Identification of Cerebral Infarction-Specific Antibody Markers from Autoantibodies Detected in Patients with Systemic Lupus Erythematosus

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Abstract

Background: Systemic lupus erythematosus (SLE) is an autoimmune disease which may be caused by development of the autoantibodies. On the other hand, SLE is a high-risk group of atherosclerosis, so it is possible that some of autoantibodies in SLE are the result of atherosclerosis-related diseases such as cerebral infarction (CI), cardiovascular disease (CVD) and diabetes mellitus (DM).

Methods: The initial screening of autoantibodies was performed using the protein array method. AlphaLISA was used to analyze the serum antibody levels using synthetic polypeptides as antigens.

Results: After the initial screening using protein array, we identified 67 antigens that were recognized by IgG antibodies in sera of patients with SLE. In the second screening, 170 peptides derived from amino acid sequences of 67 antigens were synthesized and used as antigens for analysis of serum antibody levels by AlphaLISA. The antibody levels for ten peptides were significantly higher in the sera of patients with SLE than in those of healthy donors. Further AlphaLISA analysis of sera of patients with CI, CVD or DM revealed that the serum antibody levels for four peptides derived from SOSTDC1, CTNN1, CLDN1 and CCNG2 were elevated in patients as compared to those of healthy donors.

Conclusions: Serum antibody levels against peptide antigens of SOSTDC1, CTNN1, CLDN1 and CCNG2 are useful markers for diagnosis of the progression of CI, CVD and/or DM.

Keywords: Systemic lupus erythematosus; Cerebral infarction; Cardiovascular disease; Diabetes mellitus; Antibody biomarker
Introduction

Systemic lupus erythematosus (SLE) is a chronic inflammatory disorder characterized by damage to multiple organ systems caused by the production of many autoantibodies, generation of immune complexes, and activation of the complement system [1-3]. Dysfunction of T cells and accelerated activation of B cells in SLE patients [4] enables the development of various autoantigens such as the anti-nuclear antibody [5]. SLE-specific autoantibodies thus far reported were the anti-Sm antibody [6], anti-double-stranded DNA antibody [7], anti-U1RNP antibody [8], anti-SSA/Ro antibody [9,10] and the anti-P ribosomal protein antibody [11], yet the pathogenic role of these antibodies remains to be proven.

Accelerated atherosclerotic diseases have been recognized as major causes of mortality in SLE. In the study of large case series of patients with SLE, 6-20% and 4-15% of deaths were due to cardiovascular disease (CVD) and cerebrovascular disease, respectively [12-14]. To estimate the onset risk of accelerated atherosclerosis in SLE patients, several markers have been introduced including C-reactive protein [15], lipoprotein (a) [16], homocysteine [17], inflammatory cytokines [18,19], yet the satisfactory results have not been obtained.

On the other hand, recent studies have revealed that specific autoantibodies exist in the sera of patients with atherosclerosis, such as autoantibodies for phospholipid (Antiphospholipid syndrome) [20,21], apolipoprotein A-1 [22] and oxidized low-density lipoprotein [23]. We have also reported that the antibody levels against RPA2 were associated with the onset of ischemic stroke [24]. These antibody markers might be useful for evaluation of the onset of lethal atherosclerotic disease in patients with SLE.

In the present study, we have comprehensively screened autoantigens which were recognized by IgG antibodies in the sera of patients with SLE by the protein array method. We then selected and identified autoantigens specific for cerebral infarction (CI), CVD and/or diabetes mellitus (DM).

Materials and Methods

Patients and healthy donor sera

This study was approved by the Local Ethical Review Board of the Chiba University, Graduate School of Medicine as well as that of the National Hospital Organization, Shimosizhu Hospital and Chiba-East hospitals. Sera were collected from patients after they had given written informed consent. Each serum sample was centrifuged at 3,000 x g for 10 min, and then the supernatant was stored at -80°C until use.

