DNA AND CELL BIOLOGY
Volume 16, Number 1, 1997
Mary Ann Liebert, Inc.
Pp. 73–83

Human Uteroglobin Gene: Structure, Subchromosomal Localization, and Polymorphism

ZHONGJIAN ZHANG,[1] DRAZEN B. ZIMONJIC,[2] NICHOLAS C. POPESCU,[2] NADIA WANG,[1] DANIELA S. GERHARD,[3] EDWIN M. STONE,[4] NANCY C. ARBOUR,[4] H.G. DE VRIES,[5] H. SCHEFFER,[5] J. GERRITSEN,[6] J.M. COLLE'E,[7] L.P. TEN KATE,[7] and ANIL B. MUKHERJEE[1]

ABSTRACT

Human uteroglobin (hUG) or Clara cell 10-kD protein (cc10 kDa) is a steroid-dependent, immunomodulatory, cytokine-like protein. It is secreted by mucosal epithelial cells of all vertebrates studied. The cDNA encoding hUG and the 5′ promoter region of the gene have been characterized previously. Here, we report that the structure of the entire hUG gene is virtually identical to those of rabbit, rat, and mouse. It is localized on human chromosome 11q12.3–13.1, a region in which several important candidate disease genes have been mapped by linkage analyses. Our data indicate that candidate genes for atopic (allergic) asthma and Best's vitelliform macular dystrophy are in closest proximity to the hUG gene. To determine whether hUG gene mutation may be involved in the pathogenesis of these diseases, we studied two isolated groups of patients, each afflicted with either atopy or Best's disease, respectively. We detected a single base-pair change in the hUG gene in Best's disease patients and normal controls but no such change was detected in atopy patients. This alteration in hUG gene-sequence in Best disease family appears to be a polymorphism. Although the results of our investigation did not uncover mutations in hUG gene that could be causally related to the pathogenesis of either of these diseases, its conservation throughout vertebrate phyla implies that this gene is of physiological importance. Moreover, the close proximity of this gene to several candidate disease genes makes it an important chromosomal marker in cloning and characterization of those genes.

INTRODUCTION

ALMOST THREE DECADES AGO, two laboratories independently discovered a steroid-dependent secretory protein in the rabbit uterus during early pregnancy that was named blastokinin (Krishnan and Daniel, 1967) or uteroglobin (Beier, 1968). This is probably one of the most exhaustively studied proteins from the standpoint of its physico-chemical properties, including its crystal structure and molecular biology (for review, see Miele *et al.*, 1988, 1994), but the exact physiological role of this protein still remains unclear.

Uteroglobin (UG) is a homodimer containing two disulfide bonds in its quaternary structure that covalently connect the two monomers of 70 amino acids each, which are antiparallel in orientation (Mornon *et al.*, 1980; Morize *et al.*, 1987; Bally and Delettre, 1989). Each monomer has four α-helices and one β-turn between α-helices 2 and 3, but there are no β-structures. UG is a steroid-dependent protein that has been called a uterine marker for progesterone action (Bullock, 1980). However, investigations during the past two decades have shown that expression of this protein is not restricted to the uterus but is present in almost all epithelial cells, including those of the respiratory, gastrointestinal,

[1]Section on Developmental Genetics, Heritable Disorders Branch, National Institute of Child Health and Human Development, The National Institutes of Health, Bethesda, MD 20892.
[2]Laboratory of Experimental Carcinogenesis, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892.
[3]Departments of Genetics and Psychiatry, Washington University School of Medicine, St. Louis, MO 63110.
[4]Department of Ophthalmology, The University of Iowa College of Medicine, Iowa City, IA 52242.
[5]Department of Medical Genetics, University of Groningen, The Netherlands.
[6]Beatrix Child's Hospital, University Hospital Groningen, The Netherlands.
[7]Department of Human Genetics, Free University of Amsterdam, The Netherlands.

and genito-urinary systems of both sexes (for review, see Miele *et al.*, 1988, 1994). The tissue and organ-specific expression of the UG gene is due to the presence of hormone-responsive elements in its 5′ promoter region (Jantzen *et al.*, 1987). Thus, estrogen and progesterone regulate the UG gene expression in the fallopian tube and in the uterus respectively, while prolactin further stimulates progesterone-induced UG production in the uterus of immature (Randall *et al.*, 1991) as well as ovariectomized rabbits (Daniel and Juncja, 1989). Glucocorticoids and testosterone, on the other hand, appear to regulate UG production in the lungs and in the prostate, respectively.

UG-like protein has also been detected in the human uterus (Cowan *et al.*, 1986; Kikukawa *et al.*, 1988), lung (Dhanireddy *et al.*, 1988), and prostate (Manyak *et al.*, 1988). The immunomodulatory role of this protein has long been recognized (Mukherjee *et al.*, 1982, 1983, 1988). The cDNA encoding Clara cell 10-kD protein, which is the human counterpart of rabbit UG, has been cloned and characterized (Singh *et al.*, 1988a). More recently, the expression and characterization of rabbit (Miele *et al.*, 1990) and human (Mantile *et al.*, 1993) recombinant UGs in *Escherichia coli* and analysis of the crystallographic (Mathews *et al.*, 1994) and solution (Carlomagno *et al.*, in press) structures of recombinant and natural hUG (Umland *et al.*, 1994) have been accomplished. The results of these studies suggested that the Clara cell 10-kD protein (Singh *et al.*, 1988a,b) and human UG (Mantile *et al.*, 1993) are identical.

Human (h) UG, like its counterpart in the rabbit, has a wide-ranging tissue distribution (Peri *et al.*, 1993). It has also been detected in the urine and has been called urine protein-1 (Jackson *et al.*, 1988). This protein possesses varied biochemical and biological properties including phospholipase A_2(PLA_A)-inhibitory (Levin *et al.*, 1986), immunomodulatory (Mukherjee *et al.*, 1982, 1983, 1988)/antiinflammatory properties (Levin *et al.*, 1984; Miele *et al.*, 1988; Mukherjec et al., 1988; Camussi *et al.*, 1990a,b,c; Chan *et al.*, 1990, 1991; Di Rosa and Ialenti, 1990; Ialenti *et al.*, 1990; Facchiano *et al.*, 1991; Tetta *et al.*, 1991; Perretti *et al.*, 1991; Cabre' *et al.*, 1992; Moreno *et al.*, 1995). It binds xenobiotic agents such as progesterone (Beato, 1976, 1977; Fridlansky and Milgrom, 1976; Atger *et al.*, 1980; Bochskanl and Kirchner, 1981; Peter, 1992), polychlorinated biphenyls (Gillener *et al.*, 1988; Nordlund-Moller *et al.*, 1990), and retinol (Lopez de Haro *et al.*, 1994). Because of its potent PLA_2-inhibitory, immunomodulatory/antiinflammatory activities and its high level of constitutive expression in the pulmonary system, it has been suggested that this protein may play critical roles in controlling inflammation in the airway (Mukherjee *et al.*, 1988). Thus, the possibility has been raised that hUG may be a candidate gene for atopic asthma (Mukherjee *et al.*, 1994). Moreover, the recent demonstration that in the developing lung and in the uterus the level of UG gene expression is inversely related to the level of certain eicosanoids (Peri *et al.*, 1995) warranted a thorough characterization of the human gene encoding this protein. The 5′ region of the hUG gene has already been characterized (Wolf *et al.*, 1992). Here, we report that (i) hUG gene structure, like that of the rabbit and the mouse, contains 3 exons and 2 introns; (ii) this gene localizes in chromosome 11q12.3–13.1, a region in which several important disease genes, such as allergic asthma (atopy) and Best's macular dystrophy have been localized by linkage analyses; and (iii) a 1-bp change in this gene sequence, which appears to be a polymorphism, may be detected in patients with Best's macular dystrophy and in unaffected family members but not in those with atopic asthma. The conservation of this gene throughout vertebrate phyla suggests its physiological importance. Moreover, the close proximity of this gene to several candidate disease genes makes it an important genetic marker on human chromosome 11.

MATERIALS AND METHODS

Detection of UG-like immunoreactivity in the lungs of different vertebrates by immunofluorescence

The trachea and lungs of different vertebrate phyla, such as amphibia (frog), reptilia (turtle), avis (chicken), and mammalia (rabbit) were obtained from Analytical Biological Services (Wilmington, DE). These tissues were immediately fixed in 4% buffered paraformaldehyde and paraffin blocks were prepared. The histological sections were used for immunofluorescence using goat anti-hUG immunoglobulin G (IgG) and fluoroisothiocyanate (FITC)-conjugated anti-goat antibody according to published procedure (Peri *et al.*, 1995). The photomicrographs (400×) were taken using a Zeiss photomicroscope fitted with epifluorescence monitor.

Isolation of the hUG genomic clones

A genomic library derived from human placental DNA cloned into the *Xho* I site of the FIX II was obtained from Strategene (La Jolla, CA). The titer of this library was 8.4×10^9 pfu/ml. Human UG cDNA was labeled with [α-^{32}P]dATP and used as the probe. Approximately 2×10^6 recombinants were screened using a ^{32}P-labeled 367-bp hUG cDNA probe. Duplicate lifts were made to confirm positive clones. Hybridization was performed at 65°C. Three primary positive clones were detected and plaque purified. The clones were designated AM4-1, AM4-2, and AM4-3. Clones AM4-1 and AM4-2 were chosen for further characterization. Bacteriophage DNA containing the hUG gene from these two clones was isolated and digested with *Bam* HI, *Hind* III, *Eco* RI and *Not* I. Clones AM4-1 and AM4-2 exhibited different restriction patterns with *Bam* HI, *Hind* III, *Eco* RI, and *Pst* I digests. The *Not* I digest cuts out the insert intact, leaving a small portion of the vector on either end. The sizes of inserts in AM4-1 and AM4-2 are approximately 19 kb and 16.5 kb, respectively.

