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Chapter VIII

Blood Based Gene Expression: Useful Biomarkers for Alzheimer's and Parkinson's Diseases

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Abstract

There is today an expressed need for approved biomarkers that both can aid the diagnosis of Alzheimer's and Parkinson's diseases and guide the development of new treatments for these diseases. There are today two tests approved in Europe to aid the diagnosis of Alzheimer's disease at the dementia stage and both are based on the expression of several genes in blood that together generates a model that can predict the disease. At the same time no biomarker based on proteins or metabolites in blood has yet been approved. This chapter will review the current state of development of novel biomarkers for Alzheimer's and Parkinson's diseases based on the expression of genes in blood. The chapter will also try to explain why using gene expression likely will be a viable approach for the development of novel blood based biomarkers for neurodegenerative diseases.

1. Introduction

Alzheimer's disease (AD) and Parkinson's disease (PD) are the two most common neurodegenerative diseases and are with the ageing population becoming major health problems in the western world. AD is estimated to afflict 0.9 % among those 65 years of age and increases with age to 45% among persons 85 years of age [1] while PD has a prevalence of approximately 0.5 to 1 percent among persons 65 to 69 years of age, rising to 1 to 3 percent among persons 80 years of age and older [2]. With an ageing population the number of people with these diseases is expected to increase in the years to come. In 2006 the

worldwide prevalence of AD was 26.6 million and in 2050 it is expected that more than 100 million will be affected (1) by the disease unless an effective treatment will be available. For PD the number of people affected has by some been estimated to be between 5 to 6 million worldwide [3, 4].

Although only symptomatic treatment is available today for AD and PD it is anticipated that new disease-modifying drugs and treatments will become available in a not too far future. When Alzheimer's disease has developed into dementia an extensive loss of neurons and synapses in the cerebral cortex and certain subcortical regions are apparent [5] and for PD it is estimated that about 70% of the dopaminergic neurons in the substantia nigra have died when the disease become apparent for the patient and the clinician and can be diagnosed [6]. It is therefore of key importance for optimal treatment to have reliable diagnostic tools that can detect Alzheimer's and Parkinson's diseases at the earliest possible stage. At the same time reliable diagnostic tools can also be important for the development of novel treatments of the diseases since they can help reduce the number of false positives and by that aid to enrich the study populations with individuals having the disease and can respond to the treatment. Without an enriched study population a greater treatment response is required to be able to detect when part of the response is masked by the non-response from those included in the study while not having the disease. Reliable diagnostic tools can also be important to detect change in disease progression. Biomarkers have the potential to aid in a correct diagnosis and important progress has been made in the discovery and development of potentially useful markers in recent years. Interesting and novel trends in biomarker development can now be seen. There is now a trend toward biomarkers in blood and a trend from single to multi-component biomarkers can now be seen. The logic behind these trends is clear. A blood sample is much more convenient to collect than a cerebrospinal fluid tap or a tissue biopsy and a multi-component biomarker is more likely to detect heterogeneous diseases like AD and PD. In addition to protein based biomarkers there are now also an exciting and rapid development of biomarkers based on RNA and gene expression and a couple of blood based gene expression markers also have been CE marked in Europe to aid in the diagnosis of AD in the dementia stage. This review will describe the current state of the development of novel blood based gene expression tests for the early detection of AD and PD.

2. Biomarkers

A biomarker can be defined as an indicator of a biological state. It is a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention [7]. Surrogate biomarkers are a subset of biomarkers that serve as a substitute in a study for a clinically meaningful end point that is expected to predict the effect of a given therapeutic intervention [7]. In medicine biomarkers can be used in different clinical areas and functionally they are often divided into three main categories: diagnostic biomarkers, classification biomarkers and prognostic biomarkers.

a) Diagnostic biomarkers are biomarkers intended to aid early clinical or pre-clinical diagnosis of disease. Although highly debated for its value, a typical example is the

prostate specific antigen (PSA) for prostate cancer. Although highly debated for its value where no cut point of PSA concentration yields a simultaneous high sensitivity and high specificity [8] the biomarker is widely used together with complementary examination to aid the diagnosis of prostate cancer.

