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Biomarkers of neurodegenerative disorders: How good are they?

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ABSTRACT

Biomarkers are very important indicators of normal and abnormal biological processes. Specific changes in pathologies, biochemistries and genetics can give us comprehensive information regarding the nature of any particular disease. A good biomarker should be precise and reliable, distinguishable between normal and interested disease, and differential between different diseases. It is believed that biomarkers have great potential in predicting chances for diseases, aiding in early diagnosis, and setting standards for the development of new remedies to treat diseases. New technologies have enabled scientists to identify biomarkers of several different neurodegenerative diseases. The followings, for instance, are only a few of the many new biomarkers that have been recently identified: the phosphorylated tau protein and aggregated β-amyloid peptide for Alzheimer's disease (AD), α-synuclein contained Lewy bodies and altered dopamine transporter (DAT) imaging for Parkinson's disease (PD), SOD mutations for familial amyotrophic lateral sclerosis (ALS), and CAG repeats resulted from Huntington's gene mutations in Huntington's disease (HD). This article will focus on the most-recent findings of biomarkers belonging to the four mentioned neurodegenerative diseases.

Keywords: Alzheimer's disease, Parkinson's disease, Amyotrophic Lateral Sclerosis, Huntington's disease, biomarkers.

INTRODUCTION

Neurodegenerative diseases are a varied assortment of central nervous system disorders characterized by the progressive loss of neural tissues. These disorders do not have cures because the neurons of the central nervous system cannot regenerate on their own after cell death or damage. Current advances in stem cell research may help in neuroregeneration or neuronal cell replacement as indicated by several new studies [1, 2].

Tremendous efforts have been made in recent years to identify the neuropathological, biochemical, and genetic biomarkers of the diseases so that the diagnosis could be established in earlier stages. Biomarkers are basically biological substances that can be used to indicate the presence or onset of a certain disorder. Although a neuropathologic diagnosis is a currently gold standard, it can usually be done in the form of an autopsy after the patient is dead. Therefore, it will be critical for physicians to identify disease specific biomarkers at early stages, so that patients have a chance to get an early treatment which may curb the disease progression. Furthermore, the biomarkers should not only be used to help in predicting the onset of the diseases, but also to help in overseeing the rate of progression, or in responding to treatment. For example, for Alzheimer's disease (AD), biomarkers are required to distinguish normal aging from dementia, one disorder from another with dementia, as well as to assist in identifying the exact cause of a dementia. Therefore, the biomarkers for AD, and other neurodegenerative diseases such as Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), and Huntington's disease (HD), must be reliable and specific, and they should be useful in guiding us to make more accurate diagnosis and better treatment of the diseases.

ALZHEIMER'S DISEASE (AD)

AD is the most prevalent cause for dementia in the elderly population today. In the United States over four million people are suffering from AD. It is an irreversible, progressive disease characterized by the death of neurons

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and synapses in the brain (mainly in cerebral cortex and hippocampus regions), resulting in the deterioration of cognitive functions such as memory, language, judgment and reasoning, and movement coordination. The majority of the neurons that degenerate in this disease communicate with other neurons using the neurotransmitter acetylcholine. Acetylcholine depletion results in the most striking of symptoms, as the cholinergic system functions in the processes of memory, attention, and learning [3]. Apart from acetylcholine depletion, alterations in acetylcholinesterase (AChE) and butirylcholinesterase (BuChE) can also be observed in AD patients [4]. Combined use of these two markers provides over 90% sensitivity and specificity in the detection of AD [4].

In 1998, a consensus report listed out the specific criteria and characteristics for the biomarkers of AD [5]. Primarily, biomarkers must be precise, reliable, and inexpensive, while simultaneously detecting a fundamental feature of neuropathology. Secondly, they must be validated in neuropathologically confirmed cases. Also, the markers should be extremely sensitive to any changes in the disease (> 80% sensitivity). And lastly, the marker specificity should be high enough to distinguish AD from other neurodegenerative disorders. Based on these criteria, scientists have been able to eliminate many substances that do not signify the disease and therefore are of no use to the ultimate goal of obtaining those markers that greatly contribute to improving the diagnosis of AD [6].

The key neuropathological hallmarks consist of the extracellular senile plaques and intracellular neurofibrillary tangles (NFTs) [3]. The major components of the senile amyloid plaques include the infamous β-amyloid peptides (Aβ) and the non-Aβ component of AD amyloid (NAC) [7], while the neurofibrillary tangles are fundamentally made up of hyperphosphorylated insoluble forms of the tau protein. The NFTs account for the synaptic degeneration or the atrophy of nerve cells following damage to the axons that they are synaptically connected with. Aβ forms as a result of enzymatic cleavage of the parent Amyloid Precursor Protein (APP), which is a transmembrane protein encoded by a gene on chromosome 21. The proteases that are involved in this breakdown of APP are α-, β-, and γ-secretases (Fig. 1). Aβ42 aggregates to form oligomers faster than Aβ40 because its 42-amino acid peptide contains two more hydrophobic amino acid residues. NFTs are composed of paired helical filaments (PHF), which are mainly made up of insoluble hyperphosphorylated tau protein [8]. The biological markers of AD (conveniently summarized in Tab. 1) include both the proposed genetic and biochemical markers which have been carefully studied in recent years.

