度は，95施設が参加し，2,336人が一次検診を受診した．うち1,037人が二次検診を受診，117人が三次検診の対象となった．未受診や受診拒否の42名を除き，三次検診受診者75名の内訳は，アルツハイマー型認知症26名，脳血管性認知症6名，そのほかの認知症6名，軽度認知障害17名，うつ病4名，異常なし16名であった．一次検診受診者のうち2.1％が認知症あるいは軽度認知障害であった[3]（Level 3）．

徳島県徳島市医師会では，2005（平成17）年度40歳以上基本検診対象者53,290名のうち希望者3,643名がもの忘れ検診を受診した．一次検診は問診表を用いており，1,061人が精査が必要とされ，755人が二次検診を受診した．二次検診にはMini mental state examination（MMSE）を用い，医師が実施した．その結果755人中210人が認知症を疑われ，精密検査を実施し，アルツハイマー型認知症97名，脳血管性認知症31名，の計128名が認知症と診断された．受診者の3.5％が認知症であった（Level 2）．

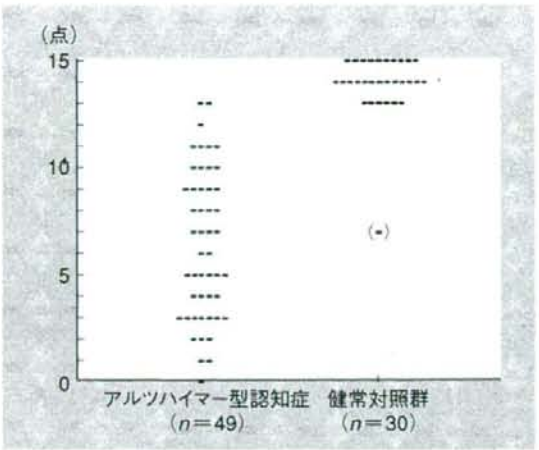
群馬県の検診は，60歳以上を対象として，一次スクリーニングとして20項目からなる自記式アンケートを事前配布しチェックして基本検診会場へ持参してもらう．5項目未満は異常なしと判定し，5項目以上あると二次スクリーニングとしてMMSEを保健師など専門職が実施する．MMSE 25点以上は異常なし，MMSE 24点以下はかかりつけ医あるいは専門医へ診察依頼を行う．2003年度は群馬県内の新町，妙義町，北橘村，粕川村，黒保根村，榛名町を対象として実施し，一次スクリーニング受診者5,139名，5項目以上のチェックがあり二次スクリーニングを受診した者1,633名（31.8％）で，うちMMSE 24点以下が281名（5.5％）で，精査の結果異常なしが111名（2.2％），かかりつけ医で経過観察が119名（2.3％），専門医で精検が53名（1.0％）であった．2004（平成16）年度は，新町，妙義町，北橘村，粕川村，黒保根村，榛名町，大間々町を対象として実施し，一次スクリーニング受診者6,921名，5項目以上のチェックがあり二次スクリーニングを受診した者1,429名（20.6％）で，うちMMSE 24点以下が228名（3.3％）で，精査の結果異常なしが88名（1.3％），かかりつけ医で経過観察が70名（1.0％），専門医で精検が70名（1.0％）であった[4]（Level 3）．

ファイブコグを用いる方法

65歳以上の茨城県利根町民約3,000人の対象者に，一次スクリーニングとして気分状態〔GDS（geriatric depression scale）〕，ADL（activity of daily living）・IADL（instrumental ADL）評価，認知機能テスト（ファイブコグ）を実施した．ファイブコグとは前駆状態診断に特化した認知テストで（記憶，注意，推論，言語，視空間認知），プロジェクターで表示，最大50名までの集団で施行可能，所要時間は30分である．二次調査としては，構造化面接と個別テストを行う．GDSからうつが疑われる者はすべて，ほかはランダムに対象を選別：精神科医師が認知機能，精神状態の診断を行う．対象者の70％が参加し，集団テストのほかに個別訪問，施設調査，介護保険申請書により調査を行った．結果として，認知症の頻度は，65歳以上町民の10％と推定（従来のわが国調査では6％程度），前駆状態の頻度はMCI（mild cognitive impairment）：3％（5歳幅の各年齢層で一定），AACD（age-assciated cognitive decline）1 memory：7％であった[5]（Level 3）．

タッチパネル式コンピュータを使った認知症スクリーニング機器を用いる方法

アルツハイマー型認知症49例，健常対照群30例を対象とした．タッチパネル式コンピュータは音声と映像による対話形式で，質問に答えながらゲーム感覚で検査を受けることができる．言葉や日時に関する質問，立方体を識別する質問など合計5問で構成し，所要時間は結果の印刷まで含め



❶タッチパネル式認知症スクリーニング検査をアルツハイマー型認知症患者と健常対照者に施行した結果

❷タッチパネル式認知症スクリーニング機器（物忘れ相談プログラム®）の実物

て合計5分以内で可能である．15点満点でアルツハイマー型認知症ではほとんどの例が12点以下であり（❶），専門医への受診が望まれる．感度（疾患がある場合，検査が陽性になる割合）96％，特異度（疾患がない場合，検査が陰性になる割合）97％と高い信頼性を示した[6]．この信頼性に加えて，この方法の利点としては，質問者による差がない，精神的，身体的ストレスが少ない，どこでも簡単に施行できる，などがあげられる．このようなことから，タッチパネル式コンピュータを用いた認知症のスクリーニング機器は，"物忘れ相談プログラム®" という商品名で日本光電から販売され，一般に利用することが可能となっている（❷）．現在定期的に行うことで，確実に認知症の早期発見に役立てることが可能である．この機器を用いて行われた検診を以下に紹介する．

山口県周防大島町での検診

山口県周防大島町は瀬戸内海に浮かぶ人口約2万2,000人の町である．ここでの検診は65歳以上高齢者すべてを対象とし，一次スクリーニングテストとしてタッチパネル式コンピュータによる認知症スクリーニング機器（物忘れ相談プログラム®）を用いている．15点満点のうち13点未満のものを二次検診の対象としている．二次検診ではMMSE，IADL，健康生活調査を行っている．MMSE 24点以下を要精密検査とし，専門医療機関へ紹介としている．2004年度は979名が一次検診を受診され，13点以下が237名（24.2％）であった．この237名に二次検診を実施し，29名（13.8％）が要精密検査となった．2005年度は，724名が一次検診を受診され，13点以下が163名（22.5％）であった．この163名に二次検診を実施し，56名（35.6％）が要精密検査となった．2005年度は，MCIを落とさないようにするためMMSEの二次検診でのカットオフ値を26点以下としたため，56名（35.6％）と要精密検査が著増した[7]．いずれにしても，一次検診でタッチパネル式コンピュータによる認知症スクリーニング機器を用いると，この段階での漏れが少ないことが容易に考えられる（Level 3）．

鳥取県東伯郡琴浦町での検診

2004年9月1日に東伯町と赤碕町が合併して，琴浦町となった．人口20,119人で，65歳以上人口5,782人，高齢化率28.7％である．一次スクリーニングテストとしてタッチパネル式コンピュータ

による認知症スクリーニング機器（物忘れ相談プログラム®）を用いている．15点満点のうち13点未満のものを二次検診の対象としている．二次検診ではタッチパネル式コンピュータを用いたADAS（TDAS）を行っている[8]．2004年度（旧東伯地区）対象者2,767名，受診者558名（受診率20％），二次検診受診者208人（37.3％）で，MCIあるいは軽度認知症と考えられた者が93名（16.7％）であった（Level 3）．

認知症検診の方法に関する考察

問診表を用いる方法は費用がかからず検診を行う際の負担も少ないが，検出率が低いように思われる．認知症患者は病識がなくなるため，進行するともの忘れを自覚しなくなる．このために問診に記載しないということが起こる．また，初期の段階であれば，もの忘れを自覚していながら，わざとチェックをしないということもある．われわれの地域でも問診表の段階で異常なしと判定された人に，タッチパネル式コンピュータによる認知症スクリーニング機器を実施したところ，異常者を多く検出した．ファイブコグは，直接検査を行うので，問診表よりよく，ファイブコグはデータからみると認知症の検出力は高く，優れた方法と考えられる．ただ，問題点としては，集団検査であること，検査時間が30分と長いことである．タッチパネル式コンピュータによる認知症スクリーニング機器を用いる方法は，個別に検査でき，時間も短く（約3分間）最も優れた方法と考える．ただ，この方法の問題点はこの機器の購入に費用がかかることである．

今後，認知症予防は大変重要な課題であり，より簡便でかつ精度のよい方法論を構築し，エビデンスを出していくことが求められる．

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随想

認知症は治療可能な時代に―― 認知症早期発見・予防の課題と展望

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浦上克哉

認知症は現在65歳以上の高齢者の10人に1人の頻度でみられる極めて“ありふれた疾患“である。また、認知症の中で最も頻度の多いアルツハイマー型認知症は20人に1人で存在する。アルツハイマー型認知症には現在塩酸ドネペジル（商品名：アリセプト）が治療薬として使用可能であり、有効性が広く報告されている。さらに、現在アミロイドβ蛋白のワクチン療法などの根本治療薬となりうる薬剤が急速な勢いで開発され、おそらく10年以内には使用可能となることが期待される。しかし、根本治療薬ができても早期に診断ができなければ効果は期待できない。そこで、今後の対策としては、来るべき治療可能な時代に向けて、認知症の早期検診を実施する必要があると考える。そこで、現在認知症早期発見のための検診そして認知症予防教室がいろいろな地域で行われている。予防というと病気にならないようにすることとのみを考える人が多いが、予防の概念は広く、このような1次予防のことのみではない。病気の早期発見、早期治療は2次予防で、病気の悪化、進展を防止するのが3次予防である。軽度認知障害（MCI）と考えられるケースに対して、認知症予防教室を実施する（1次予防）。認知症を早期発見できれば、アリセプトによる早期治療開始が可能である（2次予防）。認知症の進行したケースであったが合併症や環境の改善を図ることにより、症状の悪化、進展が防げる（3次予防）。

問題点としては、地域での認知症への偏見が根強く、認知症予防検診及び予防教室への参加率がまだ高くないことである。認知症への正しい理解を広めて、早期発見、早期治療、予防が行われることが望まれる。特に認知症予防検診を地域で実践して感じることは、若い世代（30歳～65歳代）への啓蒙活動の必要性である。検診で認知症の軽度と思われる方を見つけても、家族の理解が得られないとなかなか受診につながらない。また、受診されて治療に入っても、家で対応される家族が認知症を理解しているか否かで臨床経過が大きく異なります。このような家族が若い世代に該当します。著者は、これまで市民フォーラム、地域の公民館での講演会などで認知症の正しい理解を得ていただくための啓蒙活動を行っているが、対象のほとんどが高齢者（65歳以上）である。これからの啓蒙活動として、若い世代へ向けたものが必要と考える。地域において認知症に関するフォーラムなどが多く企画されているが、若い世代が多く来ていただけるような企画を考える必要があると思う。

物忘れ相談プログラムによる認知症検診

タッチパネル式のスクリーニングで予防教室を展開

鳥取県下の認知症対策事業

高齢化に伴い増加しつづける認知症に対する施策は重要かつ急務です。初期・発症以前の段階での脳の活性化によるトレーニングや生活習慣の改善等によって、進行や発症を遅らせる予防効果を得られることが、明らかにされてきています。