b) Classification biomarkers are used to differentiate between subtypes of a disease with a similar clinical presentation, but with different response to treatment or course. The gene expression assays for cancers of unknown primary (CUP) are good examples for classification biomarkers [9-12]. Based on the expression of a selected set of genes in tumor tissue the primary origin from which the tumor has metastasized is predicted. The test classifies to which tissue a tumor originated and can help define an optimal treatment for the cancer. The HER2 test is another well-known classification biomarker used to identify breast tumors with an elevated level of HER2 receptors [13] and where treatment with trastuzumab is efficient when the cancer has metastasized.

c) Prognostic biomarkers are biomarkers used to predict disease progression with or without the influence of therapy. Oncotype and Mammaprint are examples of prognostic biomarkers for breast cancer. Based on the expression of 21 and 70 genes, respectively, in formalin fixed tumor tissue the risk of recurrence is predicted [14, 15]. With a low score the prognosis of a benefit of chemotherapy after surgery is small and will not outweigh the risks of side effects. With a high score the prognosis of a benefit of chemotherapy is greater than the risks of side effects.

A single biomarker can in theory be used within all three functional categories but in practice it is expected that different biomarkers will perform well in only one or two [16].

3.1. Blood Based Gene Expression Biomarkers for AD and PD

The physiology of the blood-brain barrier limits potential biomarkers that are closely associated to brain pathophysiology to small molecules, lipophilic molecules, and molecules with specific transporters [17]. Brain derived proteins and metabolites that pass into the plasma will also become markedly diluted in a biochemically complex medium [17]. Moreover, it is not known if there are any direct pathophysiological processes associated with AD or PD in blood cells. The cerebrospinal fluid (CSF) biomarkers Aβ42, total-Tau and phospho-Tau associated with AD neuropathology are well recognized biomarkers for the disease and α-synuclein is a CSF biomarker for PD. However, none of these biomarkers and no other protein biomarker have been found useful in blood and no protein based diagnostic biomarker for either AD or PD in blood have been approved for use in any western world country. The promising results with an accuracy of 90% to predict AD and an accuracy above 80% to predict MCI due to AD for an 18 protein biomarker [18] has later been shown not possible to reproduce. In a recent study the same 18 proteins had a low diagnostic precision with an ROC of 0.63 [19].

The uniform chemical nature of RNA makes transcriptome studies less of a challenge than either proteome or metabolome studies and the potential use of blood-based gene expression profiling in the diagnosis of brain disorders have been described by independent groups [20-22]. Extensive studies have shown that with careful control in the experimental

design the gene expression microarray technology is reproducible both between labs and between experiments within a lab (23). This is true also for real time quantitative real-time PCR (q-PCR)[24].

3. Alzheimer's Disease

Alzheimer's disease (AD) is multifactorial and heterogeneous in both its clinical and histopathological appearance. In more than 99% of all cases the cause of the disease is not understood. Independent of its cause AD is clinically characterized by a developing dementia and histopathologically characterized by neuronal degeneration with the presence of neurofibrillary tangles (NFTs) and neurotic (senile) plaques. At autopsy AD histopathology shows considerable qualitative and quantitative heterogeneity. It can be of the neocortical, limbic, or plaque-dominant type and it may present with numerous NFTs exclusively confined to the hippocampus and entorhinal cortex. The clinical heterogeneity of the disease means that the diagnosis remains uncertain until post mortem when a histopathological examination can be performed. Therefore, the diagnosis today is made primarily by excluding other causes of dementia [25]. The two most common confounding dementias are vascular dementia and dementia with Lewy bodies.

As life expectancy increases, AD is becoming a major health problem in the western world afflicting about 27 million people worldwide today [1]. The prevalence of AD is expected to rise dramatically in the next few decades and it is expected that more than 100 million people will be living with the disease by the year 2050 [1] if this trend not is interrupted by novel treatments that affects the development of the disease. Intensive work is ongoing to identify reliable cures or preventive measures for the disease. To facilitate these investigations biomarkers are critically needed that can detect the disease at an early enough stage [26].

In a recent study to determine the accuracy for an AD diagnosis of currently used clinical diagnostic methods, clinical and neuropathologic data of 919 subjects with a clinical dementia diagnosis were collected between 2005 and 2010. Sensitivity and specificity were determined based on ‘‘probable’’ and ‘‘possible’’AD levels of clinical confidence and 4 levels of neuropathologic confidence based on varying neuritic plaque densities and Braak neurofibrillary stages. It was found that sensitivity ranged from 70.9% to 87.3% and specificity from 44.3% to 70.8% [27]. The results from this study show that the clinical diagnosis of AD among people with dementia not is perfect and can be improved. It is evident that well validated biomarkers can be a valuable tool to aid the clinician in the diagnosis.