Genetic markers

For the most part, scientists are able to link the uncommon early-onset familial AD (FAD) to certain genetic mutations, such as: *APP* gene mutations and *presenilin* gene mutations or the late-onset sporadic type to apolipoprotein E (ApoE).

βAPP gene mutations

The *βAPP* gene, the first AD susceptibility gene to be identified, encodes a transmembrane protein that is glycosylated and its longest isoform contains 770 amino acids. The first genetic mutation linked to AD was found on the *βAPP* gene on chromosome 21 [9]. This finding was confirmed by the fact that patients with trisomy 21 (Down's syndrome) also developed similar plaques and suffered Alzheimer encephalopathy in their later years [10]. *βAPP* is thought to be responsible for a few neuroprotective functions; however, according to the amyloid cascade hypothesis, a mutation on the *APP* gene will lead to an increase in the expression of the protein, eventually leaving more of the protein to be cleaved and form the harmful Aβ peptides [11].

About twelve missense mutations in the *APP* gene have all been found to be related to AD in chronically elevated levels of Aβ42 peptide. Mutational studies of the *βAPP* gene first identified a Glu693Gln missense mutation of the

Tab. 1 Summary of useful genetic and biochemical markers for Alzheimer's disease

Early-onset, familial type	Late-onset, sporadic type
Genetic markers	Genetic markers
-*Presenilin-1* gene mutations	-ApoE isoforms
-Amyloid precursor protein gene mutations	-ApoE polymorphisms
-*Presenilin-2* gene mutations	
Biochemical markers	Biochemical markers
-Plasma/CSF $A\beta_{1-42}$ peptide	-CSF $A\beta_{1-42}$ peptide
-CSF tau protein	-CSF tau protein
-Phospho-tau	

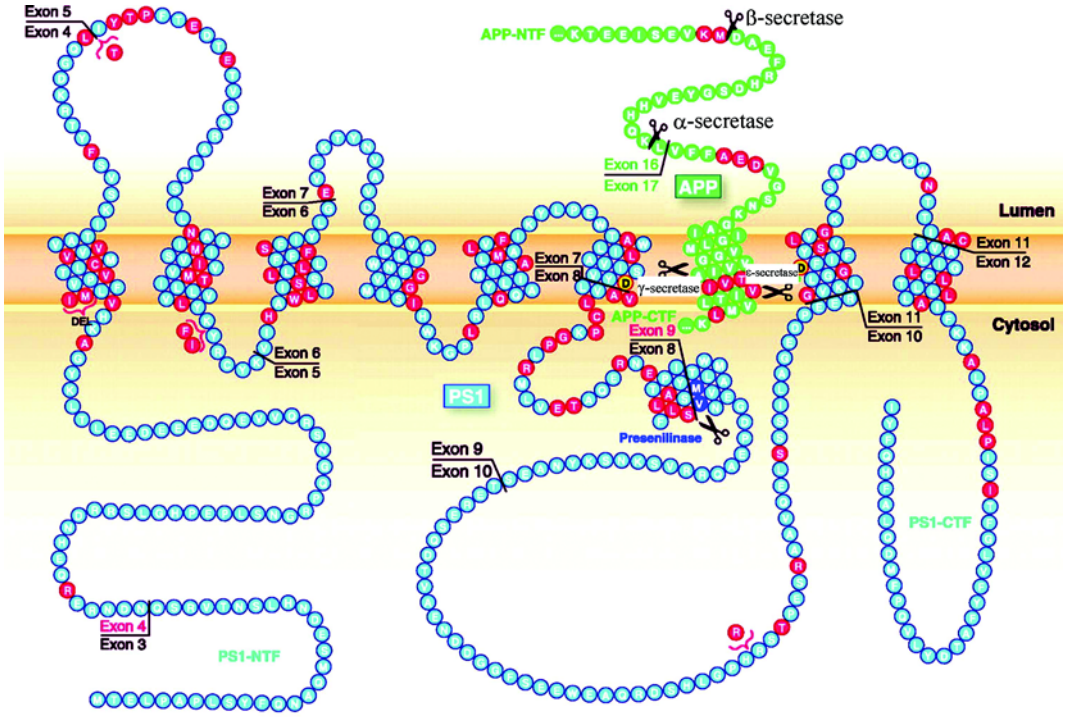

Fig. 1 Shown is the entire amino acid sequence of PS1 (blue circles) and a portion of the COOH-terminal sequence of APP (green circles). Mutations in each molecule known to cause familial AD are depicted in red. The principal sites at which the β-, α-, γ-, and e-protease cleavages of APP occur are indicated by small scissors. Replicated with permission.

