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Urinary metabolic profiling and identification of non-invasive markers for the progression of Huntington's disease

Biomarkører i urin

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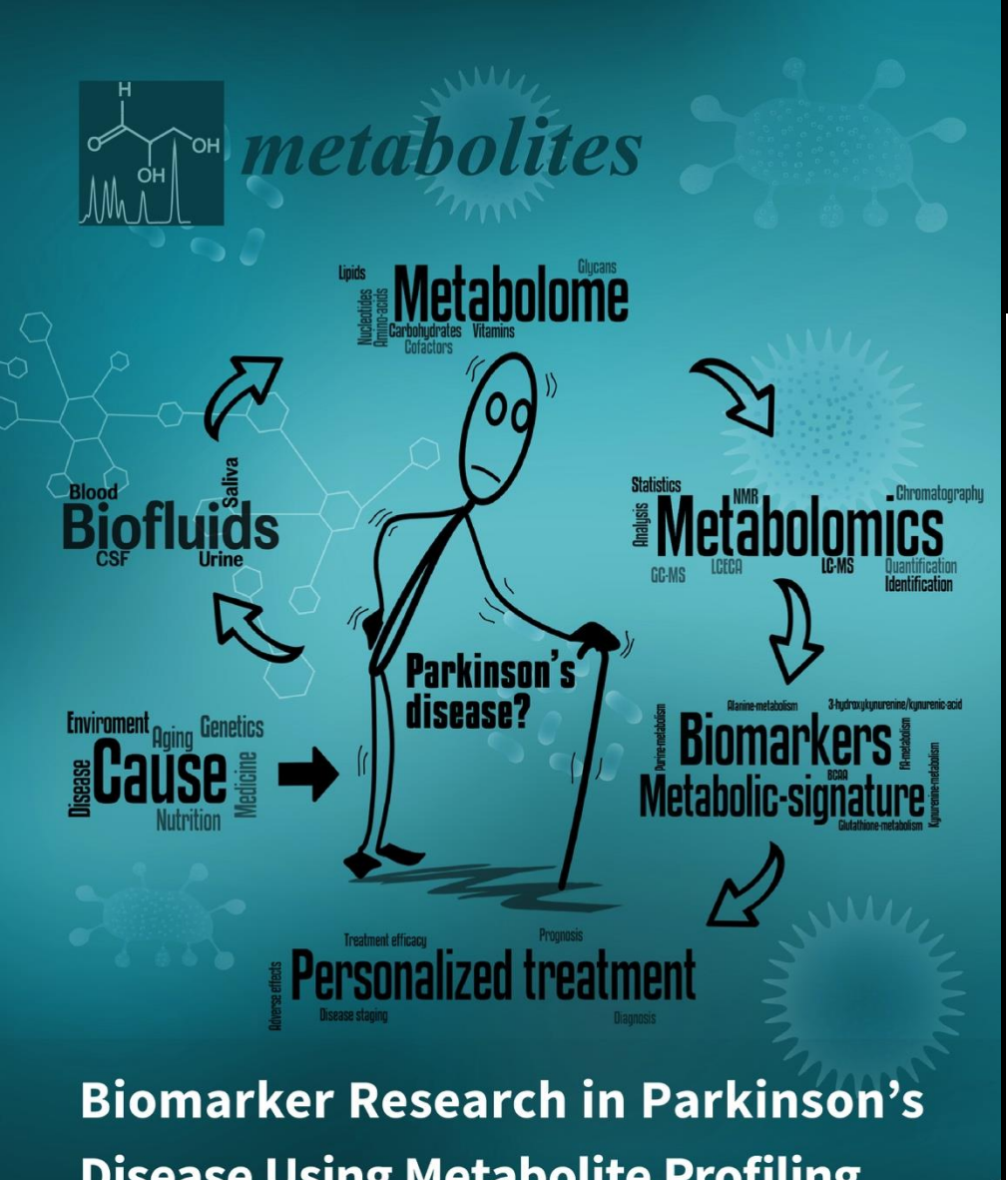
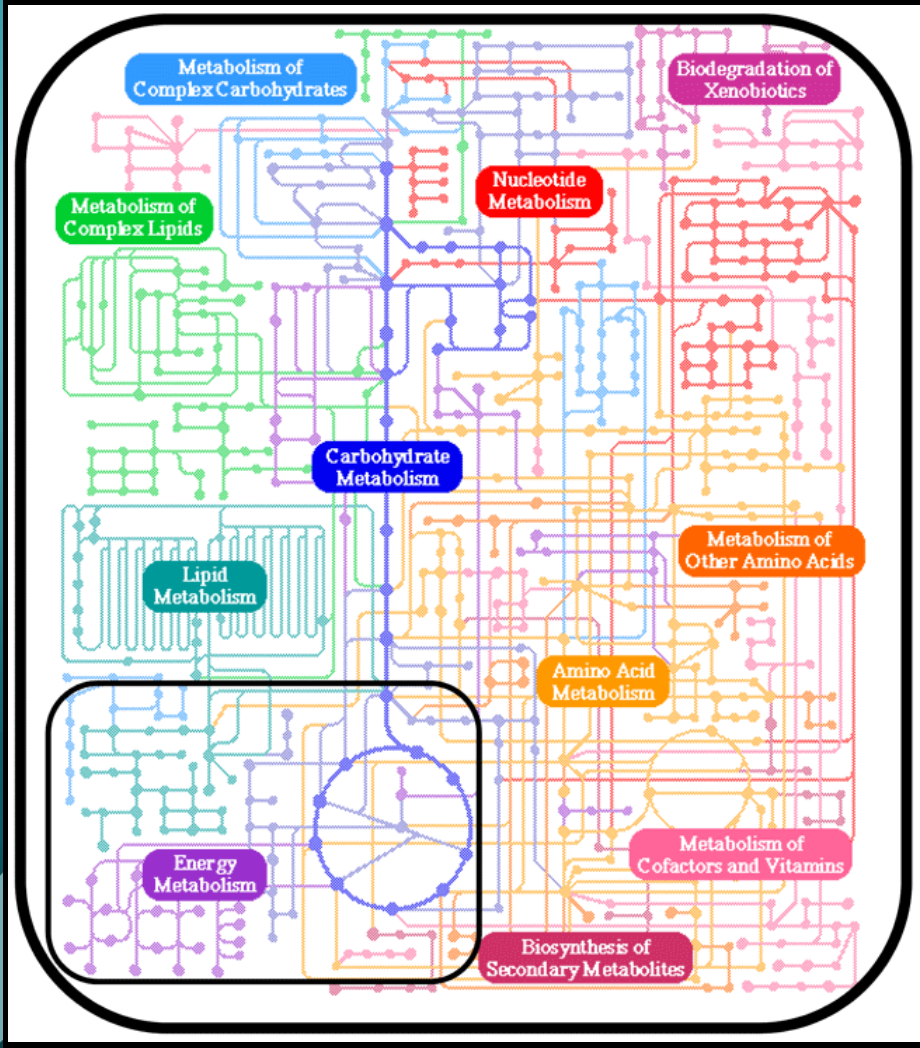
Forskningsdagen Huntingtonforening, København, 4. nov. 2017



