### ORIGINAL ARTICLE

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# **Clinical and serological associations of autoantibodies to GW bodies and a novel cytoplasmic autoantigen GW182**

Received: 13 May 2003 / Accepted: 8 September 2003 / Published online: 4 November 2003 © Springer-Verlag 2003

Abstract A novel autoantigen named GW182 was recently identified when the serum from a patient with a sensory ataxic polyneuropathy was used to immunoscreen a HeLa cDNA library. Unique features of the GW182 protein include 39 repeats of glycine (G) and tryptophan (W) residues, binding to a subset of messenger RNA and localization to unique structures within the cytoplasm that were designated GW bodies (GWBs). The goal of the present study was to identify the clinical features of patients with anti-GW182 antibodies and to characterize the B cell anti-GW182 response by defining the epitopes bound by human autoantibodies. The most common clinical diagnosis of patients with anti-GW182 antibodies was Sjögren's syndrome followed by mixed motor/ sensory neuropathy, and systemic lupus erythematosus. Of interest, 5 (28%), 9 (50%), and 3 (17%) of the 18 sera that react with GWBs had autoantibodies to the GW182 and the 52 kDa and 60 kDa SS-A/Ro autoantigens, respectively. Epitopes bound by the human autoantibodies were mapped to the GW-rich middle part of the protein, the non-GW rich region, and the C-terminus of GW182 protein. None of the GW182 epitopes had significant sequence similarities to other known proteins. GW182 represents a new category of ribonucleoprotein autoantigens.

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**Keywords** Autoantibodies · mRNA · Autoimmunity · Sjögren's syndrome · Neurological disease

Abbreviations *GWB*: Glycine tryptophan-rich cytoplasmic structure · *IIF*: Indirect immunofluorescence · *IP*: Immunoprecipitation · *NET2*: NaCl, EDTA, Tris buffer · *SDS*: Sodium dodecyl sulfate · *SjS*: Sjögren's syndrome · *SLE*: Systemic lupus erythematosus · *TBS*: Tris-buffered saline · *TnT*: Transcription and translation

### Introduction

The sera from patients with systemic autoimmune disease have been used to isolate and identify novel autoantigens that are components of macromolecular complexes that have a variety of cellular functions including transcription, translation, and ribosomal processing [1, 2, 3]. In addition, the identification of autoantigens and the characterization of their respective epitopes are used as diagnostic tools to assist in the clinical evaluation of autoimmune diseases [4, 5, 6]. For example, the presence of autoantibodies to double-stranded DNA and the Sm small nuclear ribonucleoproteins (RNPs) are highly specific serological markers for systemic lupus erythematosus (SLE) [7]. Sjögren's syndrome (SjS) is characterized by the presence of autoantibodies to SS-A/Ro, and/or SS-B/la [8]. In addition, the identification of autoantigens and their association with autoimmune disease is a key approach to understanding the autoimmune disease state [9, 10].

Recently a novel autoantigen named GW182 was discovered when the serum from a patient with ataxic sensory polyneuropathy was used to immunoscreen a HeLa cDNA library [11]. Interesting features of the GW182 protein include 39 repeats of glycine (G) and tryptophan (W) residues and its localization in unique cytoplasmic structures that have been designated as GW bodies (GWBs). The GW182 protein, which has an RNA recognition motif and binds specific mRNAs, is thought to be part of a mRNA-protein macromolecular complex. It has been postulated that GWBs provide an additional level of posttranscriptional gene regulation and function in mRNA processing in a cell compartment referred to as the ribosome or posttranscriptional operon [12, 13]. More recent evidence implicates the GW182 protein and GWBs in mRNA degradation pathways [14]. The goal of the present study was to characterize the B-cell immune response in patients with antibodies to GWBs and the GW182 protein which resides within the GWBs and to assess the clinical features of these patients. This is the first report of the clinical features of patients with anti-GWB antibodies and a description of the GW182 epitopes bound by these sera.

### **Materials and methods**

Patient serum and antibodies

All human sera used in this study were obtained from serum banks at the Advanced Diagnostics Laboratory (University of Calgary, Calgary, Canada), the W.M. Keck Autoimmune Disease Center (Scripps Research Institute, La Jolla, Calif., USA), and Juntendo University (Tokyo, Japan). The index human serum used in this study was selected based on its reactivity to an apparently unique cytoplasmic domain and its reactivity with the native and recombinant GW182 protein [11]. Clinical information was obtained by contacting the referring physician and retrospective chart review. Indirect immunofluorescence

The presence of anti-GW182 antibodies in the human sera were initially tested by Indirect immunofluorescence (IIF) using HEp-2 cell substrates (Immuno Concepts, Sacramento, Calif., USA) and had a cytoplasmic staining pattern that was characteristic of anti-GWB antibodies [11]. Reactivity with GWBs was confirmed by IIF colocalization studies on HEp-2 cells where a monoclonal antibody (4B6) to the recombinant GW182 protein which stains GWBs was used as the marker antibody [15]. Secondary antibodies for colocalization studies were fluorescein isothiocyanate conjugated anti-mouse IgG (Jackson ImmunoResearch, West Grove, Pa.,USA) and fluorescein isothiocyanate or Cy3-conjugated anti-human IgG (Jackson ImmunoResearch). Nuclei in the cell substrates were stained with 4',6-diamidino-2-phenylindole that was included in the glycerol mounting medium (VectaShield, Vector, Burlingame, Calif., USA).

In vitro transcription/translation and immunoprecipitation

Reactivity of the sera with recombinant GW182 protein was confirmed by immunoprecipitation (IP) of the recombinant protein. The full-length GW182 cDNA was used as a template to synthesize the protein in an in vitro transcription and translation (TnT) protocol that used a rabbit reticulocyte lysate kit (TnT, Promega Biotec, Madison, Wis., USA) in the presence of [35S]methionine at 30°C for 3-4 h as previously described [11, 16]. To confirm the presence of TnT products 2- to 5-µl samples were separated by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis and analyzed by autoradiography. The TnT products were then used in IP reactions by combining 100 µl of a 10% protein A Sepharose bead suspension (Sigma, catalog no. P-3391), 10 µl human serum, 500 µl NET2 buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 5 mM EDTA, 0.5% Nonidet P-40, 0.5% deoxycholic acid, 0.1% SDS, 0.02% sodium azide), and 10 µl of radiolabeled protein product. After incubation for 1 h at 4-8°C the suspension was washed five times in NET2, the proteins eluted in 10 µl sample buffer, and analyzed by 10% gel SDS polyacrylamide gel electrophoresis as described [17].

#### Epitope mapping

Epitope mapping employed sequential peptides of 15 amino acids offset by five amino acids, representing the full-length GW182 protein, were synthesized on membranes using the SPOT technology as previously described [18, 19, 20]. The membranes containing the peptides were processed for immunoblotting by soaking the membrane in Tris-buffered saline (TBS; 10 mM Tris-HCl pH7.6, 150 mM NaCl) for 10 min and then blocking with 2% milk/TBS for 1 h at room temperature. The human sera were diluted 1/100 in 2% milk/TBS and applied to the membrane. After 2 h of incubation at room temperature the membrane was washed three times with TBS. A horseradish-peroxidase conjugated goat anti-human IgG (Jackson ImmunoResearch) was diluted according to the manufacturer's protocol, and reactivity was visualized using enhanced chemiluminescence western blotting detection reagents (Amersham International). After reactive epitopes were identified a BLAST search of the GenBank using the reactive sequences as the query was conducted to identify homologous sequences in other proteins.

#### Purified recombinant GW182

The GW182 cDNA insert encoding a partial length of the GW182 protein was subcloned into pET28 (Novagen, Madison, Wis., USA). *Escherichia coli* JM109 (DE3) was transformed with this subclone, and the recombinant protein produced was purified using Ni<sup>2+</sup> affinity chromatography as per the manufacturer's instructions

(Qiagen, Valencia, Calif., USA). This recombinant protein was subsequently used in the laser bead immunoassay described below.

#### Laser bead immunoassay

A set of addressable beads bearing laser reactive dyes (Luminex, Austin, Tex., USA) were selected to couple the recombinant purified GW182 protein. Unless otherwise specified, all incubations and reactions were conducted at room temperature. Ten micrograms of 1-ethyl-3-(3-dimethylaminopropyl) carboiimide hydrochloride (Pierce, Rockford, Ill., USA) and N-hydroxysuccinimide (Pierce) was placed in separate microcentrifuge tubes (USA Scientific) and dissolved in 200 µl activation buffer (0.1 M sodium phosphate, pH 7.2). Of the laser bead suspension 100 µl was placed into a microcentrifuge tube and centrifuged at 10,000 rpm in a microcentrifuge for 1 min, and the fluid was decanted. Forty microliters of activation buffer was added to the pelleted beads, and they were gently resuspended by brief sonication and vortexing. Five microliters of 1-ethyl-3-(3-dimethylaminopropyl) carboiimide hydrochloride and N-hydroxysuccinimide was added in sequence to the resuspended microspheres, followed by brief sonication and vortexing. The suspended spheres were incubated in the dark for 20 min before the purified recombinant GW182 protein, dissolved in coupling buffer (0.14 M NaCl, 0.01 M NaPO4, pH approx. 7.2: PBS), was added to the mixture. After an additional incubation in the dark for 3 min, the suspension was centrifuged at 13,000 rpm for 3 min. The fluid was decanted and 125 µl coupling buffer added. The spheres were resuspended by sonication and vortexing as above before repelleting by centrifugation at 13,000 rpm for 3 min. The supernatant was decanted, 125 µl the protein solution (50 µg/ml) added, and the beads resuspended by sonication and vortexing. The protein and sphere suspension were incubated for 1 h at room temperature in the dark. The protein-coupled microspheres were pelleted by centrifugation at 10,000 rpm for 2 min and then resuspended in 125 µl washing buffer (PBS pH 7.2, 0.05% Tween 20). After two cycles of resuspension and pelleting in 125 µl blocking/storage buffer (0.5% BSA in PBS), the beads were stored as a suspension in 100 µl of blocking buffer at 2-8°C until required for use.