βAPP gene in affected and at-risk members of families with hereditary cerebral hemorrhage with amyloidosis of the Dutch type [12]. Certain mutations have been hypothesized to influence the processing of *βAPP*. Mutations at codons 716 and 717 lead to a selective increase in the production of Aβ peptides ending at residue 42/43 [13]. The Lyc670Asn/Met671Leu mutation, on the other hand, appears to augment the production of both Aβ40 and Aβ42/43 [14], whereas the Ala692Gly mutation exerts a more complicated effect on *βAPP* process causing impaired α-secretase cleavage, increased heterogeneity of secreted Aβ species, and increased the hydrophobicity of the Aβ. The Ala692Gly mutation also has clinical features in some cases similar to those of hereditary cerebral hemorrhage with amyloidosis-Dutch type, and in other cases more similar to AD but with somewhat subtle differences in the size of the amyloid cores. The Glu693Gln mutation causes an increased propensity for Aβ to form fibrils [15].

Presenilin gene mutations

Mutations on the two homologous presenilin genes: *presenilin 1 (PS1)* – located on chromosome 14, and *presenilin 2 (PS2)* – located on chromosome 1, are responsible for over half of the known familial AD cases. As illustrated in Fig. 1 [11], the *presenilin* genes code for proteins known as presenilins, which initiate and control the APP proteolysis into smaller peptides [16]. A missense mutation on one of these genes would cause an abnormal increase in the overall proteolytic activity of the APP, thus producing more Aβ peptides or more specifically Aβ42 [17].

To date, more than 120 different mutations have been discovered in the *PS1* gene. The majority of these mutations are missense mutations giving rise to the substitution of a single amino acid. These mutations are predominantly located at highly conserved transmembrane domains, at/near putative membrane interfaces, or in the N-terminal hydrophobic or C-terminal hydrophobic residues of the putative TM6-TM7 loop domain. Two splicing defect mutations have been identified. One involves a point mutation in the splice acceptor site at the 59-end of exon 10 (in some exon numbering systems, exon 10 is labeled as exon 9) [18], and the other arises from the deletion of a G nucleotide from the splice donor site at the 39-end of exon 5 [19]. The wide scattering of missense mutations leads to the speculation that most of FAD-related mutations end up with specific functional alteration.

Mutational analyses uncovered eight different missense

mutations in the *PS2* gene in families segregating early onset forms of AD. For the first mutation (Asn141Ile), which was detected in a proportion of families of Volga German ancestry, the FAD locus was independently mapped by genetic linkage studies to chromosome 1. The second (Met239Val) was discovered in an Italian pedigree [20]. Compared with mutations in *PS1*, screening of large data sets reveals that *PS2* mutations are likely to be less frequent [21]. However, *PS1* and *PS2* seem to have similar or overlapping functions. This hypothesis was supported by the fact that *PS2* mutations, like *PS1* mutations, could increase the secretion of long-tailed Aβ peptides [22].

ApoE polymorphisms

The Apolipoprotein (ApoE) itself is a plasma protein that is involved in the transport of cholesterol. In the central nervous system, *ApoE* is implicated in the growth and repair of the nervous system during development or after injury [23]. *ApoE* gene is located on chromosome 19 and presents as 3 alleles: ε2, ε3, ε4. The ε4 allele of the *ApoE* is associated with the early-onset familial and late-onset sporadic forms of AD. It has been hypothesized that the ε4 allele is involved in enhanced aggregation and/or decreased clearance of Aβ. A ε4 allele was detected in about 40-50% of all AD patients [24]. However, based on the criteria for biomarkers, given the high sensitivity (93%) and low specificity (55%), ApoE alone could not serve as an absolute diagnostic marker [25]. Therefore, ApoE is regarded as a risk factor indicator rather than an actual genetic marker of AD.

Biochemical markers

The most promising biochemical markers of AD have been found through the analysis of cerebrospinal fluid (CSF). For instance, in all forms of AD, CSF tau levels were found to be increased [26]. In addition to CSF markers, positron emission tomography (PET) imaging with the [^{18}F]FDDNP-PET appears to be very promising for AD [27, 28].

CSF Aβ42

As stated earlier, Aβ is processed through the proteolytic activity of the APP. Aβ42 along with Aβ40 is secreted into the extracellular space and biological fluids, including CSF. However a decrease in the concentrations of CSF-Aβ42 has been observed in many patients, thus making CSF-Aβ42 a considerable indicator of AD [26]. The sensitivity of CSF-Aβ42 to AD is around 80-90%, allowing it to distinguish AD from normal aging and depression. It is known that CSF-Aβ42 also increases in some patients with frontotemporal dementia, dementia with Lewy bodies, vascular dementia, and Creutzfeldt-Jakob disease; but in AD, there exists a decrease in levels of Aβ in CSF, which reflects cerebral amyloid deposition. The reason for that may lie in that Aβ peptide from the CSF aggregates to form plaques in the brain as AD progresses, thereby, lowering its concentration in the CSF. CSF-Aβ42 cannot be solely used as a biomarker for AD, but when used in combination with other AD biomakers, the sensitivity and specificity increase and it appears to be remarkable for diagnosis.

CSF total tau

Many studies showed that the total levels of the tau protein in the cerebrospinal fluid (CSF-tau) were found to have dramatically increased in AD patients [26]. The tau protein have been proved to be responsible for binding to microtubules in neuronal axons, thus promoting the microtubule stability and assemblage. However, so far it remains unclear as to why this abnormal increase in CSF-tau occurs. Although the CSF-tau seems to provide a very high sensitivity for AD, it lacks specificity against other dementias [29].

