Evaluation of Factors of Importance for Clinical Dementia Diagnosis

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Key Words

Alzheimer's disease · Apolipoprotein E · Homocysteine · Vascular dementia · Glutathione S-transferase · Sulfotransferase 1A1 · Diagnosis · Partial least squares discriminant analysis

Abstract

Diagnosing clinical dementia is based on an assessment of different variables, such as the patient's medical history, known risk factors, and biochemical features. Partial least squares discriminant analysis was used to evaluate variables of importance for diagnosing dementia in a clinical dementia population. Polymorphism for genotypes of glutathione S-transferase (GST) and sulfotransferase 1A1, hypothetically of importance in dementia disorders, was also included in the analysis. The study population consisted of 73 patients with Alzheimer's disease (AD), 14 with mixed dementia, 75 patients with vascular dementia, and 28 control cases. We found that several of the variables, such as the presence of ApoE4 allele, high cerebrospinal fluid levels of total tau protein, low levels of β -amyloid_(1–42), and a low score on the Mini-Mental State Examination, facilitated a discrimination between the diagnoses compared with the controls. The different diagnoses overlapped. There were indications that genotypes of GSTs contributed to a subgrouping within AD.

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Introduction

The basis for clinical dementia diagnosis is mainly a thorough clinical investigation, including the medical history of the patient, combined with an interview with an informant living close to the patient. Other factors considered to be of importance for the diagnostic considerations are neuroimaging [1, 2], ApoE4 genotyping [3–5] as well as the cerebrospinal fluid (CSF) markers, protein tau [6–9] and β -amyloid_(1–42) (A β 42) [10–12]. The search for solid, safe diagnostic markers has intensified as treatment alternatives have emerged.

It has been suggested that oxidative stress might play an important role in the pathogenesis of neurodegeneration [13–17]. Glutathione is an important intracellular antioxidant that protects against a variety of oxidative species. A disturbance in glutathione homeostasis might lead to, or result from, oxidative stress in neurodegenerative disorders [17]. A recent finding of decreased glutathione levels in lymphoblasts in Alzheimer's disease (AD) cases has been interpreted as an indication of impaired antioxidative capacity [18].

Glutathione S-transferases (GSTs) are a group of detoxification enzymes found in the cytosol that are involved in the metabolism of endogenously and exogenously generated toxins [17, 19, 20]. For AD, autopsy studies have found decreased levels of GST in the brain

and in ventricular CSF [17] as well as lower GST activity in different brain areas [19].

The GST family of genes can be grouped into 6 classes [20]. The μ class expresses 5 different genes, M1–5. Genetic polymorphisms of GSTM1 loci of the μ class and GSTT1 loci of the θ class are described. There are 3 alleles at the GSTM1 locus: GSTM1*A, GSTM1*B, and GSTM1*0, the latter being a deletion or null genotype [21, 22]. For the GSTT1 gene, the variant allele is a deletion of the gene. Therefore GSTT*0 allele expresses no enzyme [21–23]. For the GST π , 2 polymorphic alleles and 2 variations are reported, GSTP1*B and GSTP1*C, in addition to the wild-type allele, GSTP1*A [24]. Homozygous deletion of the GSTM1 and GSTT1, (GSTM1*0, GSTT1*0, respectively) can lead to an absence of the enzyme activity [23]. In addition, a correlation between deletion in GSTT1 and susceptibility to AD has been reported [25], whereas no association was found between polymorphism of GSTM1 and AD or other neurodegenerative disorders, such as Parkinson's disease [26].

Another mechanism involved in the defence of the cell metabolism is sulfation of endogenous and exogenous chemicals [27, 28]. Sulfation is a detoxification mechanism in the transformation of a substrate to a less harmful compound [27]. The sulfotransfer is catalyzed by the cytosolic sulfotransferase enzyme [29, 30]. Sulfotransferases are divided into two major families, Sult1A1 and Sult1A2. The Sult1A1 consists of at least four different subfamilies of enzymes [31]. A previous study found that platelet sulfotransferase activity was significantly higher in dementia of Alzheimer type compared with controls and that the increased activity was positively correlated with the severity of dementia [32].

Elevated homocysteine (Hcy) levels have been found to be an independent risk factor for the development of dementia disorders [33], and for the presence of white matter changes in AD patients [34, 35] and vascular dementia (VaD) patients [35].

