METHOD FOR DIAGNOSING ALZHEIMER’S DISEASE

A method is provided for diagnosing Alzheimer’s disease using human blood platelets wherein the presence or absence of functioning calcium–dependent potassium channels in blood platelets are determined by employing potassium channel blockers such as apamin or charybdotoxin, the absence of functioning calcium–dependent potassium channels indicating a positive diagnosis for Alzheimer’s disease.
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METHOD FOR DIAGNOSING ALZHEIMER'S DISEASE

Field of the Invention

The present invention relates to a method for diagnosing Alzheimer's disease by detecting the presence or absence of functioning calcium-dependent potassium channels in human blood platelets, the absence of such potassium channels indicating a positive diagnosis of Alzheimer's disease.

Background of the Invention

Alzheimer's disease is a progressive neurodegenerative disorder which causes irreversible damage to brain cells leading to dementia and ultimately death. It is characterized by formation of amyloid plaques and neurofibrillary tangles in the brain. Currently, it is primarily diagnosed by exclusion of other known causes of dementia. Diagnosis at an early stage prior to irreversible changes is practically non-existent.

In order for a therapeutic intervention to be significantly effective, it will have to be administered very early on prior to irreversible changes.

Accordingly, a non-invasive diagnostic test for early diagnosis of Alzheimer's disease would be a most welcomed addition to the diagnostician's armamentarium.

Calcium-dependent potassium channels have been found to be implicated with Alzheimer's disease. Abnormalities of potassium (K⁺) channel function have been reported in cultured cells in Alzheimer's disease (AD).

Depending upon the single channel conductance, a calcium-dependent potassium (KCa) channel is termed high-conductance or maxi-K (100-250 picosiemens (pS)), intermediate-conductance (18-50 pS), or low-conductance (10-14 pS)KCa.

The high conductance KCa is present in neurons, cardiac cells and various types of smooth muscles. The intermediate-conductance channel has been shown to be
present in red blood cells\textsuperscript{4}, and in smooth muscle\textsuperscript{5}. The low-conductance channel is present in a variety of cell types\textsuperscript{6}.

The most important tools to distinguish between low- and high-conductance $K_{Ca}$ are the toxins apamin\textsuperscript{7}, and charybdotoxin\textsuperscript{8}.

Atwal, footnote 2 at page 581, points out that "while charybdotoxin specifically blocks maxi-K, apamin is a potent blocker of the low conductance $K_{Ca}$. However, in certain tissues, for example, rat brain, charybdotoxin may block $K_{Ca}$ of all three types."

Mahaut-Smith\textsuperscript{8a} discloses that blood platelets contain a 30pS conductance charybdotoxin-sensitive channel.

It is also known that iberiotoxin specifically inhibits maxi-K channels (Elelvez et al\textsuperscript{8b}).

A 113 pS $K^+$ channel sensitive to tetraethylammonium has been described as being absent or not functional in cultured fibroblasts from patients with AD\textsuperscript{9}, and this defect was mimicked in normal fibroblasts by the addition of amyloid beta-protein (A\textsubscript{B})\textsuperscript{10}, which is also plentiful in platelets\textsuperscript{11,12}. However, tetraethylammonium is not a selective inhibitor of $K^+$ channels, and so the pharmacological identity of the abnormal channel in cultured fibroblasts is not clear.

U.S. Patent No. 5,580,748 to Alkon et al (issued December 3, 1996) discloses a method for the diagnosis of Alzheimer's disease using human cells such as fibroblasts, buccal mucosal cells, neurons, and blood cells such as erythrocytes, lymphocytes and lymphoblastoid cells, wherein the absence of a functional 133 pS potassium channel in the test cells indicates the presence of Alzheimer's disease. Tetraethylammonium is employed as a potassium channel blocker to aid in detecting the presence of the functioning 113 pS potassium channel.
Description of the Invention

In accordance with the present invention, a method is provided for diagnosing Alzheimer's disease which includes the steps of

(a) obtaining a sample of platelets from a human subject, and

(b) detecting the presence or absence in such platelets of functioning calcium-dependent potassium (K_{Ca}) channels,

the absence of such functioning K_{Ca} channel indicating a positive diagnosis for Alzheimer's disease.

Detection of the absence of the functioning calcium-dependent potassium channel will be indicated by lack of inhibition by a potassium channel blocker which has the ability to block the functioning K_{Ca} channel, and may, for example, include apamin, charybdotoxin, or a combination thereof, depending upon the specific functioning calcium-dependent potassium channel involved.