Combination of CSF-Aβ42 and CSF-tau markers

As opposed to being used alone, scientists have found that the combination use of the CSF biomarkers may greatly improve their effectiveness—increasing both their sensitivity and specificity for picking out AD patients. In certain studies, the sensitivity of the combination went up to as much as a little over 90% [30]. The specificity of the combined test was about 86%, well meeting the criteria set for reliable biomarkers of AD. For example: The specificity is 55% for Aβ42 alone or 65% for tau alone, indicating that the combination of the two should be the ideal biochemical markers set for AD [31]. In fact, the combination of several CSF biomarkers in conjunction with other diagnostic methods could be generally used to ensure high accuracy of the clinical diagnosis.

CSF Phospho-tau (P-tau)

The human tau contains many hosphorylation sites. Tau hyperphosphorylation leads to tau aggregation which subsequently results in the formation of NFTs. The ability of P-tau to discriminate between AD and normal aging shows a mean sensitivity of 70% and specificity of 94%, while the mean level of increase in AD compared with controls is approximately 250% [32]. Data on the specificity of CSFphospho-tau is sparse. However, it is believed that P-tau may be a big help in discriminating between AD and these dementias. Further, it has been noted that after acute stroke, there was a marked increase in CSF total tau, while CSF phospho-tau did not change [33], indicating rather than a marker for neuronal damage, as CSF total tau, CSF phospho-tau specifically reflects the phosphorylated tau,

and in turn the formation of NFTs.

p97/Melanotransferrin (Mtf)

The expression of p97 or melanotransferrin (Mtf) was initially detected at high levels as a marker of malignant melanoma cells. Later on it came to be known that due to its high sequence homology with human lactoferrin and serum transferrin, Mtf belongs to the group of iron binding proteins. Studies showed that Mtf content in serum was elevated in AD patients, which lead to the potential use of Mtf as an biochemical marker [34, 35]. However, one study showed that when analyzed by SDS-PAGE under non-reducing conditions, Mtf contents in serum were not significantly altered between controls and patients with mild or moderate stages of AD [36]. Further studies should be carried out to confirm that claim.

PET Molecular imaging of AD

2-(1-(6-[(2-[18F]fluoroethyl)(methyl)amino]-2-naphthyl)ethylidene)malononitrile ([^{18}F]FDDNP)-PET has been found to be able to cross the Blood-Brain-Barrier (BBB) due to its high lipophilicity, permitting it to determin the locolization and load of neurofibrillary tangles and senile amyloid plaques *in vivo* in the human brain. The discovery of a new binding site to Aβ40 fibrils as a result of FDDNP binding also opens a new opportunity for early treatment of AD. PET imaging *in vivo* may improve current clinical approaches with accuracy rates of 70-90%. The combined use of the [^{18}F]FDDNP-PET labeling system and other diagnostic tests give support to early diagnosis of AD [28]. A recent study using this method examined the *in vivo* characteristics of [^{18}F]FDDNP-PET in patients with AD and found the relative residence time of the probe in brain regions affected by AD was significantly greater than in control groups [37]. Indeed, [^{18}F]FDDNP-PET scanning in AD patients show sensitivity at early stages of the disease, before clinical evidence of cognitive decline [27]. [^{18}F]FDDNP-PET imaging of at-risk populations early and throughout the progression of the disease could help in elucidating the etiology of AD by correlating neuropathology with functional loss. Early and longitudinal detection of SPs and NFTs will allow for expedient clinical trials on patients early in the development of AD—a population that would benefit most from promising treatments geared toward reversing the effects of AD. However, an understanding of the molecular requirements of FDDNP binding may help in the optimization of the Aβ anti-aggregation potency of experimental drugs [27].

PARKINSON'S DISEASE (PD)

PD is a neurodegenerative disorder, which affects a little over one percent of all people over the age of 55. It is pathologically hallmarked by the degeneration of a neural connection, more specifically dopaminergic neurons between the substantia nigra (SN) and the striatum. Scientists determined that a great majority of dopamine-producing cells in the substantia nigra are lost in patients with PD. As these neurons are destroyed, the clinical signs which characterize PD such as the slowed movements, rigidity and tremors start to appear.

Another key neuropathological mark of PD is the formation of Lewy bodies, which are cytoplasmic inclusions primarily composed of the α-synuclein protein. Lewy bodies are present in the dopaminergic neurons of the SN and other brain regions like the cortex and magnocellular basal forebrain nuclei.

Genetic markers

In a small number of families, PD is inherited in a Mendelian autosomal dominant or autosomal recessive fashion. Genetic linkage and positional cloning studies in some of these families have identified several causative single gene mutations (namely six genes: *α-synuclein, Parkin, UCH-L1, PINK1, DJ-1 and NR4A2)*. Furthermore, four loci (PARK3, PARK8, PARK9, PARK10) across the human genome conceal unknown genes (as can be seen in Tab. 2) [38, 39]. These loci can only be characterized by the phenotypes they produce. These findings lead to intense research efforts to discover how these mutations cause neurodegeneration. The most important of these genes have been discussed as below.