予防効果を高めるために不可欠なのが、対象者の的確なスクリーニングです。正常と認知症との中間的と考えられるMCI(軽度認知機能障害)や初期の認知症の人を高い精度で判別し適切な予防サービスを提供し、認知症と予防に対する意識を地域でどう深めるかという課題に取り組む鳥取県内の事例を紹介します。

画面の指示とヘッドフォンからの音声にしたがってパネルに指で触れて設問に答えていく。

手軽なタッチパネル方式

MCIまたは初期の認知症の人を判別することを目的に、鳥取大学医学部保健学科の浦上克哉教授の指導で開発されたタッチパネル式の認知症スクリーニングは、検査の簡易さと検出率の高さから導入する市町村が鳥取県内外で増えています。

1次検診の設問内容は、広く用いられているHDS-R(改訂長谷川式簡易認知症評価スケール)を簡略化し、図形認識テストを加えたもの。設問を示す画面に軽く触れればよく、スタッフは、必ずしも専門職である必要はなく講習を受けたボランティア等でも十分に対応可能です。

検査時間は一人あたり3~5分程度、2時間で1台あたり30人前後のスクリーニングが可能です。手軽に実施でき対面式にありがちな心理的マイナス要因がないうえ、検者による違いが出にくく信頼度の高い検査結果を得ることができます。

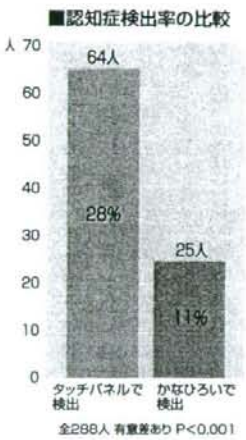
「正常な人からすでに認知症が進行している人まで同じプログラムで行う予防教室では、効果を上げるのはむずかしい。予防教室の対象者の選定と事業前後の評

価の的確さが何よりも大切です。被検者が検査されることにストレスを感じることも避けたい、最小限のマンパワーでより多くのスクリーニングが可能となる方法の確立が必要でした」（浦上教授）

検出率は約30%近くに

タッチパネル方式による1次検診のスクリーニングで13点以下（15点満点）の人は、2次検診の対象者と判定されます。認知症のスクリーニング法の一つ、かなひろいテストではおもに前頭葉、タッチパネル方式ではおもに側頭・頭頂葉の機能を反映するとされています。平成15・16年度に鳥取県下の一町一村で認知症予防教室を実施する際、鳥取大学医学部を中心とする研究グループが計228人の高齢者を対象に行ったテストでは、かなひろいでは25人（11%）が、タッチパネル方式では64人（28%）が検出されました。

MCIやアルツハイマー病の初期は前頭葉よりむしろ側頭・頭頂葉の機能が関わっているため検出率の差につながったとみられています。



2次検診を経て予防教室へ

2次検診もタッチパネル方式によって行われ、その設問内容にはADAS（アルツハイマー症評価スケール）が一部改変されたうえで組み込まれています。

ADASの検査には臨床心理士等の専門家が必要で地域の認知症予防教室等での実施はむずかしいのが実情でしたが、タッチパネル方式によるADASは、1人あたりの検査時間を20分程度に短縮でき必ずしも専門家を要しないことから普及が可能となりました。

判定基準は6点以下が正常範囲、14点以上ならば専門医療機関での精密検査などの受診勧奨、予防教室は7点以上13点以下が対象です。2次検診後は専門医が個別に診察とアドバイスを行います。

講演会と1次検診をセットに

鳥取県琴浦町（人口約2万人、高齢化

1次検診の設問内容

これから言う3つの言葉を次のうちから選んで下さい。 あとでまた聞きますからよく覚えておいて下さい。	うめ　ねこ　でんしゃ きく　うし　ひこうき さくら　いぬ　じどうしゃ わからない	（3点満点）
今日は何年の何月何日ですか。何曜日ですか。	月曜日　金曜日 火曜日　土曜日 水曜日　日曜日 木曜日	（4点満点）
先ほど覚えてもらった言葉を次のうちから選んで下さい。	うめ　ねこ　でんしゃ きく　うし　ひこうき さくら　いぬ　じどうしゃ わからない	（6点満点）
見本の図形を違う角度から見たものを、右の図形から選んで下さい。	わからない	（2点満点）
合　計		点／15点満点

（カットオフ値：12点以下）

引用資料：浦上克哉（Jpn J Cancer Chemother 30(suppl.I): 49-53,December,2003より改編）
（資料の使用許可を得ています）

楽しくからだと頭を使って脳の活性化を図る。教室での1コマ。

率約28％、平成16年9月に東伯町と赤碕町が合併）では、平成16年度からタッチパネル式の検診によって対象者を選び、認知症予防教室を展開してきました。

「近年、目立ってきたのが認知症の相談ですが、すでに深刻化しているケースが多く、早期発見・対応、予防対策の必要性を痛感せざるを得ませんでした」（健康福祉課地域包括支援センター係長・藤原静香さん）

こうした実態を踏まえ、平成15年度には認知症予防対策委員会を立ち上げ、予防検診・予防教室を中心とした事業への本格的な取り組みをスタートさせました。

平成16年度には、「ひらめきはつらつ教室」を半年かけて順次開催しました。対象者は旧東伯町内の要介護・要支援認定者以外の高齢者2768人。老人クラブを通じたチラシ配布等による呼びかけにより、対象者の20％にあたる558人が参加しました。1次検診受診者の37％にあたる208人に2次検診が勧奨され156人が受診（受診率75％）、専門医の診察を経て128人（2次受診者の82％）が介護予防教室「ほほえみの会」に参加しました。「会」は約3カ月、週1回、旧東伯町内13会場で開催されました。

翌平成17年度には、前年9月の合併で琴浦町となった旧赤碕町エリア13会場で「ひらめきはつらつ教室」を開催。老人クラブ役員や民生委員に対する説明会では、チラシと声かけの徹底による協力を要請し、対象者1957人のうち442人が参加し、参加率は前年度を上回る23％となりました。1次検診による要フォロー者は173人（参加者の39％）、2次検診受診者は130人（受診率75％）、「ほほえみの会」への参加は99人（2次受診者の76％）でした。

「『教室』は、認知症についての理解を深める講演と検診をセットにしています。講演では、認知症は誰でもおこる可能性のある病気であること、予防検診の大切さが強調されます。チラシでもきちんと明示しているので検診が受け入れやすく、受けずに帰られる参加者は一人もいません」（藤原さん）

特定高齢者施策として位置づける

平成18年度は、再び旧東伯町エリアで同内容の「ひらめきはつらつ教室」が開催され、2次検診を経て、「ほほえみの会」が実施されています。前年度と異なるのは、「ほほえみの会」が地域支援事業の特定高齢者施策事業として位置づけられ、月2回・6カ月間の日程となったこと。実際の運営には、地域包括支援センターが立案した計画に基づき、事業を受託した介護予防サービス事業所があたります。参加者には個別の予防プログラムが立てられ、終了時の評価によって、さらに6カ月間継続する人と卒業生とに分かれます。平成18年度の前期には、84人（7会場）が参加して行われ、卒業生によるOB会にあたる「悠遊クラブ」の活動も始まっています。

「ボランティアを含めマンパワーの底上げが課題。『教室』の参加率は基本検診の受診率も30％台であることから土台の数字としてけっして低い数字ではないと思います。さらに浸透を図っていきたい」（藤原さん）

楽しく継続できるプログラムを

プログラムは「みんなで楽しく」を基本に、「リラックス」「からだを動かす」「脳の活性化」を三本柱に組み立てます。

実際どのように行われているかを見学しました。血圧測定などのバイタルチェックの後、得点の合計点を足し算するボール投げ、記憶テスト、唱歌・童謡の歌詞のなぞり書きと合唱などのメニューが

「銭太鼓」は発表の場もあり、参加者のモチベーションがあがる。会での練習風景。

実施され、参加者たちには笑い声が絶えません。

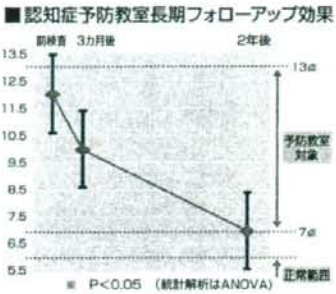
県内の米子市でもタッチパネル方式による検診で対象者を選ぶ認知症予防教室が市の独自事業として5会場で実施されています。オリジナルの体操、回想法、料理実習など多彩なプログラムが展開され、蚊屋地区では、ボランティアの協力による「銭太鼓」と呼ばれる民俗芸能などのプログラムを盛り込むといった地域を巻き込んだ工夫がみられます。

「的確なスクリーニングにより的を絞ったサービス提供を行えば、全12回程度で6カ月間か3カ月間の実践で効果が得られる。具体的に個別のプログラムに対するエビデンスを確認するには至っていないが、プログラム全体の効果は明らか。まずは参加者が楽しく継続していけることが大切です。終了後の長期的なフォローアップによる効果もはっきりしており、継続していくための場を用意することも重要です」（浦上教授）

■認知症予防教室のTDASによる評価

平均2.1点の低下（改善傾向）

■認知症予防教室長期フォローアップ効果



※ P<0.05　（統計解析はANOVA）

認知症対策を軸にしたまちづくり

誤解に基づくうわさ、2次検診の通知を受けたことを家族に内緒にする、世間体を気にした家族が本人の教室参加を反対するなどの偏見は、現在でも完全になくなったわけではありません。

「専門機関の受診が必要な人のうち病院等で受診する割合は5割程度。本人の遠慮や家族の無理解から受診に至らないことも多いはず。中年・若年世代も含め地域全体への啓発が急務です」（浦上教授）

町では、町医師会に協力を求め「認知症を考える会」（年2回）の開催や、認知症に対する正しい理解と意識のあり方の普及・啓発に向けた取り組みも進めています。平成16年度から開催されている一般町民向けの「認知症をささえるまちづくりフォーラム」もその一つです。

平成18年3月開催のフォーラムでは、「ほほえみの会」参加者と家族による体験発表も行われ、500人にのぼる参加者の前で堂々と語りました。認知症の講演や座談会、町内の小学生による認知症の絵本の朗読など盛り沢山で、大盛況となりました。このフォーラムは「認知症サポーター講座」として位置づけられ「サポーターとして今後取り組んでみたいか」の質問に「協力したい」と答えた人は59%にのぼりました。

認知症予防教室等の財政面での事業効果については、参加者群と不参加者群の要介護認定申請率の違いなどから推計が行われ「ひらめきはつらつ教室」が年約2360万円、「ほほえみの会」が年約3600万円もの給付費抑制につながったとの試算が出ています。介護保険特別会計の平成17年度予算規模が約15・6億円であることから考えると、かなり大きな経済効果を生んでいることになります。ただし、これら不参加群には「引きこもり」等の問題を抱える人が多いとも予測され、不参加群へのアプローチと効果の精査なども今後の課題となっています。