Restriction fragment analysis

Purified cloned DNAs from AM4-1 and AM4-2 were digested to completion with *Not* I, followed by complete and partial digests with *Bam* HI, *Eco* RI, *Sac* I, and *Xba* I. Digests were electrophoresed in 0.7% agarose gels, transferred by Southern blotting, and hybridized sequentially with T7, T3, hUG 5′ flanking region-derived nucleotides and a 367-bp *Pst* I fragment hUG probe, individually. Restriction maps were constructed for both clones based upon the fragments hybridized with T7 and T3 probes. Specific restriction fragments of clone AM4-1 hybridized to hUG 5′-flanking region and 367-bp *Pst* I fragment probes. Neither probe hybridized to AM4-2 restriction fragments. This was confirmed by dot blot analysis where hUG 5′-

flanking region and 367-bp *Pst* I fragment probes bound to AM4-I DNA but not to AM4-2 DNA.

DNA sequencing

An 8-kb *Bam* HI fragment of genomic DNA containing the 5′-flanking region and all three exons of hUG gene was isolated from AM4-1 clone. This fragment was subcloned into a pGEM7z cloning vector (Promega Biotec, Madison, WI) and partially sequenced by the dideoxynucleotide chain-termination method using the USB Sequenase, version 2.0, DNA sequencing kit (United States Biochemicals, Cleveland, OH). The primers used for sequencing were designed on the basis of overlapping sequences of the UG gene, which were determined with the universal primers designed from vector sequences flanking the cloned DNA fragment and the primers based on the human UG cDNA sequence.

Mapping of the hUG gene by somatic cell hybrids

Somatic cell and radiation-reduced hybrids were described by Gerhard *et al.* (1992) and Smith *et al.* (1995a). The hUG cDNA probe or primers specific for the 5′ end of the gene were used to map the gene by Southern analysis and PCR, respectively.

Fluorescence in situ *hybridization*

To localize the hUG gene, a genomic fragment of 4.5 kb was labeled by nick translation in the presence of either biotin-11-dUTP or digoxigenin-11-dUTP. Fluorescence *in situ* hybridization (FISH) was performed as previously described (Popescu *et al.*, 1985; Popescu *et al.*, 1994; Zimonjic *et al.*, 1994). Briefly, chromosomes obtained from methotrexate-synchronized normal peripheral leukocyte cultures were pretreated with RNase, denatured for 2 min at 70°C in 2× SSC, 70% (vol/vol) formamide and hybridized with the cDNA probe (200 ng) in 2× SSC, 50% (vol/vol) formamide, 10% (wt/vol) dextran sulfate, 2× Denhardt's solution, 1% Tween 20 (vol/vol), and 50 μg of human Cot-1 DNA (BRL) probe for 18 hr at 37°C. Post hybridization washing was carried out in 50% formamide–2× SSC at 42°C (3 × 6 min each) and in 0.1 × SSC at 60°C (3 × 6 min each). Biotin-labeled cDNA was detected by FITC-conjugated avidin DCS and anti-avidin antibodies (Vector Laboratories). The chromosomes were counterstained with propidium-iodide and examined under an Olympus BH2 epifluorescence microscope. Digital image acquisition, processing, and analysis as well as the procedure for direct visualization of fluorescent signals to banded chromosomes were carried out as previously described in detail (Popescu *et al.*, 1985, 1994; Zimonjic *et al.*, 1994, 1995).

Profile of patients with atopic asthma and Best's disease

Patients with Atopy: Genomic DNA from 26 sib-pairs, from the northern parts of The Netherlands, affected with allergic (atopic) asthma was studied. The inclusion criteria have been previously described (Collee' *et al.*, 1993; ten Kate *et al.*, 1994). All patients met at least two or more criteria of a modified Dutch version of the MRC/ECCs questionnaire on respiratory symptoms and positive IgE tests. More specifically, an IgE level of 0.35 PRU/ml or more was used as a criterion for inclusion into the study. The total serum IgE level for children under 10 years of age was used as standard as described by Kjellmann *et al.* (1976). The microsatellites and polymorphic markers used in the analysis were 17bTA (an intragenic marker in the cystic fibrosis gene on 7q31); D11S527, D11S534, D11S97, PYGM, D11S480, and FcεRI, all of which are located (from telomere to centromere) on chromosome 11q13. A marker D14S51 (a CA repeat close to the α_1-antitrypsin gene) was also used. The results of this study revealed that while the markers on 7q31 and 14q32 were not shared, there was significant sharing of markers (*e.g.*, D11S97, PYGM, and D11S480) on chromosome 11q13. These data suggested the presence of a candidate gene for atopic asthma in this region of chromosome 11 to which hUG gene is also localized.

Patients with Best's vitelliform macular dystrophy

Three patients from each of three separate families (9 patients) affected with Best's disease were studied. The affected phenotype in each family was independently shown by linkage analysis to map to chromosome 11q. In addition, at least one affected individual from each family was shown to have an abnormal electrooculogram. Two patients from each family exhibited classic vitelliform lesions of the macula while one individual from each family was a clinically normal spouse. The individuals performing the SSCP analysis were masked to the clinical phenotype and family relationships of these patients.

Single-strand conformation polymorphism

Genomic DNAs from atopy and Best's disease patients were isolated and examined for hUG gene mutation using single-strand conformation polymorphism (SSCP) analysis with five pairs of primers. Three exons of hUG gene were amplified by SSCP using oligonucleotide primers as follows: exon 1, 5′-CTC CAC CAT GAA ACT CGC TG (forward) and 5′-CTG AGA CTC AGC ATG CCC AG (reverse); exon 2, 5′-CTT CTC TCC TCT GTG TTG CA (forward) and 5′-TAC CAT GAG CTT AAT GAT GC (reverse); exon 3, 5′-TCC TCC TAG AGT TGA CTG CA (forward) and 5′-CGG GAT CTT CAG CTT CTA AA (reverse). For the 5′-flanking region, two sets of primers were used. One set of primers, 5′-GCC AAT GCC AAG TAA ATA GT (forward) and 5′-TGA CAG CGA GTT TCA TGG T (reverse), were used for examining the promoter region from −97 to +54. Another set of primers, 5′-GTT CAG TGA GTG ACA CAG GC (forward) and 5′-AAG GGC TCT ACA TAA GAT ACT G (reverse), were used for checking the 5′-flanking region from −265 to −97. Unlabeled primers were used for SSCP analysis, and 0.25 μl of [^{32}P]dATP (5,000 Ci/mmole; New England Nuclear, Boston, MA) were added in the reaction mixture. The polymerase chain reaction (PCR) conditions were as follows: denaturation at 95°C for 2 min for one cycle; 30 cycles of denaturation at 94°C for 1 min, annealing at 59°C for 1 min, and extension at 72°C for 1 min; final extension was at 72°C for 5 min. Following the amplification, 1 μl of the reaction mixture was mixed with 9 μl of loading buffer, which consisted of 95% formamide, 0.05% of bromophenol blue, 0.05% xylene cyanol, 10 m*M* NaOH, and 20 m*M* EDTA. After boiling for 3 min, the mixture was chilled immediately and elec-

trophoresis was carried out on an MDE gel (AT Biochem, Malvern, PA) at 3 Watts for 12–18 hr at room temperature.

RESULTS

UG-like immunoreactivity in the airways of different vertebrates

As shown in Fig. 1, an hUG-like immunoreactivity in the tracheobronchial epithelia of the frog, turtle, chicken, and the rabbit is easily detected. The pattern of distribution of this immunoreactivity is virtually identical to rabbit, rat, mouse, and human tissues. Because our antibody is highly specific for hUG and this antibody only cross-reacts with rabbit, rat, and mouse UG, we believe that the detection of high level of immunofluorescence on the epithelia of these animals indicate that a protein antigenically identical to UG is present in the tracheobronchial epithelia of all vertebrates. Moreover, the use of nonspecific IgG or preimmune serum as the first antibody or omission of hUG-antibody failed to produce detectable immunofluorescence (data not shown). This suggests that the observed hUG-like immunoreactivity is specific.

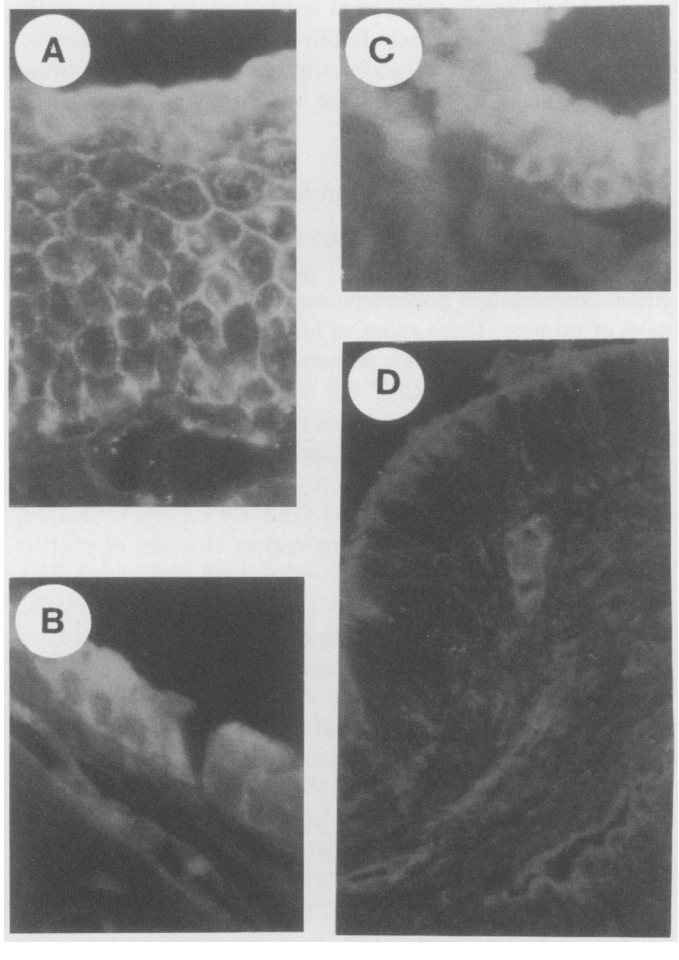

FIG. 1. Immunofluorescence of tracheobronchial tissues of animals from different vertebrate phyla: a, amphibia (frog); b, reptilia (turtle); c, aves (chicken); and d, mammalia (rabbit). Note the high level of immunofluorescence localized over the tracheobronchial epithelia of all animals, suggesting the presence of a UG-like antigen in these tissues.