α-synuclein gene

Mutations on the *α-synuclein* gene, which is located on chromosome 4, are a characteristic of Parkinson's disease and they occur in most forms including the rare early-onset familial form of PD. Polymeropoulos and his colleagues first reported linkage of autosomal dominant PD to chromosome 4q21-23 in an Italian-American family (Contursi family) with typical clinical and pathological features of PD [40]. Further analysis of this region identified a mutation of G to A (G209A) in the *α-synuclein* gene that plays an important role in dopamine associated oxidative damage. Several lines of evidence suggest a toxic mechanism caused by the aggregated protein. However, this mutation was found to be a very rare case of familial PD, having been found in only three other families with autosomal dominant PD. Another mutation of the *α-synuclein* gene was identified in a German family in which there was a single base-pair change at position 88 from C to G (C88G) which resulted in an alanine to proline substitution at position 30 of the α-synuclein protein [41]. To date, this mutation has not been detected in any other family.

Tab. 2 Genes for familial Parkinson's disease

Gene/locus	Location	Inheritane	Onset	Distinctive clinical features	Lewy bodies
parkin=PARK2	6q25	AR	Early-juvenile	Frequent dyskinesia/dystonia Slow progression	No*
DJ-1=PRRK7	1p36	AR	Early	Focal dystonia Slow progression Psychiatric symptoms	–
PINK1=PARK6	1p35–36	AR	Early	Slow progression	–
PARK9	1p36	AR	Juvenile	Spasticity Dementia Supranuclear ophthalmoparesis	–
α-synuclein=PARK1	4q421	AD	Late	Lower prevalence tremor More rapid progression	Yes
UCH-L1=PARK5	4p14	AD	Late	None	–
NR4A2	2q22–23	AD	Late	None	–
PARK3	2p13	AD	Late	Dementia in some patients	Yes
Triplication of *α-synuclein =PARK4*	4p14–16.3	AD	Late	Postural tremor in some relatives Autonomic dysfunction Dementia Weight loss early in disease	Yes
PARK8	12p11.2–q13	AD	Late	None	No
PARK10	1p32		Late	None	–

AR: Autosomal recessive; AD: Autosomal dominant

Recently, Singleton *et al* [42] identified α-synuclein locus triplication as a cause to PD in a large family (originally designed as PARK 4 mapped to 4p14163) with autosomal dominant PD, ranging clinically from dementia with Lewy bodies to typical PD. The frequency of the α-synuclein locus triplication in general population of familial PD is unknown.

Parkin gene

The *parkin* gene is a large gene consisting of over 1.5 Mb and about 12 exons, which was mapped to chromosome 6q25.2-27 [43]. A mutation in this gene (homozygous exon depletion) was first identified as an autosomal recessive early-onset trait in a Japanese family [43, 44].

Until now, more than 70 mutations on this gene have been associated with the early-onset form of parkinsonism. These mutations may account for PD in as many as 50% of familial cases of autosomal recessive juvenile parkinsonism [45, 46]. Clinically, the disease usually begins when the patient is in his/her 20s, and is prominently associated with dystonia and diurnal fluctuations, progressing slowly while accompanied by early and severe levodopa-induced dyskinesis, but no dementia. Pathologically, there is severe neuronal loss in the substantia nigra pars compacta and locus ceruleus; however, intriguing Lewy bodies which are ubiquitinated and thought to be part of the degradation process are absent in brains of parkin patients, suggesting that the mechanism of neurodegeneration may differ from other forms of PD.

The parkin protein product comprises 465 amino-acids was identified as an ubiquitin ligase involved in the process of protein degradation pathway [47], indicating that ubiquitin-mediated proteolysis may play an important role in the pathophysiology of idiopathic Parkinson's disease. Being associated with recessively inherited PD, these mutations in *Parkin* will lead to the loss or deprived function of E3 ubiquitin ligase in the nigra and striatum of the patients, resulting in the abnormal accumulation of its substrate proteins. A specific form of α-synuclein has been found to be a substrate of the *parkin* gene, thereby linking the two through the ubiquitin system [48].

In conclusion, mutations in the *parkin* gene represent a frequent cause of familial early-onset and isolated juvenile parkinsonism, thus making it one of the best genetic markers of familial early onset PD yet.

UCH-L1 gene

The *ubiquitin carboxy-terminal hydrolase L1 (UCH-L1)* gene on chromosome 4 encodes a protein which belongs to the family of deubiquitinating enzymes. These enzymes are involved in the ubiquitin/26S proteasome degradation pathway and are also found to be present in Lewy bodies. Researchers have identified a missense mutation in exon 4 of the gene in 2 siblings from a German family with Ileu to

Met substitution at position 93 (Ile 93Met) on the UCH-L1 protein. However, mutations in *UCH-L1* gene have not been found in any other families. UCH-L1 protein is richly expressed in brain, constituting possibly 1% of brain protein [49]. Its function is unknown, though it is presumed to act to recycle ubiquitin by hydrolyzing the ubiquitinated peptides, the products of the proteasome. The capability of cleaving ubiquitin carboxyl-terminal amides was damaged to 50% by mutations in the *UCH-L1* gene [50]. It is tempting to speculate that the enzyme plays a role in modifying damaged or aggregated proteins that otherwise might accumulate to toxic levels in the neuron.

