

## Identification of a Novel Autoantigen UACA in Patients with Panuveitis

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**To identify the target autoantigens in Vogt-Koyanagi-Harada disease, we made use of an immunoscreening of a bovine uveal cDNA expression library with serum samples obtained from patients with Vogt-Koyanagi-Harada disease. We identified a novel bovine antigen and homologous human autoantigen and designated it as UACA (uveal autoantigen with coiled coil domains and ankyrin repeats). mRNA of human UACA is expressed most abundantly in skeletal muscles and in various human tissues, including choroid, retina, and epidermal melanocytes. IgG autoantibodies were quantitated in an ELISA, using recombinant C-terminal 18.0% fragment of human UACA. The prevalence of IgG anti-UACA autoantibodies in patients with panuveitis (Vogt-Koyanagi-Harada disease, Behçet's disease, sarcoidosis) was significantly higher than that in healthy controls (19.6–28.1% vs 0%,  $P < 0.05$ ) indicating that autoimmunity directed against UACA is a common phenomenon in these diseases. © 2001**

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**Key Words:** Vogt-Koyanagi-Harada disease (VKH); Behçet's disease (BD); sarcoidosis; serological analysis of recombinant cDNA expression libraries (SEREX);

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Abbreviations used: VKH, Vogt-Koyanagi-Harada disease; BD, Behçet's disease; SEREX, serological analysis of recombinant cDNA expression libraries; UACA, uveal autoantigen with coiled coil domains and ankyrin repeats; EST, expressed sequence tag; ORF, open reading frame.

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### uveal autoantigen with coiled coil domains and ankyrin repeats (UACA).

Vogt-Koyanagi-Harada disease (VKH) is an autoimmune systemic disorder. In VKH, inflammatory disorders occur in multiple organs containing melanocytes, including uvea (resulting in acute bilateral panuveitis), skin (resulting in vitiligo and alopecia), central nervous system (resulting in meningitis) and inner ears (resulting in hearing loss and tinnitus). These inflammatory aspects are attributed to the destruction of melanocytes through immunological mechanisms. Several findings suggest that T helper 1 type autoreactive CD4<sup>+</sup> T cells are involved in the development of VKH (1–6). The strong association between HLA-DR4 (DRB1\*0405)-DQ4 (DQA1\*0302-DQB1\*0401) haplotype and the susceptibility to VKH has been noted in the Japanese and Brazilian patients, hence, HLA-linked genetic background is related to the development of VKH (7–10).

Identification of target autoantigen is important to understand the etiology of autoimmune diseases, and for development of antigen-specific immuno-modulation therapy. In experimental autoimmune uveitis, S-antigen and interphotoreceptor retinoid-binding protein are used as autoantigens (11, 12). However, the relationship between these autoantigens and human autoimmune uveitis is uncertain. Studies have been done to elucidate the exact etiology and target autoantigen in VKH, but much remains to be investigated. To identify target autoantigens in autoimmune diseases, serological analysis of recombinant cDNA expression libraries (SEREX) has been done. SEREX is an immunoscreening method which makes use of prokaryotically expressed cDNA libraries prepared from target organs of the autoimmune diseases and sera from patients. This strategy proved effective to identify

disease-related autoantigens, in type 1 diabetes, and systemic lupus erythematosus (13, 14). When we used this approach to identify the target autoantigen of VKH, we identified a novel autoantigen UACA (uveal autoantigen with coiled coil domains and ankyrin repeats). The prevalence of IgG anti-UACA autoantibodies in VKH patients was significantly higher than in healthy control samples. However, these antibodies were observed also in patients with Behçet's disease (BD) and sarcoidosis known as the major diseases that cause panuveitis in the Japanese population. A novel UACA protein appears to be a possible target autoantigen shared by VKH, BD, and sarcoidosis that cause different types of panuveitis.

## MATERIALS AND METHODS

**Patients.** Forty-six patients (age 19–80 years; 18 men and 28 women) with VKH were studied. All these patients underwent complete ophthalmologic and related examinations to determine the clinical diagnosis. Seven patients (age 30–62; 6 men and one woman) with sympathetic ophthalmia, 32 patients (age 15–64 years; 20 men and 12 women) with BD complicated with panuveitis, 19 patients (age 16–67 years; 2 men and 17 women) with sarcoidosis complicated with panuveitis, and 36 healthy unrelated donors (age 22–55 years; 21 men and 15 women) served as controls. All subjects are Japanese living in Japan and informed consent was obtained from all subjects prior to blood sampling. This study was done according to the tenets of the Declaration of Helsinki.