The methylenetetrahydrofolate reductase (MTHFR) enzyme is involved in the methylation of Hcy to methionine [36]. A genetic polymorphism exists for the MTHFR. This polymorphism, also called the thermolabile variant, is a C to T substitution at nucleotide 677 [37] and leads to a reduction in enzyme activity [38] and higher Hcy levels [39]. It has been reported to increase the risk of VaD, and perhaps also dementia poststroke [40], but not the risk of AD [41].

We used the multivariate statistical method, partial least squares discriminant analysis (PLS-DA), to evaluate information from previous studies and added data, such

as medical history and polymorphisms for genotypes that might be of importance for the evolution of dementia. The aim of the study was to investigate which of the available factors contribute to the different clinical dementia diagnoses.

Materials and Methods

The study included 162 in- and outpatients referred for evaluation of dementia to the Department of Geriatric Medicine at Linköping University Hospital, Sweden. Diagnostic classification was based on medical history, clinical examination, Mini-Mental State Examination (MMSE) [42], CT of the brain, EEG, routine blood and CSF samples, and a clinical descriptive neuropsychological evaluation, as previously described [43]. All diagnoses were made without knowledge of the results of the CSF analyses, and vice versa. The patients were divided into different diagnostic categories according to the ICD-10 [44].

The study group consisted of 73 patients with AD, 14 with mixed dementia (AD in combination with relevant cerebrovascular disorder), 75 with VaD, and 28 control cases.

The control group was recruited at the Orthopedics Clinic at Linköping University Hospital. Twenty-eight patients over 60 years of age undergoing planned surgical orthopedic intervention with spinal anesthesia constituted the control group. The controls were interviewed concerning their medical history and were tested with the MMSE in order to exclude patients with unknown cognitive disturbance. The control cases all had an MMSE score of 26 points or higher.

Serum creatinine was determined using routine laboratory methods. Plasma Hcy was determined by fluorescence polarization immunoassay with IMX [45–47] at the Laboratory of Clinical Chemistry, University Hospital, Linköping, Sweden, as previously described [48]. The ELISA method (Innotest hTAU-Ag; Innogenetics, Ghent, Belgium) was used to determine CSF levels of total tau (T-tau) [8], phospho-tau (P-tau; tau phosphorylated at threonine 181) [49], and A β 42 (Innotest β -amyloid_(1–42); Innogenetics) [10] at the Institute of Clinical Neuroscience, Sahlgrenska University Hospital, Mölndal, Sweden, as previously described [43]. ApoE genotyping was performed by PCR and solid-phase mini-sequencing [50]. MTHFR genotyping was performed by PCR and solid-phase mini-sequencing [51]. The genotyping of the GSTs and Sult1A1 was performed by PCR at the Clinical Research Centre, Linköping University, Linköping, Sweden, as previously described [52].

Statistics

Statistical analysis was performed with the SIMCA-P statistical package [53].

The multivariate projection methods, principal component analysis and PLS projection to latent structures, were used to reveal the underlying structure in the test sample. These methods allow information from several variables to be used simultaneously, and any interdependency between the variables is also handled. The principal components analysis is normally a straightforward tool for revealing major variance structure and clustering [54]. In cases where the clustering is subtle, however, retrospective knowledge of

expected clustering can be introduced, e.g. clinical diagnosis, to enhance the power of the principal components analysis by regressing it to a discrete variable comprised of such information, i.e. PLS-DA [55]. The models were validated by using the cross-validation procedure [56].

In PLS-DA the X variables represent predictor variables, and the Y variables represent the response variables. Variable influence on projections, VIP, is a parameter that summarizes the importance of the X variables, for both the X and Y models [57]. The variables used in the PLS-DA models are listed in table 1. To identify the most important variables for group discrimination, those with a VIP value below 0.7 were excluded (table 1) from the analysis in each model [57], and PLS-DA was re-run, resulting in new models (fig. 1–3). Some of the variables did not have any variance in one of the two groups in the respective models and were removed for mathematical reasons. They were GTM null, peptic ulcer, non-steroidal anti-inflammatory drug (NSAID) treatment, and cobalamin treatment in the mixed dementia model, and NSAID treatment in the VaD model.

Five moderate outliers in the AD group, 1 in the mixed dementia group, and 2 in the VaD group were identified by analysis of residuals. However, as removing the outliers changed the results only to a small extent (data not shown), these cases were kept in the data set.

Ethics

The local ethical committee approved the study, and informed consent was obtained from all participants and/or their relatives.

