Universitat de Girona
Facultat de Medicina

INTESTINAL MICROBIOTA IN PATIENTS WITH MULTIPLE SCLEROSIS

A pilot case-control study

AUTHOR: Esther Cilveti Gómez

TUTOR: Lluís Ramió i Torrentà

FINAL GRADE PROJECT 2015

Neuroimmunology and Multiple Sclerosis Unit

Dr. Josep Trueta University Hospital

INDEX

1. LIST OF ABBREBIATIONS

MS	Multiple Sclerosis
CNS	Central Nervous System
AI	Autoimmune
DIS	Dissemination in Space
DIT	Dissemination in Time
MRI	Magnetic imaging Ressonance
RRMS	Relapsing-Remiting Multiple Sclerosis
CIS	Clinically Isolated Syndrome
SPMS	Secondary-Progressive Multiple Sclerosis
PPMS	Primary-Progressive Multiple Sclerosis
IS	Immune System
IBD	Inflammatory Bowel Disease
GI	Gastrointestinal
DNA	Deoxyribonucleic Acid
EAE	Experimental Autoimmune Encephalomyelitis
EDSS	Expanded Disability Status Scale
HC	Healthy Control
UNIEM	Unitat de Neuroinmunologia i Esclerosis Múltiple
HUJT	Hospital Universitari Dr.Josep Trueta
CFA	Cuestionario de Frecuencia de Consumo Alimentario
BMI	Body Mass Index
CEIC	Comité Ètic i d'Investigació Clínica
IdiBGi	Institut d'investigació biomédica de Girona Dr Josep Trueta

2. ABSTRACT

Background: Multiple sclerosis is an autoimmune chronic demyelinating disease of the central nervous system. It is the main cause of non-traumatic disability in young adults and its incidence is increasing the last years. For these reasons, it is a major health problem. Nowadays, its aetiology and pathogenesis are not well known. There is a need of performing new studies in order to get a better understanding of the disease and also to improve the present treatments or to find out new therapeutical targets. In the last years, there has been an increasing interest about the role of the microbiota in some autoimmune disorders and specifically, in the regulation of central nervous system disorders.

Objective: The aim of this study is to analyse the composition of gut microbiota in patients diagnosed of multiple sclerosis and compare the results with healthy subjects in order to investigate if there is a specific faecal microbiota profile associated with this disease.

Study design: The design selected is a pilot case-control study.

Participants: Cases: Multiple Sclerosis patients diagnosed at the Neuroimmunology and Multiple Sclerosis Unit of Dr. Josep Trueta University Hospital. Controls: Healthy subjects selected from the census of Girona and age-sex matched in a 1:1 ratio with cases.

3. INTRODUCTION

3.1. Background

3.1.1. MULTIPLE SCLEROSIS

❖ **Epidemiology**

Multiple Sclerosis (MS) is the most common chronic demyelinating disease of the central nervous system (CNS), representing the main cause of non-traumatic disabling disorders in young adults (1).

According to 2013 data there are 2'3 million people affected of MS, with a global median prevalence of 33 cases for 100.000 inhabitants (2). In Spain, the prevalence vary from 32 to 125 cases for 100.000 inhabitants depending on the region where the studies were conducted, with a tendency to an increase in the incidence regarding most recent studies (3–5). MS is usually diagnosed in young adults aged between 25 and 35 years old. It affects more women than man, with a female-to-male ratio that varies from 2:1 to 3:1 according to different studies (1,2).

❖ **Risk Factors**

MS is a complex and multifactorial autoimmune (AI) disorder targeted at the CNS that causes progressive loss of myelin and neuronal and glia cell damage. Even though the precise aetiology remains largely unknown, it resemble improbable to be caused by a single causative agent. Studies have revealed that genetically susceptibility in conjunction with the exposure to certain

environmental factors could trigger a process of immune dysregulation that may lead to MS (1,6,7).

- Genetic factors: According to different data, genetics play a role in a person's susceptibility to MS, although the heritable component explains only a small part of the disease (8). Present studies suggest that HLADRB1*15:01 has the greatest evidence to be the main susceptibility allele form, notwithstanding it is hard to define specific genes and probably multiple haplotypes would be involved (9).

- Environmental factors: As stated above, heredity only explains the disease partially; hence it is believed that different environmental factors have to be involved to trigger the disease. The most important known environmental factors nowadays are: sunlight and ultraviolet radiation exposure/latitude, vitamin D3, tobacco and Epstein-Barr virus (EBV) infection (1,6,7,10).

❖ **Pathogenesis**

The pathogenesis of MS is thought to be an immune dysregulation involving unrevealed antigens located in the myelin sheaths that generates an inflammatory response into the CNS. This inflammation leads to demyelination and axonal dysfunction, which is widely believed to be responsible for the symptoms and progression of the irreversible disability (6,11,12). By analyzing MS pathology lesions in an active stage, four main patterns of demyelination and axonal injury were distinguished among patients, which turn out to indicate

that the pathogenic mechanisms of the disease are heterogeneous (13). Nonetheless, different evidences postulate that MS is an autoreactive T-cells mediated disease (12).

a) Inflammatory phase

Autoreactive CD4 T-cells are activated in peripheral blood by an unknown factor. Thereafter, they penetrate into the brain due to a disruption of the blood-brain barrier (BBB). Once there, these T-cells recognize target antigens shown by antigen-presenting cells (APC) and both form a complex, leading to a release of proinflamatory cytokines mainly composed by TNF-α and interferon-γ. Then an amplification of the inflammatory response occurs through the recruitment of the CD8 T-cells, machrophages and NK cells, together with the activation of the B cells that favors the production of auto-antibodies, responsible for tissue injury through complement activation. The course of the above process gives rise to oligodendropathy and, consequently, to a demyelination process (11,12,14).

b) Remyelination phase

It is understood that exists an ephemeral period where myelin is able to be re-synthesize through oligodendrocyte-precursor cells migration into the area where the inflammation has occurred, even though, the specific mechanism whereby remains unclear (14).

c) Axonal Injury

After multiple demyelination injuries, the capacity of remyelination diminishes and yields to internal cell processes that cause an activation of different factors that contribute to a cumulative axonal injury (14).

❖ **Diagnosis and Clinical Course**

The diagnosis of MS is based on 2010 McDonnald Criteria ***(Annex I)***, which encompasses mainly clinical criteria and laboratory assessments that should fulfil dissemination of lesions in time (DIT) and dissemination in space (DIS), besides ensuring the exclusion of alternative diagnosis (15). The main tools we can perform to reach a diagnosis comprise chiefly an accurate clinical history and magnetic imaging resonance (MRI). Even so, there exist other medical tests that can be useful in order to reach the diagnosis, such as evaluation of oligoclonal bands in cerebrospinal fluid and/or visual evoked potentials (11,15).

Clinically, the onset and course of the disease are highly variable and difficult to prognosticate at the beginning. The 85-90% of patients start with a relapsing-remitting multiple sclerosis form (RRMS), which consists on presentation of typical signs and symptoms, over at least 24 hours, in the absence of fever, infection or encephalopathy. After the relapsing episode, there is subsequent improvement of these symptoms, often with a complete remission, but unfortunately, over time, they could leave sequels. It is estimated that approximately 80% of patients with RRMS start with an acute focal clinical episode, affecting the optic nerve, the spinal cord or the brainstem, with

features suggestive of MS but isolated in time and also generally in space, which is called clinically isolated syndrome (CIS). Among 40-70% of patients diagnosed as CIS, would convert in the future to a RRMS form (12,16,17). After 10 years of progression, 50%-65% of the RRMS patients will develop a secondary-progressive multiple sclerosis (SPMS), consisting on accumulative progressive dysfunction of CNS between relapses. The remaining 10-15% of patients start from the beginning with a gradually progressive form of the disease without relapses that is called the primary-progressive multiple sclerosis form (PPMS) of the disease (11,16).

- **Management and treatment:**

The management of MS requires a multidisciplinary approach, involving professionals from different scopes with expertise in chronic neurological diseases. The first-line disease modifying drugs available are β-interferon and glatiramer acetate, which are indicated for patients with RRMS. They have shown a decrease of 30% of relapses during 2-3 years of follow up. Conversely, they have not demonstrated valuable effects on SPMS or PPMS forms, with the exception of those patients who suffer relapses despite having a SPMS form. The second-line disease modifying drugs are natalizumab and fingolimod. Due to their potential and serious adverse effects (progressive multifocal leukoencephalopathy and cardiotoxicity), its use is reserved in case of failure or intolerance to the first-line treatments. The patients with chronic impairments or sequels received treatments to ameliorate the symptoms in order to improve the quality of everyday life. Treatment with high-dose methyl-prednisolone during

relapsing periods could yield to a transient recovering of the symptoms during the relapses (11,16).

3.1.2. HUMAN MICROBIOTA

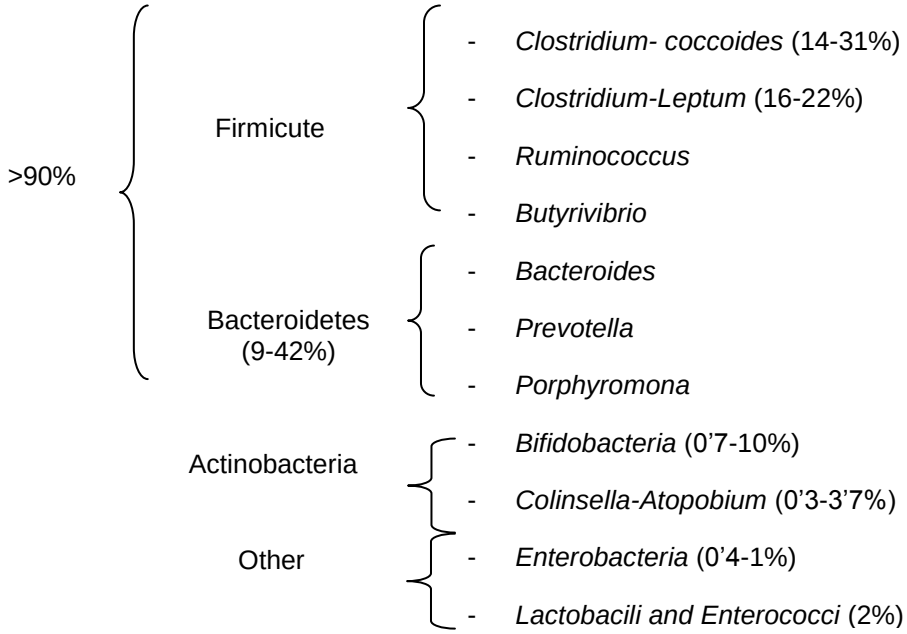
❖ **Prelude**

In MS unfortunately, as in many other AI diseases, the processes which are responsible of activation of autoaggresive cells in the peripheral blood are poorly understood, even though many hypotheses have been postulated. Over the last decade, there has been an increasing interest on the role in the AI disorders of microbial cells residing on the human body, known as microbiota. The aggregate genome that microbiota encodes are referred to as microbioma. The vast majority of this complex ecosystem lies into the intestinal tract, mainly in the colon, setting up the gut microbiota, composed of at least 10^4 microbial cells (18–21). A new approach, based on data that postulates the interaction of gut microbiota with the immune system (IS), has recently emerged and denotes the existence of imbalances on the faecal microbial profile in patients with AI or inflammatory diseases when they are compared with healthy controls. Furthermore, it is known that the normal gut microbiota balance state, called eubiosis, orchestrate essential functions related with human health and well being. Conversely, an unbalanced composition or abnormal activation of gut microbiota, defined as dysbiosis, has been strongly associated with many and varied pathological conditions, such allergic diseases, inflammatory bowel disease (IBD), cardiovascular diseases, metabolic disorders, cancer, diabetes, obesity or autism, etc.… (20–23).