Metabolomics

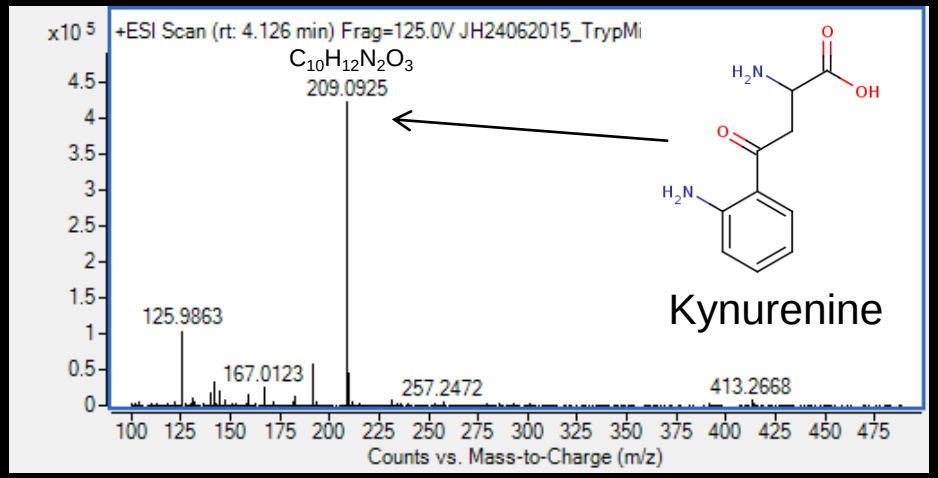
Systematic study of the metabolome



University of Rochester
https://www.urmc.rochester.edu/labs/Munger-Lab/

Metabolomics

- **Systematic study of the metabolome**
  - Mass spectrometry
    - Meassure m/z



Mass spectrum

Q-TOF mass spectrometer

Metabolomics

- **Systematic study of the metabolome**
  - Mass spectrometry
    - Measure m/z
    - Separate metabolite using (HP)LC

Q-TOF mass spectrometer

HPLC

Journal of Neurochemistry JNC
JOURNAL OF NEUROCHEMISTRY | 2017 doi: 10.1111/jnc.14104

ORIGINAL ARTICLE

Changes in kynurenine pathway metabolism in Parkinson patients with L-DOPA-induced dyskinesia

