Int J Mol Epidemiol Genet 2012;3(1):30-38
www.ijmeg.org /ISSN1948-1756/IJMEG1111009

Original Article

Genetic variation in MME in relation to neprilysin protein and enzyme activity, Aβ levels, and Alzheimer's disease risk

Scott Miners[1], Zoë van Helmond[1], Rachel Barker[1], Peter A Passmore[2], Janet A Johnston[2], Stephen Todd[2], Bernadette M McGuinness[2], Francesco Panza[3,4], Davide Seripa[4], Vincenzo Solfrizzi[3], Seth Love[1], Jonathan A Prince[5], Patrick G Kehoe[1]

[1]Dementia Research Group, University of Bristol, Institute of Clinical Neurosciences, Frenchay Hospital, Bristol, UK; [2]Department of Geriatric Medicine, The Queen's University of Belfast, Belfast, UK; [3]Department of Geriatrics, Center for Aging Brain, Memory Unit, University of Bari, Bari, Italy; [4]Geriatric Unit and Gerontology-Geriatrics Research Laboratory, Department of Medical Sciences, IRCCS, Casa Sollievo della Sofferenza, San Giovanni Rotondo, Foggia, Italy; [5]Center for Genomics and Bioinformatics, Karolinska Institute, Stockholm, Sweden

Received November 29, 2011; accepted December 12, 2011; Epub February 5, 2012; Published February 28, 2012

Abstract: Neprilysin (NEP), also known as membrane metalloendopeptidase (MME), is considered amongst the most important β-amyloid (Aβ)-degrading enzymes with regard to prevention of Alzheimer's disease (AD) pathology. Variation in the NEP gene (MME) has been suggested as a risk factor for AD. We conducted a genetic association study of 7MME SNPs – rs1836914, rs989692, rs9827586, rs6797911, rs61760379, rs3736187, rs701109 - with respect to AD risk in a cohort of 1057 probable and confirmed AD cases and 424 age-matched non-demented controls from the United Kingdom, Italy and Sweden. We also examined the association of these MME SNPs with NEP protein level and enzyme activity, and on biochemical measures of Aβ accumulation in frontal cortex – levels of total soluble Aβ, oligomeric $A\beta_{1-42}$, and guanidine-extractable (insoluble) Aβ – in sub-group of AD and control cases with post-mortem brain tissue. On multivariate logistic regression analysis one of the MME variants (rs6797911) was associated with AD risk (P = 0.00052, Odds Ratio (O.R. = 1.40, 95% confidence interval (1.16-1.70)). None of the SNPs had any association with Aβ levels; however, rs9827586 was significantly associated with NEP protein level (p=0.014) and enzyme activity (p=0.006). Association was also found between rs701109 and NEP protein level (p=0.026) and a marginally non-significant association was found for rs989692 (p=0.055). These data suggest that MME variation may be associated with AD risk but we have not found evidence that this is mediated through modification of NEP protein level or activity.

Keywords: Neprilysin, MME, gene, association, β-Amyloid, alzheimer disease, polymorphism

Preview

Introduction

Neprilysin (NEP, EC3.4.24.11; also known as neutral endopeptidase, encephalinase, common acute lymphocytic leukaemia antigen, and CD10) is a type II metalloproteinase widely expressed in many tissues [1]. Within the brain, NEP is expressed by pyramidal neurons within the neocortex and by smooth muscle vascular cells in the cerebral vasculature [2, 3]. Several lines of evidence suggest a role for NEP in the pathobiology of AD and cerebral amyloid angiopathy (CAA) [3-5]. NEP cleaves Aβ *in vitro* [6] and protects against Aβ deposition *in vivo* in human APP transgenic mice [7-9]. NEP mRNA and protein levels were reported to be reduced in post-mortem human tissue in association with AD and CAA [3, 4].

The gene which encodes NEP (MME) is located on chromosome 3q25.1-q25.2 (MIM:120520) and has previously been reported as a putative linkage region (3q12.3-q25.31) in familial forms of AD [10, 11]. Four different MME mRNA transcripts with varying 5' sequences have been identified which result from alternative transcription sites, the type 1 isoform being expressed predominantly in neurons [12]. To date,

Table 1. Demographic information for each of the four starting cohorts

Cohort	Diagnosis	No. of cases	No. of females	Age range (yrs)*	Mean (±SD) age (yrs)*
Total	AD	1057	634	-	-
	Control	424	232	-	-
Bristol	AD	289	165	57-99	80. 5±8.0
	Control	90	36	62-96	79.3±8.2
Belfast	AD	638	398[a]	54-95[b]	76.4±7.8
	Control	215	146[c]	40-100[d]	74.4±9.0
Italy I	AD	15	6	53-84	70.5±10.4
	Control	24	7	59-85	77.1±5.3
Italy II	AD	115	65[e]	51-82[f]	69.9±7.1
	Control	95	43	60-100[g]	73.2±8.6

*For the Bristol cohort the ages indicated are the age at death. For the three clinical cohorts the age is age at disease onset for the AD cases and age that blood was taken for analysis for control cases. [a]Gender information missing from 13 cases, [b]Age information missing from 127 cases, [c]Gender information missing from 1 control, [d]Age information missing from 3 controls, [e]Gender information missing from 18 cases, [f]Age information missing from 21 cases, [g]Age information missing from 2 controls. The absence of data in relation to age-range and mean age for the total cohort is because it combines both age at death and age at disease onset.

