in the electrophoretic conditions used in each study. In both studies the short *MUC2* alleles are very rare in patients as well as controls (6/136 UK patients and 3/142 UK controls, 2/114 USA patients and 4/106 USA controls), and there is no significant difference in the numbers of "small" alleles (smaller than 5.0 kb) and "large" alleles (greater than 5.0 kb) between patients and controls (Fisher's exact test, UK data p=0.29, USA p=0.31). We also analysed the samples separately after exclusion of the Jewish subjects because of the suggestion of different genetic risk in Jewish and non-Jewish groups,[25 26] but there was no difference in distribution (not shown). One subject in the USA group had one unusually large allele. The significance of this rare allele is not known though an allele approaching this in size (12 kb) is found in one of the CEPH families. Careful scrutiny of the large allele distribution shows slight but statistically non-significant difference in distribution in the patients and controls (Mann-Whitney U test, UK data set p=0.11, USA data set p=0.36) which is in the opposite direction in both groups.

This negative evidence seems to rule out the idea that short *MUC2* allele length predisposes to ulcerative colitis, but does not exclude the possibility that other variations in the *MUC2* gene, such as "within repeat" sequence differences, or the final fully glycosylated *MUC2* mucin may play a role. Furthermore, in view of the recent results of Cho *et al*[12] it may be worth studying *MUC2* in Crohn's disease since some evidence has been obtained for linkage of Crohn's disease rather than ulcerative colitis to 11p.

LV was supported by the British Lung Foundation, MM was supported by the Sir Halley Stewart Trust, and JCWL was supported by the National Association for Colitis and Crohn's Disease and by the Crohn's Disease in Childhood Research Association.

DALLAS M SWALLOW
LYNNE E VINALL
MRC Human Biochemical Genetics Unit, UCL Galton Laboratory, Wolfson House, 4 Stephenson Way, London, NW1 2HE, UK

JAMES R GUM
YOUNG S KIM
Department of Medicine, University of California Service, Gastrointestinal Research Laboratory (151M2), VA Medical Center, San Francisco California 94121, USA.

HUIYING YANG
JEROME I ROTTER
Division of Medical Genetics, Departments of Pediatrics and Medicine, Steven Spielberg Pediatric Research Center, Cedars-Sinai Research Institute and UCLA School of Medicine, Los Angeles, California, USA

MUDDASSAR MIRZA
Division of Medical & Molecular Genetics, GKT School of Medicine, 7th Floor, Guy's Tower, Guy's Hospital, London SE1 9RT, UK

JOHN C W LEE
JOHN E LENNARD-JONES
St Mark's Hospital, Harrow, Middlesex HA1 3UT, UK