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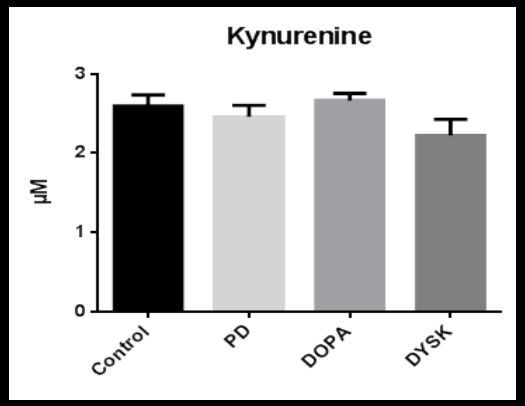
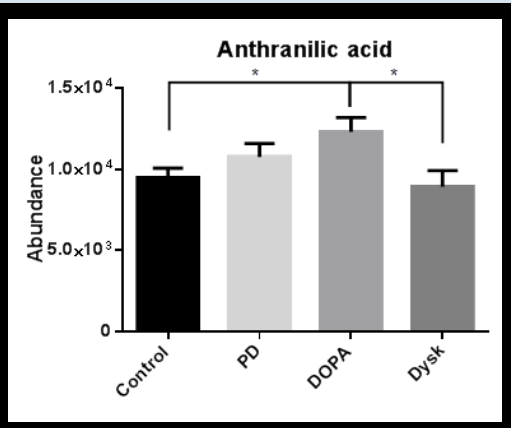
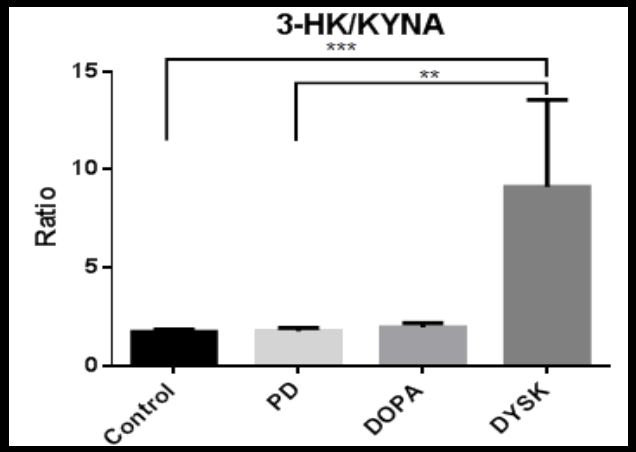
Michael Binzer,‡§ Morten Blaabjerg,††‡‡ Niels H.H. Heegaard,‡‡§§¶¶
Egon Stenager,‡§ Nils J. Færgeman† and Jan Bert Gramsbergen*