PINK1 gene

The *PTEN-induced putative kinase 1* (*PINK1*) gene contains eight exons spanning ~1.8 Kb and encodes a 581 amino acid protein. Two homozygous mutations in *PINK1* gene associated with PD were identified recently. G309D was found in a Spanish family and W437OPA substitutions were found in two Italian families [39]. Studies show that *PINK1* mutations are associated with *PARK6*, a locus relating with autosomal recessive, early-onset PD on chromosome 1p35-p36 by autozygosity mapping in a large consanguineous family from Sicily [51]. Subsequent identification of two additional consanguineous families provided additional evidence of the relationship with *PARK6* [52].

DJ-1 gene

The *DJ-1* gene encodes a ubiquitous highly conserved protein. It was shown that mutations on *DJ-1* are associated with PARK7, a monogenic form of human parkinsonism. It was first linked to the *PARK7* loucs in a genetically isolated population in the southwest of the Netherlands (it was later confirmed in an Italian family) [53]. The Dutch kindred carried a homozygous deletion of DJ-1, and the affected individuals in the Italian family were homozygous for the L166P mutation. Clinical characteristics of the DJ-1 parkinsonism include a slow progression of symptoms with sustained response to levodopa treatment [54]. The function of the DJ-1 protein remains unknown, but evidence suggests its involvement in the oxidative stress response [55]. Also some findings demonstrate that loss of DJ-1 function leads to neurodegeneration. The observation that DJ-1 may be involved in the oxidative stress response links a genetic defect in this pathway to the development of parkinsonism, with possible implications for understanding the pathogenesis of the common forms of PD [56]. Revealing the physiological role of DJ-1 may promote the understanding of the mechanisms of brain neuronal maintenance.

NR4A2 gene

NR4A2, or *NURR1* gene which encodes a member of nuclear receptor superfamily, is absolutely necessary for the differentiation and maintenance of the nigral dopaminergic neurons. One study showed two specific heterozygous mutations (–291Tdel and –245T → G) were found in European descent [57, 58]. The phenotypes of patients with mutations in the NR4A2 gene are actually identical to those with late-onset PD lacking atypical features.

Biochemical markers

Two major biochemical markers that have been very helpful to recognize the onset of PD are: the loss of the dopamine transporter detected by PET imaging and the presence of the α-synuclein protein located in the Lewy body lesions, which are characteristic of PD. Several other biochemical markers in blood and CSF hare also been proposed. The validity of these markers in clinic application is currently under investigation.

Loss of DAT

Dopamine transporter (DAT) mediates uptake of dopamine (DA) into dopaminergic neurons by an electrogenic, Na^+- and Cl^--transport-coupled mechanism. DA and the uptake blockers such as cocaine would bind to both the shared and separate domains on the transporter, which is influenced dramatically by the presence of cations. Regulation of the DAT occurs both by chronic occupancy with blocker and by acute effects of D_2 DA receptors or second messengers such as diacylglycerol (protein kinase C) and arachidonic acid. The DAT is involved in the uptake of toxins generating Parkinson's syndrome.

The localization of DAT provides the best marker for the integrity of the pre-synaptic dopaminergic systems that are most affected in PD. The concentration of DAT begin to diminish when the neuronal depopulation is over than 50%, which also explains the appearance of neurological deficit symptoms. This is why the concentration of striatal, preferentially putamen DAT concentration is a high sensitivity parameter for the detection of early phases of PD [59].

Although conventional high-resolution imaging techniques such as MRI and CT are not so beneficial in the diagnosis of PD, they are helpful in the differential identification of some other types of parkinsonisms. Scientists are interested in developing highly sensitive diagnostic techniques for early PD by assessing of DAT concentration in the striatum. PET is a technique which measures the emission of positrons from the brain after a small amount of radioactive isotopes, or tracers, have been injected into the blood stream. Till now PET is considered

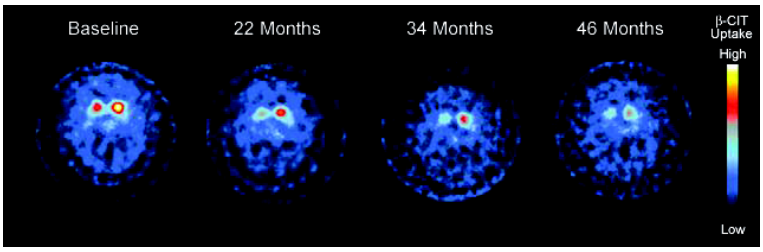

Fig. 2 ^{123}I-β-CIT SPECT images show progressive decline of nigro-striatal DAT function in patients with Parkinson's disease (PD). Replicated with permission.

to be the most useful tool for PD diagnosis, namely Fl 18 Fluorodopa [60]. Another useful imaging technology for diagnosing PD is single photon emission computed tomography (SPECT), which is similar to PET except for that it uses isotopes with longer half-lives that can be stored on site. Currently, for DAT imaging, there are several available radiopharmaceuticals. For instance, an SPECT image (Fig. 2) of the brains of a patient with PD was taken using the DAT ligand 123I-β-CIT [61] to show the progressive deterioration of nigro-striatal DA system. As can be observed from the image, reduced DAT signals were detected in the striatum of the patient with PD. Longitudinal studies including early cases of PD are needed for a better understanding of the course of the loss of striatal DAT function in PD.