In addition, in accordance with the present invention, a method is provided for diagnosing Alzheimer's disease which includes the step of detecting the presence or absence of one or more functioning small-conductance calcium-dependent potassium (SK_{Ca}) channels in blood platelets of a human subject, the absence of a functioning SK_{Ca} channel in such platelets indicating a positive diagnosis for Alzheimer's disease.

Detection of the absence of the functioning SK_{Ca} channel will be indicated by lack of inhibition by a SK_{Ca} channel blocker which has the ability to block the functioning SK_{Ca} channel, and may, for example, include apamin, charybdotoxin, or a combination thereof, depending upon the specific SK_{Ca} channel involved.

In addition, in accordance with the present invention, a method is provided for diagnosing Alzheimer's disease which includes the step of detecting the presence or absence of a functioning charybdotoxin-sensitive potassium (K_{Ch}) channel in blood platelets of a human
subject, the absence of a functioning $K_{Ca}$ channel in such platelets indicating a positive diagnosis for Alzheimer's disease.

Detection of the absence of the functioning $K_{Ch}$ channel will be indicated by lack of inhibition by charybdotoxin which has the ability to block the functioning $K_{Ch}$ channel.

Detection of the presence or absence of the functional $K_{ca}$ channel, $SK_{Ca}$ channel and/or $K_{Ch}$ channel may be determined by conventional techniques for measuring electrical currents in cells such as the patch clamp technique disclosed by Sakmann, B. et al \(^{28}\). The presence or absence of the functional potassium channel may also be detected by (1) loading blood platelets with $^{86}$Rb$^+$, (2) stimulating $^{86}$Rb$^+$ efflux (from the platelets via $K_{Ca}$ channels) with thrombin or ionomycin, (3) subjecting the thrombin- or ionomycin-stimulated $^{86}$Rb$^+$ efflux to the action of an appropriate potassium channel blocker, such as apamin, charybdotoxin or a combination thereof, depending upon the particular functional $K_{Ca}$ channel involved, and (4) determining if the $K_{Ca}$ channel blocker significantly inhibits the thrombin- or ionomycin-stimulated $^{86}$Rb$^+$ efflux to cause significant reductions in the $^{86}$Rb$^+$ efflux, a lack of significant inhibition and significant reduction in the $^{86}$Rb$^+$ efflux indicating a positive diagnosis for Alzheimer's disease.

Thus, where the $K_{Ca}$ channel is a small-conductance calcium dependent potassium (SK$_{Ca}$) channel, the potassium channel blocker employed will preferably be apamin or a combination of apamin and charybdotoxin (weight ratio apamin:charybdotoxin from about 4:1 to about 1:1, preferably from about 3:1 to about 1.5:1).

Where the $K_{Ca}$ channel is a charybdotoxin-sensitive potassium ($K_{Ch}$) channel, the potassium channel blocker employed will be charybdotoxin or a combination of
charybdotoxin and apamin (weight ratio charybdotoxin:apamin from about 4:1 to about 1:1, preferably from about 3:1 to about 1.5:1).

Brief Description of the Figures

Figures 1a and 1b are graphs showing the effects of apamin and charybdotoxin on thrombin-stimulated $^{86}$Rb$^+$ efflux in Control subjects (Figure 1a) and in patients with Alzheimer's disease (Figure 1b);

Figures 2a and 2b are graphs showing the effect of $\alpha$-dendrotoxin on thrombin-stimulated $^{86}$Rb$^+$ efflux in Control subjects (Figure 2a) and in patients with Alzheimer's disease (Figure 2b); and

Figures 3a and 3b are graphs showing the effect of apamin and charybdotoxin on ionomycin-stimulated $^{86}$Rb$^+$ efflux in Control subjects (Figure 3a) and in patients with Alzheimer's disease (Figure 3b).

Example

The following clinical experiments were carried out to determine if there is abnormal function of potassium channels in the platelets of patients with Alzheimer's disease.

Methods

Patient selection. Subjects with and without cognitive dysfunction were rigorously assessed annually, with a full range of blood tests to exclude metabolic or other causes of dementia and to assess cognitive function using the Cambridge Examination for Mental Disorders of the Elderly (CAMDEX)$^{25}$.

