EXPERIMENTAL PROTOCOL

STUDY TITLE

"Nasal brushing for the diagnosis and understanding of telomeropathies"

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1. INTRODUCTION

Telomere biology is a growing research field. New genes and/or genomic variants of established telomere-related genes are still being identified as modulators of telomere length. While long telomeres are considered as a sign of good cellular health associated with a better regenerative potential of the tissues, abnormally short telomeres are responsible for premature ageing syndromes, collectively known as telomeropathies, that can be very severe and life threatening. The diagnosis of telomeropathy is complicated by the fact that patients can present with a variety of symptoms at various ages, including aplastic anemia, pulmonary fibrosis, liver cirrhosis, unexplained liver regenerative nodular hyperplasia or myelodysplasia. If, to date, there is no treatment yet to these genetic diseases, identifying the telomeric origin of the disease is important to avoid inappropriate therapeutic treatments. Developing new and poorly invasive diagnostic tools for telomeropathy is one of our research objectives. Our second objective is to better understand the genetics of telomeropathies through functional studies of new disease-causing mutations. Together, these two objectives will contribute to a better detection and understanding of telomere-associated pathologies.

TELOMEROPATHIES: DIAGNOSIS

Telomeropathies are premature ageing diseases resulting from pathogenic germline mutations in telomere biology-related genes. Most telomeropathy patients have telomeres that are too short for their age. Telomere length (TL) can be measured in blood cells using a technique called Flow-FISH (FF) that combines fluorescent *in situ* hybridization (FISH) with flow cytometry (Baerlocher et al. 2006). To provide a diagnostic tool for telomeropathies in Belgium, the laboratory of **A. Decottignies, in collaboration with CUSL,** recently implemented the FF technique and measured TL in a cohort of 491 healthy volunteers aged 0-99 years. In combination with FF, additional genetic and phenotypic studies on patients' cells are needed to tell whether the disease is caused by prematurely defective telomeres. Knowing this is important to i) exclude any other cause of the disease, ii) avoid the use of inappropriate treatments, iii) better manage the immunosuppressive regimens that are associated with hematopoietic stem cell or lung transplantation in some telomeropathy patients and iv) enable possible targeted therapies. For instance, while pulmonary fibrosis carriers with *TOLLIP* polymorphism may benefit from N-acetylcysteine treatment, those with defective telomeres may possibly benefit from Danazol, a telomerase-activating drug previously associated with increased TL in PBMCs and stability of lung function in idiopathic PF (IPF) patients (Townsley et al. 2016).

To date, mutations in 15 telomere biology-related genes have been linked to telomeropathies and include *TERC* and *TERT* telomerase core components, various chaperone genes with roles in *TERC* maturation and stability, shelterin complex genes, the CST complex with important function in telomere replication and *RTEL1* with multiple roles at telomeres, including replication (Savage 2018). There are however many patients with abnormally short telomeres and still unidentified mutation. On the other hand, in patients with *CTC1* or *POT1* mutations, presenting with severe telomeropathies, telomeres

are defective but not abnormally shortened (Gu et al. 2018), underlying the need to combine TL measurements with additional phenotypic and genetic analyses for the proper diagnosis of telomeropathies.

One way to evaluate telomere dysfunction, independently of TL measurement in blood cells by FF, is to perform a combination of telomeric FISH and immunofluorescence (FISH/IF) against DNA damage markers to quantify the damaged telomeres, a conserved feature of cells from telomeropathy patients. The evaluation of cellular senescence, using the well-established Senescence-Associated β-Galactosidase (SA-β-Gal) assay (Dimri et al. 1995) is yet another assay to evaluate a premature ageing cellular phenotype. Importantly however, neither FISH/IF nor SA-β-Gal assays can be performed on blood cells. The cellular diagnosis therefore requires other cell types. Usually, a skin biopsy is performed that requires to establish a primary culture of fibroblasts for further analyses. The skin biopsy is however invasive, leaving permanent scars. A skin biopsy can also be used to identify the genetic mutations at the origin of the disease. This type of cell is usually preferred over blood cells because of the known ability of blood cells to undergo somatic genetic rescue that can offset the pathogenic effect of germline mutations (Revy et al. 2019). In this respect, although intragenic compensatory mutations are observed in blood cells from telomeropathy patients, in the majority of the cases, indirect somatic genetic rescue is obtained through the acquisition of well-defined activating point mutations in the promoter of h*TERT* gene that codes for the catalytic subunit of telomerase (Revy et al. 2019). Since this has already been observed in cases of heterozygous germline mutations in *PARN*, *NHP2* or *TERC* genes (all involved in maturation and activity of telomerase), this suggests that the detection of these point mutations in blood cells may be a strong indicator of the existence of a telomeropathy, prior to the exome-seq analysis.