Preview

numerous single nucleotide polymorphisms (SNPs) and other variants within MME have been reported to have varying degrees of positive or negative association with risk of AD [2, 5, 13-24]. Ongoing meta-analysis of SNP-based association studies in the online AlzGene (www.Alzgene.org) database has shown little to no overall association between MME variants and AD. However it remains unclear whether such variants might alter the protein levels or activity of neprilysin, potentially modifying the disease by affecting Aβ degradation.

This study investigated the potential genetic association between 7 MME variants (rs1836914, rs989692, rs9827586, rs6797911, rs61760379, rs3736187, rs701109) and AD. From this cohort of 1057 AD and 424 control cases (derived from 4 contributing AD research centres in the UK, Northern Ireland and Southern Italy), a sub-group of post-mortem samples was also assessed for association between the MME variants and NEP protein level and enzyme activity, previously found to be altered in AD [3], and for association with previously obtained quantitative measures of Aβ accumulation: levels of total soluble Aβ, oligomeric $A\beta_{1-42}$ and total guanidine-extracted (insoluble) Aβ [25, 26].

Materials and methods

Study cohort

DNA was extracted from brain tissue obtained from the South West Dementia Brain Bank (SWDBB) in Bristol (Human Tissue Authority license 12273) from cases with a post-mortem diagnosis of probable or definite AD according to CERAD criteria [27] or from normal control brains (Table 1). The neuropathological assessment of these cases was previously reported {van Helmond, 2010 #2629}. The remaining samples of the cohort (Table 1) comprised DNA that was extracted from blood taken from living patients attending four clinical research centres (Belfast, Northern Ireland; Bari, Italy (Italy I) and San Giovanni Rotondo, Italy (Italy II)) constituting a further 334 controls and 768 AD samples (Table 1) with an NINCDS-ADRDA diagnosis of probable AD [28]. Together 424 controls and 1057 cases with probable or definite AD were investigated for MME variation and AD risk.

Other data retrieved for each case included gender, age and APOE genotype. The manner in which age was recorded differed for the post-mortem and clinical cohorts. Age at death was the value recorded for the post-mortem cohort whilst that used for the three clinical cohorts (Belfast and Italy I and II) was age at assessment (i.e. when blood was taken) for non-demented controls and the age of disease onset for the AD cases.

Genetic analysis

Genomic DNA was extracted from brain tissue and blood by use of commercial DNA extraction kits (Nucleon ST Extraction kit, Nucleon Biosciences, Manchester, UK) and diluted to 10 ng/μl in Sigma® water (Sigma Aldrich, St Louis, USA)

as described elsewhere [1]. Following the provision of sequence information relevant to our SNPs of choice, the SNP genotyping was conducted at KBiosciences (www.kbiosciences.co.uk) using their proprietary in-house KASPar genotyping technology, a PCR-based genotyping method that is combined with allele-specific amplification followed by fluorescence detection. All assays were performed according to the manufacturers' quality controls and protocols.

Seven SNPs from across MME were selected for genotyping based either on their previous reported associations with AD (see Alzgene: www.alzgene.org for data summaries of all studies) or which served as 'tag-SNPs' according to HAPMAP (build 35) (www.hapmap.org) to give some coverage across the gene. All reported SNPs can be found in the dbSNP database (www.ncbi.nlm.nih.gov/SNP/) under their respective ID numbers.

All cases had previously been APOE genotyped, following an established protocol [29].

NEP protein and enzyme activity measurements

NEP protein level was measured by sandwich ELISA as reported previously [30] and NEP enzyme activity level was measured by an immunocapture-based fluorogenic activity assay [31]. Both parameters had previously been determined in brain tissue homogenates within a subset of the SWDBB cases (N=95 AD, 52 controls) examined here. NEP protein level and enzyme activity were adjusted for neuronal content on the basis of measurements of the neuronal marker (neuron-specific enolase (NSE)), as previously described [30], to allow for variation in neuronal number between samples.