**cDNA library.** Total RNA was extracted from bovine uvea using TRIZOL reagent (GIBCO BRL, Rockville, MD) and mRNA was purified on an oligo(dT) column (Oligotex-dT30 "Super" TaKaRa, Kyoto, Japan). First-strand cDNA synthesis was done using random primers. cDNA was ligated to *EcoRI*-*NotI*-*Bam*HI adaptors and digested with *EcoRI*. Double-strand cDNA fragments were cloned into the bacteriophage expression vector  $\lambda$ ZAPII (Stratagene, La Jolla, CA), then packaged into packaging extract (Stratagene), resulting in  $1.5 \times 10^6$  primary recombinants in this library.

**Immunoscreening.** The immunoscreening method used was a modification of published methods (15, 16). Sera were diluted in 1% bovine serum albumin/Tris-buffered-saline (TBS), and adsorbed on Sepharose 4B columns coupled with lysates of *E. coli* Y 1090 and bacteriophage-infected *E. coli* BNN97 (5 Prime 3 Prime, Boulder, CO) to remove antibodies reactive to the vector system. Recombinant phages at a concentration of  $1 \times 10^4/15$  cm plate were amplified for 6 h at 42°C, then covered with nitrocellulose filters Hybond-c extra (Amersham, Buckinghamshire, England), pretreated with isopropyl  $\beta$ -D-thiogalactoside (Wako, Osaka, Japan), and incubated for an additional 3 h at 37°C to transfer the encoded proteins onto the filter membranes. Membranes were then blocked with 5% (w/v) skim milk/TBS. After washing with TBS containing 0.05% Tween 20 (TBS-T), membranes were incubated in prepared sera for 15 h at 4°C. Followed by incubation in horseradish peroxidase (HRP)-conjugated mouse anti-human IgG (Southern Biotechnology Associates, Inc., Birmingham, AL) for 2 h at 4°C. The membranes were washed in TBS-T and TBS and incubated with ECL RPN 2106 (Amersham) for 1 min, and exposed to autoradiographic film to detect antibody-reactive phage plaques. Positive recombinant clones were picked up and purified by an additional cycle of plating and screening. To determine the reactivity of other serum samples to positive clones, plates containing equal numbers of sero-positive clones and seronegative control plaques were similarly processed. A total of  $7.4 \times 10^5$  phage plaques were screened, using sera from four VKH patients.

**Sequence analysis of identified cDNA clones.** Immunoreactive phage clones were subjected to *in vivo* excision of pBluescript phagemids, using the ExAssist helper phage/SOLR strain system (Stratagene). Plasmid DNA was purified, using ABI Prism Miniprep Kits (PE Applied Biosystems, Foster, CA). cDNA inserts were sequenced, using an ABI Prism (Perkin-Elmer, Norwalk, CT) automated DNA sequencer and for sequence alignments we used BLAST software (GenomeNet, Japan).

**Cloning and sequencing of human UACA cDNA.** To isolate the human UACA cDNA, an 815-bp cDNA fragment of human EST clone IMAGE:1527412 (GenBank Accession No. AA916992) digested by *Bgl*II and *Eco*RI was used as a probe to screen a Jurkat cell cDNA library, this human cDNA clone was highly homologous to the isolated bovine immunoreactive cDNA clone (87% identical to nucleotide position 1130–1944 of bovine cDNA clone). One positive clone was subjected to an *in vivo* excision. Subsequently, the plasmid DNA was purified and sequenced. The sequence of this human cDNA fragment was highly homologous to the sequence of identified immunoreactive bovine cDNA clone, exhibiting 87% homology at nucleotide sequence level. PCR cloning was also done to isolate the human UACA cDNA, using a human uveal cDNA as a template. Forward primers F-1, F-2 and reverse primers R-1, R-2 corresponding to nucleotide position 4450–4474, 19788–19813, 19728–19753, and 20922–20936 respectively of *Homo sapiens* chromosome 15 clone RP11-64K10 map 15q24, WORKING DRAFT SEQUENCE (GenBank Accession No. AC010076), and forward primer corresponding to nucleotide position 80–100 of *Homo sapiens* cDNA clone DKFZp434E2235 (GenBank Accession No. AL044711) were used for DNA amplification. The amplified fragments were subcloned into pGEM-T vector (Promega, Madison, WI) and sequenced by using an ABI Prism automated DNA sequencer. Secondary structure analysis was done using the software program COILS (17).