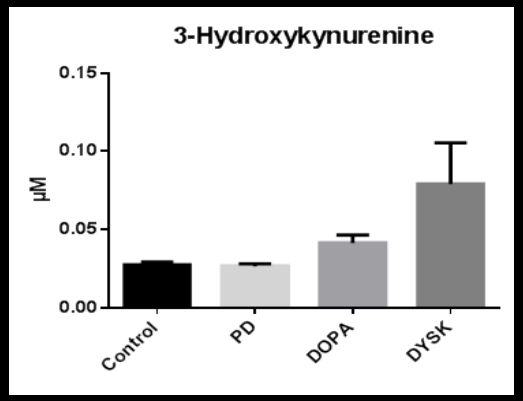
Parkinsonforeningen

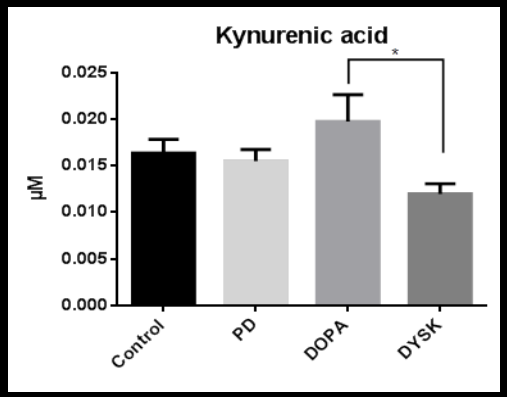
Plasma & CSF samples











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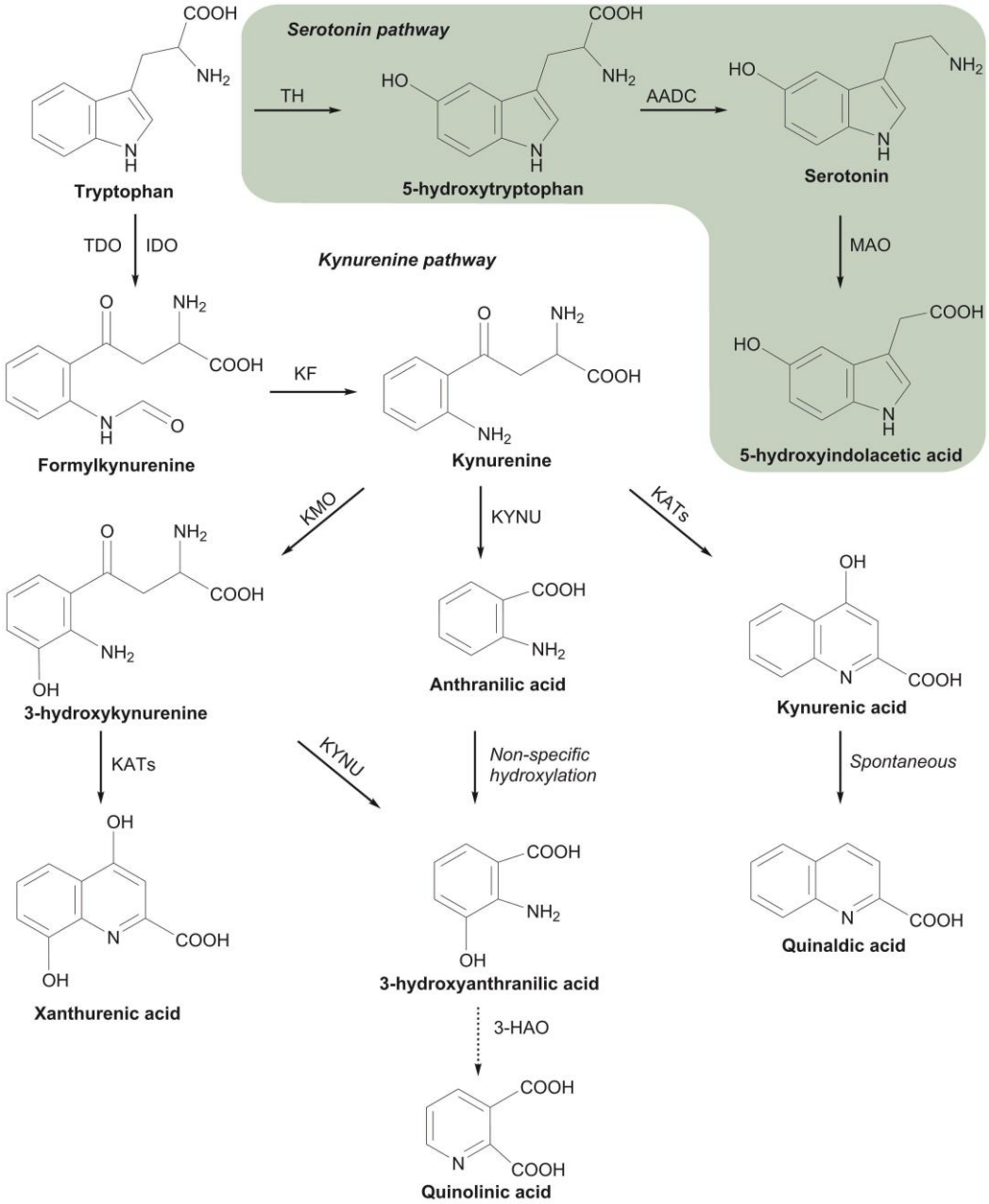
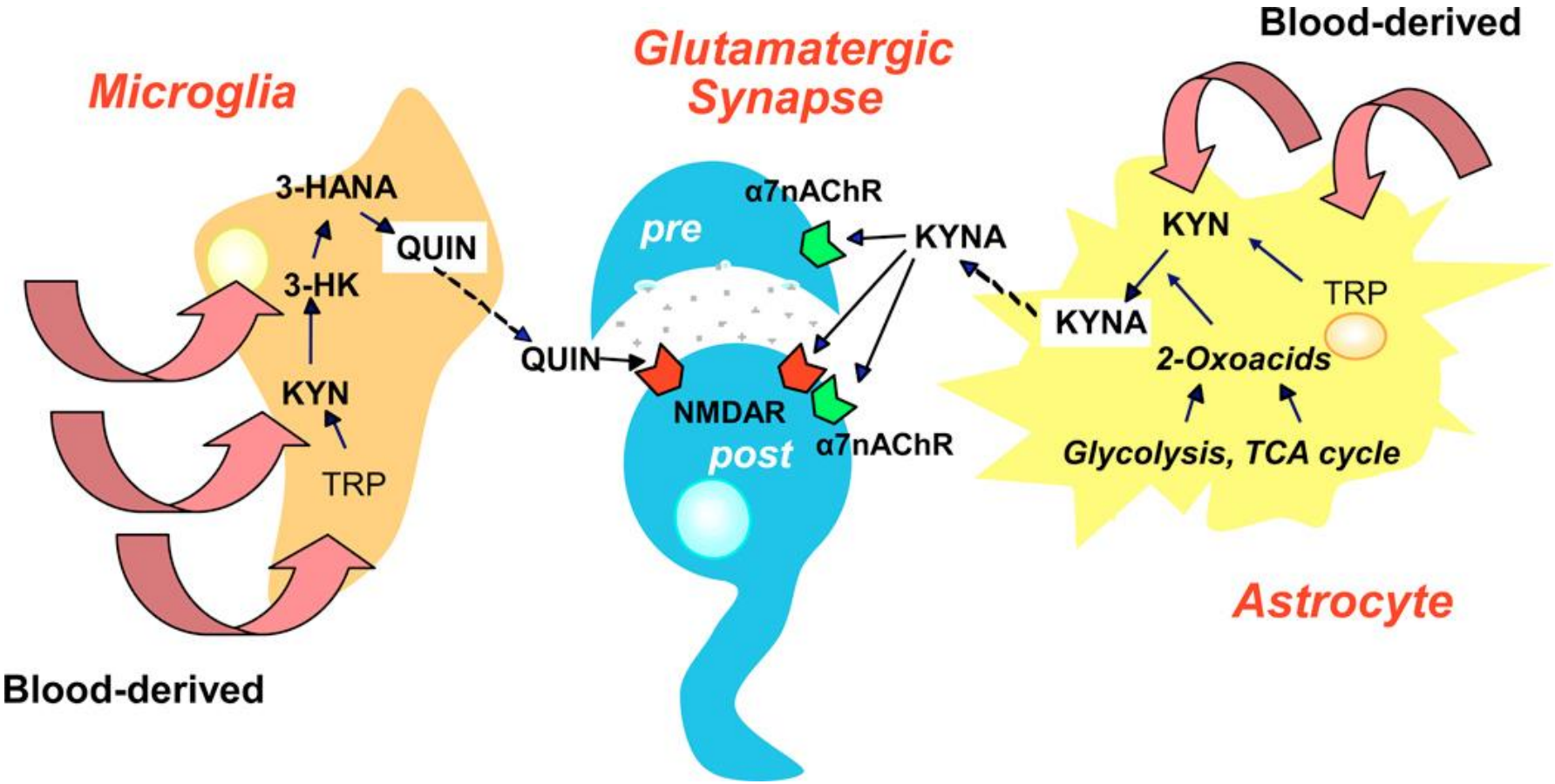
Tryptophan Metabolism: Serotonin and Kynurenine Pathways