Aβ measurements

Tissue preparation and ELISA methods used to measure total soluble and guanidine-extractable (insoluble) Aβ levels in the same SWDBB cases as those for NEP assays were also reported previously [25]. In summary, soluble and insoluble fractions were prepared from fresh frozen human brain tissue and measured by sandwich ELISA in which monoclonal anti-Aβ (clone 4G8, raised against amino acids 18-22; Millipore, Durham, UK) was used for the capture step and biotinylated anti-human β-amyloid monoclonal antibody (clone 10H3; Thermo Fisher Scientific, Northumberland, UK) for the detection step [25]. The development and validation of the oligomeric $A\beta_{1-42}$ ELISA, and the tissue preparation methods and measurement of oligomeric $A\beta_{1-42}$ levels in the SWDBB subjects included in this study were reported previously [25]. A sandwich ELISA was designed in which a rabbit polyclonal pan-$A\beta_{1-42}$ antibody (Millipore, Durham, UK) was used for the capture step and monoclonal mouse anti-oligomeric Aβ antibody (clone 7A1a; New England Rare Reagents, ME, USA) for the detection step [25].

Statistical analysis

The chi-squared statistic (χ^2) was used to assess deviation from Hardy-Weinberg equilibrium (HWE) for genotypes at individual loci as well as to assess differences in genotype distributions between demented and non-demented groups. Multivariate tests factoring in age, gender, APOE ε4 status and cohort for the association of markers with disease risk were also conducted by logistic regression in an additive model. Tests for association between single marker genotypes and quantitative traits were performed by analysis of variance (ANOVA). STATA v11.1 (www.stata.com) was used to perform all of the above statistical analyses. Sample size calculations were performed a priori to ensure a suitable sample size was present to detect a 20% shift in allele frequency at a significance level of $p<0.05$. Haplotype frequencies in the MME region were estimated after linkage disequilibrium (LD) block definition in individual blocks, by use of Haploview v4.1 [32]. LD blocks were defined by solid spines. LD between marker pairs within MME was estimated using the r^2 metric [33]. The calculation of empirical p-values for haplotypes in case-control tests was performed using 1000 permutations.

Results

Genetic association analysis

All of the polymorphisms examined were in Hardy-Weinberg equilibrium when assessed in cases and controls separately. Genotype frequencies for each MME SNP are displayed in **Table 2**. All of the genotypes except for rs6797911 were not seen to differ between AD and control. For rs6797911, a p-value of 0.035 was found. After further logistic regression analysis in which gender, age and APOE ε4 were used as co-variants, as well as identifiers for the different population, rs6797911 frequency re-

Preview

Table 2. Genotype frequencies for MME SNPs in AD

rsID/sample Chromosome location	Case/control (N)	Polymorphism			Genotypes	Logistic
rs1836914		AA	AG	GG		
	Controls	385	19			
	(404)	(.95)	(.05)	0	P = 0.943	P = 0.993
	AD	992	48			
Chr3:154 796 360	(1040)	(.95)	(.05)	0		
rs989692		CC	CT	TT		
	Controls	108	183	107		
	(398)	(.27)	(.46)	(.27)	P = 0.127	P = 0.572
	AD	254	528	236		
Chr3:154 801 365	(1018)	(.25)	(.52)	(.23)		
rs9827586		GG	AG	AA		
	Controls	151	178	61		
	(390)	(.39)	(.46)	(.15)	P = 0.494	P = 0.987
	AD	355	482	168		
Chr3:154 825 385	(1005)	(.35)	(.48)	(.17)		
rs6797911		TT	AT	AA		
	Controls	172	169	53		
	(394)	(.44)	(.43)	(.13)	P = 0.035	P = 0.00052*
	AD	362	474	162		
Chr3:154 862 390	(998)	(.36)	(.48)	(.16)		
rs61760379		CC	CT	TT		
	Controls	364	8			
(IVS17-294)	(372)	(.98)	(.02)	0	P = 0.876	P = 0.444
	AD	972	20			
Chr3:154 877 885	(992)	(.98)	(.02)	0		
rs3736187		AA	AG	GG		
	Controls	322	52	1		
	(375)	(.85)	(.14)	(.01)	P = 0.802	P = 0.764
	AD	860	126	4		
Chr3:154 886 278	(984)	(.87)	(.12)	(.01)		
rs701109		AA	AG	GG		
	Controls	71	173	158		
	(402)	(.18)	(.43)	(.39)	P = 0.406	P = 0.192
	AD	154	466	420		
Chr3:154 898 407	(1040)	(.15)	(.45)	(.40)		

Genotype counts (frequencies) are shown for various markers in MME in AD cases and controls. Percentages given were expressed relative to the total number of samples successfully assayed for each SNP where the total numbers for ADs and controls successfully assay are denoted in parentheses. Chi-square statistics and associated P values were obtained from 2x3 (genotype) contingency tables. Additionally, logistic regression statistics are presented for additive (linear) genetic models and include gender, age, and APOE-e4 status as co-variates, as well as a cohort identifier (italian, english, swedish) to control for population stratification. The Odds Ratio (OR) for rs6797911 was 1.40 with a 95% confidence interval of 1.16-1.70.

mained significantly different between AD and controls (p=0.00052). This significant association generated an OR = 1.40 (95% CI = 1.16-1.70). However, and perhaps not surprisingly, haplotype analysis did not reveal any evidence of association with any of the resultant haplotypes and AD risk (data not shown).