To analyze reactivity of the sera with the bound GW182, patient sera were diluted in Quanta Plex sample diluent (INOVA, San Diego, Calif., USA) to a final dilution of 1/1,000. To each well 40  $\mu$ l of bead stock (1 part microspheres in blocking buffer to 40 parts Quanta Plex sample diluent) and 10  $\mu$ l of diluted patient sera were added and incubated for 30 min on an orbital shaker. Then 50  $\mu$ l phycoerythrin-conjugated goat anti-human IgG (Jackson ImmunoResearch) diluted 1/50 was added to each well and incubated on the orbital shaker for an additional 30 min. The reactivity of the antigen-coated beads was determined on a Luminex 100 dual-laser flow cytometer (Luminex). Control negative and standard positive sera were included in each assay. The tests were semiquantitative, and the results were expressed as median fluorescent intensity of the test sample.

#### Line immunoassay

The serum samples were tested for reactivity to other autoantigens using a "line" assay that includes recombinant and native SmB, SS-A/Ro52, SS-A/Ro60, SS-B/la, U1-RNP, ScI-70, ribo P antigens located on a solid phase strip (INNO-LIA, Innogenetics, Norcross, Ga., USA). The assays were performed according to the manufacturer's instructions, and at the completion of the assay the strips were dried and were interpreted based on visual comparison of the intensity of the bands on the test strip to the cutoff control on another strip.

### Results

IIF using the index human serum on HEp-2 cells showed a pattern of distinctive cytoplasmic dots and what was previously described as GWBs (Fig. 1a). The number of GWBs present in HEp-2 cells varied from zero in mitotic cells to more than 30 in interphase cells. Previously it was shown that GWBs containing the GW182 autoantigen are distinguished from other cytoplasmic organelles, including the Golgi complex, lysosomes, endosomes, and proteasomes [11]. Over a 14-month period the clinical reference laboratory (Advanced Diagnostics Laboratory, University of Calgary) received approximately 5,000 sera for autoantibody analysis as requested by physicians who were investigating the presence of autoimmune disease, such as SLE and SjS, in their patients. From these 5,000 serum samples approx. 200 sera showed a cytoplasmic speckled staining pattern on HEp-2 cells. Of these 200 sera 18 (9%) had autoantibodies to the GWBs as determined by colocalization with the monoclonal antibody 4B6 that reacts with the GW182 protein and stains

**Fig. 1a–e** IIF colocalization studies using human and murine monoclonal anti-GWB antibodies. Cytoplasmic bodies in HEp-2 cells detected with the index patient serum diluted 1/100 (**a**, **b**) colocalized with the staining of a monoclonal antibody 4B6 to GW182 (**c**). The nuclei are stained blue with 4',6-diamidino-2-phenylindole (**d**) and the merged images are shown in **e**. *White bar* (**b**) 5 µm



**Table 1** Demographic, clinical, and serological features of patients with anti-GWB antibodies (*AMA* anti-mitochondrial antibodies, *mAb* monoclonal antibody, *NHS* normal human serum, *PBC* primary biliary cirrhosis, *Pt* patient serum, *IP* immunoprecipitation,

*SjS* Sjögren's syndrome, *SLE*, systemic lupus erythematosus, *SS-A/ B* Sjögren's syndrome antigen A/B, *TnT* in vitro transcription and translation, *UCTD* undifferentiated connective tissue disease)

Patient	Age (years)	Sex	Diagnosis	IIF colocalized with mAb 4B6	Line assay				Laser bead	TnT IP
no.					SmB	SS-A 52 kDa	SS-A 60 kDa	SS-B	assay GW182 antibodies <sup>a</sup>	
Group A	1									
1	73	F	Ataxic sensory polyneuropathy	+	_	+	_	_	7766 <sup>b</sup>	+
2	75	F	Sensory neuropathy, arthritis, granuloma, silicon breast implants	+	-	+	-	-	72	-
3	38	F	Sensory neuropathy, granulomatous lymph nodes	+	-	+	-	-	1209 <sup>b</sup>	+
4	46	F	Malar rash, arthralgia	+	+	_	_	-	189	_
5	64	Μ	AMA negative PBC	+	-	+	_	-	209	_
6	43	F	UCTD	+	-	_	_	-	123	-
7	51	F	Lymphoma	+	-	_	_	-	161	-
8	67	F	Renal failure, hypergammaglobulinemia	+	-	-	-	-	98	+
9	85	F	Diabetes, heart block	+	-	_	_	-	1014 <sup>b</sup>	-
Group E	3									
10	77	F	SjS, ataxia	+	_	+	_	_	5999 <sup>b</sup>	+
11	54	F	SjS, ataxia	+	-	+	_	-	123	_
12	48	F	SjS, motor neuropathy	+	-	+	_	-	287	-
Group C	2									
13	51	F	SLE	+	_	_	_	_	226	_
14	46	F	SLE	+	_	_	_	_	139	_
15	47	F	SLE, SiS	+	_	_	+	_	112	_
16	51	F	SLE, SiS	+	+	+	+	+	84	_
17	70	F	SiS	+	_	_	_	-	109	_
18	57	F	SjS, interstitial pneumonitis	+	-	+	+	+	116	-

<sup>a</sup> The results of the addressable laser bead immunoassay for antibodies to GW182 are expressed as median fluorescence units <sup>b</sup> Sera with a positive test

the GWBs (Fig. 1). The other sera had antibodies to early endosome antigen 1 [21], ribosomal RNP [22], mitochondria [23], cytoplasmic linker protein (CLIP-170) [24], and other as yet unknown endosome or lysosome antigens. None of the 18 sera that bound GWBs had antibodies to dsDNA, chromatin, U1-RNP, topoisomerase I (Scl-70), fibrillarin (U3 RNP), or centromeres/kinetochores [10]. The immunoglobulin isotype of all sera with antibodies to GWBs was IgG as shown by isotypespecific staining of HEp-2 cells, immunoblotting, and protein A Sepharose immunoprecipitation of recombinant GW182 protein. The anti-GWB titers as determined by IIF on HEp-2 cell substrates ranged from 1/320–1/5,120. A study of 2500 healthy female blood donors showed that none of these samples contained anti-GWB antibodies as determined by IIF using HEp-2 cells [25].

Although all 18 sera had antibodies to the GW body, the multiplexed laser bead assay indicated that 4 of the 18 sera (nos. 1, 3, 9, 10) recognized the recombinant GW182 protein which is one of several proteins found within GWBs (Table 1). When the reactivity of the 18 sera was also tested by IP using in vitro transcribed/translated protein, it was observed that 4 sera (nos. 1, 3, 8, 10) IP the GW182 protein (Fig. 2). Therefore when the data of the two assays that used recombinant protein are combined, 5 of the 18 sera recognized GW182.



**Fig. 2** Immunoprecipitation of the <sup>35</sup>S-labeled GW182 TnT recombinant GW182 protein with patient sera. Four sera (*lanes 1–4*) that stained GWBs, immunoprecipitation IP the recombinant GW182 (*TnT*), but normal human serum (*NHS*) did not. MW <sup>14</sup>C molecular weight markers

The clinical data obtained on the 18 patients who had the GWB staining pattern are summarized in Table 1. Of the 18 patients 17 (39%) with autoantibodies to the GWBs were women and ranged in age from 46 to 85 years (mean 58). The clinical diagnoses could be stratified into three groups: group A composed of 9 patients had predomiPatient 1



Pateint 3



## Patient 10



**Fig. 3** Epitope mapping obtained using sequential 15mer peptides offset by five amino acids that represented the full-length GW182 protein were spotted on membranes and then probed with a normal human serum (*NHS*) and three sera with anti-GWB antibodies (*patients 1, 3, 10*)

nantly mixed motor and/or sensory neuropathy, although other disease manifestations were also noted; 3 patients in group B had SjS in addition to some neurological features that overlapped with group A; in group C there were 6 patients who had SLE and/or SjS without documented evidence of neurological disease. When the various diagnoses or clinical conditions were tabulated individually, SjS was the most common, seen in 7 of 18 (39%), followed by patients with neurological disease (motor and sensory neuropathy and/or ataxia) in 6 (33%), followed by SLE in 4 (22%).

When it was observed that some of the patients had SLE and SjS, we were interested to determine whether autoantibodies to known autoantigens that are typical markers of SLE and SjS were present. Autoantibodies to SS-A/Ro and SS-B/la were correlated with the diagnosis of SjS in 6/7 patients diagnosed with SjS (Table 1). However, four patients in group A had anti-SSA/Ro52 antibodies but did not have a clinical diagnosis of SjS or

SLE. Interestingly, 9 sera had antibodies to the 52-kDa SS-A/Ro antigen, but 7 did not have coexisting antibodies to the 60 kDa SS-A/Ro antigen. One patient (no. 4) had a malar rash, arthralgia, and antibodies to the SmB protein but did not fulfill criteria [26] for classification as definite SLE.

Only 4 of the 18 patient sera (22%; nos. 1, 3, 8, 10) with anti-GWB antibodies as defined by colocalization, IP the GW182 protein. Three of these four sera (nos. 1, 3, 10; Table 1) were used for epitope mapping due to limited quantity available for the fourth serum (no. 8). Multiple epitopes over the entire length of GW182 were recognized by the patient sera (Figs. 3, 4). Four overlapping reactive peptides were shared between patient no. 1 and patient no. 10: amino acids 666-695, 951-970, 1676-1690, 1691-1705. Several peptides were in common between patient no. 1 and patient no. 3: amino acids 431-450, 766-780, 921-945, 951-970, 1101-1115, 1161-1185, 1191–1205, 1391–1410, 1431–1445, 1616–1630. Interestingly, only one peptide (1511–1525) was bound by both patient no. 10 and patient no. 3. The reactive epitopes mapped to the GW-rich, the middle portion, the non-GW rich, and the C-terminal domains of the GW182 protein (Fig. 4). When the reactive peptides were subjected to a BLAST analysis, only the published GW182 protein and related EST clones, KIAA1460, KIAA1582, and KIAA1093 showed more than 60% amino acid sequence identity. The KIAA1460 EST is known to be partial-length GW182 [11].

