Calpastatin autoantibodies: detection, epitope mapping, and development of a specific peptide ELISA

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Autoantibodies against calpastatin (CAST) were detected in a 53-year-old female patient with a history of arthritis and thrombosis. The specificity of the autoantibodies was determined by screening expression cDNA libraries, sequence analysis of positive clones, and subsequent Western blotting against recombinant antigen. Because the Western blot lacked satisfactory reproducibility, an ELISA for anti-CAST antibodies was established. The major epitope recognized by 24 Western blot-positive sera was located within the C-terminal 27 amino acids. The ELISA was therefore based on a synthetic peptide representing these amino acids. The assay was calibrated with serial dilutions of a positive reference serum. Intraassay precision is high with a CV of ~4% for low- and high-titer samples. Interassay precision (CV) was 5.6-8.2% for sera with low and intermediate titers [10-60 arbitrary units (AU)], where 1:100 dilution of the positive reference serum = 100 AU, 1:1000 dilution = 10 AU, etc.), which increases with higher titers (>60 AU). Among 205 healthy blood donors, the mean + 3 SD (after logarithmic transformation) was 30 AU; higher values were seen in 2.9% of 138 hospitalized patients. The newly developed ELISA will be a useful tool for further clinical studies on the association of anti-CAST antibodies to disease, because it permits rapid and reproducible analysis of patient sera.

INDEXING TERMS: calpains • rheumatoid arthritis • thrombosis • Western blot • autoimmunity

Autoantibodies against calpastatin (CAST) have been detected in the sera of very different patients by screening cDNA expression libraries. Five groups independently identified these autoantibodies in patients with thrombosis [1], infertility [2, 3], and rheumatoid arthritis (RA) [4, 5]. The diagnostic value of these autoantibodies as well as their role in pathophysiology remain to be elucidated even though two small studies suggest an association with RA [4, 5].

CAST is the endogenous inhibitor of the intracellular calcium-activated cysteine proteases calpain I and II (EC 3.4.22.17) [6, 7]. The inhibitor is colocalized with the calpains and is ubiquitous. The cDNA sequence of human CAST predicts a 708 amino acid protein with a calculated molecular mass of 77 kDa [8]. CAST consists of five domains, four of which are homologous (20-35% identity). In these four repeated domains a heptapeptide is found with a consensus sequence TIPPXYR, which is thought to be responsible for calpain inhibition [9-11]. The unique N-terminal domain may vary in different tissues and cells [7].

The calpains function by limited hydrolysis of specific proteins. These include cytoskeletal proteins such as spectrin and talin, cellular receptors such as the epidermal growth factor receptor, protein kinase C, and perhaps many as yet unknown substrates [6]. The calpains have been implicated in numerous regulatory processes including cytoskeletal rearrangement in the early phase of platelet aggregation or neutrophil degranulation, intracellular signal transduction (or modulation), and altered protein expression. Therefore, the calpain system is likely to take part in a number of pathological processes. These might be caused by uncontrolled activation of the proteases, for example in platelet aggregation [12] and cartilage destruction in arthritic synovia [13-15]. Furthermore, extracellular sites of action for the calpains and a role in tissue destruction, e.g., after hypoxia, have been discussed [16, 17].

These biochemical and cell biological data provide potential links to a pathophysiologic role of autoantibodies directed against a part of this ubiquitous protease system. Further investigations into this novel facet of autoimmunity are clearly needed. A first step in this direction will be a thorough analysis of potential disease associations of anti-CAST antibodies. Since reliable Western blot assays with recombinant protein are difficult to establish, time consuming, not quantitative, and in
some cases hard to interpret, they are not suitable for clinical studies or routine diagnostics. Therefore, an ELISA was developed that will permit the reliable analysis of large series of samples, which is a prerequisite for the further elucidation of the relevance of anti-CAST autoantibodies.

**Materials and Methods**

**PATIENTS AND PROBANDS**

Sera (205) of healthy blood donors and sera from 138 consecutive patients, which were analyzed in the daily routine clinical chemistry of our hospital, were stored in aliquots at −20 °C. Procedures concerning the use of human subjects in this study were in accordance with the Helsinki Declaration of 1975, as revised in 1983.