1 Satsangi J, Jewell DP, Rosenberg WMC, Bell JI. Genetics of inflammatory bowel disease. *Gut* 1994;**35**:696-700.
2 Yang H, Rotter JI. In: Kirsner JB, Shorter RG, eds. *Inflammatory bowel disease*. Baltimore: Williams and Wilkins, 1995:301-31
3 Yang H, Rotter JI. In: Rimoin DL, Connor RE, Pyeritz RE. *Emery and Rimoin's principles and practice of medical genetics*. Edinburgh: Churchill Livingstone, 1997:1533-53.
4 Hugot JP, Laurent-Puig P, Gower-Rousseau C, *et al*. Mapping of a susceptibility locus for Crohn's disease on chromosome 16. *Nature* 1996;**379**: 821-3.
5 Toyada H, Wang SJ, Yang H, *et al*. Distinct associations of HLA class II genes and inflammatory bowel disease. *Gastroenterology* 1993;**104**:741-8.
6 Naom I, Lee J, Ford D, *et al*. Analysis of the contribution of HLA genes to genetic predisposition in inflammatory bowel disease. *Am J Hum Genet* 1996;**59**:226-33.
7 Satsangi J, Welsh KI, Bunce M, *et al*. Contribution of genes of the major histocompatibility complex to susceptibility and disease phenotype in inflammatory bowel disease. *Lancet* 1996;**347**:1212-17.
8 Satsangi J, Grootscholten C, Holt H, Jewell DP. Clinical patterns of familial inflammatory bowel disease. *Gut* 1996;**38**:738-41.
9 Satsangi J, Parkes M, Louis E, *et al*. Two stage genome wide search in inflammatory bowel disease provides evidence for susceptibility loci on chromosomes 3, 7, and 12. *Nat Genet* 1996;**14**:199-202.
10 Mirza MM, Lee J, Teare D, *et al*. Evidence of linkage of the inflammtory bowel disease susceptibility locus on chromosome 16 (IBD1) to ulcerative colitis. *J Med Genet* 1998;**35**:218-21.
11 Ohmen JD, Yang HY, Yamamoto KK, *et al*. Susceptibility locus for inflammatory bowel disease on chromosome 16 has a role in Crohn's disease, but not in ulcerative colitis. *Hum Mol Genet* 1996;**5**:1679-83.
12 Cho JH, Nicolae DL, Gold LH, *et al*. Identification of novel susceptibility loci for inflammatory bowel disease on chromosomes 1p, 3q and 4q: evidence for epistasis between 1p and IBD1. *Proc Natl Acad Sci USA* 1998; **95**:7502-7.
13 Corfield AP, Myerscough N, Bradfield N, *et al*. Colonic mucins in ulcerative collitis: evidence for loss of sulfation. *Glycoconj J* 1996;**13**:809-22.
14 Hinoda Y, Akashi H, Suwa T, *et al*. Immunohistochemical detection of MUC2 mucin core protein in ulcerative colitis. *J Clin Lab Anal* 1998;**12**:150-3.
15 Podolsky DK, Isselbacher KJ. Glycoprotein composition of colonic mucosa: specific alterations in ulcerative colitis. *Gastroenterology* 1984;**87**:991-8.
16 Probert CSJ, Warren BF, Perry T, *et al*. South Asian and European colitics show characteristic differences in colonic mucus glycoprotein type and turnover. *Gut* 1995;**36**:696-702.
17 Tytgat KM, van der Wal JW, Einerhand AW, Buller HA, Dekker J. Quantitative analysis of MUC2 synthesis in ulcerative colitis. *Biochem Biophys Res Commun* 1996;**224**:397-405.
18 Pullan RD, Thomas GAO, Rhodes M, *et al*. Thickness of adherent mucus gel on colonic mucosa in humans and its relevance to colitis. *Gut* 1994;**35**: 353-9.
19 Vinall LE, Hill AS, Pigny P, *et al*. Variable number tandem repeat polymorphism of the mucin genes located in the complex on 11p15.5. *Hum Genet* 1998;**102**:357-66.
20 Toribara NW, Gum JJ, Culhane PJ, *et al*. MUC-2 human small intestinal mucin gene structure. Repeated arrays and polymorphism. *J Clin Invest* 1991;**88**:1005-13.
21 Griffiths B, Mathews DJ, West L, *et al*. Assignment of the polymorphic intestinal mucin gene (MUC2) to chromosome 11p15. *Ann Hum Genet* 1990;**54**:277-85.
22 Pladdet IE, Pena AS, Oudejans CBM. Mucins genes as markers for the genetic predisposition of ulcerative colitis. *Proc Am Gast Assn May* 1995:A-310.
23 Gum JR, Hicks JW, Toribara NW, Siddiki B, Kim YS. Molecular cloning of human intestinal mucin (MUC2) cDNA-identification of the amino-terminus and overall sequence similarity to pre-pro von-Willibrand. *J Biol Chem* 1994;**269**:2440-6.
24 Vinall L, Pratt WS, Swallow DM. In: Corfield AP, ed. *Mucin methods and protocols.* New York: Humana Press, 1998.
25 Roth MP, Petersen GM, McElree C, *et al*. Familial empiric risk estimates of inflammatory bowel disease in Ashkenazi Jews. *Gastroenterology* 1989;**96**: 1016-20.
26 Yang H, McElree C, Roth MP, *et al*. Familial empirical risks for inflammatory bowel disease: differences between Jews and non-Jews. *Gut* 1993;**34**:517-24.

