

Molecular genetic heterogeneity in autosomal dominant drusen

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Table 1 Haplotype data in families linked to the chromosome 2p16 dominant drusen locus. *Italicised area indicates common haplotype and thus common ancestry. Bar indicates EFEMP1 intragenic markers*

| Pedigree | EFEMP1 | | | | | | | | | |
|----------|---------|---------|-----------|----------|----------|----------|----------|----------------|----------|--------|
| | D2S2739 | D2S2352 | D2S2251 | 133018CA | Intron 4 | Intron 8 | Intron 9 | R345W mutation | 322A4AAT | D2S378 |
| UK1 | 7 | 2 | <i>11</i> | 2 | 1 | 2 | U | Yes | 3 | 3 |
| UK2 | 10 | 5 | <i>11</i> | 3 | 1 | 2 | U | Yes | 3 | 3 |
| UK3 | 10 | 5 | <i>11</i> | 3 | 1 | 2 | U | Yes | 3 | 3 |
| UK4 | 3 | 5 | <i>11</i> | 3 | 1 | 2 | U | Yes | 3 | 3 |
| US1 | 5 | 4 | <i>11</i> | 3 | U | 2 | 2 | Yes | 3 | 3 |
| US2 | 10 | 2 | 8 | 3 | 1 | 2 | 2 | Yes | 2 | 2 |
| US3 | 6 | 2 | 10 | 3 | 1 | 2 | 2 | Yes | 3 | 2 |
| UK5 | 7 | 2 | 8 | 3 | U | 2 | 2 | No | 3 | 9 |
| UK6 | 6 | 7 | 6 | 1 | U | 2 | 2 | No | 2 | 8 |

U = uninformative. Families UK5 and UK6, despite linkage to the region, do not share the common haplotype or have the R345W mutation.

Abstract

Objective—Autosomal dominant drusen is of particular interest because of its phenotypic similarity to age related macular degeneration. Currently, mutation R345W of *EFEMP1* and, in a single pedigree, linkage to chromosome 6q14 have been causally related to the disease. We proposed to investigate and quantify the roles of *EFEMP1* and the 6q14 locus in dominant drusen patients from the UK and USA.

Design—Molecular genetic analysis.

Participants—Ten unrelated families and 17 young drusen patients.

Main outcome measures—Exons 1 and 2 of *EFEMP1* were characterised by 5' rapid amplification of cDNA ends and direct sequencing. Exons 1–12 of *EFEMP1* were then investigated for mutation by direct sequencing. A *HpaII* restriction digest test was constructed to detect the *EFEMP1* R345W mutation. Marker loci spanning the two dominant drusen linked loci were used to generate haplotype data.

Results—Only seven of the 10 families (70%) and one of the 17 sporadic patients (6%) had the R345W mutation. The *HpaII* restriction digest test was found to be a reliable and quick method for detecting this. No other exonic or splice site mutation was identified. Of the three families without *EFEMP1* mutation, two were linked to the 2p16 region.

Conclusions—*EFEMP1* R345W accounts for only a proportion of the dominant drusen phenotype. Importantly, other families linked to chromosome 2p16 raise the possibility of *EFEMP1* promoter sequence mutation or a second dominant drusen gene at this locus. Preliminary haplotype data suggest that the disease

gene at the 6q14 locus is responsible for only a minority of dominant drusen cases.

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Drusen deposits in the macular region of the retina are found in an important set of inherited blinding conditions. These may be subdivided into those conditions where drusen are the earliest and principle finding occurring before 50 years of age and those conditions where drusen are a minor secondary feature, for example, Sorsby's fundus dystrophy. All pedigrees documented to date within the former subgrouping have been reported to exhibit autosomal dominant inheritance and so are said to express a "dominant drusen" phenotype. It is not yet known whether the dominant drusen retinopathy in these different pedigrees represents one or a number of distinct disease entities.¹ Eponymous types of dominant drusen include Doyme's honeycomb retinal degeneration (DHRD) and malattia leventinese (ML). In DHRD, large drusen deposits have been described at the macula and around the edge of the optic nerve head.^{2,3} In ML, as well as macular drusen, small, discrete drusen are also seen radiating into the peripheral retina. These radial deposits are described as continuous with or internal to the basement membrane of the retinal pigment epithelium.⁴ Macular drusen are also a hallmark of age related macular degeneration (ARMD), a complex genetic disorder that accounts for 50% of blind registration in the developed world.⁵ Characteristically, drusen are seen clinically before the sixth decade of life in dominant drusen pedigrees, but later than this in ARMD.