**Northern blot analysis and reverse transcription-PCR (RT-PCR).** For Northern blot analysis, the mRNA blot from CLONTECH (Palo Alto, CA) was hybridized with the <sup>32</sup>P-labeled cDNA fragment of human EST clone IMAGE:1527412 (GenBank Accession No. AA916992) as described in the manufacturer's instructions. For RT-PCR, total RNA was isolated from human epidermal melanocytes (Cell Systems Corp., Kirkland) and normal human choroidal and retinal tissues, using TRIZOL reagent. Subsequently, poly(A)<sup>+</sup> RNA was purified with the Dynabeads mRNA Purification Kit (DYNAL, Oslo, Sweden). These tissues were obtained from the enucleated eye from a patient with mycosis of supermaxilla. Informed consent was obtained after explanation of nature of the research was explained following the tenets of the Declaration of Helsinki. Poly(A)<sup>+</sup> RNA were purified from 10  $\mu$ g of each total RNA and subjected to cDNA synthesis, using random hexamer primers and Superscript reverse transcriptase (GIBCO/BRL). Gene-specific PCR primers were designed to amplify fragments of 505 bp and used in the RT-PCR (95°C for 1 min, 58°C for 1 min and 72°C for 1 min, 30 cycles). PCR primers for UACA were forward, 5'-GAGAAAGAAGTTGGAATCATAA-3' and reverse, 5'-TTGTGTAGTGAGTTGGAAAG-3'. PCR using cDNA-specific beta-actin primers was done as control (18).

**Preparation of glutathione-S transferase (GST) fusion protein.** A 783-bp DNA fragment digested from *Homo sapiens* cDNA clone IMAGE 608930 (GenBank Accession No. AA197064) corresponding to nucleotide position 3462–4245 of UACA cDNA was inserted into a pGEX-4T-2 vector to produce glutathione-S-transferase (GST)-UACA fusion protein. This UACA fragment covers C-terminal 261 amino acids (18.0%) of whole UACA consisting of 1449 amino acids (Fig. 1A). Plasmids with this construct were transformed in *E. coli* (DH 5 $\alpha$ ) and incubated in 500 ml of Luria broth medium for 8 h at 37°C with shaking. Then IPTG was added at a final concentration of 0.1 mM and the preparation was incubated for 16 h at 25°C with shaking. This suspension was centrifuged and the pellet was suspended in 20 ml of lysis buffer (50 mM Tris-HCl [pH 7.5], 25% Sucrose). Then we added 100  $\mu$ l of Nonidet P-40 (10%), MgCl<sub>2</sub> (1 M)

on ice. The lysate was sonicated and centrifuged, then the supernatant was incubated with 2 ml of a slurry of glutathione-Sepharose 4B for 2 h at 4°C. This suspension was centrifuged and the pellet was washed in WE buffer (20 mM Tris-HCl [pH 7.5], 2 mM MgCl<sub>2</sub>, 1 mM DTT). The fusion protein was eluted with G buffer (5 mM GSH, 50 mM Tris-HCl [pH 9.6]).

**Enzyme-linked immunosorbent assay (ELISA).** Detection and titration of antibody to a fragment of UACA were done, using indirect ELISA. GST-UACA fusion protein and GST protein were prepared and used as antigens. Microtiter plates (96-well) (NUNC, Denmark) were coated with GST-UACA fusion protein in PBS (pH 7.4) for 15 h at 4°C. GST protein was also coated in different wells, as a control. The plates were then washed with PBS containing 0.05% Tween 20 (PBS-T) and blocked with 5% skim milk/PBS for 2 h at room temperature. The plates were washed with PBS-T and incubated for 15 h at 4°C with serum samples diluted at 1:50 with 1% skim milk/PBS. The plates were washed in PBS-T and 100  $\mu$ l of HRP-conjugated mouse anti-human IgG diluted at 1:2000 with 1% skim milk/PBS were added to each well followed by incubation at room temperature for 2 h. The plates were washed with PBS-T, and 100  $\mu$ l solution of *o*-phenylenediamine (Sigma Fast; Sigma Chemical Co., St. Louis, MO) was added to each well. After 30 min, the reaction was stopped by adding 50  $\mu$ l of 3 M H<sub>2</sub>SO<sub>4</sub>, and OD 490 nm was determined, using a Model 550 microplate reader (Bio-Rad, Hercules, CA). The specific corrected OD of an individual sample was calculated by subtracting the OD value of GST protein coated well from that of GST-UACA fusion protein.