J Med Genet 1999;**36**:860–862

Mutation analysis of the *DKC1* gene in incontinentia pigmenti

EDITOR—There are a number of monogenic diseases with complex phenotypes which are clinically distinct but also overlap in phenotype with one or more other syndromes. If mutations in the same gene are responsible for causing the related syndromes, the diseases are allelic. Two diseases linked to Xq28, incontinentia pigmenti (IP, MIM 308310, Bloch-Sulzberger syndrome) and dyskeratosis congenita (DKC, MIM 305000, Zinsser-Cole-Engmann syndrome) show similarities in phenotype, although the modes of expression differ. Whereas IP is X linked dominant with embryonic lethality in males, the major form of DKC is X linked recessive. The gene responsible for causing DKC, *DKC1*, was recently identified[1] and maps about 20 kb proximal to the factor VIII gene, *F8C*.[2] Linkage analyses have provided evidence that the IP gene is located in the telomeric 2 Mb region of Xq28 distal to DXS52[3] and lod scores of highest significance were found around *F8C*.[4 5] The physical map position of *DKC1* and genetic linkage of the IP locus, together with the overlap in the DKC and IP phenotypes (table 1), raised the possibility that these two diseases could be allelic.

Table 1 Comparison of the IP and DKC phenotypes affecting ectodermal tissues and the haemopoietic system

	Incontinentia pigmenti (IP)	*Dyskeratosis congenita (DKC)*
Skin		
Reticulate hyperpigmentation	+ (late stage)	+ (early stage)
Hypopigmentation	+ (reticulate or linear)	+ (scattered macules)
Alopecia	+ (scarring)	+ (non-scarring)
Epidermal atrophy	+ (late stage)	+
Pigment incontinence	+	+
Teeth		
Hypodontia	+ (prominent)	+ (occasional)
Eyes		
Retinal involvement	+ (retinal detachment, vascular proliferation)	−
Epiphora	+ (rare, one case)	+ (frequent)
Haemopoietic system		
Pancytopenia	−	+
Bone marrow failure	−	+

The IP and DKC phenotypes share abnormalities in ectodermal derivatives, such as nail dystrophy, alopecia, hypodontia, and skin manifestations[6 7] (table 1). Both IP and DKC are characterised by the early appearance of reticulate skin pigmentation, although this manifests differently in the two diseases. In IP the clinical signs affecting the skin are initially apparent as an erythematous, inflammatory vesicular rash. The rash later becomes verrucous and streaks of hyperpigmentation follow. The pigmentation then fades in the second decade of life often leaving scarred and atrophic hypopigmented areas. In DKC patients the inflammatory and verrucous stages do not occur and the appearance of hyper- and hypopigmentation is progressive. The overlap in the skin abnormalities is confirmed by microscopic examination of skin biopsies from IP and DKC patients, which show common histological features such as epidermal atrophy and pigment migration.[8] In both disorders a defect in the immune system may be causing the skin manifestations. In IP the inflammatory vesicular rash points to an involvement of the immune system and is supported by observations that the rashes are associated with constitutional eosinophilia and may recur during feverish infections. Further, it has been suggested that the skin phenotype in IP resembles that observed in patients with graft versus host (GVH) disease.[9] A GVH-like pathogenesis suggestive of an involvement of the immune system in the skin also occurs in some DKC cases.[10 11]

DKC patients develop progressive pancytopenia of one or more cell lines and bone marrow failure is the main cause of death in the first or second decade of life in 90% of the cases.[12] This is accompanied by humoral and cellular disturbances of the immune system.[13] Pancytopenia and bone marrow failure are not associated with IP. There have been reports, however, of decreases in lymphocyte number and both neutrophil and lymphocyte dysfunction in IP.[14–16] Another abnormality of the peripheral blood system suggesting an involvement of the immune system is the occurrence of leucocytosis with eosinophilia in a substantial proportion of newborn females with IP in the absence of infection. A report on a male IP patient who died postnatally and showed excessive haemorrhaging and haemolysis at birth further indicates a defect in the haematological system.[17]

Extreme skewing of X chromosome inactivation has been observed in the blood cells of most DKC carrier females[18 19] as well as in the skin and haemopoietic cells of affected IP females.[20 21] The non-random inactivation of the X chromosome carrying the mutant allele in the skin cells of IP females is responsible for the disappearance of the clinical signs because of a positive selection for cells expressing the normal allele.[20 21] It is conceivable that a defect in the haemopoietic system leading to bone marrow failure as is observed in DKC males is not apparent in IP females because of a similar selective pressure favouring cells carrying the active normal X chromosome. The skewed X chromosome inactivation in IP females could in part explain the difference in female presentation and the more severe phenotype observed in hemizyous IP males.