❖ Development and composition of gut microbiota

It has been shown that the biggest changes in composition of microbiota occur during childhood and it has been hypothesized that during early life this changes could initiate and lead to the development of future diseases (24). After the birth, facultative anaerobic microbes colonize the newborn, showing initial differences depending on the delivery mode (caesarean or vaginal delivery). When milk feeding starts, the most important microbial specie turns to be *Bifidobacterium*. Afterwards, with the introduction of solid food intake, a conversion towards a more adult-like composition begins, with an increasing of *Bacteroides, Clostridium* and *Ruminococcus,* and a decrease of levels of *Bifidobacterium* and *Enterobacterium*. This process is not considered complete until the subjects reach 2'5-3 years old (20,24). Throughout adulthood the microbial community remains almost stable within healthy individuals over time, although marked inter-individual variations can occur. Due to this variability, there is much controversy in establishing what could be a healthy microbiota composition profile, even so, it has been possible to define a dominant human gut microbiota, called "core microbiota", composed mainly by 2 phylotypes: *Firmicutes* and *Bacteroidetes*, that represent the 90% of all species (18,21). During the elderly, there is a decrease of *Bifidobacterium*, *Lactobacillus*, *Eubacterium* and *Bacteroides,* while aerobe microbial increases. The causes of this variation are not certainly known, notwithstanding, it is thought that poly-drug intake could be the main implied factor (25).

Figure 1: The normal microbiota composition (18)**:**



Several factors have been determined to result in changes of microbial gut composition. The best known are host genetics, age, delivery mode, sedentary life style, diet shifts, improved sanitation, antibiotic use or disease, among many others (23). On the other hand, regardless of different microbiota composition profiles, a further important fact, is to understand its function, since despite the inter-individual variation, there is a shared functionality (21–23). In recent years, the Deoxyribonucleic Acid (DNA) sequencing technology to identify microbiota composition has improved considerably. With the scientific advance in the amplification process of the 16S ribosomal DNA from microbial cells, it is possible to define the phylotypes in faecal samples and obtaining the microbiome.

- **Relationship between microbiota and CNS**

Most microbial cells develop and establish a beneficial and homeosthatic relationship with their hosts. While gut microbiota allows nutrition, development of IS and prevention of enteropathogen's infection, the intestinal epithelium mucus of the host, supplies the bacterial cells with nutrients (22). Focusing on CNS, it has been described a symbiotic relationship between microbiota and the CNS, named as the *"gut-brain-axis"*, which is an integrative concept of the bi-directional neurohumoral interplay between CNS and the gastrointestinal (GI) system (26–28).

Due to the existence of this inter-communication and its importance on the regulation of normal functionality, in the recent years, some studies have been carried out to explore the role of microbioma in the regulation of CNS disorders, and specifically in MS, a few *"in-vitro"* studies have been conducted (29).

A current study performed in experimental autoimmune encephalomyelitis (EAE), the animal model of MS, has shown that the gut microbiota is necessary to trigger the immune processes driven by myelin-specific CD4 T-cells. In this study using $MOG_{92\text{-}106}$ T-cell antigen receptor in a transgenic mice, was observed that whereas 80% of mice developed spontaneously EAE in the following 3-8 months, astoundingly, germ-free mice were protected throughout their life, probably due to weaker Th17 and autoreactive B cell response. It was also seen that germ-free mice develop EAE in the 6-12 months after being re-colonized with commensal microbiota (30). One more study conducted on EAE has shown a less aggressive EAE outcome in mice under germ-free conditions

compared with colonized mice, probably due to the release of less proinflammatory cytokines and production of higher levels of regulatory T cells (31). Moreover, another study showed that oral administration of non-absorbing antibiotics 1 week before the sensitization of mice that were induced to develop EAE, changed the gut flora profile and also improved the progress of EAE in association with decreasing levels of released proinflammatory cytokines (32). A different study conducted on human has shown higher percentages of antibodies against GI antigens in patients with demyelinating diseases when they were compared to healthy controls (33).

Indeed, it remains clear that microbiota plays an essential role in the development of spontaneous EAE. Despite the fact that there are differences between EAE and MS, both share comparable pathogenesis cascade mechanisms. The main difference is that MS does not need an induced artificial sensitization to autoantigens but despite this, EAE is the main used experimental model for human MS, and is the animal model used for testing new drugs (34). For that reason, it would be logical to think that the microbiota influence could also occur somewhat similar in MS.

3.2. Justification

MS is increasingly becoming a major illness condition, especially in developed countries, since the last years the incidence increased and more people are affected by this chronic disorder. Notwithstanding the fact that over the last years a lot of progress has been achieved in the knowledge of MS disease, much remains to be discovered around this disease, since the causes and pathogenesis mechanisms have not been well established yet, and unfortunately, it remains very hard to understand and also to predict the different evolution and prognosis of patients. In addition, cost of MS, as a chronic disease affecting young people, is very high both for the health care system and for people who suffer it. MS is estimated to cause an "*annual loss of 0'28 quality-adjusted life-year per patient"* and *"a total mean annual cost of 33.465€ per patient"* (35). From this data, the 56% is due to informal care and indirect costs, whereas the direct health-care costs represent the 43%. Taking into account the both clinical and cognitive disabilities that MS cause, mainly in young people, the diagnosis should be done as soon as possible, in order to manage and treat it and try to delay the possible sequels and progression.

The interest of the gut microbiota implication on the IS maturation, the control of a balanced inflammatory response, and consequently, the relationship with different diseases, is increasing. Much research is focused on the demonstration that microbiota is also involved in CNS disorders. Based on this, it is understandable the interest in exploring the role of gut microbiota specifically in MS. A different composition of microbiota profile could be

detected on faecal samples in patients with MS compared to healthy individuals and this dysbiosis could become, in the future, used as a biomarker of prognosis or drug response in MS.

The main point of this protocol is the lack of studies performed in humans, since the available evidences are from EAE studies, the animal model of MS. For that reason, it is necessary to go further on this field and continue exploring this new approach in humans, characterizing changes in stool samples, in order to have data to future new studies, which may represent a better understanding of MS pathogenesis, or could lead, in the future, to explain the role of that dysbiosis in MS pathogenesis and, even, investigate if changing the gut microbial pathological composition could lead to an effective way to treat the disease.

Based on this data the aim of this study is to evaluate the microbial flora in patients with MS and see if there are differences when they are compared to population controls.

4. BIBLIOGRAPHY

1. Romero SO, Batlle J, Bonaventura I. Situación epidemiológica actual de la esclerosis múltiple: pertinencia y puesta en marcha de un registro poblacional de nuevos casos en Cataluña. Rev Neurol. 2010;50(10):623–33.

2. Multiple Sclerosis International Federation. Atlas of MS 2013 [Internet]. multiple sclerosis international federation. 2013 [cited 2014 Nov 19]. Available from: http://www.msif.org/wp-content/uploads/2014/09/Atlas-of-MS.pdf

3. Tola MA. Prevalence of multiple sclerosis in Valladolid,northern Spain. J Neurol. 1999;246:170–4.

4. Latorre MAP, López A, Errea JM. Prevalence of multiple sclerosis in the province of Teruel,Spain. J Neurol. 1997;244:182–5.

5. Fernández O, Fernández V, Guerrero M, León A, López-Madrona JC, Alonso A, et al. Multiple sclerosis prevalence in Malaga, Southern Spain estimated by the capture-recapture method. Mult Scler [Internet]. 2012 Mar [cited 2014 Nov 6];18(3):372–6. Available from: http://search.proquest.com/docview/925631395?accountid=15295

6. Ramagopalan S V, Dobson R, Meier UC, Giovannoni G. Multiple sclerosis: risk factors, prodromes, and potential causal pathways. Lancet Neurol [Internet]. http://www.thelancet.com/pdfs/journals/laneur/PIIS1474-4422%2810%2970094-6.pdf; 2010 Jul [cited 2014 Oct 15];9(7):727–39. Available from: http://ac.els-cdn.com/S1474442210700946/1-s2.0-S1474442210700946-main.pdf?_tid=8c8b34e8-9a8b-11e4-bb13-00000aab0f6b&acdnat=1421088667_0a077d74df112e851ada4b1bf8db538b

7. Milo R, Kahana E. Multiple sclerosis: geoepidemiology, genetics and the environment. Autoimmun Rev [Internet]. 2010 Mar [cited 2014 Sep 27];9(5):A387–94. Available from: http://ac.els-cdn.com/S1568997209001888/1-s2.0-S1568997209001888-main.pdf?_tid=a45ca4fe-9a8a-11e4-ada9-00000aab0f01&acdnat=1421088278_70987ae86b996831c90f0a8739a9a571

8. Carton H, Vlietinck R, Debruyne J, De Keyser J, D'Hooghe MB, Loos R, et al. Risks of multiple sclerosis in relatives of patients in Flanders, Belgium. J Neurol Neurosurg Psychiatry. 1997 Apr 1;62(4):329–33.

9. Hafler DA, Alastair Compston, F.Med.Sci. PD, Stephen Sawcer, M.B., Ch.B., Ph.D. E, S. Lander, Ph.D., Mark J. Daly PD, Philip L. De Jager, M.D., Ph.D. PIW, de Bakker, Ph.D., Stacey B. Gabriel PD, et al. Risk Alleles for Multiple Sclerosis Identified by a Genomewide Study - ProQuest. N Engl J Med [Internet]. 2007 [cited 2014 Dec 11];357:851–62. Available from: http://www.nejm.org/doi/pdf/10.1056/NEJMoa073493

10. Krementsov DN, Teuscher C. Environmental factors acting during development to influence MS risk: insights from animal studies. Mult Scler [Internet]. 2013 Nov [cited 2014 Nov 6];19(13):1684–9. Available from: http://search.proquest.com/docview/1450013027?accountid=15295

11. Noseworthy JH. Medical Progress: Multiple Sclerosis. N Engl J Med [Internet]. 2000 [cited 2014 Nov 4];343(September 28):938–53. Available from: http://search.proquest.com/docview/223954419/fulltextPDF?accountid=15295

12. Fernández Fernández F, Fernández V. Esclerosis multiple. Concepto. Etiopatogenia. Fisiopatología. Manifestaciones clínicas. Investigaciones paraclínicas. Diagnóstico. Historia natural. Med - Programa Form Médica Contin Acreditado. Elsevier; 2007 Apr;9(76):4867–77.