Both ML and DHRD have been mapped to chromosome 2p16^{6,7} and a single mutation

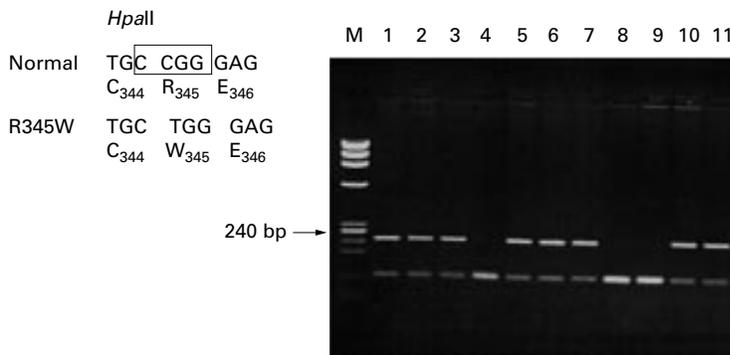


Figure 1 Restriction enzyme digestion assay for the arginine to tryptophan mutation at codon 345 of the *EFEMP1* gene. Eleven members of pedigree US1 are presented. The mutation abolishes a *HpaII* site. PCR was used to amplify a 240 bp fragment spanning exon 10 from patient DNA. The PCR products were digested with the restriction enzyme *HpaII* and run out on agarose gels. Patients 4, 8, and 9 are unaffected and therefore do not have the mutation. Patients 1-3, 5-7, and 10-11 are all affected and have the Arg345Trp mutation. M represents the DNA marker *PhiX174/HaeIII*.

(R345W) has been identified in the *EFEMP1* gene.⁸ This was found in pedigrees with both ML and DHRD and it has been suggested that this mutation originates from a common ancestor and that perhaps most cases of dominant drusen derive from this. More recently, a single dominant drusen pedigree has been mapped to chromosome 6q14.^{9,10} In this study, we proposed to determine whether these molecular genetic localisations are relevant to a large cohort of dominant drusen pedigrees identified in the UK and USA.

Methods

FAMILY AND CLINICAL DATA

Ten unrelated families expressing a dominant drusen phenotype and 17 young cases without a family history (16 British and one American patient) were enrolled into the study. Informed consent for clinical and genetic assessment was obtained in accordance with the Declaration of Helsinki. The cohort comprised seven families from the UK (UK1 to UK7) and three families from the USA (US1 to US3) (table 1). Families UK1 and US2 have previously been

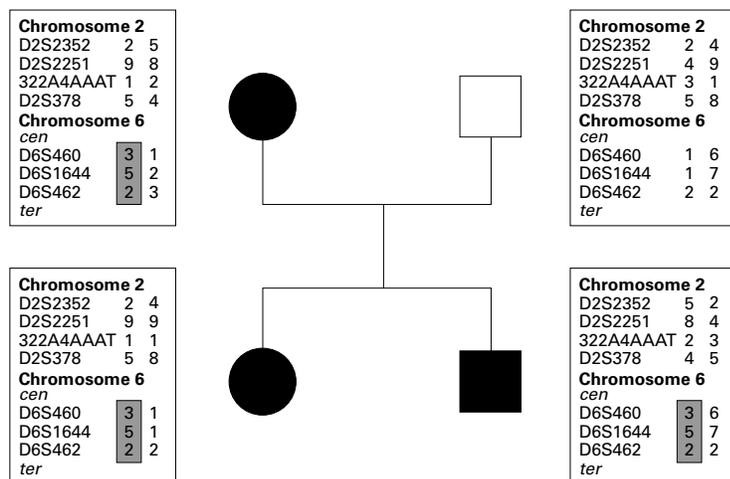


Figure 2 Haplotype data in family UK7 at chromosomes 2p16 and 6q14 linked to the dominant drusen phenotype. Chromosome 2 data show a recombination event suggesting exclusion from linkage. Shaded areas show the common haplotype for the chromosome 6q14 dominant drusen locus in affected children inherited from the affected parent.