## RESULTS

**Identification of an immunoreactive bovine cDNA clone, and cloning of its human homologous gene.** cDNA expression library was prepared from bovine uvea and  $1.0 \times 10^5$  to  $2.0 \times 10^5$  phage plaques were immunoscreened with sera obtained from four VKH patients. Eleven positive immunoreactive clones were identified by serum from one patient HN (age 48 years, woman). These clones were purified and their partial DNA sequences revealed that they were derived from one kind of cDNA. One clone ~6 kbp was sequenced and the analysis of this sequence showed an initial ATG preceded by an in-frame stop codon suggesting that the open reading frame (ORF) has been identified. The ORF encodes for a protein of 1405 amino acids with a predicted molecular weight of 161182 daltons (Fig. 1A). A homology search using BLAST software revealed that the sequence of this cDNA shared homology with several human EST clones obtained from various tissues, including melanocytes, retina and cochlea. To isolate a human cDNA homologous to the bovine gene, a cDNA fragment isolated from a human EST clone IMAGE:1527412 (GenBank Accession No. AA916992), one of the human homologous cDNA clones, was used as a probe to screen a Jurkat cell cDNA library. However the sequence of isolated human cDNA did not contain the complete 5' end of the ORF. Next we did PCR cloning to isolate the complete human cDNA. We found that this sequence encodes for a protein of 1449 amino acids with a predicted molecular weight of 165,778 Da. Analysis of the amino acid sequence showed that this gene contains 6 repeats with the consensus sequence for the ankyrin motif (19), the

leucine zipper pattern (20), and coiled coil domains (Figs. 1A and 1B).

A homology search revealed that the sequence of this human cDNA shares the sequence of a human cDNA clone (mRNA for KIAA1561 protein, GenBank Accession No. AB046781) (21), and several human EST clones, but there was no homology shared with the characterized and published human proteins among reported genes in the database. In other species, the protein sequence encoded by a mRNA that is overexpressed in dog thyroid tissue following TSH stimulation (GenBank Accession No. X99145) was 85% identical to the protein sequence of this human gene (22). Thereby we concluded that the isolated bovine and human cDNAs encode novel proteins and we designated it as UACA (uveal autoantigen with coiled coil domains and ankyrin repeats) (GenBank Accession Nos. AF322915 and AF322916). These two proteins show a 86% amino acid homology. The amino acid sequence of human UACA has 47% similarity (27% identity) with the protein sequence of NOPREG (novel retinal pigment epithelial cell gene) (23) that also contains 6 ankyrin repeats and coiled coil domains, suggesting that there is a structural similarity between these two proteins. The amino acid sequence of UACA also showed similarity with several proteins containing coiled coil domains, including myosin heavy chain (24). The peptide fragment <sup>1029</sup>ENDKLLKKE<sup>1036</sup> of UACA was 7/8 identical to the peptide fragment <sup>40</sup>ENAKLLKKE<sup>47</sup> of segment VP6 of Banna virus (25).

**Comparison of the prevalence of IgG anti-human UACA autoantibodies in sera from patients with panuveitis and healthy controls.** To determine if autoimmunity directed against human UACA is specifically associated with VKH, serum samples obtained from patients with panuveitis (VKH, BD, sympathetic ophthalmia, sarcoidosis) and healthy controls were tested for their reactivity to a C-terminal 18.0% fragment of recombinant human UACA protein produced as GST fusion protein (Fig. 1A). We evaluated the titer of IgG anti-UACA autoantibody in sera, using ELISA. To exclude the effect of reactivity to GST protein, we used GST-UACA fusion protein and GST protein for ELISA, respectively. Evaluation of IgG anti-UACA autoantibodies was determined by subtracting the reactivities to GST from those to GST-UACA. The ratio of the OD values for GST-UACA fusion protein to GST protein in 22 positive samples was  $9.57 \pm 0.99$  (mean  $\pm$  SE), and in 118 negative samples was  $3.52 \pm 0.15$  (mean  $\pm$  SE). Figure 2 shows a scattergram of individual serum titers. The results were expressed as the relative value of OD unit where the OD value of VKH patient HN was defined as 100 OD unit. The cutoff level for positivity of anti-UACA IgG autoantibodies, 99.7 was defined as the mean value plus 3 SD of the autoantibody titers in healthy controls. The prevalence of autoantibodies in

**A**

Human 60 MKSLKSLRRQDVPGPASSGAAAASAHAAADWNKYDDRMLKAAERGDVEKVTSLAKKGVN  
 Bovine -M-CWFSCAPK\*\*\*\*\*NRQ-----S-----

PGKLDVHGRSVFHVVTSGKNLECLNAILIHGVDITTSITPAGRNALHLLAAKYGHALCLOKL 120  
 -----A-----A-----

LYNCPTEHADLQGRATALQKKAMADCPSSIQLLCDHGASVNAKDKVGRTPPLVLATOMSKP 180  
 -----V-----HDA-----D-----C-----

TICOLLIDRGADVNSRDKQNRRTALMLGCEYGCRAVEVLIKNGADISLLDAI<sup>1</sup>GH<sup>2</sup>DSSYYA 240  
 -----R-----I-----K-----V-----