Human UG gene sequence and organization

The hUG gene consists of three exons spanning 3.3 kb from the start of exon 1 to the end of exon 3. The open reading frame of the first exon has 54 bp that encode 18 amino acids forming most of the hydrophobic amino-terminal leader peptide. The second exon, which encodes 63 amino acids, is the largest of the three exons. The first three amino acids in exon 2 belong to the terminal end of the leader peptide. This exon harbors the putative active site amino acids of UG (residues 39–47) responsible for PLA_2-inhibitory activity. A 3.1-kb intron separates exon 1 from exon 2. The third exon is located 0.9 kb downstream from the end of exon 2. This exon encodes the last 10 amino acid residues of the hUG protein. The exon boundaries are shown in Table 1.

A comparison of the hUG gene sequences with those of mouse and rabbit reveals that there are several highly conserved areas in the 5′-flanking regions. The TATA boxes and two Oct regions of UG genes beart a high degree of homology (Fig. 2). In addition to the portion of exon 2 that encodes the putative antiinflammatory peptide known as "antiflammin" (Miele *et al.*, 1988), there are two very highly conserved regions detectable in the UG gene in all three species. The first region starts at nucleotide −247 of the hUG gene and ends at nucleotide −261. In this region, the UG genes of all three species share greater than 93% structural identity. The second region is located between nucleotides −267 to −290, which is located just before the start of the first Oct region. In this region, there is more than 79% identity between rabbit and hUG genes as well as between those of human and mouse, whereas there is 75% identity between mouse and rabbit UG genes. The functions of these highly conserved regions are not clear at this time. The exon–intron boundaries in all three species are virtually identical (Fig. 2). The deduced polypeptide sequence encoded by all three genes suggests that the most highly conserved region is the antiflammin (Miele *et al.*, 1988) region (amino acid residues 39–47) encoded by exon 2 in all three species.

Chromosomal localization of hUG gene

Normal human prometaphase and metaphase chromosomes were hybridized with a biotinylated hUG probe. A fluorescent signal was detected in 67 (83.75%) of 80 randomly examined spreads with low nonspecific FITC background. Signal consisting of two symmetrical fluorescent spots on both chromatids at the long arm of at least one medium size submetacentric chromosome was observed in 51% (76.25%) of the spreads. In 49 metaphases (61.25%), two apparently homologous chromosomes exhibited fluorescent doublets. A symmetrical signal on sister chromatids was not observed on other chromosomes. Similar results were obtained in an independent experiment. The G-banding pattern of the chromosomes with specific FITC labels identified them as chromosome 11. To demonstrate the identity of the chromosomes unequivocally, a set of slides with recorded signals were rehybridized (Xu and Wang, 1994) with a chromosome 11 painting probe. Without exception, FITC-labeled chromosomes reacted exclusively with a whole chromosome 11 painting probe. To localize precisely the position of the signal, a total of 40 LUT-inverted and contrast-enhanced digital images of DAPI-banded metaphases (Zimonjic *et al.*, 1995) with both chromosomes 11 labeled were analyzed and the signal was localized at region 11q12.3–13.1 (Fig. 3), where we assign the locus of hUG gene.

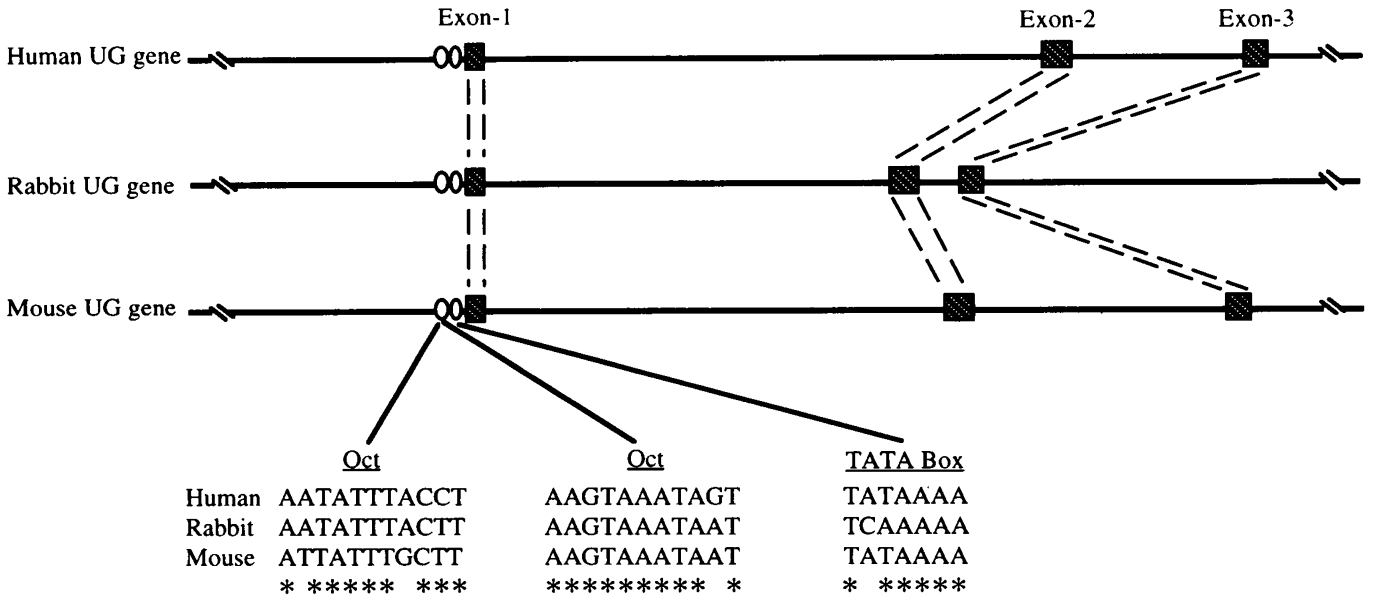

FIG. 2. Schematic diagram representing the organizations of the human, rabbit, and mouse gene structures. Hatched boxes show the exons of the UG genes. Top. Open ellipses represent *Oct* promoter regions whereas solid ellipses are the TATA box regions. Bottom. Sequence alignment of homologous regions of human, rabbit, and mouse uteroglobin genes. Partial *Oct* promoter and TATA box gene sequences are presented.

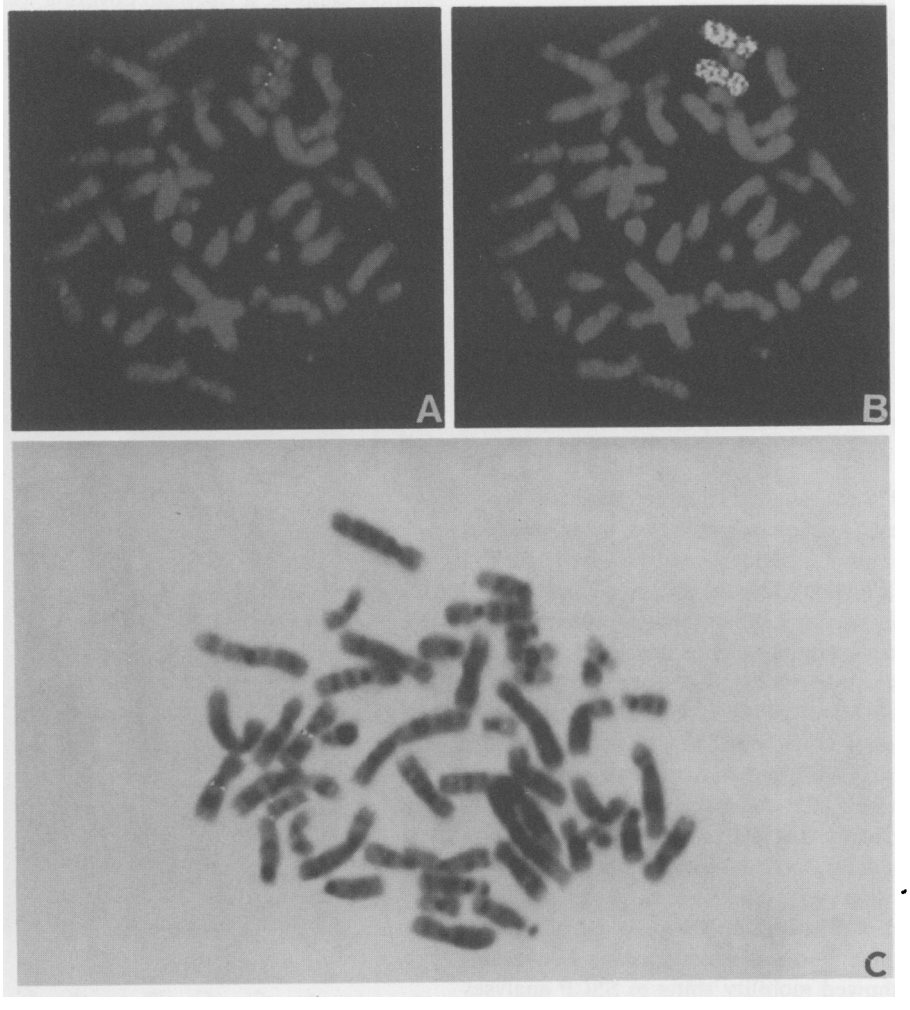

FIG. 3. Chromosome localization of hUG gene by FISH. A. Digital image of the metaphase from a normal donor 46, XY hybridized with a biotin-11-dUTP-labeled hUG genomic probe. Two medium-sized, apparently homologous submetacentric chromosomes have symmetrical fluorescent label on the long arms. B. The same metaphase after rehybridization with a whole chromosome 11 painting probe. Both labeled chromosomes are identified as chromosome 11. C. Regional FISH localization of the hUG locus at 11q12.3–13.1 contrast-enhanced, LUT-inverted, and digital image of DAPI-banded chromosomes.