Segregation of the two Kynurenine Pathway Branches in the Brain



Schwarcz et al. 2012

Indsamling af urin fra Huntingtons patienter, bærer og kontroller (pårørende)

HD Patient (eller bærer) ID	Fødselsdato	Køn (M/K)	Dato	PrøveID (PatintID+Dato)	Tidspunkt sidste måltid	Tidspunkt urinprøve	UHDRS Motorscore	Psykopharmaka (neuroleptica, SSRI, etc) (ja/nej)	Anden medicin (ja/nej)
P1	0912-1988	K	2504-2016	P1-2504-2016	09.00	11.00	0	Nej	p-piller
			0604-2017	P1-0604-2017	08.12	10.40	0	Nej	p-piller
P2	2107-1972	K	2804-2016	P2-0804-2016	08.30	10.37	28	Sertralin, Seroquel	Ja
			1805-2017	P2-1805-2017	8.30	12.30	35	Sertralin, Seroquel	Ja
P3	0707-1940	M	1005-2016	P3-1005-2016	07.30	10.07	30	sperdal, Oxapax, Seroxat,Remer	Ja
			1108-2017	P3-1108-2017	11.30	13.10	31	perdal, Oxapax, Sertralin,Reme	Ja
P4	0704-1944	K	1005-2016	P4-1005-2016	11.30	13.53	40	Sertralin, Tolmin	Ja
			1905-2017	P4-1905-2017	07.30	11.00	39	Sertralin, Tolmin	Ja
P5	0210-1958	M	1905-2016	P5-1905-2016	07.00	10.20	0	Nej	Nej
			1007-2017	P5-1007-2017	07.30	09.15	0	Nej	Nej
P6	2805-1465	K	1905-2016	P6-1905-2016	08.00	12.18	15	Mirtazapin	Nej
			0607-2017	P6-0607-2017	06.45	09.25	13	Mirtazapin	Vitaminer
P7	0610-1970	M	1606-2016	P7-1606-2016	09.00	14.25	66	Nej	Omega3 Vitaminer
			0806-2017	P7-0806-2017	07.00	10.50	67	Nej	Omega3 Vitaminer
P8	1311-1996	K	3006-2016	P8-3006-2016	10.00	12.39	0	Nej	lorfin, PCM, bumitin
			0510-2017	P8-0510-2017	07.30	09.58	0	nej	nej
P9	3004-1973	K	3006-2016	P9-3006-2016	08.30	14.25	0	Nej	Nej
			0806-2017	P9-0806-2017	07.00	09.32	0	Nej	Vitaminer
P10	3101-1941	K	1108-2016	P10-1108-2016	08.00	10.00	34	Nitoman, Sertralin	Ja

HD gruppen: 44

Urine samples:
HD-bærer: n=26
HD-sympt: n=29

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Indsamling af urin fra Huntingtons patienter, bærer og kontroller (pårørende)