RIGDNLDLTLTKTASENTNKGRELVWKKGPSLQQRNLTHMQDEVNVKSHQREHQNIQDLE 300  
 -----S-----SQ-L-----T-N-----

IENEDLKERLRKIQEQRILLDKVNGLQLLNNEEVMVADDLESEREKKLSLLAAKEKQHE 360  
 -----K-----

ESLRTIEALKNRFKYFESDHLGSGSHFSNRKEDMLLKQGMYMADSQCTSPGIPAHMQSR 420  
 -----S-----\*\*\*\*\*-----T-M-V-----

SMLRPLELSLPSQTSYSENEILKKELEAMRTFCESAKQDRLLQNELAHKVAECKALALE 480  
 -----A-N-A-----D-----

CERVKEDSDEQIKQLEDALKDVQKRMYESEGKVKQM<sup>3</sup>THFLALKEHLTSEAAAGNHRLTE 540  
 -----D-----T-----M-----

ELKDQLKDLKVKYEGASAEV<sup>4</sup>GKLRNQIKQNEMIVBEFKRDEGKLEENKRLQKELSMCEM 600  
 -----M-----L-----M-----L-----

EREKKGKRVTEMEGQAKELSAKLALSIPA<sup>5</sup>EKFENMKSSLSNEVNEKAKKLVEMEREHEKS 660  
 -----R-----L-----L-D-----L-----L-----IDV-----Y-R-----

LSEIRQLKRELENVKAKLAQHVKPEEHEQVKS<sup>6</sup>LEQKSGE<sup>7</sup>GKKIT<sup>8</sup>PLK<sup>9</sup>Q<sup>10</sup>QKEIE 720  
 -----N-T-P-----L-----L-----R-----S-----M-----

KVYLDNKLKEQAHLNLT<sup>11</sup>EMKNHYVPLK<sup>12</sup>VSEDMK<sup>13</sup>KSHDAI<sup>14</sup>DDLNRKLLDV<sup>15</sup>TQY<sup>16</sup>TEKKL 780  
 -----C-----TQ-VN-----T-----E-----V-V-----K-S-H-----

EMEKLLENDLSKDVSRLETVPPEKHEKEIIALKSNIVELKKQSELKKKCGEDQEK 840  
 -----M-A-----N-----I-----R-----MM-----T-----N-----

IHALTSENTNLKMMNSQYVPVKT<sup>17</sup>HEEVKMTLND<sup>18</sup>TLAKTNRELLDVKKK<sup>19</sup>FEDINQEFVKI 900  
 -----YS-M-----ND-----T-H-----I-TA-SS-D-----V-----C-----

KDKNEILKRNLENTQ<sup>20</sup>NQIKA<sup>21</sup>EYISLAEHEAKMSSLSQSMR<sup>22</sup>KVQDSNAEILAN<sup>23</sup>YRKGQEEI 960  
 -----E-----V-----R-----E-G-RK-K-----NS-----K-S-----

VTLHAEIKAQK<sup>24</sup>KELDTIQECIKV<sup>25</sup>KYAPIVSFE<sup>26</sup>ECERKFKATEKELKDQ<sup>27</sup>LSBQ<sup>28</sup>TQKYSVSE 1020  
 -----E-A-R-----L-----I-L-----E-Q-----NT-----

EEVKNKQENDK<sup>29</sup>LKKEIF<sup>30</sup>TLQKDLRDKTVLIEK<sup>31</sup>SHEMERALS<sup>32</sup>RKTDELNK<sup>33</sup>QLK<sup>34</sup>DSQ<sup>35</sup>Y<sup>36</sup>T 1080  
 -----A-C-----L-----K-N-H-N-Y-T-----E-R-L-----

EVKNVKEKLVEENAKQTSEILAVQ<sup>37</sup>NLLQK<sup>38</sup>QH<sup>39</sup>VPLEQ<sup>40</sup>VEALKKSLNGT<sup>41</sup>IENLKEELKSMQR 1140  
 -----A-KE-----A-T-----S-----S-----T-----TK-----

CYEKEQQTVTKLHQLLENQK<sup>42</sup>SSVPLAEHLQ<sup>43</sup>IKAEFEKEVGI<sup>44</sup>IKASLREKEEESQNK<sup>45</sup>MEE 1200  
 -----Q-R-M-----V-----T-----

