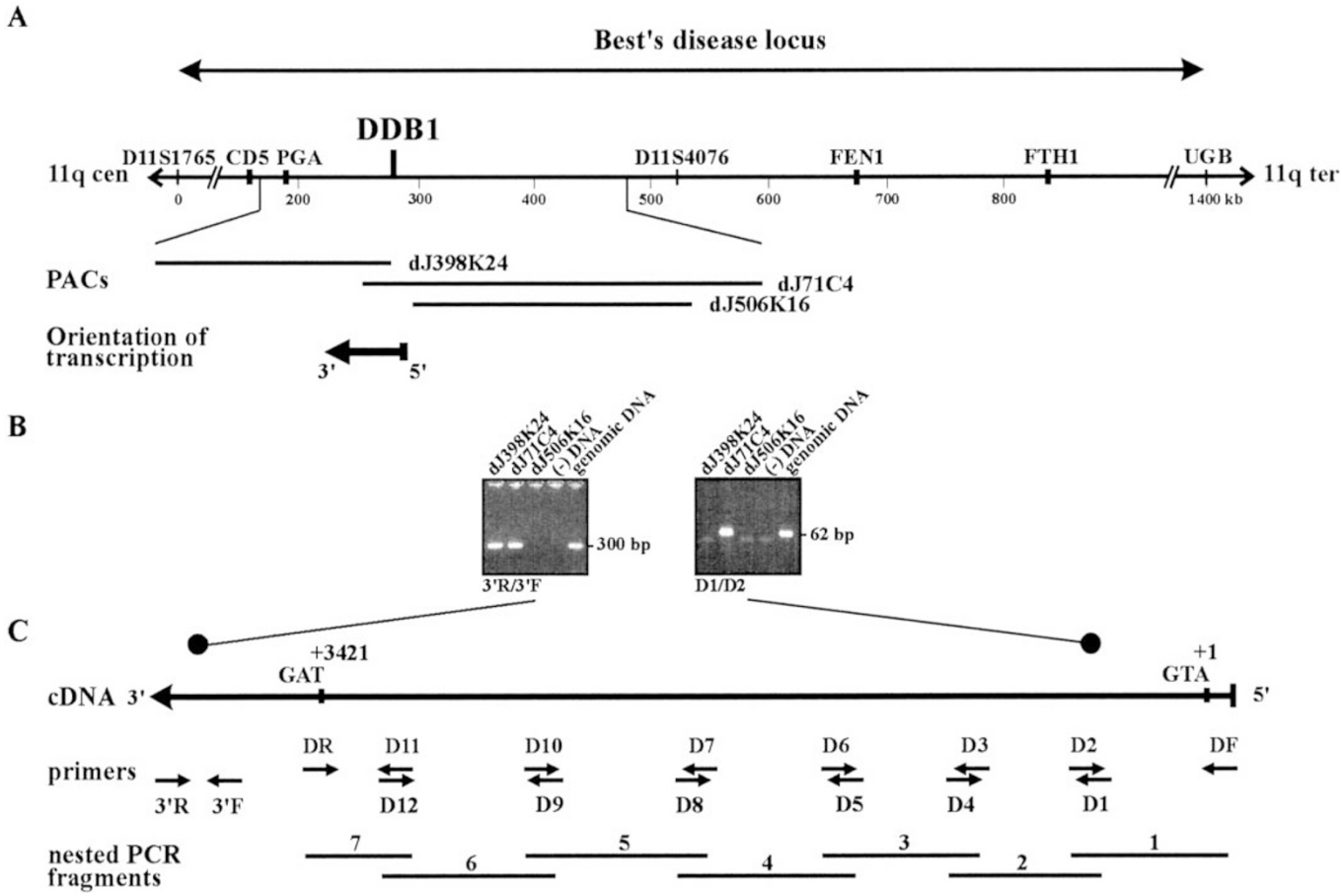

Figure 1 **A** *Schematic representation of the Best's disease locus on chromosome 11q12-q13.1 showing the localisation of known genes within the critical interval. Overlapping PAC clones dJ398K24, dL71C4 and dJ506K16 are part of a high-coverage PAC contig described elsewhere.*[9]. **B** *PCR mapping of the 5'- and 3'-UTR of the* DDB*1 gene using primer pairs D1/D2 and 3'R/3'F, respectively. The results demonstrate the orientation of transcription to be from the telomere to the centromere (arrow).* **C** *Location of oligonucleotide primers within the full length DDB1 cDNA used in the present study. Nested PCR fragments were analysed utilising SSCA and direct sequencing. The location of primers used for establishing the orientation of transcription within the established PAC contig is indicated by filled circles.*

Expression Analysis of DDB*1*

Northern blot analysis of *DDB*1 identifies a 4.4 kb transcript in total RNA of RPE, lung, cerebellum, retina and the RPE cell line, ARPE-19 (Figure 2). A comparison of the signal intensities relative to the β-actin control hybridisation reveals an abundance of the *DDB*1 transcript in retinal tissue (Figure 2).

Genetic Analysis

To investigate linkage in Best's disease families R, H and W to chromosome 11q12–q13.1, we determined the genotypes of all family members at polymorphic loci D11S1765, D11S4076 and UGB (Figure 1a). In family R, the four affected individuals in three generations share haplotype 6-1-5 (D11S1765-D11S4076-UGB). Similarly, in family H, the five patients in three generations all carry a 6-1-5 haplotype. Finally, in family W, the five patients in three generations share a 7-2-5 haplotype (data not shown). Together, these analyses suggest a chromosome 11q12–q13.1 location of the disease gene in these three families.

