Isolation of skin fibroblasts from MS patients for reprogramming into induced pluripotent stem cells

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Ethical review	Approved WMO
Status	Recruitment stopped
Health condition type	Demyelinating disorders
Study type	Observational invasive

Summary

ID

NL-OMON37835

Source
ToetsingOnline

Brief title
MSIPS

Condition

- Demyelinating disorders

Synonym
demyelination disorder, Multiple sclerosis

Research involving
Human

Sponsors and support

Primary sponsor: Universitair Medisch Centrum Groningen
Source(s) of monetary or material Support: Ministerie van OC&W,Dutch MS Research Foundation

Intervention

Keyword: differentiation, oligodendrocytes, pluripotent stem cells, reprogramming

Outcome measures

Primary outcome

- IPS cells can be generated from skin fibroblasts of MS patients and

differentiated into OPCs and functional oligodendrocytes

- OPCs and oligodendrocytes derived from MS patients via IPS reprogramming show

differences in expression profiles and in responses to stress in comparison to

those of healthy controls.

Secondary outcome

not applicable

Study description

Background summary

Multiple sclerosis (MS) is an inflammatory, neurodegenerative disease initially characterized by relapsing remitting (RR) clinical episodes. The relapses reflect the progression of lesions within the CNS featuring inflammation, myelin destruction and axonal loss. After each relapse, axonal damage is restricted by the remyelination activity of local OPCs. The transition of RRMS to the secondary progressive form of MS marks the limit of brain plasticity where OPCs are no longer able to remyelinate axons and axonal loss can no longer be compensated by parallel connections. In primary progressive MS (PPMS), a steady neurological deterioration occurs already from the moment of diagnosis suggesting that OPC recruitment and proper remyelination fails from the beginning. Although cell-mediated immune mechanisms play a prominent role in disease progress, it is suggested that intrinsic aberrations in oligodendrocyte physiology in MS patients may be a primary factor. The groundbreaking discovery that somatic cells (e.g. skin fibroblasts) can be reprogrammed into pluripotent stem cells (IPS cells) that can be differentiated in any cell type has offered an unprecedented source for patient-derived cells. Such patient-derived IPS cells appear not only a promising source for cell replacement therapies, but particularly for disease-modeling and, so far, IPS

cells have been generated from patients with neurological disorders (e.g. Huntington, ALS, SCA). MS is not a genetic disorder with known single or multiple gene mutations. However, a specific (epi) genetic profile present in MS patients is suggested to underlie an aberrant physiology and behavior of oligodendrocytes resulting in abnormal responses to local stress and/or abnormal myelin production. IPS technology now offers a unique opportunity to examine this with MS patient derived OPCs in vitro.

Study objective

In this study we aim to address the hypothesis that oligodendrocytes and oligodendrocyte precursor cells from MS patients exhibit epigenetically determined differences in protein expression profiles and functional behavior in comparison to those from healthy controls. To investigate this we make use of MS patient-derived IPS cells.
We have the following specific objectives:
Aim 1: To isolate skin fibroblasts from MS patients by punch biopsy under local anesthesia and store them after in-vitro multiplication.
Aim 2: To reprogram the patient-derived skin fibroblasts into induced pluripotent stem (IPS) cells and, after full IPS characterization/verification, differentiate them in-vitro into oligodendrocyte precursor cells (OPCs) and oligodendrocytes with a protocol developed in our lab.
Aim 3: To compare the expression profiles of MS patient-derived OPCs and oligodendrocytes with control ones using RNA sequencing, proteomics and epigenetic characterization.
Aim 4: To expose MS patient and control-derived OPCs and oligodendrocytes to different types of stress (excitotoxic, oxidative and inflammatory) in vitro and compare their responses by analyzing their behavior and expression profiles.
Aim 5: To co-culture MS patient and control-derived OPCs with rat DRG neurons, a well-known model to examine myelin formation in detail. In this set-up, myelin production by MS patient-derived oligodendrocytes will be analyzed in detail with techniques mentioned above.

Study design

For the whole study, fibroblasts from two groups of MS patients will be used: 5 RRMS patients, 5 PPMS patients (as controls IPS cells from 5 healthy age-matched persons will be directly obtained from the IPS facilities, LUMC, Leiden and from the department Neuroscience, UMCG).
After patient selection, a skin punch biopsy will be performed according to standard procedures under sterile conditions and after local anesthesia at the department of Dermatology, UMCG. The skin biopsies will be collected in cold sterile basic culture medium and transported to the lab of the dept. Neurosciences, UMCG. After tissue dissociation, fibroblasts will be cultured and multiplied under fibroblast specific/selective culture conditions. Part of

these fibroblasts will be stored frozen, another part will be used for reprogramming into IPS cells and subsequently differentiated into OPCs according to procedures developed in the dept. Neurosciences, UMCG. Subsequently, the IPS derived OPCs will be subjected to extensive analyses, comprising mRNA sequencing, proteomics and epigenetic profiling (e.g. DNA methylation patterns). Myelination capacity of the various IPS derived oligodendrocytes will be examined and compared in vitro.

Intervention

After patient selection, a skin punch biopsy (diameter 4mm) will be performed according to standard procedures under sterile conditions and after local anesthesia at the department of Dermatology, UMCG.

Study burden and risks

From the MS patients a small (6mm) skin biopsy will be taken from the innerside of the upper arm using a standard punch technique under local anesthesia. The only minimal risk involved in particpation may be the occurrence of infection (despite intense desinfection of the site of biopsy) (<1% of cases) ; effective treatment with antibiotic ointment.

Contacts

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Trial sites

Listed location countries

Netherlands

Eligibility criteria

Age
Adults (18-64 years)
Elderly (65 years and older)

Inclusion criteria

- Age 20 years or above.
- Diagnosis of RRMS or PPMS according to the revised McDonald criteria independently confirmed by 2 neurologists .
- Informed consent is obtained.

Exclusion criteria

- Patients under 20 years of age.
- Unclear diagnosis of stage/type of the disease
- Recent (less than 4 weeks) treatment with high dose intravenous methylprednisolone
- Ongoing treatment with immunomodulating drugs other than interferon-beta or copaxone
- Extensive skin disorder precluding a biopsy from unaffected skin area
- Known allergy for local anesthetics
- Informed consent can, for whatever reason, not be obtained (f.i. the presence of neurological deficits that preclude a reliable verbal communication or lead to cognitive disability).

Study design

Design

Study type:	Observational invasive
Intervention model:	Other
Allocation:	Non-randomized controlled trial
Masking:	Open (masking not used)
Control:	Active
Primary purpose:	Basic science

Recruitment

NL

Recruitment status:	Recruitment stopped
Start date (anticipated):	01-09-2012
Enrollment:	10
Type:	Actual

Ethics review

Approved WMO

Date:	17-07-2012
Application type:	First submission
Review commission:	METC Universitair Medisch Centrum Groningen (Groningen)

Study registrations

Followed up by the following (possibly more current) registration

No registrations found.

Other (possibly less up-to-date) registrations in this register

No registrations found.

In other registers

Register	ID
CCMO	NL40425.042.12