The DSM-IV-TR and NINCDS–ADRDA criteria for the diagnosis of AD have been validated against neuropathological gold standards with diagnostic accuracy ranging from 65–96% [28-31]. However, the specificity of these diagnostic criteria against other dementias is only 23–88% [27-29]. To improve specificity and at the same time also include the prodromal phase of the disease as an early non-dementia stage of AD a revision of the diagnostic criteria has been suggested including the use of well validated biomarkers as supportive features [32]. For the first time the use of biomarkers has been suggested to be included as a central criterion for the diagnosis of AD.

Criteria for a useful diagnostic biomarker have been proposed by an international consensus group on molecular and biochemical markers of AD 1998 [33]. According to these guidelines, a biomarker for AD should detect a fundamental neuropathology and be validated in neuropathologically confirmed cases. Biomarker sensitivity for AD should exceed 80% and its specificity in differentiating between AD and other dementias should be higher than 80%. An ideal biomarker should also be reliable, reproducible, non-invasive, simple to perform and inexpensive. Since this consensus paper was written, the understanding of the disease has improved with the realization that an ability to detect the disease at the earliest possible stage is an additional and important feature required by an AD biomarker. Nevertheless, these criteria present many of the challenges in identifying useful AD biomarkers. There is currently only limited knowledge of how AD is manifested in blood, which is a body fluid readily available for biomarker discovery and use. Until we know more of how the disease affects other parts of the body than the brain it will remain a challenge for potential biomarkers today to fulfill the first criteria, the detection of a fundamental neuropathologic feature. An additional and significant challenge presented by these criteria is that the AD biomarker should be validated in neuropathologically confirmed cases, which currently means post mortem confirmed cases. To properly confirm the value of any given biomarker in line with these criteria would take many years. This is a significant and challenging task and in this review there are no examples where the diagnosis has been confirmed with post mortem histopathology.

AD is associated with profound biochemical and pathological alterations in the brain including aberrant amyloid precursor protein (APP), amyloid β-protein (Aβ) metabolism, tau protein phosphorylation, oxidative stress, inflammation and lipid dysregulation. In AD, the main cause of dementia is assumed to result from the progressive loss of synaptic function and neurological degeneration [34]. Neurofibrillary tangles (NFT's) and senile plaques are the neuropathologic hallmarks of AD and were described by Alois Alzheimer already in 1906. Senile plaques and NFT's, although not individually unique to AD, have a characteristic spreading and density in the diseased brain [35].

AD is a challenging disease for the development of biomarkers. The assessment of AD biomarkers is complicated by several factors. In addition to the variability in clinical features and multiple molecular etiologies that have been mentioned, the development of AD biomarkers is also burdened with a diagnostic imprecision since confirmation of the disease preferentially has to await post mortem histopathological examination. The long asymptomatic and prodromal stages, rates of progression, and complex disease genetics complicate the picture further.

3.1. Single Gene Biomarkers for AD

Several genes have been identified that are differentially expressed in blood or blood fractions of AD patients. They can all be potentially useful as blood based diagnostic, classification and prognostic biomarkers based on the expression of a single gene has been described [36-42]. However, except for TOMM40, the differential expression has not been confirmed in independent studies.

In an extensive study showing that clusterin in plasma is associated with brain atrophy in AD a significantly increased gene expression in blood was found in AD compared to both

mild cognitive impairment (MCI) and cognitively healthy controls [41]. Since clusterin is genetically strongly associated with AD [43] and is a known chaperone regulating amyloid formation and clearance [44] it would be highly interesting if the differential expression could be confirmed in independent studies.

In a validation of differently expressed genes in leukocytes between AD and healthy controls and utilizing pathway-based functional enrichment 34 genes where enriched [37]. When comparing AD, MCI and healthy controls 8 genes significantly associated with purine metabolism and ABC transporters where identified. However, when validated using qPCR only ABCB1 had a significant positive correlation with MMSE score among AD, MCI and healthy controls [37].