Mutational Analysis of DDB*1*

In order to assess the *DDB*1 gene for mutations in the six Best's disease patients we used reverse transcriptase (RT)-PCR to amplify seven overlapping cDNA fragments together encompassing the entire coding region of the *DDB*1 gene (Figure 1c). Subsequently, restriction enzyme-digested PCR fragments were subjected to SSCA using two conditions to increase sensitivity. No abnormal migration of single stranded cDNAs were observed for any of the six probands in all seven cDNA fragments analysed. In addition, approximately 60% of the coding region of DDB1 was directly sequenced in four of the six Best's disease patients. Comparison to the published cDNA sequence of *DDB*1 (GenBank Acc. No. U18299) has not revealed any sequence alterations.

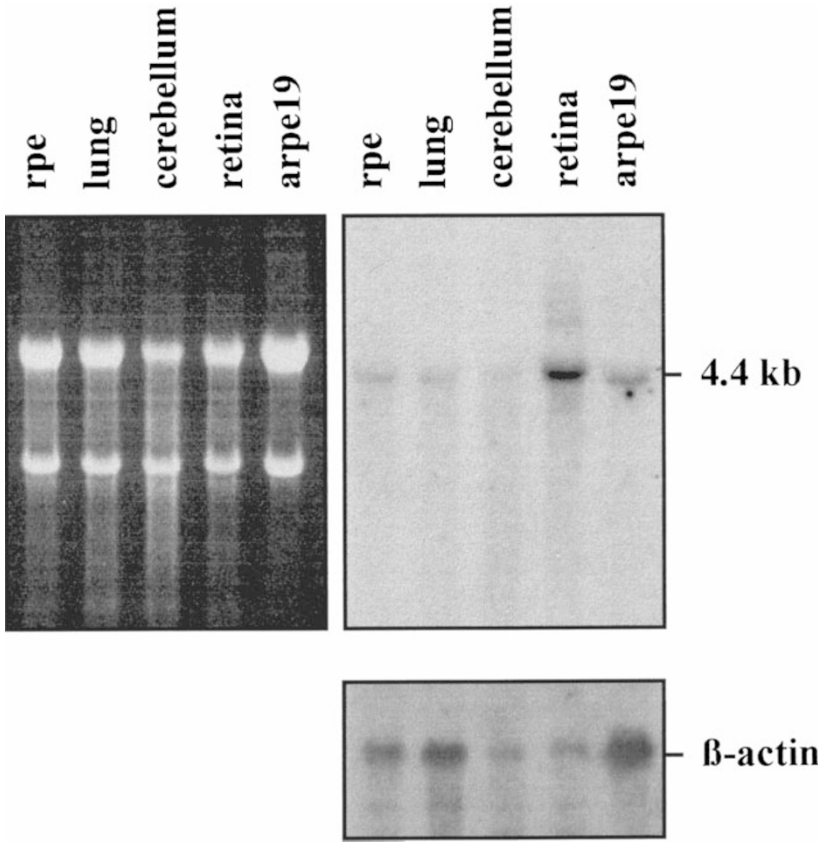

Figure 2 *Northern blot analysis of DDB1 in total RNA from retinal pigment epithelium (rpe) lung, cerebellum, retina and cell line RNA of retinal pigment epithelium (arpe19). Loading and RNA integrity was determined by ethidium-bromide stained gel analysis (left) and control hybridisation with a human β-actin probe (below). Although the 4.4 kb transcript is present in all tissues tested a preferential expression in retina is apparent.*

Discussion

Although the autosomal dominant Best's vitelliform macular dystrophy was already described in 1905,[1] the primary cause leading to the disease phenotype still remains elusive. The availability of an increasing number of genes and knowledge of their probable functions makes it now feasible to assess attractive candidates that map within the chromosomal region known to harbour the disease locus. In this study, we have performed a detailed localisation of the *DDB*1 gene within the critical Best's disease region. The 127 kDa DDB1 subunit together with the 48 kDa DDB2 subunit form a functional heterodimer, DDB, that is presumed to participate in UV-damaged DNA recognition and nucleotide excision repair.[9] Our northern blot analysis has shown that the 4.4 kb *DDB*1 transcript is expressed in all tissues examined consistent with previous findings that have demonstrated an ubiquitious expression in many tissues including pancreas, kidney, skeletal muscle, liver, lung, heart, placenta and brain.[13] In addition, our analysis has revealed a striking abundance of the DDB1 transcript in the human retina suggesting that the corresponding protein may play an important role in this tissue.

Aside from its proposed role in UV-damaged DNA repair, DDB is thought to exert alternate but as yet unknown functions.[9] This may particularly be true in tissues such as the retina that normally are protected from the most DNA-damaging UV light, UV-B, by the filtering capacity of cornea and lens.[14] In light of our Northern blot results the retina seems to be an excellent tissue in which DDB functions should be further analysed.

To assess the 3420 bp of coding sequence of the DDB1 transcript in Best's disease we have developed an RT-PCR assay. However, a combination of SSCA and direct sequencing has not revealed any sequence alterations in our six patients. Nevertheless, it should be pointed out that some minor types of mutations may have been missed using our assay, eg deletion of a complete allele or mutations in the promotor region of the gene. Also, it is commonly believed that SSCA detects on average approximately 80% of sequence variations. To further exclude the possibility of locus heterogeneity in Best's disease, we have performed linkage analyses in three out of six families where DNA samples of several affected and unaffected members were available. In each case, our results are consistent with a location of the disease locus to chromosome 11q12–q13.1. This is in excellent agreement with a series of earlier reports that demonstrate genetic homogeneity in Best's disease.[2,3,7,11] Together, our results make it unlikely that *DDB*1 is a major primary cause in the pathogenesis of Best's disease.

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