Importantly as well, having non-blood cells in culture, like fibroblasts, allows functional studies of the mutations to increase our knowledge of the telomeropathies at the cellular level.

Altogether, this underlines the interest of having access to non-blood cells from telomeropathy patients for proper diagnosis, mutation identification and functional studies. In that context, developing alternative and less invasive approaches to collect non-blood cells so as to replace skin biopsies would be highly beneficial to patients.

THE OLFACTORY EPITHELIUM: AN ALTERNATIVE SOURCE OF CELLS FOR TELOMEROPATHY DIAGNOSIS AND UNDERSTANDING?

The olfactory neuroeptithelium (ONE), located at the upper part of nasal fossae in a region named the olfactory cleft, is considered as a unique structure owing to its sustained regenerative capacity. Indeed, mature olfactory sensory neurons are continuously produced from a population of stem cells (horizontal and globose cells) throughout adulthood age (Rawson and Ozdener 2013). Consequently, ONE is considered as the unique source of regenerating neuronal cells that can be easily obtained from living humans.

ONE cells can be easily collected using a technique called nasal brushing. In this technique, ONE is sampled using a cytology brush that is introduced in the olfactory cleft at the level of the head of the middle turbinate and gently moved by circular movements to collect epithelial cells. It can easily be performed under endoscopic control and has no known adverse effect (Benitez-King et al. 2011; Orru et al. 2014). This non-invasive technique was found to be efficient in obtaining mature olfactory sensory neurons as well as neuronal cell precursors and stem cells from healthy subjects and patients (Benitez-King et al. 2011). Importantly, neuronal precursors can be propagated in culture (Benitez-King et al. 2011). Studies have shown that the collected neuronal precursors possess an undifferentiated state, the capability of self-renewal and the capacity to proliferate and differentiate into neuronal and glial cells without genetic reprogramming (Idotta et al. 2019; Jimenez-Vaca et al. 2018; Matigian et al. 2010; McCurdy et al. 2006). Moreover, despite a superficial exfoliation, it is important to mention that nasal brushing allows the collection of a significant number of cells, Brozzetti et al. reporting that the number of cells ranged up to 2 millions, with 40% of those being neuronal cells (Brozzetti et al. 2020). Although non-invasive, the brushing technique thus represents a valuable tool to collect cells from the ONE and is thought to be an excellent surrogate model to study pathophysiological neural mechanisms.

Besides that, it is also important to underline the link between aging and dysfunction of the olfactory system. Indeed, from a behavioral point of view, it is well known that aging is associated to a progressive impairment of olfactory function (Huart et al. 2013). Although the decrease of olfaction is physiological with age, aged people having a more severe olfactory dysfunction are at higher risk to develop neurodegenerative disease, such as Alzheimer's and Parkinson's disease (Mesholam et al. 1998), and are also at higher risk of 5-years mortality (Devanand et al. 2015; Pinto et al. 2014). This relationship being specific to the sense of smell, these findings suggest that the olfactory system is particularly sensitive to aging. Currently, olfactory impairment is thus considered as an emerging biomarker of biological aging. Among the diverse hypotheses explaining the link between olfactory impairment and aging, it has been postulated that olfactory impairment might reflect a decline of this continuous neurogenesis, through environmental exposure or brain aging. Loss of this necessary process could be seen more globally as an indicator of lowered physiologic repair function which may reflect senescence.

Therefore, we think that ONE could provide an interesting cellular model for the diagnosis and study of telomeropathies and replace the invasive skin biopsies that are currently being performed for genetic and phenotypic analyses of telomere dysfunction and senescence. Moreover, assessment of olfactory function in patients with telomeropathy could bring new interesting insights.

2. OUTLINE OF OBJECTIVES AND PROJECT DESIGN

2.1 OBJECTIVES

The main objective of this study is to develop new tools for the diagnosis and understanding of telomeropathies. Our more specific aims are:

- To assess the suitability of nasal brushing analyses and blood tests for the diagnosis of telomeropathies
- To assess whether primary cell cultures obtained from nasal brushing could constitute a valuable tool for functional studies of new germline mutations

2.2 STUDY DESIGN

This study is a monocentric prospective interventional study on 250 subjects (150 patients and 100 controls). The promotor of this study is the Cliniques universitaires Saint-Luc (Brussels, Belgium).