### Discussion

In this study we report the clinical features of 18 patients who have autoantibodies to a unique structure within the cytoplasm which we previously named GW bodies or GWBs. Five of the 18 sera that colocalized with the murine monoclonal antibody to GW182 (monoclonal antibody 4B6) which stains the GW bodies, reacted with the GW182 protein in two different immunoassays. This suggests that either the epitopes reactive by IIF are not present in the recombinant proteins used in these assays, or that GWBs contain target autoantigens that remain to be defined. Studies are underway to define additional autoantigens in GWBs.

The incidence of anti-GWB antibodies reported in this study was 0.36%. In the Advanced Diagnostics Laboratory at the University of Calgary, this approaches the incidence of autoantibodies to Sm and Golgi antigens (0.4% and 0.5%, respectively) and was higher than antibodies to proliferating cell nuclear antigen (0.1%), Jo-1 (histidyl t-RNA synthetase (0.1%) and Scl-70 (topoisomerase I, 0.3%; unpublished data).

It is not clear whether the autoantibodies directed against GWBs and GW182 are pathogenic. The clinical diagnosis of patients with GWB autoantibodies included SjS, motor or sensory neuropathies, SLE, and a variety of other clinical conditions. Observations of mRNA processing may be relevant because particles containing

Number         Sequence         Position         #1         #5         #10           6         EKDGLKNSTGLGSQN         26-40         98         EKDGLKNSTGLGSQN         98-1005           7         RNSTGLGSQNKIVVG         31-45         99         198         MERNAWGUYSSSSN         66-100         200         GOVREVGKGPOSKE         100-1015         200         200         200         100         100         200         GOVREVGKGPOSKE         100-1015         200         100         100         200         100         100-1015         200         200         100         100         200         100         100         200         100         100         200         100         100         200         100         100         200         100         100         100         100         100         200         100         200         100         200         100         200         100 <td< th=""><th></th><th>PEPTIDE</th><th></th><th></th><th>PATIEN</th><th>Г</th><th>191</th><th>FVKQFSNISFSRDSP</th><th>951-965</th><th></th><th></th></td<>		PEPTIDE			PATIEN	Г	191	FVKQFSNISFSRDSP	951-965		
Number         Sequence         Position         ff         ff3         ff3         ff3         ff3         ff4							192	SNISFSRDSPEENVQ	956-970		
6         EKDGLRNSTGLGSQN         26-40         109         HSLNGDVRTVGKG         991-1005           7         RNSTGLGSQNKFVVG         31-45         200         GOVNRTVGKGC         991-1005           20         STLSNASNIGAWPVL         106-120         201         TVGKAPORSPISKE         1001-1015           21         STLSNASNIGAWPVL         106-120         201         TVGKAPORSPISKE         1001-1015           20         QCSTIGMVFNSQNS         144-155         202         POSRPIGNERAVPCQV         1101-1115           30         GYUNSYGKVSSQG         176-190         221         PLSSSQPNLRAQVPPS         1136-1150           213         TQSVSTGKVSSSQK         266-280         233         SQLQRLAQQCRAQS         1161-1175           214         LLAQQQRAQQRSPV         1161-1175         234         LLAQQQRAQQSRNP         1171-1185           215         AVGNYSGDKCSGA         311-325         234         LLAQQQRAQQSRNP         1171-1185           216         SRVSGANSGANSGS         311-345         235         GRFISVQQOMMOQSR         1171-1185           216         SRVSGANSGANSGS         311-345         235         GRFISVQQQMNQQSR         1161-1175           217         GRKTINOWKSTILED         311-335         236 </td <td>Number</td> <td>Sequence</td> <td>Position</td> <td>#1</td> <td>#3</td> <td>#10</td> <td>198</td> <td>MEIDKHSLNIGDYNR</td> <td>986-1000</td> <td></td> <td></td>	Number	Sequence	Position	#1	#3	#10	198	MEIDKHSLNIGDYNR	986-1000		
6         EKDGLRNSTGLGSQN         2-640           7         RNSTGLGSQNKTVVG         31-45           18         NNRMAWGTVSSSSN         86-100           23         STLNASNHGAWPV1         106-120           24         STLNASNHGAWPV1         106-120           25         STLNASNHGAWPV1         106-120           26         OCYNTYCKORCORSPP         106-110           27         OKYNSTORYNSKOR         141-155           28         STLNSSYSGOPNILR         106-110           29         OCSTICQMPMNQSINK         146-160           21         TMKYSSGOPNILR         113-1145           21         STLNSSOPNILRAQUPP         1101-115           21         STLNSSOPNILRAQUPP         1101-115           21         STLNSSOPNILRAQUPP         1111-115           21         STLNSSOPNILRAQUPP         1111-125 <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td>199</td> <td>HSLNIGDYNRTVGKG</td> <td>991-1005</td> <td></td> <td></td>							199	HSLNIGDYNRTVGKG	991-1005		
7       RNSTGLGSQNKFVG       31-45         18       NRNMAWGTUSSSS       86-100         22       STLSASASHIGAWPVL       106-120         23       QCSTIGQMENNQSINSKVSG       146-160         20       GQMENNQSINSKVSG       146-160         21       PLSSGPUSKES       100-1015         22       QPAQPESSSPPNLR       100-1105         23       GCSTIGQMENNQSINSKVSG       146-160         24       FLEPSSTGAWENSGOR       176-190         25       ATGNSYGDKSCGROP, 176-190       221         25       ATGNSYGDKSCGROP, 277-285       233         26       GTIWGAYGNNYSGDK, 266-280       233         25       ATGNSYGDKSCGA, 311-325       234         264       KGGGWWESGA, 331-345       235         265       WESGANSQSTSWGS       31-345         266       WESGANSQSTSWGS       31-345         27       GKTINGWKSTIEED 381-395       264         264       SPYPYDPHNN       131-125         270       SPKNESSWNPPFER       136-130         271       SSNMWPFER       136-130         272       PELFWOPMNN       131-1325         273       MCIGSQSNRDRKH       451-450         274<	6	EKDGLRNSTGLGSQN	26-40				200	GDYNRTVGKGPGSRP	996-1010		
18       NNRMAWGTVSSSSN       86-100       202       PGSRVDSKESSNER       1006-1020         22       STLNSSNIG4WPL       106-120       201       MRGVGNTAGRMQU       106-1005         29       QCSTIGQMPNNOSIN       141-155       202       OPRADROMQU       106-1105         36       SEVSCTOKVSPSGOP       175-190       221       OPRADROMQU       106-1105         37       TQKVSFSGOP(DUTT       181-195       221       PNSGLGNPLFGPQQV       1131-1145       221         45       ELPSSNTGAWRVSTM       221-235       231       SQLORALOQOS       1161-1175       223       224       LLAQAQSORSVPSORP       1161-1175       224       124, GAGSORSVSORP       1161-1175       226       226       67       75       GKGSNANSGGSKGG       313-35       236       67       78, GKANSGSTRWG       211-125       226       227       PLSKGSVQQMMQOSR       111-1125       226       227       PLSKGSVQQMMQOSR       111-125       226       227       PLSKGSVQQMMQOSR       111-125       226       227       PLSKGSVQQMMQOSR       111-125       226       227       PLSKGSVQVPPDFNNSTIT       131-130       226       227       PLSKTGKQVPPDFNNSTIT       131-135       226       227       PLSKTGKQVPPDFNNSTIST       136-1330	7	RNSTGLGSQNKFVVG	31-45				201	TVGKGPGSRPQISKE	1001-1015		
22       STLNASNIGAWPVL       106-120       217       MCGYGTAAQPROMQ       106-110         30       GQMPNOQSINSKVSG       146-160       221       PLSSOPNLR       100-1115         30       SEVSOTOKVSTSOQCOP       176-190       221       PLSSOPNLR       100-1115         37       TQKVSTSOQCOPNTT       181-195       221       PLSSOPNLR       100-1115       221         34       ELPSSNTGAWRSTM       221-235       223       100-1115       223       101-1175         54       GTWSGANSQSOR       246-380       233       SQLQRAQSR VP       116-1175       233       116-1180       234       101-1175       235       235       0RASORSVSORAP       21-285       234       102-00CA3QS0RSVS       116-1180       235       0RASORSVSORAP       21-285       235       0RASORSVSORAP       21-216-290       236       0REISVDQOMMQOR       111-125       <	18	NNRMNAWGTVSSSSN	86-100				202	PGSRPQISKESSMER	1006-1020		
29         QCSTIGQMIPNOSIN         141-155         220         QPAOPLSSOPNLR         1006-1110           36         GVMPNOSINSKYSKS         146-160         221         PNSGLONPLFCRQQV         1131-1145           36         SEVSCTCKVSPSGOP         176-190         227         PNSGLONPLFCRQQV         1131-1145           37         TQKVSPSGOP(DNTT         181-195         228         PNSGLONPLFCRQQV         1131-1145           45         ELPSSNTGAWRVSTM         221-235         231         LSQLORQAS         1161-1175           53         AYGSNYSGDCSGPN         271-285         234         LLAQQQSQSNPSNP         1161-1175           64         KGGGWESGAANSQS         316-330         236         GREPSQRLRKTVTDS         127-61-290           67         TSWGSGNANSGGSR         331-345         256         GREPSQRLRKTVTDS         127-125           77         GKTFTNGWKSTEEDD         381-395         264         SPFVPDFWNSTTD         121-125           78         NGWKSTEEDDQGAST         386-400         270         SPSWNBPFER         136-1330           79         WDTETSPRCEWKSTS         316-430         271         SPSWNBPFER         136-130           79         WDTETSPRGERKTDN         481-4455         270	22	STLNSASNHGAWPVL	106-120				217	MFGVGNTAAQPRGMQ	1081-1095		
30         GQMPNNQSINSKYG         146-160         221         PLSSSPPNLRAQVPP         1101-115           36         SEVSGRVSTSRSQCP         176-190         221         PLSSSPPNLRAQVPP         1101-115           37         TQKVSPSOQPQNITT         181-195         228         INPLGPQVVAILAQVPP         1101-115           36         GYGNSGRVSCRSQP         271-285         233         SQUQRLAQQQRAQS         1161-117           36         QVNTNKGGVVESCA         311-325         233         SQUQRLAQQQRAQS         1166-1180           36         QVNTNKGGVVESCA         311-325         233         SQUQRLAQQQRAQS         1161-1175           36         MYGNSCNCANSQGS         313-345         235         GRPLSVQQOMMQOSR         1161-1175           37         TSWGSONCANSQGS         313-345         256         REPSVPDYDNMN         1311-1325           38         ROWSSTEED         313-345         256         RPLSVQQOMMQOSR         1271-1185           38         ROGONPERD         336-530         270         SPVPVDYDNNN 1311-1325         270           39         DPKVLSNSQWCOTPI         461-475         270         SPVNPORNN 1316-1325         270           39         DPKVLSNSQWCONDYN         461-475         270	29	QCSTIGQMPNNQSIN	141-155				220	QPPAQPLSSSQPNLR	1096-1110		
36         SEVSGTQKVSFSGQP         176-190         227         PNRGGLNFLGRQQV         1131-1145           37         TQKVSFSGQPNIT         181-195         231         LSQUAVAMINQ         1136-1150           45         GTTMCAGVGSNYSGDK         266-280         231         LSQUAVAMINQ         1136-1150           55         AYGSNYSGDKCSGPN         271-285         231         LSQUAVAMINQ         1161-1175           64         KGGOVMESGANSOSS         316-330         234         LLAQOQRAQS REGVERSGRP         1166-1180           65         MESGANSOSTSWGS         213.35         236         CRASOSSWFSGRRP         1166-1180           66         MESGANSOSTSWGS         313-35         236         CRASOSSWFSGRRP         1166-1180           67         TSWCSOGANSOSGR         313-35         256         KEPOSILRKWTTVDS         1276-1290           68         GNGANSOSGRRGWQT         336-350         266         PRLEESPT VPDFNAN         1311-1325           76         KTITNOWKSTEEED         381-395         270         SPVVDFWDSTSTS         1316-1330           78         ROGKSKNDQMTKH         414-445         271         SSVNWPPEFR         1346-1360           79         DPRVLSNSQWODPKKK         464-475         270	30	GQMPNNQSINSKVSG	146-160				221	PLSSSQPNLRAQVPP	1101-1115		
37       TQKVSFSQQPQNITT       181-195       228       LNPLFCPQQVAILNQ       113-1150       228         54       GTTWGAYGSNYSGDK       266-280       233       SQLQRLLAQQQRAQS       115-1150       223         55       AYGGSNYSGDK CSPN       271-285       233       SQLQRLLAQQQRAQS       1161-1175         63       QVNTNKGGWESGA       311-325       235       236       GRAQSQRSVFSCNPP       1171-1185         64       KGGOWESGA ANSOS       331-345       235       GRAQSQRSVFSCNPP       1171-1185         65       WESGAANSQCSTSWGG       321-335       236       GRAQSQRSVFSCNPP       1171-1185         66       GNGANSGGSRKWGT       336-350       237       RLIRW TIVDSISWNT       121-1205       236         77       GKTTTNGWKSTEEED       381-395       237       RLIRW TIVDSISWNT       121-1205       244         88       ESQSRDRRKIDUTL       434-445       271       SSWWPPEER       1361-1350       272         92       NRTDLDPRVLSNSGW       456-470       271       SSWWPPEERFGEPW       1351-1365       272       272       PFERRGEPW GTSNIL       136-1370       272       272       PFERRGEPW GTSNIL       136-1370       272       272       PFERRGEPW GTSNIL       136-1370	36	SEVSGTQKVSFSGQP	176-190				227	PNNGGLNPLFGPQQV	1131-1145		
