"RT-QulC assay on olfactory brushings in asymptomatic carriers of E200K PRNP mutation: an explorative study for establishing when a preventative therapy should be started "

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Human PrP gene ($PRNP$) mutations correlate to distinct disease phenotypes such as:
- Genetic Creutzfeldt-Jakob Disease (gCJD)
- Fatal Familial Insomnia
- Gerstmann Straussler Scheincker syndrome
- PrP systemic amyloidosis

The disease phenotype of gCJD is characterized by a rapidly progressive neurological disorder.

Mutation carriers of E200K develop a gCJD. E200K is diffuse worldwide with clusters in Slovakia, Italy (Calabria region), and among Libyan Jews in Israel.

For unknown reasons, the penetrance of E200K mutation varies among clusters and the probability to develop CJD in Calabrians is 50% at the age of 60 and 61% at the age of 70 (D’ Alessandro M. et al. Lancet 1998)
Prion Conversion and Replication in Genetic Prion Diseases

PrP gene Mutation

Mutated PrP

Pathological PrP

Conversion and Propagation

Brain accumulation
Neuronal damage

Preclinical Stage (30-60 years)

Clinical overt (few months)
Pre-clinical Diagnosis of Prion Disease?
Pre-symptomatic diagnosis in fatal familial insomnia: serial neurophysiological and $^{18}$FDG-PET studies

Pietro Cortelli, Daniela Perani, Pasquale Montagna, Roberto Gallassi, Paolo Tinuper, Provini Federica, Patrizia Avoni, Franco Ferrillo, Davide Anchisi, Rosa Maria Moresco, Ferruccio Fazio, Piero Parchi, Agostino Baruzzi, Elio Lugaresi and Pierluigi Gambetti
Prion Conversion and replication in Genetic Prion Diseases

PrP gene Mutation

PrP\textsuperscript{M}

PrP\textsuperscript{Sc}

Conversion and Propagation

Slow

Fast

Brain accumulation
Neuronal damage

Preclinical Stage (30-60 years)

Potential therapeutetic window

Clinical overt
(few months)
A Test for Creutzfeldt–Jakob Disease Using Nasal Brushings

Christina D. Orrú, Ph.D., Matilde Bongianni, Ph.D., Giovanni Tonoli, M.D., Sergio Ferrari, M.D., Andrew G. Hughson, M.S., Bradley R. Groveman, Ph.D., Michele Fiorini, Ph.D., Maurizio Pocchiari, M.D., Salvatore Monaco, M.D., Byron Caughey, Ph.D., and Gianluigi Zanusso, M.D., Ph.D.
Short movie of Nasal Brushing Procedure
Diagnosis Accuracy of OM brushing coupled with RT-QuIC

Olfactory Mucosa brushing is:

- **Easily performed**: It does not require trained otolaryngologists
- **Non-invasive**: No medical or surgical complications or a smell disorder
- **Highly efficient**: It collects around 300,000 olfactory neurons with a single sampling
- **Highly sensitive**: RT-QuIC assay of OM detects pathological PrP seeding activity in OM of CJD patients with a 97% sensitivity and 100% specificity
RT-QuIC assay in Olfactory Brushings of patients with sporadic CJD and E200K using Hamster PrP 23-231 at 42°C

![Graph showing ThT fluorescence over time for sCJD and E200K]
Migration of E200K family members from rural region of Calabria to the Northern Italy

E200K cluster where the preclinical OM testing was setting up

Rural region of Calabria 142 km²
Subjects enrollment

30 subjects

42 subjects

OM brushing and blood collection from E200K family members

First group

Second group

Collection and RT-QuIC setting

PRNP Gene sequencing

Set up of «Improved» RT-QuIC experimental conditions

(Presumably, during the preclinical stage, the amount of PrP oligomers is consistently lower than the clinical overt)

3 months

6 months

8 months

12 Months

Completed Data analyses

In case of RT-QuIC positive OM starting a preventive therapy with doxycycline

RT-QuIC testing of all OM samples combining the results with genetics

Testing and data analyses

Each subject was informed about the study and voluntarily participated
Study Plan: Second Part

Subjects enrollment

Each subject was informed about the study and voluntarily participated.

OM brushing and blood collection from E200K family members

First group

30 subjects

8 Months

Second group

42 subjects

Collection and RT-QuIC setting

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Completed Data analyses

In case of RT-QuIC positive OM starting a preventive therapy with doxycycline

PRNP Gene sequencing

Set up of «Improved» RT-QuIC experimental conditions

(Presumably, during the preclinical stage, the amount of PrP oligomers is consistently lower than the clinical overt)

RT-QuIC testing of all OM samples combining the results with genetics
72 subjects enrolled: 30 Male and 42 Female

Mean age: 51.2 (range 19-90)

All undewent to OM brushing (except two) and blood withdrawal

No complications were observed and all OM samples were suitable for the test

Genetic analysis: 21 carriers of E200K mutation
End Point Dilution of OM from patients with E200K PRNP mutation
(Hamster PrP 23-231 at 42°C)
RT-QuIC testing of OM
Using Standard Conditions
(Hamster PrP 23-231 at 42°C)
RT-QuIC testing of OM using «Improved»
RT-QuIC Conditions
(Hamster PrP 90-231 at 55°C)

ThT Fluorescence

Time (h)

E200K Patient

Neurological controls (AD, PD, LBD)
RT-QuIC testing of OM using «Improved» RT-QuIC Conditions
(Hamster PrP 23-231 at 55°C)

ThT Fluorescence

Time (h)

E200K Patient

Neurological controls (AD, PD, LBD)
RT-QuIC testing of OM using «Improved»
RT-QuIC Conditions
(Vole PrP 90-231 109M at 55°C)
Nasal brushing procedure was well tolerated by all subjects;

The end-point dilution of OM from patients with E200K, using RT-QuIC standard conditions (Hamster PrP 23-231 at 42°C), was $10^{-6}$. No positive sample in OM from healthy family members;

We tested different «Improved» RT-QuIC experimental conditions, changing PrP substrates of reaction and temperatures, for increasing the sensitivity without compromising the specificity of the test;

We determined that the most appropriate «Improved condition» was Hamster PrP 23-231 at 55°C (OM resulted positive after 5 hours in E200K patient without false positive in other neurodegenerative disorders).