CD36 is a glycoprotein and a scavenger receptor of class B, and appears to play a key role in the pro-inflammatory events associated with AD. The expression of the CD36 gene in leukocytes was found to be significantly ($p<0.05$) decreased in AD and even more so in MCI [39]. Since it is expected that not all individuals with MCI will progress to an AD dementia diagnosis the more pronounced decrease in the MCI is surprising but is not discussed in the paper. It should be noted that gender ratio between the cohorts did vary and the old controls included in the study also were clearly younger than the AD and MCI individuals [39]. If this has affected the results is not known.

Increased expression of the CD44 gene, encoding a cell surface glycoprotein expressed by cells of the immune and central nervous system, was found in lymphocytes of 16 AD patients compared to 19 healthy controls [42]. However, the results have to be interpreted with some caution since also in this study there is a clear imbalance both between genders and in age between the two groups.

The TOMM40 gene is genetically closely linked to the ApoE gene. This makes it difficult to separate the specific genetic association to AD. However, a variable length polymorphism of TOMM40 has been described that predicts the age of late-onset AD [45]. It was recently shown that the TOMM40 is significantly down-regulated in AD [40]. This significant down-regulation was not only seen in an initial discovery study but also in two independent validation studies.

A functional polymorphism in the gene encoding brain-specific glutathione S-transferase Mu 3 (GSTM3) has been associated with late-onset AD [46]. Interestingly, the GSTM3 protein co-localizes with amyloid-β plaques in the brain. In a study including 347 AD, 291 MCI and 146 healthy age matched controls the expression of GSTM3 in AD blood where found to be reduced compared to healthy age matched controls [47]. The expression was reduced in the presence of the GSMT3 risk allele for both AD and healthy age matched controls. Although, a large set of MCI samples were included in the study gene expression data for the MCI cohort were unfortunately not presented.

Based on the expression pattern in post mortem AD brains compared to non-AD brains 33 genes were selected for a study to find gene expression alterations in blood in individuals with and without AD [48]. The study identified five genes (SNX2, COG2, GRIK, CNR2, HIST1H3E) that each showed significant ($p<0.05$) correlation with MMSE score [48]. However, no correlation with AD were presented making it uncertain how useful any of these genes might be as a biomarker for the disease.

3.2. Multi Gene Biomarkers for AD

Most of the studies that have tried to identify useful AD biomarkers have focused on only one or very few potential candidate markers. Given the multiplicity of pathophysiological processes implicated in AD, the diagnostic accuracy may be further improved by combining several markers. An approach using a pattern of gene expression from many informative genes will cover more of the multifactorial nature of AD than any single gene biomarkers and can potentially create a more robust marker profile characteristic for the disease.

There are a few studies published on AD biomarker discovery combining the expression of several genes and using blood as the clinical sample. A pilot study of 16 AD patients and controls that was published already in 2005 using a cDNA microarray including probes for 3200 genes identified a set of 20 candidate probes that showed an altered expression in AD [49]. At this time microarray technology was only at its infancy and neither the quality of arrays or array techniques were properly optimized making achieved results hard to reproduce unless differences in expression were extensive. The results from this study have also not been repeated. A few years later a set of 6424 cDNA clones representing unique genes was screened using RNA isolated from blood mononuclear cells from 14 AD and 14 controls. The group identified 19 up- and 136 down-regulated genes common to both males and females [50]. Clear gender differences were seen and many genes were differentially expressed in either males or females. No model for AD prediction using these genes was however generated.

A whole genome screen to identify a characteristic gene expression pattern in blood for the detection of AD used a training set of 94 AD, 73 healthy age matched controls and 21 young controls to generate a predictive model. The model based on the expression of 1239 genes was then validated on a balanced set of 31 AD, 25 healthy age matched controls, 7 young controls, 27 with a diagnosed PD and 10 with MCI [51]. The model developed predicted AD with an accuracy of 87% both in the test set and in the validation set and with an area under the receiver curve (AUC) of 0.93 and 0.94, respectively [51]. Of the 27 PD in the validation set 24 were predicted as non-AD indicating that the model did not predict another neurodegenerative disease but had a specificity for AD [51]. A set of 96 genes from this study was selected and a predictive model was developed based the expression in blood of a calibration set of 103 AD and 105 healthy controls [52]. The model predicted disease class in the calibration set with an agreement to clinical diagnosis of 71.6%. In two independent test sets the locked model predicted disease class with a similar agreement to clinical diagnosis of 71.6% and 71.5% respectively [52]. The model and the set of 96 genes are now included in ADtect®, a test that is CE marked in Europe to aid the diagnosis of AD.