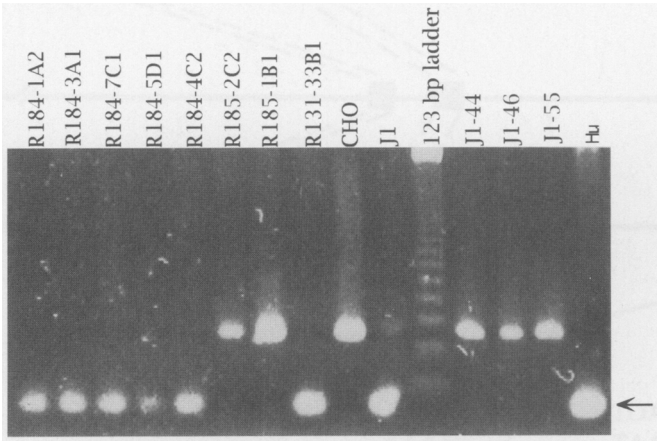

FIG. 4. Sublocalization of phUG by radiation-reduced and somatic cell hybrids. Primers specific for the hUG-flanking region were used to amplify DNA from three somatic cell hybrid cell lines (J1-44, J1-46, and J1-55), the eight radiation-reduced cell lines (R184-1A2, R184-3A1, R184-7C1, R184-5D1, R184-4C2, R185-2C2, R185-1B1, and R131–33B1), human, and Chinese hamster ovary (CHO). The product was electrophoresed on 1:1% NuSieve:/Sea-KemLE agarose gel. A human-specific fragment of 115 bp was obtained. In some cell lines an ~320-bp CHO band was also observed.

Mapping of the hUG gene by somatic cell and radiation hybrids

We used a series of somatic cell and radiation-reduced somatic cell hybrids described previously (Smith *et al.*, 1995a) to map the hUG gene with respect to other genes in 11q12.1–13.2. The UG hybridization pattern (Fig. 4) indicates that it is between pepsinogen A and D11S480, a region of about 4 cM (Smith *et al.*, 1995b).

SSCP analyses of hUG gene from patients with Best's disease and atopic asthma

Atopic Asthma: SSCP analysis for all three exons and the 5′ promoter region of the UG gene was carried out using genomic DNA samples from atopic patients as described earlier. Genomic DNA from a total of 52 patients and 52 parents belonging to 26 well-characterized atopic families were included in this analysis. The same primer pairs as described for Best's disease (see Materials and Methods) were used. No abnormal SSCP pattern was found in any of the exons or the 5′ promoter region which covered nucleotides −265 to −97 and nucleotides −97 to +54, respectively (data not shown). Thus, it was concluded that there was no SSCP-detectable mutation in the region of the gene covered by this analysis.

Best's Disease: Genomic DNA samples from 9 patients representing three Best's disease families were examined by SSCP analysis with five pairs of primers (see Materials and Methods). It was found that of nine Best's disease genomic DNA samples, seven samples showed mobility shifts in SSCP analyses covering the 5′-flanking region of hUG gene from nucleotides −97 to +54. No SSCP pattern shifts were found in all three exons and the 5′-flanking region of hUG gene from nucleotides −265 to −97. Among the seven shifted SSCP pattern samples, five samples, A, E, F, G, and H, were heterozygous for a shift,

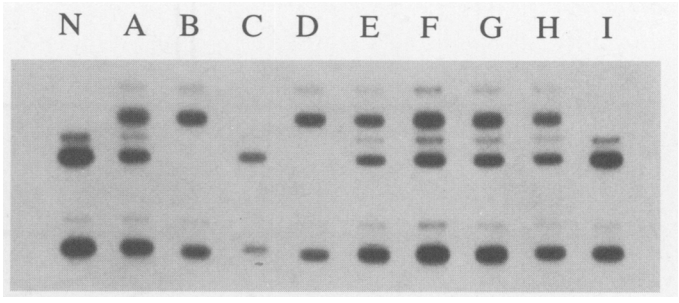

FIG. 5. SSCP analyses of amplified DNAs from Best's macular dystrophy patients. Lanes A–I, DNA samples from Best's macular dystrophy patients; lane C, normal control DNA sample. Abnormal patterns of electrophoretic mobility of DNA bands were detected in lanes A, B, D, E, F, G, and H. In lanes B and D, an arrow indicates a missing band.

two samples, B and D, were homozygous for the shift, whereas the normal control samples were homozygous for the normal allele (Fig. 5).

To analyze further the mutation in the seven abnormal band-shifted samples, a fragment covering the UG gene 5′-flanking region from −97 to +54 was amplified by PCR and subcloned into TA vector. DNA sequencing revealed that there is a single basepair change at position +33, in which a base C was substituted with a base T (Fig. 6). This single basepair change (C → T) was found in all subclones in Best's disease genomic DNA samples B and D. Even though this alteration in nucleotide sequence was detected in all patient samples A, E, F,

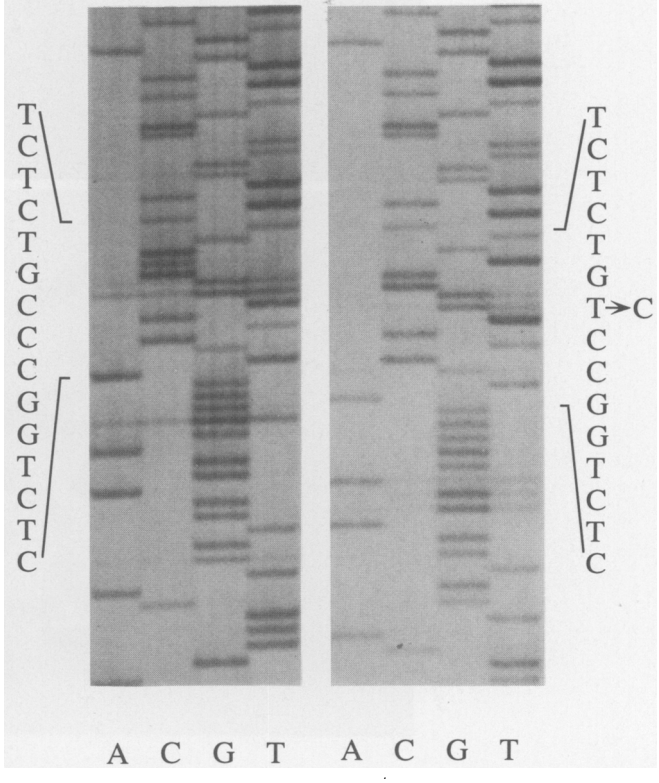

FIG. 6. Autoradiographs representing partial nucleotide sequences of the 5′-flanking region of hUg gene from normal and Best's macular dystrophy patients. Note that at position +33, the base C was substituted with the base T.

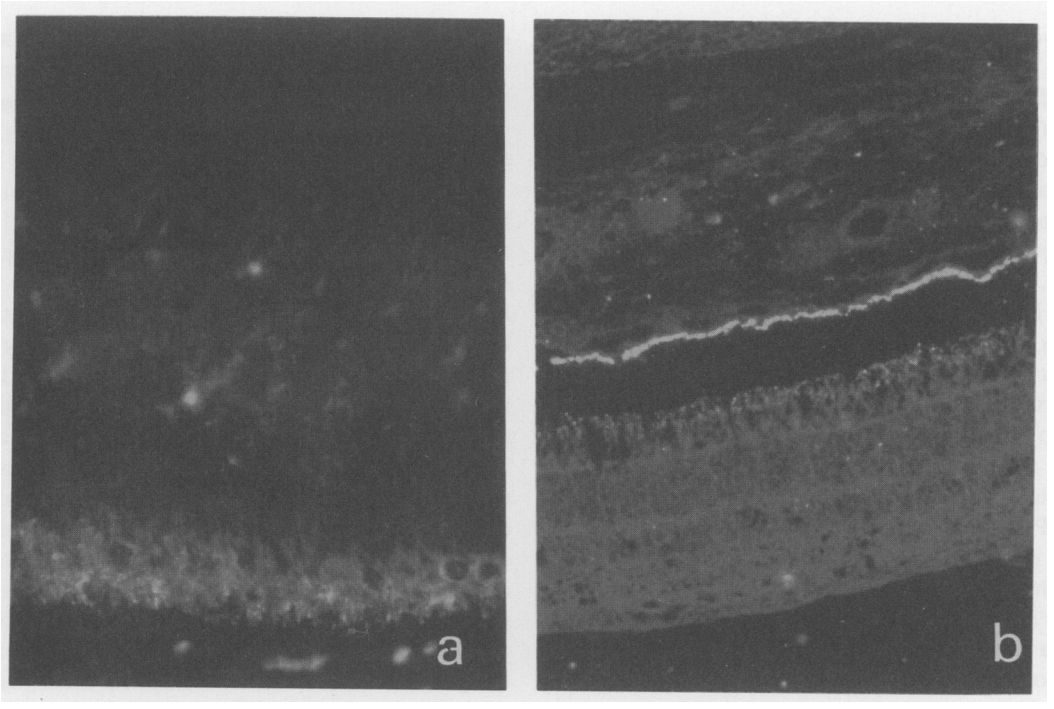

FIG. 7. Immunofluorescence of mouse (a) and human (b) retina. Note the presence of intense UG-specific immunofluorescence in both mouse and human retina.

