

CLAIMS

1. Use of an antibody, or an antigen-binding fragment thereof, specifically binding to APRIL in the manufacture of a medicament for prevention and/or treatment of hypertriglyceridemia, metabolic syndrome, non-alcoholic steatohepatitis, diabetes mellitus type 2, atherogenic dyslipidemia, abdominal aortic aneurysm, cardiovascular events and/or atherosclerosis.
2. The use according to claim 1, for the treatment and or prevention of hypertriglyceridemia, wherein preferably

the hypertriglyceridemia is at least one selected from the group of hypertriglyceridemia in metabolic syndrome, hypertriglyceridemia in non-alcoholic steatohepatitis-related, hypertriglyceridemia in diabetes mellitus type 2, hypertriglyceridemia in atherogenic, cardiovascular events with a history of hypertriglyceridemia and atherosclerosis with hypertriglyceridemia.

3. The use according to claim 1, for the treatment and or prevention of abdominal aortic aneurysm.
4. The use according to claim 1, wherein cardiovascular events comprise myocardial infarction, stroke, peripheral artery disease, angina pectoris and/or urgent hospitalization for angina leading to revascularization.
5. The use according to claim 1 to 4, wherein the binding of the antibody or antigen-binding fragment thereof to APRIL results in an increased interaction of APRIL with proteoglycans, wherein preferably the proteoglycans are arterial and vascular proteoglycans, wherein more preferably

the proteoglycans are comprised in an extracellular matrix.

6. The use according to any one of claims 1 to 5, wherein the binding of the antibody or antigen-binding fragment thereof to APRIL modulates the interaction of APRIL with at

least one of its endogenous receptors, wherein preferably the binding of the antibody or antigen-binding fragment thereof to APRIL blocks the interaction of APRIL with the receptors TACI and BCMA.

7. Use of an antibody, or antigen-binding fragment thereof or the antibody, or antigen-binding fragment thereof, in the manufacture of a medicament for the prevention and/or treatment according to any one of claims 1 to 6, wherein the antibody or antigen-binding fragment thereof
 - (a) comprises a variable heavy (VH) chain comprising CDR3 as defined in SEQ ID NO:10 and a variable light (VL) chain comprising CDR3 as defined in SEQ ID NO:13;
 - (b) comprises a variable heavy (VH) chain comprising CDR3 as defined in SEQ ID NO:17 and a variable light (VL) chain comprising CDR3 as defined in SEQ ID NO:20;
 - (c) comprises a variable heavy (VH) chain comprising CDR3 as defined in SEQ ID NO:24 and a variable light (VL) chain comprising CDR3 as defined in SEQ ID NO:27;
 - (d) comprises a variable heavy (VH) chain comprising CDR3 as defined in SEQ ID NO:31 and a variable light (VL) chain comprising CDR3 as defined in SEQ ID NO:34; or
 - (e) comprises a variable heavy (VH) chain comprising CDR3 as defined in SEQ ID NO:38 and a variable light (VL) chain comprising CDR3 as defined in SEQ ID NO:41.
8. The use according to any one of claims 1 to 9, wherein the antibody or antigen-binding fragment thereof
 - (a) comprises a variable heavy (VH) chain comprising CDR1 as defined in SEQ ID NO:8, CDR2 as defined in SEQ ID NO:9 and CDR3 as defined in SEQ ID NO:10 and a variable light (VL) chain comprising CDR1 as defined in SEQ ID NO:12, CDR2 as defined in the sequence: YAS and CDR3 as defined in SEQ ID NO:13;
 - (b) comprises a variable heavy (VH) chain comprising CDR1 as defined in SEQ ID NO:15, CDR2 as defined in SEQ ID NO:16 and CDR3 as defined in SEQ ID NO:17 and a variable light (VL) chain comprising CDR1 as defined in SEQ ID NO:19, CDR2 as defined by the amino acid sequence: AAS and CDR3 as