clinically described.^{11,12} UK1 is descended from the patients originally described by R W Doyme.² All subjects classified as affected exhibited soft drusen at the macula before 45 years of age. Unaffected status was assigned to those patients with a normal ophthalmic examination and over 55 years of age. Since no other type of inheritance has been described, simplex cases under 45 years of age, with retinal drusen for no other apparent cause, were classified as examples of dominant drusen.

CHARACTERISATION OF 5' END OF *EFEMP1* AND MUTATION SCREEN

The structure of the 5' end of the *EFEMP1* gene including 5' untranslated exons 1 and 2 has not been fully established. In order to be able to screen the entire *EFEMP1* gene for mutations, we therefore characterised the 5' end of the gene. Two PAC clones containing the *EFEMP1* gene were isolated from the RPCII PAC library (de Jong), obtained from the HGMP Resource Centre, Hinxton, Cambridge. A 5 kb *EcoRI* subclone containing exon 3 of the gene was identified. A combination of 5' rapid amplification of cDNA ends (RACE) and genomic sequencing was used to characterise the region upstream of exon 3 including exon 1 (GenBank accession number AY027910) and exon 2 (GenBank accession number AY027911). Using this new information, intronic primers were designed to amplify exons 1 and 2 for mutation screening. These were 1F: AACGCTGGGCT-CAGCGCTC and 1R: CATCCCAACAGAT-GTCACC with an annealing temperature of 60°C and 2F: ACTGGAAGATTGCTCTCC and 2R: TCCATTTCAAAGGGGACGG at 55°C.

Genomic DNA was extracted from peripheral blood lymphocytes from family members using the Nucleon II kit (Nucleon Biosciences). Exons 1 to 12 were then amplified by PCR using primers as listed above for exons 1-2 and using previously described primers for exons 3-12 under standard conditions.⁸ Both strands of each amplified fragment were examined for mutation by direct sequencing using standard protocols.¹³

Since the R345W mutation removes a *HpaII* restriction site in exon 10, we investigated whether this could be used as an initial diagnostic test for this mutation. PCR was used to amplify a 240 bp fragment spanning exon 10 from patient DNA. The PCR products were then digested with the restriction enzyme *HpaII* and run out on agarose gels.

HAPLOTYPE ANALYSIS

Five microsatellite marker loci, D2S2739, D2S2352, D2S2251, D2S2153, and D2S378, spanning approximately 4 cM (Marshmed map)¹⁴ of the chromosome 2p16 region linked to dominant drusen, were used to generate haplotype data. Six further marker loci were also used in the chromosome 2p16 analysis, 133018CA, 322A4AAAT, and four intragenic polymorphisms derived from introns 4, 8, 9, and 11 of the *EFEMP1* gene.⁸ Six microsatel-