Lewy bodies

The Lewy bodies are found to be a characteristic of PD which contains α-synuclein. Human α-synuclein protein is relatively abundant in brain and predominantly expressed in pre-synaptic nerve terminals. The protein was first implicated in the pathogenesis of a neurodegenerative disease as a novel peptide, which was unrelated to α-amyloid peptide. However, it was isolated from purified amyloid of AD brains and thus named non-Aβ component of AD amyloid (NAC) [7]. Analysis of its amino-acid sequence revealed that more than half the molecule consists of six imperfect eleven amino-acids repeats, each of which possesses a conserved six-amino-acid core [62]. Two mutations in *α-synuclein* gene were found to be associated with the rare inherited forms of PD, which led to the discovery of Lewy bodies and Lewy neurites, the characteristic lesions in brains of patients with PD and dementia with Lewy bodies [63]. Both mutations of result in amino acid substitutions within this repeated region. However, it still remains unclear how the mutant encoded proteins assist fibril formation, thus leading the way to the discovery of Lewy bodies. Given that α-synuclein is also found in other synucleinopathies such as dementia with Lewy bodies (DLB) and multiple system atrophy, it should be used in the aid of other diagnostic methods to increase the specificity and sensitivity for PD [64].

Biochemical markers in blood and CSF

An alternative hematogenous biomarker for PD may arise from studies in the periphery. Several potential markers of oxidative stress such as malondialdehade, superoxide radicals, the coenzyme Q10 redox ratio, and 8-hydroxyguanosine from RNA oxidation have been measured in blood and the levels of these markers tend to be abnormally higher in PD compared with control groups [65]. DAT immunoreactivity in lymphocytes is decreased and MAO-B activity in platelet is increased [65]. Elevated plasma homocystein level, a risk factor associated with cardiovascular disease and AD, is also detected in PD [66]. The CSF levels of 8-hydroxy-2'-deoxyguanosine and 8-hydroxyguanosine are moderately higher while β-phenylethylamine is lower in PD [65]. CSF specimens from PD patients, regardless of therapy, contain factors that cause specific dopaminergic (DAergic) cell injury in *in vitro* studies [67]. It has been demonstrated proinflammatory factor tumor necrosis factor (TNF-α) in CSF is 3-4 fold higher in PD vs normal and disease controls [67]. These findings provide valuable insight into the nature of the pathogenesis of the disease, but none of them are sufficient to be used as a diagnostic biomarker for PD in clinical practice.

OTHER DISEASES

Currently, PD and AD are the two main neurodegenerative disorders in which significant amounts of knowledge to gain better understand the nature of these disorders have been acquired through the application of the respective biomarkers. However, there are still a few other neurodegenerative diseases in which biomarkers are not yet fully understood, in spite of the fact that some progress has been made. In this section of other Diseases, biomarkers of ALS and HD will be examined.

Amyotrophic Lateral Sclerosis (ALS)

ALS is an age-dependent motorneuron neurodegenerative disease characterized by the neuronal death of the upper and lower motor neurons, skeletal muscle atrophy, paralysis, and death. The primary goal for scientists with regards to the biomarkers of ALS is to show direct evidence of motor neuronal degeneration within the brain or spinal cord. Approximately 2% of all ALS and 20% of familial cases are associated with mutations in the gene for copper/zinc superoxide dismutase, *SOD1* [68]. Animal models (especially experiments done on rodents) have shown that those transgenic ones for mutant human *SOD1* develop progressive skeletal muscle atrophy, paralysis and death, similar to human cases. This gene appears to be the best biological marker presently available for familial ALS [69].

ALS is clinically and genetically heterogeneous, and as genetic advances are made, phenotypic and genotypic classification will improve. For example, there is a specific phenotype associated with the recessive *SOD1* mutation, D90A, of predominantly UMN [70]. This mutation remains an enigma and is one of the few with a consistent phenotype. Thus, a genetic molecular diagnosis can be made with some confidence from the clinical presentation.

Two more genetic mutations: *ALS2* and *NEFH* have been found to be associated with ALS, leading to speculation of two more possible genetic markers. However, studies have shown that these two mutations are not common causes of the disorder [71].

