DIFFERENTIAL EXPRESSION OF ALPHA-SYNUCLEIN ISOFORMS: A POSSIBLE PERIPHERAL BLOOD MARKER OF PARKINSON DISEASE

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Duration: 3 years
1. Summary of the project

I.1. OBJECTIVES

1. To determine reactive SNCA 140, SNCA 112, and SNCA 126 mRNA expression levels in lymphocytes from Parkinson disease (PD) patients with different disease stage and controls.
2. Data analysis and comparison of the results obtained in peripheral blood with the corresponding expression levels in brain of PD patients and controls.
3. To confirm differences in expression levels observed at the mRNA level with protein expression analysis by Western blotting and to test for the presence of different alpha-synuclein isoforms in serum.
4. To determine butyrylcholinesterase (BChE) expression levels in brain and blood from patients with Lewy body diseases.
5. To study BChE promoter sequence in patients with Lewy body diseases and to define polymorphisms and/or genotype combinations for at least one of these diseases.
6. To correlate data obtained in objectives 4 and 5. To establish a genetic marker.

I.2. DESIGN, PROCEEDINGS AND METHODS

2.1 Patients with PD will be diagnosed at the Department of Neurology of the Hospital Clinic, Barcelona and control individuals will be also identified. Frozen samples of brains with Lewy body diseases will be provided by the Neurological Tissue Bank of the Hospital Clinic, Barcelona.
2.2 Various lymphocyte aliquots will be obtained for the subsequent RNA or protein extraction. Furthermore, whole blood for DNA extraction and two serum aliquots will be stored for later analysis.
2.3 After RNA extraction cDNA will be obtained by RT-PCR and the relative expression levels of four SNCA isoforms will be determined by real time PCR. Relative expression levels will be evaluated the to detect differential expression of different isoforms in different stages of PD. Furthermore, the data will be correlated with the results obtained for the expression of these isoforms in brain.
2.4 To detect the different isoforms at the protein level, proteins will be extracted from lymphocytes and their concentration will be determined. After separation by electrophoresis, the isoforms will be detected by Western blotting by the use of isoform-specific antibodies. To determine the presence of different isoforms of alpha-synuclein in serum immunoprecipitation with isoform-specific antibodies will be performed.

2.5 BChE expression levels will be determined in brain, the promoter sequence will be analyzed by sequencing and the corresponding genotypes of all samples with Lewy body diseases and controls will be determined. Specific genotypes for at least one of the diseases will be established. Specific genotypes will be correlated with the expression levels.

2.6 The results will be corroborated in a group of patients with clinical diagnosis and controls.

3. WORKING PLAN
1. Diagnosis of PD patients, detection of control subjects and blood collection. DURATION: months 1-12
2. Analysis of BChE expression in brain, analysis of the BChE promoter sequence. DURATION: months 1-12
3. Extraction of RNA, determination of concentration and integrity, reverse transcription, protein extraction, determination of concentration. DURATION: months 4-15
4. Real-time PCR to determine the relative expression of alpha-synuclein isoforms in blood and data analysis. DURATION: months 16-20
5. Western blotting for alpha-synuclein isoforms in blood by the use of isoform-specific antibodies, immunoprecipitation. DURATION: months 21-25
6. Establishment of a specific marker from the BChE genotypes for at least one of the diseases with Lewy bodies in post-mortem samples. Confirmation of the results in a group of patients with clinical diagnosis. DURATION: months 26-30
7. Data Analysis: Correlation of all alpha-synuclein isoforms expression results in peripheral blood with genotypic data and BChE expression. Writing the doctoral thesis attached to the project. Preparation of manuscripts. DURATION: months 31-36
2. Results

1. DIFFERENTIAL EXPRESSION OF ALPHA-SYNUCLEIN TRANSCRIPTS AS A POSSIBLE MARKER FOR PARKINSON'S DISEASE DIAGNOSIS

The analysis of four new transcripts revealed that SNCAtv4 is specifically expressed in brain. SNCAtv1 and SNCAtv2 are the major transcripts and show a slight overexpression in advanced stages of PD. In contrast, SNCAtv3, the minor transcript, was significantly decreased in the first two stages of the disease.

This last result therefore represents solid data to expand this part of the study to broader groups of patients and also to test the expression of SNCAtv3 as a potential biomarker in groups of individuals at risk for developing PD.

2. ALPHA-SYNUCLEIN ISOFORMS AT THE PROTEIN LEVEL

There were no significant differences in protein levels for alpha-synuclein 140 (isoform majority) among the different stages of PD and controls. Isoforms alpha-synuclein-126 and 112 were not detectable at the protein level, probably because of their low expression in blood.