**LIBRARY SCREENING AND ANALYSIS OF RECOMBINANT CLONES**

A human kidney cDNA expression library in Agt11 (Clontech, Palo Alto, CA) was screened according to standard protocols [18] with the index patient’s serum diluted 1:300 as source of primary antibody. The second antibody was a peroxidase-conjugated rabbit anti-human IgG (Dako, Hamburg, Germany) diluted 1:1000. Immunoreactive plaques were picked and purified to homogeneity by several rescreenings. Phage DNA was isolated by using a commercially available kit (Quiagen, Hilden, Germany). The insert DNA was purified after cleavage of the phage DNA with EcoRI and subcloned into pUC18 for sequencing. All sequence analyses were performed on an automated DNA sequencer (A.L.F.; Pharmacia, Uppsala, Sweden). Sequencing reactions were done with fluorescently labeled primers suitable for pUC18 or by incorporation of fluorescently labeled dATP (Boehringer Mannheim, Mannheim, Germany), if internal primers were used. Sequencing reagents were from Pharmacia.

**EXPRESSION OF RECOMBINANT CAST FUSION PROTEINS**

CAST fragments were generated either directly by restriction enzyme cleavage of the cloned original cDNA fragment in pUC18 or by PCR amplification. They were inserted into the prokaryotic expression vector pXa1 (Boehringer Mannheim) [19] in frame with the β-galactosidase gene and propagated in *Escherichia coli* XL1 blue [20]. The primers used to generate the different CAST fusion proteins are shown in Fig. 1. In addition to the insert of the original cDNA clone, several smaller inserts were generated to map the relevant epitope(s) on CAST. Furthermore, two clones with single amino acid exchanges were constructed. Insert sequences and the correct insertion into the expression vector were verified for all clones by sequence analysis with a pXa1-specific oligonucleotide (pXa1/5938–598) as described above. Expression of the β-galactosidase/CAST fusion protein was induced by adding 2 mmol/L isopropyl β-d-thiogalactopyranoside (IPTG) to a 50-mL culture at an absorbance of 600 nm of 0.4 to 0.6, and growth was continued for 3 to 5 h [18]. The cells were harvested by centrifugation and lyzed in reducing sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer for 7 min at 95 °C. Cell debris was removed by centrifugation for 5 min at 10 000 g.

**WESTERN BLOT DETECTION OF ANTI-CAST ANTIBODIES**

Total protein derived from recombinant *E. coli* was separated by SDS-PAGE in 7.5% acrylamide gels under reducing conditions as described [21]. Gels were either stained in Coomassie Blue G250 [22] or used for Western blotting to polyvinylidene fluoride (PVDF) membranes (Immobilon-P, 0.45 μm; Millipore, Bradford, UK). Protein transfer was performed with SemiDry-Blotting in a discontinuous buffer system as described earlier [23]. For the 7.5% acrylamide gels 0.8 A/cm² were applied for 45 min. Unspecific binding sites on the PVDF membranes were blocked with 50 g/L nonfat milk powder in phosphate-buffered saline (PBS: 137 mmol/L NaCl, 2.7 mmol/L KCl, 4.3 mmol/L Na₂HPO₄, 1.4 mmol/L KH₂PO₄, pH 7.4) for 1 h at room temperature or overnight at 4 °C. Thereafter the membrane was cut into 17 vertical strips (0.5 × 6 cm) for further incubation with individual patient or proband sera. These sera were diluted 1:100 in 2 mL of blocking buffer and incubated for 1 h at room temperature with the blot strips, followed by three washings, 15 min each, with 1 mL/L Tween 20 in PBS. Bound IgG was detected with a peroxidase-conjugated anti-human IgG (Dako), diluted 1:800 in blocking buffer. After three washings the blot was stained with the peroxidase substrate 3-amino-9-ethylcarbazole (Sigma, St. Louis, MO).

**ELISA PLATES**

Microtiter plates (Maxisorb; Nunc, Roskilde, Denmark) were coated overnight at room temperature with a synthetic peptide representing the C-terminal 27 amino acids of human CAST (Progen Biotechnik, Heidelberg, Germany) dissolved in PBS at 60 μg/L (100 μL/well). Plates were subsequently rinsed with blocking buffer (PBS, 10 mL/L Tween 20, 50 g/L sucrose). They were then soaked for at least 10 min in blocking buffer. After removal of excess buffer, the plates were dried by lyophilization sealed under vacuum and stored at 4 °C. For the analysis of the precision of the ELISA as well as larger patient studies, a batch of 50 microtiter plates was prepared as described.
above by Progen Biotechnik. This batch has been used for >3 months without an apparent change in autoantibody detection.