VSKLQSEVQNTKQALKKLE<sup>46</sup>TREVVDLSKYKATKSDLETQ<sup>47</sup>ISSLNEKLANLN<sup>48</sup>RKYBEVCEE 1260  
 -----I-----D-----

VLHAKKKEISAKDEKELLHFSIEQ<sup>49</sup>EIKDQKERCDKSLTTITELQ<sup>50</sup>RRIQESAKQIEAKDNK 1320  
 -----L-----Q-----

ITELLNDVERLKQALNGLS<sup>51</sup>QLTYTS\*GNPTK<sup>52</sup>RQSQLIDT<sup>53</sup>LQH<sup>54</sup>QVKSLEQQLADADR<sup>55</sup>HQ<sup>56</sup>E 1379  
 -----AHLMEV-S-S-----S-Q-R-----\*

VIAIYRTHLSAAQGHMDE<sup>57</sup>DVQ<sup>58</sup>EAL<sup>59</sup>LADHTNAAGACV<sup>60</sup>LAVSTDCQYLFYLAGAEHSLCNS 1439  
 -----A-T-----D-----R-V\*G-PQPTVAFP-----VR-F-----

MVFLGLTVLV 1449  
 -----A-----A-----

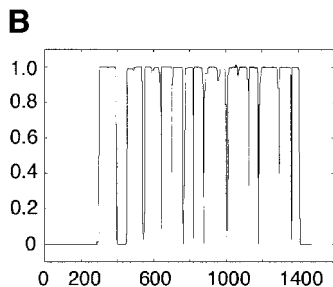


FIG. 1—Continued

patients with panuveitis and healthy controls is shown in Table 1. We found IgG anti-UACA reactivities in 19.6% (9/46) of VKH patients, and 0% (0/36) of the healthy controls. The prevalence of IgG anti-UACA auto-antibodies in VKH patients was significantly higher than that in healthy controls ( $P < 0.005$ ). Anti-UACA reactivities were found in 0% (0/7) of patients with sympathetic ophthalmia, 28.1% (9/32) of patients with BD and 21.1% (4/19) of patients with sarcoidosis. The differences in prevalence of autoantibodies were also statistically significant between BD and healthy controls ( $P < 0.001$ ), and between sarcoidosis and healthy controls ( $P < 0.05$ ).

*Expression of UACA gene in human tissues determined by Northern blot analysis and RT-PCR.* To examine the expression of UACA gene, we performed Northern blot analysis, using Human MTN Blot and Human MTN Blot II (CLONTECH). These membranes were probed with radiolabeled UACA specific cDNA probe which was also used to isolate UACA from a Jurkat cell cDNA library. As shown in Fig. 3, the highest level of expression was observed in skeletal muscle, and all tissues analyzed showed the expression in various levels. As it was impossible in Japan to obtain sufficient amounts of RNA from the human eye to examine Northern blot analysis, we did RT-PCR analysis. As shown in Fig. 4, a 505 bp band corresponding to nucleotide position 3529–4033 of UACA cDNA was observed in all materials, thereby indicating transcription of the UACA gene in human epidermal melanocytes, retina and choroidal tissue.

## DISCUSSION

We used the SEREX method to search for autoantigens associated with VKH. It is well established that specific autoantibodies are present in sera from pa-

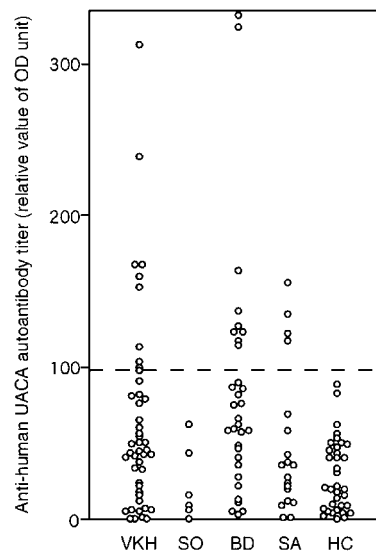


FIG. 2. Distribution of the titer of anti-UACA IgG autoantibodies in patients with Vogt-Koyanagi-Harada disease (VKH), Sympathetic ophthalmia (SO), Behçet's disease (BD), Sarcoidosis (SA), and healthy controls (HC). The reactivities of autoantibodies are expressed as relative value of the OD unit in which the OD value for serum sample of patient HN was estimated to be 100 OD units. Broken line indicates a cutoff level for the autoantibody positivity. The ratio of the OD values for GST-UACA fusion protein to GST protein in 22 positive samples was  $9.57 \pm 0.99$  (mean  $\pm$  SE), and in 118 negative samples was  $3.52 \pm 0.15$  (mean  $\pm$  SE).

tients with autoimmune diseases, including type-1 diabetes mellitus and multiple sclerosis in which autoreactive T cells play major roles in the pathogenesis (26–33). Therefore, we expected that autoantibody to the target autoantigen may also be present in the VKH patients, although VKH disease is considered to be caused by T helper 1 type T cells recognizing uveal autoantigens. We expected that isotype switches of autoantibodies occur in the presence of antigen specific activated  $CD4^+$  T cells, hence we used mouse anti-human IgG as the second antibody to detect IgG autoantibodies.