13. Lassmann H. Recent neuropathological findings in MS: implications for diagnosis and therapy. J Neurol. 2004 Sep;251 Suppl:IV2–5.

14. Frohman E, Racke M, Raine C. Multiple Sclerosis. The Plaque and Its Pathogenesis. N Engl J Med [Internet]. 2006;(354):942–55. Available from: http://search.proquest.com/docview/223938699/fulltextPDF?accountid=15295

15. Polman CH, Reingold SC, Banwell B, Clanet M, Cohen J, Filippi M, et al. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. Ann Neurol [Internet]. 2011 Feb [cited 2014 Jul 10];69(2):292–302. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3084507/pdf/ana0069-0292.pdf

16. Compston A, Coles A. Multiple sclerosis. Lancet [Internet]. 2008 Oct 25 [cited 2014 Jul 11];372(9648):1502–17. Available from: http://www.thelancet.com/pdfs/journals/lancet/PIIS0140-6736%2808%2961620-7.pdf

17. Miller DH, Chard DT, Ciccarelli O. Clinically isolated syndromes. Lancet Neurol [Internet]. 2012 Feb [cited 2014 Dec 10];11(2):157–69. Available from: http://www.thelancet.com/pdfs/journals/laneur/PIIS1474-4422%2811%2970274-5.pdf

18. Doré J, Corthier G. [The human intestinal microbiota]. Gastroentérologie Clin Biol [Internet]. 2010 Sep [cited 2014 Oct 9];34 Suppl 1:S7–15. Available from: http://ac.els-cdn.com/S0399832010700154/1-s2.0-S0399832010700154-main.pdf?_tid=089b8774-9a8f-11e4-97b2-00000aacb35d&acdnat=1421090164_08bd82fbe22b3085af9f9dcfba4f44de

19. Corthier G, Doré J. [A new era in gut research concerning interactions between microbiota and human health]. Gastroentérologie Clin Biol [Internet]. 2010 Sep [cited 2014 Oct 21];34 Suppl 1:S1–6. Available from: http://ac.els-cdn.com/S0399832010700142/1-s2.0-S0399832010700142-main.pdf?_tid=9eefb72c-9a8f-11e4-a272-00000aab0f26&acdnat=1421090416_f57e2e43df7a1ea7ddc46df402bd589a

20. Clemente JC, Ursell LK, Parfrey LW, Knight R. The impact of the gut microbiota on human health: an integrative view. Cell [Internet]. 2012 Mar 16 [cited 2014 Jul 9];148(6):1258–70. Available from: http://download.bioon.com.cn/view/upload/201301/29141412_8723.pdf

21. Robles Alonso V, Guarner F. Linking the gut microbiota to human health. Br J Nutr [Internet]. 2013 Jan [cited 2014 Dec 16];109 Suppl:S21–6. Available from: http://search.proquest.com/docview/1285158094/fulltextPDF?accountid=15295

22. Lewis GDW and JD. Analysis of the Human Gut Microbiome and Association With Diseaseblic Access. Clin Gastroenterol Hepatol [Internet]. 2013 [cited 2014 Dec 16];11(7):774–7. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3822013/pdf/nihms484854.pdf

23. Lozupone C a, Stombaugh JI, Gordon JI, Jansson JK, Knight R. Diversity, stability and resilience of the human gut microbiota. Nature [Internet]. 2012 Sep 13 [cited 2014 Jul 14];489(7415):220–30. Available from: http://www.nature.com/nature/journal/v489/n7415/pdf/nature11550.pdf

24. Arrieta M-C, Stiemsma LT, Amenyogbe N, Brown EM, Finlay B. The Intestinal Microbiome in Early Life: Health and Disease. Front Immunol [Internet]. 2014 Sep 5 [cited 2014 Sep 10];5(September):1–18. Available from: http://journal.frontiersin.org/Journal/10.3389/fimmu.2014.00427/full

25. Tiihonen K, Ouwehand AC, Rautonen N. Human intestinal microbiota and healthy ageing. Ageing Res Rev [Internet]. 2010 Apr [cited 2014 Sep 30];9(2):107–16. Available from: http://ac.els-cdn.com/S1568163709000725/1-s2.0-S1568163709000725-main.pdf?_tid=2c496220-9a91-11e4-bd2d-00000aab0f6b&acdnat=1421091083_2d65ec6aea2c52afe25fd6dd84272843

26. Wang Y, Kasper LH. The role of microbiome in central nervous system disorders. Brain Behav Immun [Internet]. Elsevier Inc.; 2014 May [cited 2014 Jul 14];38:1–12. Available from: http://ac.els-cdn.com/S0889159113006004/1-s2.0-S0889159113006004-main.pdf?_tid=126ec9ea-9a81-11e4-ad50-00000aacb362&acdnat=1421084168_dbd7f5b5338809a16226421739942dcb

27. Collins SM, Surette M, Bercik P. The interplay between the intestinal microbiota and the brain. Nat Rev Microbiol [Internet]. 2012 Nov [cited 2014 Nov 28];10(11):735–42. Available from: http://search.proquest.com/docview/1112379928?accountid=15295

28. Clarke G, Grenham S, Scully P, Fitzgerald P, Moloney RD, Shanahan F, et al. The microbiome-gut-brain axis during early life regulates the hippocampal serotonergic system in a sex-dependent manner. Mol Psychiatry [Internet]. 2013 Jun [cited 2014 Nov 28];18(6):666–73. Available from: http://spofscc.com/uploads/The_microbiome-gut-brain_axis_from_bowel_to_behavior_1_.pdf

29. Ochoa-Repáraz J, Mielcarz DW, Begum-Haque S, Kasper LH. Gut, bugs, and brain: role of commensal bacteria in the control of central nervous system disease. Ann Neurol [Internet]. 2011 Feb [cited 2014 Dec 8];69(2):240–7. Available from: http://onlinelibrary.wiley.com/doi/10.1002/ana.22344/pdf

30. Berer K, Mues M, Koutrolos M, Rasbi Z Al, Boziki M, Johner C, et al. Commensal microbiota and myelin autoantigen cooperate to trigger autoimmune demyelination. Nature [Internet]. 2011 Nov 24 [cited 2014 Oct 17];479(7374):538–41. Available from: http//www.nature.com/nature/journal/v479/n7374/pdf/nature10554.pdf

31. Lee YK, Menezes JS, Umesaki Y, Mazmanian SK. Proinflammatory T-cell responses to gut microbiota promote experimental autoimmune encephalomyelitis. Proc Natl Acad Sci U S A [Internet]. 2011 Mar 15 [cited 2014 Dec 20];108 Suppl:4615–22. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3063590/pdf/pnas.201000082.pdf

32. Yokote H, Miyake S, Croxford JL, Oki S, Mizusawa H, Yamamura T. NKT cell-dependent amelioration of a mouse model of multiple sclerosis by altering gut flora. Am J Pathol [Internet]. 2008 Dec [cited 2014 Dec 17];173(6):1714–23. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2626383/pdf/JPATH173001714.pdf

33. Banati M, Csecsei P, Koszegi E, Nielsen HH, Suto G, Bors L, et al. Antibody response against gastrointestinal antigens in demyelinating diseases of the central nervous system. Eur J Neurol [Internet]. 2013 Nov [cited 2014 Dec 18];20(11):1492–5. Available from: http://onlinelibrary.wiley.com/doi/10.1111/ene.12072/pdf

34. Constantinescu CS, Farooqi N, O'Brien K, Gran B. Experimental autoimmune encephalomyelitis (EAE) as a model for multiple sclerosis (MS). Br J Pharmacol [Internet]. 2011 Oct [cited 2014 Jul 14];164(4):1079–106. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3229753/pdf/bph0164-1079.pdf

35. Kobelt G, Berg J, Lindgren P, Izquierdo G, Sánchez-Soliño O, Pérez-Miranda J, et al. Costs and quality of life of multiple sclerosis in Spain. Eur J Health Econ. 2006 Sep;7 Suppl 2:S65–74.

5. HYPOTHESIS

Patients with MS have differences in the composition of their intestinal microbiota compared to healthy controls.

6. OBJECTIVES

6.1. Principal objective

To analyse the composition of gut microbiota in patients diagnosed of MS and assess whether there is a specific faecal microbiota profile associated with MS.

6.2. Secondary objective

To determine if there are differences in the composition of gut microbiota depending on the degree of neurological impairment of the disease, evaluated according to the Kurtzke Expanded Disability Status Scale (EDSS) ***(Annex II).***

7. METHODS

7.1. Study design

The study is designed as a pilot case-control study. Cases will be diagnosed of MS according to McDonnald 2010 criteria and will be identified and age-sex matched in a 1:1 ratio with healthy controls (HC) selected from the provincial census of Girona.

7.2. Study Population

The study population is composed of adult patients diagnosed with MS according to McDonnald 2010 criteria and followed in the Neuroimmunology and Multiple Sclerosis Unit (UNIEM) of Dr. Josep Trueta University Hospital (HUJT) in Girona.

7.3. Inclusion and Exclusion Criteria

Inclusion Criteria	Exclusion Criteria
Patients diagnosed of MS according to McDonnald criteria 2010 (cases) Participants not diagnosed of any disease of the CNS (controls)	Individuals who are in treatment with immunosuppressive drugs (cases and controls)
Individuals aged between 18-65 years old (cases and controls)	Individuals who have been treated with antibiotics during the last month (cases and controls)

Individuals who are able to cooperate and agree to participate in the study by signing the consent form (cases and controls).	Individuals who suffer from IBD or diabetes mellitus I or II (cases and controls)

7.4. Sample and sampling

7.4.1. SAMPLING

Cases will be selected once they are diagnosed and followed by UNIEM at HUJT, using a consecutive non-probabilistic sampling of patients. All patients that meet inclusion and exclusion criteria will be informed, using the information sheet ***(Annex III)***, about the aim of the study, as well as the necessary additional tests (microbiological test of stool) to which it should be submitting, and they will be asked to take part into the study. If the patient agrees, the principal investigator will give the consent form to be signed ***(Annex IV)***.

The controls will be selected using a consecutive non-probabilistic volunteer sampling from the provincial census of Girona.

7.4.2. SAMPLE SIZE

As this study is a first phase aimed to demonstrate association but not causality, it is considered that twenty patients (n=20) should be enough to this exploratory study. As the study is aimed to match cases with controls, twenty healthy individuals (n=20) from similar age-sex than patients participating in the study will be selected. The final total sample size will be forty individuals (n=40). If the

sample sized proposed were sufficient to demonstrate association between faecal microbial composition and MS, a second phase analysis will be done aimed to determine whether a causal relationship exists. If the association is not well established, an extended sample size will be collected.