The samples of SLE were obtained from Shimosizhu Hospital, and those of CI and transient ischemic attack (TIA) were obtained from Sawara Hospital, Rosai Hospital, Aoba Hospital and Chiba Medical Center. Samples of CVD and DM were obtained from Chiba University Hospital, and those of healthy donors were from Chiba University, Kashiwado Clinic and Fujikura Kasei Co.

Protein array screening

Initial screening was performed using ProtoArrays™ Human Protein Microarrays v4.0 (Thermo Fisher Scientific, Waltham, MA), which were loaded with 9,480 species of proteins. A total of 11 sera, 6 from patients and 5 from healthy donors, were used to detect antigens recognized specifically by IgG antibodies in the sera of patients.

Peptide synthesis

Three epitope sites in the candidate antigen proteins were predicted using the program ProPred (http://www.imtech.res.in/raghava/propred/). N-terminal biotinylated 15mer peptides without purification were synthesized and used in the second screening. For the third screening, synthetic peptides were purified by HPLC. The purity of each peptide was determined to be higher than 90%.

AlphaLISA (Amplified Luminescence Proximity Homogeneous Assay)

To evaluate the serum antibody levels, AlphaLISA was used. AlphaLISA was performed in 384-well microtiter plates (white opaque OptiPlate™ from Perkin Elmer) containing 2.5 μL of 1/100-diluted serum and 2.5 μL of biotinylated synthetic peptides (400 ng/mL) in AlphaLISA buffer (25 mM HEPES, pH 7.4, 0.1% casein, 0.5% Triton X-100, 1 mg/mL dextran-500, and 0.05% Proclin-300). The reaction mixture was incubated at room temperature for 6-8 h, then anti-human IgG-conjugated acceptor beads (2.5 μL at 40 μg/mL) and streptavidin-conjugated donor beads (2.5 μL at 40 μg/mL) were added and incubated at room temperature in the dark for another 1 - 14 days. The plate was read on an EnSpire Alpha microplate reader (PerkinElmer).

Statistical analyses

Fisher’s exact (two-sided) probability test and the Mann-Whitney U test were used to determine the significance of the differences between the two groups. All statistical analyses were carried out using the GraphPad Prism 5 (GraphPad Software, La Jolla, CA). P values lower than 0.05 were considered statistically significant.

Results

Initial screening of SLE-specific antigens by protein array

By using protein microarrays loading with 9,480 proteins, we examined 6 sera from SLE patients and 5 sera from healthy controls to identify SLE-associated antigens. Sixty-seven proteins such as SOSTDC1, CTNND1 were selected as antigens by reacting with more than 5 sera from SLE patients and not with any of the sera from healthy donors (Table 1). These proteins may include not only antigens specific for SLE but also those specific for the complication such as CI, CVD and DM.

| Name   | Protein   |
|--------|-----------|
| ZIC4   | C3orf52   |
| SDHB   | DKFZp762  |
| MGC17553 | SOSTDC1  |
| RARS2  | RNPC3     |
| IPO11  | CDC45L    |
| SLC25A24 | ZNF649    |
| OTX1   | ABAT      |
| MKRN2  | CLIC5     |
| KCNS3  | H2AFY     |

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Table 1: List of Protein array-selected antigens recognized by serum antibodies of SLE patients.