The hypothesis that a different spectrum of mutations in the *DKC1* gene causes IP is compatible with the ubiquitous expression pattern of *DKC1*, its high degree of conservation, and the putative function of the peptide dyskerin in rRNA biogenesis.[1 22–24] Seventeen different mutations have been identified in DKC patients of which 82% are missense mutations.[25] To date no premature stop codon mutations, frameshifts, or whole gene deletions have been identified. Taken together, these observations strongly suggest an essential function for dyskerin and that complete loss of function mutations would not be viable. It appears likely that a null mutation in *DKC1* could explain the prenatal lethality observed in IP males and that the same mutation in an IP female might result in the clinical signs observed.

The genomic structure of the *DKC1* gene has been determined.[25] The coding sequence is split into 15 exons and the gene extends over 15 kb (accession numbers AJ0101395, AJ0101396). As intronic primers flanking each of the 15 exons had been designed for mutation screening of DKC patients, it was possible to screen the *DKC1* gene efficiently for mutations in IP patients. The analysis of a large number of IP patients of different nationalities was possible because of the collaborative efforts of five research groups. Thirteen of these families have been described previously.[4 5 21 26] All 15 exons of 23 female IP patients and one spontaneously aborted male fetus carrying the mutant allele[5] were subjected to SSCP analyses. SSCP protocols that had previously been shown to be efficient for mutation detection were used and the conditions for each exon were determined to allow good resolution of the two single strands.[25 27] No shifts were observed for any of the patients. To exclude point mutations which may have been missed by SSCP, all exons from two spontaneously aborted male patients were PCR amplified and sequenced, but no mutations were found. Furthermore, 18 of the 24 DNA samples analysed by SSCP plus 32 additional IP females and three additional IP males were analysed by Southern hybridisation using the full length *DKC1* cDNA as a probe. The following restriction enzyme digests were analysed: *Xba*I, *Bam*HI, *Eco*RI, *Pst*I, *Hin*dIII, *Sac*I, *Nco*I, *Bgl*II, and *Taq*I. No differences in dosage and no aberrant bands were detected when compared with DNA samples from normal males and females. The results from Southern hybridisations and the fact that all exons were amplifiable for two IP male patients indicate that a partial or whole gene deletion of *DKC1* as a general mechanism for causing IP is unlikely. Moreover, no mutations were identified in the coding region or at the exon-intron boundaries of the two IP male patients. Owing to the difficulty of obtaining sufficient cells with an active IP mutation bearing X chromosome from female patients and because very few IP male patients with a normal XY karyotype exist, no analyses were carried out at the RNA level. It therefore cannot entirely be ruled out that there may be mutations in the promoter region or in the 5' and 3' untranslated regions (UTR) which could alter the levels of *DKC1* mRNA directly or alter the stability of the transcript in IP patients. However, we consider this to be a very unlikely possibility and conclude that IP and DKC are not allelic.

Sincere appreciation is extended to Susanne Emmerich (National Incontinentia Pigmenti Foundation) and the participating families for their willing and continuing cooperation in these investigations. We are grateful to Tracy Jakins, Helen Stewart, and Dian Donnai. We thank John Dean for providing DNA samples. Parts of this work were supported by grants from the Deutsche Forschungsgemeinschaft (DFG), the Genome Analysis Program, Telethon Italy, grant E526 to MD, the UK MRC, and the Foundation Fighting Blindness, Hunt Valley, Maryland, USA. Dr Lewis is a Senior Scientific Investigator of Research to Prevent Blindness, New York, NY, USA.