Results

The mean $\pm$ SD age in the VaD group was significantly higher (79.0 ± 5.6 years) than in the AD group (75.8 ± 6.5 years, one-way ANOVA, $p < 0.01$) and the controls (74.6 ± 9.9 years, one-way ANOVA, $p < 0.01$), but not compared with the mixed dementia group (77.8 ± 4.9 years, one-way ANOVA, NS). There was no significant difference in gender distribution (female/male) between the groups (AD: 44/29; mixed dementia: 12/2; VaD: 45/30, and controls: 20/8, χ^2 test, NS). When the mean $\pm$ SD MMSE scores were compared, the AD group had a lower score (20.0 ± 5.4 , one-way ANOVA, $p < 0.001$) than the controls (28.6 ± 1.2), as did the mixed dementia group (18.6 ± 5.8 , one-way ANOVA, $p < 0.001$) and the VaD group (21.3 ± 5.1 , one-way ANOVA, $p < 0.001$). There were, however, no significant differences between the dementia groups (one-way ANOVA, NS).

The R²Y(cum) values represent the cumulative sum of squares of all the Ys explained by the extracted components in the PLS-DA model, whereas the Q²(cum) values stand for the cumulative fractions of the total variation for all the Ys that can be predicted by the extracted components (i.e. goodness of prediction) [57]. The scores

Table 1. Variables used in PLS-DA models

Sex	Female/Male
Age ^c	Years
Fam ^{a,b,c}	Family history of dementia disorder
Edu	Education
Duration	Years
Hypertension ^b	Medical history – Hypertension
Cardiovasc ^c	Medical history – Cardiovascular disorder
CVS ^{b,c}	Medical history – Cerebrovascular disorder
Psych ^{a,b,c}	Medical history – Psychiatric disorder
Inflam	Medical history – Inflammatory disorder
Peptic	Medical history – Peptic ulcer
Diabetes ^a	Medical history – Diabetes mellitus
Antidepr ^{a,b,c}	Actual medication – Antidepressive treatment
NSAID ^a	Actual medication – NSAID treatment
Cortisone	Actual medication – Cortisone treatment
Cobalamin ^{a,c}	Actual medication – Cobalamin treatment
Folate	Actual medication – Folate treatment
S-Crea	Serum – Creatinine
P-Hcy ^c	Plasma – Homocysteine
CSF-MMA	CSF – Methylmalonic acid
T-Tau ^{a,b,c}	CSF – Total tau protein
A β 42 ^{a,b,c}	CSF – β -amyloid _(1–42)
P-Tau ^{a,b}	CSF – Phospho-tau protein
MMSE ^{a,b,c}	Mini-Mental State Examination
ApoE4 ^{a,b,c}	Apolipoprotein E4 ϵ 4 alleles
MTHFR ^a	Methylenetetrahydrofolate reductase 677C→T allele
GT del ^{a,b}	Glutathione S-transferase T1 deletion
GT pres ^{a,b}	Glutathione S-transferase T1 genotype present
GM del ^{a,b}	Glutathione S-transferase M1 deletion
GM pres ^{a,b}	Glutathione S-transferase M1 genotype present
GP A/A ^{b,c}	Glutathione S-transferase P1, genotype A/A
GP A/BC ^{a,b,c}	Glutathione S-transferase P1, genotype A/BC
GP BC/BC ^b	Glutathione S-transferase P1, genotype BC/BC
GTM M/M	Glutathione S-transferase T1M1, genotype M/M
GTM M/T ^{a,b}	Glutathione S-transferase T1M1, genotype M/T
GTM T/T	Glutathione S-transferase T1M1, genotype T/T
GTM null ^{a,c}	Glutathione S-transferase T1M1 null genotype
Sult 1/1	Sulfotransferase 1A1, genotype 1/1
Sult 1/2 ^{b,c}	Sulfotransferase 1A1, genotype 1/2
Sult 2/2 ^{b,c}	Sulfotransferase 1A1, genotype 2/2

^a Variable with VIP value above 0.7 in PLS-DA model for AD.

^b Variable with VIP value above 0.7 in PLS-DA model for mixed dementia.

^c Variable with VIP value above 0.7 in PLS-DA model for VaD.

of the R²Y(cum) for the calculated models ranged from 0.63 to 0.82, and the Q²(cum) scores ranged from 0.51 to 0.63.

