cïëN
Fundación Centro Investigación
Enfermedades Neurológicas

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ANNUAL REPORT

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01.

Profile and presentation

1. Profile and presentation

1.1. Who we are

A FOUNDATION FROM THE PUBLIC SECTOR

The Research Center for Neurologic Diseases (CIEN, for its acronym in Spanish) Foundation was established on December 27, 2002, by virtue of a resolution of the Council of Ministers. It is defined as a non-profit public sector Foundation, with State-wide scope and competence. Currently, it depends on the Ministry of Science and Innovation through the Carlos III Institute of Health.

Among its founding objectives are supporting, promoting, and coordinating research in neurological diseases, especially neurodegenerative disorders. It also highlights its unifying and coordinating role of leading Spanish research groups in this field.

The CIEN Foundation is based at the Queen Sofia Foundation Alzheimer Center, whose construction was called the Alzheimer Project. Once the Alzheimer Center was built, it was divided into a nursing home for the healthcare and follow-up of the patients and a Research Center.

COLLABORATION WITH THE QUEEN SOFIA FOUNDATION

Since its establishment, the CIEN Foundation manages and coordinates the Alzheimer's Research Project, created by the Queen Sofia Foundation, and located in the Alzheimer Center that bears her name.

The CIEN Foundation is a pioneer center in Spain in which to comprehensively address the consequences that Alzheimer's disease causes both on patients and their family environment. Since its opening in 2007, the CIEN Foundation, located at the neighborhood of Vallecas, Madrid, seeks to respond to the social health project proposed by the Alzheimer Project of the Queen Sofia Foundation.
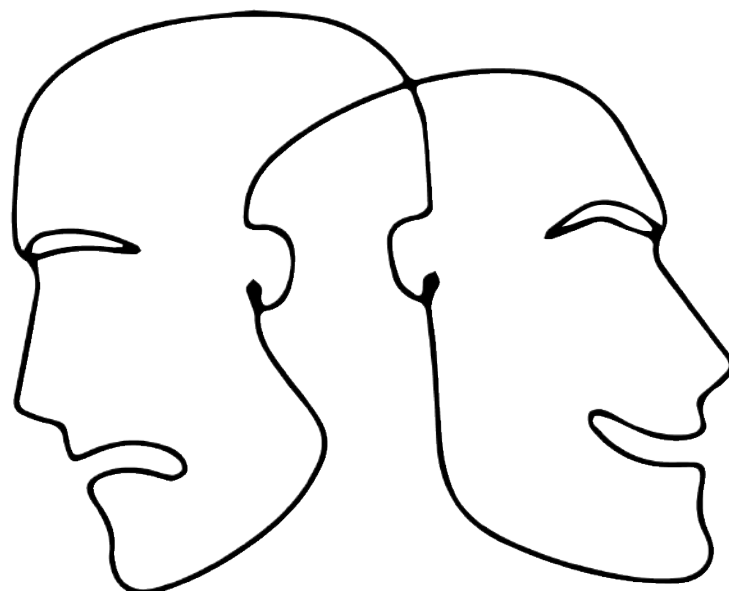
A REFERENCE CENTER IN EUROPE ON ALZHEIMER'S DISEASE RESEARCH

Only two institutions in Spain participate in the European Union Joint Programming for Disease Neurodegenerative Diseases (JPND): CIEN Foundation and CIBERNED. Its excellent infrastructures, modern methodologies, and cutting-edge technologies at their disposal as well as the available critical mass of researchers were the criteria most valued by representatives of this organization after being proposed by the Carlos III Institute of Health.

In addition, both CIEN Foundation as CIBERNED are integrated into the international Network of Centers of Excellence in Research on Neurodegeneration (COEN), mostly composed by European research centers.