Diagnoses were made according to criteria of the National Institutes of Neurology and Communicative Disorders-Alzheimer's Disease and Related Disorders Association Work Group (NINCDS-ADRDA)$^{13}$ and the criteria described in the Diagnostic and Statistical Manual of Mental Disorders, Third Edition, Revised (DSM-III-R)$^{14}$ in
addition to data from CT scans, and the SPET regional cerebral blood flow data. Those with an OPTIMA (Oxford Project to Investigate Memory and Aging) diagnosis of 'probable AD' not only had no evidence of any other significant metabolic or psychiatric process that was thought to contribute to the dementia, but also had evidence of both significant medial temporal lobe atrophy on CT, without evidence of moderate or greater white matter change on axial images, and moderate or greater parietotemporal blood flow deficits on SPET. If there was clinical evidence of another process in addition to or other than AD, which the clinician considered could have contributed to the clinical presentation or have given rise to it, then a diagnosis of 'possible AD' was made (four cases, one with ischaemia and three with possible frontal lobe dementia). If, however, the clinical presentation could have been attributed to either AD or another dementia, but the imaging and longitudinal data were highly suggestive of AD on the basis of previously reported necropsy-confirmed cases, and overall the clinician's impression was of AD, then a diagnosis of 'probable AD' was made (four cases, two of frontal lobe dementia, one of vascular disease, and one of hypoxia). In six cases the diagnosis was clearly 'probable AD'.

In a previous necropsy-confirmed cohort, the use of these criteria, using CT and SPET changes alone, without taking into account the clinical history or cognitive profile, had a sensitivity of over 85% and a specificity of over 95%. In the cases with 'probable AD' studied in this series, all of whom remain alive, the likelihood that AD accounted for the dementia is thought to be extremely high. Controls were selected to be age- and sex-matched (Table 1), had no evidence of cognitive dysfunction, i.e. their CAMCOG scores were over 79/108, complained of no memory problems, and did not have the combination of a minimum medial temporal lobe width on CT of less than the fifth centile for controls of the same age with a moderate
or greater parietotemporal perfusion deficit on SPET. Subjects recruited as part of OPTIMA were 14 patients with dementia of the Alzheimer type and 14 non-demented age- and sex-matched controls (details in Table 1); each experiment was performed on 11 or 12 of these individuals.

**Platelet perfusion**. Blood was drawn by venepuncture and anticoagulated with acid-citrate-dextrose (ACD). Platelets were prepared by centrifugation within an hour of venesection and incubated with $^{86}$Rb$^+$ for 2 h. The platelet suspension ($2.5 \times 10^7$ platelets per ml) was then injected into perfusion chambers, where the cells settled and became immobilized on inert filters (Millipore). The platelets were then continuously perfused with Krebs solution, to allow them to stabilize for 20 min before the start of each experiment. The perfusion buffer was then changed for 5 min to Krebs solution containing thrombin (0.3 IU/ml) or ionomycin (1 μM), after which the perfusion was switched to the original solution and continued for another 20 min. From 0 to 10 min after the addition of thrombin or ionomycin the perfusate was collected at 1-min intervals and thereafter at 2-min intervals. The solutions used for perfusion were bubbled with 95% O$_2$ and 5% CO$_2$ throughout the experiment (pH 7.4). At the end of the experiment the polycarbonate filters were retrieved and placed in scintillation vials, to which 3.5 ml of Aquasafe 500 scintillation fluid was added. The radioactivity in the perfusates and filters was determined by liquid scintillation counting in a Beckmann LS 6000 SE counter and the loss of radioactivity from the cells was calculated. The amount of $^{86}$Rb$^+$ (pmol) in each fraction of perfusate collected was determined using the specific activity of the isotope. The radioactivity was measured in each fraction and the results were plotted as the cumulative efflux of $^{86}$Rb$^+$ against time.

**Buffers and drugs.** ACD contained citric acid (15 g), trisodium citrate (25 g), and dextrose (20 g) in 1 l of distilled water. Krebs buffer contained (mmol/l): NaCl
(119); KCl (4.6); CaCl₂ (1.5); Na₂H₄PO₄ (1.2); MgCl₂ (1.2); NaHCO₃ (15); and glucose (11). Apamin, charybdotoxin, α-dendrotoxin, iberiotoxin, ionomycin, and human thrombin were purchased from Sigma Chemical Company, Poole, Dorset.

86RbCl was purchased from Amersham International plc (Amersham, Bucks).

**Thrombin and ionomycin.** Solutions of thrombin were prepared freshly in distilled water for each experiment and further diluted to a concentration of 0.3 IU/ml in Krebs solution. Stock solutions of ionomycin (10 mM in dimethylsulphoxide) were prepared and stored in aliquots at 4°C. On the day of the experiment ionomycin was further diluted in Krebs solution to a concentration of 1 μM.