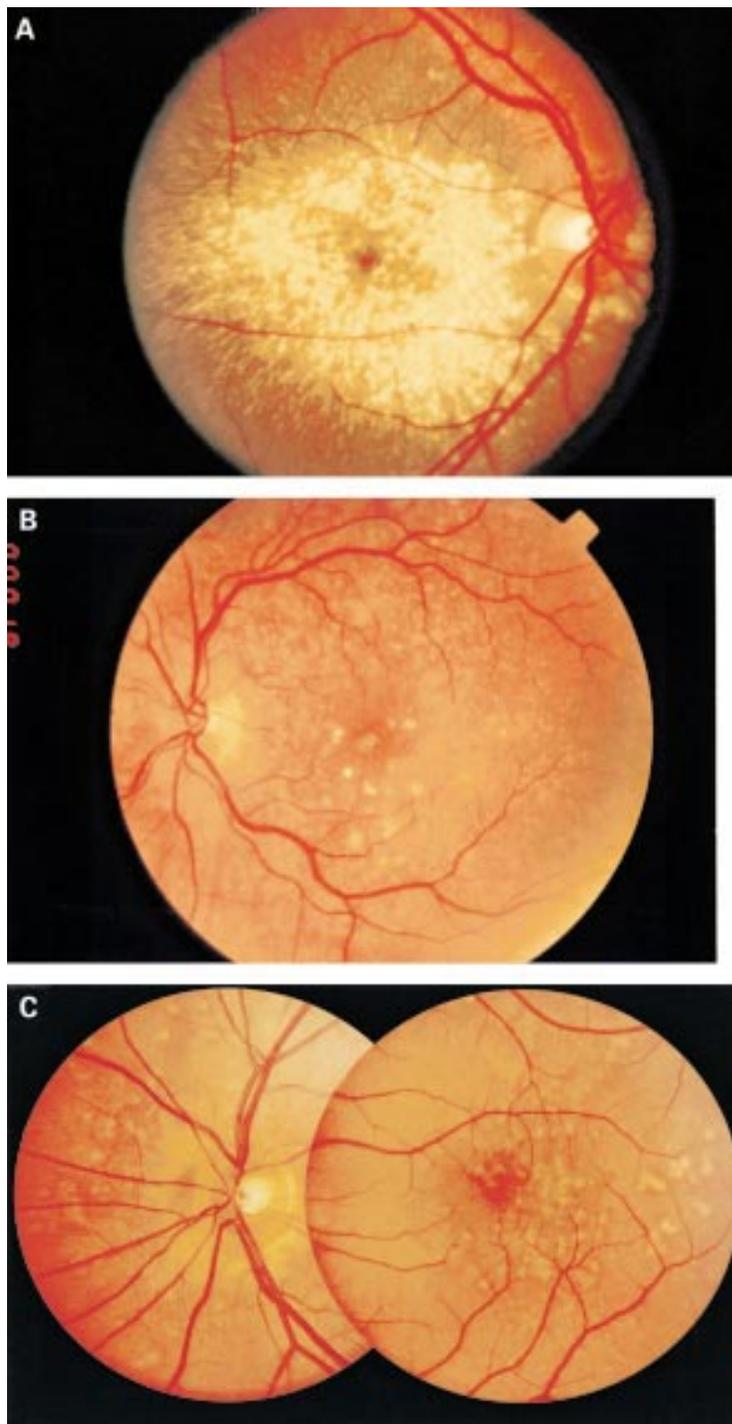


Figure 3 Fundus photographs of patients affected by dominant drusen. (A) A 44 year old patient from pedigree US1 with R345W *EFEMP1* mutation. (B) A 63 year old patient (drusen noted from 45 years of age) from pedigree UK6, linked to chromosome 2p16 but without *EFEMP1* mutation. (C) A 39 year old sporadic drusen patient who does not have *EFEMP1* mutation.

lite marker loci, D6S460, D6S1652, D6S2258, D6S1644, D6S462, and D6S1056, mapping to the 6q14 dominant drusen linked region, were also used in selected pedigrees.

Marker loci were amplified by PCR incorporating [³³P]- α -dATP and visualised by autoradiography following electrophoresis on 6% denaturing polyacrylamide gels.

Results

Mutation screening established that seven of the 10 families (70%, four British, three American) and one of the 17 simplex cases (6%, one British patient) had the *EFEMP1* R345W mutation. This was further established using the *HpaII* restriction enzyme digestion assay (fig 1). Haplotype data generated at marker loci spanning the 4 cM linked interval of chromosome 2p16 showed that these patients all shared a common genetic haplotype across the 1 cM region D2S2251 to D2S378, including intragenic *EFEMP1* polymorphisms (table 1). This suggests a founder mutation. It was of particular note that the intragenic marker loci were often uninformative, for example, marker locus intron 11 was uninformative in all families studied.