AN INNOVATIVE, INTEGRATED VISION OF THE FIGHT AGAINST ALZHEIMER'S DISEASE

The CIEN Foundation and the Queen Sofia Foundation share a common perspective on action in relation to Alzheimer's disease: This disease requires a comprehensive approach model where research should be one of the fundamental pillars. The leading exponent of this integrative model is the Queen Sofia Foundation Alzheimer Center, where the main backbones of Alzheimer Project converge.

1. A live-in residence for 156 Alzheimer's patients.

2. A day-care outpatient center for 40 Alzheimer's patients.

3. An Alzheimer's research center: the so-called Alzheimer's Project Research Unit (UIPA), managed by the CIEN Foundation.

4. A training center for healthcare staff, relatives, and volunteers.

The management model implemented by the Queen Sofia Foundation Alzheimer Center has sought to summon the will and interests of all parties involved: Administration (State, Regional and Local) and civil society. For this reason, the management of UIPA, devoted to research, was assigned to the CIEN Foundation, while the Ministry of Family and Social Affairs of the Region of Madrid is responsible for healthcare and training activities.

FOCUSED ON RESEARCH IN NEURODEGENERATIVE DISEASES

The CIEN Foundation is in charge of the following tasks: implement a model of translational research that allows to effectively and efficiently transfer the scientific advances achieved in basic research to clinical practice; promote the continuous training of professionals involved with the research in neurodegenerative diseases through seminars, lectures and doctoral the-

ses; disseminate the calls launched by funding agencies, both nationally and internationally, promoting participation; and encourage the implementation of coordinated research projects in neurodegenerative diseases.

The Vallecas Project is the main research project carried out by the CIEN Foundation, both in terms of the resources used and their social impact. At the same time, the CIEN Foundation develops other research projects on neurodegenerative diseases, among which the following stand out: the Research Program of the Queen Sofia Foundation Alzheimer Center and the National Network of Biobanks.

The CIEN Foundation currently has four departments devoted to scientific research: a department of Biochemistry and Molecular Genetics, for the processing and analysis of biological samples; a Clinical department, in which specialists in Neurology and Neuropsychology carry out the assessment and clinical follow-up of both patients treated at the center itself, as well as those volunteers from research projects who attend it; a Neuroimaging department, with a 3T Magnetic Resonance; and a Neuropathology department whose main activity corresponds to the CIEN Tissue Bank (BT-CIEN, for its acronym in Spanish) for the extraction, processing, classification and distribution of tissue.

From the administrative point of view, the CIEN Foundation is also responsible for managing other centers related to research in neurodegenerative diseases such as the Network Center for Biomedical Research in Neurodegenerative Diseases (CIBERNED, for its acronym in Spanish), maintaining collaboration agreements with the Carlos III Institute of Health for carrying out those management activities.

A SUSTAINABLE CENTER FOR THE BENEFIT OF RESEARCH

Until December 2020, the Queen Sofia Foundation Alzheimer Center has avoided emitting a total of 140,195 kg of CO_2 since its inauguration in 2007 thanks to the power generation from its solar thermal and photovoltaic panels. In total, they have produced 264,519 kWh, which has saved 134,756.36€ that have been allocated to research on this disease.

In its design and construction 18 years ago at the initiative of Her Majesty Queen Sofia, "special care" was taken that the project maintained a "deep respect for the environment", incorporating bioclimatic measures, an orientation adapted to its location, actions against the overheating, natural ventilation, water treatment or use of ecological covers. For this reason, it was recognized with the "Green Light" emblem of the European Commission and obtained the highest qualification in its energy certification by the Institute for Diversification and Energy Savings (IDEAE, for its acronym in Spanish).

CAFRS PHOTOVOLTAIC ENERGY

Billing since the opening of the Center	124.816,06 €
Accumulated production	264.519 kWh
CO_2 emissions avoided	140.195 kg

1.2. The CIEN Foundation in 2020

- CIEN Foundation researchers have published 24 scientific papers in specialized journals (20% more than in 2019), of which 20 (83.33%) have been published in journals classified in the first and second quartiles, with an average impact factor of 6,937.