田中満雄町長は「認知症対策はまちづくりそのものともいえる重要な課題。予防事業の展開やフォーラムの成功により、認知症への誤解や偏見は以前に比べるとかなり払拭されてきたと思います。認知症についてこれからも啓発に努める一方、家族や自分がなった場合でも安心して暮らしていける社会をどうつくるのか、町民みんなで考えていきたい」と今後のまちづくりに向けて抱負を語っています。

Human Molecular Genetics, 2007, Vol. 16, No. 23 **2854–2869**
doi:10.1093/hmg/ddm244
Advance Access published on August 30, 2007

Genetic association of *CTNNA3* with late-onset Alzheimer's disease in females

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Received July 18, 2007; Revised and Accepted August 22, 2007

Alzheimer's disease (AD), the most common form of dementia in the elderly, was found to exhibit a trend toward a higher risk in females than in males through epidemiological studies. Therefore, we hypothesized that gender-related genetic risks could exist. To reveal the ones for late-onset AD (LOAD), we extended our previous genetic work on chromosome 10q (genomic region, 60–107 Mb), and single nucleotide polymorphism (SNP)-based genetic association analyses were performed on the same chromosomal region, where the existence of genetic risk factors for plasma Aβ42 elevation in LOAD was implied on a linkage analysis. Two-step screening of 1140 SNPs was carried out using a total of 1408 subjects with the *APOE-ε3*3* genotype: we first genotyped an exploratory sample set (LOAD, 363; control, 337), and then genotyped some associated SNPs in a validation sample set (LOAD, 336; control, 372). Seven SNPs, spanning about 38 kb, in intron 9 of *CTNNA3* were found to show multiple-hit association with LOAD in females, and exhibited more significant association on Mantel–Haenszel test (allelic *P*-values$_{MH\text{-}F}$ = 0.000005945–0.0007658). Multiple logistic regression analysis of a total of 2762 subjects (LOAD, 1313; controls, 1449) demonstrated that one of the seven SNPs directly interacted with the female gender, but not with the male gender. Furthermore, we found that this SNP exhibited no interaction with the *APOE-ε4* allele. Our data suggest that *CTNNA3* may affect LOAD through a female-specific mechanism independent of the *APOE-ε4* allele.

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INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disorder clinically characterized by progressive cognitive deterioration and is the most common form of dementia in the elderly. Its neuropathological features are amyloid plaques [extracellular deposition of amyloid β-protein (Aβ)] and neurofibrillary tangles (intracellular aggregation of highly phosphorylated microtubule-associated protein tau), which finally lead to synaptic loss and/or neuronal death.

Recent epidemiological studies on AD revealed gender-related differences in its prevalence (1–3) and incidence (4–6). Compared with males, females are more likely to develop AD, although results contradicting this gender difference have been reported (7–9). In blood mononuclear cells in AD, there are substantial gender differences in gene expression (10). The plasma level of amyloid beta-protein 42 (Aβ42), a major constituent of senile plaques, is significantly increased in females with mild cognitive impairment, a transitional state between normal aging and mild dementia (11). In transgenic animal models of AD, gender-dependent accumulation and deposition of Aβ42 and Aβ40 have been observed (12–15). Moreover, there has been increasing research on gender-related genetic risk factors in AD: *ACT* (16), *MPO* (17,18), *ACE* (19), *ESR2* (20), *DSC1* (21) and *ABCA1* (22). Therefore, based on these findings, we hypothesized that gender-related genetic risk factors that modify Aβ metabolism in late-onset AD (LOAD), which accounts for 95–99% of AD, could exist.

We have paid a great deal of attention to chromosome 10q, especially because the existence of genetic risk factors for plasma Aβ42 elevation in it was implied on linkage analysis of LOAD families (23). Furthermore, through other genetic approaches, including genome-wide linkage screening of affected sib pairs (24) and candidate gene-based analysis of multiplex AD families (25), chromosome 10q was strongly suggested to be the most prominent one for LOAD. Therefore, regarding a genomic region on chromosome 10q (60–107 Mb), we previously performed large-scale single nucleotide polymorphism (SNP)-based screening of a Japanese population to identify additional genetic risk factors to *APOE* (19q13.2), which is universally recognized as a major risk gene for the development of LOAD (OMIM +107741). Consequently, we found that *DNMBP*, which is involved in synaptic vesicle recycling, was associated with LOAD with the *APOE-ε3*3* genotype or lacking the *APOE-ε4* allele in several sample sets (26).

Interestingly, replicated evidence for a parent-of-origin effect of chromosome 10q was recently reported for LOAD (27,28), which suggests that gender-related genes such as imprinting genes could be responsible for the disease development. Here, in order to determine whether or not gender-related loci associated with LOAD are present, our previous genetic work on chromosome 10q (26) was extended. Two sample sets for screening, *Exploratory* and *Validation*, comprising only *APOE-ε3*3* subjects were prepared, which were used for a case–control association study after being stratified as to gender. We first genotyped the Exploratory set, and then genotyped some significantly associated SNPs in the Validation set. Through this stepwise screening, among the 1140 SNPs subjected to the exploratory screening, we finally found seven SNPs located in intron 9 of *CTNNA3* that showed reproducible association with LOAD in females. These replicated SNPs were further examined by means of genotyping of all the subjects with all *APOE* genotypes (*ε2*2*, *ε2*3*, *ε2*4*, *ε3*3*, *ε3*4* and *ε4*4*), i.e. 1526 LOAD patients (female, 1103; male, 423) and 1666 controls (female, 998; male, 668), some of them exhibiting significance only in a female sub-sample set. In terms of biological functions, *CTNNA3* (29,30), encoding α-T catenin, is thought to be a promising candidate for LOAD because it is a binding partner of β-catenin, which interacts with PSEN1 (31), and because it was recently shown to be associated with the level of plasma Aβ42 in a set of families with LOAD (32). Multiple logistic regression analysis in a total of 2762 subjects (LOAD, 1313; controls, 1449) revealed that one (SNP rs713250) of the seven associated SNPs exhibits a significant interaction with the female gender, but not with the male gender and the *APOE-ε4* allele. Our data suggest that *CTNNA3* could affect LOAD through a female-specific mechanism independent of the *APOE-ε4* allele.

RESULTS

Allelic association

To determine whether gender-related loci associated with LOAD on chromosome 10q (60–107 Mb) exist or not, we stratified the Exploratory sample set (Table 1) by gender, resulting in female and male subsets. An allelic contingency table (2×2)-based χ^2 test was performed using already-obtained genotype data (26) for 1140 SNPs for the Exploratory set. Calculation of allelic *P*-values and odds ratios (ORs) with 95% confidence interval (CI) was carried out to examine the genetic association of these SNPs. In a Japanese population, these SNPs were actually polymorphic and showed a *P*-value >0.05 in exact tests of Hardy–Weinberg equilibrium (HWE) in both cases and controls of the Exploratory set (details given under Materials and Methods). The results of χ^2 tests for the gender-stratified sets are presented in Fig. 1. In the female group (LOAD, 249; controls, 223), 106 of the 1140 SNPs had significant allelic *P*-values <0.05, and 34 of these 106 showed more significant values (allelic *P*-values <0.01). In the male group (LOAD, 114; controls, 114), 53 of the 1140 SNPs showed allelic *P*-values <0.05, and 7 of these 53 showed more significant association with allelic *P*-values <0.01.

A total of 41 SNPs (34 and 7 SNPs in female and male Exploratory sets, respectively) showing allelic *P*-values <0.01 were further analyzed by means of χ^2 tests to determine whether or not these SNPs actually exhibit reproducible allelic association using another sample set, Validation, sub-grouped as to gender (Table 1). In the male Validation set (LOAD, 94; controls, 159), three of the above-mentioned seven SNPs showed reproducible association (allelic *P*-values = 0.0342 – 0.046). Among these three SNPs, only SNP rs1000280 exhibited a significant value on Mantel–Haenszel test (allelic $P\text{-value}_{\text{MH-M}} = 0.0009112$). This SNP is located in the intergenic region between *LOXL4* (100.00–100.02 Mb) and *C10orf33* (100.13–100.16 Mb); therefore, we did not

Table 1. Subject information

Sample set ID		Number of subjects	AAO/AAE Mean (SD)	Range	MMSE Mean (SD)	Range	*APOE* Genotype						Allele		
							*2*2*	*2*3*	*2*4*	*3*3*	*3*4*	*4*4*	*ε2*	*ε3*	*ε4*
Overall set															
All	Female														
	LOAD	1103	73.5 (6.6)	60–93	15.7 (7.0)	0–30	0	31	13	491	465	103	44	1478	684
	Control	998	73.0 (7.9)	60–96	28.0 (1.8)	24–30	2	77	9	748	152	10	90	1725	181
	Male														
	LOAD	423	73.3 (6.6)	60–93	18.4 (6.6)	0–30	1	18	4	208	148	44	24	582	240
	Control	668	73.1 (7.7)	60–95	28.1 (1.8)	24–30	1	55	6	495	104	7	63	1149	124
Subsets															
Negative-ε4	Female														
	LOAD	522	74.6 (7.0)	60–93	15.1 (7.4)	0–30	0	31	—	491	—	—	31	1013	—
	Control	827	73.1 (7.9)	60–96	28.0 (1.8)	24–30	2	77	—	748	—	—	81	1573	—
	Male														
	LOAD	227	73.6 (7.2)	60–93	17.9 (7.3)	0–30	1	18	—	208	—	—	20	434	—
	Control	551	73.0 (7.8)	60–95	28.1 (1.8)	24–30	1	55	—	495	—	—	57	1045	—
Positive-ε4	Female														
	LOAD	581	72.6 (6.0)	60–92	16.3 (6.6)	0–30	—	—	13	—	465	103	13	465	684
	Control	171	72.7 (7.6)	60–90	28.0 (1.9)	24–30	—	—	9	—	152	10	9	152	181
	Male														
	LOAD	196	72.9 (5.8)	60–86	18.9 (5.7)	1–30	—	—	4	—	148	44	4	148	240
	Control	117	73.7 (7.4)	60–91	27.9 (1.9)	24–30	—	—	6	—	104	7	6	104	124
*ε3*3*	Female														
	LOAD	491	74.7 (7.0)	60–93	15.1 (7.3)	0–30	—	—	—	491	—	—	—	982	—
	Control	748	73.1 (7.9)	60–96	28.0 (1.8)	24–30	—	—	—	748	—	—	—	1496	—
	Male														
	LOAD	208	73.7 (7.3)	60–93	18.0 (7.3)	0–30	—	—	—	208	—	—	—	416	—
	Control	495	73.0 (7.8)	60–95	28.1 (1.8)	24–30	—	—	—	495	—	—	—	990	—
Screening sets															
Exploratory	Female														
	LOAD	249	74.3 (6.2)	62–90	15.7 (7.2)	0–30	—	—	—	249	—	—	—	498	—
	Control	223	80.2 (4.1)	75–96	28.0 (1.9)	24–30	—	—	—	223	—	—	—	446	—
	Male														
	LOAD	114	74.6 (6.8)	62–93	19.2 (7.6)	0–30	—	—	—	114	—	—	—	228	—
	Control	114	80.6 (4.0)	75–95	28.0 (2.0)	24–30	—	—	—	114	—	—	—	228	—
Validation	Female														
	LOAD	242	75.0 (7.7)	60–93	14.7 (7.3)	0–29	—	—	—	242	—	—	—	484	—
	Control	213	75.5 (4.7)	70–94	27.8 (1.9)	24–30	—	—	—	213	—	—	—	426	—
	Male														
	LOAD	94	72.6 (7.6)	60–92	16.8 (6.9)	0–29	—	—	—	94	—	—	—	188	—
	Control	159	75.7 (4.5)	70–92	28.1 (1.8)	24–30	—	—	—	159	—	—	—	318	—

