

CLAIMS

1. *In vitro* method for the detection of Apolipoprotein E isotype 4 (ApoE4) or fragments thereof in a blood sample of a subject comprising the steps of:

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- a. contacting said sample with a solid phase, wherein the solid phase is not covered with any binder or capture molecule,

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contacting said sample simultaneously or sequentially with at least one binder binding specifically to ApoE4, thereby forming an ApoE4-binder-complex, wherein said binder is an antibody or a functional antibody fragment or non-IgG protein scaffold, wherein ApoE4 or fragments thereof is immobilized to said solid phase and the binder is bound to ApoE4 and, wherein binding of ApoE4 or the ApoE4-binder-complex to said solid phase occurs in the presence of a detergent, and

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- b. detecting the ApoE4-binder-complex, ~~wherein said ApoE4 binder complex is immobilized to said solid phase, wherein the solid phase is not covered with any binder or capture molecule and wherein binding of ApoE4 or the ApoE4-binder complex to said solid phase occurs in the presence of a detergent,~~

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wherein the fragments of ApoE4 comprise at least 6 amino acids in length with the proviso that the amino acid 158 (Arginine) is encompassed within said fragment, whereby the sequence of the amino acid 158 refers to Seq ID NO.1;

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~~wherein ApoE4 or fragments thereof is immobilized to said solid phase and the binder is bound to ApoE4.~~

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2. The method according to claim 1, wherein the ApoE4-binder-complex is detected qualitatively or quantitatively.
3. The method according to claims 1 to 2, wherein a quantitative detection of the ApoE4-binder-complex indicates the concentration of ApoE4 in said sample.

4. The method according to claims 1 to 3, wherein the binder is an antibody or a functional antibody fragment or non-IgG protein scaffold with a binding affinity constant of at least 10^7 M^{-1} , preferred 10^8 M^{-1} , preferred affinity constant is greater than 10^9 M^{-1} , most preferred greater than 10^{10} M^{-1} .
5. The method according to claims 1 to 4, wherein the binder is labelled with a label in order to be detected, wherein said label is selected from the group comprising chemiluminescent label, enzyme label, fluorescence label or radioiodine label.
6. The method according to claims 1 to 5, wherein the detection of ApoE4 is used for a classification of a subject to be ApoE4 positive or ApoE4 negative, when the level of the detected ApoE4 is above a certain threshold, wherein said threshold is determined by a specific internal positive control.
7. The method according to claim 6, wherein the ApoE4 positive group can be divided into two groups which corresponds to subjects which are homozygous or heterozygous for ApoE4 alleles using a specific threshold, wherein said specific threshold is determined by measuring genotyped homozygous or heterozygous samples.
8. The method according to claims 1 to 7, wherein the solid phase is selected from the group of polymeric surface (e.g. polystyrene), glass surface, cellulose and cellulose derivatives (e.g. nitrocellulose, polyvinylidene fluoride, nylon).
9. The method according to claims 1 to 8, wherein the solid phase is selected from the group ~~comprising~~ of glass or polymeric surface, wherein polymeric surface comprises ~~but is not limited to~~ polystyrene, poly(divinylbenzene), styrene-acrylate copolymer, styrene-butadiene copolymer, styrene-divinylbenzene copolymer, poly(styrene-oxyethylene), polymethyl methacrylate, polyurethane, polyglutaraldehyde, polyethylene imine, polyvinylpyrrolidone, N,N'-methylene bis-acrylamide, polyolefins, polyethylene, polytetrafluoroethylene, polyethylene terephthalate, polypropylene, polyvinylchloride, polyvinylacetate, polyacrylonitrile, polysulfone, poly(ether sulfone), polydimethylsiloxane, pyrolyzed materials, block copolymers, and copolymers of the foregoing, silicones, or silica.

10. The method according to claims 1 to 9, wherein said blood sample is contacted with an unsaturated solid phase.
- 5 11. The method according to claims 1 to 10, wherein a stabilizing protein, ~~optionally in the reaction buffer~~, stabilizes the binding of ApoE4 and fragments thereof in the blood sample to the solid phase by blocking unspecific binding sites and interacting with the conformation of ApoE4, wherein the stabilizing protein is selected from the group comprising bovine serum albumin (BSA), albumin from human serum, casein, milk proteins, immunoglobulins and stabilizing amino acids (e.g. Alanine, Arginine, 10 Glycine, Isoleucine, Lysine, Proline, Serine), preferably the stabilizing protein is bovine serum albumin (BSA).
12. The method according to claim 11, wherein the stabilizing protein is in a reaction buffer.
- 15 ~~12.13.~~ The method according to claim 11, wherein the concentration of BSA as stabilizing protein is in the range of 0-10 %, preferably 0.2-5%, most preferably 0.5-2.5 %.
- 20 ~~13.14.~~ The method according to claims 1-~~12~~13, wherein the detergent is selected from the group of anionic detergents (e.g. sodium dodecylsulfate (SDS)), cationic detergents (e.g. Cetyltrimethylammonium bromide (CTAB)), zwitterionic detergents (e.g. CHAPS) and/ or non-ionic detergents e.g. Tween-20, Triton X-100.
- 25 ~~14.15.~~ The method according to claim ~~13~~14, wherein the Tween-20 concentration is selected from a range of 0.001-10%, preferably 0.01-1 %, most preferably 0.2-0.5 %.
- 30 ~~15.16.~~ The method according to claim ~~13~~14, wherein the Triton X-100 concentration is selected from a range of 0.001-0.5 %, preferably 0.01-0.2 %, most preferably 0.05-0.1 %.
- 35 ~~16.17.~~ The method according to any of the preceding claims, wherein the blood sample is selected from the group of whole blood, serum and citrate plasma, heparin plasma, EDTA-plasma.
- ~~17.18.~~ The method according to any of the preceding claims, wherein the subject is selected from the group of living human or human-organism, preferably a human subject,

wherein the subject is healthy, apparently healthy, diseased with cognitive impairment dementia, in particular AD.

~~18-19.~~ A Kit comprising:

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a. one binder directed against an epitope comprised in the sequence of ApoE4, wherein the binder is labelled with a detectable label,

b. a solid phase,

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c. a detergent containing reaction buffer.

~~19-20.~~ Use of a kit according to claim ~~18~~19 for the *in vitro* detection and/or quantification of ApoE4 in a blood sample of a subject.

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~~20-21.~~ Use of the method according to any one of claims 1 to ~~17~~18 or the use of claim ~~19~~20 for the risk stratification, or stratification for application of therapeutic measures of a disease or condition associated with elevated ApoE4 levels, wherein the disease or condition is selected from the group of cognitive impairment and neurodegenerative diseases including dementia and/or Alzheimer's disease (AD)

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~~21.~~ ~~The use according to claim 20, wherein the disease or condition is selected from the group of cognitive impairment and neurodegenerative diseases including dementia and/or Alzheimer's disease (AD).~~

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22. A method according to any one of claims 1 to ~~17~~18 or the use of any one of claims ~~19~~20 to 21 for detecting the risk to develop AD, wherein an elevated level of ApoE4 above a certain threshold is predictive for an enhanced risk of developing AD.

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23. The method according to any one of claims 1 to ~~17~~18 or the use of any one of claims ~~19~~20 to ~~22~~21, wherein said presence of ApoE4 or the ApoE4-binder-complex is correlated with a risk of developing/incidence of AD.