HD Patient (eller bærer) ID	Fødselsdato	Køn (M/K)	Dato	PrøveID (PatintID+Dato)	Tidspunkt sidste måltid	Tidspunkt urinprøve	EVT. Sygdom/medicin
K1	0112-1988	M	2504-2016	K1-2504-2016	09.00	12.30	Nej
K2	2305-1971	M	2804-2016	K2-2804-2016	08.30	13.55	Nej
K3	1509-1943	K	1005-2016	K3-1005-2016	07.30	11.40	Diabetes, får Metformin, Victoza Insulatard
K4	0505-1945	M	1005-2016	K4-1005-2016	11.30	13.20	Forhøjet Cholesteroltal
K5	2909-1963	K	1905-2016	K5-1905-206	07.00	08.45	Nej Har menses
K6	2601-1973	K	1606-2016	K6-1606-2016	09.00	14.35	Nej
K7	2602-1991	K	3006-2016	K7-3006-2016	10.00	10.55	Gravid 12. uge
K8	1004-1980	M	1808-2016	K8-1808-2016	18.00 (17/08)	08.30	Nej
K9	1710-1949	M	0109-2016	K9-0109-2016	09.00	12.30	Forhøjet blodtryk
K10	0111-1958	K	2209-2016	K10-2209-2016	09.00	13.00	Nej
K11	1905-1972	K	3009-2016	K11-3009-2016	06.45	11.45	Kunstig hjerteklap Forhøjet blodtryk
K12	1907-1977	K	1310-2016	K12-1310-2016	08.00	10.41	Nej
K13	2608-1973	K	2010-2016	K13-2010-2016	06.45	09.40	Nej

Kontrol N=24

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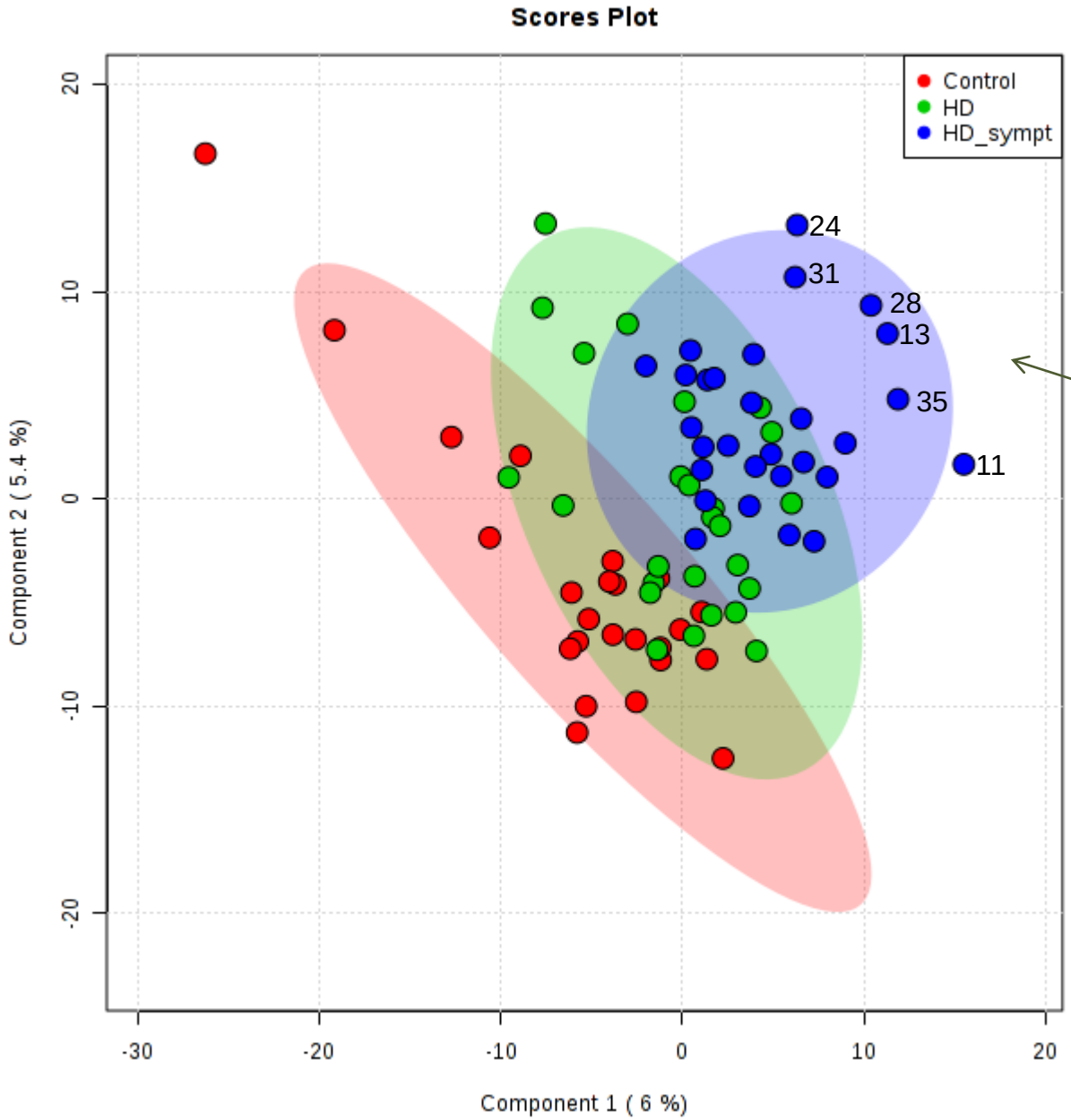