The sample set IDs used in this study, i.e. single SNP case–control study, linkage disequilibrium and case–control haplotype analyses, and multiple logistic regression analysis, are shown in italics.

investigate this SNP further. In the female Validation set (LOAD, 242; controls, 213), 16 of the above-mentioned 34 SNPs exhibited allelic association with *P*-values <0.05. These SNPs exhibited significance on Mantel–Haenszel test of the two female sets (allelic $P\text{-values}_{\text{MH-F}} = 0.000005945 - 0.0008809$). These allelic $P\text{-values}_{\text{MH-F}}$ remained at significant levels even after Bonferroni's correction for 34 tests (allelic $P\text{-values}_{\text{MH-F(B)}} = 0.0002021 - 0.02995$). Of the 16 SNPs, 9 (rs911541, rs3740066, rs11190302, rs35715207, rs3758394, rs3740058, rs3740057, rs11190315 and rs6584331) are located in a locus between *ENTPD7* and *DNMBP* recently reported by our group (26). The remaining seven, rs7909676, rs2394287, rs4459178, rs10997307, rs12258078, rs10822890 and rs713250, spanning about 38 kb, are encompassed by intron 9 of *CTNNA3*, which consists of 18 exons (Fig. 2A and C). The allelic *P*-values of these seven SNPs in the two sample sets, Exploratory and Validation, are presented in Table 2, and marker information on them is summarized in Table 3. The genotypic and allelic distributions are presented in the Supplementary Material, Table S1.

To examine the gender-specific effects of the seven *CTNNA3* SNPs on LOAD, we additionally performed joint analysis regarding gender (Table 2). For this analysis, female and male allelic contingency tables were combined for the Exploratory and Validation sets, respectively (Supplementary Material, Table S1). χ^2 tests based on the combined 2×2 allelic contingency tables and calculation of the ORs with 95% CI were carried out. In the Exploratory set comprising both genders, none of these seven SNPs showed more significant association (allelic *P*-values $= 0.00005431 - 0.0235$) in comparison with the Exploratory set only including females (allelic *P*-values $= 0.00004614 - 0.008$). The ORs exhibited a tendency to decrease; for example, for SNP rs10822890, from 1.72 to 1.55. A similar trend for both the allelic

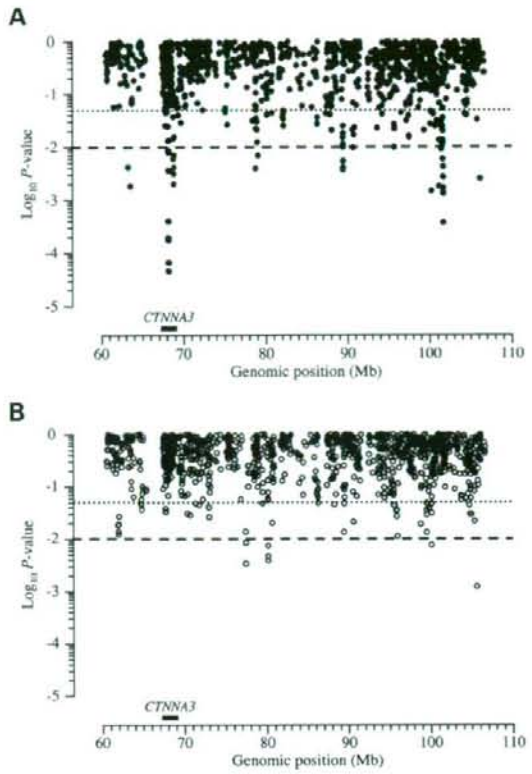

Figure 1. Allelic *P*-values of 1140 SNPs for the Exploratory set comprising female (**A**) (LOAD, 249; control, 223) or male (**B**) (LOAD, 114; control, 114) *APOE-ε3*3* subjects. Dotted and dashed lines indicate allelic *P*-values at the 0.05 and 0.01 levels, respectively. The significantly associated locus focused on in this study is indicated by the thick line, which is labeled '*CTNNA3*'. The genomic position conformed to NCBI build 35.1.

P-values and ORs of these seven SNPs was observed on Mantel–Haenszel test.

The reproducible seven SNPs on *CTNNA3* were further examined by means of stratified analysis, based on the carrier status of the *APOE-ε4* allele, with the χ^2 test (Table 4). The genotypic and allelic distributions are presented in the Supplementary Material, Table S2. We used the overall sample set, *All*, including all subjects (LOAD, 1526; controls, 1666) with all *APOE* genotypes (*2*2*, *2*3*, *2*4*, *3*3*, *3*4* and *4*4*), and two sub-sample sets, *Negative-ε4* and *Positive-ε4*, which were stratified as to the presence (*2*4*, *3*4* and *4*4)* or absence (*2*2*, *2*3* and *3*3*) of the *APOE-ε4* allele (Table 1). As shown in Table 4, in the All set, five (rs7909676, rs2394287, rs4459178, rs10822890 and rs713250) of the seven SNPs were statistically significant in females (allelic *P*-values = 0.0009719 – 0.00126). In the Negative-*ε4* set, all seven SNPs exhibited more significant association with LOAD in females (allelic *P*-values = 0.00001019 – 0.002555). No evidence was found of association with any of the seven SNPs in males in any sample set.

For joint analysis concerning gender, female and male contingency tables (2 × 2) with the allelic distributions were combined for the All, Negative-*ε4* and Positive-*ε4* sample sets, respectively (Supplementary Material, Table S2). Allelic *P*-values and ORs (95% CI) derived from the combined contingency tables were used to evaluate the gender-specific effects on LOAD (Table 4). This analysis revealed that in the All set including both genders, the ORs of significant SNPs (rs7909676, rs10822890 and rs713250) tended to be lower, compared with those in the female All set; for example, from 1.23 to 1.11 for SNP rs713250. A similar decreasing tendency for ORs of significant SNPs (rs7909676, rs2394287, rs4459178, rs10997307, rs12258078, rs10822890 and rs713250) in the Negative-*ε4* set including both genders was also observed in comparison with those in the female Negative-*ε4* set; for example, from 1.42 to 1.24 for SNP rs10822890.

Multiple logistic regression analysis, involving *APOE-ε4*, gender, age, the seven replicated SNPs on *CTNNA3* and their interactions as independent variables, was performed to assess the potential effects of these variables on the association with LOAD, using 2762 subjects [LOAD, 1313 (female, 949; male, 364); controls, 1449 (female, 877; male, 572)] (Table 5). In this analysis, the subjects used were not sub-grouped as to gender and/or carrier status of the *APOE-ε4* allele. Initially, we carried out multiple logistic regression analysis with a forward stepwise method without interaction terms to elucidate which variables explained an association with LOAD independently. Model 1 in Table 5 shows significant risk factors selected by this analysis. Expectedly, the *APOE-ε4* allele, gender and age, which are well-known risk factors for LOAD, had significant effects on the LOAD risk. Among the seven associated SNPs, SNP rs713250 was chosen as representative and selectively entered in this model [for genotype CC: OR (95% CI), 1.36 (1.08–1.71); *P*-value = 0.009]. Following this primary analysis, we further assessed second-order interaction terms created by the four significant risk factors including the SNP rs713250 (Model 2 in Table 5). Six interactions were tested by means of a forward stepwise method in addition to *APOE-ε4*, gender, age and the SNP rs713250. It was demonstrated that the SNP rs713250 exhibited significant interaction with the female gender in a dose-dependent manner as to the allele C [TC_female, OR (95% CI) = 1.68 (1.12–2.54); CC_female, OR (95% CI) = 2.57 (1.59–4.17)].

Linkage disequilibrium and case–control haplotype analyses

To reveal genetic relationship between each significant SNP on *CTNNA3*, linkage disequilibrium (LD) and haplotype estimation analyses were performed. For these analyses, we used four sample sets (All as the overall sample set, and Negative-*ε4*, Positive-*ε4* and *ε3*3* as sub-sample sets) after being sub-grouped as to gender (Table 1). From the Japanese HapMap genotype data (JPT), these SNPs were found to be encompassed by a highly structured LD block extending about 80 kb from 68.10 to 68.18 Mb (Fig. 2B). They were in strong LD: the robust LD block structures did not differ between females and males or between LOAD and controls in any sample set (Supplementary Material, Fig. S1).

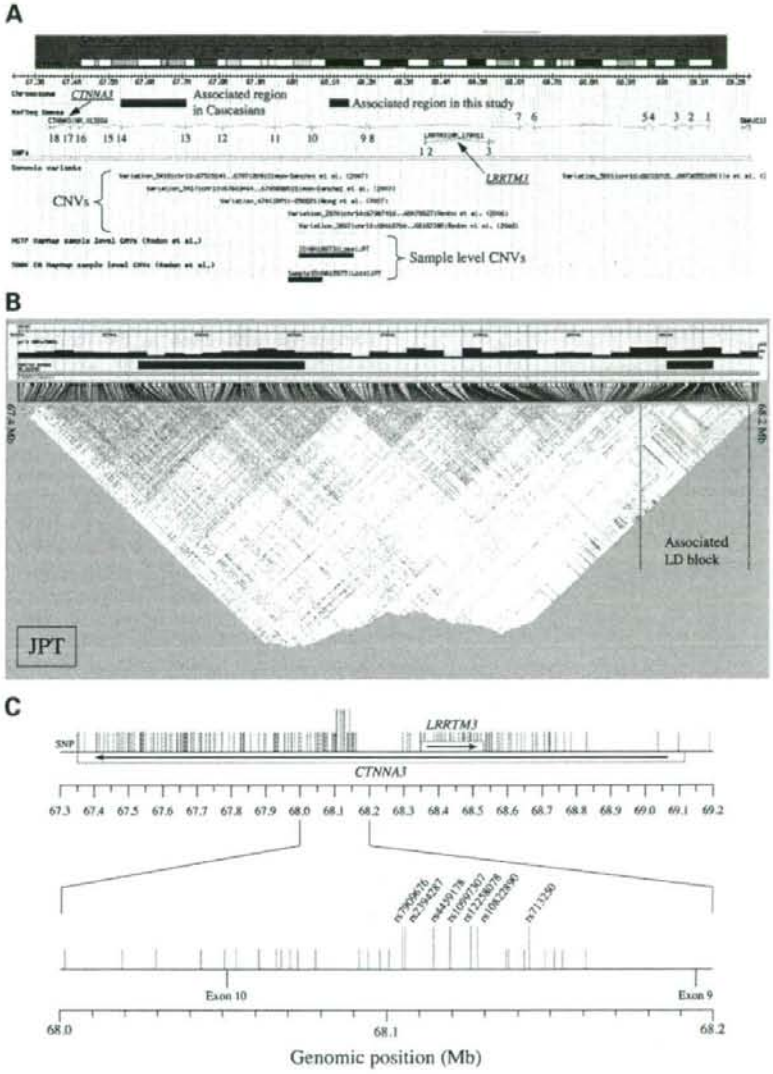

Figure 2. Genomic position and LD block structure of an associated locus within *CTNNA3*. (**A**) Genomic region including *CTNNA3* from web site Database of Genomic Variation (http://projects.tcag.ca/variation/). Boxes filled in green and black, also used in Fig. 2B, represent the associated genomic regions identified here and in studies on Caucasians (32,35,37), respectively. Each exon of *CTNNA3* and *LRRTM3* is numbered; CNV, copy number variation. (**B**) Overview of the LD pattern between 67.4 and 68.2 Mb in Japanese. HapMap genotype data (1768 SNPs) on 45 unrelated Japanese in Tokyo (JPT) were used as calculation of LD measures, D'. (**C**) Physical positions of the seven replicated SNPs. Vertical lines indicate the SNPs used in this study: significantly associated SNPs are indicated by the long labeled vertical lines. Horizontal arrows within open boxes indicate the transcription orientations of individual genes. The mapping position of each SNP is according to dbSNP build 125 on NCBI build 35.1.