45       ELPSNTGAVENYSTM       221-235         54       GTTWGAYGSNYSORDK 26-280       30         55       AYGSNYSORDKCSQPN       271-285         64       KOGGVWESGA       311-325         64       KOGGVWESGA       311-325         65       WESGANSQSR WSGS       316-330         67       TSWGSOGANSGOSR       313-345         68       GNGANSGOSRROWT       336-350         77       GKTTTKGWKSTEEED       381-395         78       NGWKSTEEEDQGSAT       386-400         78       NGWKSTEEEDQGSAT       386-400         79       WDTENROGEVKENT       431-445         88       ESQSKURKKIDQHTIL       431-445         80       DPRVLSNSGWQOTPI       461-475         93       DPRVLSNSGWOOPKP       521-535         105       GNDTSSVSGWGDPKP       521-535         117       KNGQGWGDGXSSQG       581-595         118       WCDQGKSQQGWSSA       566-600         118       WCDQGKSQQGWSSA       566-600         118       WCDQGKSQQGWSSA       566-600         134       SQGWGPPKSNSDLG       661-675         135       DPPKSNQSLGWGDSS       671-689         136	37	TQKVSFSGQPQNITT	181-195				228	LNPLFGPQQVAMLNQ	1136-1150		
54       GTTWGAYQSNYSODK       266-280       233       SQLQRLLAQOQRAQS       1161-1175         63       QVNTNKGGGVWESGA       311-325       234       LLAQOQRAQSCRNP       1161-1175         64       KGGWESGANSQS       311-325       235       QQRAQSQRNP       1161-1175         65       WESGANSQSTWGS       321-335       236       GRPSQDQCMAQORXP       1161-1175         66       GNGANSQSTWGS       321-335       236       GRPSQDQCMAQORXP       1161-1175         67       TSWGSGNGANSQS 311-345       236       GRPSQDQCMAQORXP       1171-1185       236         68       GNGANSGSRNGWGT       336-350       257       RLRKWTTVDS       1276-1290       257         77       GKTFTNGWKSTEEED       381-395       264       SPKVPDENNSSTEP       1316-130       271         87       EKGGGESQRDRRKI       431-445       271       SSVNWPPERPGEPW       1361-1375       272       PFEVPDENNSSTEP       1361-1375       276       DPYVTDENNSSG       1391-1405       276 <td< td=""><td>45</td><td>ELPSSNTGAWRVSTM</td><td>221-235</td><td></td><td></td><td></td><td>231</td><td>LSQLNQLSQISQLQR</td><td>1151-1165</td><td></td><td></td></td<>	45	ELPSSNTGAWRVSTM	221-235				231	LSQLNQLSQISQLQR	1151-1165		
55       AYGSNYSGDKCSGPN       271-285       234       LLAQQQRAQSQRSYP       1166-1180         64       KGGGWWESGAANSQS       316-330       235       QRPLSVQQDNMQQSR       1191-1205         65       WESGAANSQTSWGS       321-335       235       QRPQSVSPSORNP       1171-1185         66       GNGANSGGSRR       331-345       236       GRPLSVQQQNMQQSR       1191-1205         67       TSWGSONGANSGGSR       331-345       256       KEPQSRLRKWTTVDS       1281-1295         68       GNGANSGGSRRWGT       336-530       256       RELESPFVPTDENN       1311-1325         78       NGWKSTEEED       381-495       266       SPFVDPMNSSTSP       136-130       270         92       NRTDLPRVLSNSGW 456-470       271       SSVNWPPETR       1346-130       271       SSVNWPPETR       1346-130       272       PPERVGEPWKGYPN       135-137       273       PGEWKGYPNIDET       136-1410       273       PGEWGYNNISLS       1376-137       273       POEWKGYPNIDET       136-1410       273       PFERCEPWKGYPNIDET       136-1410       274       PFERVERGEWKGYPNIDET       136-1410       275       276       NYTREVDHLRDNSGSSSSL       1396-1410       275       276       NYTREVDHLRDNSGSSSSL       136-1410       276       NYT	54	GTTWGAYGSNYSGDK	266-280				233	SQLQRLLAQQQRAQS	1161-1175		
63       QVNTKGGGVWESGA       311-325         64       KGGVWESGANSQS       316-330         65       WESGANSQSTSWGS       321-335         66       GRGANSGGSRGWGT       336-350         67       TSWGSGNGANSGGSR       331-345         68       GNGANSGGSRGWGT       336-350         77       GKTTFNOWKSTEEED       381-395         78       NGWKSTEEEDQGSAT       386-400         87       EKGTGESQSRDRRKI       431-445         88       ESQSRDRRKDUPHTI       436-430         92       NRTDLDPRVISNSGW       456-470         92       NRTDLDRVISNSGW       456-470         93       DPRVISNSGWGDPHT       436-435         94       MOTETSPRGERKTDN       481-495         105       GNDTSSVSGWGDPKP       525-540         106       SVSGWGDPKPALRWG       526-540         118       WGDQKSSQGWSNSA       586-690         133       SKPTPSQUWODQPKS       661-675         134       SQGWGDPKSNQSLG       666-680         135       DPPKSNQSLGWGDS       671-685         136       NQSLGWGDSSKPYSS       676-690         137       WGDSSKPVSSPBYNNK       661-675         138	55	AYGSNYSGDKCSGPN	271-285				234	LLAQQQRAQSQRSVP	1166-1180		
64         KGGGWESGAANSQS         316-330         239         GRPLSVQQQMMQQSR         1191-1205         200           65         WESGAANSQSTSWGS         321-335         200         257         RLEKWTTVDS         276-1290         201           67         GKTFINGWKSTEEED         381-345         261         FRLESPFVPTDFMN         131-1325         201           78         NGWKSTEEED         381-345         264         SPFVPTDFMNSTSP         1316-1330         201           78         NGWKSTEEED         381-430         201         SPNCSSVNWPEFER         1346-1360         201           79         WDTLDPRVLSNSGW         456-470         271         SPNUMPEFER         1346-1360         201           90         WDTLDPRVLSNSGW         456-470         272         PEFRPGEPW KGYPNIDET         136-1370         201           97         WDTETSPRGERKTDN         481-495         201         276         DPYVTPGSVSINKLSI         1376-1390         201           105         GNDSKSVGSQG         526-540         201         276         DPYVTPGSVSINKLSI         136-1450         201         201         21-315         201         201         201         201         201         201         201         201         20	63	QVNTNKGGGVWESGA	311-325				235	QRAQSQRSVPSGNRP	1171-1185		
65       WESGANSQSTSWGS       321-335       256       KEPQSRLRKWTYDS       1276-1290       281-1295         68       GNGANSGGSRRGWGT       336-350       263       FRLESPFVPJDFMN       131-1325       263       FRLESPFVPJDFMN       131-1325       263       FRLESPFVPJDFMN       131-1325       263       FRLESPFVPJDFMN       131-1325       263       FRLESPFVPJDFMNSTSP       136-1300       264       SPFVPYDDFMNSTSP       136-1300       264       SPFVPYDFMNSTSP       136-1300       263       FRLESPFVPGEFW       135-1370       270       SPGEPWKGPNNDSPFT       136-1370       272       PEFRPGEFW       135-1370       270       SPGEPWGGVPNDSPFT       136-1370       273       PGEPWGGVPNDSPG       270       NTREVDHLRDRNSG       1376-1390       270       SPGEPWGGVPNDSPG       270       NTREVDHLRDRNSG       376-1390       270       NTREVDHLRDRNSG       376-1390       270       NTREVDHLRDRNSGSSSSL       136-1410       280       280       VDHLRDRNSGSSSSSL       136-1410       280       281       281       281       281       281       281       281       281 <td< td=""><td>64</td><td>KGGGVWESGAANSQS</td><td>316-330</td><td></td><td></td><td></td><td>239</td><td>GRPLSVQQQMMQQSR</td><td>1191-1205</td><td></td><td></td></td<>	64	KGGGVWESGAANSQS	316-330				239	GRPLSVQQQMMQQSR	1191-1205		
67       TSWGSUNGANSGOSR       331-345       257       RLRKWTVDSISVNT       1281-1295       263         68       GNGANSGOSROWGT       336-350       263       FRLESPFVPJOFMN       1311-1325       263         77       GKTFTNGWKSTEEED       381-395       264       SPVVPJOFMNSTSP       1316-1330       264         78       NGWKSTEEEDQGSAT       386-400       270       SPNGSSSVNWPPEFR       1346-1360       271         92       NRTDLDPPVLSNSGW       456-470       271       SSVNWPPEFR       1361-1375       272         93       DPRVLSNSGWGQTPI       461-475       273       PGEPWGCYPNUDPT       1361-1375       276         97       WDTETSPRGERKTDN       481-495       276       DPVVTPGSVINNLSI       1376-1390       276         106       SVSGWODPKPALRWG       526-540       278       PGEWGCYPNUDPT       1361-1375       278         118       WGDQQKSSQG 681-505       288       AQSTSARNSDSKTIW       1431-1445       292       297       NTSLAHELWKVPLP       1436-1470       292       292       293       AHELWKVPLPKNIT       1461-1475       293       294       KVPLPKNITN       1461-1475       293       294       KVPLPKNITN       1466-1480       297       297       <	65	WESGAANSQSTSWGS	321-335				256	KEPQSRLRKWTTVDS	1276-1290	11200000000	
68       GNGANSGGSRGWGT       336-350       263       FRLESPFVPYDFMN       1311-1325         78       NGWKSTEEEDQGSAT       386-400       264       SPFVPYDFMNSSTSP       136-1330         87       EKGTGESQSRDRRKI       431-445       264       SPFVPYDFMNSSTSP       1316-1330         88       ESQSKDRKKIDQHTL       436-450       270       SPNGSSSVNWPPEFR       1346-1360       271         92       NRTDLDPRVLSNSGW       456-470       272       PPEFRRGEPW       1351-1355       272         93       DPRVLSNSGWOGTPH       461-475       276       DPYVTROSVINLSI       1376-1390       276         96       SVSGWGDPKPALRWG       521-535       280       VDHLRDRNSGSSSL       1396-1410       280       280       VDHLRDRNSG       1391-1405       280       287       PLSSTAQSTARNSDSKLTW       1431-1445       286       287       PLSTAQSTARNSDSKLTW       1436-1440       287       280       VDHLRDRNSGSSSSL       1396-1410       292       284       AQSTSARNSDSKLTW       1436-1440       293       AHELWKVPLPKNT       1461-1475       293       AHELWKVPLPKNT       1461-1475       293       AHELWKVPLPKNT       1461-1475       293       294       KVPLPFKNTTPGSSWGC       296       QKVPLPSKNT       1461-1475	67	TSWGSGNGANSGGSR	331-345				257	RLRKWTTVDSISVNT	1281-1295		
77       GKTFTNGWKSTEEED       381-395       264       SPFVPYDFMNSSTSP       1316-1330       28         87       EKGTGESQSRDRRKI       431-445       270       SPNGSSSVNWPPER       1346-1360       270         92       NRTDLDPRVLSNSGW       456-470       271       SSVNWPPERPGEPWK0YPN       1351-1365       28         93       DRVLSNSGWGQTPI       461-475       273       PGEPWK0YPNIDPET       1361-1375       29         97       WDTETSPRGERKTDN       481-495       279       NTVREVDHLRDRNSG       1391-1405       270       NTVREVDHLRDRNSG       1391-1405       270       SVNWPPERNQE       271       SVNWPERNQE       280       VDHLRDRNSG       1391-1405       270       270       NTVREVDHLRDRNSG       1391-1405       270       NTVREVDHLRDRNSG       1391-1405       270       270       NTVREVDHLRDRNSG       <	68	GNGANSGGSRRGWGT	336-350				263	FRLEESPFVPYDFMN	1311-1325		
78       NGWKSTEEEDQGSAT       386-400       270       SPNGSSSVNWPPEFR       1346-1360       286         87       EKGTGESQSRDRRKI       431-445       271       SSVNWPPEFRGEPW       1351-1365       272         92       NRTDLDPRVLSNSGW       456-470       273       PGEPWKGYPN       1356-1370       273         93       DPRVLSNSQWGOTPI       461-475       276       DPYTFROSVINLIS       1376-1390       279         97       WDTETSPRGERKTDN       481-495       270       NTVREVDHLRDRNSG       1391-1405       279       NTVREVDHLRDRNSG       1391-1405       279       NTVREVDHLRDRNSG       1391-1405       279       NTVREVDHLRDRNSG       1391-1405       279       136       279       NTVREVDHLRDRNSG       1391-1405       270       288       AQSTSARNSDRLTW       1436-1450       287       1431-1445       287       288       AQSTSARNSDRLTW       1436-1450       292       1715LAHELWKVPLPKNIT       1461-1475       293       294       KVPLPFNNTAPSRP       1466-1480       297       293       294       KVPLPFNNTAPSRP       1466-1480       297       298       GQKPLSTWDNSPLR       1486-1500       300       301       GGGWGNSDARYTPG       1510-153       304       303       RYTPGSWGGSSGR       1511-1525       304 <td>77</td> <td>GKTFTNGWKSTEEED</td> <td>381-395</td> <td></td> <td></td> <td></td> <td>264</td> <td>SPFVPYDFMNSSTSP</td> <td>1316-1330</td> <td> </td> <td></td>	77	GKTFTNGWKSTEEED	381-395				264	SPFVPYDFMNSSTSP	1316-1330	 	
87       EKGTGESQSRDRRKI       431-445       271       SSVNWPPERPOEPW       1351-1365         88       ESQSRDRRKIDQHTL       436-470       272       PPERPGEPWKGYPN       1356-1370         93       DPRVLSNSGW       456-470       272       PPERPGEPWKGYPN       1351-1365         97       WDTETSPRGERKTDN       481-495       276       DPYVTPGSVINNLSI       1376-1390       279         105       GNDTSSVSGWGDPKP       521-535       276       DPYVTPGSVINNLSI       1376-1390       279         106       SVSGWGDPKPALRWG       526-540       278       NTVREVDHLRDRNSG SSSL       1396-1410       280         117       KNKQGWGDQGKSSQG 581-555       286       405       287       PLSSTAQSTSARNSD       1431-1445       292         118       WGDQGKSSQGWSVSA       586-600       292       TNTSLAHELWKVPLP       1436-1470       293       AHELWKVPLPNNIT       1461-1475       293       AHELWKVPLPNNIT       1461-1475       293       294       KVPLPPNNIT       1461-1475       294       297       PPGLTGQKPLSTWD       1481-1495       297       297       PPGLTGQKPLSTWD       1481-1495       297       297       PPGLTGQKPLSTWD       1481-1495       298       GQKPPLSTWDNSLR       1460-1475       298       <	78	NGWKSTEEEDQGSAT	386-400				270	SPNGSSSVNWPPEFR	1346-1360		