NINA S HEISS
ANNEMARIE POUSTKA
Department of Molecular Genome Analysis, Deutsches Krebsforschungszentrum (DKFZ), Im Neuenheimer Feld 280, 69120 Heidelberg, Germany

STUART W KNIGHT
Department of Haematology, Imperial College School of Medicine, London, UK

SWAROOP ARADHYA
DAVID L NELSON
Department of Molecular & Human Genetics, Baylor College of Medicine, Houston, Texas, USA

RICHARD A LEWIS
Departments of Ophthalmology, Medicine, Pediatrics, and Molecular and Human Genetics, Texas, Houston, USA

TERESA ESPOSITO
ALFREDO CICCODICOLA
MICHELE D'URSO
International Institute of Genetics and Biophysics (IIGB), Via G Marconi 10, 80125 Naples, Italy

ASMAE SMAHI
SOLANGE HEUERTZ
ARNOLD MUNNICH
Hopital des Enfants-Malades, Unité des Recherches sur les Handicaps Génétiques de l'Enfant, Paris, France

PIERRE VABRES
Service de Dermatologie, Centre Hospitalier Universitaire, Paris, France

HAYLEY WOFFENDIN
SUSAN KENWRICK
University of Cambridge, Department of Medicine, Cambridge Institute for Medical Research, Cambridge, UK

1 Heiss NS, Knight SW, Vulliamy TJ, *et al*. X-linked dyskeratosis congenita is caused by mutations in a highly conserved gene with putative nucleolar functions. *Nat Genet* 1998;**19**:32-8.
2 Tantravahi U, Murty VV, Jhanwar SC, *et al*. Physical mapping of the factor VIII gene proximal to two polymorphic DNA probes in human chromosome band Xq28: implications for factor VIII gene segregation analysis. *Cytogenet Cell Genet* 1986;**42**:75-9.
3 Sefiani A, M'rad R, Simard L, *et al*. Linkage relationship between incontinentia pigmenti (IP2) and nine terminal X long arm markers. *Hum Genet* 1991;**86**:297-9.
4 Smahi A, Hyden-Granskog C, Peterlin B, *et al.* The gene for the familial form of incontinentia pigmenti (IP2) maps to the distal part of Xq28. *Hum Mol Genet* 1994;**3**:273-8.
5 Jouet M, Stewart H, Landy S, *et al*. Linkage analysis in 16 families with incontinentia pigmenti. *Eur J Hum Genet* 1997;**5**:168-70.
6 Landy SJ, Donnai D. Incontinentia pigmenti (Bloch-Sulzberger syndrome). *J Med Genet* 1993;**30**:53-9.
7 Dokal I. Dyskeratosis congenita: an inherited bone marrow failure syndrome. *Br J Haematol* 1996;**92**:775-9.
8 Griffiths WA. Reticulate pigmentary disorders - a review. *Clin Exp Dermatol* 1984;**9**:439-50.
9 Person JR. Incontinentia pigmenti: a failure of immune tolerance? *J Am Acad Dermatol* 1985;**13**:120-4.
10 Esterly NB. Nail dystrophy in dyskeratosis congenita and chronic graft-vs-host disease. *Arch Dermatol* 1986;**122**:506-7.
11 Ling NS, Fenske NA, Julius RL, Espinoza CG, Drake LA. Dyskeratosis congenita in a girl simulating chronic graft-vs-host disease. *Arch Dermatol* 1985;**121**:1424-8.
12 Knight S, Vulliamy T, Copplestone A, Gluckman E, Mason P, Dokal I. Dyskeratosis Congenita (DC) Registry: identification of new features of DC. *Br J Haematol* 1998;**103**:990-6.
13 Sölder B, Weiss M, Jager A, Belohradsky BH. Dyskeratosis congenita: multisystemic disorder with special consideration of immunologic aspects. *Clin Pediatr* 1988;**37**:521-30.
14 Jessen RT, Van Epps DE, Goodwin JS, Bowerman J. Incontinentia pigmenti. Evidence for both neutrophil and lymphocyte dysfunction. *Arch Dermatol* 1978;**114**:1182-6.
15 Ment L, Alper J, Sirota RL, Holmes LB. Infant with abnormal pigmentation, malformations, and immune deficiency. *Arch Dermatol* 1978;**114**:1043-4.
16 Brunquell PJ. Recurrent encephalomyelitis associated with incontinentia pigmenti. *Pediatr Neurol* 1987;**3**:174-7.
17 Roberts JL, Morrow B, Vega-Rich C, Salafia CM, Nitowsky HM. Incontinentia pigmenti in a newborn male infant with DNA confirmation. *Am J Med Genet* 1998;**75**:159-63.
18 Devriendt K, Matthijs G, Legius E, *et al.* Skewed X-chromosome inactivation in female carriers of dyskeratosis congenita. *Am J Hum Genet* 1997;**60**:581-7.
19 Vulliamy TJ, Knight SW, Dokal I, Mason PJ. Skewed X-inactivation in carriers of X-linked dyskeratosis congenita. *Blood* 1997;**90**:2213-16.
20 Migeon BR, Axelman J, de Beur SJ, Valle D, Mitchell GA, Rosenbaum KN. Selection against lethal alleles in females heterozygous for incontinentia pigmenti. *Am J Hum Genet* 1989;**44**:100-6.
21 Parrish JE, Scheuerle AE, Lewis RA, Levy ML, Nelson DL. Selection against mutant alleles in blood leukocytes is a consistent feature in incontinentia pigmenti type 2. *Hum Mol Genet* 1996;**5**:1777-83.
22 Meier UT, Blobel G. NAP57, a mammalian nucleolar protein with a putative homolog in yeast and bacteria. *J Cell Biol* 1994;**127**:1505-14.
23 Cadwell C, Yoon HJ, Zebarjadian Y, Carbon J. The yeast nucleolar protein Cbf5p is involved in rRNA biosynthesis and interacts genetically with the RNA polymerase I transcription factor RRN3. *Mol Cell Biol* 1997;**17**:6175-83.
24 Lafontaine DLJ, Bousquet-Antonelli C, Henry Y, Caizergues-Ferrer M, Tollervey D. The box H + ACA snoRNAs carry Cbf5p, the putative rRNA pseudouridine synthase. *Genes Dev* 1998;**12**:527-37.
25 Knight SW, Heiss NS, Vulliamy TJ, *et al*. X-linked dyskeratosis congenita is predominantly caused by missense mutations in the DKC1 gene. *Am J Hum Genet* 1999;**65**:50-8.
26 Woffendin H, Jakins T, Jouet M, *et al*. X-inactivation and marker studies in three families with incontinentia pigmenti: implications for counselling and gene localisation. *Clin Genet* 1999;**55**:55-60.
27 Jouet M, Moncla A, Paterson J, *et al*. New domains of neural cell-adhesion molecule L1 implicated in X-linked hydrocephalus and MASA syndrome. *Am J Hum Genet* 1995;**56**:1304-14.