Figure 1 shows the scores (t) and loadings (w*c), respectively, from the PLS-DA model, including all AD

Fig. 1. **a** PLS-DA model for AD and controls, scores. C = Controls; A = Alzheimer's disease. **b** PLS-DA model for AD and controls, loadings.

cases and the controls. The graphs can be considered as coordinate systems, where observations that are closely situated are similar and those that are distant are different in many respects. The score plot (fig. 1a) shows which variables produced the differentiation between AD and control cases, with an overlap of some cases. The influ-

ence of the different variables can be studied in the loading plots (fig. 1b), where the most influential variables are situated far from origo, in either direction, along a vector direction passing through origo and the Y-loading, i.e. the DA vector for either having AD diagnosis or being a control.

Fig. 2. **a** PLS-DA model for mixed dementia and controls, scores. C = Controls; M = mixed dementia. **b** PLS-DA model for mixed dementia and controls, loadings.

Ranked according to diminishing positive correlation with dementia caused by AD as the dependent variable, the significant predictors were: presence of the ApoE4 ϵ 4 allele, high CSF levels of T-tau, family history of dementia, high CSF levels of P-tau protein, current treatment with cobalamin, current antidepressive treatment, and

the presence of GSTT1M1 M/T alleles, GSTT1 allele, and GSTP1 A/BC alleles (fig. 1b). The predictors, in diminishing order, that were positively correlated with controls in the same model were: high CSF levels of A β 42, high MMSE score, deletion of GSTT1 allele, and current treatment with NSAIDs (fig. 1b).

Fig. 3. **a** PLS-DA model for VaD and controls, scores. C = Controls; V = vascular dementia. **b** PLS-DA model for VaD and controls, loadings.

There were two significant components in the model for the AD diagnosis. The pattern of the second component ($w^*c[2]$) might suggest that the presence or deletion of GSTT1 or GSTM1 alleles can be involved in the subgrouping within the AD cluster.

MTHFR, family history of dementia, history of psychiatric disorder, and current treatment with cobalamin or antidepressive drugs formed a cluster indicating that these variables were correlated to one another. Cobalamin treatment clearly indicates diagnosed cobalamin deficiency, and this state can be linked to the thermolabile

MTHFR variant of the enzyme, leading to reduced enzyme activity. A disease history reporting previous psychiatric disorder in these cases most often includes depression and anxiety problems. Hence, a correlation between a history of psychiatric disorder and antidepressive treatment is an expected finding. Finally, a possible link between depression and cobalamin deficiency has previously been reported [58].

Figure 2 shows the scores and loadings from the PLS-DA models, including the mixed dementia cases and controls. In diminishing positive correlation the predictors for the mixed dementia dependent variable were: high CSF levels of T-tau protein, presence of the Sult1A1 2/2 alleles, high CSF levels of P-tau, presence of the ApoE4 ϵ 4 allele, family history of dementia, presence of GSTT1M1 M/T alleles, and presence GSTM1 allele (fig. 2b). Predictors for the controls were high MMSE score, high CSF levels of A β 42, presence of GSTP1 A/A alleles, and deletion of GSTM1 allele (fig. 2b).

The clustered mixed dementia cases and the controls were well separated (fig. 2a). The mixed dementia group was a small, but rather homogeneous group. As only one significant component could be extracted in this model, any putative subgrouping within the cluster of mixed dementia was not indicated.

Finally, figure 3 shows the scores and the loadings for the PLS-DA models, including all cases of VaD and controls. The predictors for a VaD-dependent variable, in diminishing positive correlation, were: previous CVS, current antidepressive treatment, high age, high CSF levels of T-tau protein, high plasma levels of Hcy, presence of the ApoE4 ϵ 4 allele, presence of Sult1A1 2/2 alleles, previous cardiovascular disorder, current treatment with cobalamin, and presence of GSTP1 A/BC alleles (fig. 3b). The predictors for the controls in the same model were: high CSF levels of A β 42, high MMSE score, presence of Sult1A1 1/2 alleles, or presence of GSTP1 A/A alleles. The VaD and the controls was rather well separated, although some overlap occurred (fig. 3a). Two significant components could be extracted from the models including VaD patients. However, as this group seems to be more heterogeneously distributed, the pattern of loadings is more difficult to interpret.