2.2.1 Study population

This study will be performed in 150 subjects with confirmed or suspected telomeropathy and recruited through Cliniques universitaires Saint-Luc (CUSL) (Brussels, Belgium).
We will include patients with suspicion of telomeropathy: patients with idiopathic pulmonary fibrosis, adult or pediatric medullar dysplasia or myelodysplasia, unexplained liver cirrhosis or unexplained liver regenerative nodular hyperplasia.
100 age-matched healthy controls will be recruited from CUSL.

2.2.2 Study endpoints

Endpoint #1: To assess the suitability of nasal brushing analyses and blood tests for the diagnosis of telomeropathies.

Rationale
To date, the complete diagnosis of telomeropathies, including the identification of responsible mutations, is based on blood samples and fibroblast cultures obtained through skin biopsies. Cells obtained through nasal brushing offer the opportunity to detect mutations involved in telomeropathies, in a mildly invasive way. We will thus assess a population of patients with a suspicion of telomeropathy, using a combination of blood tests and nasal brushings, and will compare their nasal brushing results to those of age-matched healthy controls. If we confirm that the nasal brushing offers the opportunity to i) detect damaged telomeres and premature cellular senescence and ii) identify mutations related to telomeropathies, this technique could become a non-invasive clinical tool for the diagnosis work-up of telomeropathies

Assessments
We will collect blood samples in each patient and will perform a nasal brushing to each subject (patients and controls) included in this study. One sample of blood will be collected for each patient and processed following the protocol further described (see chapter 3.1.).

For nasal brushing, one collection will be performed in the most accessible nostril. We will evaluate if it is possible to detect damaged telomeres and premature cellular senescence in patient, in comparison to healthy controls.
In the patients group, collected cells will be split into three parts, two will be devoted to Endpoint#1, and the other for the Endpoint #2, following the protocol further described (see chapter 3.2.).
For patients recruited from the pediatric consultation, if possible, this sample will be collected during another medical procedure planned under general anesthesia (eg. marrow puncture). Similarly, pediatric controls will be recruited among patients undergoing elective otorhinolaryngological surgery (eg. grommets, tonsillectomy).

Endpoint #2: To develop primary cell cultures for the functional study of new germline mutations.

Rationale
To date, various germline mutations have already been identified in telomeropathy patients, in a total of 15 genes. Understanding how these mutations were affecting telomere biology relied on *in vitro* studies with either patient-derived fibroblasts or engineered human cell lines recapitulating the mutation. This was a mandatory step towards the molecular understanding of these pathologies. Because olfactory neural precursors have the capacity to grow in culture, this offers the additional possibility to perform functional studies on primary cultures of nasal brushing-derived cells for novel mutations, with still unknown impact on telomeres, that would be identified. Again, this could advantageously replace patients' fibroblast cultures.

Assessments
The second part of cells harvested from the olfactory epithelium will be cultured, according to protocol described in chapter 3.2.

Endpoint #3: To evaluate whether patients with telomeropathies have impaired olfactory function.

Rationale
Olfactory function is decreased in several diseases and is increasingly recognized as an indicator of biological aging. To date, no data exist regarding the impact of telomeropathies on olfactory function. Therefore, we aim to psychophysically assess olfactory function in patients with telomeropathies, in comparison to age-matched healthy controls. This study will be perfomed in a subset of subjects (see section 7.1).

Assessment
For each participant older than 6 years old, olfactory function will be assessed with the validated test.

3. EXPERIMENTAL PROCEDURES AND DATA ACQUISITION

3.1. BLOOD SAMPLES

In patients with suspicion of telomeropathy, Flow-FISH analyses are already used for the diagnosis in routine. These standard of care procedures will therefore not be associated with any additional cost.
Blood tests will be performed by nurses at the blood collection center of the CUSL. One EDTA tube of 7.5 ml is sufficient for Flow-FISH. Blood tests will be performed while the patient is at the clinic and will not require a supplementary consultation.
We will measure leukocyte telomere length using a validated Flow-FISH technique, developed by Prof A. Decottignies (UCL), and available at CUSL.
Blood sample for Flow-Fish analysis is performed as standard of care procedure for the patients involved in the study. Therefore, these analyses will not be associated to additional costs, neither in term of material of personal.
A Figure is enclosed to this document to summarize experimental procedures and samples flow.
Residual material will be stored in the UCLouvain biobank, at Prof. A. Decottignies' lab. They will be kept for 10 years and then will be destroyed.