3. BUTYRYLCHOLINESTERASE - GENOTYPE COMBINATION AS A MARKER FOR THE DIFFERENTIAL DIAGNOSIS OF DEMENTIA WITH LEWY BODIES

A study of genetic association between different BChE polymorphisms (BChE) and Lewy body diseases (LBD), including PD and dementia with Lewy bodies (DLB) was carried out. Four polymorphisms, not described before, were detected in the promoter of the BChE gene and genotype combinations composed of the four promoter polymorphisms and the K variant polymorphism located in exon 4 of the BChE gene were analyzed.

To start with, we examined post-mortem brain samples with neuropathological diagnosis. A group of samples with these characteristics and postmortem
neuropathological diagnosis makes it possible to obtain sound preliminary data for the study of larger clinical groups. The first analysis revealed that one of the 29 genotype combinations found in a total of 85 samples, could be a specific marker for LBD. In the clinical study the specificity of this genotype combination was confirmed and an additional specific genotypic combination was also detected.

Taking into account these results, the following diagnostic algorithm was established as a guideline for the differential diagnosis of DLB against Alzheimer disease (AD). In the near future the study will be completed with groups of clinical DLB samples and the implementation of this analysis as a diagnostic tool in clinical practice will be proposed.

Figure: (A) Diagnostic logarithm for DLB diagnosis. In patients with clinical diagnosis of possible/probable AD or possible/probable DLB the five BChE genotypes will be determined. If genotype combination AAAGCC8+K+ or AAAAC+77KW is being carried, the patient will be diagnosed as having DLB. The presence of other genotype combinations will be considered.
combinations will require further testing. (B) BChE expression is decreased in brains of carriers of these genotype combinations compared with the rest of the patients with DLB.

The results of this part of the project have been patented under the title "Genetic marker for the diagnosis of dementia with Lewy bodies" (P1589EP01). Our patent provides:

1. The first biomarker for DLB, and, at the same time, the first genetic marker for this disease.
2. The differential diagnosis of one of the subgroups of DLB vs. AD following a simple diagnostic logarithm.
3. A considerable reduction in diagnostic cost because of a simple blood genotyping.
4. An important step towards personalized medicine, enabling the application of adequate treatment from the beginning of the disease.

In addition, the PhD student Montserrat Domingo Sàbat, who is attached to the project, has defended her doctoral thesis with the title: "Genetics of butyrylcholinesterase in synucleinopathies: Establishment of a differential diagnostic tool for dementia with Lewy bodies".

4. BETA-SYNUCLEIN: THE DRASTIC DECREASE OF EXPRESSION DEFINES A MOLECULAR SUBGROUP IN DEMENTIA WITH LEWY BODIES

Alpha-synuclein is the major component of Lewy bodies and its oligomerization and aggregation are key events in the development of Lewy body diseases. Beta-synuclein belongs to the same protein group, shows 78% homology with alpha-synuclein, and is capable of inhibiting alpha-synuclein aggregation.

We analyzed beta-synuclein expression in different brain areas of samples from different Lewy body diseases. The correlation of results with clinical and neuropathological data enabled the detection of a molecular subgroup of DLB, characterized by a drastic decrease of beta-synuclein expression in the cerebral cortex, a short disease course and the presence of pure Lewy body pathology.
We have characterized for the first time one of these subgroups, describing the associated molecular defect. Due to the impact of this finding, the results were published in Brain.

3. Relevance and possible clinical implications of the final results

The identification of two BChE genotype combinations as a genetic biomarker for the differential diagnosis of DLB is an important finding. Besides being the first marker for DLB, this biomarker can be a useful tool for clinical diagnosis of DLB against AD.

a- The use of the biomarker will establish the clinical diagnosis of DLB with certainty in approximately 60% of patients (see Figure Diagnostic Algorithm). These patients can receive appropriate treatment from the beginning of the disease, slowing their cognitive decline and thereby improving their quality of life.

b- The patients carrying the genotype combinations exhibit have decreased BChE levels in the cerebral cortex. For the success of a clinical trial it is important to have homogeneous groups of patients to obtain comparable data in regard to drug response. Our biomarker can be very useful to include patients pertaining to a specific DLB subgroup to test new drugs.
4. Publications

Beyer K, Domingo-Sàbat M, Santos C, Tolosa E, Ferrer I, Ariza A.  
*The decrease of beta-synuclein in cortical brain areas defines a molecular subgroup of dementia with Lewy bodies.*  
Brain 2010, 133(Pt 12): 3724-33. IF: 9.490

Beyer K, Domingo-Sàbat M, Ariza A.  

Beyer K, Ariza A.  
*The therapeutical potential of alpha-synuclein antiaggregatory agents for dementia with Lewy bodies.*  

*Differential expression of alpha-synuclein, parkin, and synphilin-1 isoforms in Lewy body disease.*  

Beyer K, Domingo-Sàbat M, Lao JI, Carrato C, Ferrer I, Ariza A.  
*Identification and characterization of a new alpha-synuclein isoform and its role in Lewy body diseases.*  

Beyer K, Ariza A.  