88         ESQSRDRRKIDQHTL         436-450         272         PPETRPGEPWKGYPN         1356-1370           92         NRTDLDPRVLSNSGW         456-470         273         PGEPWKGYPNIDPET         136-1375           93         DPRVLSNSGWOQTPI         461-475         276         DPYVTGSVINLSI         1376-1390           97         WDTETSPRGERKTDN         481-495         279         NTVREVDHLRDRNSG         1391-1405           106         SVSGWGDPKPALRWG         526-540         280         VDHLRDRNSGSSSL         1396-1410           117         KNKQGWGDQKSSQG         581-595         288         AQSTSARNSD         1431-1445           118         WGDGQKSSQGWSYSA         586-600         292         TNTSLAHELWKVPLP         1436-1470           133         SKPTPSQGWGDPKS         661-675         293         AHELWKYPLPNIT         1461-1475           134         SQGWGDPKSNQSLG         661-685         294         KVPLPPKNIT APSRP         1466-1480           135         DPPKSNQSLGWGDSS         676-690         298         GQKPPLSTWDNPLR         1486-1500           136         MQSLGWGDQALSKSG         681-695         301         IGGGWGNSDAR 1496-1510         302           137         WGDSKPVSSRWIKEDD         736-750	87	EKGTGESQSRDRRKI	431-445				271	SSVNWPPEFRPGEPW	1351-1365		
92         NRTDLDPRVLSNSGW         456-470         273         PGEPWKGYPNIDET         1361-1375         288           97         WDTETSPRGERKTDN         481-495         276         DPYVTPOSVINLSI         1376-1390         276           105         GNDTSSVSGWGDPKP         521-535         276         DPYVTPOSVINLSI         1376-1390         276           106         SVSGWGDPKP         521-535         288         AQSTSARNSDSKL         1390-1405         280           118         WGDGQKSSQGWSNSA         586-600         287         PLSSTAQSTSARNSD         1431-1445         288           133         SKPTPSQGWGDPPKSN         661-675         292         TNTSLAHELWKVPLP         1456-1470         292           133         SKPTPSQGWGDPSKS         661-675         293         AHELWKVPLPKNIT         1461-1475         294           134         SQGWGDPPKSNQSLG         666-680         294         KVPLPFKNITAPSRP         1466-1480         297         PCLTGQKPPLSTWDNSPLR         1486-1500         300         187         301         IGGGWGNSDAR 1496-1510         301         303         171         304         SSWGESSGRITSW         150-1515         303         303         1511-1525         303         313         LPHGNALVRYSKEE         561-	88	ESQSRDRRKIDQHTL	436-450				272	PPEFRPGEPWKGYPN	1356-1370	 	
93       DPRVLSNSGWGQPIPI       461-475       276       DPYVTPGSVINNLSI       1376-1390       279         97       WDTETSPRGERKTDN       481-495       279       NTVREVDHLRDRNSG       1391-1405       279         106       SVSGWGDPKPL       521-535       280       VDHLRDRNSG       1391-1405       281         106       SVSGWGDPKPALRWG       526-540       287       PLSSTAQSTSARNSD       1431-1445       282         117       KNKQGWGDQKSSQG       581-595       288       AQSTSARNSDSKLTW       1436-1470       283         133       SKPTPSQGWGDPKS       661-675       293       AHELWKVPLPKNIT       1461-1475       293         135       DPPKSNQSLG       666-680       294       KVPLPFKNITAPSRP       1466-1480       294         136       NQSLGWGDSKPVSS       676-690       293       AHELWKVPLPKNIT       1481-1495       294         137       WGDSSKPVSSPDWNK       681-695       300       NSPLRIGGGWGNSDA       1496-1510       301         138       KPVSSPDWNKQODIV       691-705       302       GNSDARYTPG 1510-1515       304       304       SWGESSSGR       1511-1525       304       304       SWGESSSGR       1511-1525       304       304       SWGESSSGR	92	NRTDLDPRVLSNSGW	456-470				273	PGEPWKGYPNIDPET	1361-1375		
97       WDTETSPRGERKTDN       481-495         105       GNDTSSVSGWGDPKP       521-535         106       SVSGWGDPKPALRWG       526-540         117       KNKQGWGDQKSSQG       581-595         118       WGDGQKSSQGWSVSA       586-600         113       SKPTPSQWGDPKS       661-675         134       SQCWGDPKSNQSLG       666-680         135       DPPKSNQSLG       666-690         136       NQSLGWGDSSK PVSS       676-690         137       WGDSSKPVSSPDWNK       681-695         138       KPVSSPDWNKQQDIV       686-700         139       PDWNKQQDIVCSWGI       691-705         144       EPSPESIRRKMEIDD       736-750         150       MEIDDGTSAWGDSKS       746-760         154       VNMWNKNVPNGNSRS       766-780         177       PGNRPTGWEEEDVE       881-895         189       NKQEEAWINPFVKQF       931-945         189       NKQEEAWINPFVKQF       941-955         190       AWINPFVKQFSNISF       946-960	93	DPRVLSNSGWGQTPI	461-475				276	DPYVTPGSVINNLSI	1376-1390		
105       GNDTSSVSGWGDPKP       521-535       280       VDHLRDRNSGSSSL       1396-1410         106       SVSGWGDPKPALRWG       526-540       287       PLSSTAQSTSARNSD       1431-1445         117       KNKQGWGDPKS       661-675       288       AQSTSARNSDSKLTW       1436-1450       292         118       WGDGQKSSQG       581-595       288       AQSTSARNSDSKLTW       1436-1470       293         133       SKPTPSQGWGDPPKS       661-675       293       AHELWKVPLPPKNIT       1461-1475       294         134       SQGWGDPKSNQSLG       676-690       294       KVPLPPKNITAPSRP       1466-1480       297         137       WGDSSKPVSSPDWNK       681-695       298       GQKPPLSTWDNSPLR       1481-1495       298         138       KPVSSPDWNKQQDIV       686-700       301       IGGGWGNSDARYTPG       1501-1515       302         148       EPSPESIRRKMEIDD       736-750       303       RYTPGSSWGE       1506-1520       303       303       RYTPGSSWGE       150-1530       304       SSWGSSQRIGKSS       313       LPHGNALVRYSSKEE       151-1525       304       304       SSWGESSGRITMUL       1516-1530       313       313       LPHGNALVRYSSKEE       314       ALVRYSSKEEVVKAQ       1566-1580 </td <td>97</td> <td>WDTETSPRGERKTDN</td> <td>481-495</td> <td></td> <td></td> <td></td> <td>279</td> <td>NTVREVDHLRDRNSG</td> <td>1391-1405</td> <td></td> <td></td>	97	WDTETSPRGERKTDN	481-495				279	NTVREVDHLRDRNSG	1391-1405		
106       SVSGWGDPKPALRWG       526-540       287       PLSSTAQSTSARNSD       1431-1445         117       KNKQGWGDQKSSQG       581-595       288       AQSTSARNSDSKLTW       1436-1450         133       SKPTPSQGWGDPPKS       661-675       292       TNTSLAHELWKVPLP       1456-1470       288         134       SQGWGDPPKSNQSLG       666-680       292       TNTSLAHELWKVPLPRNIT       1461-1475       293         135       DPPKSNQSLGWGDSS       671-685       294       KVPLPKNIT APSRP       1466-1480       294         136       NQSLGWGDSSKPVSS       676-690       298       GQKPPLSTWDNSPLR       1486-1500       298       GQKPPLSTWDNSPLR       1486-1500       298       GQKPPLSTWDNSPLR       1486-1510       300       NSPLRIGGGWGNSDA       1496-1510       301       10GGWGNSDARYTPG       1501-1515       302       303       RYTPGSSWGE       1506-1520       303       304       SSWGESSSGR       1511-1525       304       SSWGESSQRWGE       304       SSWGESQRWGE       304       SSWGESQRWGE       304       334 <td>105</td> <td>GNDTSSVSGWGDPKP</td> <td>521-535</td> <td></td> <td></td> <td></td> <td>280</td> <td>VDHLRDRNSGSSSSL</td> <td>1396-1410</td> <td></td> <td></td>	105	GNDTSSVSGWGDPKP	521-535				280	VDHLRDRNSGSSSSL	1396-1410		
117       KNKQGWGDGQKSSQG       581-595       288       AQSTSARNSDSKLTW       1436-1450       292         118       WGDQKSSQGWSVSA       586-600       292       TNTSLAHELWKVPLP       1456-1470       293         133       SKPTPSQGWGDPKSNQSLG       666-680       293       AHELWKVPLPPKNIT       1461-1475       293         134       SQGWGDPKSNQSLG       666-680       293       AHELWKVPLPPKNIT       1461-1480       293         135       DPPKSNQSLGWGDSS       671-685       293       AHELWKVPLPPKNIT       1481-1480       293         136       NQSLGWGDSSKPVSS       676-690       294       KVPLPPKNITAPSRP       1486-1500       293         137       WGDSSKPVSSPDWNK       681-095       298       GQKPPLSTWDD       1486-1510       293         138       KPVSSPDWNKQQDIV       686-700       300       NSPLRIGGGWGNSDA       1496-1510       204         139       PDWNKQQDIVGSWGI       691-705       302       GNSDARYTPG       1501-1515       302         154       VNMWNKNVPRGNSRS       766-780       304       SSWGESSSGR       1511-1525       304         171       WGSSSVGPQALSKSG       851-865       314       ALVRYSSKEE       1561-1575       314       315 </td <td>106</td> <td>SVSGWGDPKPALRWG</td> <td>526-540</td> <td></td> <td></td> <td></td> <td>287</td> <td>PLSSTAQSTSARNSD</td> <td>1431-1445</td> <td></td> <td></td>	106	SVSGWGDPKPALRWG	526-540				287	PLSSTAQSTSARNSD	1431-1445		
118       WGDQGKSSQGWSVSA       586-600       292       TNTSLAHELWKVPLP       1456-1470       201         133       SKPTPSQGWGDPKS       661-675       203       AHELWKVPLPFKNIT       1461-1475       201         134       SQGWGDPKSNQSLG       666-680       201       203       AHELWKVPLPFKNIT       1461-1475       201         135       DPPKSNQSLGWGDSS       671-685       201       203       AHELWKVPLPFKNIT       1461-1475       201         136       NQSLGWGDSSKPVSS       676-690       201       297       PPGLTGQKPPLSTWD       1486-1500       201         138       KPVSSPDWNKQQDIV       686-700       201       1GGGWGNSDA       1496-1510       203       201       1GGGWGNSDA       1496-1510       203       201       1GGGWGNSDA       1496-1510       201       203       301       IGGGWGNSDA       1496-1510       203       203       RYTPGSSWGE       150-1510       203       203       RYTPGSSWGE       150-1510       204       303       RYTPGSSWGE       150-1520       204       304       SSWGESSGRITINWL       151-1525       204       304       SSWGESSGRITINWL       1516-1530       204       313       LPHGNALVRYSSKEE       1561-1575       204       323       QSLTPSPGWQSLGSS <td>117</td> <td>KNKQGWGDGQKSSQG</td> <td>581-595</td> <td></td> <td></td> <td></td> <td>288</td> <td>AQSTSARNSDSKLTW</td> <td>1436-1450</td> <td></td> <td></td>	117	KNKQGWGDGQKSSQG	581-595				288	AQSTSARNSDSKLTW	1436-1450		
133       SKPTPSQGWGDPPKS       661-675         134       SQGWGDPPKSNQSLG       666-680         135       DPPKSNQSLGWGDSS       671-685         136       NQSLGWGDSSKPVSS       676-690         137       WGDSSKPVSSPDWNK       681-695         138       KPVSSPDWNKQQDIV       686-700         139       PDWNKQQDIVGSWGI       691-705         148       EPSPESIRRKMEIDD       736-750         150       MEIDDGTSAWGDPSK       746-760         154       VNMWNKNVPNGNSRS       766-780         177       PGNRPTGWEEEEDVE       881-895         187       RRERGMMKGGNKQEE       921-935         189       NKQEEAWINFYKQF       941-955         190       AWINPFVKQFSNISF       946-960         190       AWINPFVKQFSNISF       946-960	118	WGDGQKSSQGWSVSA	586-600				292	TNTSLAHELWKVPLP	1456-1470		
134       SQGWGDPPKSNQSLG       666-680       294       KVPLPPKNITAPSRP       1466-1480       297         135       DPPKSNQSLGWGDSS       671-685       298       GQKPPLSTWD       1481-1495       298         136       NQSLGWGDSSKPVSS       676-690       298       GQKPPLSTWDNSPLR       1486-1500       298       GQKPPLSTWDNSPLR       1486-1510       298       GQKPPLSTWDNSPLR       1486-1510       298       GQKPPLSTWDNSPLR       1486-1510       298       GQKPLSTWDNSPLR       1486-1510       298       301       IGGGWGNSDAR       179       174       746-760       301       IGGGWGNSDARVTPG       1516-1520       304       SSWGESSSGR       1511-1525       304       SSWGESSSGR       313       LPHGNALVRYSSKEE       1561-1575       314       ALVRYSSKEE       1561-1575       314       314       ALVRYSSKEEVVKAQ       1566-	133	SKPTPSQGWGDPPKS	661-675				293	AHELWKVPLPPKNIT	1461-1475		
135       DPPKSNQSLGWGDSS       671-685       297       PPGLTGQKPPLSTWD       1481-1495       298         136       NQSLGWGDSSKPVSS       676-690       298       GQKPPLSTWDNSPLR       1486-1500       298         137       WGDSSKPVSSPDWNK       681-695       200       NSPLRIGGGWGNSDA       1496-1510       201         138       KPVSSPDWNKQQDIV       686-700       201       IGGGWGNSDARYTPG       1501-1515       201         148       EPSPESIRRKMEIDD       736-750       203       RYTPGSSWGESSSGR       1511-1525       203         150       MEIDDGTSAWGDPSK       746-760       201       304       SSWGESSSGR       1511-1525       201         171       WGSSSVGPQALSKSG       851-865       201       314       ALVRYSSKEE       1561-1575       201         177       PGNRPTGWEEEEDVE       881-895       213       LPHGNALVRYSSKEE       1511-1625       223       224       SPGWQSLGSS       1611-1625       224       236       335       YSSLWGPSSDPR       236       233       337       SSDPRGISSPP INAFLSVD       1686-1700       233       337       SSDPRGISSPSPINA       1681-1695       233       338       GISSPSPINAFLSVDHLGGGG       1691-1705       336       336       337	134	SQGWGDPPKSNQSLG	666-680				294	KVPLPPKNITAPSRP	1466-1480		