| No. | Name       | Sequence       | No. | Name       | Sequence       | No. | Name       | Sequence       |
|-----|------------|----------------|-----|------------|----------------|-----|------------|----------------|
| 1   | ZIC4-3     | YKTSLVMRKRL   | 44  | SIAH1-267  | FAENGNLGINVTISM| 87  | MYBBP1A-1134| LLYWQAMKTLGVQRPK|
|     | CTRB1      |               |     | ORC3L      |                |     | ERp27      |                |
|     | MAPK13     |                |     | MAP4K4     |                |     | TUFM       |                |
|     | MIER3      |                |     | GLCE       |                |     | UX51       |                |
|     | C3orf15    |                |     | ENG        | HAPLN1         |     | ETV3       |                |
|     | PRKCH      | C3orf157      |     | NOLA1      | RNF32          |     |            |                |
|     | ANK1       | MIER3         |     | RPS15A     | GLCE           |     |            |                |
|     | C15orf15   | ERp27         |     | C15orf15   | UX51           |     |            |                |
|     | RBMS3      | ETV3          |     | CSNK1A1    |                |     |            |                |
|    | Gene   | Position | Description |
|---|---------|----------|-------------|
| 2 | ZIC4-185 | FKAKYKLVNHVR | VHT | 45 TAS2R13-30 INCIDWVSKREL SSV |
| 3 | ZIC4-269 | RGDCKYTHPS SLRKL | 46 TAS2R13-11 KIAFSSPPAFLY KEL |
| 4 | SDHB-238 | FSLYRCHTINC TRT | 47 TAS2R13-17 VKFTMTMFSLT PFTV |
| 5 | MGC17553-17 | PSKENWFQRL SQAV | 48 TAS2R13-28 GNAKLQAFLL VAAK |
| 6 | MGC23985-18 | LTCYADDKPDK PDDK | 49 FGFR23-6 LRLWVCALCSV CMS |
| 7 | RARS2-2 | ACGRFRAIAQ LSREY | 50 FGFR23-40 IHLYTATARNY HLS |
| 8 | RARS2-179 | GLLGTFQFOLFG YEEQ | 51 FGFR23-85 ITGMVSRRLY MDFR |
| 9 | RARS2-359 | QMLKIMGYDWA EROCF | 52 FGFR23-131 QHYFLVSLGRA KRA |
| 10 | RARS2-402 | LRMLQNMAK ILE | 53 TFAM-5 RSMVGVLSAlg SEFGA |
| 11 | RARS2-500 | QHLLHRDEVLY KSNG | 54 TFAM-38 LPRWFSVLAS CPITK |
| 12 | IPO11-52 | HTLDNVRWHV LYSF | 55 TFAM-231 LRTIRIKKORKG AEE |
| 13 | IPO11-143 | QRHARRALTFFY VHVT | 56 CLDN1-12 ACVLSCIYIMA |
| 14 | IPO11-215 | LKVLRLTVNGF VEP | 57 CLDN1-69 FRYNVTGVLWR KCHG |
| 15 | IPO11-320 | CMNLIKIVMKV NTK | 58 CLDN1-177 HIIAGLCTLSV SCY |
| 16 | IPO11-526 | DQDLVVRGGAT TLK | 59 MAFG-34 VRELNQHRLG SEKE |
| 17 | IPO11-579 | HVHLHNSCIVER YNM | 60 CCNG2-84 LDRFALMKVK MKH |
| 18 | IPO11-708 | KIINGYFILSSTE FL | 61 CCNG2-130 QCCCTASDIK MKH |
| 19 | SLC2A24-113 | OSLQTLTGLTISE VEQ | 62 CCNG2-181 SLDKLEAOQKA CNKR |
| 20 | SLC2A24-248 | RSLWVRGNTN VIKA | 63 CCNG2-231 KHSHKINODETEF YWR |
| 21 | SLC2A24-389 | LQGGALSSTCG QLAS | 64 CCNG2-270 WIVRSRTAQNL HS |
| 22 | SLC2A24-430 | LFRRISKEGIPG FLY | 65 ACTLB8-146 FFLCKTAVTLAF SAG |
| 23 | SLC2A24-444 | YRIGITPNNKVL PSF | 66 ACTLB8-376 KLIASTNIMERK FSP |
| 24 | OTX1-68 | REEVALKINLPE SRV | 67 APEX1-168 VTVAPNAGRG LVR |
| 25 | MKRN2-109 | LDRNLSGMAE RKTO | 68 APEX1-189 DEAFRRKLKGL ASRK |

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The selected useful antigen peptides are shown in bold. Numbers of peptide names represent the first amino acid number of the original proteins.