3.2. NASAL BRUSHINGS

The nasal brushings will be performed by Prof. C. Huart or Prof. Valérie Hox, trained ENT specialists, during their scientific time. For patients and controls recruited among patients from the clinic, nasal brushings will be performed while these subjects are at the clinic for their standard cares. For controls recruited outside the clinic, the nasal brushing will require a specific study consultation.
A local vasoconstrictor (Xylometazolin, 1mg/ml) will be applied at the level of the head of the inferior turbinate with the use of a nasal tampon to facilitate the visibility and the access to the olfactory cleft. Each nostril will be assessed, using a 30° rigid endoscope of 4mm (Karl Storz®, Tuttlingen, Germany), in order to identify the nostril having the most accessible olfactory cleft. This nostril will be chosen to receive the brushing. A sterile disposable cytology brush (i.e. FLOQSwabs, Copan®, Italy) will be gently rolled on the mucosal surface, at the level of the head of the middle turbinate, as described previously (Orrù et al., 2014). Two brushings will be done for each subject. The brush will then be immerged in a conical centrifuge tube containing DMEM-F12 medium supplemented with GlutaMAX. Cellular material will then be dissociated from the brush using disposable micropipette tips.
In the patients group, cellular material will be split into three parts for specific processing. A first part will be cultured (Prof A. Decottignies' lab, de Duve Institute) (Endpoint #2), a second one will be processed by Cytospin for future stainings (Endpoint #1) (Prof A. Decottignies' lab, de Duve Institute) and the last part will be kept frozen and further used for whole exome sequencing (Endpoint #1), if the suspicion of telomeropathy has been confirmed. This latter step will be performed at the laboratory of genetics of CUSL (Prof. A de Leener)
In the control group, cellular material will be exclusively used for the staining evaluating the degree of senescence and damages to telomeres.
A Figure is enclosed to this document to summarize experimental procedures and samples flow.