Association of MME variants with Aβ concentration and neprilysin level and activity

To evaluate effects of MME sequence variants on Aβ levels, multi-locus ANOVA models were fit using four SNP markers (rs989692, rs9827586, rs6797911, rs701109). All of these were intronic except for rs701109 which is mapped to the 3'UTR of MME. These SNPs were selected for a number of reasons. rs6797911 had previously been demonstrated to be genetically associated with AD; rs989692 had been reported to be associated with age at onset of AD [13]; rs701109 is one of the more commonly studied SNPs to date (see Alzgene: http://www.alzgene.org/meta.asp? geneID=73); and rs9827586 is a HapMap defined tag SNP that is located between rs989692 and

Preview

Table 3. Quantitative trait associations for MME

rsID/sample	Trait	Genotypes			Significance
rs989692		C/C	C/T	T/T	
	NEP protein	126.2 ± 18.11 (12)	220.3 ± 17.72 (35)	164.0 ± 29.32 (17)	P = 0.058
	NEP enzyme activity	77.4 ± 9.23 (12)	76.87 ± 5.76 (35)	70.63 ± 7.61 (17)	NS
rs9827586		GG	G/A	A/A	
	NEP protein	178.75 ± 19.96 (20)	221.21 ± 22.97 (33)	150.17 ± 18.95 (14)	P = 0.014
	NEP enzyme activity	67.48 ± 4.92 (21)	80.45 ± 6.64 (33)	74.63 ± 8.10 (14)	P = 0.006
rs6797911		T/T	T/A	A/A	
	NEP protein	187.02 ± 22.56 (26)	229.15 ±21.52 (32)	188.72 ± 34.12 (11)	NS
	NEP enzyme activity	72.11 ± 6.56 (28)	79.89 ± 5.97 (31)	64.24 ± 5.28 (12)	NS
rs701109		A/A	G/A	G/G	
	NEP protein	322.45 ± 65.69 (7)	194.22 ± 18.90 (31)	190.35 ± 21.93 (32)	P = 0.026
	NEP enzyme activity	67.99 ± 11.86 (8)	70.41 ± 4.45 (31)	79.63 ± 6.52 (38)	NS

Genotypic means ± SEM of NEP protein levels and NEP enzyme activity levels (P values <0.05 considered significant). Genetic association was observed between MME rs9827586 and both NEP protein level (p = 0.014) and strongly significant with enzyme activity (p = 0.0006). There was also some evidence of association between rs701109 and NEP protein levels p=0.026. The fact that both rs9827586 and rs701109 have similar patterns of effect is perhaps reflected by the fact that only rs701109 was in weak linkage disequilibrium with rs9827586 ($r^2 < 0.5$). There was also suggestive although non-significant evidence of association between rs989692 and NEP protein levels p=0.058.

Preview

rs6797911. All analyses were adjusted for post-mortem delay, age, gender and disease status.

There was evidence of an association of rs9827586 with both NEP enzyme activity and protein expression (Table 3), particularly after adjustment for the level of neuronal specific enolase (NSE), a marker of neuronal content, allowing adjustment for neuronal loss in AD [30]. Genetic associations were also observed for rs989692 and rs701109 but these did not survive correction for multiple testing. We investigated the presence of genetic association between these variants and levels of total soluble and insoluble Aβ and soluble oligomeric Aβ1-42, previously measured for the brain bank samples [26, 34-36]. For none of these variables was there evidence of association with any of the 4 selected SNPs in MME (data not shown).

Discussion

Excessive accumulation of Aβ is thought to initiate the development AD and to lead also to CAA. Levels of Aβ reflect the balance between Aβ production and clearance; age- and disease-related reduction in enzyme-mediated degradation of Aβ is one potential mechanism that may account for the abnormal accumulation of Aβ in AD and CAA [34].

Three MME SNPs located within the vicinity of the 3'UTR (rs989692 and rs701109) and the 5'UTR (rs373187) were previously been shown to be associated with AD in Spanish and Finnish populations [37, 38]. However these associations were not replicated in recent studies, including a large American Caucasian study [24], a recent Swedish population study [13] and our data from West-Central and Southern Europe. Furthermore, these three SNPs were not associated with biochemical measures of AD (CSF Aβ42 and tau levels) or mini-mental state examination (MMSE) scores in the Swedish cohort, although there was a suggestion of association between AD age-at-onset and rs989692 [13]. Our present study has not provided evidence of an association between any of the selected 4 MME variants and total soluble Aβ, oligomeric Aβ1-42 and total insoluble Aβ within the frontal cortex. A dinucleotide repeat polymorphism (GT) in the MME promoter region was reported to be associated with AD [20] and CAA risk [39] but this association has not since been

reproduced in most other studies [17, 18, 22, 24]. A large number of other SNPs within MME were not associated with AD [24].