Table 2: List of amino acid sequences of synthetic peptides used for the second screening. A total of 170 peptides were predicted as epitopes from Autoantibodies Detected in Patients with Systemic Lupus Erythematosus. J Mol Biomark Diagn 6: 219. doi: 10.4172/2155-9929.1000219

| Peptide Name | Sequence |
|--------------|----------|
| SOSTDC1-156  | KITVTVTACKR YLRFY |
| CTNN1D-21    | TSVPELVPKVVA NHT |
| CLDN1-69     | SOSTDC1-156  |
| CCNG2-231    | CTNN1D-21    |
| TFAM-231     | CLDN1-69     |
| TOP3B-628    | CCNG2-231    |
| MYBBP1A-1134 | TOP3B-628    |
| MYBBP1A-1306 | MYBBP1A-1134 |

| Sequence | Length | Positive No. | Total No. | HD | SD | Cut-off value |
|----------|--------|--------------|-----------|----|----|---------------|
| 1,730    | 2,302  | 5            | 3         | 2,613 | 884 | 9,176 |
| 4,518    | 2,053  | 5            | 3         | 1,799 | 1,739 | 2,505 |
| 2,532    | 2,606  | 6            | 6         | 5    | 5   | 3            |
| 3,374    | 433    | 5            | 5         | 1,799 | 2,505 | 676 |
| 1,799    | 433    | 5            | 5         | 1,799 | 2,505 | 676 |
| 2,505    | 2,505  | 5            | 6         | 6    | 5   | 3            |

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### Table 3: Comparison of serum antibody levels between HD and SLE patients examined by AlphaLISA.

|          | SOSTDC1-156 | CTNND1-211 | CLDND1-69 | CCNG2-231 | TFAM-231 | TOP3B-628 | MYBBP1A-1134 | MYBBP1A-1306 |
|----------|--------------|------------|-----------|-----------|-----------|-----------|--------------|--------------|
| HD       | 2,970        | 2,233      | 2,948     | 1,804     | 4,694     | 2,386     | 2,074        | 3,808        |
| SD       | 1,187        | 739        | 1,691     | 442       | 1,392     | 757       | 703          | 1,060        |
| Cut-off value | 5,344        | 3,711      | 6,331     | 2,688     | 7,479     | 3,900     | 3,479        | 5,928        |
| Total No. | 128          | 128        | 127       | 128       | 125       | 128       | 128          | 128          |
| Positive No. | 6           | 6          | 3         | 7         | 5         | 6         | 6            | 7            |
| Positive (%) | 4.70%        | 4.70%      | 2.40%     | 11.90%    | 1.20%     | 13.10%    | 11.90%       | 13.10%       |
| P (vs HD) | 0.000048     | 0.029      | -         | 0.109     | 0.089     | 0.045     | 0.065        | 0.042        |

Table 4: Comparison of serum antibody levels among HD, CI patients and CVD patients examined by AlphaLISA.

### Discussion

There are various types of autoantibodies in the sera of SLE patients due to the dysfunction of T cells and the accelerated activation of B cells. Available data suggest that young women with SLE are at a substantially increased risk of AMI, congestive heart failure, and cerebrovascular accidents [12-14]. If autoantibodies develop during the progress of CI and CVD, they can be amplified in patients with SLE due to their dysregulated immune systems. Thus, we performed the first screening using SLE sera and then the second and third screenings using CI and CVD samples. Through the first screening by protein array method followed by second screening using crude peptide antigens and validation tests using three sets of control HD and patients' sera, we identified SOSTDC1, CTNND1, CLDND1 and CCNG2 as novel useful markers for the diagnosis of atherosclerosis-related diseases such as CI, CVD and DM.
Table 5: Comparison of serum antibody levels among HD, CI patients and DM patients examined by AlphaLISA.