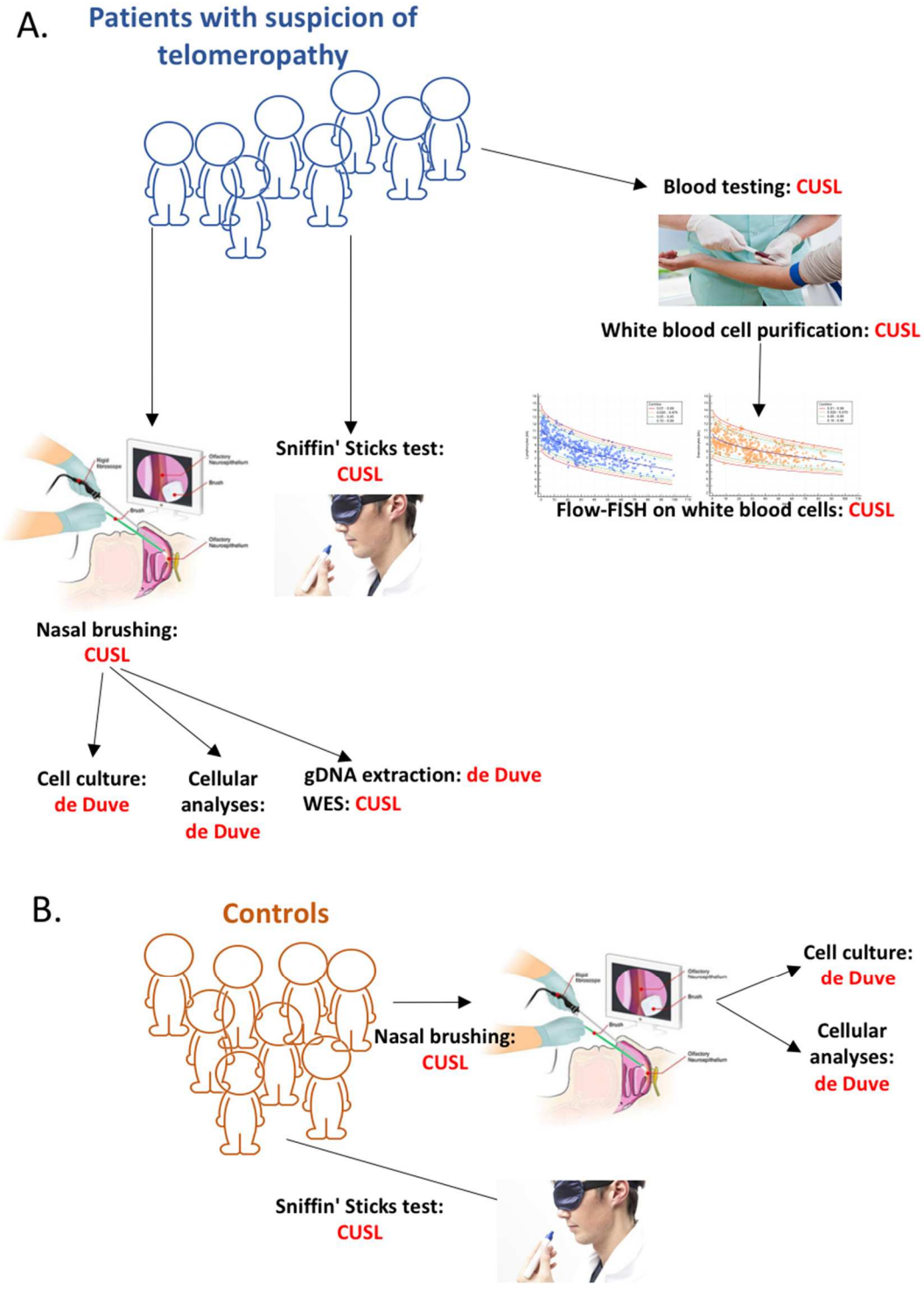

Figure summarizing experimental procedures and samples flow in patients (A) and controls (B)

3.3. SNIFFIN' STICKS TEST

Olfactory function will be evaluated in the department of Otorhinolaryngology of CUSL (Prof. C. Huart).
For patients and controls recruited among patients from the clinic, olfactory tests will be performed while these subjects are at the clinic for their standard cares. For controls recruited outside the clinic, olfactory testing will require a specific study consultation (at the same time than the nasal brushing).
For adult participants, we will use the Sniffin' Sticks test which is a validated psychophysical testing method (Hummel et al. 2007). Sniffin' Sticks test is based on pen-like odor dispensing devices that will be presented to the patients. The extended version of this test will be used and consists of three tests of olfactory function, namely tests for odor threshold, odor discrimination and odor identification. Odor threshold for n-butanol will be assessed using a single-staircase, three alternative forced choice procedure. Odor discrimination will be assessed using again a three alternative forced choice procedure. Triplets of pens will be presented to the patients, with two containing the same odorant and one a different odorant. Odor identification will be assessed for 16 common odors using a multiple-choice identification from lists of four descriptors each. In order to quantify olfactory function, results from the three subtests will be presented as a composite "TDI score" (/48) which is the sum of the results obtained for each subtest: threshold (/16), discrimination (/16), identification (/16). TDI score will be used to classify patients in three categories: normosmic, hyposmic or anosmic.
For the pediatric population, we will use an adapted version of the Sniffin' Sticks test, the Sniffin' Kids test. This test is a 14-item odor identification test and has been validated in children between 6-17 years of age (Schriever et al. 2014). Therefore, considering Endpoint #3, olfactory function will be assessed only in participants >6 years old. Olfactory function will be assessed during the hospitalization, before the general anesthesia.

4. RECRUITMENT OF PARTICIPANTS

Patients with a suspicion of telomeropathy (i.e. idiopathic pulmonary fibrosis, medullar aplasia, myelodysplasia, unexplained liver cirrhosis, unexplained liver regenerative nodular hyperplasia) will be recruited through pneumology (Prof. A. Froidure), adult hematology (Prof. X. Poiré), pediatric hematology (Prof. B. Brichard) and adult hepathology (Prof. B. Delire) consultations.

Adult healthy controls will be recruited through the consultation of otorhinolaryngology (Prof. C. Huart, Prof. V. Hox) or through advertising. Notably, pediatric controls will be recruited from children patients planned for elective otorhinolaryngologic surgery under general anesthesia.

The physician in charge of the patients will briefly outline the objectives of the study to the potential participants and/or their parents. With their permission, the potential participants will then be contacted by one of the investigators who will provide detailed explanation of the project's objectives. If interested, the patients and/or their parents will receive a letter of invitation and information sheet will be given to the putative participants. They will be free to contact us

should they require further clarification or information concerning inclusion and exclusion criteria. It will be clearly explained that accepting or refusing to participate will have absolutely no impact on present or future care. Subject and/or their parents will benefit from at least 24h and enough time to agree to participate or not to the study.