Apamin, charybdotoxin, iberiotoxin, and α-dendrotoxin. Apamin, charybdotoxin, iberiotoxin, and α-dendrotoxin were freshly prepared for each experiment. Apamin, (100 nM), charybdotoxin (300 nM), iberiotoxin (300 nM), and α-dendrotoxin (200 nM) were reconstituted in distilled water and stored in aliquots at -20°C. On the day of the experiment the toxins were further diluted in Krebs solution. Apamin was pre-incubated with the platelets (added at time -20 min) and charybdotoxin, iberiotoxin,, and α-dendrotoxin were added with thrombin (at 0 min) for a period of 5 min.

**Data presentation and analysis.** The results in Figures 1-3 are shown as cumulative effluxes of 86Rb⁺ from 0 to 14 min. The data are shown as means ± SEMs (n = number of experiments with platelets obtained from different volunteers). The efflux data were analyzed using analysis of variance with repeated measures.

**K⁺ channel fluxes.** Platelets were prepared and their K⁺ channel fluxes studied as described by DeSilva, H.A. et al17, by loading fresh platelets with 86Rb⁺ (used as a radioactive analogue of K⁺)18,19 and stimulating 86Rb⁺ efflux with thrombin and ionomycin. It has already been shown that thrombin and ionomycin stimulate 86Rb⁺ efflux from platelets via K⁺ channels, and that the efflux occurs
via $K_{Ca}$ channels, sensitive to the highly selective inhibitors apamin and charybdotoxin (i.e. small-conductance calcium-dependent, $SK_{Ca}$, channels and charybdotoxin-sensitive, $K_{Ch}$, channels), and via voltage-gated ($K_{V}$) channels, sensitive to $\alpha$-dendrotoxin20.21.

**Uptake of rubidium by platelets.** The uptake of $^{86}\text{Rb}^+$ by the platelets was the same in both groups (not shown). Since over 90% of this uptake in platelets is inhabitable by ouabain and attributable to the sodium/potassium pump (de Silva & Aronson, unpublished observations), this result suggests that the sodium/potassium pump functions normally in AD.

**Non-stimulated $^{86}\text{Rb}^+$ efflux.** Non-stimulated cumulative $^{86}\text{Rb}^+$ efflux was linear with time (open circles; Figures 1-3) and did not differ between AD patients and controls. This efflux is partly mediated by the Na$^+$/K$^+$/2Cl$^-$ co-transport system17.

**Thrombin-stimulated $^{86}\text{Rb}^+$ efflux.** Figures 1a and 1b show the effects of apamin and charybdotoxin on thrombin-stimulated $^{86}\text{Rb}^+$ efflux. Figure 1a relates to Control subjects, and shows that thrombin 0.3 IU/ml (4-filled circles) increased $^{86}\text{Rb}^+$ efflux over the non-stimulated efflux (1-open circles). Apamin 100 nM (3-open squares) and charybdotoxin 300 nM (2-open triangles) inhibited the stimulated $^{86}\text{Rb}^+$ efflux ($n=11$; $P < 0.0001$).

Figure 1b relates to patients with Alzheimer's disease and shows that thrombin 0.3 IU/ml (4-filled circles) increased $^{86}\text{Rb}^+$ efflux over the non-stimulated efflux (1-open circles) to the same extent as in controls ($P = 0.996$). Apamin 100 nM (3-open squares) and charybdotoxin 300 nM (2-open triangles) had no significant effect on the stimulated $^{86}\text{Rb}^+$ efflux ($n=12$; $P = 0.941$).

Figures 2a and 2b show the effect of $\alpha$-dendrotoxin on thrombin-stimulated $^{86}\text{Rb}^+$ efflux. Figure 2a relates to Control subjects and shows that thrombin 0.3 IU/ml (filled circles) increased $^{86}\text{Rb}^+$ efflux over the non-stimulated
efflux (open circles). α-dendrotoxin 200 nM (open triangles) inhibited the thrombin-stimulated efflux (n=11; P < 0.0001). Figure 2b relates to patients with Alzheimer's disease and shows that thrombin 0.3 IU/ml (filled circles) increased $^{86}$Rb$^+$ efflux over the non-stimulated efflux (open circles). α-dendrotoxin 200 nM (open triangles) inhibited the thrombin-stimulated efflux (n=12; P < 0.0001).