- Celebration of the II edition of the Memorables Film Festival, a short film festival dedicated to Alzheimer's disease, whose main objective is to promote the creation and dissemination of short films that have this disease as a common thread.

- The ISCIII Research Professor and CIBERNED Principal Investigator, Miguel Calero, is appointed Scientific Director of the CIEN Foundation.

- Commemoration of the 10th anniversary of the inauguration of the CIEN Foundation Tissue Bank (BT-CIEN). During these 10 years, BT-CIEN's own activity has experienced sustained growth, both in the registration of donors and the collection of donated brain tissue, as well as in the transfer of samples to researchers.

- Dr. Bryan Strange, director of the CTB-UPM Clinical Neuroscience Laboratory and head of the Neuroimaging area of the CIEN Foundation, is awarded the 2020 Research Excellence Incorporation award from the Polytechnic University of Madrid.

- In May, Dr. Miguel Medina is appointed head of the Department of Biochemistry and Molecular Genetics.

- The acquisition of a digital ELISA kit (Simoa, Quanterix) for the determination of biomarkers was approved in November.

- Adaptation of the work to the COVID-19 scenario. New work organizational systems have been introduced to adapt as best as possible to the new situation. E.g.: implementation of hygiene and prevention measures in the workplace included in the action plan against COVID-19, flexible hours, promotion of working from home, organization of the shifts and new shift registration system.

- Incorporation of the beneficiary of the Queen Sofia Foundation-Mapfre 2020 fellowship, to carry out stays at the CIEN Foundation and at the Cologne University Hospital in Germany.

- A survey of the entire Vallecas Project cohort was carried out to determine how the SARS-CoV-2 pandemic had impacted the elderly population. Subsequently, with the information collected, a scientific study was carried out, recently published in the journal

Gerontology.

- By virtue of the replacement rate assigned to the entity and the job stabilization process, included in the General State Budget Law for 2018, 11 permanent jobs have been called, something very positive, since it allows to generate stability in structural positions in the CIEN Foundation, reducing the temporary nature of contracts in the entity from 70% to 10%.

- The CIEN Foundation seeks to ensure that management is in line with the needs and demands of society, and for this reason the commitment has been made to draw up the 1st Plan for Equal Opportunities between women and men.

- To increase knowledge and experience in the area of pathological anatomy of the Medical Intern Residents (MIR) program, during 2020 two MIR rotations were accepted from the 12 de Octubre University Hospital and the Integrated Care Management of Albacete in the area of Neuropathology of the CIEN Foundation.

- · Thanks to the current agreements between different universities, educational centers and the CIEN Foundation, a total of 2 students from the Autonomous University of Madrid and the King Juan Carlos University have carried out internships at our center.

- A psychosocial evaluation of the entity has been carried out. The main objective of this action is to provide information that allows the psychosocial diagnosis of an entity

or partial areas of it, in order to be able to establish improvement actions appropriate to the risks detected and the environment in which they must be carried out.

- During 2020 we have continued to promote activity on social networks, creating a growing community. In the last year, the number of followers on the CIEN Foundation's social profiles has increased by 2.7%.

- A Senior Graduate is hired for the Department of Biochemistry and Molecular Genetics in December.

- Beginning of clinical evaluations within the project "Family history of Alzheimer's disease in older people: influence of subjective cognitive impairment" (RTI2018-098762-A-C32) funded by the State Program of R+D+i Oriented to the Challenges of the Society of the Ministry of Science and Innovation.