G, and H, it only can be found in some of the subclones for each patient genomic DNA samples. This is because patient samples A, E, F, G, and H were heterozygous, whereas patients B and D were homozygous for this mutation based on the mobility-shift patterns in SSCP and the DNA sequencing information. Because the point mutation was found at the position outside of the promoter region and it was found in samples from unaffected spouses (patients E, F, and H), it is most likely a polymorphism rather than a mutation pathogenetically related to this disease.

Expression of UG-specific immunoreactivity in the mouse and human retina

To determine whether UG is expressed in the mouse and human retina, we used antibodies against mouse and human UG, respectively, to detect UG immunoreactivity by immunofluorescence. As shown in Fig. 7 the presence of UG-specific immunoreactivity is clearly demonstrated on both mouse (Fig. 7A) and human (Fig. 7B) retina. Preimmune antibody failed to produce any immunofluorescence (data not shown), suggesting that the observed immunofluorescence reflects the presence of UG in the retina of both species.

DISCUSSION

In this study, we demonstrate that hUG gene structure is very similar to that of the mouse (Ray *et al.*, 1993; Singh *et al.*, 1993), rat (Hagen *et al.*, 1990), and the rabbit (Bullock, 1980; Mornon *et al.*, 1980; Atger *et al.*, 1981; Sneed *et al.*, 1981; Menne *et al.*, 1982; Morize *et al.*, 1987). Although the cDNAs or genes encoding this protein have not been cloned and characterized from any other phyla other than mammals, the results of our present study demonstrate that a UG-like protein is detectable in four representative vertebrates (Fig. 1). Previously, we reported that recombinant rabbit and hUGs (Miele *et al.*, 1990, Mantile *et al.*, 1993) are virtually identical both structurally (Mornon *et al.*, 1980; Morize *et al.*, 1987) and functionally (Miele *et al.*, 1990; Mantile *et al.*, 1993). The many and varied biological properties of UG and hUG described so far (Miele *et al.*, 1988, 1994) are suggested to be related to either their PLA_2-inhibitory property (Levin *et al.*, 1986; Miele *et al.*, 1988; Singh *et al.*, 1988a,b; Miele *et al.*, 1990; Facchiano *et al.*, 1991; Mantile *et al.*, 1993) or due to their ability to bind various xenobiotic agents (Beato, 1976, 1977; Fridlansky and Milgrom, 1976; Atger *et al.*, 1980; Bochskenal and Kirchner, 1981; Gillener, 1988; Nordlund-Moller *et al.*, 1990; Peter *et al.*, 1992; Lopez de Haro and Neato, 1994). However, some of the biological properties of UG, such as its ability to inhibit chemotaxis, phagocytosis, thrombin-induced platelet aggregation, and cell proliferation may not be explainable on the basis of the known physicochemical properties of this protein.

It is known, however, that this protein is secreted by the uterine endometrium and crosses the trophoblast layer of the im-

TABLE 1. EXON BOUNDARIES OF THE HUMAN UG GENE

5′ Intron boundary	*Intron number and size (kb)*	*3′ Intron boundary*
AGCTCC/ggtggag	3.1	gttgca/GCTTCT
CTCATG/gtaacc	0.66	gttttg/GAAAAA

Note: Exon sequences are shown in capitalized letters whereas intron sequences are shown in lowercase letters.

planting embryo to reach the blastocoele cavity (Kirshnan and Daniel, 1967). The mechanism of this transmigration from the uterus to the blastocoele is suggested to involve a carrier-mediated process (Robindon *et al.*, 1989). Recently, we (Kundu *et al.*, 1996) and others (Diaz Gonzalez and Nieto, 1995) discovered that high-affinity binding sites for hUG are present on the surface of various cell types, including the human trophoblasts and NIH-3T3 cells. Moreover, hUG appears to regulate cellular invasion *via* this putative receptor (Kundu *et al.*, 1996). Most interestingly, while the human trophoblast cells express the UG-binding protein, it is not detectable on human choriocarcinoma cells (Kundu *et al.*, 1996), a highly invasive malignant cancer, suggested to arise from the trophoblasts. A similar cell-surface binding protein for rabbit UG has recently been reported by Diaz-Gonzalez and Nieto (1995). Thus, UG appears to exert its biological effects both by catalytically inactivating secretory group I and II PLA_2 activities (Levin *et al.*, 1986; Miele *et al.*, 1988; Singh *et al.*, 1988b; Camussi *et al.*, 1990a,b,c; Ialenti *et al.*, 1990; Facchiano *et al.*, 1991; Cabre *et al.*, 1992; Lloret and Moreno, 1994) as well as by binding to its receptor on the cell surface (Kundu *et al.*, 1996; Diaz-Gonzalez and Nieto, 1995). Therefore, it would be logical to assume that mutation of either the ligand (*i.e.*, UG) or its receptor or both could lead to pathological conditions.

In previous reports, Wolf *et al.* (1992) have determined that the hUG gene is localized to chromosome 11. Our results of metaphase FISH in addition to confirming this finding further demonstrate that the hUG gene is located precisely on human chromosome 11q12.3–13.1 (Fig. 3). This result is also supported by our recent results with radiation-reduced somatic cell hybrids (Smith *et al.*, 1995a). Interestingly, the region covered by chromosome 11q12–14 has been shown to harbor a number of candidate disease genes such as atopic asthma, Best's macular dystrophy, McArdle's syndrome, B-cell lymphomas, Bardet-Biedl syndrome, and multiple endocrine neoplasia-1. Because hUG is a potent anti-inflammatory/immunomodulatory protein that appears to have receptor-mediated functions, we sought to determine whether hUG gene is mutated in atopic asthma and Best's macular dystrophy.

We searched for a hUG gene mutation in patients with atopy first because the pathophysiologic manifestations of this disease, especially those of atopic asthma, may be closely related to the cellular inflammatory response to foreign antigens in the respiratory epithelia. IgE has been known to activate PLA_2 and release arachidonic acid, which may be used for the synthesis of proinflammatory lipid mediators such as eicosanoids and platelet-activating factor (PAF) (Urata and Siraganian, 1985; McGivney *et al.*, 1981). Accumulation of basophils and eosinophils in the respiratory system is one of the hallmarks of atopic asthma. Mast cell degranulation in response to IgE binding to its receptor may also be involved in the pathogenesis of this disease. In addition, the results of recent investigations suggest that a group I PLA_2 (PLA_2-I) may be associated with the secretory granules of these cells (Chock *et al.*, 1994). Furthermore, cDNAs encoding a high-affinity receptor for PLA_2-I have recently been cloned and characterized (Ishizaki *et al.*, 1994; Lambeau *et al.*, 1994; Ancian *et al.*, 1995). Through this receptor, PLA_2-I exerts several cellular effects, including bronchial smooth muscle contraction (Sommers *et al.*, 1992), a critical feature of asthma. Most importantly, an inverse relationship between the production of proinflammatory eicosanoids and the level of hUG has been reported in the nasopharyngeal lavage fluid obtained from children with viral infections (Volvovitz *et al.*, 1988). Additionally, genetic linkage data suggest that a candidate gene for atopy may be located on human chromosome 11q13 (Young *et al.*, 1992; Sanford *et al.*, 1993). Furthermore, hUG is a potent antiinflammatory protein. Results of site-directed mutagenesis of the hUG gene indicate that a mutation in the nonapeptide region (active site responsible for PLA_2 inhibition) of this protein leads to a total lack of PLA_2-inhibitory activity (manuscript in preparation). The mutated protein may also lack other biological functions of this protein such as its antichemotactic, antichemokinetic, and antiphagocytic properties. Thus, it is conceivable that hUG gene mutation may alter: (i) the function of hUG as an inhibitor of PLA_2 activity; (ii) its function as a ligand of its receptor and consequent blockage of signal transduced *via* this pathway; or (iii) a complete deficiency of this protein, which may lead to dysregulation of the homeostatic mechanisms that regulate the integrity of the mucosal epithelia. As a result, one would expect the release of high levels of arachidonic acid, itself a potent chemoattractant, causing migration of immunocytes into the pulmonary wet mucosa where the hUG gene is constitutively expressed at a high level under normal physiological conditions. In addition, high arachidonic acid release may eventually lead to increased production of proinflammatory lipid mediators [*e.g.*, prostaglandins, leukotrienes, thromboxanes, platelet activity factor (PAF), *etc.*]. Thus, a vicious cycle could ensue by initiating and propagating an inflammatory response in the respiratory tract or aggravating existing inflammatory processes. In the tracheobronchial tree and in the lung, the vascular bed as well as the smooth muscles may respond to the inflammatory reaction and cause bronchospasm, which clinically manifests as respiratory distress, the hallmark of asthma. In the epithelia of the upper respiratory tract, including the nasopharyngeal epithelia, the symptoms of inflammation may be manifested as rhinitis and mucosal edema, commonly encountered in atopic asthma. Although we have uncovered no mutation in the hUG gene in atopic patients, it is possible that a mutation in its receptor gene may be associated with this disease. This could only be ascertained when the cDNA and the gene for the receptor is cloned and characterized.