Ours is the first study to report genetic association between AD and rs6797911. This same variant had a marginally significant association with AD in the Translational Genomics Research Institute (TGEN) Genome Wide Association Study (GWAS) [19, 40], the direction of effect being the same as in the present study. Further support for this association comes from one of the first large-scale GWAS meta-analyses by Lambert and colleagues who found assocation of SNP rs2016848 with AD, with a p-value of 0.0066; rs2016848 is a perfect proxy for rs6797911 [41]. The reason why we found no further associations within MME is likely to be because rs6797911 was in weak linkage disequilibrium (LD) with only rs101109 ($r^2 < 0.5$) with little LD with any of the others studied.

We have found that rs9827586 is strongly associated with NEP protein level and enzyme activity. One might have expected this to modulate AD risk given the function of NEP as an Aβ degrading enzyme, yet there was no evidence of association between this SNP and the post-mortem measures of Aβ load. It is possible that the observed association with NEP level and activity is simply a chance finding. However, we previously showed NEP enzyme activity to be increased in AD in relation to disease severity [30]. Variation at rs9827586 may influence the upregulation of NEP in AD in response to Aβ accumulation without having an effect on the development of the disease.

Elevated NEP activity may also help to limit the vasoconstrictive action of angiotensin II in the renin angiotensin system (RAS). There is now a growing body of evidence supporting the role of the RAS in the pathogenesis of AD [42]. We previously showed that the activity of angiotensin-converting enzyme, responsible for the synthesis of angiotensin II, is increased in the brain in AD [43, 44]. NEP cleaves angiotensin I (the precursor of angiotensin II) to generate angiotensin 1-7, which serves to counteract the function of angiotensin II. NEP, like ACE, is elevated in AD [30, 43, 44] - both perhaps upregulated by Aβ as a physiological response to prevent its continuing accumulation but both also affecting the regulation of arterial tone and cerebral blood flow and potentially thereby contributing to cognitive decline [45, 46].

In summary, we report genetic association between the MME variant rs6797911 and AD. We also provide evidence that other variants of MME, particularly rs9827586, or variants in linkage disequilibrium, may influence NEP level and activity and thereby have disease modifying effects in AD. Further research is needed to clarify the nature of the relationship between NEP activity, Aβ metabolism, angiotensin II and cerebral blood flow, and the role that genetic variation in MME plays in these interactions and in the progression of AD.

Acknowledgements

This work was part-supported by a BRACE programme grants and an Alzheimer's Research UK post-doctoral research fellowship to Dr Scott Miners.

Address correspondence to: Dr. Patrick G Kehoe, Dementia Research Group, John James Laboratories, School of Clinical Sciences, University of Bristol, Day Hospital, Frenchay Hospital, Bristol, BS16 1LE, UK Tel: +44-117-340-3070; Fax: +44-117-340-6665; E-mail: Patrick.Kehoe@bristol.ac.uk

References

[1] Chalmers KA, Barker R, Passmore PA, Panza F, Seripa D, Solfrizzi V, Love S, Prince JA and Kehoe PG. LRP-1 variation is not associated with risk of Alzheimer's disease. Int J Mol Epidemiol Genet 2010; 1: 104-113.

[2] Carpentier M, Robitaille Y, DesGroseillers L, Boileau G and Marcinkiewicz M. Declining expression of neprilysin in Alzheimer disease vasculature: possible involvement in cerebral amyloid angiopathy. J Neuropathol Exp Neurol 2002; 61: 849-856.

[3] Miners JS, Van Helmond Z, Chalmers K, Wilcock G, Love S and Kehoe PG. Decreased expression and activity of neprilysin in Alzheimer disease are associated with cerebral amyloid angiopathy. J Neuropathol Exp Neurol 2006; 65: 1012-1021.

[4] Murray IVJ, Proza JF, Sohrabji F and Lawler JM. Vascular and metabolic dysfunction in Alzheimer's disease: a review. Experimental Biology and Medicine 2011; 236: 772-782.

[5] Iwata N, Tsubuki S, Takaki Y, Shirotani K, Lu B, Gerard NP, Gerard C, Hama E, Lee HJ and Saido TC. Metabolic regulation of brain Abeta by neprilysin. Science 2001; 292: 1550-1552.