We identified a novel protein, UACA, as a candidate target autoantigen in VKH. UACA contains 6 ankyrin repeats and coiled coil domains, including a motif of leucine zipper pattern. Ankyrin repeats is a 31–33 amino acid motif present in a number of proteins and it is implicated with protein to protein interactions (19). For example, in GABP $\beta$ , ankyrin repeats mediate stable interaction with GABP $\alpha$  and enhance the specificity of the DNA binding activity of the  $\alpha$  subunit (34).

FIG. 1. Predicted amino acid sequences and structural characteristics of human and bovine UACA. (A) Predicted amino acid sequences for human and bovine UACA proteins. – indicates identical amino acid residues; \* indicates the gap between human and bovine amino acid sequence; open box indicates the consensus sequence of ankyrin repeat and open ellipses indicate the leucine zipper motif; underline indicates the amino acid sequence of a recombinant UACA protein produced as GST fusion protein. (B) Coiled coil domains in human UACA protein analyzed using the secondary structure analyzing program COILS.

TABLE 1

Prevalence of IgG Anti-UACA Autoantibodies Evaluated by ELISA in Sera from Patients with Panuveitis and Healthy Controls

Disease	Anti-human UACA IgG-positive donors	<i>P</i> value*
Vogt-Koyanagi-Harada disease	9/46 19.6%	<i>P</i> < 0.005
Sympathetic ophthalmia	0/7 0.0%	
Behçet's disease	9/32 28.1%	<i>P</i> < 0.001
Sarcoidosis	4/19 21.1%	<i>P</i> < 0.05
Healthy controls	0/36 0.0%	

\* Differences in prevalence of IgG anti-UACA autoantibodies are statistically significant between patients and healthy controls.

And in NF- $\kappa$ B, ankyrin repeats play important roles in protein-protein interactions that regulate localization and activity of this subunit (35). On the other hand, coiled coil domains are involved in self-aggregation or interaction with other proteins and have been noted in many autoantigens (36–39). The leucine zipper motif promotes dimerization through  $\alpha$  helical coiled coil formation, and it is able to bind to DNA (20, 40, 41). These observations suggest that UACA have the potential for protein-protein interactions and may interact with other proteins.

The prevalence of IgG anti-UACA autoantibodies in VKH patients was significantly higher than that in healthy controls. The increased incidence of serological response to UACA in VKH patients indicates a possible relationship between the breakdown of immunological tolerance to the molecule and etiology of the disease. In the Japanese population, VKH, BD and sarcoidosis are the major diseases that cause panuveitis. BD, a systemic inflammatory disorder of unknown etiology, is characterized and diagnosed by four major symptoms, oral aphthous ulcers, ocular symptoms, including iritis and chorioretinitis, skin lesion including erythema nodosum, and genital ulcerations. Sarcoidosis is a systemic granulomatous disease of unknown etiology. VKH, BD and sarcoidosis cause different types of panuveitis. The clinical symptoms of eye lesion are mainly granulomatous iritis and serous retinal detachment in VKH, non-granulomatous iritis and chorioretinitis, including retinal vasculitis (arteritis), exudates and hemorrhage in BD, granulomatous iritis, vitreous opacity and periphlebitis in sarcoidosis. Several autoantibodies had been detected in sera from patients with BD and sarcoidosis (42–46) suggesting that the immunological disorder may be concerned with the development of these diseases. In the present study, the presence of anti-UACA IgG antibodies was observed in BD and sarcoidosis patients as well as in VKH patients. This finding suggests that (1) a similar immunological disorder is involved in VKH, BD and