ELISA PROCEDURE
The wells of the coated microtiter plate were filled with 100 μL of patient sera diluted 1:200 in washing buffer (PBS, 0.5 mL/L Tween 20) and incubated at 37 °C for 45 min. The plate was washed four times with washing buffer (200 μL/well), incubated with 100 μL of anti-human IgG-peroxidase (Dianova, Raboisen, Germany) diluted 1:50 000 for another 45 min at 37 °C, and washed again four times. The peroxidase activity retained in the wells was assayed by addition of 100 μL of tetramethylbenzidine substrate solution (Progen). After 10 min at room temperature, reactions were stopped by adding 50 μL of 0.5 mol/L H₂SO₄ to each well and absorbances were measured within 30 min at 450 nm (reference filter 630 nm) in a microtiter plate reader (MR 3000; Dynatech, Denkendorf, Germany). A calibration curve was generated with an anti-CAST-positive reference serum from a blood donor in dilutions from 1:10 to 1:1000. Optimal curve fitting was performed with Sigma Plot software (Jandel Scientific, Erkrath, Germany).

Results
PATIENT DESCRIPTION AND IDENTIFICATION OF CAST AS AN AUTOANTIGEN
The index patient, a 53-year-old white woman (H.M.), presented to the rheumatology clinic with arthritic pain in her hands. She had a history reminiscent of the antiphospholipid syndrome with deep vein thrombosis, recurrent cerebral ischemic events, and abortion with two other unremarkable pregnancies. However, neither antibodies to cardiolipin or phosphatidylserine nor lupus anticoagulant was detected. Indirect immunofluorescence with her serum revealed an unusual fluorescence pattern on hep-2 cells and primate liver and kidney slices, reminiscent of liver kidney microsomal antibodies (Fig. 2). The antibody was of the IgG type. The patient’s serum was subsequently used for immunoscreening of a human kidney cDNA expression library in Agt11. Several immunoreactive clones were identified. From one clone a cDNA fragment of ~0.6 kb was isolated and subcloned into pUC18. Sequence analysis showed 100% identity to the C-terminal part of human CAST, the endogenous inhibitor of the calpain proteases. The isolated CAST fragment was 618 bp in length and coded for the C-terminal 97 amino acids of the protein.

For expression of the 97 C-terminal amino acids (CAST_{612-708}) we cloned the cDNA fragment in frame into the prokaryotic expression vector pXa1, which results in a β-galactosidase fusion protein of ~126 kDa. Western blot analysis showed immunoreactivity of the patient’s serum with the expressed fusion protein but not with β-galactosidase alone (Fig. 3), indicating that the serum contains antibodies specific for CAST. To further verify the specificity, the patient’s serum was preincubated with soluble recombinant CAST. This abolished the reactivity in Western blots completely (Fig. 4). The immunofluorescence pattern of the serum is unchanged by preincubation with recombinant CAST; this indicates that the fluorescence pattern of the patient’s serum is not caused by CAST autoantibodies.

ANALYSIS OF PATIENTS’ SERA
To obtain an estimate of the frequency of CAST autoantibodies, healthy blood donors and several patient groups were analyzed by Western blots against proteins from E. coli lysates with the expressed CAST fusion protein. Among >400 patients and blood donors, >20 additional positive sera were detected. However, the interpretation of the Western blot assay was

![Image](image-url)

Fig. 2. Indirect immunofluorescence with index patient’s serum.
Slides were incubated according to standard procedures with a 1:100 dilution of the patient’s serum. On both hep-2 cells (A) and primate kidney sections (B) a cytoplasmic fluorescence was seen. The nuclei were spared. In the kidney section, primarily the cells of the proximal tubuli were stained.

![Image](image-url)

Fig. 3. Recombinant β-galactosidase/CAST_{612-708} on Coomassie Blue-stained SDS-PAGE (A) and Western blot (B).
Index patient’s serum diluted 1:100 was used as source of primary antibody. In both panels total soluble protein from E. coli was loaded onto the gel. Lane 1 contains protein from pXa1 that expresses only β-galactosidase. In lanes 2 and 3 protein from a clone containing a fusion protein between β-galactosidase and the C-terminal 97 amino acids of CAST is loaded (lane 2 not induced, lane 3 induced with IPTG). The lower arrows indicate the position of β-galactosidase; the upper arrows the position of the CAST fusion protein.
Fig. 4. Preincubation of the index patient's serum with soluble recombinant CAST.