Four haplotypes were estimated in each LD block consisting of the seven SNPs: three major haplotypes (frequency >0.1), [H1]C-A-T-T-T-A-T, [H2]A-G-C-C-G-G-C and [H3]A-G-C-T-T-G-C, and one minor haplotype, [H4]C-A-T-T-T-A-C (Table 6). H1 exhibited the highest frequency (range 0.4363–0.5356) and H4 the lowest (range 0.0084–0.031). Haplotypes H1, H2 and H3 were always estimated with the expectation-maximization (EM) algorithm in the four sample sets examined. Haplotype H4 was not inferred in either Negative-*ε4* or *ε3*3* consisting of male subjects.

Table 2. Statistics for seven reproducible SNPs found on two-step screening involving *APOE-ε3*3*

Sample set	Exploratory		Validation		Exploratory + Validation[a]	
Female						
Number of subjects						
LOAD	249		242		491	
Control	223		213		436	
dbSNP	Allelic *P*-value	OR (95% CI)	Allelic *P*-value	OR (95% CI)	Allelic *P*-value	OR (95% CI)
rs7909676	***0.0004042***	1.61 (1.23–2.09)	***0.0132***	1.40 (1.07–1.82)	***0.00002087***	1.50 (1.24–1.81)
rs2394287	***0.0001782***	1.64 (1.27–2.13)	***0.0427***	1.31 (1.01–1.71)	***0.00004311***	1.47 (1.22–1.77)
rs4459178	***0.0001939***	1.64 (1.26–2.13)	***0.0296***	1.34 (1.03–1.75)	***0.00002885***	1.49 (1.23–1.79)
rs10997307	***0.008686***	1.45 (1.10–1.91)	***0.0372***	1.34 (1.02–1.76)	***0.0008809***	1.39 (1.15–1.69)
rs12258078	***0.008***	1.44 (1.10–1.89)	***0.0352***	1.34 (1.02–1.76)	***0.0007658***	1.39 (1.15–1.68)
rs10822890	***0.00004614***	1.72 (1.32–2.23)	***0.0266***	1.35 (1.04–1.75)	***0.000008277***	1.52 (1.27–1.83)
rs713250	***0.00006663***	1.69 (1.31–2.20)	***0.0162***	1.38 (1.06–1.80)	***0.000005945***	1.53 (1.27–1.84)
Male						
Number of subjects						
LOAD	114		94		208	
Control	114		159		273	
dbSNP	Allelic *P*-value	OR (95% CI)	Allelic *P*-value	OR (95% CI)	Allelic *P*-value	OR (95% CI)
rs7909676	0.2961	1.22 (0.84–1.77)	0.1933	0.78 (0.54–1.13)	0.8507	0.98 (0.75–1.27)
rs2394287	0.3418	1.20 (0.83–1.74)	***0.0183***	0.64 (0.44–0.93)	0.3125	0.87 (0.67–1.14)
rs4459178	0.3456	1.20 (0.82–1.74)	***0.0209***	0.65 (0.45–0.94)	0.3231	0.88 (0.67–1.14)
rs10997307	0.9594	1.20 (0.69–1.48)	0.2477	0.79 (0.54–1.17)	0.4350	0.90 (0.68–1.18)
rs12258078	0.8457	1.01 (0.71–1.52)	0.1901	0.77 (0.52–1.14)	0.4308	0.90 (0.68–1.18)
rs10822890	0.2235	1.26 (0.87–1.83)	***0.0237***	0.65 (0.45–0.95)	0.4534	0.91 (0.70–1.18)
rs713250	0.2588	1.24 (0.85–1.79)	0.0578	0.70 (0.49–1.01)	0.5761	0.93 (0.72–1.20)
Female + male						
Number of subjects						
LOAD	363		336		699	
Control	337		372		709	
dbSNP	Allelic *P*-value	OR (95% CI)	Allelic *P*-value	OR (95% CI)	Allelic *P*-value	OR (95% CI)
rs7909676	***0.0004364***	1.47 (1.19–1.82)	0.1668	1.16 (0.94–1.44)	***0.0005443***	1.30 (1.12–1.52)
rs2394287	***0.0002861***	1.48 (1.20–1.84)	0.6336	1.05 (0.85–1.30)	***0.003812***	1.25 (1.07–1.45)
rs4459178	***0.0003147***	1.48 (1.20–1.84)	0.5868	1.06 (0.86–1.31)	***0.003417***	1.25 (1.08–1.46)
rs10997307	***0.0321***	1.28 (1.02–1.60)	0.2511	1.14 (0.91–1.42)	***0.02028***	1.20 (1.03–1.41)
rs12258078	***0.0235***	1.29 (1.03–1.61)	0.2757	1.13 (0.91–1.40)	***0.01778***	1.21 (1.03–1.41)
rs10822890	***0.00005431***	1.55 (1.25–1.92)	0.4934	1.08 (0.87–1.33)	***0.0008589***	1.29 (1.11–1.50)
rs713250	***0.00008381***	1.53 (1.24–1.89)	0.2786	1.12 (0.91–1.39)	***0.0003985***	1.31 (1.13–1.52)

Allelic *P*-values and ORs, with 95% CI in parentheses, are indicated. Boldface indicates statistically significant results (allelic *P*-value <0.05). The genotypic and allelic distributions are shown in the Supplementary Material, Table S1.
[a]Computed by the method of Mantel and Haenszel.

Table 3. Summary of seven associated SNPs within intron 9 of *CTNNA3*

dbSNP	Genomic position (bp)[a]	Alleles[b]	Exploratory				Validation			
			GSR	Frequency[c]	HWE[d]		GSR	Frequency[e]	HWE[f]	
					LOAD	Control			LOAD	Control
rs7909676	68 104 803	C/A	96.43	0.507/0.493	0.3377	0.8206	96.75	0.506/0.494	0.6566	0.9161
rs2394287	68 105 668	A/G	97.57	0.521/0.480	0.5934	0.5760	97.88	0.517/0.483	0.741	1.0000
rs4459178	68 114 303	T/C	96.71	0.512/0.488	0.9137	0.5778	96.47	0.514/0.486	0.5784	0.9159
rs10997307	68 119 438	T/C	95.00	0.633/0.367	0.9107	0.6173	97.74	0.640/0.360	1.0000	0.1316
rs12258078	68 125 734	T/G	99.29	0.641/0.359	0.9116	0.6219	99.01	0.641/0.359	1.0000	0.1686
rs10822890	68 127 819	A/G	97.71	0.516/0.484	0.5208	0.5757	97.60	0.515/0.486	0.5055	1.0000
rs713250	68 143 405	C/T	98.43	0.501/0.499	0.5211	0.6579	98.45	0.503/0.497	0.7415	0.9170

GSR, genotyping success rate.
[a]Based on dbSNP build 125 on NCBI build 35.1.
[b]Nucleotides of the major allele/minor allele.
[c]The major allele/minor allele frequency, calculated using genotype data obtained for 363 LOAD patients and 337 controls with *APOE-ε3*3* in the Exploratory set.
[d]*P*-values were calculated with exact tests of HWE using both 363 LOAD patients and 337 controls with *APOE-ε3*3* in the Exploratory set.
[e]The major allele/minor allele frequency, calculated using genotype data obtained for 336 LOAD patients and 372 controls with *APOE-ε3*3* in the Validation set.
[f]*P*-values were calculated with exact tests of HWE using both 336 LOAD patients and 372 controls *APOE-ε3*3* in the Validation set.

Table 4. Allelic association of seven associated SNPs, encompassed by intron 9 of *CTNNA3*, in the overall sample set, All, and two sub-sample sets, Negative-ε4 and Positive-ε4, stratified as to the presence or absence of the *APOE-ε4* allele

Gender	Female		Male		Female + male	
Sample set	All[a]					
Number of subjects						
LOAD	1103		423		1526	
Control	998		668		1666	
dbSNP	Allelic *P*-value	OR (95% CI)	Allelic *P*-value	OR (95% CI)	Allelic *P*-value	OR (95% CI)
rs7909676	***0.001646***	1.22 (1.08–1.38)	0.2558	0.90 (0.76–1.08)	***0.0472***	1.11 (1.00–1.22)
rs2394287	***0.001696***	1.22 (1.08–1.38)	0.1906	0.89 (0.75–1.06)	0.0512	1.10 (1.00–1.22)
rs4459178	***0.002843***	1.21 (1.07–1.37)	0.2085	0.89 (0.75–1.07)	0.0681	1.10 (0.99–1.21)
rs10997307	0.2316	1.08 (0.95–1.23)	0.4329	0.93 (0.77–1.12)	0.517	1.03 (0.93–1.15)
rs12258078	0.2307	1.08 (0.95–1.23)	0.5439	0.94 (0.79–1.13)	0.4422	1.04 (0.94–1.16)
rs10822890	***0.00126***	1.22 (1.08–1.38)	0.2137	0.89 (0.75–1.07)	***0.0402***	1.11 (1.00–1.23)
rs713250	***0.0009719***	1.23 (1.09–1.39)	0.1358	0.88 (0.73–1.04)	***0.0439***	1.11 (1.00–1.22)
Sample set	Negative-ε4[b]					
Number of subjects						
LOAD	522		227		749	
Control	827		551		1378	
dbSNP	Allelic *P*-value	OR (95% CI)	Allelic *P*-value	OR (95% CI)	Allelic *P*-value	OR (95% CI)
rs7909676	***0.00001471***	1.42 (1.21–1.66)	0.6951	0.96 (0.76–1.20)	***0.0008525***	1.24 (1.09–1.41)
rs2394287	***0.00005357***	1.38 (1.18–1.62)	0.4346	0.91 (0.73–1.14)	***0.003869***	1.21 (1.06–1.37)
rs4459178	***0.00005308***	1.39 (1.18–1.62)	0.4728	0.92 (0.74–1.15)	***0.003415***	1.21 (1.07–1.38)
rs10997307	***0.002555***	1.28 (1.09–1.51)	0.8393	0.98 (0.77–1.23)	***0.0163***	1.18 (1.03–1.34)
rs12258078	***0.001978***	1.29 (1.10–1.52)	0.8693	0.98 (0.78–1.24)	***0.0129***	1.18 (1.04–1.35)
rs10822890	***0.00001019***	1.42 (1.22–1.67)	0.5198	0.93 (0.74–1.16)	***0.001046***	1.24 (1.09–1.41)
rs713250	***0.00001576***	1.41 (1.21–1.65)	0.5154	0.93 (0.74–1.16)	***0.001162***	1.24 (1.09–1.40)
Sample set	Positive-ε4[c]					
Number of subjects						
LOAD	581		196		777	
Control	171		117		288	
dbSNP	Allelic *P*-value	OR (95% CI)	Allelic *P*-value	OR (95% CI)	Allelic *P*-value	OR (95% CI)
rs7909676	0.8115	0.97 (0.76–1.24)	0.1917	0.80 (0.58–1.12)	0.3764	0.92 (0.75–1.11)
rs2394287	0.8995	0.98 (0.77–1.26)	0.4275	0.87 (0.63–1.22)	0.7096	0.96 (0.79–1.17)
rs4459178	0.7375	0.96 (0.75–1.23)	0.2844	0.84 (0.60–1.16)	0.438	0.93 (0.76–1.12)
rs10997307	***0.0409***	0.77 (0.60–0.99)	0.4491	0.88 (0.62–1.24)	0.0528	0.82 (0.67–1.00)
rs12258078	***0.0306***	0.76 (0.59–0.97)	0.6752	0.93 (0.66–1.31)	0.0727	0.83 (0.68–1.02)
rs10822890	0.582	0.93 (0.73–1.19)	0.2816	0.84 (0.60–1.16)	0.3617	0.91 (0.75–1.11)
rs713250	0.9234	0.99 (0.77–1.26)	0.0784	0.74 (0.54–1.03)	0.3245	0.91 (0.75–1.10)