Score

Efter normalisering kan vi adskille grupperne

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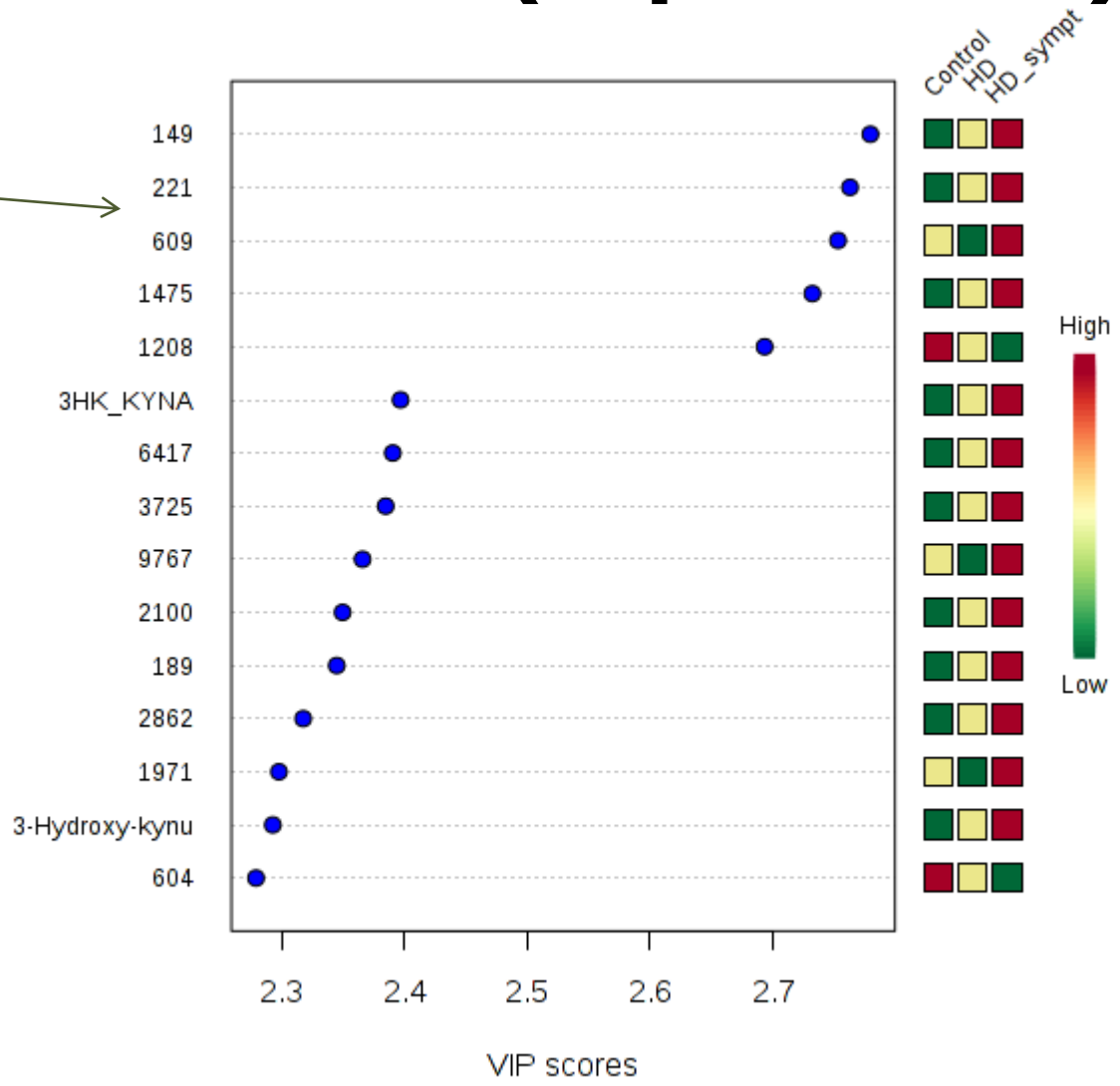
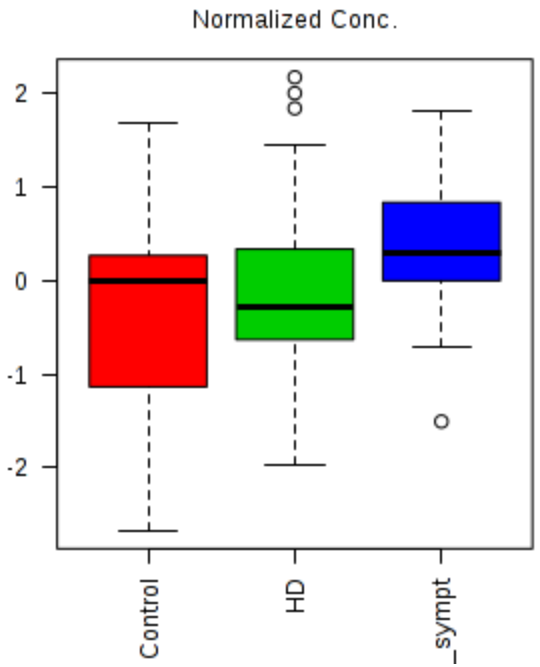
Stoffer der har størst påvirkning på adskillelse (top 15 vist)