136       NQSLGWGDSSKPVSS       676-690       298       GQKPPLSTWDNSPLR       1486-1500       1486-1500         137       WGDSSKPVSSPDWNK       681-695       300       NSPLRIGGGWGNSDA       1496-1510       1496-1510         138       KPVSSPDWNKQQDIVGSWGI       691-705       301       IGGGWGNSDA       1496-1510       301       302       GNSDARYTPGSWGE       1501-1515       1496-1510       1496-1510       303       RYTPGSSWGE       1501-1515       303       303       RYTPGSSWGE       1501-15130       140       1511-1525       304       SSWGESSGRITINWL       1516-1530       151       151       151       151       151       151       151       151       151       151       151       151       151       151       151       151       151       151       151       151       151       151       151       151       151       151       151       151       151       151       151       151       151       151       151       151       151       151       151       151       151       151       151       151       151       151       151       151       151       151       151       151       151       151       151       151       <	135	DPPKSNQSLGWGDSS	671-685				297	PPGLTGQKPPLSTWD	1481-1495		
137       WGDSSKPVSSPDWNK       681-695         138       KPVSSPDWNKQQDIV       686-700         139       PDWNKQQDIVGSWGI       691-705         148       EPSPESIRRKMEIDD       736-750         150       MEIDDGTSAWGDPSK       746-760         154       VNMWNKNVPNGNSRS       766-780         171       WGSSSVCPQALSKSG       851-865         177       PGNRPTGWEEEEDVE       881-895         187       RRERGMMKGGNKQEE       921-935         189       NKQEEAWINFVKQF       941-955         190       AWINPFVKQFSNISF       946-960         333       GISSPSPINAFLSVDH       1681-1695         334       GISSPSPINAFLSVDHLGGGG       168-1700         335       SISDRGSGSS       1691-1705         340       AFLSVDHLGGGGESM       1691-1705	136	NQSLGWGDSSKPVSS	676-690				298	GQKPPLSTWDNSPLR	1486-1500		
138       KPVSSPDWNKQQDIV       686-700       301       IGGGWGNSDARYTPG       1501-1515         139       PDWNKQQDIVGSWGI       691-705       302       GNSDARYTPGSSWGE       1506-1520         148       EPSPESIRRKMEIDD       736-750       303       RYTPGSSWGE       1506-1520       303         150       MEIDDGTSAWGDPSK       746-760       303       RYTPGSSWGE       1511-1525       304         154       VNMWNKNVPNGNSRS       766-780       313       LPHGNALVRYSSKEE       1561-1575       313         171       WGSSSVGPQALSKSG       851-865       314       ALVRYSSKEEVVKAQ       1566-1580       314         185       SSKGLSGKKRRERG       921-935       323       QSLTPSPGWQSLGSS       1611-1625       324         187       RRERGMMKGGNKQEE       931-945       335       YSTSLWGPPSSDPR       1676-1690       335       336       WGPPSSSDPRGISSP       1676-1690       337       SSDPRGISSPSPINA       1681-1695       338       GISSPSPINAFLSVD       1686-1700       339       SPINAFLSVDHLGGGG       1691-1705       340       AFLSVDHLGGGGGESM       1691-1705       340       340       AFLSVDHLGGGGGESM       1691-1705       340       340       AFLSVDHLGGGGGESM       1691-1705       340       340	137	WGDSSKPVSSPDWNK	681-695				300	NSPLRIGGGWGNSDA	1496-1510		
139       PDWNKQQDIVGSWGI       691-705       302       GNSDARYTPGSSWGE       1506-1520         148       EPSPESIRRKMEIDD       736-750       303       RYTPGSSWGESSGR       1511-1525         150       MEIDDGTSAWGDPSK       746-760       304       SSWGESSSGR       1511-1525         154       VNMWNKNVPNGNSRS       766-780       314       ALVRYSSKEE       1561-1575       314         171       WGSSSVGPQALSKSG       851-865       314       ALVRYSSKEEVVKAQ       1566-1580       314         177       PGNRPTGWEEEDDVE       881-895       314       ALVRYSSKEEVVKAQ       1661-1630       323         185       SSKGLSGKKRRERG       921-935       324       SPGWQSLGSSS RILG       1616-1630       335       YSTSLWGPPSSDPR       1677-1685         189       NKQEEAWINFFVKQF       941-955       336       WGPPSSSDPR       1671-1685       336       337       SSDPRGISSPSPINA       1681-1690       337       SSDPRGISSPSPINA       1681-1695       338       GISSPSPINAFLSVD       1686-1700       339       SPINAFLSVDHLGGGG       1691-1705       340       AFLSVDHLGGGGGESM       1691-1705       340       340       AFLSVDHLGGGGGESM       1691-1705       340       340       AFLSVDHLGGGGGESM       1691-1705       <	138	KPVSSPDWNKQQDIV	686-700				301	IGGGWGNSDARYTPG	1501-1515		
148       EPSPESIRRKMEIDD       736-750         150       MEIDDGTSAWGDPSK       746-760         154       VNMWNKNVPGNSRS       766-780         171       WGSSSVGPQALSKSG       851-865         177       PGNRPTGWEEEEDVE       881-895         185       SSKGLSGKKRRRERG       921-935         187       RRERGMMKGGNKQEE       931-945         190       AWINPFVKQFSNISF       946-960         303       RYPPGSSWGESSSGR       1511-1525         304       SSWGESSSGRITNWL       1516-1530         313       LPHGNALVRYSSKEE       1561-1575         323       QSLTPSPGWQSLGSSS       1611-1625         324       SPGWQSLGSSQSRLG       1616-1630         335       YSTSLWGPPSSSDPR       1671-1685         336       WGPPSSSDPRGISSP       1676-1690         337       SSDPRGISSPSPINA       168-1700         338       GISSPSPINAFLSVD       1686-1700         339       SPINAFLSVDHLGGGG ESM       1691-1705         340       AFLSVDHLGGGGESM       1691-170	139	PDWNKQQDIVGSWGI	691-705				302	GNSDARYTPGSSWGE	1506-1520		
150         MEIDDGTSAWGDPSK         746-760         304         SSWGESSSGRITNWL         1516-1530         308           154         VNMWNKNVPNGNSRS         766-780         313         LPHGNALVRYSSKEE         1561-1575         313         124           171         WGSSSVGPALSKSG         881-895         314         ALVRYSSKEEVVKAQ         1566-1580         314         323         QSLTPSPGWQSLGSS         1611-1625         323         224         SPGWQSLGSSQSRLG         1616-1630         335         324         SPGWQSLGSSQSRLG         1616-1630         336         336         WGPPSSSDPR         1671-1685         336         337         SSDPRGISSP         1671-1690         338         GISSPSPINAFLSVD         1686-1700         339         SPINAFLSVDHLGGGG         1691-1705         340         AFLSVDHLGGGGESM         1691-1705         340         AFLSVDHLGGGGESM         1691-1705         340         340         AFLSVDHLGGGGESM         1691-1705         340         340         AFLSVDHLGGGGESM         1691-1705         340         340         340         1651-570         340         340         1651-1700         340         340         340         1641-1645         340         340         340         1641-1645         340         340         340         1641-1705 <td>148</td> <td>EPSPESIRRKMEIDD</td> <td>736-750</td> <td></td> <td></td> <td></td> <td>303</td> <td>RYTPGSSWGESSSGR</td> <td>1511-1525</td> <td></td> <td></td>	148	EPSPESIRRKMEIDD	736-750				303	RYTPGSSWGESSSGR	1511-1525		
154       VNMWNKNVPGNSRS       766-780       313       LPHGNALVRYSSKEE       1561-1575         171       WGSSSVGPQALSKSG       851-865       314       ALVRYSSKEEVVKAQ       1566-1580         177       PGNRPTGWEEEEDVE       881-895       323       QSLTPSPGWQSLGSS       1611-1625         185       SSKGLSGKKRRERG       921-935       324       SPGWQSLGSSQSRLG       1616-1630         187       RRERGMMKGGNKQEE       931-945       335       YSTSLWGPPSSDPR       1671-1685         189       NKQEEAWINPFVKQF       941-955       336       WGPPSSSDPRGISSP       1676-1690         337       SSDPRGISSPSPINA       1681-1695       338       GISSPSPINAFLSVD       1686-1700         339       SPINAFLSVDHLGGG       1691-1705       340       AFLSVDHLGGGGESSM       1696-1710	150	MEIDDGTSAWGDPSK	746-760				304	SSWGESSSGRITNWL	1516-1530		
171       WGSSSVGPQALSKSG       851-865       314       ALVRYSSKEEVVKAQ       1566-1580         177       PGNRPTGWEEEDDVE       881-895       323       QSLTPSPGWQSLGSS       1611-1625         185       SSKGLSGKKRRERG       921-935       324       SPGWQSLGSSQSRLG       1616-1630         187       RERGMMKGGNKQEE       931-945       335       YSTSLWGPPSSDPR       1671-1685       336         190       AWINPFVKQFSNISF       946-960       337       SSDPRGISSP       1668-1700       338       GISSPSPINAFLSVD       1686-1700         330       SPINAFLSVDHLGGGG       1691-1705       340       AFLSVDHLGGGGGESM       1691-1705       340	154	VNMWNKNVPNGNSRS	766-780				313	LPHGNALVRYSSKEE	1561-1575		
177       PGNRPTGWEEEDVE       881-895       323       QSLTPSPGWQSLGSS       1611-1625         185       SSKGLSGKKRRERG       921-935       324       SPGWQSLGSSQSRLG       1616-1630         187       RERGMMKGONKQEE       931-945       335       YSTSLWGPPSSSDPR       1671-1685         189       NKQEEAWINPFVKQF       941-955       336       WGPPSSSDPRGISSP       1676-1690         190       AWINPFVKQFSNISF       946-960       337       SSDPRGISSPSPINA       1681-1695         338       GISSPSPINAFLSVD       1686-1700       339       SPINAFLSVDHLGGGG       1691-1705         340       AFLSVDHLGGGGESM       1696-1710       1691-1705       1690	171	WGSSSVGPQALSKSG	851-865				314	ALVRYSSKEEVVKAQ	1566-1580		
185       SSKGLSGKKRRERG       921-935       324       SPGWQSLGSSQSRLG       1616-1630         187       RERGMMKGGNKQEE       931-945       335       YSTSLWGPPSSDPR       1671-1685         189       NKQEEAWINPFVKQF       941-955       336       WGPPSSSDPRGISSP       1676-1690         190       AWINPFVKQFSNISF       946-960       337       SSDPRGISSPSPINA       1681-1695         338       GISSPSPINAFLSVD       1686-1700       339       SPINAFLSVDHLGGGGESM       1691-1705         340       AFLSVDHLGGGGESM       1696-1710       1696-1710       1696-1710	177	PGNRPTGWEEEEDVE	881-895				323	QSLTPSPGWQSLGSS	1611-1625		
187         RERGMMKGGNKQEE         931-945         335         YSTSLWGPPSSSDPR         1671-1685         1671-1685           189         NKQEEAWINPFVKQF         941-955         336         WGPPSSSDPRGISSP         1676-1690         336           190         AWINPFVKQFSNISF         946-960         337         SSDPRGISSPSPINA         1681-1695         338         GISSPSPINAFUND         1686-1700         339         SPINAFLSVDHLGGG         1691-1705         340         AFLSVDHLGGGGESM         1696-1710         340         AFLSVDHLGGGGESM         1696-1710         340         340         340         340         340         340         340         340         340         340         340         340         340         340         340         340         340         340         340         340         340         340         340         340         340         340         340         340         340         340         340         340         340         340         340         340         340         340         340         340         340         340         340         340         340         340         340         340         340         340         340         340         340         340         3	185	SSKGLSGKKRRRERG	921-935				324	SPGWQSLGSSQSRLG	1616-1630		
189         NKQEEAWINPFVKQF         941-955         336         WGPPSSSDPRGISSP         1676-1690           190         AWINPFVKQFSNISF         946-960         337         SSDPRGISSPSPINA         1681-1695           338         GISSPSPINAFLSVD         1686-1700         339         SPINAFLSVDHLGGG         1691-1705           340         AFLSVDHLGGGGESM         1696-1710         1696-1710         1696-1710	187	RRERGMMKGGNKQEE	931-945				335	YSTSLWGPPSSSDPR	1671-1685		
190         AWINPFVKQFSNISF         946-960         337         SSDPRGISSPSPINA         1681-1695           338         GISSPSPINAFLSVD         1686-1700         338           339         SPINAFLSVDHLGGG         1691-1705         340           340         AFLSVDHLGGGGESM         1696-1710         1681-1695	189	NKQEEAWINPFVKQF	941-955				336	WGPPSSSDPRGISSP	1676-1690		
338         GISSPSPINAFLSVD         1686-1700           339         SPINAFLSVDHLGGG         1691-1705           340         AFLSVDHLGGGGESM         1696-1710	190	AWINPFVKQFSNISF	946-960				337	SSDPRGISSPSPINA	1681-1695		
339         SPINAFLSVDHLGGG         1691-1705           340         AFLSVDHLGGGGESM         1696-1710							338	GISSPSPINAFLSVD	1686-1700		
340 AFLSVDHLGGGGESM 1696-1710							339	SPINAFLSVDHLGGG	1691-1705		
							340	AFLSVDHLGGGGESM	1696-1710		