Control subjects. In control subjects, thrombin stimulated an increase in $^{86}$Rb$^+$ efflux from platelets (Figures 1a and 2a). In 8 of 11 control subjects, apamin and charybdotoxin inhibited the thrombin-stimulated $^{86}$Rb$^+$ efflux by at least 18% and 16% respectively, while in the other three subjects these toxins had minimal effects (less than 10% inhibition). When the data from all the control subjects were pooled, both apamin and charybdotoxin caused significant reductions in $^{86}$Rb$^+$ efflux (Figures 1a; Table 2). In addition, α-dendrotoxin inhibited thrombin-stimulated $^{86}$Rb$^+$ efflux from the platelets of all the controls (Figure 2a; Table 2). These results are similar to the effects of these toxins on thrombin-stimulated $^{86}$Rb$^+$ efflux in the platelets of young volunteers$^{20,21}$, and they confirm that there are $\text{SK}_\text{Ca}$, $\text{K}_\text{Ch}$, and $\text{KV}$ channels in normal human platelets.

Alzheimer's disease. Thrombin also stimulated $^{86}$Rb$^+$ efflux from the platelets of 12 patients with AD (Figures 1b and 2b), to the same extent as in controls (cf. Figures 1a and 2a with Figures 1b and 2b). In contrast to the results in controls, apamin and charybdotoxin had minimal effects on thrombin-stimulated $^{86}$Rb$^+$ efflux (less than 10% inhibition) in 9 of 12 patients with AD, while in the other three patients each toxin inhibited the thrombin-stimulated efflux by at least 16%. When the data from all the patients with AD were pooled, neither apamin nor charybdotoxin caused significant reductions in $^{86}$Rb$^+$ efflux (Figure 1b; Table 2). In contrast, α-dendrotoxin inhibited the thrombin-stimulated $^{86}$Rb$^+$ efflux from the platelets of all the patients with AD (Figure 2b; Table 2).
Ionomycin-stimulated \(^{86}\text{Rb}^+\) efflux.

Figures 3a and 3b show effect of apamin and charybdotoxin on ionomycin-stimulated \(^{86}\text{Rb}^+\) efflux.

As seen in Figure 3a, **Control subjects**: Ionomycin 1 \(\mu\text{M}\) (5-filled circles) increased \(^{86}\text{Rb}^+\) efflux over the non-stimulated efflux (1-open circles). Apamin 100 nM (4-open squares) and charybdotoxin 300 nM (3-open triangles) inhibited the stimulated \(^{86}\text{Rb}^+\) efflux (P < 0.0001). Apamin and charybdotoxin combined (2-filled squares) inhibited the stimulated efflux more than either toxin alone (\(n=11\); P < 0.0001).

As seen in Figure 3b, **Alzheimer's disease**: Ionomycin 1 \(\mu\text{M}\) (5-filled circles) stimulated \(^{86}\text{Rb}^+\) efflux over the non-stimulated efflux (1-open circles) to the same extent as in controls (P = 0.960). Apamin 100 nM (4-open squares) and charybdotoxin 300 nM (3-open triangles), either alone or in combination (2-filled squares), had no significant effect on the stimulated \(^{86}\text{Rb}^+\) efflux (\(n=11\); P = 0.883).

**Control subjects.** In 9 of 11 control subjects, apamin and charybdotoxin inhibited the ionomycin-stimulated \(^{86}\text{Rb}^+\) efflux by at least 18% and 22% respectively, while in two subjects these toxins had minimal effects (less than 12% inhibition). When the data from all the controls were pooled, both apamin and charybdotoxin caused significant reductions in \(^{86}\text{Rb}^+\) efflux (Figure 3a; Table 2). The two toxins combined had a greater effect than either toxin alone (Figure 3a; Table 2). These results are similar to those seen in young volunteers\(^{20,21}\), and they confirm the presence of \(\text{SK}_{\text{Ca}}\) and \(\text{K}_{\text{Ch}}\) channels in human platelets.

**Alzheimer's disease.** Ionomycin also stimulated \(^{86}\text{Rb}^+\) efflux from the platelets of 11 patients with AD (Figure 3b), to the same extent as in controls (cf. Figures 3a and 3b). However, apamin, charybdotoxin, and their combination had minimal effects on the ionomycin-stimulated \(^{86}\text{Rb}^+\) efflux in 8 of 11 patients with AD (less than 12% inhibition). When the data from all the patients with AD
were pooled, apamin and charybdotoxin, either alone or in combination, had minimal effects on $^{86}\text{Rb}^+$ efflux (Figure 3b; Table 2).