|                | SOSTDC1-156 | CTNND1-211 | CLDND1-69 | CCNG2-231 | TFAM-231 | TOP3B-628 | MYBBP1A-1134 | MYBBP1A-1306 |
|----------------|-------------|------------|-----------|-----------|----------|-----------|-------------|-------------|
| HD             | r value     | P value    | r value   | P value   | r value  | P value   | r value     | P value     |
| Gender         | -0.079      | 0.0408     | 0.019     | 0.6341    | -0.019   | 0.6226    | 0.057       | 0.1448      |
| Age            | 0.182       | <0.0001    | 0.157     | <0.0001   | 0.102    | 0.0089    | 0.057       | 0.1420      |
| Height         | -0.062      | 0.1131     | -0.054    | 0.1639    | -0.009   | 0.8115    | -0.028      | 0.4770      |
| Weight         | -0.008      | 0.8351     | -0.125    | 0.0013    | -0.065   | 0.0932    | -0.044      | 0.2595      |
| Body mass index| 0.039       | 0.3227     | -0.111    | 0.0043    | -0.074   | 0.0586    | -0.027      | 0.4849      |
| Intima media thickness (IMT) | 0.218 | <0.0001 | 0.117 | 0.0127 | 0.040 | 0.3920 | 0.019 | 0.6819 |
| Diabetes       | 0.110       | 0.0045     | 0.013     | 0.7397    | -0.036   | 0.3614    | 0.017       | 0.6708      |
| Hypertension   | 0.160       | <0.0001    | 0.066     | 0.0919    | 0.038    | 0.3346    | 0.035       | 0.3678      |
| Albumin/globulin ratio | 0.011 | 0.7883 | -0.005 | 0.9026 | -0.001 | 0.9827 | 0.066 | 0.0962 |
| Aspartate transaminase | 0.004 | 0.9241 | 0.009 | 0.8197 | 0.016 | 0.6736 | -0.011 | 0.7763 |
| Alanine transaminase | -0.013 | 0.7353 | 0.015 | 0.7042 | -0.051 | 0.1903 | -0.006 | 0.8714 |
| Alkaline phosphatase | 0.046 | 0.2624 | 0.007 | 0.8733 | -0.031 | 0.4473 | -0.042 | 0.2991 |
| Lactate dehydrogenase | -0.016 | 0.6972 | 0.061 | 0.1269 | -0.015 | 0.7089 | 0.025 | 0.5356 |