Fig. 4 Amino acid sequence and position of the GW182 protein synthetic peptides and their reactivity with three patient sera with anti-GWB antibodies. *Gradient of white to black* Increasing intensity of reaction of antibodies with peptide

mRNA and the human protein staufen have been observed in neurons [27, 28]. Staufen binds double-stranded RNA and was visualized in RNA containing particles in rat hippocampal neurons after transient transfection experiments [28, 29]. It may be relevant that the GW182 autoantigen was also shown to bind mRNA through its RNA binding motif [11]. It is interesting to speculate that the storage of mRNA by GWBs may be an important process in maintenance of neurons and neurotransmission and that disruption of GW182 function by autoantibodies may affect neural integrity and subsequent motor/sensory neurological disease. This view is supported by preliminary data suggesting that GW182 is highly expressed in neural tissues (unpublished observations). Recent evidence suggests that GW182 and GW bodies are involved in mRNA decapping and subsequent mRNA degradation [14]. It is interesting to speculate that disruption of the GW182 protein and/or GW bodies by the presence of autoantibodies affect one aspect of the mRNA degradation pathway vital in the overall maintenance and function of the cell. Although we have not determined whether mRNA degradation in the GWBs is directly related GW182 function in nonstop [30] or nonsense-mediated mRNA decay [31, 32], the failure to degrade problematic mRNAs with no stop codons or premature termination codons may have pathological consequences on the function of the cell and subsequently be manifest as a disease state.