J Med Genet 1999;**36**:862–865

A nonsense mutation in the retinal specific guanylate cyclase gene is the cause of Leber congenital amaurosis in a large inbred kindred from Jordan

EDITOR—Leber congenital amaurosis (LCA) (MIM 204000) has the earliest onset and is the most severe form of retinal dystrophy.[1–3] It is an autosomal recessive condition that is recognised within the first few months of life because of impaired vision and an extinguished electroretinogram.[4] Nystagmus, specifically pendular, and eye poking are frequently observed early on,[5] while hypermetropia and keratoconus may develop later during the course of the disease.[6 7] Genetic heterogeneity was confirmed when the first gene of LCA was mapped to chromosome 17p13.1 (*LCA1*) by homozygosity mapping in consanguineous Arab families.[8 9] Four different mutations in the retinal specific guanylate cyclase gene (*RETGC*) were found in four unrelated probands and thus *LCA1* was assumed to result from homozygous alterations in this gene.[10]

We report here a nonsense mutation in the *RETGC* gene, which in the homozygous state is responsible for LCA in a large inbred tribe from Jordan. We had already identified a large, highly inbred family from the Jordan valley consisting of about 2000 living subjects, in which affected members have LCA.[11] A 31 member subset of this family was investigated (fig 1). All members were examined by an ophthalmologist and a paediatrician. Four patients had ERG performed (Nos 3, 9, 13, 14). Blood samples were collected from 28 family members after obtaining informed consent from them or their legal guardian.

DNA was extracted from peripheral blood samples by standard procedures.[12] Seventeen different dinucleotide repeat markers reported to be linked to *LCA1* on chromosome 17 were used to test for linkage.[8 9] Amplification of these markers was performed according to the manufacturer's conditions (Research Genetics). Products