Two identical Western blot strips with the β-galactosidase/CAST<sub>612-708</sub> fusion protein were produced and incubated with the patient's serum either preincubated with soluble fusion protein (lane 1) or not (lane 2). The signal was abolished by preincubation.

complicated by a less-than-satisfactory reproducibility. Even though the majority of positive sera could be defined as either anti-CAST positive or negative, several sera were detected that were positive on one occasion and negative when the immunoblot was repeated. These results suggested to us that the Western blot assay is not suitable for the analysis of large patient groups and clinical studies, even though it may be a useful tool to initially identify patients with autoantibodies to CAST.

EPITOPE MAPPING

The most obvious alternative to Western blot assays is a specific ELISA. The development of an ELISA requires the availability of pure antigen preparation. The purification of large amounts of pure CAST from natural sources or from recombinant bacteria is technically difficult, particularly with respect to reproducibility of the preparation. Therefore, we decided to use a synthetic peptide or a mixture of peptides representing the major epitope(s) in CAST. In a first step the relevant epitopes on the protein had to be characterized.

The anti-CAST-positive sera were not able to precipitate the recombinant fusion protein in immunodiffusion assays, whereas a polyclonal anti-β-galactosidase antibody precipitated the β-galactosidase fusion protein (data not shown). This suggested to us that there might be only one epitope on the CAST fragment. For further epitope mapping, small CAST cDNA fragments were generated by PCR, subcloned into pXa1, and expressed as β-galactosidase fusion proteins in E. coli (Fig. 5). In Western blot analysis, only clones that contained the C-terminal amino acids reacted with the index patient's serum (Figs. 5 and 6). The C-terminal fragment CAST<sub>682-708</sub> reacted with all 16 sera tested, whereas only one serum also recognized another CAST fragment (CAST<sub>545-689</sub>). This indicates that the major epitope is located within the C-terminal 27 amino acids of the protein.

To further characterize the epitope, fusion proteins with a modified CAST C-terminus were generated by PCR with mutant primers. The β-galactosidase fusion protein contained residues 649–708 of human CAST. In one clone Lys<sub>706</sub> was substituted by Ile (CAST<sub>K706I</sub>); in another, Asp<sub>707</sub> was substituted by Ala (CAST<sub>D707A</sub>). The exchange of either of the two charged amino acids by an aliphatic residue abolished binding of the index patient's serum antibodies to the fusion protein (Figs. 5 and 7). Furthermore, antibody binding of 23 of 24 anti-CAST-positive sera was abolished. The serum that was not affected was identical to the one that had an additional binding site in fragment CAST<sub>645-689</sub>. This experiment provided further evidence that the C-terminus of CAST contains the major epitope for autoantibodies. In addition, it suggested that the two amino acids Lys<sub>706</sub> and Asp<sub>707</sub> are part of the antibody binding site.

Fig. 5. Schematic representation of CAST fragments generated for epitope mapping.

Numbers indicated for each fragment represent the CAST amino acids present in this particular fragment. The mutant clones CAST<sub>T706</sub> and CAST<sub>D707A</sub> are also shown. In the left-hand column the primers used to generate the specific subclone are indicated. Only the clone CAST<sub>E612-645</sub> was generated by restriction endonuclease digestion with EcoRI/PstI from pUC18 containing the original insert. In the first column to the right, "+" indicates a positive reaction in Western blots with the index patient's serum, and "0" indicates a negative reaction. In the second column a summary of the reactivities of the other Western blot-positive sera is given. n.d., not done.