Allelic *P*-values and ORs, with 95% CI in parentheses, are indicated. Boldface indicates statistically significant results (allelic *P*-values <0.05). The genotypic and allelic distributions are shown in the Supplementary Material, Table S2.

[a]All *APOE* genotypes (*APOE-ε2*2*, *2*3*, *2*4*, *3*3*, *3*4* and *4*4*) comprising those of 1526 LOAD patients (female, 1103; male, 423) and 1666 controls (female, 998; male, 668).

[b]Non-carriers of the *APOE-ε4* allele (*2*2*, *2*3* and *3*3*) comprising 749 LOAD patients (female, 522; control, 227) and 1378 controls (female, 827; male, 551).

[c]Carriers of the *APOE-ε4* allele (*2*4*, *3*4* and *4*4*) comprising 777 LOAD patients (female, 581; male, 196) and 288 controls (female, 171; male, 117).

Because multiple SNPs may increase the risk of LOAD in combination, we carried out a case–control haplotype analysis (Table 6). In the All set, haplotypes H1 (permutation *P*-value = 0.0029) and H3 (permutation *P*-value = 0.0043) exhibited significant association in females. In both the Negative-ε4 and ε3*3 sets, haplotypes H1, H2 and H3 exhibited significance in females (permutation *P*-value H1 < H2 < H3). In the All, Negative-ε4 and ε3*3 sets, the frequency of haplotype H1 was decreased in LOAD, suggesting it is a protective haplotype for LOAD. On the other hand, haplotypes H2 and H3 were increased in LOAD, implying that they are risk haplotypes for LOAD. In males, each haplotype showed no significant difference in any sample set.

Of the four sample sets of females, three showed significant association in global tests: All (global permutation *P*-value = 0.0006), Negative-ε4 (global permutation *P*-value = 0.0008), and ε3*3 (global permutation *P*-value = 0.001). We did not detect significance in any haplotype in the female sub-sample set Positive-ε4 (global permutation *P*-value = 0.3323).

Relationship between the Aβ40/42 ratio and genetic variation on *CTNNA3*

The levels of plasma Aβ40 and Aβ42 and their ratio (Aβ40/42) were compared between LOAD patients ($N = 456$) and control subjects ($N = 147$) within different gender groups (Fig. 3A–C). The Mann–Whitney *U*-test was adopted as a non-parametric method for this analysis. In both the female and male groups, the Aβ40 levels (Fig. 3A) and Aβ40/42 ratio (Fig. 3C) were significantly higher in LOAD in comparison with those in controls. The Aβ42 levels were significantly lower in LOAD compared with those in controls (Fig. 3B).

Table 5. Multiple logistic regression analysis

Variables[a]	Category	OR (95% CI)
Model 1		
APOE	*ε4* (−) (Ref)	1.00
	ε4 (+)	**5.00 (4.20–5.96)***
Gender	Male (Ref)	1.00
	Female	**1.64 (1.38–1.94)***
SNP rs713250[b]	TT (Ref)	1.00
	TC	1.13 (0.92–1.37)
	CC	**1.36 (1.08–1.71)****
Age	—	**1.01 (1.00–1.02)*****
Model 2		
APOE	*ε4* (−) (Ref)	1.00
	ε4 (+)	**5.74 (3.62–9.10)***
Gender	Male (Ref)	1.00
	Female	0.88 (0.62–1.26)
SNP rs713250[b]	TT (Ref)	1.00
	TC	0.81 (0.58–1.12)
	CC	0.75 (0.51–1.10)
Age	—	**1.02 (1.01–1.03)****
SNP rs713250_gender[b]	Others (Ref)	1.00
	TC_Female	**1.68 (1.12–2.54)*****
	CC_Female	**2.57 (1.59–4.17)***
Age_*APOE*	Age_*ε4* (−) (Ref)	1.00
	Age_*ε4* (+)	**0.97 (0.95–1.00)*****
Gender_*APOE*	Others (Ref)	1.00
	Female_*ε4* (+)	**1.49 (1.03–2.15)*****

Ref, reference.
*P-value <0.001; **P-value <0.01; ***P-value <0.05.
[a]'_' signifies the interaction between variables.
[b]Global P-value <0.05.

To determine whether or not the difference in the Aβ40/42 ratio between LOAD and the controls is due to the SNPs identified here, two-way ANOVA was performed across diagnosis (LOAD and control) and three genotypic groups (major homozygotes, heterozygotes and minor homozygotes) within different gender and their combined groups (Fig. 3D–F). SNP rs713250 was used as a representative of the seven associated SNPs because it showed the most significant association with LOAD on Mantel–Haenszel test (allelic P-value$_{MH\text{-}F} = 0.000005945$), as shown in Table 2. The log-transformed Aβ40/42 ratio values [$\log_2$(Aβ40/42 ratio + 1)] were used in this analysis. Before two-way ANOVA, the Kolmogorov–Smirnov (KS) normality test and Bartlett's test for equal variances were performed for the each dataset as to gender. Almost every sub-group examined passed the KS normality test. Both the female–male (Fig. 3D) and female (Fig. 3E) groups passed the Bartlett's test, but not the male group (Fig. 3F, $P = 0.01178$). Through two-way ANOVA, a significant effect of diagnosis was observed for every group (P-values <0.0001). However, we did not detect any genotype-dependent effect of this SNP on the Aβ40/42 ratio, and no interaction between the SNP, Aβ40/42 ratio and diagnosis.

DISCUSSION

In this study, we extended our previous work on chromosome 10q (26), and thoroughly reanalyzed the genotype data for 1140 SNPs in order to discover gender-related genetic loci for LOAD. In a single SNP-based case–control study, we found seven SNPs on *CTNNA3* showing genetic association with LOAD in females with the *APOE-ε3*3* genotype or without the *APOE-ε4* allele. Furthermore, multiple logistic regression analysis revealed that one (SNP rs713250) of these seven SNPs directly interacted with the female gender, but not with the male gender, and did not show any interaction with the *APOE-ε4* allele at all. These are the first findings constituting evidence that *CTNNA3* may affect the development of sporadic LOAD through a novel female-specific mechanism independent of the *APOE-ε4* allele. We consider the genetic association identified here to reflect one single signal. The reasons are: (1) the seven significant SNPs span only ~38 kb and are clustered in intron 9 of *CTNNA3* (Fig. 2A and C), which suggests a multiple-hit genomic region of SNPs associated with LOAD; (2) solid linkage disequilibrium was observed between all of these seven SNPs ($D' > 0.9$) (Supplementary Material, Fig. S1); and (3) the associated region was encompassed by a tight structured LD block extending ~80 kb (Fig. 2B).

Janssens *et al.* (29,30) cloned full-length *CTNNA3* cDNA as a novel member of the α-catenin gene family and determined its genomic structure. *CTNNA3* contains 18 exons and spans ~1.78 Mb (67.35–69.13 Mb), being the longest of all genes located on chromosome 10. The chromosomal location of *CTNNA3* is 10q21 (30), which includes the suggestive linkage region between microsatellite markers D10S1227 (57.20 Mb) and D10S1211 (66.39 Mb) in LOAD (24). Ertekin-Taner *et al.* (23) found a linkage with a maximum LOD score of 3.93 at 81 cM close to D10S1225 (64.43 Mb) using the plasma Aβ42 level as a surrogate trait in a set of LOAD families, and the same chromosomal region was identified by Myers *et al.* (24) by means of genome-wide screening of sibling pairs with LOAD. To date, there have been six papers on the genetic association of *CTNNA3* with LOAD (32–37). In the first report (32), it was demonstrated that two SNPs located in intron 13 of *CTNNA3* are associated with familial LOAD with high levels of plasma Aβ42, which was used as an intermediate phenotype related to AD. These intronic SNPs, spanning 423 bp, are rs12357560 and rs7070570: the former lies 1174 bp upstream, and the latter 1597 bp downstream from exon 14, respectively. They are in strong LD: $D'=1$ in all four populations, CEU, CHB, JPT and YRI, used in the HapMap project (38). A genotype-dependent correlation between SNP rs7070570 and the plasma Aβ42 level has also been detected: the major homozygote (TT) is associated with the highest level of Aβ42, the heterozygote (TC) with an intermediate level and the minor homozygote (CC) with the lowest level (32). Martin *et al.* (34). found that SNP rs7074454 located in intron 13 of *CTNNA3*, lying 355 bp upstream from SNP rs7070570, was significantly associated with both familial and sporadic cases of LOAD. Non-synonymous SNP rs4548513 (AGC → AAC, Ser596Asn) located in exon 13 of *CTNNA3*, lying 175 721 bp upstream from SNP rs7070570, has been shown to be associated with familial AD (37). All of these four SNPs, rs7070570, rs12357560, rs7074454 and rs4548513, lie in a genomic region extending from exons 13 to 14 (Fig. 2A), which has been shown to be located within a large LD block spanning around 310 kb (67.43–67.74 Mb)