Table 5: Comparison of serum antibody levels among HD, CI patients and DM patients examined by AlphaLISA.
|                       |     |     |     |     |     |     |     |     |
|-----------------------|-----|-----|-----|-----|-----|-----|-----|-----|
| Total bilirubin       | 0.046 | 0.2502 | -0.017 | 0.6647 | 0.026 | 0.5049 | 0.015 | 0.7068 |
| Choline esterase      | -0.039 | 0.3834 | 0.009 | 0.8342 | 0.018 | 0.6893 | -0.001 | 0.9749 |
| gamma-GTP             | 0.027 | 0.4988 | -0.004 | 0.9311 | -0.019 | 0.6432 | 0.003 | 0.9400 |
| Total protein         | -0.044 | 0.2729 | -0.073 | 0.0656 | -0.011 | 0.7861 | 0.002 | 0.9596 |
| Albumin               | -0.024 | 0.5439 | -0.065 | 0.0994 | -0.013 | 0.7397 | 0.054 | 0.1743 |
| Blood urea nitrogen   | -0.019 | 0.6331 | -0.038 | 0.3306 | 0.000 | 0.9816 | -0.040 | 0.3009 |
| Creatinin             | 0.010 | 0.7904 | -0.021 | 0.5848 | 0.023 | 0.5547 | -0.007 | 0.8603 |
| Estimated glomerular filtration rate | -0.004 | 0.9326 | 0.023 | 0.5866 | -0.010 | 0.8060 | -0.004 | 0.9214 |
| Uric acid             | -0.019 | 0.6690 | 0.030 | 0.5104 | 0.011 | 0.7992 | 0.025 | 0.5729 |
| Amylase               | -0.084 | 0.0875 | -0.015 | 0.7540 | 0.017 | 0.7362 | -0.074 | 0.1322 |
| Total cholesterol     | -0.067 | 0.1131 | 0.033 | 0.4346 | -0.054 | 0.2030 | -0.022 | 0.5983 |
| HDL cholesterol       | -0.002 | 0.9599 | 0.000 | 0.9931 | 0.038 | 0.4340 | 0.087 | 0.0694 |
| Triglyceride          | -0.031 | 0.5086 | 0.013 | 0.7773 | -0.028 | 0.5419 | -0.035 | 0.4594 |
| Na                    | -0.001 | 0.9811 | 0.002 | 0.9500 | 0.003 | 0.9370 | 0.077 | 0.0507 |
| K                     | -0.025 | 0.5245 | 0.043 | 0.2779 | 0.031 | 0.4397 | 0.058 | 0.1393 |
| Cl                    | 0.005 | 0.8985 | 0.061 | 0.1236 | 0.007 | 0.8680 | 0.036 | 0.3583 |
| C-reactive protein    | 0.047 | 0.3018 | -0.046 | 0.3182 | 0.056 | 0.2241 | -0.050 | 0.2732 |
| LDL cholesterol       | -0.119 | 0.0275 | 0.043 | 0.4254 | -0.070 | 0.1940 | -0.091 | 0.0913 |
| White blood cell      | 0.015 | 0.7028 | -0.041 | 0.2992 | 0.030 | 0.4382 | -0.036 | 0.3629 |
| Red blood cell        | -0.005 | 0.9062 | -0.049 | 0.2110 | 0.030 | 0.4471 | -0.009 | 0.8185 |
| Hemoglobin            | 0.013 | 0.7468 | -0.059 | 0.1360 | 0.034 | 0.3896 | 0.007 | 0.8621 |
| Hematocrit            | 0.017 | 0.6567 | -0.047 | 0.2325 | 0.039 | 0.3262 | 0.031 | 0.4225 |
| Mean cell volume      | 0.072 | 0.0647 | 0.026 | 0.5057 | -0.005 | 0.896 | 0.049 | 0.2145 |
| Mean corpuscular hemoglobin | 0.050 | 0.1988 | -0.018 | 0.6498 | 0.009 | 0.6220 | -0.002 | 0.9513 |
| Mean corpuscular hemoglobin concentration | -0.021 | 0.6015 | -0.070 | 0.0737 | 0.019 | 0.6314 | -0.067 | 0.0891 |
| Red cell distribution width | 0.021 | 0.5894 | -0.011 | 0.7837 | -0.002 | 0.9551 | -0.030 | 0.4462 |
| Platelet              | -0.031 | 0.4254 | -0.027 | 0.4944 | 0.027 | 0.4896 | 0.010 | 0.8202 |
| Mean platelet volume  | 0.005 | 0.8969 | 0.025 | 0.5186 | -0.019 | 0.6356 | 0.025 | 0.5217 |
| Procalcitonin         | -0.020 | 0.6023 | -0.016 | 0.6912 | 0.035 | 0.3677 | 0.031 | 0.4303 |
| Platelet distribution width | -0.002 | 0.9667 | -0.002 | 0.9601 | -0.037 | 0.3433 | 0.006 | 0.8785 |
| Blood sugar           | 0.047 | 0.2467 | 0.011 | 0.7832 | -0.069 | 0.0909 | -0.063 | 0.1215 |
| HbA1c                 | 0.016 | 0.7264 | 0.015 | 0.7405 | -0.067 | 0.1310 | -0.042 | 0.3409 |
| Smoking habit         | 0.152 | <0.0001 | -0.058 | 0.1368 | -0.010 | 0.8047 | -0.036 | 0.3532 |
| Alcohol drinking habit | 0.058 | 0.1386 | -0.053 | 0.1762 | 0.029 | 0.4552 | -0.033 | 0.3934 |
| Green tea drinking habit | -0.017 | 0.6664 | 0.018 | 0.6377 | -0.014 | 0.7178 | 0.054 | 0.1690 |
| Coffee drinking habit | -0.064 | 0.1022 | -0.005 | 0.8913 | -0.008 | 0.8346 | 0.021 | 0.5904 |
The following information is known for these selected markers: SOSTDC1/sclerostin domain containing 1 (Accession No.: NM_015464) is a member of bone morphogenetic protein (BMP) of TGF-β superfamily [25,26]. It works as a BMP antagonist and suppresses cell proliferation, differentiation or cell death induced by BMP. BMPs also play important parts in the development of atherosclerosis [27]. CTNND1/catenin (cadherin-associated protein), delta 1 (Accession No.: NM_001085458) is a member of the Armadillo protein family and mediates the signaling from the cell-adhesion molecule cadherin onto cells [28]. CLDND1/claudin domain containing 1 (Accession No.: NM_001040181) contains the domain of claudin which is involved in tight junction, but its function is not known [29]. CCNG2/cyclin G2 (Accession No.: NM_004354): It is a member of the cyclin family and induced by DNA damaging agents [30].