It should be noted that recently Marsh *et al.* (1994) and Postma *et al.* (1995) have reported that a susceptibility gene for asthma may be localized to chromosome 5q31-q33. It would be interesting to determine whether the hUG receptor gene localizes in this region of chromosome 5 and as well as its relationship to the putative asthma gene in this locus. Atopy has been linked to markers in 11q13, specifically between D11S97 (a marker distal to PYGM) and D11S451 (Sanford *et al.*, 1993), with the highest location scores being between D11S480 and FcϵRI-β. FcϵRI-β is within 1.5 megabase (mb) of PGA (Stafford *et al.*, 1994) and proximal to UG. Therefore, UG is a very attractive candidate for atopy, especially because of its chromosomal location and its potent antiinflammatory function.

The second candidate disease we investigated is Best's macular dystrophy (Best, 1905). Best's disease has been mapped to chromosome 11q13 (Stone *et al.*, 1992; Forsman *et al.*, 1992; Weber *et al.*, 1994). More precisely, this disease gene has been further sublocalized by genetic analysis proximal to D11S480 and distal to FcϵRI-β (Graff *et al.*, 1994) and proximal to a tetranucleotide sequence that is located 5′ from the hUG gene

(Stoehr and Weber, 1995). Because the orientation of transcription is not known, the coding sequences of hUG could be in the candidate region. In Best's disease, the mechanism of macular degeneration is not clear. We discovered that in murine as well as in human retina a high level of UG-like immunoreactivity could be readily detected (Fig. 7). Thus, hUG was a reasonable candidate gene from both positional and tissue expression standpoints. However, our study revealed a sequence change that did not segregate with the disease phenotype. This basepair change appears to be a polymorphism in the hUG gene because it occurs in both affected and unaffected members of Best's disease family. Admittedly, the patient population we studied is small and our results underscore the need for further investigation, including a larger population of patients with this disease, to determine if other hUG gene mutations are detectable.

In sum, we have delineated the structure and subchromosomal localization of hUG gene and uncovered a hitherto unreported single base-pair change in the 5′ region of this gene in Best disease families that we believe is a polymorphism.

ACKNOWLEDGMENTS

We thank Drs. J. Butler, S.W. Levin, and J.B. Sidbury, Jr., for critical reviews of the manuscript and valuable suggestions. Two of us (D.S.G., E.M.S.) are being supported by a grant from the Foundation Fighting Blindness.

REFERENCES

ANCIAN, P., LAMBEAU, G., MATTEI, M.G., and LUZDUNSKI, M. (1995). The human 180-kDa receptor for secretory phospholipase A_2. J. Biol. Chem. **270,** 8963–8970.

ATGER, M., MORNON, J.P., SAVOURET, J.F., LOOSFELT, H., FRIDLANSKY, F., and MILGROM, E. (1980). Uteroglobin: A model for the study of the mechanism of action steroid hormones. In *Steroid Induced Uterine Proteins: Developments in Endocrinology*, vol. 8. M. Beato, ed. (Elsevier, Amsterdam) pp. 341–346.

ATGER, M., ATGER, P., TIOLLAIS, P., and MILGROM, E. (1981). Cloning of rabbit genomic fragments containing the uteroglobin gene. J. Biol. Chem. **256,** 5970–5972.

BALLY, R., and DELETTRE, J. (1989). Structure and refinement of the oxidized P21 form of uteroglobin at 1.64 Å-resolution. J. Mol. Biol. **206,** 153–170.

BEATO, M. (1976). Binding of steroids to uteroglobin. Steroid Biochem. **7,** 327–334.

BEATO, M. (1977). Physico-chemical characterization of uteroglobin and its interaction with progesterone. In *Development in Mammals*, vol. 2. M.H. Johnson, ed. (Elsevier, Amsterdam) pp. 173–185.

BEIER, H.M. (1968). Uteroglobin: A hormone sensitive endometrial protein involved in blastocyst development. Biochim. Biophys. Acta **160,** 289–291.

BEST, F.Z. (1905). Uber eine hereditare Maculaaffektion: Beitrage zur Vererbungslehre. Augenheilkd. **13,** 199–212.

BOCHSKANL, R., and KIRCHNER, C. (1981). Uteroglobin and the accumulation of progesterone in the uterine lumen in the rabbit. Wilhem Roux's Arch. Dev. Biol. **190,** 127–135.

BULLOCK, D.W. (1980). Uterine proteins as markers of progesterone action. In *Steroid-Induced Proteins. Developments in Endocrinology*, vol. 8. M. Beato ed. (Elsevier-North Holland, Amsterdam) p. 315.

CABRE', F., MORENO, J.J., CARABAZA, A., ORTEGA, E., MOULEON, D., and CARGANICO, G. (1992). Antiflammins. Anti-inflammatory activity and effect on human phospholipase A2. Biochem. Pharmacol. **44,** 519–525.

CAMUSSI, G., TETTA, C., BUSSOLINO, F., and BAGLIONI, C. (1990a). Antiinflammatory peptides (antiflammins) inhibit synthesis of platelet-activating factor, neutrophil aggregation and chemotaxis, and intradermal inflammatory reactions. J. Exp. Med. **171,** 913–927.

CAMUSSI, G., TETTA, C., and BAGLIONI, C. (1990b). Antiflammins inhibit synthesis of platelet-activating factor and intradermal inflammatory reactions. Adv. Exp. Med. Biol. **279,** 161–172.

CAMUSSI, G., TETTA, C., TURELLO, E., and BAGLIONI, C. (1990c). Anti-inflammatory peptides inhibit synthesis of platelet-activating factor. In *Cytokines and Lipocortins in Inflammation and Differentiation*. M. Melli and L. Parente, eds. (Wiley Liss, New York) p. 69.

CARLOMAGNO, T., MANTILE, G., PAOLOLLO, L., MIELE, L., MUKHERJEE, A.B., and BARBATO, G. (1996). Secondary structure determination of uteroglobin by heteronuclear multidimensional NMR and CD spectroscopy. ^{1}H complete and ^{15}N, ^{13}C assignments of the backbone. J. Biomol. NMR (in press).

CHAN, C.C., NI, M., MIELE, L, CORDELLA-MIELE, E., MUKHERJEE, A.B., and NUSSENBLATT, R.B. (1990). Antiflammins: Inhibition of endotoxin-induced uveitis in Lewis rats. In *Ocular Immunology Today*. M. Usui, S. Ohono, and K. Aoki, eds. (Elsevier Science, New York), pp. 467–470.

CHAN, C.C., NI, M., MIELE, L., CORDELLA-MIELE, E., FERRICK, M., MUKHERJEE, A.B., and NUSSENBLATT, R.B. (1991). Effects of antiflammins on endotoxin-induced uveitis in rats. Arch. Ophthalmol. **109,** 278–281.

CHOCK, S.P., SCHMAUDER-CHOCK, E.A., CORDELLA-MIELE, E., MIELE, L., and MUKHERJEE, A.B. (1994). The localization of phospholipase A_2 in the secretory granule. Biochem. J. **300,** 619–622.

COLLE'E, J.M., TEN KATE, L.P., DE VRIES, H.G., KLIPHUIS, J.W., BOUMAN, K., SCHEFFER, H., and GERRITSEN, J. (1993). Allele sharing on chromosome 11q13 in sibs with asthma and atopy. Lancet **242,** 936.

COWAN, B.D., NORTH, D.H., WHITWORTH, N.S., FUJITA, R., SCHUMACHER, U.K., and MUKHERJEE, A.B. (1986). Identification of a uteroglobin-like antigen in human uterine washings. Fertl. Steril. **45,** 820–823.

DANIEL, J.C., JR., and JUNCJA, S.C. (1989). Amplification of uteroglobin secretion by alternating prolactin-progesterone administration. J. Endocrinol. **122,** R5–6.

DHANIREDDY, R., FUJITA, R., and MUKHERJEE, A.B. (1988). Detection of uteroglobin-like protein in the human neonatal lung. Biochem. Biophys. Res. Commun. **152,** 1447–1454.

DI ROSA, M., and IALENTI, A. (1990). Selective inhibition of inflammatory reactions by vasocortin and antiflammin 2. Prog. Clin. Biol. Res. **349,** 81–90.

DIAZ-GONZALEZ, K., and NIETO, A. (1995). Binding of uteroglobin to microsomes and plasmatic membranes. FEBS Lett. **361,** 255–258.

FACCHIANO, A., CORDELLA-MIELE, E., MIELE, L., and MUKHERJEE, A.B. (1991). Inhibition of phospholipase A2 by uteroglobin and derived peptides: possible mechanism of action. Life Sci. **48,** 453–463.

FRIDLANSKY, F., and MILGROM, E. (1976). Interaction of uteroglobin with progesterone 5α-pregnane-3,20-dione and estrogens. Endocrinology **99,** 1244–1251.

FORSMAN, K., GRAFF, C., NORDSTROM, S., JOHANSSON, K., WESTERMARK, E., GUSTAVSON, K.-H., WADELIUS, C., and HOLMGREN G. (1992). The gene for Best's macular dystrophy is located at 11q13 in a Swedish family. Clin. Genet. **42,** 156–159.