Table 6. Case–control haplotype analysis

Sample set	Gender	Number of subjects		Haplotype[a]	Frequency		Number of estimated alleles		Permutation *P*-value (10 000)	OR (95% CI)
		LOAD	Control		LOAD	Control	LOAD	Control		
All	Female	1103	998	**[H1]C-A-T-T-T-A-T**	0.4717	0.5174	1041	1033	**0.0029**	0.83 (0.74–0.94)
				[H2]A-G-C-C-G-G-C	0.3592	0.3375	792	674	0.1538	1.10 (0.97–1.25)
				[H3]A-G-C-T-T-G-C	0.1406	0.1110	310	222	**0.0043**	1.31 (1.09–1.57)
				[H4]C-A-T-T-T-A-C	0.0196	0.0169	43	34	0.5632	1.15 (0.73–1.81)
				Others[b]	0.0089	0.0172	20	33	—	—
				Sum	1.0000	1.0000	2206	1996	—	—
				Global	—	—	—	—	**0.0006**	—
	Male	423	668	[H1]C-A-T-T-T-A-T	0.5293	0.4973	448	664	0.145	1.14 (0.96–1.35)
				[H2]A-G-C-C-G-G-C	0.3344	0.3415	283	456	0.7739	0.97 (0.81–1.16)
				[H3]A-G-C-T-T-G-C	0.1179	0.1314	100	176	0.3927	0.88 (0.68–1.15)
				[H4]C-A-T-T-T-A-C	0.0084	0.0131	7	18	0.3117	0.61 (0.25–1.47)
				Others[b]	0.01	0.0167	8	22	—	—
				Sum	1.0000	1.0000	846	1336	—	—
				Global	—	—	—	—	0.2273	—
Negative-ε4	Female	522	827	**[H1]C-A-T-T-T-A-T**	0.4430	0.5228	462	865	**< 0.0001**	0.72 (0.62–0.85)
				[H2]A-G-C-C-G-G-C	0.3888	0.3273	406	541	**0.0008**	1.31 (1.11–1.54)
				[H3]A-G-C-T-T-G-C	0.1418	0.1132	148	187	**0.0323**	1.30 (1.02–1.63)
				[H4]C-A-T-T-T-A-C	0.0206	0.0185	22	31	0.6661	1.13 (0.65–1.96)
				Others[b]	0.0058	0.0182	6	30	—	—
				Sum	1.0000	1.0000	1044	1654	—	—
				Global	—	—	—	—	**0.0008**	—
	Male	227	551	[H1]C-A-T-T-T-A-T	0.5240	0.5039	238	556	0.5078	1.08 (0.87–1.35)
				[H2]A-G-C-C-G-G-C	0.3479	0.3456	158	381	0.9532	1.01 (0.80–1.27)
				[H3]A-G-C-T-T-G-C	0.1167	0.1289	53	142	0.5618	0.89 (0.64–1.25)
				Others[b]	0.0114	0.0216	5	23	—	—
				Sum	1.0000	1.0000	454	1102	—	—
				Global	—	—	—	—	0.7917	—
ε3*3	Female	491	748	**[H1]C-A-T-T-T-A-T**	0.4363	0.5179	428	775	**0.0002**	0.72 (0.61–0.85)
				[H2]A-G-C-C-G-G-C	0.3919	0.3305	385	494	**0.0019**	1.31 (1.11–1.55)
				[H3]A-G-C-T-T-G-C	0.1436	0.1151	141	172	**0.0405**	1.29 (1.02–1.64)
				[H4]C-A-T-T-T-A-C	0.0219	0.0178	22	27	0.4617	1.25 (0.71–2.20)
				Others[b]	0.0063	0.0187	6	28	—	—
				Sum	1.0000	1.0000	982	1496	—	—
				Global	—	—	—	—	**0.001**	—
	Male	208	495	[H1]C-A-T-T-T-A-T	0.5214	0.4995	217	491	0.383	1.11 (0.88–1.39)
				[H2]A-G-C-C-G-G-C	0.3459	0.3525	144	349	0.8585	0.97 (0.76–1.24)
				[H3]A-G-C-T-T-G-C	0.1202	0.1300	50	129	0.6659	0.91 (0.64–1.29)
				Others[b]	0.0125	0.0220	5	21	—	—
				Sum	1.0000	1.0000	416	990	—	—
				Global	—	—	—	—	0.8879	—
Positive-ε4	Female	581	171	[H1]C-A-T-T-T-A-T	0.4976	0.4907	577	168	0.9006	1.02 (0.80–1.30)
				[H2]A-G-C-C-G-G-C	0.3327	0.3870	387	132	0.0799	0.79 (0.62–1.02)
				[H3]A-G-C-T-T-G-C	0.1396	0.1009	162	35	0.0797	1.42 (0.96–2.09)
				[H4]C-A-T-T-T-A-C	0.0187	0.09	22	3	0.2313	2.18 (0.65–7.33)
				Others[b]	0.0114	0.0124	14	4	—	—
				Sum	1.0000	1.0000	1162	342	—	—
				Global	—	—	—	—	0.3323	—
	Male	196	117	[H1]C-A-T-T-T-A-T	0.5356	0.4638	210	109	0.0961	1.32 (0.96–1.83)
				[H2]A-G-C-C-G-G-C	0.3188	0.3238	125	76	0.934	0.97 (0.69–1.38)
				[H3]A-G-C-T-T-G-C	0.1193	0.1459	47	34	0.3988	0.80 (0.50–1.29)
				[H4]C-A-T-T-T-A-C	0.0129	0.0310	5	7	0.1429	0.42 (0.13–1.34)
				Others[b]	0.0134	0.0355	5	8	—	—
				Sum	1.0000	1.0000	392	234	—	—
				Global	—	—	—	—	0.0728	—

Statistically significant haplotypes and permutation *P*-values are highlighted in bold.
[a]The SNP order, from left to right, is as follows: rs7909676, rs2394287, rs4459178, rs10997307, rs12258078, rs10822890 and rs713250.
[b]Haplotypes with frequencies <0.01 in both LOAD and control subjects.

in CEU subjects (37) (Supplementary Material, Fig. S2). They have a tendency to exhibit selective association with familial rather than sporadic LOAD (32,35,37). Therefore, it is likely that the large LD block region contributes to a specific form of familial LOAD in Caucasians. We also assessed these four SNPs and SNPs neighboring them in our Japanese sporadic LOAD subjects, however, none of these SNPs exhibited significant association (data not shown). In the genomic

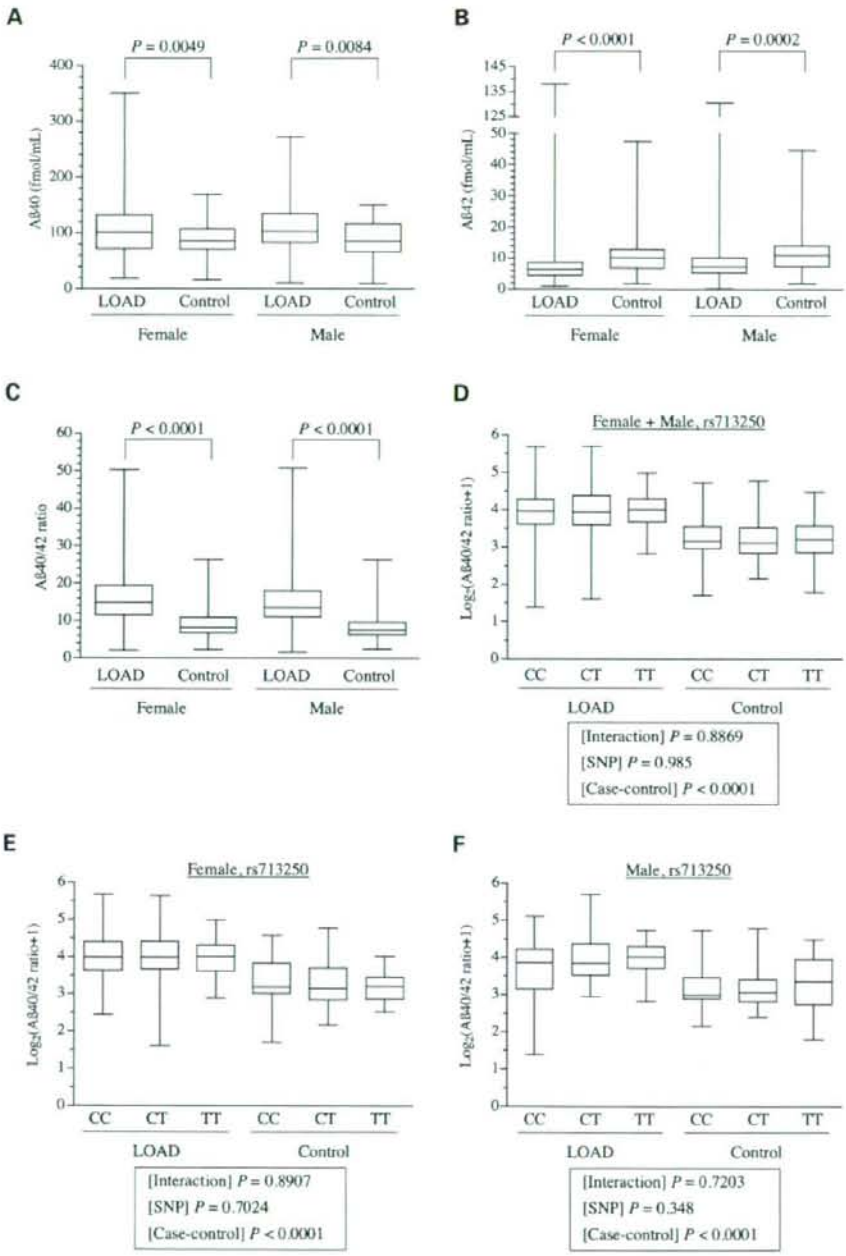

Figure 3. Comparison of the plasma levels of Aβ40 and Aβ42, and the Aβ40/42 ratio. The differences in the relative amounts of Aβ40 (**A**) and Aβ42 (**B**), and the Aβ40/42 ratio (**C**) were compared between LOAD patients and controls by means of Mann–Whitney's *U*-test within different gender groups. (**D**, **E**, **F**) Correlation between the Aβ40/42 ratio, an associated SNP on *CTNNA3*, and the diagnosis (LOAD or control). Using log-transformed Aβ40/42 ratio values, two-way ANOVA tests were performed after Bartlett's test for the homogeneity of variances and the KS normality test. The results for SNP rs713250 are presented here as being representative of the seven associated SNPs identified in this study. The horizontal line inside each box denotes the median value. The box extends from the 25th and 75th percentiles. The error bars extend down to the lowest value and up to the highest. Genotypes CC, CT and TT represent major-allele homozygotes, heterozygotes and minor-allele homozygotes, respectively.

region including the four SNPs, different LD block structures were observed in Japanese and CEPH subjects (Fig. 2B and Supplementary Material, Fig. S2). As one of the reasons why reproducible association could not be detected for these four SNPs, we mainly consider that an ethnic difference may exist.