If the subject and/or his parents agree to participate to the study, an appointment will be fixed, if possible, on the same day that a routine medical visit to the CUSL. Written informed consent will be collected that day by the principal investigator or one of the medical co-investigator. After giving their written informed consent, subjects will be enrolled in the study. The informed consent sheet will list the specific experimental procedures and tests required to conduct the study, as well as the maximum duration of the experiment/testing.

5. INFORMED CONSENT

Before any screening or study-specific procedure is performed, the subject will be asked to sign and date the subject information sheet and accompanying informed consent form. Importantly, since the present study involves genetic testing in patients, patients will be explicitly informed that they are able to participate to the study although refusing to take part to the genetic testing. A specific informed consent form for genetic testing will be signed as well. The informed consent will also mention if volunteers will have access or not to their own results. Specifically, for genetic analysis, we will ask the patients whether they want to be informed about the results or not.
For minor subjects, both parents will be asked to sign and date a specific information sheet and accompanying informed consent. Pediatric patients older than 6 years will also be asked whether they agree to participate to this study. Pediatric patients will receive adapted information, both through oral explanations and through age-adapted informed consent.
Therefore, we will propose specific informed consents to (1) adult patients, (2) adult controls, (3) patients' parents, (4) controls' parents, (5) 6-11 years old patients, (6) 6-11 years old controls, (7) 12-14 years old patients, (8) 12-14 years old controls, (9) 15-17 years old patients, (10) 15-17 years old controls.

6. PRE-STUDY SCREENING

After signing the informed contents, investigators will screen the subject to ensure the eligibility of the subject.

Demographics. The date of birth and gender of the subject will be recorded.

Medical and surgical history. Subjects will be asked whether or not they suffer from any known disease, notably from sinonasal disorders, psychiatric or neurological disease, history of olfactory dysfunction (i.e. following upper respiratory tract infection, head trauma). We will also record medical and surgical ENT history. Additional questions will be asked to ensure correct screening for inclusion and exclusion criteria, as detailed in section 7.

Physical Examination. Subjects will undergo a rhinological endoscopic examination to assess olfactory cleft, notably to rule out any skull base abnormality such as meningocele or abnormalities that may lead to bleeding.

At the end of the screening visit, and taking into consideration the inclusion and exclusion criteria, the eligibility of the subject will be decided.

7. SELECTION AND WITHDRAWAL OF VOLUNTEERS

7.1. Inclusion and exclusion criteria

Endpoints #1 and #2

Study population		Inclusion criteria	Exclusion criteria
Adults (>18 years)	Patients	- Having a suspicion of or a confirmed telomeropathy - Ability to provide written informed consent	- No access to the olfactory cleft - Abnormal endoscopic finding (i.e. meningocele, vascular ectasia)
	Controls	- Ability to provide written informed consent	- No access to the olfactory cleft - Abnormal endoscopic finding (i.e. meningocele, vascular ectasia)
Children (<18 years)	Patients	- Written informed consent obtained from the parents and participant if she/he is older than 6 years old	- No access to the olfactory cleft - Abnormal endoscopic finding (i.e. meningocele, vascular ectasia)

	Controls	- Written informed consent obtained from the parents and participant if she/he is older than 6 years old	- No access to the olfactory cleft - Abnormal endoscopic finding (i.e. meningocele, vascular ectasia)

Endpoint #3

Study population		Inclusion criteria	Exclusion criteria
Adults	Patients	- Having a suspicion of or a confirmed telomeropathy - Ability to provide written informed consent	- History of neurological or psychiatric disorder known to interfere with olfactory function - History of olfactory trouble (postinfectious, posttraumatic, toxic) or chronic rhinosinusitis
	Controls	- Ability to provide written informed consent	- History of neurological or psychiatric disorder known to interfere with olfactory function - History of olfactory trouble (postinfectious, posttraumatic, toxic) or chronic rhinosinusitis
Children	Patients	- Age: 6-18 - Written informed consent obtained from the parents and participant	- History of neurological or psychiatric disorder known to interfere with olfactory function - History of olfactory trouble (postinfectious,

			posttraumatic, toxic) or chronic rhinosinusitis
	Controls	- Age: 6-18 - Written informed consent obtained from the parents and participant	- History of neurological or psychiatric disorder known to interfere with olfactory function - History of olfactory trouble (postinfectious, posttraumatic, toxic) or chronic rhinosinusitis

7.2. Discontinuation criteria

The subject may be excluded for the reasons given in section 7.1 of this protocol. The excluded subjects will be replaced.
Participants and/or their parents will be informed of the fact that they have the right to withdraw from the experiment at any time and for any reason, without prejudice, and without being obliged to give the reasons for doing so.
In the interest of the subject's health and well-being, the investigator may withdraw the subject at any time. In addition, the subject may be withdrawn for any of the following reasons: administrative decision by the investigator, ineligibility, subject non-compliance with study requirements, and occurrence of any adverse event which results in the inability to continue to comply with study procedures.
The reason for withdrawal will be recorded. If the subject is withdrawn due to an adverse event, the investigator will arrange for follow-up visits or telephone calls until the adverse event has resolved or stabilized.