Although abnormalities of $K^+$ channels have been reported in AD in neural cells (reduced post-mortem binding of $^{125}$I-apamin to hippocampal neurones$^{22}$) and in fibroblasts (absence of a 113 pS channel sensitive to tetraethylammonium$^3$), this study is the first to show functional abnormalities of $K^+$ channels in the platelets of patients with AD. The $^{86}\text{Rb}^+$ effluxes in platelets in response to both thrombin and ionomycin were quantitatively normal in AD, and the thrombin-stimulated efflux showed normal sensitivity to inhibition with $\alpha$-dendrotoxin, suggesting that the $K_v$ channels are normal in platelets in AD. However, the lack of inhibition of both thrombin-stimulated and ionomycin-stimulated effluxes by apamin and charybdotoxin suggests either that the $SK_{Ca}$ and $K_{Ch}$ channels are not present in platelets in AD or, if they are present, that they are not sensitive to inhibition by these toxins.

If $SK_{Ca}$ and $K_{Ch}$ channels are present in platelets in AD, but unresponsive to inhibition, that would be consistent with the observation that the binding of $^{125}$I-apamin is reduced in post-mortem hippocampal neurones in AD$^{22}$. This might be due to a change in the structure of the binding sites of the inhibitors. Alternatively, it might be due to an abnormality of the specific interaction of calcium with the channels, since the efflux stimulated by ionomycin, which increases the intracellular concentration of calcium, was not inhibitable.

Furthermore, the $K_v$ channels were not affected, suggesting that $K_{Ca}$ channels are selectively impaired in AD.

Alternatively, $SK_{Ca}$ and $K_{Ch}$ channels may not be present at all in AD. However, if that is so, then $^{86}\text{Rb}^+$ efflux must be occurring through other $K^+$ channels, since the thrombin-stimulated and ionomycin-stimulated effluxes were quantitatively normal. However, normal human
platelets do not contain large-conductance calcium-dependent (BK\textsubscript{Ca}) channels\textsuperscript{21}, and iberiotoxin, a selective inhibitor of BK\textsubscript{Ca} channels, had no effect on ionomycin-stimulated effluxes in platelets from any individual (Table 2), while the only other type of K\textsuperscript{+} channel found in platelets, K\textsubscript{v} channels\textsuperscript{20,21}, responded normally to α-dendrotoxin. These observations argue against upregulation of other normal channels in AD. On the other hand, metabolites of the beta-amyloid precursor protein (β-APP) are capable of de novo formation of K\textsuperscript{+} channels\textsuperscript{23} that are insensitive to some inhibitors\textsuperscript{1}, and the \textsuperscript{86}Rb\textsuperscript{+} efflux detected in AD might be via such channels.

Absence or non-functionality of the K\textsubscript{Ca} channels might also explain the observation\textsuperscript{24} that platelets from patients with AD had a higher thrombin-stimulated rise in intracellular Ca\textsuperscript{2+}, since that might represent an exaggerated attempt to switch on non-existent or non-functional channels.

The frequencies of alleles ε2, ε3, and ε4 of the apolipoprotein E gene in the patients and controls are shown in Table 3. As expected, significantly more patients with AD had one or two ε4 alleles. Nine of the 13 individuals (patients and controls) who had one or two ε4 alleles had abnormalities of inhibition of K\textsubscript{Ca} channels, compared with only five of the 15 who had no ε4 alleles (P=0.06), suggesting a link between the K\textsuperscript{+} channel abnormalities and the presence of the ε4 allele. No such relationship was found between the K\textsuperscript{+} channel abnormalities and either the ε2 or the ε3 allele.

In conclusion, there were abnormalities of inhibition of SK\textsubscript{Ca} and K\textsubscript{Ch} channels in the platelets of patients with AD compared with matched controls. Thus, K\textsubscript{Ca} channel abnormalities provide a marker for patients with AD.
Table 1. Patient data. The data are given as mean (sd). There were no significant differences between the groups.