[6] Shirotani K, Tsubuki S, Iwata N, Takaki Y, Harigaya W, Maruyama K, Kiryu-Seo S, Kiyama H, Iwata H, Tomita T, Iwatsubo T and Saido TC. Neprilysin degrades both amyloid beta peptides 1-40 and 1-42 most rapidly and efficiently among thiorphan- and phosphoramidon-

Preview

sensitive endopeptidases. J Biol Chem 2001; 276: 21895-21901.
[7] Eckman EA, Adams SK, Troendle FJ, Stodola BA, Kahn MA, Fauq AH, Xiao HD, Bernstein KE and Eckman CB. Regulation of steady-state beta -amyloid levels in the brain by neprilysin and endothelin-converting enzyme, but not angiotensin-converting enzyme. J Biol Chem 2006; 281: 30471-30478.
[8] Hemming ML, Selkoe DJ and Farris W. Effects of prolonged angiotensin-converting enzyme inhibitor treatment on amyloid beta-protein metabolism in mouse models of Alzheimer disease. Neurobiol Dis 2007; 26: 273-281.
[9] Marr RA, Rockenstein E, Mukherjee A, Kindy MS, Hersh LB, Gage FH, Verma IM and Masliah E. Neprilysin gene transfer reduces human amyloid pathology in transgenic mice. J Neurosci 2003; 23: 1992-1996.
[10] Butler AW, Ng MY, Hamshere ML, Forabosco P, Wroe R, Al-Chalabi A, Lewis CM and Powell JF. Meta-analysis of linkage studies for Alzheimer's disease-a web resource. Neurobiol Aging 2009; 30: 1037-1047.
[11] Hamshere ML, Holmans PA, Avramopoulos D, Bassett SS, Blacker D, Bertram L, Wiener H, Rochberg N, Tanzi RE, Myers A, Wavrant-De Vrieze F, Go R, Fallin D, Lovestone S, Hardy J, Goate A, O'Donovan M, Williams J and Owen MJ. Genome-wide linkage analysis of 723 affected relative pairs with late-onset Alzheimer's disease. Hum Mol Genet 2007; 16: 2703-2712.
[12] Iwata N, Higuchi M and Saido TC. Metabolism of amyloid-beta peptide and Alzheimer's disease. Pharmacol Ther 2005; 108: 129-148.
[13] Blomqvist ME, McCarthy S, Blennow K, Andersson B and Prince JA. Evaluation of neprilysin sequence variation in relation to CSF beta-Amyloid levels and Alzheimer disease risk. Int J Mol Epidemiol Genet 2010; 1: 47-52.
[14] Fu Y, Li AF, Shi JJ, Tang MN, Guo YB and Zhao ZH. Lack of association of neprilysin gene polymorphisms with Alzheimer's disease in a southern Chinese community. Int Psychogeriatr 2009; 21: 354-358.
[15] Giedraitis V, Kilander L, Degerman-Gunnarsson M, Sundelof J, Axelsson T, Syvanen AC, Lannfelt L and Glaser A. Genetic analysis of Alzheimer's disease in the Uppsala Longitudinal Study of Adult Men. Dement Geriatr Cogn Disord 2009; 27: 59-68.
[16] Li H, Wetten S, Li L, St Jean PL, Upmanyu R, Surh L, Hosford D, Barnes MR, Briley JD, Borrie M, Coletta N, Delisle R, Dhalla D, Ehm MG, Feldman HH, Fornazzari L, Gauthier S, Goodgame N, Guzman D, Hammond S, Hollingworth P, Hsiung GY, Johnson J, Kelly DD, Keren R, Kertesz A, King KS, Lovestone S, Loy-English I, Matthews PM, Owen MJ, Plumpton M, Pryse-Phillips W, Prinjha RK, Richardson JC, Saunders A, Slater AJ, St George-Hyslop PH, Stinnett SW, Swartz JE, Taylor RL, Wherrett J, Williams J, Yarnall DP, Gibson RA, Irizarry MC, Middleton LT and Roses AD. Candidate single-nucleotide polymorphisms from a genomewide association study of Alzheimer disease. Arch Neurol 2008; 65: 45-53.
[17] Lilius L, Forsell C, Axelman K, Winblad B, Graff C and Tjernberg L. No association between polymorphisms in the neprilysin promoter region and Swedish Alzheimer's disease patients. Neurosci Lett 2003; 337: 111-113.
[18] Oda M, Morino H, Maruyama H, Terasawa H, Izumi Y, Torii T, Sasaki K, Nakamura S and Kawakami H. Dinucleotide repeat polymorphisms in the neprilysin gene are not associated with sporadic Alzheimer's disease. Neurosci Lett 2002; 320: 105-107.
[19] Reiman EM, Webster JA, Myers AJ, Hardy J, Dunckley T, Zismann VL, Joshipura KD, Pearson JV, Hu-Lince D, Huentelman MJ, Craig DW, Coon KD, Liang WS, Herbert RH, Beach T, Rohrer KC, Zhao AS, Leung D, Bryden L, Marlowe L, Kaleem M, Mastroeni D, Grover A, Heward CB, Ravid R, Rogers J, Hutton ML, Melquist S, Petersen RC, Alexander GE, Caselli RJ, Kukull W, Papassotiropoulos A and Stephan DA. GAB2 alleles modify Alzheimer's risk in APOE epsilon4 carriers. Neuron 2007; 54: 713-720.
[20] Sakai A, Ujike H, Nakata K, Takehisa Y, Imamura T, Uchida N, Kanzaki A, Yamamoto M, Fujisawa Y, Okumura K and Kuroda S. Association of the Neprilysin gene with susceptibility to late-onset Alzheimer's disease. Dement Geriatr Cogn Disord 2004; 17: 164-169.
[21] Shi J, Zhang S, Tang M, Ma C, Zhao J, Li T, Liu X, Sun Y, Guo Y, Han H, Ma Y and Zhao Z. Mutation screening and association study of the neprilysin gene in sporadic Alzheimer's disease in Chinese persons. J Gerontol A Biol Sci Med Sci 2005; 60: 301-306.
[22] Sodeyama N, Mizusawa H, Yamada M, Itoh Y, Otomo E and Matsushita M. Lack of association of neprilysin polymorphism with Alzheimer's disease and Alzheimer's disease-type neuropathological changes. J Neurol Neurosurg Psychiatry 2001; 71: 817-818.
[23] Vepsalainen S, Helisalmi S, Mannermaa A, Pirttila T, Soininen H and Hiltunen M. Combined risk effects of IDE and NEP gene variants on Alzheimer disease. J Neurol Neurosurg Psychiatry 2009; 80: 1268-1270.
[24] Wood LS, Pickering EH, McHale D and Dechairo BM. Association between neprilysin polymorphisms and sporadic Alzheimer's disease. Neurosci Lett 2007; 427: 103-106.
[25] van Helmond Z, Heesom K and Love S. Characterisation of two antibodies to oligomeric Abeta and their use in ELISAs on human brain tissue homogenates. J Neurosci Methods 2009; 176: 206-212.
[26] van Helmond Z, Miners JS, Kehoe PG and Love

