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Quantification of catecholamine metabolites in CSF biomarker to validate its use for early diagnosis, disease severity and explore its utility as disease progression biomarker for de novo PD from the PPMI cohort

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PPMI Project ID: 122

Summary or Scientific Abstract

Please describe the analysis performed (Should not exceed 200 words)
Different pattern of neurotransmitter metabolites in PD are hypothesized given its established role in the disease process. Former studies confirmed consistent signatures, eg significant decrease of the main dopamine metabolite homovanillic acid (HVA)[1]. However, its utility for use in clinics has been questioned, mainly due to results of the DATATOP study in which repeated CSF measurements of dopamine metabolites suffered from high variability and a lack of correlation with disease severity[1, 2]. Recent studies using optimized high-performance liquid chromatography (HPLC) revived interested but its robustness for use in clinics to measure longitudinal samples is questionable due to complex sample processing and analysis[3, 4]. We previously performed mass-spectrometry based absolute quantification of monoamine metabolites including catecholamines in CSF samples from the DeNoPa cohort and identified monoamine metabolites with superior performance to classify the disease and to correlate with disease severity compared to established CSF protein biomarker. CSF samples from PPMI will be used to a) confirm utility to classify and predict early, unmedicated PD subjects, b) confirm correlation with disease severity scores and c) assess utility to monitor disease progression in absence/presence of symptomatic medication.

Method

Describe the methods used. Sufficient information should be provided to enable investigators to understand the methodology and interpret the data

Concentrations of catecholamines and related metabolites will be determined by Metanomics Health, Berlin/Germany (see www.metanomics-health.com). Metanomics Health has developed a mass spectrometry-based analysis platform that enables robust detection and quantification of low abundance monoamines and related metabolites. The platform implements an analytical procedure described in detail by Yamada et al.[5] and has been adapted for use with catecholamines in CSF. In brief, samples will be subjected to ultracentrifugation and derivatization prior to online solid-phase extraction and LC-MS/MS analysis (Symbiosis Pharma

(Spark, Emmen, Netherlands) coupled to an Applied Biosystems API4000 MS/MS-System). After comprehensive analytical validation, the sample data will be normalized against an internal standard. Internal standards used for quantification are isotope labeled catecholamines. They are added to the standard calibration curve as well as to each sample before extraction to correct for random and systematic errors. Each analyte is normalized to its appropriate internal standard resulting in relative areas. Following, relative areas are used for quantification via external calibration curve. This is a best practice approach for LC-MS-quantification methods. Internal standards are required to meet two criteria: 1) they are not present in the analyzed sample type or present at very low abundancies and 2) they should be similar in structure to the analyte to be quantified. The latter criterion assures that the internal standard compound is underlying the same effects as the metabolite to be quantified such as matrix effects. Therefore, isotope labeled standards are considered to be the 'ideal' internal standards. The use of internal standards in LC-MS quantification methods result in higher precision and higher accuracy of the quantification.

Additionally, the application of calibration standards will allow for absolute quantification. The limit of detection or quantification will be determined from the daily calibration curve and absolute quantification will be performed using stable isotope-labelled standards. A highly controlled sample workflow and QC will ensure robustness and reliability of MS-based detection. The established procedure has contributed to publications in neurodegenerative and pharmacological research[6, 7].

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Limits of detection

MET_CHEM_NO	METABOLITE_NAME	LOD [ng/ml]	ULOQ [ng/ml]
79400032	3,4-Dihydroxymandelic acid	0.3	25
79400006	3,4-Dihydroxyphenylacetic acid (DOPAC)	0.05	100
79400008	3,4-Dihydroxyphenylglycol (DOPEG)	0.01	40
79400030	3-Methoxytyrosine	0.4	300
79400027	3-O-Methyldopamine	0.02	7.5
79400025	4-Hydroxy-3-methoxymandelic acid	0.3	400
79400033	4-Hydroxy-3-methoxyphenylglycol (HMPG)	0.03	150
79400001	Homovanillic acid (HVA)	0.5	500
79400014	Metanephrine	0.015	7.5
79400012	Normetanephrine	0.02	35
79400024	3,4-Dihydroxyphenylalanine (DOPA)	0.05	19.3
79400022	Adrenaline (Epinephrine)	0.01	2.5
79400016	Dopamine	0.005	1.8
79400018	Noradrenaline (Norepinephrine)	0.04	10
79400035	Histamine	0.05	200
79400004	5-Hydroxy-3-indoleacetic acid (5-HIAA)	0.5	128
79400010	Serotonin (5-HT)	0.1	87.5