7.5. Variables and data collection

In this study protocol, causality will not be possible to be demonstrated; only association information would be obtained. To that end, it is not feasible to define the variables as independent and dependent due to the lack of temporal sequence, and so, the variables will be designated as variable A and variable B instead of independent and dependent.

7.5.1. VARIABLE OF STUDY A: Diagnosis of MS

The diagnosis will be performed by a neurologist at UNIEM according to 2010 McDonnald Criteria. A meticulous neurological exam will be performed in order to obtain EDSS score. The data related with the diagnosis will be expressed in the form of a qualitative dichotomous variable (Yes or Not). EDSS score will be presented as a quantitative discrete variable (from 1 to 10).

7.5.2. VARIABLE OF STUDY B: Gut microbiological profile on faeces by stool samples

To obtain this data, a sequencing of 16S-rDNA from the bacteria obtained in stool samples will be conducted. Participants themselves will

collect their own stool samples in containers for stool samples that will be given to them by the nursing team. Patients will bring their samples at the UNIEM in HUJT. The samples will be labelled with a code of numbers from 1 to 40 before being stored for their correct conservation inside an -80ºC freezer. The DNA extraction of the stool samples will be performed with Nucleospin® Soil Kit (Macherey-Nagel GmbH&Co) according to the instructions recommended by the manufacturer. The DNA concentration, will be quantified by Qubit®BR assay Kit in the Fluorometer Qubit® 2.0. Once the DNA will be extracted, 16S rDNA will be amplified by Polymerase Chain Reaction using universal bacterial 27F (5'-AGA GTT TGA TCM TGG CTC AG-3') and 1492R (5'-CGC TTA CCT TGT TAC GAC TT-3') with thermocycler GeneAmp ® 2700 Applied Biosystems. The results, will be visualized by electrophoresis on agarose gels. The bacterial genetic profile will be obtained by a massive sequencing of the sample done by next-generation sequencing (NGS) using Illumina MiSeq® System MySeq Desktop Sequencer. By doing this analysis the most prevalent bacteria could be sequenced and consequently quantified, leading to the identification of differences among patients and controls. The data result will be presented as a quantitative continuous variable form represented in relative numeric indexes.

7.5.3. CO-VARIABLES

These co-variables have been demonstrated to be associated with possible changes in gut microbial composition and for that reason they

will be collected to avoid confusion and to be able to obtain interpretable results. Some of these variables (gender, sex, ethnicity and other AI diseases) will be obtained by the clinical history in the cases group and by the data collection sheet in the controls group ***(Annex VI)***. The other co-variables (diet and body mass index) will be obtained by qualified personnel.

- **Gender:** The gender will be presented as a qualitative dichotomous variable (male or female)

- **Age:** The age measured in years will be expressed as a quantitative discrete variable.

- **Ethnicity:** Defined as the human community with similar physical characteristics, genetic features, common history, language and cultural traits. The ethnicity will be presented as a qualitative nominal variable with 5 categories: Caucasian, Asian, Black African, Maghreb or Others.

- **Diet:** An expertise nutritionist will conduct the collection of diet information. Individual interview about usual long-term diet will be performed using a *"Cuestionario de Frecuencia de consumo de alimentos" (CFA)* ***(Annex VII)***. The nutritional information obtained will be analysed with a computer-based-nutritional assessment program ("Nutritionist Pro Diet Analysis"). This program allows to obtain the average of total kilocalories (Kcal) and the grams of

macronutrients and micronutrients consumed every day. With this information, a conversion from grams to Kcal will be performed (1 gram of carbohydrates, proteins or fibre is 4 Kcal and 1 gram of fat is 9 Kcal). The macro-nutrients of the same food groups will be clustered by Euclidean distance as carbohydrates, fats, proteins and fibre. Therefore, the participants will be categorized depending on the predominant macronutrient in their diet. In case of any macronutrient increase equal or greater than 10% of the expected proportion for a balanced diet according to *"Sociedad Española de Nutrición",* the participant will be considered of having a high predominant diet on that macronutrient. On account of that, this variable will be expressed as a qualitative nominal variable with 5 predominant nutrient categories: balance diet, high fat diet, high fibre diet, high carbohydrate diet or high protein diet.

- **Body Mass Index (BMI):** Defined as the weight measured in kilograms divided by the square of the height measured in meters (kg/m^2). The patient must be weighed without shoes and underwear, on 100 g precision balance. The result will be presented in the form of a qualitative ordinal variable, thereby, patients will be classified as: underweight (<18'50), normal weight (18'50-24'99), overweight (25'00-29'99) or obese (≥30'00).

- **Other AI diseases:** The presence of other AI diseases (hipothyroidism, hiperthyroidism, systemic lupus erythematosus,

psoriasis and rheumatoid arthritis) will be presented in the form of a qualitative dichotomous variable (Yes or Not).

8. STATISTICAL ANALYSIS

As it is stated above, in this study it is not attainable to define independent or dependent variables. On the other hand, in order to perform the appropriate statistical analysis it is necessary to define the variables as independent or dependent, so it is, that microbiota composition profile in the stool samples of the two studied groups will be considered as our independent variable, and the presence of MS disease will be considered as our dependent variable.

8.1. Descriptive analysis

The input data will be expressed as a quantitative continuous variable (number of bacteria present in each phylotipe group), and the median will be obtained, as it is not expected to follow a normal distribution. For the principal objective, the outcome variable will be expressed as a categorical dichotomous variable (presence or absence of MS diagnosis) and will be measured as percentages. For the secondary objective, the outcome variable will be expressed as a quantitative discrete variable (EDSS score from 1 to 10) and will be measured as means +/- standard deviation (SD).

8.2. Bivariate analysis

For the principal objective, to compare the bacterial phylotipes among cases and controls, the qualitative variable expressed in percentages will be represented on a frequency table and a Chi- Square test will be performed. To analyse the quantitative variable (nº of bacteria in each phylotipe) a Wilcoxon test will be used, as they follow a non-normal distribution.

For the secondary objective, to determine if there are differences depending on the disability degree, the quantitative continuous variable (nº of bacteria in each phylotipe) and the quantitative discrete variable (EDSS score from 1 to 10) will be analysed using a Pearson Correlation test.

8.3. Multivariate analysis

To adjust our variables for co-variables in order to avoid potential confounders and obtain interpretable results from the study, a multinomial logistic regression analysis will be performed to analyse the principal objective, whereas for the secondary objective a multiple linear regression model will be used.

All the statistical analysis of the variables will be performed using the Statistical Package for the Social Sciences programme (SPSS) 19.0.

9. FEASIBILITY

9.1. Work plan

The study will be achieved in 1 year and 6 months and the distribution of the phases will be as following:

1. **Preparation of the study:** The management committee, composed by two researchers, will conduct this first phase of the study, which include the drafting of the definitive protocol, the coordination and training of all the research team and the ethic committee evaluation and further approval.

2. **Data Collection and preservation:** Patients will be proposed to take part in the study by the neurologists involved in the study. Subsequently, they will be distributed into the case group taking the personal and disease information from their clinical history. The controls will be selected and age-sex matched from the census of Girona through telephone calls made by an administrative. The neurologists will inform personally about all the procedures to both group of participants. Afterwards, each participant will be given the information sheet, the inform consent and a stool sample container by the nursing team involved in the study. The participants will have to bring the informed consent signed as well as their sample container to UNIEM, where the researchers will label all the obtained samples with a code of numbers from 1 to 40 before sending them to the laboratory in order to storage

them under -80ºC. If possible, the same day, the diet interviews will be performed by an expertise dietician and a data collection sheet will be given to the control group in order to fill up their clinical and personal information. Once all the samples are collected they will be sent to the Microbiological department located in "*Scientific and Technological Park of the UdG"* where the sample processing will be performed as explained above.

3. **Data Evaluation:** After processing the stool samples, the phylogenetic profile composition will be obtained and will be quantified for each group and also for the EDSS score in the cases group.

4. **Statistical analysis, publication and dissemination:** The results obtained will be analysed by the steering committee and a statistician. The managing researchers will write a final report and will disseminate it in national and international congresses and different written publications.

9.2. Chronogram

TASKS	TIME																					
	Phase 0			1rst YEAR of study													2nd YEAR of study					
	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A
1. Preparation of the study:																						
Elaboration of the protocol																						
Ethic committee evaluation and approval																						
Coordination and training of the members of the team																						
2. Data collection:																						
Cases and control recruitment																						
Sample collection																						
Sample processing - Setting up of PCR - DNA extraction and amplification																						
Data evaluation:																						
Analysis of the phylogenetic microbiological profile (NGS)																						
Quantification (qPCR)																						
3. Statistical analysis, publication and dissemination:																						
Statistical analysis and interpretation of the results																						
Final report																						
Dissemination of the results																						

10. ETHICAL CONSIDERATIONS

This study will be conducted according to the ethical principles established by World Medical Association in the "*Declaration of Helsinki of Ethical Principles for Medical Research Involving Human Subjects"* (last revised in October 2013). The research protocol will be presented to the *Clinical Research Ethical Committee* (*CEIC*, "*Comitè Ètic d'Investigació Clínica"*) at HUJT to be evaluated.

The confidentiality of personal and clinical information of all participants involved in the study will be guaranteed, according to the "*Ley Orgánica 15/1999 de Protección de Datos de Carácter Personal".* All the information will be only used for the purpose of the research. Patients will always be allowed to modify or destruct any of their collected data study.

According to the *"Ley 41/2002 Básica reguladora de la autonomía del paciente y de derechos y obligaciones en materia de información y documentación clínica" a*ll participants interested on being part of the study, will be asked to sign voluntarily the informed consent. Before being included in it, they will receive all the appropriate information about the study through a personal conversation with the researchers and using the information sheet.

Participants will also be asked to authorize the inclusion of their stool sample into the *"Institut d'investigació biomedical de Girona Dr Josep Trueta"* (IdibGi) BioBank. A specific information sheet and informed consent will be given to them ***(Annex V)***.

11. STUDY LIMITATIONS

The main limitations of this study are the following:

- The background that justifies this study comes mainly from research in the animal models of EAE and so, the results that would be obtained are difficult to predict.
- Probably, MS patients participating in the study, will receive immunomodulatory treatment for their disease and it is not well established the possible role of MS drugs in the microbiota composition profile.
- As the sampling method is non-probabilistic and the participants will be selected on the basis of their availability or because of the researcher's personal judgement, an unknown portion of the population will be excluded.
- There are not previous data about the microbiota composition of the participants, and we will not know if it will be pathological. In consequence, the results of this study may provide only association information but they cannot provide causality.
- This study will be conducted with a small sample size. This implies that the results may not be statistically significant and need for further validation study with a larger sample.