Preview

S. Oligomeric Abeta in Alzheimer's Disease: Relationship to Plaque and Tangle Pathology, APOE Genotype and Cerebral Amyloid Angiopathy. Brain Pathol 2010; 20: 468-480.
[27] Mirra SS, Heyman A, McKeel D, Sumi SM, Crain BJ, Brownlee LM, Vogel FS, Hughes JP, van Belle G and Berg L. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part II. Standardization of the neuropathologic assessment of Alzheimer's disease. Neurology 1991; 41: 479-486.
[28] McKhann G, Drachman D, Folstein M, Katzman R, Price D and Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology 1984; 34: 939-944.
[29] Wenham PR, Price WH and Blandell G. Apolipoprotein E genotyping by one-stage PCR. Lancet 1991; 337: 1158-1159.
[30] Miners JS, Baig S, Tayler H, Kehoe PG and Love S. Neprilysin and insulin-degrading enzyme levels are increased in Alzheimer disease in relation to disease severity. J Neuropathol Exp Neurol 2009; 68: 902-914.
[31] Miners JS, Verbeek MM, Rikkert MO, Kehoe PG and Love S. Immunocapture-based fluorometric assay for the measurement of neprilysin-specific enzyme activity in brain tissue homogenates and cerebrospinal fluid. J Neurosci Methods 2008; 167: 229-236.
[32] Barrett JC, Fry B, Maller J and Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 2005; 21: 263-265.
[33] Hill WG. Estimation of linkage disequilibrium in randomly mating populations. Heredity 1974; 33: 229-239.
[34] Miners JS, van Helmond Z, Kehoe PG and Love S. Changes with Age in the Activities of beta-secretase and the Abeta-degrading Enzymes Neprilysin, Insulin-degrading Enzyme and Angiotensin-converting Enzyme. Brain Pathol 2010; 20: 794-802.
[35] Miners JS, van Helmond ZK, Chalmers K, Wilcock GK, Love S and Kehoe PG. Downregulation of neprilysin in Alzheimer's disease is associated with cerebral amyloid angiopathy and APOE ε4. JNEN 2006; submitted.
[36] van Helmond Z, Miners JS, Kehoe PG and Love S. Higher Soluble Amyloid beta Concentration in Frontal Cortex of Young Adults than in Normal Elderly or Alzheimer's Disease. Brain Pathol 2010; 20: 787-793.
[37] Clarimon J, Munoz FJ, Boada M, Tarraga L, Sunyer J, Bertranpetit J and Comas D. Possible increased risk for Alzheimer's disease associated with neprilysin gene. J Neural Transm 2003; 110: 651-657.
[38] Helisalmi S, Hiltunen M, Vepsalainen S, Iivonen S, Mannermaa A, Lehtovirta M, Koivisto AM, Alafuzoff I and Soininen H. Polymorphisms in neprilysin gene affect the risk of Alzheimer's disease in Finnish patients. J Neurol Neurosurg Psychiatry 2004; 75: 1746-1748.
[39] Yamada M, Sodeyama N, Itoh Y, Takahashi A, Otomo E, Matsushita M and Mizusawa H. Association of neprilysin polymorphism with cerebral amyloid angiopathy. J Neurol Neurosurg Psychiatry 2003; 74: 749-751.
[40] Coon KD, Myers AJ, Craig DW, Webster JA, Pearson JV, Lince DH, Zismann VL, Beach TG, Leung D, Bryden L, Halperin RF, Marlowe L, Kaleem M, Walker DG, Ravid R, Heward CB, Rogers J, Papassotiropoulos A, Reiman EM, Hardy J and Stephan DA. A high-density whole-genome association study reveals that APOE is the major susceptibility gene for sporadic late-onset Alzheimer's disease. J Clin Psychiatry 2007; 68: 613-618.