![Image of a diagram showing the schematic representation of CAST fragments with numbers indicating the amino acids and positive or negative reactions in Western blots with sera from patients.](image-url)
DEVELOPMENT OF AN ANTI-CAST ELISA

Since the major epitope recognized by >90% of the sera with CAST autoantibodies could be located to the C-terminal 27 amino acids and perhaps even to the C-terminal 10 amino acids, we decided to develop an ELISA based on a synthetic peptide that consisted of these 27 amino acids (CAST\textsubscript{62-70}). This peptide was coated to microtiter plates as described in Materials and Methods. Sera were incubated at a dilution of 1:200 and the binding of autoantibodies to the peptide was detected by a peroxidase-conjugated anti-human IgG antibody. By varying the concentrations of the conjugate, the serum dilution, the blocking reagent, and the substrate, the background absorbance could be reduced to <0.010, while several positive samples had absorbances >1.000, so that a reasonable slope of the calibration curve is achieved (Fig. 8). The assay is calibrated with a CAST autoantibody-positive recalcified plasma (donor H.K.) used at different concentrations from 1:50 to 1:1000. Arbitrary units (AU) were defined such that the absorbance at a dilution of 1:100 equals 100 AU and the absorbance at 1:1000 equals 10 AU, and so on. In the range 20–200 AU, the absorbance is linear to the logarithm of the antibody titer (Fig. 8). An even better curve-fitting can be achieved by a nonlinear procedure.

The calibration of the ELISA with serum of donor H.K. was confirmed by two other high-titer sera (H.B., H.M.) used as a calibrator. A total of 14 positive sera, ranging from 26 to 164 AU, were tested. Absorbances were expressed respective to each calibrator used. Excellent correlation in this range between the results by the different calibrators was obtained, as shown in Fig. 9 ($r = 0.9981$ for H.K./H.B. and 0.9968 for H.K./H.M.).

To further characterize the newly designed test, the precision of the ELISA was determined. The within-run precision ($CV\text{\textsubscript{intra}}$) was assessed by repeatedly analyzing two specimens, diluted 1:200, one with high CAST antibody titer and one with a low titer. Under standard assay conditions 20 analyses of the
specimens yielded mean antibody titers of 142 AU for the high-titer serum with an SD of 5.7 AU (CV = 4.0%) and 25 AU for the low-titer serum with an SD of 0.9 AU (CV = 3.7%). The variability of absorbance values between runs (CV\textsubscript{inter}) was also determined by analysis of two samples with high and low antibody titer on 20 different days. For the high-titer sample the mean was 134 ± 20.2 AU (CV = 15.1%). In a medium-titer sample the mean was 52 ± 4.2 AU (CV = 8.2%), and in a low-titer serum the mean was 11 ± 0.6 AU (CV = 5.6%). This shows that the assay is reproducible and precise. Particularly, in the normal range and the low abnormal range the CV\textsubscript{inter} and CV\textsubscript{inter} are well within the range for comparable assays.

We next estimated a reference range of the assay for healthy blood donors. The mean age of this group was 26 years with a range from 18 to 55 years (n = 205). There were 113 men and 92 women in this group. As expected, the distribution of the antibody titers was highly skewed to the left. Therefore the data were transformed logarithmically before further analysis. The cutoff was defined as the mean + 3 SD (derived from the transformed data), which represents 30 AU. When male and female blood donors were analyzed separately, slight differences were found for the calculated cutoff (28 AU and 32 AU, respectively). However, these differences were due to a larger SD in women. This is related to the presence of five anti-CAST-positive sera in women compared with only two in men. A significantly larger cohort would be needed to establish a difference in the cutoff between men and women. With this cutoff, seven (3.4%) blood donors were determined anti-CAST positive, which is lower than the frequency estimated from Western blot analysis (5.4%). Interestingly, there were probands that were positive by ELISA but negative on Western blots. The sera that behaved differently in the two assays all recognized the C-terminal peptide CAST\textsubscript{382-708} on Western blots. This might be interpreted as evidence that the sera that do not react in the ELISA recognize an epitope dependent on the structure of the fusion protein. Furthermore, this problem reflects the at best semiquantitative nature of Western blot assays.

We then analyzed 138 patient sera from our hospital. These samples were taken from the daily routine clinical chemistry laboratory. Most of the patients are from the departments of internal medicine and surgery. Aside from an attempt to include ~50% of patients older than 60 years, no selection criterion was used. The diagnoses of the patients were unknown to the laboratory at the time of sampling. The patient group had a mean age of 52 with a range of 11–88 years. The gender ratio was 79 men to 59 women. In this group four patients (2.9%) tested positive. This was not significantly different from the healthy blood donors. Linear regression of the antibody units plotted against the respective age of the patients and blood donors revealed no age dependency of CAST autoantibodies (r = 0.0815). Furthermore, these data provide preliminary evidence that there is no increased prevalence of these autoantibodies in an unselected patient population compared with healthy controls.