- defined in SEQ ID NO:20;
- (c) comprises a variable heavy (VH) chain comprising CDR1 as defined in SEQ ID NO:22, CDR2 as defined in SEQ ID NO:23 and CDR3 as defined in SEQ ID NO:24 and a variable light (VL) chain comprising CDR1 as defined in SEQ ID NO:26, CDR2 as defined by the amino acid sequence: GTN and CDR3 as defined in SEQ ID NO:27;
 - (d) comprises a variable heavy (VH) chain comprising CDR1 as defined in SEQ ID NO:29, CDR2 as defined in SEQ ID NO:30 and CDR3 as defined in SEQ ID NO:31 and a variable light (VL) chain comprising CDR1 as defined in SEQ ID NO:33, CDR2 as defined by the amino acid sequence:GTS and CDR3 as defined in SEQ ID NO:34; or
 - e) comprises a variable heavy (VH) chain comprising CDR1 as defined in SEQ ID NO:36, CDR2 as defined in SEQ ID NO:37 and CDR3 as defined in SEQ ID NO:38 and a variable light (VL) chain comprising CDR1 as defined in SEQ ID NO:40, CDR2 as defined by the amino acid sequence: LVS and CDR3 as defined in SEQ ID NO:41.
9. The use according to any one of claims 1 to 8, wherein the antibody or antigen-binding fragment thereof
- (a) comprises a variable heavy (VH) chain sequence comprising the amino acid sequence of SEQ ID NO:7 or a sequence having 90%, preferably 95% sequence identity to SEQ ID NO:7; and
a variable light (VL) chain sequence comprising the amino acid sequence of SEQ ID NO:11 or a sequence having 90%, preferably 95% sequence identity to SEQ ID NO:11;
 - (b) comprises a variable heavy (VH) chain sequence comprising the amino acid sequence of SEQ ID NO:14 or a sequence having 90%, preferably 95% sequence identity to SEQ ID NO:14; and
a variable light (VL) chain sequence comprising the amino acid sequence of SEQ ID NO:18 or a sequence having 90%, preferably 95% sequence identity to SEQ ID NO:18;
 - (c) comprises a variable heavy (VH) chain sequence comprising the amino acid sequence of SEQ ID NO:21 or a sequence having 90%, preferably 95% sequence identity to SEQ ID NO:21; and

- a variable light (VL) chain sequence comprising the amino acid sequence of SEQ ID NO:25 or a sequence having 90%, preferably 95% sequence identity to SEQ ID NO:25;
- (d) comprises a variable heavy (VH) chain sequence comprising the amino acid sequence of SEQ ID NO:28 or a sequence having 90%, preferably 95% sequence identity to SEQ ID NO:28; and
a variable light (VL) chain sequence comprising the amino acid sequence of SEQ ID NO:32 or a sequence having 90%, preferably 95% sequence identity to SEQ ID NO:32; or
- (e) comprises a variable heavy (VH) chain sequence comprising the amino acid sequence of SEQ ID NO:35 or a sequence having 90%, preferably 95% sequence identity to SEQ ID NO:35; and
a variable light (VL) chain sequence comprising the amino acid sequence of SEQ ID NO:39 or a sequence having 90%, preferably 95% sequence identity to SEQ ID NO:39.
10. The use according to any one of claims 1 to 9, wherein the antibody is an IgM, IgG1, IgG2a or IgG2b, IgG3, IgG4, IgA or IgE antibody.
11. The use according to any one of claims 1 to 10, wherein the antigen-binding fragment is a Fab fragment, an F(ab') fragment, an Fv fragment or an scFv fragment.
12. A polynucleotide that encodes the antibody, or an antigen-binding fragment thereof, as defined in any one of claims 7 to 11, wherein preferably the polynucleotide encodes the antibody, or an antigen-binding fragment thereof, as defined in claim 9.
13. A pharmaceutical composition comprising the antibody, or antigen-binding fragment thereof, as defined in any one of claims 7 to 11 and a pharmaceutically acceptable carrier, preferably comprising a further therapeutic agent, wherein more preferably the further therapeutic agent is selected from a group consisting of: fibrates, statins, agents that inhibit the expression of Angiopoietin-like protein 3 (ANGPTL3) or Apolipoprotein C-III (apoC-III), and agents that prevent the binding of auto-antibodies to GPIHBP1.
14. A method for quantifying the concentration of non-canonical APRIL (nc-APRIL) in a

sample, the method comprising the steps of:

- a) contacting the sample comprising nc-APRIL with a first monoclonal antibody specifically binding to a first epitope of nc-APRIL, wherein said first monoclonal antibody is an immobilized antibody;
- b) contacting the mixture of step (a) with a second monoclonal antibody, wherein said second monoclonal antibody specifically binds to a second epitope of nc-APRIL;
- c) detecting the binding of the second monoclonal antibody to immobilized nc-APRIL; and
- d) quantifying the concentration of nc-APRIL in the sample according to the detected binding in step (c)

wherein preferably the method is a sandwich enzyme-linked immunosorbent assay (ELISA) method.

15. A method for quantifying the total concentration of c-APRIL and nc-APRIL in a sample, the method comprising the steps of:

- a) contacting a denatured sample comprising nc-APRIL and/or c-APRIL with a first monoclonal antibody specifically binding to a first epitope of denatured nc-APRIL and c-APRIL, wherein said first monoclonal antibody is an immobilized antibody;
- b) contacting the mixture of step (a) with a second monoclonal antibody, wherein said second monoclonal antibody specifically binds to a second epitope of denatured nc-APRIL and c-APRIL;
- c) detecting the binding of the second monoclonal antibody to the immobilized forms of nc-APRIL and/or c-APRIL; and
- d) quantifying the total concentration of nc-APRIL and c-APRIL in the sample according to the detected binding in step (c)

wherein preferably the method is a sandwich enzyme-linked immunosorbent assay (ELISA) method.

16. A method for quantifying the amount of c-APRIL in a sample, the method comprising the steps of:

- a) quantifying the amount of nc-APRIL in a first portion of the sample with the method according to claim 14;

- b) quantifying the total amount of nc-APRIL and c-APRIL in a second portion of the sample with the method according to claim 15, wherein the second portion of the sample has been denatured; and
- c) quantifying the concentration of c-APRIL in the sample, wherein quantifying the concentration of c-APRIL in the sample involves subtracting the concentration of nc-APRIL in the sample from the total concentration of nc-APRIL and c-APRIL in the sample

wherein preferably the method is a sandwich enzyme-linked immunosorbent assay (ELISA) method.

17. The method according to any one of claims 14 to 16, wherein the second monoclonal antibody is conjugated to a detection moiety or a binding moiety, wherein preferably the binding moiety is biotin.
18. The method according to any one of claims 14 to 17, wherein the first monoclonal antibody comprises a variable heavy (VH) chain sequence comprising the amino acid sequence of SEQ ID NO:56 or a sequence having 90%, preferably 95% sequence identity to SEQ ID NO:56; and a variable light (VL) chain sequence comprising the amino acid sequence of SEQ ID NO:60 or a sequence having 90%, preferably 95% sequence identity to SEQ ID NO:60; and wherein the second monoclonal antibody comprises
 - (a) a variable heavy (VH) chain sequence comprising the amino acid sequence of SEQ ID NO:42, or a sequence having 90%, preferably 95% sequence identity to SEQ ID NO:42; and a variable light (VL) chain sequence comprising the amino acid sequence of SEQ ID NO:46, or a sequence having 90%, preferably 95% sequence identity to SEQ ID NO:46; or
 - (b) a variable heavy (VH) chain sequence comprising the amino acid sequence of SEQ ID NO:49, or a sequence having 90%, preferably 95% sequence identity to SEQ ID NO:49; and a variable light (VL) chain sequence comprising the amino acid sequence of SEQ ID NO:53, or a sequence having 90%, preferably 95% sequence identity to SEQ ID NO:53.
19. The method according to any one of claims 14 to 17, wherein the first monoclonal antibody comprises
 - (a) a variable heavy (VH) chain sequence comprising the amino acid sequence of