12. BUDGET

EXPENSES	COSTS
Staff expenses	
Administrative (60h x 20€/hour)	**1.200 €**
Laboratory staff (150h x 20€/hour)	**3.000 €**
Nurse (60h x 20€/hour)	**1.200 €**
Dietician (30h x 20€/hour)	**600 €**
Statistical analysis (30h x 35€/hour)	**1.050 €**
Materials	
Stool containers (40 samples)	**2'30 €**
DNA extraction kits	**1.500 €**
PCR reagents	**1.800 €**
Other molecular biology reagents (proteinase K, buffers, agarose…)	**1.200 €**
Documents printing	**100 €**
Other consumables: gloves, PCR plates, test tubes, pipette tips	**1.000** €
Services Procurement	
Probes and primers design + bacterial quantification	**3.600 €**
Next Generation Sequencing + phylogenetic study (x45 samples)	**3.200 €**
Publication and dissemination expenses	
Article scientific revision and publication	**1.500 €**
MS International meeting: ACTRIMS-ECTRIMS	**1.200 €**
TOTAL COST	**22.153€**

13. ANNEXES

13.1. Annex I: The 2010 McDonald criteria for diagnosis of MS

TABLE 4: The 2010 McDonald Criteria for Diagnosis of MS

Clinical Presentation	Additional Data Needed for MS Diagnosis
≥2 attacks[a]; objective clinical evidence of ≥2 lesions or objective clinical evidence of 1 lesion with reasonable historical evidence of a prior attack[b]	None[c]
≥2 attacks[a]; objective clinical evidence of 1 lesion	Dissemination in space, demonstrated by: ≥1 T2 lesion in at least 2 of 4 MS-typical regions of the CNS (periventricular, juxtacortical, infratentorial, or spinal cord)[d]; or Await a further clinical attack[a] implicating a different CNS site
1 attack[a]; objective clinical evidence of ≥2 lesions	Dissemination in time, demonstrated by: Simultaneous presence of asymptomatic gadolinium-enhancing and nonenhancing lesions at any time; or A new T2 and/or gadolinium-enhancing lesion(s) on follow-up MRI, irrespective of its timing with reference to a baseline scan; or Await a second clinical attack[a]
1 attack[a]; objective clinical evidence of 1 lesion (clinically isolated syndrome)	Dissemination in space and time, demonstrated by: For DIS: ≥1 T2 lesion in at least 2 of 4 MS-typical regions of the CNS (periventricular, juxtacortical, infratentorial, or spinal cord)[d]; or Await a second clinical attack[a] implicating a different CNS site; and For DIT: Simultaneous presence of asymptomatic gadolinium-enhancing and nonenhancing lesions at any time; or A new T2 and/or gadolinium-enhancing lesion(s) on follow-up MRI, irrespective of its timing with reference to a baseline scan; or Await a second clinical attack[a]
Insidious neurological progression suggestive of MS (PPMS)	1 year of disease progression (retrospectively or prospectively determined) plus 2 of 3 of the following criteria[d]: 1. Evidence for DIS in the brain based on ≥1 T2 lesions in the MS-characteristic (periventricular, juxtacortical, or infratentorial) regions 2. Evidence for DIS in the spinal cord based on ≥2 T2 lesions in the cord 3. Positive CSF (isoelectric focusing evidence of oligoclonal bands and/or elevated IgG index)

If the Criteria are fulfilled and there is no better explanation for the clinical presentation, the diagnosis is "MS"; if suspicious, but the Criteria are not completely met, the diagnosis is "possible MS"; if another diagnosis arises during the evaluation that better explains the clinical presentation, then the diagnosis is "not MS."

[a]An attack (relapse; exacerbation) is defined as patient-reported or objectively observed events typical of an acute inflammatory demyelinating event in the CNS, current or historical, with duration of at least 24 hours, in the absence of fever or infection. It should be documented by contemporaneous neurological examination, but some historical events with symptoms and evolution characteristic for MS, but for which no objective neurological findings are documented, can provide reasonable evidence of a prior demyelinating event. Reports of paroxysmal symptoms (historical or current) should, however, consist of multiple episodes occurring over not less than 24 hours. Before a definite diagnosis of MS can be made, at least 1 attack must be corroborated by findings on neurological examination, visual evoked potential response in patients reporting prior visual disturbance, or MRI consistent with demyelination in the area of the CNS implicated in the historical report of neurological symptoms.

[b]Clinical diagnosis based on objective clinical findings for 2 attacks is most secure. Reasonable historical evidence for 1 past attack, in the absence of documented objective neurological findings, can include historical events with symptoms and evolution characteristics for a prior inflammatory demyelinating event; at least 1 attack, however, must be supported by objective findings.

[c]No additional tests are required. However, it is desirable that any diagnosis of MS be made with access to imaging based on these Criteria. If imaging or other tests (for instance, CSF) are undertaken and are negative, extreme caution needs to be taken before making a diagnosis of MS, and alternative diagnoses must be considered. There must be no better explanation for the clinical presentation, and objective evidence must be present to support a diagnosis of MS.

[d]Gadolinium-enhancing lesions are not required; symptomatic lesions are excluded from consideration in subjects with brainstem or spinal cord syndromes.

MS = multiple sclerosis; CNS = central nervous system; MRI = magnetic resonance imaging; DIS = dissemination in space; DIT = dissemination in time; PPMS = primary progressive multiple sclerosis; CSF = cerebrospinal fluid; IgG = immunoglobulin G.

13.2. Annex II: Kurtzke EDSS score

Kurtzke Expanded Disability Status Scale (EDSS)

- ❑ 0.0 - Normal neurological exam (all grade 0 in all Functional System (FS) scores*).
- ❑ 1.0 - No disability, minimal signs in one FS* (i.e., grade 1).
- ❑ 1.5 - No disability, minimal signs in more than one FS* (more than 1 FS grade 1).
- ❑ 2.0 - Minimal disability in one FS (one FS grade 2, others 0 or 1).
- ❑ 2.5 - Minimal disability in two FS (two FS grade 2, others 0 or 1).
- ❑ 3.0 - Moderate disability in one FS (one FS grade 3, others 0 or 1) or mild disability in three or four FS (three or four FS grade 2, others 0 or 1) though fully ambulatory.
- ❑ 3.5 - Fully ambulatory but with moderate disability in one FS (one grade 3) and one or two FS grade 2; or two FS grade 3 (others 0 or 1) or five grade 2 (others 0 or 1).
- ❑ 4.0 - Fully ambulatory without aid, self-sufficient, up and about some 12 hours a day despite relatively severe disability consisting of one FS grade 4 (others 0 or 1), or combination of lesser grades exceeding limits of previous steps; able to walk without aid or rest some 500 meters.
- ❑ 4.5 - Fully ambulatory without aid, up and about much of the day, able to work a full day, may otherwise have some limitation of full activity or require minimal assistance; characterized by relatively severe disability usually consisting of one FS grade 4 (others or 1) or combinations of lesser grades exceeding limits of previous steps; able to walk without aid or rest some 300 meters.
- ❑ 5.0 - Ambulatory without aid or rest for about 200 meters; disability severe enough to impair full daily activities (e.g., to work a full day without special provisions); (Usual FS equivalents are one grade 5 alone, others 0 or 1; or combinations of lesser grades usually exceeding specifications for step 4.0).
- ❑ 5.5 - Ambulatory without aid for about 100 meters; disability severe enough to preclude full daily activities; (Usual FS equivalents are one grade 5 alone, others 0 or 1; or combination of lesser grades usually exceeding those for step 4.0).
- ❑ 6.0 - Intermittent or unilateral constant assistance (cane, crutch, brace) required to walk about 100 meters with or without resting; (Usual FS equivalents are combinations with more than two FS grade 3+).

- ❑ 6.5 - Constant bilateral assistance (canes, crutches, braces) required to walk about 20 meters without resting; (Usual FS equivalents are combinations with more than two FS grade 3+).
- ❑ 7.0 - Unable to walk beyond approximately 5 meters even with aid, essentially restricted to wheelchair; wheels self in standard wheelchair and transfers alone; up and about in wheelchair some 12 hours a day; (Usual FS equivalents are combinations with more than one FS grade 4+; very rarely pyramidal grade 5 alone).
- ❑ 7.5 - Unable to take more than a few steps; restricted to wheelchair; may need aid in transfer; wheels self but cannot carry on in standard wheelchair a full day; May require motorized wheelchair; (Usual FS equivalents are combinations with more than one FS grade 4+).
- ❑ 8.0 - Essentially restricted to bed or chair or perambulated in wheelchair, but may be out of bed itself much of the day; retains many self-care functions; generally has effective use of arms; (Usual FS equivalents are combinations, generally grade 4+ in several systems).
- ❑ 8.5 - Essentially restricted to bed much of day; has some effective use of arm(s); retains some self-care functions; (Usual FS equivalents are combinations, generally 4+ in several systems).
- ❑ 9.0 - Helpless bed patient; can communicate and eat; (Usual FS equivalents are combinations, mostly grade 4+).
- ❑ 9.5 - Totally helpless bed patient; unable to communicate effectively or eat/swallow; (Usual FS equivalents are combinations, almost all grade 4+).
- ❑ 10.0 - Death due to MS.

---

*Excludes cerebral function grade 1.

Note 1: EDSS steps 1.0 to 4.5 refer to patients who are fully ambulatory and the precise step number is defined by the Functional System score(s). EDSS steps 5.0 to 9.5 are defined by the impairment to ambulation and usual equivalents in Functional Systems scores are provided.

Note 2: EDSS should not change by 1.0 step unless there is a change in the same direction of at least one step in at least one FS.

Sources: Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology. 1983 Nov;33(11):1444-52.

Haber A, LaRocca NG. eds. Minimal Record of Disability for multiple sclerosis. New York: National Multiple Sclerosis Society; 1985.

13.3. Annex III: Information sheet

SERVEI DE NEUROLOGIA
UNITAT DE
NEUROIMMUNOLOGIA i
Esclerosi Múltiple
Hospital Universitari Dr. Josep Trueta

FULL D'INFORMACIÓ AL PACIENT

ESTUDI: Determinació de la flora microbiana en l'esclerosi múltiple

INVESTIGADOR: Dr. Lluís Ramió i Torrentà
Unitat de Neuroimmunologia i Esclerosi Múltiple
Servei de Neurologia
Hospital Universitari Dr.Josep Trueta
Avda de França s/n
17001- Girona

1. INFORMACIÓ AL PACIENT PARTICIPANT

1.1. INFORMACIÓ GENERAL

Ens dirigim a vostè per informar-lo sobre un projecte que duu a terme la Unitat de Neuroimmunologia i Esclerosi Múltiple de l'Hospital Universitari Dr. Josep Trueta, al qual se'l convida a participar. L'estudi ha estat ja revisat i aprovat pel Comitè Ètic de Investigació Clínica (CEIC) d'aquest hospital.

La col·laboració que li sol·licitem consisteix en la recollida d'una mostra fecal, però abans de res, necessitem el seu consentiment lliure i voluntari. Per això volem proporcionar-li la informació correcta i suficient per que pugui valorar si vol o no participar en l'estudi. Per tant, llegeixi aquesta fulla informativa amb atenció i nosaltres li aclarirem els dubtes que li puguin sorgir. Pot consultar la decisió amb les persones que consideri oportunes.