Three of the 10 families and 16 of the simplex cases were found to be negative for the R345W mutation by *HpaII* restriction enzyme analysis and direct sequencing of exon 10 (fig 1). Subsequent sequencing of exons 1-9, 11, and 12 in these cases did not identify any other mutation in any exons or at splice site junctions of the *EFEMP1* gene. It was noteworthy that each of the three families without the R345W mutation also had different haplotypes at the 2p16 linked locus, both from each other and from the R345W founder population. This suggests that these families are unrelated to the original common ancestor who had the *EFEMP1* mutation (table 1). Interestingly, two of these families, UK5 and UK6, have previously been linked to the 2p16 disease associated locus.¹⁵ The other family, UK7, was too small for statistically significant linkage analysis. However, recombination events observed in family UK7 show convincing exclusion of the 2p16 locus (fig 2). Haplotype data for the chromosome 6q14 dominant drusen locus generated in family UK7 is presented in fig 2. Recombination events in families UK5 and UK6 excluded linkage to this locus (data not shown).

Clinical photographs of the retina of dominant drusen patients who do not have *EFEMP1* R345W mutation are presented in fig 3. Typically, soft macular drusen were noted in each case. It was noteworthy that clinically, in each case, these deposits were confined to the macular region and were neither juxtapapillary (abutting the optic nerve head) nor extended in to the periphery as has been described in many dominant drusen pedigrees with the R345W mutation.^{3 4 11}

Discussion

It is clear from the work presented here that *EFEMP1* mutation including R345W accounts for a large but not an overwhelming proportion of cases of dominant drusen. Only 70% of families and more strikingly only 6% of sporadic cases showed *EFEMP1* mutation. Therefore, although an *EFEMP1* mutation screen should be the initial test in the molecular diagnosis of a case of retinal drusen in a patient under 50 years of age, a negative result does not exclude this clinical diagnosis. Abolition of the *HpaII* restriction site in exon 10, as

described here for the first time, can be used to enhance the efficiency of *EFEMP1* screening for the common R345W mutation (fig 1). In addition, information on untranslated exons 1 and 2 now allows for a more complete assessment of the role of this gene in subjects expressing a drusen phenotype.

It is of particular interest that two pedigrees UK5 and UK6, although showing linkage to chromosome 2p16, did not have an *EFEMP1* mutation. This may be interpreted as evidence to suggest a second dominant drusen gene at this locus. Alternatively, these pedigrees may express mutations of the *EFEMP1* promoter sequence. No such promoter sequence mutation has yet been associated with outer retinal dystrophy, although *RB1* promoter sequence mutation has been associated with retinoblastoma.¹⁶

Another striking feature of this study was the phenotype seen in dominant drusen cases that did not have *EFEMP1* mutation. All had soft macular drusen, but none seemed to exhibit juxtapapillary or hard radial drusen (fig 3B, C). This latter feature is a cardinal sign in ML.⁴ In DHRD, juxtapapillary drusen are seen unless there is juxtapapillary atrophy and radial drusen are seen in some cases but not others.¹¹ It is of interest that these ML and DHRD pedigrees have all had R345W *EFEMP1* mutation.⁸ It is possible that the juxtapapillary drusen and radial drusen are features specific to R345W *EFEMP1* although the pedigree recently mapped to 6q also had members with radial drusen.¹⁰ This needs to be investigated in further studies.

Family UK7 also did not exhibit *EFEMP1* mutation and, significantly, haplotype analysis excluded the chromosome 2p16 locus. Chromosome 6q haplotype data, however, may suggest that the dominant drusen phenotype in UK7 is the result of mutation at this locus. The evidence for this is, however, not conclusive and other loci may be relevant. Drusen are a minor feature of a number of other mapped retinal dystrophies including Sorsby's fundus dystrophy (chromosome 22q31),¹⁷ North Carolina macular dystrophy (chromosome 6q16),¹⁸ and autosomal dominant age related macular degeneration (1q).¹⁹ Each of these loci should be included in any secondary genome search for the disease gene leading to a retinal drusen phenotype.

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