SEQ ID NO:42, or a sequence having 90%, preferably 95% sequence identity to SEQ ID NO:42; and a variable light (VL) chain sequence comprising the amino acid sequence of SEQ ID NO:46, or a sequence having 90%, preferably 95% sequence identity to SEQ ID NO:46; or

- (b) a variable heavy (VH) chain sequence comprising the amino acid sequence of SEQ ID NO:49, or a sequence having 90%, preferably 95% sequence identity to SEQ ID NO:49; and a variable light (VL) chain sequence comprising the amino acid sequence of SEQ ID NO:53, or a sequence having 90%, preferably 95% sequence identity to SEQ ID NO:53; and

wherein the second monoclonal antibody comprises a variable heavy (VH) chain sequence comprising the amino acid sequence of SEQ ID NO:56 or a sequence having 90%, preferably 95% sequence identity to SEQ ID NO:56; and a variable light (VL) chain sequence comprising the amino acid sequence of SEQ ID NO:60 or a sequence having 90%, preferably 95% sequence identity to SEQ ID NO:60

20. A kit for determining the level of nc-APRIL in a sample, the kit comprising a first and a second monoclonal antibody, wherein both monoclonal antibodies bind to different epitopes of nc-APRIL.

21. A nephelometric assay for quantifying the concentration of non-canonical APRIL (nc-APRIL) in a sample, the assay comprising the steps of:

- a) contacting a sample comprising nc-APRIL with at least one antibody, or antibody coupled to microparticles or microbeads specifically binding to nc-APRIL;
- b) transmitting light to the mixture of step (a);
- c) measuring a change in light scattering intensity of the mixture in response to the irradiation in step (b); and
- d) quantifying the concentration of nc-APRIL in the sample according to the measurement in step (c),

wherein preferably steps (a) to (c) are repeated with at least one dilution of the sample comprising nc-APRIL and/or the at least one antibody.

22. The method according to any one of claim 21, wherein the at least one antibody specifically binding to nc-APRIL comprises

- (a) a variable heavy (VH) chain sequence comprising the amino acid sequence of SEQ ID NO:42, or a sequence having 90%, preferably 95% sequence identity to SEQ ID NO:42; and a variable light (VL) chain sequence comprising the amino acid sequence of SEQ ID NO:46, or a sequence having 90%, preferably 95% sequence identity to SEQ ID NO:46; or
 - (b) a variable heavy (VH) chain sequence comprising the amino acid sequence of SEQ ID NO:49, or a sequence having 90%, preferably 95% sequence identity to SEQ ID NO:49; and a variable light (VL) chain sequence comprising the amino acid sequence of SEQ ID NO:53, or a sequence having 90%, preferably 95% sequence identity to SEQ ID NO:53; or
 - (c) a variable heavy (VH) chain sequence comprising the amino acid sequence of SEQ ID NO:56 or a sequence having 90%, preferably 95% sequence identity to SEQ ID NO:56; and a variable light (VL) chain sequence comprising the amino acid sequence of SEQ ID NO:60 or a sequence having 90%, preferably 95% sequence identity to SEQ ID NO:60
23. A method for predicting and/or diagnosing hypertriglyceridemia, metabolic syndrome, non-alcoholic steatohepatitis, diabetes mellitus type 2, atherogenic dyslipidemia, cardiovascular events and/or atherosclerosis, the method comprising the steps of:
- (a) determining the concentration of non-canonical APRIL (nc-APRIL) in a sample that has been obtained from said subject;
 - (b) comparing the concentration of nc-APRIL that has been determined in step (a) to a reference value; and
 - (c) predicting and/or diagnosing hypertriglyceridemia, metabolic syndrome, non-alcoholic steatohepatitis and diabetes mellitus type 2, atherogenic dyslipidemia, cardiovascular events and/or atherosclerosis in said subject based on the comparison made in step (b).
24. A method for predicting mortality risk in subjects suffering from hypertriglyceridemia, metabolic syndrome, non-alcoholic steatohepatitis, diabetes mellitus type 2, atherogenic dyslipidemia, cardiovascular events and/or atherosclerosis, the method comprising the steps of:
- (a) determining the concentration of nc-APRIL in a sample that has been obtained from said subject;