1.2. PARTICIPACIÓ VOLUNTÀRIA

Ha de saber que la seva participació en aquest estudi és voluntària i que pot decidir no participar o canviar la seva decisió i retirar el consentiment en qualsevol moment, sense que per això s'alteri la relació amb el seu metge ni es produeixi cap perjudici en el seu tractament.

1.3. DESCRIPCIÓ I OBJECTIU DEL ESTUDI

El projecte pel qual li sol·licitem la seva participació es centra en l'estudi de l'esclerosi múltiple, una malaltia autoimmune complexa de causa desconeguda, però en la que es creu que tant factors ambientals com genètics participen en el seu desenvolupament.
Recentment s'han descrit estudis que suggereixen que l'esclerosis múltiple podria estar relacionada amb una composició microbiana diferent a l'habitual. L'objectiu d'aquest estudi és estudiar el perfil microbiològic en mostres de femta de pacients, la qual cosa ens permetrà iniciar futures investigacions per entendre millor el paper que pot tenir en la malaltia.
Per a qualsevol estudi científic és tan important el disposar de mostres de pacients afectes de la malaltia, com de individus que no pateixen la malaltia (grup control).

Per això li demanem el seu consentiment per recollir mostres fecals, a partir de les quals obtindrem material per estudiar la seva flora microbiana. Aquestes mostres seran degudament processades i guardades.

1.4. MÈTODE D'OBTENCIÓ DE MOSTRES

El pacient obtindrà la seva mostra de femta en el recipient per recollida de femta que se li proporcionarà i seguint les instruccions recomanades. Les mostres obtingudes seràn etiquetades amb un codi per mantenir la confidencialitat del subjecte participant en l'estudi.

1.5. DESTINACIÓ MOSTRA DESPRÉS DE LA SEVA UTILITZACIÓ EN AQUEST PROJECTE DE RECERCA

Un cop finalitzada la investigació, és possible que hi hagi mostra sobrant. En relació a aquestes, se li ofereixen les següents opcions:

A- La destrucció de la mostra sobrant.

B- La seva utilització en futurs projectes relacionats amb aquesta línia de recerca.

C- La introducció de l'excedent de la mostra a un Biobanc

En aquest cas, se li facilitarà full d'informació al pacient i consentiment específic que haurà de signar i serà custodiat pel coordinador del biobanc del seu Hospital.

1.6. BENEFICIS

Probablement vostè no rebi cap benefici directe amb la seva participació en aquest projecte, però sí la satisfacció d'haver col·laborat en un projecte d'investigació que pot millorar el benestar i el tractament a pacients afectats d'esclerosi múltiple.

Qualsevol troballa que es pugui aplicar al curs de la seva malaltia li serà indicada.

Vostè té dret a conèixer els resultats dels estudis que s'obtinguin a partir de l'anàlisi de les mostres donades. I també té dret a la no informació d'aquests resultats.

1.7. CONFIDENCIALITAT

Les dades recollides seran estrictament confidencials. Només s'autoritzarà per la recollida de dades del seu historial mèdic a persones sotmeses al secret professional sempre amb el previ coneixement de l'investigador principal. Les mostres seran codificades per respectar l'anonimat. En cap cas el seu nom apareixerà en la publicació dels resultats.

El tractament, la comunicació i la cessió de les dades de caràcter personal de tots els participants s'ajustarà a la Llei Orgànica 15/1999, de 13 de desembre de protecció de dades de caràcter personal. D'acord al que estableix la legislació mencionada, vostè pot exercir els drets d'accés, modificació, oposició i cancel·lació de dades, per això s'ha de dirigir al seu metge de l'estudi. Les dades recollides per l'estudi estaran identificades mitjançant un codi i només el seu metge de l'estudi/ col·laboradors

podran relacionar aquestes dades amb vostè i amb la seva història clínica. Si vostè decideix retirar el consentiment per participar en aquest estudi, cap dada nova serà afegida a la base de dades i, pot exigir la destrucció de totes les mostres identificables prèviament retingudes per evitar la realització de nous anàlisis.

1.8. COMPENSACIÓ ECONÒMICA

La donació i la utilització de mostres biològiques humanes són gratuïtes. Per tant, per la seva participació en l'estudi no rebrà cap compensació econòmica.

En el cas que es produís un desenvolupament comercial dels coneixements generats, els possibles beneficis que es podrien rebre anirien íntegrament a cobrir els objectius científics del grup de investigació. Firmant aquest consentiment vostè renuncia als drets sobre qualsevol ús comercial amb la informació o mostres que vostè està cedint.

13.4. Annex IV: Informed consent

SERVEI DE NEUROLOGIA
UNITAT DE NEUROIMMUNOLOGIA i Esclerosi Múltiple

CONSENTIMENT INFORMAT AL PACIENT

Jo .....

- He llegit el full informatiu que se m'ha entregat
- He pogut fer preguntes sobre l'estudi
- He rebut suficient informació sobre l'estudi
- He parlat amb: (nom del investigador).....
- Comprenc que la meva participació és voluntària
- Comprenc que les mostres obtingudes seran etiquetades amb un codi per mantenir la confidencialitat de les meves dades i que, d'acord amb la Llei de Biomedicina de 2007 (llei 14/2007 de Investigació Biomèdica) la mostra sobrant de l'estudi serà utilitzada per futurs projectes relacionats amb aquest projecte o amb la seva malaltia o bé destruïda, segons la meva voluntat.
- Comprenc que puc revocar el meu consentiment en qualsevol moment, sense haver de donar explicacions i sense que això alteri la meva assistència sanitària.

Autoritzo a ser contactat en el cas de necessitar més informació o mostres biològiques addicionals: SI/No i telèfon o direcció de contacte

☐ Sí ☐ No Telèfon/ e-mail: .....

I lliurement, dono la meva conformitat per participar en l'estudi amb mostres de:

☐ Femta

Si hi hagués excedent de la mostra, afirmo haver estat advertit sobre les opcions de destinació en finalitzar el projecte de recerca.

En aquest sentit:

- Sol·licito la destrucció de la mostra excedent ☐
- Permeto que les meves mostres siguin utilitzades en investigacions futures relacionades amb la meva malaltia
  ☐ Sí ☐ No
- Permeto que les meves mostres siguin introduïdes en el biobanc de l'hospital.
  ☐ Sí ☐ No

Dono lliurement la meva conformitat per participar en l'estudi i dono el meu consentiment per l'accés i utilització de les meves dades en les condicions detallades en el full d'informació

DONANT	PERSONA QUE INFORMA	☐ TESITMONI (1) / ☐ TUTOR (2)
Nom	***Nom***	***Nom***
Cognoms	***Cognoms***	***Cognoms***
DNI	***DNI***	***DNI***
Edat		***Relació amb donant:***
Signatura	***Signatura***	***Signatura***

(1) Autoritzat pel donant
(2) Represetant legal

Data: __ __ / __ __ / __ __

13.5. Annex V: Biobank information sheet and informed consent

FULL D'INFORMACIÓ AL PACIENT

UTILITZACIÓ MOSTRES BIOLÒGIQUES I DADES CLÍNIQUES PER UN PROJECTE D'INVESTIGACIÓ I CONSERVACIÓ FINAL EN UN BIOBANC

A l'Hospital Universitari de Girona Dr Josep Trueta (HUGJT) i/o altres Centres Hospitalaris adscrits, igual que en la majoria d'hospitals, a més de l'assistència als pacients, es realitza investigació biomèdica. La finalitat d'aquesta investigació és progressar en el coneixement de les malalties i en la seva prevenció, diagnòstic i tractament. Aquesta investigació biomèdica requereix recollir dades clíniques i mostres biològiques de pacients i donants sans per a analitzar-los i obtenir conclusions amb l'objectiu de conèixer millor les malalties i avançar cap al seu diagnòstic i/o tractament.
Les mostres i dades clíniques obtingudes per al diagnòstic o control de les malalties, una vegada utilitzades amb aquesta finalitat, resulten també útils i necessàries per a la investigació. De fet, molts dels avenços científics obtinguts en aquests últims anys en medicina són fruit d'aquest tipus d'estudis.

Sol·licitem la seva autorització per la cessió de les mostres biològiques i la informació clínica associada per prosseguir amb la investigació biomèdica, una vegada hagi finalitzat el projecte d'investigació BioEM.

Seguint el que estableix la Llei 14/2007, d'Investigació Biomèdica, la Llei Orgànica 15/1999, de Protecció de Dades Personals, i les seves normes de desenvolupament, li sol·licitem que llegeixi detingudament aquest document d'informació i el consentiment informat que se li adjunta al final per a la seva firma, si està d'acord en participar en aquesta proposta.

Un Biobanc és una institució regulada per lleis específiques que facilita la investigació biomèdica, és a dir, aquella destinada a promoure la salut de les persones. Les mostres incloses en un Biobanc poden ser cedides per a la investigació en Medicina, sempre sota la supervisió d'un comitè científic i un altre d'ètica. Les mostres es cediran generalment sense informació personal associada, encara que a vegades podrà ser necessari l'accés a la història clínica o al resultats d'altres proves per a completar la investigació.

FINALITAT DE LA INVESTIGACIÓ: progressar en el coneixement de les malalties.

La finalitat de la investigació és millorar el nostre coneixement de les malalties. Les mostres, les dades clíniques i analítiques i les proves d'imatge s'utilitzaran per a la recerca biomèdica. Tot això permetrà progressar en el coneixement de la prevenció, diagnòstic, pronòstic i/o tractament de les malalties.

CONSIDERACIONS PARTICULARS DEL PROJECTE:

La **Unitat de Neuroimmunologia i Esclerosi Múltiple** (UNIEM), coordinada pel Dr. **Lluís Ramió-Torrentà** i formada per professionals sanitaris, docents i d'investigació especialitzats, està dedicada a l'atenció global i integral de pacients afectats de malalties immunològiques amb afectació del sistema nerviós com l'esclerosi múltiple i d'altres malalties. A part del treball assistencial que desenvolupa en el seu dia a dia,

també centra part dels seus esforços en la investigació biomèdica relacionada amb aquest tipus de patologies.
L'esclerosi múltiple és una malaltia autoimmune complexa de causa desconeguda, en la que tant factors ambientals com genètics participen en el seu desenvolupament. Poc es coneix, en el moment del diagnòstic, de l'evolució clínica que s'observarà en cada pacient, la qual pot presentar un ampli ventall de formes. És per això que la comunitat mèdica i, la societat en general, tenen interès en la millora del diagnòstic, pronòstic, seguiment i tractament d'aquests pacients.

MOSTRES BIOLÒGIQUES I INFORMACIÓ ASSOCIADA: una vegada finalitzat el projecte d'investigació es custodiaran i conservaran en el Biobanc IDIBGI fins la seva extinció.