8. SAFETY

8.1. Adverse event

An adverse event is defined here as a harmful and unintended occurrence in a subject participating to the study. All adverse events will be reported from the time a signed and dated informed consent form is obtained, until completion of the last study-related procedure.

8.2. Serious adverse events

A serious adverse event is an unexpected occurrence that (1) results in death, (2) is life-threatening, (3) requires hospitalization or prolongation of existing hospitalization or (4) results in persistent or significant disability or incapacity.

Given this research project consists of blood sample and collection of cells originating from the olfactory epithelium through non-invasive nasal brushing, we do not expect any prolonged or serious adverse event.

8.3. Potential adverse events

The blood sample may be perceived as slightly painful by the patient (however this feeling is temporary) and may result in a cutaneous hematoma.
The nasal brushing of the olfactory cleft may be perceived as uncomfortable or slightly painful by the patient. However, this feeling is temporary. No adverse effect has been reported in the literature.
Similarly to naso-pharyngeal swabs, it is possible that a slight bleeding occurs in some subjects with weakened mucosa. The risk of severe bleeding is extremely rare. A recent retrospective study investigating the risk of naso-pharyngeal swabs found a rate of 8 complications (4 bleeding / 4 foreign body) out of a total of 643.284 patients (Koskinen et al. 2021). These complications were related to incorrect swabbing technique. Moreover, 1 study report CSF leak after naso-pharyngeal swab, occurring in a patient with meningo-encephalocele (Sullivan et al. 2020). Because of the rarity of such skull base malformation and the fact that a nasal endoscopy will systematically be performed, we can assume that the risk of CSF leak is negligeable. Importantly, brushing will be performed by trained ENT specialists, ensuring a as atraumatic as possible brushing; and patients will be screened for factors that may predispose to severe bleeding. Moreover, if a bleeding occurs, adequate medical intervention will be ensured.
Additionally, one study demonstrated the safety of biopsy of olfactory neuroepithelium for the olfactory function (Andrews et al. 2016). Therefore, we can assume that the risk of olfactory dysfunction following a poorly invasive nasal brushing of the olfactory cleft is extremely low, especially since the procedure will be performed unilaterally. However, in the very unlikely event that a subject complains of olfactory dysfunction, specific medical measures will be taken.
Importantly, to avoid the discomfort due to blood sample and nasal brushing in pediatric population, these samplings will be performed during a general anesthesia that is planned for the routine medical management of these participants (eg. telomeropathy patients: marrow puncture; pediatric controls: tonsillectomy).

8.4. Reporting procedures for all adverse events

All adverse events occurring during the study observed by the investigator or reported by the subject, whether or not attributed to the study, will be recorded. The following information will be assigned by the investigator: description, date of onset and resolution, severity, assessment of relatedness to the experiment, and action taken. The investigator may be asked to provide follow-up information. All related adverse events that result in a subject's withdrawal from the experiment will be followed up until a satisfactorily resolution occurs.

8.5. Serious adverse event reporting procedures

A serious adverse event occurring to a research participant will be reported to the Ethics Committee that gave a favorable opinion of the study. Reports of related and unexpected serious adverse events will be submitted within 15 days after becoming aware of the event.
All serious adverse events that have not resolved by the end of the study, or that have not resolved upon discontinuation of the subject's participation in the study will be followed until any of the following occurs: (1) the event resolves, (2) the event stabilizes, (3) the event returns to baseline, if a baseline value is available, (4) the event can be attributed to agents other than the experiment, (5) when it becomes unlikely that any additional information can be obtained (subject or healthcare practitioner refusal to provide additional information, lost to follow-up after demonstration of due diligence with follow-up efforts).
Any event requiring hospitalization that occurs during the course of a subject's participation in a clinical study will be reported as a serious adverse event except hospitalizations for (1) social reasons in absence of an adverse event, (2) surgery or procedures planned before entry into the study, (3) clinic protocol procedures.
All of the medical investigators of this project are medical practitioners registered to l'Ordre des Médecins/Orde der Artsen. It will be left to their judgment to decide whether or not an adverse event is of sufficient severity to require the subject's removal from the study. A subject may also voluntarily withdraw from the study due to what he or she perceives as an intolerable adverse event.