[41] Lambert JC, Heath S, Even G, Campion D, Sleegers K, Hiltunen M, Combarros O, Zelenika D, Bullido MJ, Tavernier B, Letenneur L, Bettens K, Berr C, Pasquier F, Fievet N, Barberger-Gateau P, Engelborghs S, De Deyn P, Mateo I, Franck A, Helisalmi S, Porcellini E, Hanon O, de Pancorbo MM, Lendon C, Dufouil C, Jaillard C, Leveillard T, Alvarez V, Bosco P, Mancuso M, Panza F, Nacmias B, Bossu P, Piccardi P, Annoni G, Seripa D, Galimberti D, Hannequin D, Licastro F, Soininen H, Ritchie K, Blanche H, Dartigues JF, Tzourio C, Gut I, Van Broeckhoven C, Alperovitch A, Lathrop M and Amouyel P. Genome-wide association study identifies variants at CLU and CR1 associated with Alzheimer's disease. Nat Genet 2009; 41: 1094-1099.
[42] Kehoe PG, Miners S and Love S. Angiotensins in Alzheimer's disease - friend or foe? Trends Neurosci 2009; 32: 619-628.
[43] Miners JS, Ashby E, Van Helmond Z, Chalmers KA, Palmer LE, Love S and Kehoe PG. Angiotensin-converting enzyme (ACE) levels and activity in Alzheimer's disease, and relationship of perivascular ACE-1 to cerebral amyloid angiopathy. Neuropathol Appl Neurobiol 2008; 34: 181-193.
[44] Miners JS, Raiker M, Love S and Kehoe PG. ACE variants and association with brain Aβ levels in Alzheimer’s disease. American Journal of Translational Research 2010; 3: 8.
[45] Ruitenberg A, den Heijer T, Bakker SL, van Swieten JC, Koudstaal PJ, Hofman A and Breteler MM. Cerebral hypoperfusion and clinical onset of dementia: the Rotterdam Study. Ann Neurol 2005; 57: 789-794.
[46] Warkentin S, Erikson C and Janciauskiene S. rCBF pathology in Alzheimer's disease is associated with slow processing speed. Neuropsychologia 2008; 46: 1193-1200.
[47] Kehoe PG, Russ C, McIlory S, Williams H, Holmans P, Holmes C, Liolitsa D, Vahidassr D, Powell J, McGleenon B, Liddell M, Plomin R, Dynan K, Williams N, Neal J, Cairns NJ, Wilcock

Preview

G, Passmore P, Lovestone S, Williams J and Owen MJ. Variation in DCP1, encoding ACE, is associated with susceptibility to Alzheimer disease. Nat Genet 1999; 21: 71-72.
[48] Lehmann DJ, Cortina-Borja M, Warden DR, Smith AD, Sleegers K, Prince JA, van Duijn CM and Kehoe PG. Large meta-analysis establishes the ACE insertion-deletion polymorphism as a marker of Alzheimer's disease. Am J Epidemiol 2005; 162: 305-317.
[49] Belbin O, Brown K, Shi H, Medway C, Abraham R, Passmore P, Mann D, Smith AD, Holmes C, McGuinness B, Craig D, Warden D, Heun R, Kolsch H, Love S, Kalsheker N, Williams J, Owen MJ, Carrasquillo M, Younkin S, Morgan K and Kehoe PG. A multi-center study of ACE and the risk of late-onset Alzheimer's disease. J Alzheimers Dis 2011; 24: 587-597.
[50] Kehoe PG. Angiotensins and Alzheimer's disease: a bench to bedside overview. Alzheimers Res Ther 2009; 1: 3.
[51] Rigat B, Hubert C, Alhenc-Gelas F, Cambien F, Corvol P and Soubrier F. An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. J Clin Invest 1990; 86: 1343-1346.

Preview