A different approach to develop a blood based gene expression model has been used by Fehlbaum-Beurdeley [53, 54]. In a proof-of-concept genome-wide investigation they took into account the discriminatory power of splice variation. They identified a set of 170 oligonucleotide probes associated with 133 genes that could distinguish AD from healthy controls with a sensitivity of 100% and specificity of 96% [53]. However, the AD cohort was recruited from Tunisia and the healthy controls from one French center and through a US CRO. To what extent the different sources of samples may have had on the final results is not clear. The research team has continued the development of a test to predict AD based on the same idea of using gene splice variants. They recently presented calibration data on their predictive model [54]. The model was optimized by establishing a grey zone that discount

prediction scores near the disease status threshold [54]. In the validation of the test, AclarusDx™, 45 (21.5%) of the samples were within the grey zone and could not be predicted. When excluding these samples the model predicted disease with a sensitivity of 81.3% and a specificity of 67.1% [54]. Based on available data it is not possible to determine the accuracy of the test. The age is clearly lower in the healthy controls compared to those with AD but it is not clear if this difference had any effect on the model and prediction scores. When comparing the 96 gene set in ADtect® and the 136 gene set in AclarusDx™ it is interesting to note that they are entirely distinct from each other. No gene is found to be present in both gene sets.

The development and validation of the two CE marked tests ADtect® and AclarusDx™ show that a blood-based gene expression test for AD in the stage of dementia can be developed. It should be interesting if a similar approach can be used to develop a test that can predict the disease also in the prodromal stage. Initial proof-of-concept studies indicate that it indeed can be possible [55]. Future work will show how this work will proceed.

4. Parkinson's Disease

Parkinson's disease is the second most common neurodegenerative disorder, after Alzheimer's disease. It is characterized clinically by parkinsonism (resting tremor, bradykinesia, rigidity, and postural instability) [56] and pathologically by the loss of neurons in the substantia nigra and elsewhere in association with the presence of ubiquinated protein deposits in the cytoplasm of neurons (Lewy bodies) [57, 58] and thread-like proteinaceous inclusions within neurites (Lewy neurites). Parkinson's disease has a prevalence of approximately 0.5 to 1 percent among persons 65 to 69 years of age, rising to 1 to 3 percent among persons 80 years of age and older [2]. The diagnosis is made clinically, although other disorders with prominent symptoms and signs of parkinsonism, such as post-encephalitic, drug-induced, and arteriosclerotic parkinsonism, may be confused with Parkinson's disease until the diagnosis is confirmed at autopsy. Studies have demonstrated that movement disorder experts may misdiagnose early PD 10% or more of the time, whereas misdiagnosis may reach 50% in primary care [59, 60]. Patients misdiagnosed in primary care as having PD likely have drug-induced tremor, essential tremor (ET) or psychogenic tremor. In a patient without tremor but with other signs of parkinsonism, there may be uncertainty as to whether he or she has atypical parkinsonism or PD. Patients that develop parkinsonism while receiving dopamine-blocking agents may have drug-induced parkinsonism or PD. Biomarkers that help clinicians differentiate PD from ET, drug-induced from psychogenic tremor, and PD from atypical and drug-induced parkinsonism would be invaluable in clinical practice.

Like AD also PD shows clinicopathological heterogeneity that may confuse proper diagnosis [61]. Several studies have shown that at autopsy patients have quite variable degrees of Lewy bodies and Lewy neurites often in diverse regions of the nervous system, a finding that may account for at least some of the observed clinical variability. The clinical definition of PD encompasses a relatively broad spectrum of motor impairments, and for many patients, non-motor manifestations become considerable over time [62]. Such diverse clinical features contribute to individual patient variability [63], and recent meta-analyses have identified four main clinical phenotypes [19]. Clinicopathological correlations suggest

that more severe and more rapid progression of pathology with chronological age, as well as the involvement of additional neuropathologies, differentiates these phenotypes [19]. As is the case for AD also PD can be a challenging disease for the development of biomarkers. In addition to the variability in clinical features the long asymptomatic prodromal stages, rates of progression, and complex disease genetics complicate the picture further.

4.1. Single Gene Biomarkers for PD

Several genes with an altered expression in blood have been described that potentially can be developed to become useful biomarkers for PD [64-71]. However, their utility as biomarkers needs to be confirmed in independent studies.