The biological and surrogate markers are under evaluation for their roles in the diagnosis of ALS, in measuring disease progression during therapeutic trials in ALS, and in developing potential new therapies for the disease. Currently the primary underlying mechanisms or causes of ALS are unknown, although many biological changes resulting from the disease process have been recognized. The cascade of biochemical changes occurring within the motor system in ALS eventually leads to degeneration of the lower and upper motor neurons (LMNs and UMNs), which is responsible for the clinical symptoms and signs of the disease. Ideally, scientists should employ direct evidence of motor neuronal degeneration as the primary biological marker in studies of ALS, but brain or spinal cord biopsies are so hard to obtain that they are forced to use secondary or surrogate markers [72]. A surrogate marker is basically the substitute for another marker to disease-related epi-phenomena that nevertheless can be used in the diagnosis or measurement of progression of ALS, as no true biomarkers have yet been confirmed for the disorder.

If there is a known family history of similar diseases for a patient presenting signs and symptoms suggestive of ALS, genetic techniques should be applied to establish the diagnosis and screen for mutations of known genes, such as the *SOD1* gene in familial ALS. The diagnosis of ALS in the early stages is very difficult because there is no clear evidence indicating the involvement of UMN and LMN at multi-level damage. LMN denervation on electromyography can assist in confirming the diagnosis of ALS in a patient with clinical signs that are purely restricted to the UMN. Similarly, MRI evidence of corticospinal tract degeneration or evidence of UMN dysfunction as indicated by 1 H-magnetic resonance spectroscopy (1 H-MRS) can be used to demonstrate UMN involvement in a patient with signs restricted to the LMN [73]. Unfortunately, no technique for the detection of UMN is sensitive enough since clinical signs usually antedate the appearance of MR abnormalities. Biomarkers which have been advanced as potential surrogate markers for the diagnosis of ALS include increased levels of glutamate in the CSF and blood [74], oxidative products in the blood [66, 75], abnormal splicing variants of mRNA from EAAT2 (GLT1) in the CSF [76], and changes of 1 H-MRS in levels of N-acetyl aspartate (NAA) and glutamate [77].

Huntington's Disease (HD)

HD is an inherited disease characterized by choreiform movements, psychiatric symptoms, and slowly progressive dementia [78]. In adults, HD most often causes involuntary movements, but rigidity also can be a feature of the disease. HD is inherited as an autosomal dominant disorder with complete penetrance. A HD gene (*IT15* gene) on chromosome 4 has been identified with an abnormal protein product (huntingtin) identified in the brain [79]. However, the relationship between this protein and the selective loss of neuronal groups in the CNS remains to be established. The mutation was identified genetically as a trinucleotide repeat expansion of CAG sequences within the coding region of *IT15* gene. The normal range of CAG repeats is about 15 to 30 repeats; however, one study showed that the expanded HD alleles in Singaporeans consists 40 to 54 CAG repeats [80]. The number of CAG repeats, as one study showed, may aid in the prediction of age of onset and penetrance of HD [81]. For instance, for a 41-year-old individual who has 42 CAG repeats, it predicts a 91 % chance that the individual will have HD onset by the age of 65 [82]. Onset of the disease may be different but usually comes up in midlife, depending on both sex and age. As the expansion of CAG was documented in almost all the cases, it can be assumed that a gene mutation on the *IT15* is the cause of HD and thus it could be considered a genetic marker [79].

Similar to the biomarkers of the other neurodegenerative diseases, biomarkers for HD would facilitate an accurate

evaluation of the effectiveness of new therapies and improve the safety and efficiency of clinical trials. As the mechanism of pathogenesis in HD is not yet clear, biomarkers will be needed at all stages of the diseases and combinations of detection methods will be required. Biomarkers to detect alterations of energy metabolism and oxidative damage are available but require validation. Imaging technologies offer great promise in identifying biomarkers. These modalities include PET and nuclear magnetic resonance spectroscopy (NMRS) [82, 83]. Animal and human studies of biomarkers should be run in parallel and compared with each other in order to take advantage of advances being made in animal models to understand the mechanisms of disease. Using the MR modalities, patients could be serially imaged over months or years with minimal adverse side effects, potentially revealing a great deal of information about the natural history of the disease.

A distinction must be made between state and trait markers. For HD, the ideal trait marker already exists, i.e., the presence of the gene with a CAG expansion. State markers, in contrast, tell the state of the disease once it has become manifest and these are what scientists want to know more about.

CONCLUSION

Several of the biomarkers that have been identified in the past decade are under investigation for their potential application in the early diagnosis of respective neurodegenerative disorders along with an enormous scope for further research in the areas of both genetic and biochemical markers. Although genetic markers are only detected in certain populations, finding these markers is critical in order to pinpoint the cause and pathogenesis of the disorders. Biochemical markers may provide more valuable information regarding different diagnosis and therapeutic guidance to specific diseases. But individual biomarkers cannot and should not necessarily be used alone to diagnose disease; each biomarker has its own application for specific diseases or for specific stages of disease. Overall, a combination of several biomarkers is usually needed to enhance the accuracy, specificity and sensitivity. Again we must bear in mind that biomarkers are just a part of the clinical evaluation and should be used together with a thorough clinical work-up for best results.

Thus, the ultimate goal for scientists to achieve in the future is to find better biomarkers precisely representing the respective diseases; to maximize the use of biomarkers to detect specific biological, pathological, and biochemical abnormality for the purpose of improvement in clinical diagnosis and treatment; and to help better understand the mechanisms of these neurodegenerative disorders.

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