GERHARD, D.S., LAWRENCE, E., WU, J., CHUA, H., MA, N., BLAND, S.D., and JONES, C. (1992). Isolation of 1001 new markers from human chromosome 11, excluding the region of 11p13–11p15.5, and their sublocalization on a new set of radiation reduced somatic cell hybrids. Genomics **13,** 1133–1142.

GILLENER, M., LUND, J., CAMBILLAU, C., ALEXANDERSON, M., HURTIG, U., BERGMAN, A., KLASSON-WEHLER, E., and GUSTAFSSON, J.A. (1988). The binding of methylsulfonyl polychloro-biphenyls to uteroglobin. J. Steroid. Biochem. **31,** 27–33.

GRAFF, C., FORSMAN, K., LARSSON, C., NORDSTROM, S., LIND, L., JOHANSSON, K., SANDGREN, O., WEISSENBACH, J., HOLMGREN, G., GUSTAVSON, K.H., and WADELIUS, C. (1994). Fine mapping of Best's macular dystrophy localize the gene in close proximity to be distinct from the D11S480/ROM1 loci. Genomics **24,** 524–534.

HAGEN, G., WOLF, M., KATYAL, S.L., SINGH, G., BEATO, M., and SUSKE, G. (1990). Tissue-specific expression, hormonal regulation and 5'-flanking gene region of the rat Clara cell 10 kDa protein: Comparison to rabbit uteroglobin. Nucleic Acid Res. **18,** 2939–2946.

IALENTI, A., DOYLE, P.M., HARDY, G.N., SIMPKIN, D.S., and DI ROSA, M. (1990). Anti-inflammatory effects of vasocortion and nonapeptide fragments of uteroglobin and lipocortin I (antiflammins). Agents and Actions **1–2,** 48–49.

ISHIZAKI, J.K., HANASAKI, K., HIGASHINO, J., KISHINO, N., KIKUCHI, O., OHARA, O., and ARITA, H. (1994). Molecular cloning of pancreatic group I phospholipase A_2 receptor. J. Biol. Chem. **269,** 5897–5904.

JACKSON, P.J., TURNER, R., KEEN, J.N., BROOKSBANK, R.A., and COOPER, E.H. (1988). Purification and partial amino acid sequence of human urine protein-1. J. Chromatogr. **452,** 359–367.

JANTZEN, K., FRITTON, H.P., TGO-KEMENES, T., ESPEL, E., JANICH, S., CATO, A.C.B., and BEATO, M. (1987). Partial overlapping of binding sequences for steroid hormone receptors and DNAase I hypersensitive sites in the rabbit uteroglobin gene region. Nucleic Acids Res. **15,** 4535–4552.

KIKUKAWA, T., COWAN, B.D., TEJADA, R.I., and MUKHERJEE, A.B. (1988). Detection of rabbit uteroglobin-like protein in the human endometrium. J. Clin. Endocrinol. Metab. **67,** 315–321.

KJELLMAN, N.-I.M., JOHANNSON, S.J.O., and ROTH, A. (1976). Serum IgE levels in healthy children quantified by a sandwich technique (PRIST). Clin. Allergy **6,** 51–59.

KRISHNAN, R.S., and DANIEL, J.C. JR. (1967). Blastokinin: Inducer and regulator of blastocyst development in the rabbit uterus. Science **158,** 490–492.

KUNDU, G.C., MANTILE, G., MIELE, L., CORDELLA-MIELE, E., and MUKHERJEE, A.B. (1996). Recombinant human uteroglobin suppresses cellular invasiveness via a novel class of high-affinity cell surface binding site. Proc. Natl. Acad. Sci. USA **93,** 2915–2919.

LAMBEAU, G., ANCIAN, P., BARHANIN, J., and LAZDUNSKI, M. (1994). Cloning and expression of a membrane receptor for secretory phospholipase A_2. J. Biol. Chem. **269,** 1575–1578.

LEVIN, S.W., BUTLER, J.D., SCHUMACHER, U.K., WIGHTMAN, P.D., and MUKHERJEE, A.B. (1986). Uteroglobin inhibits phospholipase A2 activity. Life Sci. **38,** 1813–1819.

LLORET, S., and MORENO, J.J. (1994). Effect of nonapeptide fragments of uteroglobin and lipocortin I on oedema and mast cell degranulation. Eur. J. Pharmacol. **264,** 379–384.

LOPEZ-DE HARO, M.S., PEREZ MARTINEZ, M., GARCIA, C., and NIETO, A. (1994). Binding of retinoids by uteroglobin. FEBS Lett. **349,** 249–251.

MANYAK, M.J., KIKUKAWA, T., and MUKHERJEE, A.B. (1988). Expression of a uteroglobin-like protein in human prostate. J. Urol. **140,** 176–182.

MANTILE, G., MIELE, L., CORDELLA-MIELE, E., SINGH, G., KATYAL, S.L., and MUKHERJEE, A.B. (1993). Human Clara cell 10kDa protein is the counterpart of rabbit uteroglobin. J. Biol. Chem. **267,** 20343–20351.

MARSH, D.G., NEELY, J.D., BREAZEALE, D.R., GHOSH, B., FREIDHOFF, L.R., EHLICH-KAUTZKY, E., and SCHOU, C. (1994). Linkage analysis of IL4 and other chromosome 5q31.1 markers and total serum Immunoglobulin E concentrations. Science **264,** 1162–1166.

MATHEWS, J.H., PATTABIRAMAN, N., WARD, K.B., MANTILE, G., MIELE, L., and MUKHERJEE, A.B. (1994). Crystallization and characterization of the recombinant human Clara cell 10kDa protein. Proteins: Structure, Function and Genetics **20,** 191–196.

McGIVNEY, A., MORITA, Y., CREWS, F.T., HIRATA, F., AXELROD, J., and SIRAGANIAN, R.P. (1981). Phospholipase activation in the IgE-mediated and Ca^{2+} ionophore A23187-induced release of histamine from rat basophilic leukemic cells. Arch. Biochem. Biophys. **212,** 572–580.

MENNE, C., SUSKE, G., ARNEMANN, J., WENZ, M., CATO, A.C., and BEATO, M. (1982). Isolation and structure of the gene for the progesterone-inducible protein uteroglobin. Proc. Natl. Acad. Sci. USA **79,** 4853–4857.

MIELE, L., CORDELLA-MIELE, E., and MUKHERJEE, A.B. (1987). Uteroglobin: Structure, molecular biology, and new perspectives on its function as a phospholipase A2 inhibitor. Endocr. Rev. **8,** 474–490.

MIELE, L., CORDELLA-MIELE, E., FACCHIANO, A., and MUKHERJEE, A.B. (1988). Novel anti-inflammatory peptides from the region of highest similarity between uteroglobin and lipocortin I. Nature **335,** 726–730.

MIELE, L., CORDELLA-MIELE, E., and MUKHERJEE, A.B. (1990). High level bacterial expression of uteroglobin, a dimeric eukaryotic protein with two interchain disulfide bridges, in its natural quaternary structure. J. Biol. Chem. **265,** 6427–6435.

MIELE, L., CORDELLA-MIELE, E., MANTILE, G., PERI, A., and MUKHERJEE, A.B. (1994). Uteroglobin and uteroglobin-like proteins: the uteroglobin family of proteins. J. Endocrinol. Invest. **17,** 679–692.

MORIZE, I., SURCOUF, E., VANEY, M.C., EPELBOIN, Y., BUEHNER, M., FRIDLANSKY, F., MILGROM, M., and MORNON, J.P. (1987). Refinement of the C2221 crystal form of oxidized uteroglobin at 1.34 A. J. Mol. Biol. **194,** 725–739.

MORNON, J.P., FRIDLANSKY, F., BALLY, R., and MILGROM, E. (1980). X-ray crystallographic analysis of a progesterone binding protein. The C222i crystal form of oxidized uteroglobin at 2.2 Å resolution. J. Mol. Biol. **127,** 415–425.

MUKHERJEE, A.B., ULANE, R.E., and AGRAWAL, A.K. (1982). Role of uteroglobin and transglutaminase in masking the antigenicity of implanting rabbit embryos. Am. J. Reprod. Immunol. **2,** 135–141.

MUKHERJEE, D.C., ULANE, R.E., MANJUNATH, R., and MUKHERJEE, A.B. (1983). Suppression of epididymal sperm antigenicity in the rabbit by uteroglobin and transglutaminase in vitro. Science **219,** 989–991.

MUKHERJEE, A.B., CORDELLA-MIELE, E., KIKUKAWA, T., and MIELE, L. (1988). Modulation of cellular response to antigens by uteroglobin and transglutaminase. Adv. Exp. Med. Biol. **231,** 135–152.

MUKHERJEE, A.B., PERI, A., MIELE, L., CORDELLA-MIELE, E., MANTILE, G., KUNDU, G.C., ZHANG, Z., YUAN, P., and GERHARD, D.S. (1994). Could the gene coding for human uteroglobin (Clara cell 10kDa protein) be a candidate gene for atopy? Am. J. Hum. Genet. **55,** A197.

NORDLUND-MOLLER, L., ANDERSSON, O., AHLGREN, R., SCHILLING, J., GILLNER, M., GUSTAFFSON, J.A., and LUND, J. (1990). Cloning, structure, and expression of a rat binding protein for polychlorinated biphenyls. Homology to the hormonally regulated progesterone-binding protein uteroglobin. J. Biol. Chem. **267,** 12690–12693.