The expression of the α-synuclein gene SNCA measured as the SNCA/ACTB ratio was significantly ($p>0.001$) increased in peripheral blood mononuclear cells in PD patients compared to healthy controls [67]. Although the ratio also increased with age the disease dependent increase was still clear [67]. Since α-synuclein is the major component of Lewy bodies the increased expression in blood are in agreement with characteristic findings in PD brain.

In a metabolome study pyruvate where shown with partial least square discriminant analysis to be the key metabolite in plasma to separate PD from control samples [64]. Among 40 genes associated with pyruvate metabolism and that was also expressed in blood only PDHB and NPFF was significantly ($p<0.05$) deregulated with PD compared to healthy controls [64]. Gene expression analysis was done *in silico* based on data available from separate study cohort in a previously published study [3], which identified neither PDHB nor NPFF as being influential.

The circadian clock gene BMAL1 was shown to have a significantly ($p=0.002$) lower expression in a whole blood extract of PD patients compared to healthy controls [65]. Samples were collected from 17 PD and 16 healthy controls.

VMAT2 is the main protein responsible for sequestration of cytoplasmic dopamine into synaptic vesicles for storage and release and protects neurons from dopamine-induced oxidative stress. VMAT2 mRNA has been found to be reduced in post-mortem substantia nigra of PD patients compared to non-PD controls [72]. In a study it has now also been found a significant ($p<0.05$) reduction of VMAT2 mRNA levels in blood platelets from PD patients compared to both healthy controls and patients with vascular parkinsonism [68]. Since the VMAT2 protein is expressed in platelets with pharmacodynamic and pharmacokinetic characteristics highly similar to those of the brain transporter [73] the choice of blood platelets as surrogate tissue was well chosen.

Autophagy has often been suggested to have a central role in the process of PD neurodegeneration [74-76]. In accordance with this the autophagy associated gene LAMP-2 was shown to be reduced ($p<0.001$) in leukocytes of peripheral blood from PD patients compared to healthy controls while expression of LC3, another autophagy associated gene, was increased ($p<0.01$) [71].

In an analysis of 3 microarray experiments of whole blood from PD patients and healthy controls it was found that the RNA splicing gene SRRM2 was the only gene differentially up-regulated ($p<0.05$) in all three experiments [70]. SRRM2 expression was not changed in blood of other neurologically diseased patients compared to healthy controls. It was further

found a significant ($p<0.05$) up-regulation of the 5' exons of SRRM2 and a down-regulation ($p<0.05$) of the downstream exons causing a 0.7 fold down-regulation of the long isoform of the transcript. This suggest that alternative splicing might be affected in blood of PD patients and indeed it was found about hundred genes with a significant alternative splicing of their transcripts in the blood of PD patients [70].

4.2. Multi Gene Biomarkers for PD

There are a few published studies describing a multi component gene expression approach using blood as the sample source. In an effort to find perturbed biological processes in blood of early stage PD a whole genome screen for informative gene expression based markers is described [3]. In a set of 66 samples consisting of PD, healthy controls, and controls of other neurodegenerative diseases like AD and PD like diseases a molecular multigene marker associated with risk of PD was identified. The marker was then validated using 39 independent samples. A four-step supervised prediction method was used to build the risk marker and the genes in the set were rank-ordered. An optimal number of eight genes for the marker were determined including HIP2, UTX, VDR, CA12, CLTB, ACRV1, CEACAM4 and FPRL2 [3]. Since the study was designed to determine a risk score for PD no cut of value between disease and no-disease was determined and it is thus not possible do determine an accuracy of the test. Although ROC curves are shown no AUC values are presented. In addition to the eight component gene marker 22 different unique genes were found to be differentially expressed in PD versus healthy controls [3].

Based on results from previous post mortem brain gene expression profiling [77] 12 genes were selected in a pilot study to identify potential blood biomarkers for PD (78). Four significant ($p<0.05$) genes, PSMA2, LAMB2, ALDH1A and HIST1H3E, were identified. Using the expression of these four genes a sensitivity to predict PD versus healthy controls and AD of 80% (AUC=0.93) was achieved in an independent set of samples from 22 PD, 33 healthy controls and 12 AD [78]. In a recent paper the same group identified a partially overlapping set of 7 genes that in an independent set of samples with an advanced PD predicted all correctly (sensitivity 100%) and all AD samples as non-PD (specificity 100%) [79]. In addition to ALDH1A that was included also in the previous 4 gene set the predictive gene set also included SKP1, HIP2, PSMC4 and HSPA8. It should be mentioned that HIP2 was also one of the genes in the optimal 8 gene set for PD identified by Scherzer et al. [3]. There is an uncertainty in how to interpret these results. Although the results are impressing the validation has been made on a rather limited number of samples. Also no results on how the model predicted PD in dependent samples of early PD, de novo PD and healthy controls with no neurodegenerative disease where presented.