Our study shows that multiple epitopes of the GW182 protein are recognized by the human antibodies. The SPOT method of epitope mapping has been validated, and the majority of studies has shown that each patient displays an individual epitope pattern [6]. The diverse and heterogenic epitope recognition pattern among the patients observed in this study is not unlikely since the fine specificity of B-cell immune processes strongly depends on the MHC system. Epitope mapping followed by BLAST analysis confirmed that the autoantibody targets are unique to the GW182 protein because sequence similarity to other known eukaryotic or prokaryotic **Table 2** Ribonucleoprotein (*RNP*) autoantigens (*MCTD*) mixed connective tissue disease, *PM* polymyositis, *RA* rheumatoid arthritis, *RNP* ribonucleoprotein, *SjS* Sjögren's syndrome, *SLE* systemic lupus erythematosus, *SSc* systemic sclerosis)

RNP antigen	RNA: protein antigens	Associated disease
Sm UL DND	U2-U6 snRNA: SmD	SLE
Ra33	hnRNA: A2 core protein	RA
SS-A/Ro	hY RNAs: 60 and 52-kDa proteins	SLE/SjS
Ribosomal P proteins	rRNA: P0/P1/P2 proteins rRNA: L12/S10/15 proteins	SJS SLE
Ribosome		SLE
Fibrillarin Hu GWB	U3-RNA: 35 kDa fibrillarin mRNA: 37–45 kDa mRNA: 182 kDa GW182	SSC Paraneoplastic neurological syndromes SL E/SiS/neuropathy

proteins or expressed sequence tags was not observed. This suggests that the GW182 protein drives the autoimmune response and reactivity to endogenous or exogenous proteins with similar sequence motifs and molecular mimicry is less likely. This also raises the possibility that, as with many other autoantibody systems, autoreactivity to GW182 demonstrates intramolecular epitope spreading [33, 34]. A study using more sera and different methods in an extended epitope mapping study should shed more light on the epitope distribution on GW182.

The association of anti-GWB antibodies with antibodies to the 52 kDa SS-A/Ro antigen, particularly in the patients with no evidence of SjS and SLE was an unexpected finding. Although the 52-kDa SS-A/Ro antigen has been localized to both the nucleus and cytoplasm, antibodies from a variety of sources directed to the 52-kDa SS-A/Ro autoantigen do not produce a GWB staining pattern [35, 36]. The function of the 52 kDa SS-A/Ro antigen is not clear [37], and the observation that it is associated with GWB antibodies may help clarify its function.

In summary, GWBs are a novel class of RNP autoantigens that are specifically recognized by human autoantibodies. Over the past three decades several autoantigens that are part of RNP macromolecular complexes have been described, and we propose that autoantibodies to GWBs and GW182 now join this growing list (Table 2). Some of these autoantigens, including Sm, U1-RNP, and Hu, have been shown to have a central role in mRNA splicing, mRNA processing, and mRNA translation [37, 38, 39]. In this study we observe that the diseases associated with autoantibodies to GWBs overlap with those associated with other RNPs but extend to patients who appear to have primary neurological disorders.

Acknowledgements We acknowledge the technical assistance of Joan Miller, Cheryl Hanson, Jill Wenger (University of Calgary) and Dr. Zheng Yang (Scripps Research Institute). This work was supported in part by the Canadian Institutes for Health Research Grant MOP-57674 and the National Institutes of Health Grants AR42455, AI47859 and AI39645. M.J.F holds the Arthritis Society Chair at the University of Calgary.

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