PERI, A., CORDELLA-MIELE, E., MIELE, L., and MUKHERJEE, A.B. (1993). Tissue-specific expression of the gene coding for human clara cell 10-kD protein, a phospholipase A2-inhibitory protein. J. Clin. Invest. **92,** 2099–20109.

PERI, A., DUBIN, N., DHANIREDDY, R., and MUKHERJEE, A.B. (1995). Uteroglobin gene expression in the rabbit uterus throughout gestation and in the fetal lung: Relationship between uteroglobin and

eicosanoid levels in the developing fetal lung. J. Clin. Invest. **96,** 343–353.

PETER, W., DUNKEL, R., STOUTEN, P.F.W., VRIEND, G., BEATO, M., and SUSKE, G. (1992). Interchain cysteine bridges control entry of progesterone to the central cavity of the uteroglobin dimer. Protein Engineering **5,** 351–359.

POPESCU, N., ZIMONJIC, D., HATCH, C., and BONNER, W. (1994). Chromosomal mapping of the human histone H2A.Z gene to 4q24 by *in situ* fluorescence hybridization. Genomics **20,** 333–335.

POPESCU, N.C., AMSBAUGH, S.C., SWAN, D.C., and DiPAOLO, J.A. (1985). Induction of chromosome banding by trypsin/EDTA for gene mapping by *in situ* hybridization. Cytogenet. Cell Genet. **39,** 73–74.

POSTMA, D.S., BLEECKER, E.R., AMELUNG, P.J., HOLROYD, K.J., XU, J., PANHUYSEN, C.I.M., MEYERS, D.A., and LEVITT, R. (1995). Genetic susceptibility to asthma- bronchial hyperresponsiveness coinherited with a major gene for atopy. New. Engl. J. Med. **333,** 894–900.

RANDALL, G.W., DANIEL, J.C., JR., and CHILTON, B.S. (1991). Prolactin enhances uteroglobin gene expression by uteri of immature rabbits. J. Reprod. Fert. **91,** 249–257.

RAY, M.K., MAGDALENO, S., O'MALLEY, B.W., and DEMAYO, F.J. (1993). Cloning and characterization of the 5′ flanking region of 10kDa protein gene: Comparison of the 5′ flanking region with the human rat and rabbit gene. Biochem. Biophys. Res. Commun. **197,** 163–171.

ROBINDON, D.H., KIRK, K.L., and BENOIS, D.J. (1989). Macromolecular transport in rabbit blastocysts: evidence for a specific uteroglobin transport system. Mol. Cell. Endocrinol. **63,** 227–234.

SANFORD, A.J., SHIRAKAWA, T., MOFFATT, M.F., DANIELS, S.E., RA, C., FAUX, J.A., YOUNG, R.P., NAKAMURA, Y., LATHROP, G.M., COOKSON, W.O.C.M., and HOPKIN, J.M. (1993). Location of atopy and β subunit of high-affinity IgE receptor (FcϵRI) on chromosome 11q. Lancet **341,** 332–334.

SINGH, G., KATYAL, S.L., BROWN, W.E., PHILLIPS, S., KENNEDY, A.L., ANTHONY, J., and SQUEGLIA, N. (1988a). Amino-acid and cDNA nucleotide sequences of human clara cell 10 kDa protein. Biochim. Biophys. Acta **950,** 329–337.

SINGH, G., SINGH., J., KATYAL, S.L., BROWN, W.E., KRAMPS, J.A., PARADIS, I.L., DAUBER, J.H., MACPHERSON, T.A., and SQUEGLIA, N. (1988b). Identification, cellular localization, isolation, and characterization of human clara cells-specific 10 kD protein. J. Histochem. Cytochem. **36,** 73–80.

SINGH, G., KATYAL, S.L., BROWN, W.E., and KENNEDY, A.L. (1993). Mouse Clara cell 10 kDa (cc10) protein: cDNA nucleotide sequence and molecular basis for the variation in progesterone binding of cc10 from different species. Exp. Lung Res. **19,** 67–75.

SMITH, C.M., BORA, P.S., BORA, N.S., JONES, C., and GERHARD, D.S. (1995a). Genetic and radiation reduced somatic cell hybrid mapping of glutathione S-transferase, P1 gene. Cytogenet. Cell Genet. **71,** 235–239.

SMITH, C.M., WELLS, S.A., and GERHARD, D.S. (1995b). Mapping of eight new polymorphisms in 11q13 in the vicinity of multiple endocrine neoplasia type 1: Identification of a close distal marker. Human Genet. **96,** 377–387.

SNEED, R., DAY, L., CHANDRA, T., MACE, M., BULLOCK, D.W., and WOO, S.L. (1981). Mosaic structure and mRNA precursors of uteroglobin, a hormone-regulated mammalian gene. J. Biol. Chem. **256,** 11911–11916.

SOMMERS, C.D., BOBBITT, J.L., BEMIS, K.G., and SYNDER, D.W. (1992). Porcine pancreatic phospholipase A_2-induced contractions of guinea pig lung pleural strips. Eur. J. Pharmacol. **216,** 87–96.

STAFFORD, A.N., RIDER, S.H., HOPKIN, J.M., COOKSON, W.O., and MONACO, A.P. (1994). A 2.8 Mb YAC contig in 11q12–q13 localizes candidate genes for atopy: FcϵRI-β and CD20. Hum. Mol. Genet. **3,** 779–785.

STOEHR, H., and WEBER, B.H.F. (1995). A recombination event excludes the ROM1 locus from the Best's vitelliform macular dystrophy region. Hum. Genet. **95,** 219–222.

STONE, E.M., NICHOLS, B.E., STREB, L.M., KIMURA, A.E., and SHEFFIELD, V.C. (1992). Genetic linkage of vitelliform macular degeneration (Best's disease) to chromosome 11q13. Nat. Genet. **1,** 246–250.

TEN KATE, L.P., COLLEE', J.M., DE VRIES, H.G., GERRITSEN, J., KAUFFMAN, H., VAN DER MEULEN, M.A., and SCHEFFER, H. (1994). Sibs with atopy and asthma share marker alleles at 11q13, but not at 7q31 or 14q32. Am. J. Hum. Genet. **55,** A366 (abstract).

TETTA, C., CAMUSSI, G., BUSSOLINO, F., HERRICK-DAVIS, K., and BAGLIONI, C. (1991). Inhibition of the synthesis of platelet-activating factor by antiinflammatory peptides (antiflammins) without methionine. J. Pharmacol. Exp. Ther. **257,** 616–620.

UMLAND, T.C., SWAMINATHAN, S., SINGH, G., WARTY, V., FUREY, W., PLETCHER, J., and SAX, M. (1994). Structure of a human Clara cell phospholipid-binding protein-ligand complex at 1.9 A resolution. Nat. Struct. Biol. **1,** 538–545.

VOLVOVITZ, B., NATHANSON, I., DECASTRO, G., KIKUKAWA, T., MUKHERJEE, A.B., BRODSKY, L., FOX, M.J., and OGRA, P.L. (1988). Relationship between leukotriene C_4 and a uteroglobin-like protein in nasal and tracheobronchial mucosa of children: Implication in acute respiratory illness. Int. J. Allergy Appl. Immunol. **86,** 420–425.

URATA, C., and SIRAGANIAN, R.P. (1985). Pharmacologic modulation of the IgE or Ca^{2+} ionophore A23187 mediated Ca^{2+} influx, phospholipase activation, and histamine release in rat basophilic leukemic cells. Int. Arch. Allergy. Appl. Immunol. **78,** 92–100.

WEBER, B.H.F., WALKER, D., MULLER, B., and MAR, L. (1994). Best's vitelliform dystrophy (VMD2) maps between D11S903 and PYGM: No evidence for locus heterogeneity. Genomics **20,** 267–274.

WOLF, M., KLUG, J., HACKENBERG, R., GESSLER, M., GRZESCHIK, K.H., BEATO, M., and SUSKE, G. (1992). Human CC10, the homologue of rabbit uteroglobin: Genomic cloning, chromosomal localization and expression in endometrial cell lines. Hum. Mol. Genet. **1,** 371–378.

XU, J., and WANG, N. (1994). Identification of chromosomal alterations in human ovarian carcinoma cells using combined GTG-banding and repetitive fluorescence *in situ* hybridization (FISH). Cancer Genet. Cytogenet. **74,** 1–7.

YOUNG, R.P., SHARP, P.A., LYNCH, J.R., FAUX, J.A., LATHROP, G.M., COOKSON, W.O., and HOPKIN, J.M. (1992). Confirmation of genetic linkage between atopic IgE responses and chromosome 11q13. J. Med. Genet. **29,** 236–238.

ZIMONJIC, D.B., POPESCU, N.C., MATSUI, T., ITO, M., and CHIHARA, K. (1994). Localization of the human cholecystokinin-B/gastrin receptor gene to chromosome 11p15.4–15.5 by fluorescence *in situ* hybridization. Cytogenet. Cell Genet. **65,** 184–185.

ZIMONJIC, D.B., REZANKA, L., DiPAOLO, J.A., and POPESCU, N.C. (1995). Refined localization of the erb B-3 proto-oncogene by direct visualization of FISH signals on LUT-inverted and contrast-enhanced digital images of DAPI-banded chromosomes. Cancer. Genet. Cytogenet. **80,** 100–102.

Address reprint requests to:
Dr. Anil B. Mukherjee
Section on Developmental Genetics
Building 10, Room 95241
Heritable Disorders Branch
National Institute of Child Health and Development
The National Institutes of Health
Bethesda, MD 20892-1830

Received for publication May 30, 1996; accepted August 16, 1996.