**Discussion**

Autoantibodies against human CAST were detected in a patient with a history reminiscent of the antiphospholipid syndrome. The major epitope for CAST autoantibodies from 24 consecutive anti-CAST-positive sera was localized to the 27 C-terminal amino acids of the protein. A peptide ELISA, based on this CAST fragment, was developed and tested for use in clinical studies and routine diagnostics.

Analysis of 205 healthy blood donors showed a significant prevalence of anti-CAST autoantibodies (7 of 205 or 3.4%). This was observed whether these samples were analyzed by ELISA or by Western blot. In fact, the prevalence in Western blot was higher than in the ELISA. A total of 11 sera was tentatively classified as positive by Western blot. This is probably related to the fact that the definition of positive and negative samples is somewhat arbitrary in the Western blot assay. The observed relatively high prevalence of CAST autoantibodies in healthy individuals is important for the interpretation of other studies. For instance, Wang et al. isolated this autoantibody among others by immunoscreening a human testis cDNA expression library with pooled sera of infertile men [3]. The authors speculate that CAST autoantibodies might be associated with immunologically induced infertility. However, no attempt was made to show an increased prevalence of CAST autoantibodies in infertile patients. Taking into account our data with regard to the prevalence of these autoantibodies in healthy persons, it could be just by coincidence that the CAST autoantibodies were found in infertile men.

In the studies of Mimori et al. and Després et al., anti-CAST antibodies were associated with RA [4, 5]. Approximately 50% of RA patients were positive for anti-CAST by Western blot. However, both studies are small, and the study by Mimori et al. [4] does not even include a healthy control group. Furthermore, there are some substantial discrepancies regarding the association of CAST autoantibodies to other autoimmune diseases, e.g., systemic lupus erythematosus. Whereas Després et al. [5] claim that among their patients with autoimmune diseases other than RA the prevalence of anti-CAST is low, Mimori et al. [4] describe an incidence >20% for several other autoimmune diseases. However, the latter authors did not specifically describe under which conditions a sample was considered positive in their assay. These data clearly demonstrate that sufficiently large numbers of patients have to be analyzed before definitive conclusions about the disease association of CAST autoantibodies can be drawn. Furthermore, it is mandatory that reproducible tests are used that permit an unbiased determination of positive and negative samples. In our hands, the Western blot assay with lysates from recombinant bacteria did not meet these criteria. Apparently, Després et al. also had problems with the definition of a positive sample. They tried to circumvent part of this problem by performing duplicate determinations of samples and a third analysis in cases in which the two initial determinations did not yield the same result [5]. Such a procedure is clearly subject to a severe investigator bias, whereas the ELISA yields a numerical result, which permits an unbiased interpretation. The analysis of a large number of healthy persons provides an
estimate of the reference range for the assay, which can be defined by the usual criteria used in clinical chemistry.

When the sera of 138 hospital patients were analyzed, the prevalence of CAST autoantibodies was very similar to that in the healthy blood donors. Since this group was significantly older than the blood donors, these data preclude a strong age dependency of CAST autoantibodies. In addition, this second group does not provide any evidence for a nonspecific disease association of these autoantibodies. These two observations suggest that there may be reasonably specific disease associations of CAST autoantibodies, which may make clinical tests for their presence a valuable diagnostic tool. This is further supported by an apparent association of autoantibodies against CAST with venous thrombosis, which has been detected in an analysis of different patient groups (Schlosser et al., unpublished data).

Finally, the question of the role of CAST autoantibodies in disease processes needs to be analyzed further. The apparent association with RA is a first interesting observation. As for many other autoantibodies, a thorough analysis of their disease association is mandatory [24]. In addition, it is important to determine the origin of anti-CAST antibodies. The question has to be addressed of whether there are other relevant epitopes within the peptide. Clearly, we are still at the beginning of further investigations of this interesting phenomenon. Particularly, it is still unclear whether these antibodies are the cause of disease processes or only markers for a disease.

The peptide-ELISA for the detection and quantification of anti-CAST autoantibodies, described here, is probably a versatile tool for the systematic examination of clinical correlations between CAST antibodies and disease occurrence and (or) progression.

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References