High-level gene expression of *CTNNA3* is detected predominantly in heart and testis, and low-level expression in several tissues including brain (29). Coimmunoprecipitation analysis revealed that CTNNA3 binds directly to β-catenin in both a human cell line transfected with *CTNNA3* cDNA, and heart and testis tissue extracts of mouse (30). β-Catenin forms a complex with presenilin 1 (PSEN1) (31,39,40), mutations of which cause familial cases of early-onset AD (EOAD) [Alzheimer Disease & Frontotemporal Dementia Mutation Database (AD&FTDMDB), http://www.molgen.ua.ac.be/ADMutations/]. The expression level of β-catenin is reduced in the brains of EOAD patients with *PSEN1* mutations (31). Intracellular trafficking of β-catenin is affected in human cells bearing *PSEN1* mutations (41), resulting in sustained loss of Wnt/β-catenin signal transduction, which is probably followed by the onset and development of AD (42,43). Although, at present, there is no direct evidence suggesting that CTNNA3 interacts with PSEN1, it is assumed that their genetic polymorphisms or combinations in *CTNNA3* may have a negative influence on the Wnt/β-catenin signaling pathway, leading to potential involvement in the pathogenesis of AD. In this study, it was clarified that seven intronic SNPs on *CTNNA3* were significantly and reproducibly associated with sporadic female cases of LOAD without the *APOE-ε4* allele. Intronic variants are considered to have the potential to directly affect gene-expression levels in some cases (44); therefore, we performed quantitative real-time RT–PCR analysis of *CTNNA3* using the postmortem brains of 19 neuropathologically-confirmed LOAD cases and 22 control ones. Two-way ANOVA revealed that there was no statistically significant interaction between the *CTNNA3* expression level, the associated SNPs identified here and the diagnosis (data not shown). Additionally, although a genotype-dependent transition effect on the plasma Aβ42 level was observed for intronic SNP rs7070570 by Ertekin-Taner *et al.* (32), it was found that none of these SNPs influence the plasma levels of Aβ peptides (Fig. 3D–F).

However, interestingly, by means of a search of a public genome database, the Database of Genomic Variants (http://projects.tcag.ca/variation/), we discovered that there is copy number variation (CNV) (45) in the genomic region comprising the seven associated SNPs on *CTNNA3*: variation ID 3807 at Locus 2128, which was detected in a Japanese subject (ID, NA18973) (Fig. 2A). CNV, i.e. deletion, insertion and duplication with >1 kb in length of the genomic sequence (46), rather than SNP could cause phenotypic diversity and complex diseases in humans by altering the gene dose or disrupting the coding or regulatory sequences of genes, and may account for the LOAD susceptibility. Regarding our LOAD subjects, we did not examine the presence or absence of CNV within *CTNNA3*. Therefore, in a further study, it is very important to determine whether or not CNV in *CTNNA3* is associated with LOAD.

Recently, in LOAD families, notable evidence was obtained suggesting a maternal parent-of-origin effect on chromosome 10q between microsatellite markers D10S1233 (44.05 Mb) and D10S1225 (64.43 Mb) with a non-parametric LOD score >1.0: the highest LOD score of 3.73 was seen for microsatellite marker D10S1221 (57.20 Mb) (27,28). Moreover, it was found that *CTNNA3* is subject to genomic imprinting with cell-type specificity in placental tissues: biallelic and monoallelic (maternal-allele) expression is observed in extravillus and villus trophoblasts, respectively (47). Mouse *Ctnna3* (Clone ID 4933408A16 on FANTOM2), orthologous to human *CTNNA3*, has been deposited as a maternal imprinting gene on chromosome 10 in the Expression-based Imprint Candidate Organizer DataBase (48; EICO DB, http://fantom2.gsc.riken.jp/EICODB/imprinting/), provided by RIKEN (Japan). These findings led us to examine whether or not *CTNNA3* shows allele-specific expression caused by a molecular mechanism such as genomic imprinting in the brain. We conducted real-time RT–PCR analysis with allele-specific amplification using postmortem human brains heterozygous for non-synonymous SNP rs4548513 in exon 13 [LOAD, 7 (female:male = 3:4); control, 8 (female:male = 3:5)]. Unexpectedly, biallelic expression was detected in brain tissues, and there was no significant difference between LOAD patients and control subjects in the expression level of *CTNNA3* (data not shown). Since as in placental tissues, as described above, it is possible that cell-type dependent imprinting for *CTNNA3* may occur in the brain, further expression analysis should be carefully carried out using homogeneous populations of specific cells from brain tissues. Now genome-wide prediction and the discovery of imprinted genes have progressed (49,50), and 600 (2.5%) of 23 788 annotated autosomal genes have been found to be potentially imprinted in the mouse genome by computational estimation: 384 (64%) of these candidate-imprinted genes show maternal-allele expression (50). It is expected that failure of imprinted gene expression in the human brain may lead to cognition and behavior defects such as Alzheimer's disease, schizophrenia, the bipolar affective disorder and epilepsy (51–53). Therefore, it is important and interesting to actively examine imprinted genes present in the genetic linkage region of LOAD.

MATERIALS AND METHODS

Subjects

The Japanese Genetic Study Consortium for AD (JGSCAD) was organized in 2000, and blood samples were collected to survey risk genes for LOAD by means of a genome-wide association study. All individuals included in this study were Japanese. Probable AD cases met the criteria of the National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders. Control subjects who had no signs of dementia and lived in an unassisted manner in the local community were also recruited. Age at onset (AAO) is here defined as the age at which the family and/or individuals first noted cognitive problems during work or in daily activities. The Mini-Mental State Examination (MMSE), and Clinical Dementia Rating and/or the Function Assessment Staging were used for the evaluation of cognitive impairment: MMSE was used for almost every subject.

The basic demographics of the LOAD patients and non-demented control subjects are presented in Table 1. A total of 3192 subjects comprising 1526 LOAD patients [female, 1103 (72.3%); male, 423 (27.7%)] and 1666 controls [female, 998 (59.9%); male, 668 (40.1%)], which is referred to as overall sample set All in this study, were used to discover gender-related loci associated with LOAD on chromosome 10q: information on these subjects was also presented in our recent paper, Kuwano *et al.* (26). The mean AAO ± standard deviation (SD) in the 1526 LOAD patients was 73.5 ± 6.6 (range 60–93). The mean age at examination (AAE) ± SD of the control subjects was 73.1 ± 7.8 (range 60–96). There was no significant difference between AAO in LOAD patients and AAE in control subjects with the unpaired Student's *t*-test (*P*-value = 0.1239). The mean MMSE score in the 1526 LOAD patients was 16.5 (SD 7.0), which was significantly lower (*P*-value with unpaired Student's *t*-test <0.0001) than that in the 1666 controls (mean ± SD 28.0 ± 1.8). The numbers (frequency) of *APOE-ε2*2*, *ε2*3*, *ε2*4*, *ε3*3*, *ε3*4* and *ε4*4* in the 1526 LOAD subjects were 1 (0.07%), 49 (3.21%), 17 (1.11%), 699 (45.81%), 613 (40.17%) and 147 (9.63%), and those in the 1666 control subjects were 3 (0.18%), 132 (7.92%), 15 (0.90%), 1243 (74.61%), 256 (15.37%) and 17 (1.02%). The allelic distribution of *APOE* was significantly different between LOAD patients (*ε2*, 68; *ε3*, 2060; *ε4*, 924) and control subjects (*ε2*, 153; *ε3*, 2874; *ε4*, 305), as expected (*P*-value with χ^2 test using a 2×3 contingency table, <0.0001).

The present study was approved by the Institutional Review Board of Niigata University and by all participating institutes. Informed consent was obtained from all controls and appropriate proxies for patients, and all samples were anonymously analyzed for genotyping.

SNPs and genotyping

SNP information was obtained from five open databases: NCBI dbSNP (Build 125, http://www.ncbi.nlm.nih.gov/SNP/), UCSC Genome Bioinformatics (http://genome.ucsc.edu/), International HapMap Project (Rel#20/phaseII on NCBI Build 35.1 assembly and dbSNP Build 125, http://www.hapmap.org/index.html), Ensemble Human (Version 37 on NCBI Build 35.1, http://www.ensembl.org/Homo_sapiens/) and Celera myScience (Version R27 g on NCBI Build 35.1, http://myscience.appliedbiosystems.com/). We selected 1322 SNPs in the region from 60 to 107 Mb on chromosome 10q; mean intermarker distance ± SD, 34.9 ± 87.4 kb; 95% CI, 30.2–39.6 kb. The information on all SNPs, including rs or Celera IDs and genomic positions on NCBI build 35.1, used here was presented in detail elsewhere (26). These SNPs consisted of 29 missense mutations, 27 silent mutations, 6 SNPs in the 5′-UTR, 29 SNPs in the 3′-UTR, 921 SNPs in introns, 282 SNPs in intergenic regions and 28 SNPs in four loci shared by two different genes (*CTNNA3/LRRTM3*, *CDH23/C10orf54*, *C10orf55/PLAU* and *PGAM1/EXOSC1*). Among the 1322 SNPs, 28 SNPs could not be genotyped. To examine deviation from HWE of 1294 SNPs, exact tests (details given under Statistical analysis) were performed with both 363 LOAD patients and 337 control subjects (carrying *APOE-ε3*3* in the exploratory sample set, as shown in Table 1). We used 1140 SNPs that were shown to be actually polymorphic in the Japanese population and showed *P*-values >0.05 with the exact tests; mean intermarker distance ± SD, 40.5 ± 96.7 kb; 95% CI, 34.9–46.1 kb.

Genomic DNA was extracted from peripheral blood with a QIAamp DNA Blood Maxi Kit (Qiagen, Duseldorf, Germany) and examined fluorometrically with a PicoGreen dsDNA quantification kit (Molecular Probes, California, USA). SNP genotyping of individual samples was performed with an ABI PRISM 7900HT instrument using TaqMan technology, and TaqMan SNP Genotyping Assays were purchased from Applied Biosystems (California, USA).

Case–control study

To discover gender-related genetic loci on chromosome 10q (60–107 Mb on NCBI build 35.1), allelic association was assessed by means of the χ^2 test based on a 2×2 contingency table in comparison with allele frequencies in LOAD patients and control subjects within different gender groups. For screening, two independent sample sets, Exploratory and Validation, comprising case–control subjects with *APOE-ε3*3* were first used after being stratified as to gender (Table 1). Sample set Exploratory comprising 363 LOAD patients and 337 control subjects was genotyped (26), and SNPs showing significant association (allelic *P*-value <0.01) were then subjected to further examination using another sample set, Validation, comprising 336 LOAD patients and 372 control subjects. Multistage, including two-stage, genotyping designs for large-scale association surveys have been proved to be practically as well as theoretically effective for identifying common genetic variants that predispose to human disease (54–58). Therefore, we considered that replication in both the Exploratory and Validation sample sets implicates an association of particular SNPs with LOAD.

Subsequently, for stratified analysis we increased the number of subjects and constructed an overall sample set, All. Furthermore, to construct three sub-sample sets, overall sample set All was stratified as to the *APOE* carrier status: Negative-*ε4*, *APOE-ε 2*2*, *2*3* and *3*3*; *ε3*3*, *APOE-ε 3*3*; Positive-*ε4*, *APOE-ε 2*4*, *3*4* and *4*4* (Table 1). The sample numbers for LOAD patients and controls in All, Negative-*ε4*, *ε3*3* and Positive-*ε4* were 1526 and 1666, 749 and 1378, 699 and 1243, and 777 and 288, respectively. These four sample sets were used for the χ^2 test after being sub-grouped as to gender.

Case–control haplotype analysis with significant SNPs was also performed using the following sample sets: All, Negative-*ε4*, *ε3*3* and Positive-*ε4*. These four sample sets were used after being stratified as to gender.

Aβ40 and Aβ42 quantification

For Aβ40 and Aβ42 quantification, 603 subjects consisting of 456 LOAD patients (female, 332; male, 124) and 147 control subjects (female, 95; male, 52) were used. They are included in the All set. The sandwich enzyme-linked immunosorbent assay (59–61) was used to specifically quantify whole plasma Aβ species. The standardization, sensitivity and specificity of the method were described in a previous paper (61).