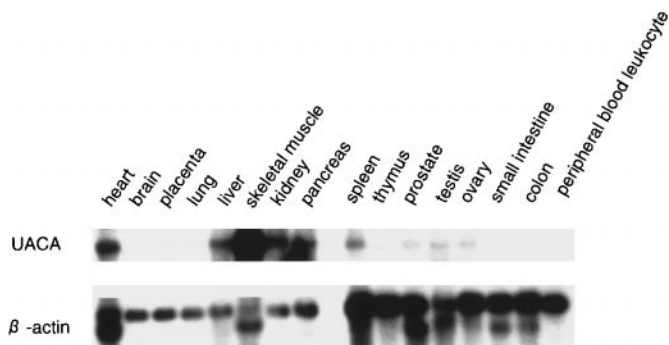


FIG. 3. Northern blot analysis shows expression of the UACA mRNA in human tissues. A unique transcript of 2.3 kb was detected in all mRNA analyzed. The highest expression was detected in skeletal muscles.

sarcoidosis, or (2) the production of anti-UACA IgG antibodies reflects a secondary phenomena related to tissue damage, or (3) the antibodies play different roles in each disease.

Because of difficulty in production of a large recombinant UACA as a whole molecule, we used the C-terminal 18.0% of UACA to detect anti-UACA autoantibodies in patients' sera. A relatively lower prevalence of anti-UACA autoantibodies in patients may be explained by usage of a small fragment of UACA for ELISA. To evaluate autoimmunity directed against the whole UACA molecule, the production of other fragments of UACA will need to be done.

As the target autoantigen of VKH, such as UACA, is present in various human tissues, it seems unlikely that autoimmunity directed against this protein causes uvea and melanocyte-specific tissue damage. However, many examples are known in which autoantibodies reactive to ubiquitously expresses proteins are de-

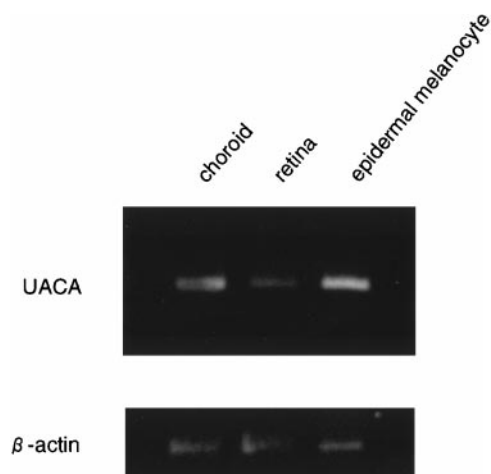


FIG. 4. RT-PCR analysis using human UACA-specific primers shows the expression of UACA mRNA in human choroid, retina and epidermal melanocyte.

ected in organ-specific autoimmune diseases (47–52). Mechanisms underlying immune-responses to ubiquitous self proteins in tissue-specific autoimmune diseases remain unclear. A possible explanation is that cryptic epitopes are exposed in target tissues of autoimmunity and activate CD4<sup>+</sup> T cell (53, 54). Cryptic epitopes can be exposed by tissue-specific protein modification, or in the local proteolytic environment, especially in cases of inflammation, then presented to CD4<sup>+</sup> T cells. The activated CD4<sup>+</sup> T cells may provide cognate or cytokine-mediated activation to B cells and cytotoxic T cells, and subsequently inducing antibody production and cytotoxic response. To elucidate the relationship between UACA and, VKH and other panuveitis, it will be of interest to determine if experimental animals immunized with UACA develop autoimmune disorders such as VKH, BD or sarcoidosis or not.

In the present study we also showed that the peptide fragment <sup>1029</sup>ENDKLLKKE<sup>1036</sup> of UACA was 7/8 identical to the peptide fragment <sup>40</sup>ENAKLLKKE<sup>47</sup> of segment VP6 of Banna virus (25). The peptide sequence ENA/DKLLKKE carries the motif for binding with HLA-DR4 (DRB1\*0405) (55), the VKH susceptible allelic product of HLA gene in the Japanese population. Banna virus belongs to coltivirus type species composed of 12 segments of double-strand RNA genomes VP1–VP12 and has been isolated from humans suffering from meningoencephalitis (56, 57), but the epidemiology of this virus is unknown. Activation of autoreactive T cells by bacterial or viral peptides that have sufficient sequence similarity with an immunodominant autoantigenic peptide (molecular mimicry) (58) is suggested to be an important event in the induction of autoimmunity. The identity of amino acids sequence between UACA and Banna virus suggests that the infection of Banna virus may be a possible trigger of VKH, BD and sarcoidosis that cause panuveitis. To elucidate the relationship between Banna virus and these diseases, it is necessary to do epidemiological studies of Banna virus, including quantitation of anti-Banna virus antibody in patients and healthy controls, and investigate T cell responses directed against these peptides.

In summary, we identified a novel protein UACA, which appears to be a possible target autoantigen in VKH, BD and sarcoidosis that cause different types of panuveitis.

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