Discussion

Classification is a way of making complex disorders comprehensible and can be considered a basis for knowledge. The PLS-DA multivariate method was used in the

present study to reveal the variables that carry class-separating information. PLS-DA made it possible to combine variables of very different kinds in the same analysis even though they might have been intercorrelated, which is often the case in clinical patient data. We found it interesting that previously unknown combinations of data in specific patterns might be able to contribute to dementia classification. In our models we demonstrated good separations between the respective diagnosis and the controls, although there was some overlap. However, as the same variables contributed to dementia of different diagnoses, their inherent discriminative power was not very large and did not possess more than partial explanatory power.

One methodological objection might be the exclusion of neuroimaging parameters from the analysis. The reason was that CT scans of the controls were not available, so these data were excluded for the dementia patients because the aim of this study was primarily to discriminate between dementia of different etiologies and neurologically healthy controls. However, we ran a PLS-DA model with data from the dementia patients only, and included CT data, to investigate the power to discriminate between the different dementia diagnoses. We found that there was a large overlap between the different diagnoses included in the model (data not shown). The R²Y(cum) representing the amount of the total variance of the Ys explained by the components for this model was just 0.282, which cannot exclude a chance model.

One explanation for some of the overlap between dementia cases and controls can be illustrated by the outliers in the model containing the AD cases. Four out of five cases were outliers due to CSF P-tau levels above the 90th percentile in the controls. We chose not to remove these cases as they might contain important information per se that could contribute to the unbiased overview. The discrimination between AD cases and controls was highly influenced by the established correlates, ApoE4, high CSF levels of T-tau protein, and low CSF levels of A β 42, which have been described during the past decade on innumerable occasions. However, these variables were also of importance for discriminating between mixed dementia or VaD and control cases. Indeed, recent research suggests a heterogeneity of the dementia syndrome [59], and there seems to be an overlap between the two most common dementia diagnoses, AD and VaD [60–62].

Other variables correlating with an AD diagnosis were certain genotypes for the GSTs, where a separation within the AD diagnosis was detected. However, this study

does not include enzyme activities. Theoretically, the GSTs could be involved in AD evolution via an impaired antioxidative capacity, which some previous studies have indicated [19, 25]. However, before making any such conclusions, experiments studying the specific enzyme activities must be conducted.

In all models, elevated levels of A β 42 and high MMSE score were positively correlated to controls as expected. The finding of an inverse relationship between AD and the use of NSAIDs also confirms previous knowledge [63, 64].

When interpreting the plots of the PLS-DA analyses, it has to be remembered that the cause or effect of the different variables must be taken under consideration. One illustration of this is the correlation between family history of dementia, history of psychiatric disorder, current treatment with cobalamin or antidepressive drugs, and the presence of the thermolabile genotype for the MTHFR enzyme. The link between dementia and depressive disorders may lie in a disturbance in the one-carbon metabolism, which involves the MTHFR. This relates to the methylation reactions involving the Hcy metabolism in the nervous system [65].

In the VaD models, the strongest predictive factor for VaD was previous CVS, which is obvious as this is one of the diagnostic criteria for the specific entity. Antidepressive treatment also seemed to be of importance for the VaD diagnosis, which might be due to the common poststroke depressive state [66, 67]. These two variables are close to each other in the loadings model, thus indicating a clear correlation between these variables. The pattern of the loadings in the VaD model was more difficult to interpret as this group was distributed more heterogeneously. One interesting factor was that the presence of Sult1A1 2/2 genotype loaded significantly on VaD

diagnosis. A similar pattern was also found in the model for dementia with features of both AD and VaD. This specific enzyme genotype did not seem to be involved at all in the AD model. One hypothesis is that the Sult1A1 enzyme might be involved in vascular damage in the genesis of dementia with vascular contributions. It has previously been found that platelet sulfotransferase activity was higher in AD compared with controls [32]. Another study indicated a possible association of this group of enzymes and protection against cell damage during aging [28]. It has also been established that the sulfotransferases are involved in the sulfate conjugation of hormones and neurotransmitters [68] which might be involved in the evolution of dementia. However, this needs to be further investigated by experiments that study the specific enzyme activities in different dementia diagnoses and in healthy controls.

We conclude that the power of classification possessed by several of the variables used in dementia evaluation is not strong enough for a certain diagnosis in individual cases. The diagnoses overlap, and there is some evidence of a common background. Other variables, however, such as genotypes of GSTs and Sult1A1, indicate that there might be different components in the etiology of AD and VaD. There is a great need for diagnostic markers with high specificity and sensitivity but also for classification studies in large clinical patient materials that include diagnostic variables of different contents.

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