Vostè pot decidir, si una vegada finalitzat el projecte d'investigació abans esmentat, les dades clíniques recollides i les mostres biològiques sobrants d'aquest projecte passen a ser custodiades i conservades al Biobanc (banc de mostres biològiques) IDIBGI, fins la seva extinció.
Aquest Biobanc és un establiment sense ànim de lucre i inscrit en el *Registro Nacional de Biobancos* dependent de l'*Instituto de Salud Carlos III* amb la referència B.0000872, que acull col·leccions organitzades de mostres biològiques i informació associada en les condicions i garanties de seguretat que exigeix la legislació anteriorment referida i els codis de conducta aprovats per els Comitès d'Ètica. Les esmentades mostres i la seva informació associada queden disponibles per aquells investigadors que ho sol·licitin al Biobanc.
Qualsevol estudi d'investigació per al qual se sol·liciti la utilització d'aquestes dades o mostres haurà de disposar sempre de l'aprovació del Comitè d'Ètica de la Investigació Clínica (CEIC) competent, que vetllarà per a què els investigadors desenvolupin els seus estudis seguint sempre les més estrictes normes ètiques i legals. A més, el comitè científic del Biobanc garantirà que els projectes siguin d'excel·lència científica. La investigació biomèdica és actualment un fenomen global, de manera que ocasionalment aquestes mostres podran ser cedides a grups d'investigació fora d'Espanya, sempre que compleixin els requisits de la legislació espanyola i ho aprovin els corresponents comitès.
En el cas de ser necessària alguna mostra addicional, la institució sanitària es podria posar en contacte amb vostè per a sol·licitar-li novament la seva col·laboració. En aquest cas se li informarà dels motius i se li sol·licitarà de nou el seu consentiment.

PROTECCIÓ DE DADES I CONFIDENCIALITAT: les mostres es conservaran codificades.

Les dades personals que es recullin seran obtingudes, tractades i emmagatzemades complint en tot moment el deure del secret, d'acord amb la legislació vigent en matèria de protecció de dades de caràcter personal.
La identificació de les mostres biològiques del Biobanc serà sotmesa a un procés de codificació. A cada mostra se li assigna un codi d'identificació, que serà l'utilitzat per els investigadors. Només el personal autoritzat pel Biobanc i el personal autoritzat pel Dr. Lluís Ramió i Torrentà podrà relacionar la seva identitat amb els citats codis. Mitjançant aquest procés els investigadors que sol·licitin mostres al Biobanc no podran conèixer cap dada que reveli la seva identitat. De la mateixa manera, encara que els resultats obtinguts de la investigació realitzada amb les seves mostres es publiquin en revistes científiques, la seva identitat no serà facilitada. En aquells estudis en els quals no es prevegin resultats potencialment útils per a la seva salut, i d'acord amb el corresponent Comitè d'Ètica, les mostres i dades podran ser anonimitzades, és a dir, no hi haurà cap possibilitat de tornar a associar la mostra amb la seva identitat.
Les seves mostres i dades clíniques associades a les mateixes passaran a formar part del fitxer del Biobanc, inscrit en l'Agència de Protecció de Dades sota la responsabilitat de l'Institut d'Investigació Biomèdica de Girona (IDIBGI).

Vostè podrà exercir els seus drets d'accés, rectificació, cancel·lació i objecció, així com obtenir informació sobre l'ús de les seves mostres i dades associades, dirigint-se a:

DIRECCIÓ DEL BIOBANC IDIBGI s/n	Avinguda de França
Hospital Universitari de Girona Dr Josep Trueta	17007 Girona
Biobanc@IDIBGI.org	Tfn 972 940 282

CARÀCTER ALTRUISTA DE LA DONACIÓ: La cessió de mostres biològiques que vostè realitza al Biobanc IDIBGI és gratuïta.

La donació té per disposició legal caràcter altruista, per la qual cosa vostè no obtindrà ni ara ni en el futur cap benefici econòmic de la mateixa, ni tindrà drets sobre possibles beneficis comercials dels descobriments que es puguin aconseguir com a resultats de la investigació biomèdica.

PARTICIPACIÓ VOLUNTÀRIA: la seva negativa NO repercutirà en la seva assistència mèdica, present o futura.

La seva participació és totalment voluntària. Si firma el consentiment informat, confirmarà que desitja participar. Pot negar-se a participar o retirar el seu consentiment en qualsevol moment posterior a la firma sense haver d'explicar els motius i que això repercuteixi en la seva assistència mèdica, present o futura.

COST i RISCOS ASSOCIATS: la seva donació no li suposa CAP cost.

L'obtenció de la mostra no suposarà cap cost econòmic per a vostè. No es realitzarà una intervenció exclusivament per a l'obtenció de mostres per a investigació sense el seu consentiment explícit.

REVOCACIÓ DEL CONSENTIMENT: si vostè decideix firmar aquest consentiment podrà també cancel·lar-lo lliurement. Això comportarà la destrucció de les seves mostres.

Si en un futur vostè volgués anul·lar el seu consentiment, les seves mostres biològiques serien destruïdes i les dades associades a les mateixes serien retirades del Biobanc. També podria sol·licitar l'anonimització de les mostres, de manera que en aquest cas s'eliminaria la relació entre les seves dades personals (que revelen la seva identitat) i les seves mostres biològiques i dades clíniques associades. Els efectes d'aquesta cancel·lació o anonimització no es podrien estendre a la investigació que ja s'hagi realitzat. Si desitgés cancel·lar el consentiment, ho hauria de sol·licitar per escrit a la Direcció del Biobanc IDIBGI, a l'adreça anteriorment mencionada.

INFORMACIÓ SOBRE ELS RESULTATS DE LA INVESTIGACIÓ: se li proporcionarà informació si vostè la desitja rebre.

En el cas que vostè ho demani expressament, el Biobanc podrà proporcionar informació sobre quines són les investigacions en què s'han utilitzat les seves mostres i dels resultats globals d'aquestes investigacions, excepte en el cas de cancel·lació o anonimització.
Els mètodes utilitzats en investigació biomèdica solen ser diferents dels aprovats per a la pràctica clínica, per el que no han de ser considerats amb valor clínic per a vostè. Malgrat això, en el cas que aquestes

investigacions proporcionin dades que poguessin ser clínica o genèticament rellevants per a vostè i interessar a la seva salut o a la seva família, li seran comunicats si així ho estima oportú. Així mateix, podria donar-se el cas que s'obtingui informació rellevant per a la seva família. En aquest supòsit, li correspondrà a vostè decidir si vol o no que aquesta informació li sigui comunicada. En cas afirmatiu, ha de consignar-ho a la casella que apareix al final d'aquest document.
Si vostè no desitja aquesta informació, tingui en compte que la llei estableix que, quan la informació obtinguda sigui necessària per a evitar un greu perjudici per a la salut dels seus familiars biològics, un Comitè d'experts estudiarà el cas i haurà de decidir si és convenient informar als afectats o als seus representants legals.

Si us plau, pregunti al personal sanitari que li ha comunicat aquesta informació sobre qualsevol dubte que pugui tenir,
ara o en el futur, en relació a aquest consentiment. Així mateix, pot comentar els seus dubtes al seu metge, que el posarà en contacte amb el personal sanitari autoritzat.

Moltes gràcies per la seva col·laboració.

BIOBANC IDIBGI

Li agraïm la seva desinteressada col·laboració amb l'avenç de la ciència i la medicina.
D'aquesta manera està col·laborant a vèncer les malalties i ajudar a multitud de malalts actuals i futurs.

Hospital Universitari de Girona Doctor Josep Trueta — Servei de Neurologia, Unitat de Neuroimmunologia i Esclerosi Múltiple — IAS Institut d'Assistència Sanitària — Bio Banc IDIBGI

CONSENTIMENT INFORMAT

UTILITZACIÓ MOSTRES BIOLÒGIQUES I DADES CLÍNIQUES PER UN PROJECTE D'INVESTIGACIÓ I CONSERVACIÓ FINAL EN UN BIOBANC

Si ha comprès la informació que se li ha proporcionat en el document informatiu, resolt qualsevol dubte que pogués tenir i decideix col·laborar amb el Biobanc IDIBGI i en els termes abans explicats, si us plau, llegeixi i firmi a continuació aquest full:

Qui signa el present document autoritza a l'HUGJT i/o altres Centres Hospitalaris adscrits a que les mostres biològiques de femta i la informació clínica associada siguin incorporades al Biobanc IDIBGI un cop finalitzat el **projecte de recerca BioEM de la Unitat de Neuroimmunologia i Esclerosi Múltiple (UNIEM)** i que puguin ser cedides des del mateix amb la finalitat de desenvolupar projectes d'investigació biomèdica, sempre que aquests comptin amb l'obligada aprovació del Comitè d'Ètica competent.

Aquesta autorització la concedeix després d'haver estat informat verbalment i haver llegit la informació adjunta sobre el consentiment informat per a la recollida de dades clíniques, analítiques, proves d'imatge i mostres biològiques sobrants per a investigació biomèdica.

Confirmo que:

1. Autoritzo a dipositar, un cop finalitzat el projecte BioEM, l'excedent de la mostra i tota la informació associada a aquesta en el Biobanc IDIBGI:
   ☐ SÍ ☐ NO
2. Autoritzo que la mostra biològica cedida i la informació clínica associada s'utilitzi en investigacions:
   Nacionals: ☐ SÍ ☐ NO Internacionals: ☐ SÍ ☐ NO
3. Desitjo que se'm comuniqui la informació derivada de la investigació que realment sigui rellevant i aplicable per a la meva salut o la de la meva família:
   ☐ SÍ ☐ NO Telèfon o email de contacte.....
4. Autoritzo a ser contactat en el cas de necessitar més informació o mostres biològiques addicionals:
   ☐ SÍ ☐ NO Telèfon o email de contacte.....
5. He expressat el meu desig de que se'm respectin les següents excepcions respecte a l'objectiu i mètodes de les investigacions:
   .....
   .....

DONANT	PERSONA QUE INFORMA	☐ TESTIMONI[1] / ☐ TUTOR[2]
Nom *Cognoms* *DNI* *Edat* *Signatura*	*Nom* *Cognoms* *DNI* *Signatura*	*Nom* *Cognoms* *DNI* *Relació amb el donant:* *Signatura*

[1] Autoritzat pel donant
[2] Representant legal

A ....., a .....de.....de.....

Arribada la majoria d'edat, el donant té dret a l'anul·lació del consentiment. En cas que no l'exerceixi, es considerarà que l'actual document de consentiment continua vigent.