<table>
<thead>
<tr>
<th>Measure</th>
<th>Alzheimer's disease (n = 14)</th>
<th>Controls (n = 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>67.1 (7.5)</td>
<td>67.9 (7.7)</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>9/5</td>
<td>9/5</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>154 (16)</td>
<td>140 (15)</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>90 (14)</td>
<td>81 (14)</td>
</tr>
<tr>
<td>Serum Sodium (mM)</td>
<td>139 (3)</td>
<td>139 (2)</td>
</tr>
<tr>
<td>Serum potassium (mM)</td>
<td>3.9 (0.4)</td>
<td>4.0 (0.3)</td>
</tr>
<tr>
<td>Serum Calcium (mM)</td>
<td>2.43 (0.10)</td>
<td>2.32 (0.13)</td>
</tr>
<tr>
<td>Serum urea (M)</td>
<td>5.7 (1.3)</td>
<td>6.3 (2.7)</td>
</tr>
<tr>
<td>Serum creatinine (µM)</td>
<td>104 (12)</td>
<td>103 (24)</td>
</tr>
<tr>
<td>Blood glucose (mM)</td>
<td>5.9 (2.1)</td>
<td>5.6 (1.1)</td>
</tr>
<tr>
<td>Platelet count (x10^9/l)</td>
<td>233 (58)</td>
<td>233 (62)</td>
</tr>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>13.9 (0.8)</td>
<td>14.2 (1.1)</td>
</tr>
<tr>
<td>Mean cell volume (fl)</td>
<td>89 (4)</td>
<td>90 (5)</td>
</tr>
<tr>
<td>White cell count (x10^9/l)</td>
<td>6.9 (1.5)</td>
<td>6.5 (1.5)</td>
</tr>
<tr>
<td>Total serum cobalamins (ng/l)</td>
<td>270 (90)</td>
<td>315 (125)</td>
</tr>
</tbody>
</table>
Table 2. Inhibition of thrombin-stimulated and ionomycin-stimulated $^{86}$Rb$^+$ effluxes by apamin, charybdotoxin, $\alpha$-dendrotoxin, and iberiotoxin in platelets of patients with AD and age- and sex-matched controls. Data are given as median (interquartile range) percentages. Statistical comparisons by rank sum tests.

**Thrombin-stimulated $^{86}$Rb$^+$ efflux**

<table>
<thead>
<tr>
<th>Toxin</th>
<th>Inhibition in controls (%)</th>
<th>Alzheimer's disease (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apamin</td>
<td>26 (20-27)</td>
<td>0 (0-0)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Charybdotoxin</td>
<td>22 (18-29)</td>
<td>0 (0-4)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>$\alpha$-dendrotoxin</td>
<td>26 (20-29)</td>
<td>19 (18-27)</td>
<td>NS</td>
</tr>
</tbody>
</table>

**Ionomycin-stimulated $^{86}$Rb$^+$ efflux**

<table>
<thead>
<tr>
<th>Toxin</th>
<th>Inhibition in controls (%)</th>
<th>Alzheimer's disease (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apamin</td>
<td>30 (20-31)</td>
<td>1 (0-20)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Charybdotoxin</td>
<td>28 (20-34)</td>
<td>4 (1-26)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Apamin + Charybdotoxin</td>
<td>51 (34-52)</td>
<td>2 (0-40)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Iberiotoxin</td>
<td>2 (0-3)</td>
<td>0 (0-4)</td>
<td>NS</td>
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</tbody>
</table>
Table 3. Numbers of normal and abnormal results in patients and controls. An abnormal result was defined as less than 15% inhibition by apamin and charybdotoxin. Statistical comparisons were by chi-square test. For comparison the distributions of apolipoprotein E alleles in the two groups are also shown.

<table>
<thead>
<tr>
<th>Agonist</th>
<th>Alzheimer's disease</th>
<th>Controls</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrombin</td>
<td>No. with an abnormal result</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>No. with a normal result</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>Ionomycin</td>
<td>No. with an abnormal result</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>No. with a normal result</td>
<td>2</td>
<td>8</td>
</tr>
</tbody>
</table>

Apolipoprotein E allele frequencies

<table>
<thead>
<tr>
<th>Allele</th>
<th>Disease</th>
<th>Controls</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ε2/2</td>
<td>0</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>ε2/3</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>ε3/3</td>
<td>4</td>
<td>8</td>
<td>&lt;0.03†</td>
</tr>
<tr>
<td>ε3/4</td>
<td>5</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>ε4/4</td>
<td>4</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

*chi-squared test
†chi-squared test for trend
References


6. R. Inoue et al, Pflügers Archiv. 405, 173 (1985);


What is claimed is:

1. A method for diagnosing Alzheimer's disease, which comprises obtaining a sample of platelets from a human subject, and detecting the presence or absence of functioning calcium-dependent potassium (K$_{Ca}$) channels of specified slope conductance in such platelets, the absence of said functioning K$_{Ca}$ channel indicating a positive diagnosis for Alzheimer's disease.

2. The method as defined in Claim 1 wherein the absence of said functioning K$_{Ca}$ channel is indicated by lack of inhibition by a potassium channel blocker which has the ability to block the functioning K$_{Ca}$ channel.