- (b) comparing the concentration of nc-APRIL that has been determined in step (a) to a reference value; and
 - (c) determining the mortality risk of said subject based on the comparison made in step (b).
25. A method for determining whether a subject is susceptible to the treatment of hypertriglyceridemia, metabolic syndrome, non-alcoholic steatohepatitis, diabetes mellitus type 2, atherogenic dyslipidemia, cardiovascular events and/or atherosclerosis, the method comprising the steps of:
- (a) determining the concentration of nc-APRIL in two or more samples that have been obtained from said subject at an earlier and a later time point;
 - (b) determining that said subject is susceptible to the treatment of hypertriglyceridemia, metabolic syndrome, non-alcoholic steatohepatitis and diabetes mellitus type 2, atherogenic dyslipidemia, cardiovascular events and/or atherosclerosis, if the concentration of nc-APRIL is higher in a sample that has been obtained at a later time point compared to a sample that has been obtained at an earlier time point; or determining that said subject is not susceptible to the treatment of hypertriglyceridemia, metabolic syndrome, non-alcoholic steatohepatitis, diabetes mellitus type 2, atherogenic dyslipidemia, cardiovascular events and/or atherosclerosis, if the concentration of nc-APRIL is similar or lower in a sample that has been obtained at a later time point compared to a sample that has been obtained at an earlier time point,
- wherein preferably the earlier time point is before the beginning of the treatment and the later time point is after the beginning of the treatment; or wherein the earlier and the later time points are after the beginning of the treatment.
26. The method according to claim 25, wherein the treatment comprises the use of the antibody, or antigen-binding fragment thereof, as defined in any one of claims 7 to 11 or the pharmaceutical composition of claim 13.
27. The method according to any one of claims 23 to 26, wherein the cardiovascular events comprise myocardial infarction, stroke, peripheral artery disease, angina pectoris and/or urgent hospitalization for angina leading to revascularization.

28. The method according to any one of claims 23 to 27, wherein the sample is or comprises human serum or human plasma.
29. The method according to any one of claims 22 to 28, wherein the concentration of nc-APRIL is determined with at least one antibody specifically binding to nc-APRIL, wherein preferably at least one antibody specifically binding to nc-APRIL binds to an epitope within the amino acid sequence SEQ ID NO:64 and/or at least one antibody specifically binding to nc-APRIL binds to an epitope within the amino acid sequence SEQ ID NO:96.
30. The kit according to claim 20 or the method for quantifying according to one of claims 14 to 18, wherein the first monoclonal antibody specifically binds to an epitope within the amino acid sequence SEQ ID NO:96 and/or the second monoclonal antibody specifically binds to an epitope within the amino acid sequence SEQ ID NO:64.
31. The kit according to claim 20 or the method for quantifying according to one of claims 14 to 17 or 19, wherein the first monoclonal antibody specifically binds to an epitope within the amino acid sequence SEQ ID NO:64 and/or the second monoclonal antibody specifically binds to an epitope within the amino acid sequence SEQ ID NO:96.
32. The nephelometric assay according to one of claims 21 to 22, wherein at least one antibody specifically binding to nc-APRIL binds to an epitope within the amino acid sequence SEQ ID NO:64 and/or at least one antibody specifically binding to nc-APRIL binds to an epitope within the amino acid sequence SEQ ID NO:96.
33. The method according to any one of claims 23 to 29, wherein the concentration of nc-APRIL is determined with the method according to any one of claims 14 or 30 to 31, the nephelometric assay according to one of claims 21 to 22 or 32, or the kit according to claim 20.
34. An antibody, or an antigen-binding fragment thereof, specifically binding to APRIL, wherein the antibody, or the antigen-binding fragment thereof, binds to an epitope within the amino acid sequence SEQ ID NO:64 or SEQ ID NO:96.