Another whole genome expression screen has recently been performed including 79 PD patients and 75 matched healthy controls (M.K.Karlsson, pers. comm.). In this study a predictive model based on the expression of 700 genes was developed that predicted PD with an overall accuracy of 88% and with an accuracy of 85% for de novo PD. However, the accuracy of the model has to be confirmed in an independent set of samples.

When any of the last two studies can be confirmed in larger, independent studies it will give further support to what has been achieved with AD multi gene biomarkers that using a model based on the expression in blood of a set of selected informative genes can be the way

to develop good diagnostic biomarkers for complex and heterogenic neurodegenerative diseases like AD and PD.

Conclusion

The introduction of acetylcholine esterase inhibitors as symptomatic treatment has highlighted the importance of diagnostic markers for AD. Increased awareness of drug availability and treatment has also made patients seek medical advice at an earlier stage of the disease. This has presented physician with a greater need for identifying the more challenging subtle and typically slow progressive memory disturbances in the early stages of disease. Different tools to aid the diagnosis would be most welcome and biomarkers could be such a tool. The pharmaceutical industry is also in need for good biomarkers faced with the challenge of including enriched cohorts of individuals with MCI that is due to AD in their clinical trials. Often the cohort is mixed with a significant fraction of individuals with an MCI that is due to something else than AD. With a mixed cohort it will require a larger number of participants to be able to detect an effect of a treatment and make the trials more expensive. The pharmaceutical industry also request biomarkers that can measure change in disease progression, preferably within a short time frame. Such a biomarker would be most helpful when measuring effect of a treatment.

However, the development of biomarkers is a challenging work and it is often not realized what is needed to generate a biomarker that is approved by relevant authorities. An initial proof-of-concept study is just the start of a long process requiring calibration of a prediction model in a study using large enough numbers of both the controls and disease samples relevant for the intended use. How the samples can be handled without affecting the prediction has to be determined and the model has to be validated in large enough independent cohorts of relevant control and disease samples. Preferably, the prediction accuracy should not be deviating too much from the prediction achieved in the calibration study. To achieve approval from relevant authorities there may be further requirements requested such as including samples from all major ethnical groups and from a determined number of different clinical sites.

Although highly requested there are currently no biomarker approved for the detection of AD at the prodromal stage of the disease. However, there are today two blood based tests CE marked in Europe to aid the diagnosis at the early (ADtect®) and the more advanced (AclarusDx™) dementia stage. It is noteworthy that both tests not are based on proteins or metabolites but instead they are both multi component biomarkers based on gene expression. There are unpublished works on the development of a test that can detect AD in the pre-dementia stage. There are promising proof-of-concept results on the further development of ADtect® to a test that can detect AD in the pre-dementia stage (55). If successful this test would be most welcome both for the clinical practitioners and for the pharmaceutical industry.

Diagnosis of PD is most often more straightforward for the clinician than a diagnosis for AD. A patient may present with asymmetric resting tremor and bradykinesia with associated features of mild facial masking and reduced arm swing with tremor. If this patient progresses gradually and responds well to levodopa over time than it is highly likely that this patient will

have PD pathology at autopsy [80]. Unfortunately, many patients with parkinsonism do not have classic features at presentation that allow a definitive diagnosis [81]. However, perhaps even more important is the need of biomarkers for developing treatments designed to slow or prevent progression of the disease. For this purpose both progression biomarkers and early, preferably pre-clinical, diagnostic PD biomarkers are needed. Non-invasive blood based biomarkers would for these purposes be a preferred choice. Contrary to AD there are to date no approved biomarkers developed for the early diagnosis of PD. Several potential biomarkers in blood have been identified but yet none has been independently validated and approved for diagnostic use. The uniform chemical nature of RNA makes a transcriptome approach likely and based on the experience with the development of approved RNA based blood markers for AD it should be possible to use the same approach also for PD.

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