Ukendte



Ca 1200 stoffer er brugt

Øget 3-HK/KYNA ratio i urin fra Huntingtons patienter med symptomer
(p<0.05 Kruskal-Wallis test)



To do

- Idendificere stoffer som viser signifikant forskel mellem grupperne
- Sammenligne 1. urinprøve med 2. urinprøve (taget 1 år senere)
- Sammenligne Huntington urindata med urindata fra Parkinson studie

Follow-up study i større gruppe HD patienter ? Funding?
Intervention med KMO hæmmer?

SDU

Current Biology 21, 961–966, June 7, 2011 ©2011 Elsevier Ltd All rights reserved DOI 10.1016/j.cub.2011.04.028

Report

The Kynurenine Pathway Modulates Neurodegeneration in a *Drosophila* Model of Huntington's Disease

Susanna Campesan,[1] Edward W. Green,[1] Carlo Breda,[1] Korrapati V. Sathyasaikumar,[2] Paul J. Muchowski,[3] Robert Schwarcz,[2] Charalambos P. Kyriacou,[1] and Flaviano Giorgini[1,*]
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[2]Maryland Psychiatric Research Center, University of Maryland School of Medicine, Baltimore, MD 21228, USA
[3]Gladstone Institute of Neurological Disease, Department of Biochemistry and Biophysics and Department of Neurology, University of California, San Francisco, San Francisco, CA 94158, USA

Summary

Neuroactive metabolites of the kynurenine pathway (KP) of tryptophan degradation have been implicated in the pathophysiology of neurodegenerative disorders, including Huntington's disease (HD) [1]. A central hallmark of HD is neurodegeneration caused by a polyglutamine expansion in the huntingtin (htt) protein [2]. Here we exploit a transgenic *Drosophila melanogaster* model of HD to interrogate the therapeutic potential of KP manipulation. We observe that genetic and pharmacological inhibition of kynurenine 3-monooxygenase (KMO) increases levels of the neuroprotective metabolite kynurenic acid (KYNA) relative to the neurotoxic metabolite 3-hydroxykynurenine (3-HK) and ameliorates neurodegeneration. We also find that genetic

to study the roles of 3-HK and KYNA in neurodegeneration (Figure 1A). Levels of these metabolites can be modulated by agents that inhibit the first step in the KP (catalyzed in mammals by tryptophan 2,3-dioxygenase [TDO], indoleamine 2,3-dioxygenase [IDO], and indoleamine 2,3-dioxygenase 2) or by inhibiting kynurenine 3-monooxygenase (KMO), which lies at the branching point between 3-HK and KYNA synthesis. Both IDO and KMO inhibitors have shown promise in preclinical studies of a variety of human disorders [12]. In flies, KMO and TDO are encoded by the genes *cinnabar* (*cn*) and *vermillion* (*v*), respectively, which are expressed in the eye and have been extensively characterized for their role in eye color pigmentation [13]. The role of the KP in eye function is conserved from flies to humans, where the pathway plays a central role in the formation of ultraviolet irradiation (UV) filters in the lens [14]. Notably, fly mutants that perturb the KP have been found to alter brain plasticity and modulate life span [15, 16]. To date, the therapeutic potential of KMO specifically, and the KP in general, has not been explored in an animal model of neurodegeneration, and the role of KP metabolites in this disease process has not been clarified. This study interrogates these questions using both genetic and pharmacological tools in a fruit fly model of Huntington's disease (HD).

HD Model Flies Exhibit Perturbed Flux through the KP

Increased flux through the central KP is correlated to pathology in HD patients, as well as in mouse and yeast models of HD [1]. To examine whether the pathway is dysfunc-

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Report

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[1]Department of Genetics, University of Leicester, University Road, Leicester LE1 7RH, UK
[2]Maryland Psychiatric Research Center, University of

to study the roles of 3-HK and KYNA in neurodegeneration (Figure 1A). Levels of these metabolites can be modulated by agents that inhibit the first step in the KP (catalyzed in mammals by tryptophan 2,3-dioxygenase [TDO], indoleamine 2,3-dioxygenase [IDO], and indoleamine 2,3-dioxygenase 2) or by inhibiting kynurenine 3-monooxygenase (KMO), which lies at the branching point between 3-HK and KYNA synthesis.

huntingtin (htt) protein [2]. Here we exploit a transgenic *Drosophila melanogaster* model of HD to interrogate the therapeutic potential of KP manipulation. We observe that genetic and pharmacological inhibition of kynurenine 3-monooxygenase (KMO) increases levels of the neuroprotective metabolite kynurenic acid (KYNA) relative to the neurotoxic metabolite 3-hydroxykynurenine (3-HK) and ameliorates neurodegeneration. We also find that genetic