3. The method as defined in Claim 2 wherein the potassium channel blocker is apamin or charybdotoxin or a combination thereof.

4. The method as defined in Claim 1 wherein the presence or absence of the calcium-dependent potassium channel is determined by (1) loading blood platelets with $^{86}$Rb$^+$, (2) stimulating $^{86}$Rb$^+$ efflux (from the platelets via K$_{Ca}$ channels) with thrombin or ionomycin, (3) subjecting the thrombin- or ionomycin-stimulated $^{86}$Rb$^+$ efflux to the action of a potassium channel blocker, and (4) determining if the potassium channel blocker significantly inhibits the thrombin- or ionomycin-stimulated $^{86}$Rb$^+$ efflux to cause significant reductions in the $^{86}$Rb$^+$ efflux, a lack of significant inhibition and significant reduction in the $^{86}$Rb$^+$ efflux indicating a positive diagnosis for Alzheimer's disease.

5. A method for diagnosing Alzheimer's disease, which comprises detecting the presence or absence of one or more functioning small-conductance calcium-dependent potassium (SK$_{Ca}$) channels in blood platelets of a human subject, the absence of a functioning SK$_{Ca}$ channel in such platelets indicating a positive diagnosis for Alzheimer's disease.
6. The method as defined in Claim 5 wherein the absence of a functioning SKCa channel is indicated by lack of significant inhibition by a SKCa channel blocker which has the ability to block the functioning SKCa channel.

7. The method as defined in Claim 6 wherein the potassium channel blocker is apamin.

8. The method as defined in Claim 6 wherein the potassium channel blocker is a combination of apamin and charybdotoxin.

9. The method as defined in Claim 5 wherein the presence or absence of the SKCa channel is determined by (1) loading blood platelets with $^{86}\text{Rb}^+$, (2) stimulating $^{86}\text{Rb}^+$ efflux from the platelets via $K_{\text{Ca}}$ channels with thrombin or ionomycin, (3) subjecting the thrombin- or ionomycin-stimulated $^{86}\text{Rb}^+$ efflux to the action of a SKCa channel blocker, and (4) determining if the SKCa channel blocker significantly inhibits the thrombin- or ionomycin-stimulated $^{86}\text{Rb}^+$ efflux to cause significant reductions in the $^{86}\text{Rb}^+$ efflux, a lack of significant inhibition and significant reduction in the $^{86}\text{Rb}^+$ efflux indicating a positive diagnosis for Alzheimer's disease.

10. A method for diagnosing Alzheimer's disease, which comprises detecting the presence or absence of a functioning charybdotoxin-sensitive potassium ($K_{\text{Ch}}$) channel in blood platelets of a human subject, the absence of a functioning $K_{\text{Ch}}$ channel in such platelets indicating a positive diagnosis for Alzheimer's disease.

11. The method as defined in Claim 10 wherein the absence of a functioning $K_{\text{Ch}}$ channel is indicated by lack of significant inhibition by charybdotoxin which has the ability to block the functioning $K_{\text{Ch}}$ channel.

12. The method as defined in Claim 10 wherein the absence of a functioning $K_{\text{Ch}}$ channel is indicated by lack of significant inhibition by a combination of charybdotoxin and apamin.
13. The method as defined in Claim 10 wherein the presence or absence of the charybdotoxin-sensitive channel is determined by (1) loading blood platelets with $^{86}$Rb$^+$, (2) stimulating $^{86}$Rb$^+$ efflux (from the platelets via $K_{Ca}$ channels) with thrombin or ionomycin, (3) subjecting the thrombin- or ionomycin-stimulated $^{86}$Rb$^+$ efflux to the action of a $K_{Ch}$ channel blocker, and (4) determining if the $K_{Ch}$ channel blocker significantly inhibits the thrombin- or ionomycin-stimulated $^{86}$Rb$^+$ efflux to cause significant reductions in the $^{86}$Rb$^+$ efflux, a lack of significant inhibition and significant reduction in the $^{86}$Rb$^+$ efflux indicating a positive diagnosis for Alzheimer's disease.
**INTERNATIONAL SEARCH REPORT**

**International application No.**
PCT/US98/23198

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(6) : G01N 33/20  
US CL : 436/56, 63, 69, 79

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 436/56, 63, 69, 79

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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[X] Further documents are listed in the continuation of Box C.  

See patent family annex.

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**Date of the actual completion of the international search**

08 MARCH 1999

**Date of mailing of the international search report**

08 APR 1999

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