13.6. Annex VI: Data Collection sheet

Full d'informació mèdica a emplenar pels participants

1) Gènere: ☐ Masculí ☐ Femení

2) Edat:

3) Ètnia: ☐ Caucàssica ☐ Asiàtica ☐ Africana ☐ Magrebí ☐ Altres

4) Historial mèdic

a. Antecedents mèdics personals

b. Malalties autoinmunitàries

☐ Hipotiroidisme o hipertiroisme ☐ Artritis reumatoide ☐ Lupus ☐ Psoriasis

5) Tractaments actuals:

13.7. Annex VII: Frequency questionnaire of food consumption

QÜESTIONARI DE FREQÜÈNCIA DE CONSUM D'ALIMENTS

Nº història _____

Nom _____

Data _____

*Un **Cuestionario de Frecuencia Alimentaria (CFA)**, basado en un cuestionario utilizado y validado por Willett en el Estudio de Salud de las Enfermeras Norteamericanas, el cual ha permitido estimar la ingesta dietética habitual de los entrevistados. Para ello el CFA se ha adaptado previamente para su uso en castellano en nuestra comunidad, habiéndose acometido también su validación y el estudio de su reproducibilidad entre un grupo de adultos voluntarios asistentes a dos centros de salud de la Comunidad Valenciana. Este cuestionario consta de un total de 93 ítems de alimentos o grupos de ellos, sobre los que se pregunta por su frecuencia de consumo habitual para una determinada cantidad, a lo largo del año anterior a la entrevista*

LEER LAS INSTRUCCIONES APARECIDAS EN EL CUESTIONARIO.

Para cada alimento, consignar <u>cuantas veces como media</u> ha tomado la cantidad que se indica durante el año pasado. Tenga en cuenta las veces que lo toma solo y las que lo añade a otros alimentos o platos (Ej.: La leche del café, huevos en las tortillas, etc)

I. LACTEOS	Nunca ó <1 mes	1-3 por mes	1 por sem	2-4 por sem	5-6 por sem	1 por día	2-3 por día	4-5 por día	6+ al día
1. Leche entera (1 vaso o taza, 200 cc)									
2. Leche descremada (1 vaso, 200cc)									
3. Leche condensada (1 cucharada)									
4. Yogurt (Uno, 125 gramos)									
5. Requesón, cuajada, queso blanco o fresco (100g)									
6. Queso cremoso o en porciones (Una porción)									
7. Queso curado o semicurado: Manchego (1 trozo, 50 g)									
8. Natillas, flan, puding (uno)									
9. Helados (1 cucurucho, vasito o bola)									
II. HUEVOS, CARNES, PESCADOS	Nunca ó <1 mes	1-3 por mes	1 por sem	2-4 por sem	5-6 por sem	1 por día	2-3 por día	4-5 por día	6+ por día
10. Huevos de gallina (uno)									
11. Pollo con piel (1 plato o pieza)									
12. Pollo sin piel (1 plato o pieza)									
13. Carne de ternera, cerdo, cordero como plato principal (1 plato o pieza)									
14. Carne de caza: conejo, codorniz, pato (1 plato)									
15. Higado de ternera, cerdo o pollo (1 plato)									
16. Visceras: callos, sesos, mollejas (1 ración, 100 g)									
17. Embutidos: jamón, salchichón, salami, mortadela (1 ración, 50g)									
18. Salchichas y similares (una mediana)									
19. Patés, foie-gras (media ración, 50 g)									
20. Hamburguesa (una, 100 g)									
21. Tocino, bacon, panceta (2 lonchas, 50 g)									
22. Pescado frito variado (un plato o ración)									
23. Pescado hervido o plancha: merluza, lenguado, sardinas, atún. (1 ración)									
24. Pescados en salazón: bacalao, anchoas (media ración, 50 g)									
25. Pescados en conservas: atún, sardinas, arenques (1 lata)									
26. Almejas, mejillones, ostras (1 ración, 100 g)									
27. Calamares, pulpo (1 ración, 100 g)									
28. Marisco: gambas, langosta y similares (1 ración, 100 g)									

(Si no se especifica, los platos para carnes y pescado son de tamaño mediano)

III. VERDURAS Y LEGUMBRES	Nunca ó <1 mes	1-3 por mes	1 por sem	2-4 por sem	5-6 por sem	1 por día	2-3 por día	4-5 por día	6+ al día
29. Espinacas cocinadas (1 plato)									
30. Col, coliflor, brocoles cocinadas (1 plato)									
31. Lechuga, endivias, escarola (1 plato)									
32. Tomates (uno mediano)									

Para alimentos que se consumen por temporadas, calcular el consumo medio para todo el año. Por ejemplo, si un alimento como la sandía se come 4 veces a la semana durante todo el verano (3 meses), entonces el consumo medio al año se marcaría en "1 vez por semana".

III. VERDURAS Y LEGUMBRES (Continuación)	Nunca ó <1 mes	1-3 por mes	1 por sem	2-4 por sem	5-6 por sem	1 por día	2-3 por día	4-5 por día	6+ al día
33. Cebolla (una mediana)									
34. Zanahoria, calabaza (una o plato pequeño)									
35. Judias verdes cocinadas (1 plato)									
36. Berenjenas, calabacines, pepinos (uno)									
37. Pimientos (uno)									
38. Espárragos (una ración o plato)									
39. Champiñones, setas (1 plato)									
40. Legumbres cocinadas: lentejas, garbanzos, judías pintas o blancas (1 plato mediano)									
41. Guisantes cocinados (1 plato)									
IV. FRUTAS	Nunca ó <1 mes	1-3 por mes	1 por sem	2-4 por sem	5-6 por sem	1 por día	2-3 por día	4-5 por día	6+ por día
42. Naranjas, pomelo, mandarinas (Una)									
43. Zumo de naranja natural (un vaso pequeño, 125 cc)									
44. Plátano (uno)									
45. Manzana, pera (una mediana)									
46. Fresas (1 plato o taza de postre)									
47. Cerezas (1 plato o taza de postre)									
48. Melocotón, albaricoques (uno mediano)									

49. Higos frescos (uno)									
50. Sandía, melón (1 tajada o cala, mediana)									
51. Uvas (un racimo mediano o plato de postre)									
52. Aceitunas (tapa o plato pequeño, aprox. 15 unidades pequeñas)									
53. Frutas en almíbar: melocotón, peras, piña (2 mitades o rodajas)									
54. Frutos secos: piñones, almendras, cacahuetes, avellanas (1 plato o bolsita pequeña)									
V. PAN, CEREALES Y SIMILARES	Nunca ó <1 mes	1-3 por mes	1 por sem	2-4 por sem	5-6 por sem	1 por día	2-3 por día	4-5 por día	6+ por día
55. Pan blanco (Una pieza pequeña o 3 rodajas de molde, 60 g)									
56. Pan integral (Pieza pequeña o 3 rodajas de molde)									
57. Picos, roscos y similares (una unidad, 3,5 g)									
58. Patatas fritas (1 ración, 100 g)									
59. Patatas cocidas, asadas (1 patata mediana)									
60. Bolsa de patatas fritas (1 bolsa pequeña, 25-30 g)									
61. Arroz cocinado (1 plato mediano)									
62. Pastas: espagueti, macarrones y similares (1 plato)									
VI. ACEITES Y GRASAS	Nunca ó <1 mes	1-3 por mes	1 por sem	2-4 por sem	5-6 por sem	1 por día	2-3 por día	4-5 por día	6+ por día
63. Aceite de oliva (1 cucharada)									
64. Otros aceites vegetales: girasol, maíz, soja (1 cucharada)									
65. Margarina añadida al pan o la comida (1 cucharada o untada)									
66. Mantequilla añadida al pan o la comida (1 cucharada o untada)									
67. Manteca (de cerdo) añadida al pan o la comida (1 cucharada o untada)									

Para cada alimento, marcar la casilla apropiada para cu consumo medio durante el año pasado. Por ejemplo si toma una cucharada de mermelada cada dos días, entonces debe marcar la casilla "2-4 veces por semana"

VII. DULCES Y PASTELESRES	Nunca ó <1 mes	1-3 por mes	1 por sem	2-4 por sem	5-6 por sem	1 por día	2-3 por día	4-5 por día	6+ al día
68. Galletas tipo María (1 galleta)									
69. Galletas con chocolate (1 galleta doble)									
70. Croissant, donuts (uno)									
71. Magdalena, bizcocho (uno)									
72. Pasteles, tarta (unidad o trozo mediano)									
73 Churros (masa frita), 1 ración									
74. Chocolate, bombones (una barrita o dos bombones, 30 g)									
75. Chocolate en polvo y similares (1 cucharada)									
VIII. BEBIDAS	Nunca ó <1 mes	1-3 por mes	1 por sem	2-4 por sem	5-6 por sem	1 por día	2-3 por día	4-5 por día	6+ por día
76. Vino blanco, tinto o rosado (1 vaso, 125 cc)									
77. Cerveza (una caña o botellín 1/5, 200 cc)									
78. Brandy, ginebra, ron, wiskey, vodka, aguardientes 40º (1 copa, 50 cc)									
79. Refrescos con gas: cola, naranja, limón (ej. cocacola, fanta, etc) (Uno, 250 cc)									
80. Zumo de frutas envasado (1 lata pequeña o vaso, 200 cc)									
81. Café (1 taza)									
82. Café descafeinado (1 taza)									
83. Té (1 taza)									
IX. PRECOCINADOS, PREELABORADOS Y MISCELANEAS	Nunca ó <1 mes	1-3 por mes	1 por sem	2-4 por sem	5-6 por sem	1 por día	2-3 por día	4-5 por día	6+ por día
84. Croquetas (una)									
85. Palitos o delicias de pescado fritos (una unidad)									
86. Sopas y cremas de sobre (1 plato)									
87. Mayonesa (1 cucharada)									
88. Salsa de tomate (media taza)									
89. Picantes: tabasco, pimienta, guindilla (1/2 cucharadita)									
90. Sal (1 pizca o pellizco con dos dedos)									
91. Ajo (1 diente)									
92. Mermeladas, miel (1 cucharada)									
93. Azucar (ej. en el café, postres, etc.) (1 cucharadita)									

1. ¿Qué hace Vd. con la grasa visible cuando come carne?

1. La quito toda 2. Quito la mayoría 3. Quito un poco4. No quito nada

2. ¿Cada cuanto tiempo come comidas fritas, fuera o dentro de casa?

1. A diario 2. 4-6 veces/sem 3. 1-3 veces/sem 4. < 1 vez/sem

3. ¿Qué clase de grasa o aceite usa para:

	Manteca/Mantequilla	Margarina	Aceite oliva	Otros ac. vegetales
ALIÑAR	_____	_____	_____	_____
COCINAR/FREIR	____	_____	_____	_____

4. ¿Toma Vd. algún producto de vitaminas? 1. Sí 2. No ¿Cual?
_____

5. ¿Ha tomado alguno en el año pasado? 1. Sí 2. No ¿Cual?
_____

6. ¿Hace algún tipo de dieta? 1. Sí 2. No ¿Cual?
_____

7. ¿Ha cambiado su dieta durante el año pasado? 1. Sí 2. No

8. ¿Ha cambiado su peso en el último año? 1. Igual 2. Aumentado 3. Disminuido