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Metabolite information

MET_CHEM_NO	METABOLITE_NAME	ONTOLOGY1_NAME	ONTOLOGY2_NAME	CAS_UID
79400032	3,4-Dihydroxymandelic acid	Hormones, signal substances and related	Catecholamine catabolites	CAS-14883-87-5
79400006	3,4-Dihydroxyphenylacetic acid (DOPAC)	Hormones, signal substances and related	Catecholamine catabolites	CAS-102-32-9
79400008	3,4-Dihydroxyphenylglycol (DOPEG)	Hormones, signal substances and related	Catecholamine catabolites	CAS-3343-19-9
79400030	3-Methoxytyrosine	Hormones, signal substances and related	Catecholamine catabolites	CAS-300-48-1
79400027	3-O-Methyldopamine	Hormones, signal substances and related	Catecholamine catabolites	CAS-554-52-9
79400025	4-Hydroxy-3-methoxymandelic acid	Hormones, signal substances and related	Catecholamine catabolites	CAS-55-10-7
79400033	4-Hydroxy-3-methoxyphenylglycol (HMPG)	Hormones, signal substances and related	Catecholamine catabolites	CAS-27391-18-0
79400001	Homovanillic acid (HVA)	Hormones, signal substances and related	Catecholamine catabolites	CAS-306-08-1
79400014	Metanephrine	Hormones, signal substances and related	Catecholamine catabolites	CAS-5001-33-2
79400012	Normetanephrine	Hormones, signal substances and related	Catecholamine catabolites	CAS-97-31-4
79400024	3,4-Dihydroxyphenylalanine (DOPA)	Hormones, signal substances and related	Catecholamine precursors	CAS-59-92-7
79400022	Adrenaline (Epinephrine)	Hormones, signal substances and related	Catecholamines	CAS-51-43-4
79400016	Dopamine	Hormones, signal substances and related	Catecholamines	CAS-51-61-6
79400018	Noradrenaline (Norepinephrine)	Hormones, signal substances and related	Catecholamines	CAS-51-41-2
79400035	Histamine	Hormones, signal substances and related	Other hormones, signal substances and related	CAS-51-45-6
79400004	5-Hydroxy-3-indoleacetic acid (5-HIAA)	Hormones, signal substances and related	Serotonin and serotonin metabolism	CAS-54-16-0
79400010	Serotonin (5-HT)	Hormones, signal substances and related	Serotonin and serotonin metabolism	CAS-50-67-9

Legend

Abbreviations

CAS_UID: Numerical identifier for chemical elements, compounds and polymers assigned by the American Chemical Society (Chemical Abstracts Service).
MET_CHEM_NO: Internal metabolite identification number used by Metanomics Health GmbH.
DICT_STRUCTURE_ELUCIDATION_ID:
1 = Identified
2 = Structure annotation is based on strong analytical evidence (combinations of e.g. chromatography, mass spectrometry, chemical reactions, deuterium-labeling, database and literature search, comparison to similar/homologous/isomeric reference compounds).
3 = Metabolite exhibits identical qualitative analytical characteristics (chromatography and mass spectrometry) compared to a "Structure Elucidation ID = 2" metabolite. Further structural and analytical investigations of this metabolite - also in comparison to structurally identified or " Structure Elucidation ID = 2 " metabolites - are still pending.
4 = Structure elucidation pending

Type of data: Quantitative: Data are normalized against internal standards and reported as absolute concentrations.

LOD: Limit of detection
ULOQ: Upper limit of quantification

About the Author

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References

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