**Table 6**: Correlation analysis between antibody marker levels and the subject’s information. Shown are correlation coefficients (r) and P values calculated by Spearman’s analysis. Significant correlations are marked in bold.

| Habit                        | SOSTDC1-156 | CTNND1-211 | CLDND1-69 | CCNG2-231 |
|------------------------------|-------------|------------|-----------|-----------|
| Chinese tea drinking habit   | -0.083      | 0.0323     | -0.025    | 0.5245    |
| Working habit                | -0.137      | 0.0005     | -0.073    | 0.0659    |
| Exercise habit               | -0.029      | 0.484      | -0.011    | 0.7888    |

The positivity was approximately 10% and 13% at most. Multiple factors can affect the progress of CI, CVD and DM. Spearman correlation analysis between the antibody levels and the information of the patients revealed that the levels of SOSTDC1-156 but not of CTNND1-211, CLDND1-69 or CCNG2-231 are correlated with IMT, hypertension and smoking (Table 6). Thus, the SOSTDC1-156 marker can predict atherosclerotic CI caused by hypertension and/or smoking habit. There are many causes that affect the progress of CI, and each antibody marker may be associated with a respective cause of CI. Thus, the positivity of each maker cannot be expected to particularly high. The development of an increasing number of such antibody markers may make the prediction of the onset of CI at a strong possibility.

We used the sera of patients with CI within two weeks of onset. Various antigens appear immediately after the onset of CI whereas the antibodies are not produced until two weeks later. Thus, the antibodies specifically detected in sera immediately after the onset are known to have been present prior to the onset. By measuring the levels of these antibodies, it is possible to predict the onset, i.e., serum antibody markers can be prediction markers for the onset of CI.

In most cases, CI is not induce suddenly but mediated frequently by health issues such as TIA and asymptomatic CI. When small infarctions occur, it is possible for antigens to leak out from infarction lesions. Repeated exposure to such antigens may raise the antibodies to detectable levels. In fact, the antibody levels against SOSTDC1-156 were found to be higher in TIA patients than that of those in HD (Figure 1). The antibody levels of CCNG2-231 were highly associated with DM (Table 5), and therefore, it may be useful for the early diagnosis of DM. If the levels of both SOSTDC1-156 and CCNG2-231 were high, the patient might suffer from CI caused by DM. CTNND1-211 and CLDND1-69 may contribute to diagnose CVD. Application of these biomarkers for the clinical use is very important and the early development of the diagnosis kit is expected.

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