9. DATA ANALYSIS

Blood samples collected at CUSL will be analyzed at the laboratory of the Cliniques universitaires Saint-Luc, where leukocyte telomere length will be analyzed using a validated Flow-FISH technique (Prof. P. Saussoy, head of biologogical hematology department), developed by Prof A. Decottignies (UCLouvain).

Cellular material harvested through the nasal brushing will be processed at the laboratory of Prof. A. Decottignies (UCLouvain) and at the laboratory of Genetics of CUSL (Prof. A. De Leener).

10. DIRECT ACCESS TO SOURCE DATA/DOCUMENTS

The investigator will allow trial-related monitoring, audits, and regulatory inspections, and provide direct access to source data and documents to authorized auditors and monitors.

11. ETHICS

11.1. Declaration of Helsinki

The investigator will ensure that the study is conducted in full conformity with the current revision of the Declaration of Helsinki (last amended October 2000, with additional footnotes added in 2002 and 2004).

11.2. Informed consent

A written version of informed consent form will be presented to the subject and /or her/his parents detailing: the exact nature of the experiment, the implications and constraints of the protocol, the known side effects, and any risks involved in taking part. It will be clearly stated that the subject is free to withdraw from the study at any time for any reason without prejudice to future care, and with no obligation to give the reason for withdrawal.
The subject and/or her/his parents will be allowed as much time as wished to consider the information, and the opportunity to question any or all investigators listed in the ethics application, their general practitioner or other independent parties to decide whether they will participate in the study.
Written Informed Consent will then be obtained by means of subject's dated signature and/or parent's dated signature, signature of the person who presented the informed consent and, if different, the principal investigator.
A copy of the signed Informed Consent will be given to the subject. The original signed form will be retained at the study site.

11.3. Independent ethics committee

A copy of the protocol, the informed consent form, subject information sheet, and any proposed advertising material will be submitted to an Independent Ethics Committee for approval.
The principal investigator will submit and, when necessary, obtain approval from the Committee for all subsequent protocol amendments and changes to the informed consent document.
The investigator will notify substantial amendments to the protocol and will notify the Committee of these in accordance with local procedures.

11.4. Subject confidentiality

The investigator will ensure that the subject's anonymity is maintained. The subject will be identified by a subject ID number. As described in section 14, all documents will be stored securely and kept in strict confidence.

12. DATA HANDLING AND RECORD KEEPING

All the data will be pseudonymized thanks to the use of a specific identification code. The subject will be identified through this identification code, which will depend on the study, on the group of subjects and on the inclusion order. Pseudonymized data will be recorded in a protected RedCap database.
Data protection will be ensured by the law of the July 30, 2018 relating to the protection of privacy and by Belgian and European regulations (European General regulation on the protection of personal data (GRPD) of May 25, 2018). These rights will also be guaranteed by the law of April 22, 2002 relating to the rights of the patients.

13. FINANCING AND INSURANCE

The study will be funded by grants obtained by Prof. A. Decottignies and Prof. C. Huart.
Blood sample analysis and genetic analysis (whole-exome sequencing) will only be performed in patients. For patients involved in the current study, Flow-Fish analysis is already performed in clinical routine as standard of care procedure. Similarly, genetic analysis on cellular material is considered as a standard of care for patients with suspicion of telomeropathy. Therefore, these analyses will not be associated to additional costs, neither in term of material of personal.
Indemnity for negligent and non-negligent harm will be provided by a specific insurance provided by the Cliniques universitaires Saint-Luc.
Company:
Police number:

14. PUBLICATION POLICY

The Principal Investigator will coordinate dissemination of data from this study in the form of publications in peer reviewed scientific journals, conference presentations, and access to raw data. In addition, research participants who are interested in directly acquiring detailed information will be encouraged to contact a named investigator listed on this application.

15. BIOBANKING

After analysis of telomere length in blood at CUSL, human samples (blood and cells) will be kept frozen at UCLouvain (de Duve Institute, room 75.01.5817).
Samples will be kept for a maximal duration of 10 years, and will then be destroyed.
Secondary use of human body material: Patients are informed that if, within the course of this study, we want to do additional analyses, not included in the present protocol, on the remaining material, this will be done after requesting the opinion of the ethical committee.

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