1.3. Organizational structure

1.3.1. Governing and Management Bodies

The CIEN Foundation is composed of three bodies, one of management, represented by Mrs. Mª Ángeles Pérez Muñoz, manager of the CIEN Foundation; another one of scientific management, represented by Dr. Miguel Calero Lara, and a third governing body, the

Board of Trustees:

a) The Board of Trustees is responsible for the government and representation of the CIEN Foundation as well as for the fulfillment of the Foundation objectives, administration and management of its capital assets. Board members represent all sectors involved in neurological diseases research: public institutions related to the field of health, research, social and industrial policy, technology, business, and education.

b) Scientific Director: represented by Dr. Miguel Calero Lara.

c) Managing Director: represented by Ms. M. Angeles Perez Muñoz.

1.3.2 Advisory and participation bodies

External Scientific Advisory Committee

In the Board meeting held on March 10, 2014, the composition of the CIEN Foundation External Scientific Advisory Committee it is presented and approved. It is aimed at improving the scientific quality of the activities, optimizing the available resources, and exploiting the singularities of the Queen Sofia Foundation and the Alzheimer Center. Since that date, the Committee is formed by the following members:

- Mr. Joaquín Arenas Barbero. BSc in Pharmacy and PhD in

Clinical Biochemistry by Faculty of Medicine of Complutense University of Madrid. He has held the positions of General Director and Deputy General Director of Networks and Cooperative Research Centers within the Carlos III Institute of Health (ISCIII). He is currently Director of the 12 de Octubre Hospital Research.

- Mr. Javier De Felipe Oroquieta. PhD in Biological Sciences by the Complutense University of Madrid. Neurobiologist at the Cajal Institute. He leads the Spanish team of the Blue Brain international project (Cajal Blue Brain) integrated by scientists from the Technical University of Madrid and CSIC. He belongs to CIBERNED and collaborates actively with the CIEN Foundation.

- Mr. Miguel Medina Padilla. PhD in Biochemistry and Molecular Biology from the Autonomous University of Madrid. Deputy Scientific Director of CIBERNED and principal Investigator of the Vallecas Project.

- Mr. José Ramón Naranjo Orovio. PhD in Pharmacy from the Complutense University of Madrid. Neurobiologist at the National Center for Biotechnology (CSIC). He belongs to CIBERNED and collaborates actively with the CIEN Foundation.

- Mr. Fernando Rodríguez Artalejo. Doctor in Medicine and Surgery from the Autonomous University of Madrid. Professor of Preventive Medicine and Public Health at the Autonomous University of Madrid.

The Board of Trustees will function in plenary session and as a Delegated Commission.

At the end of 2020 the Board of Trustees has the following members:

HONORARY PRESIDENT

Mr. Pedro Duque Duque. Minister of Science and Innovation

President

Mr. Rafael Rodrigo Montero. Secretary General for Research of the Ministry of Science and Innovation.

Vicepresident

Ms. Raquel Yotti Álvarez. Director of the Carlos III Institute of Health

Ex-officio members

Ms. Rosa Menéndez López. President of the CSIC State Agency.

Ms. Pilar Aparicio Azcárraga. General Director of Public Health of the Ministry of Health.

Mr. Cristóbal Belda Iniesta. Deputy Director General of Evaluation and Promotion of Research of the Carlos III Health Institute.

Ms. Silvia Calzón Fernandez. Secretary of State for Health of the Ministry of Health.

Ms. Nuria Lera Hervás. Director of the Department of National Affairs of the Presidency of the Government.

Ms. Margarita Blázquez Herranz. Deputy Director General of Cooperative Research Networks and Centers of the Carlos III Health Institute.

Elected members

Mr. Javier Santos Burgos. Director General of Research and High Health Inspection of the Department of Universal Health and Public Health of the Region of Valencia.

Ms. Mª Teresa Serrano Gotarredona. General Director of Research and Knowledge Transfer, of the Ministry of Economy, Knowledge, Business and University of the Andalusian Regional Government.

Secretary

Ms. Margarita Blázquez Herranz. Deputy Director General of Cooperative Research Networks and Centers of the Carlos III Health Institute.

LEGAL ADVISOR

State Attorney

Mr. José Luis Beotas López

GUESTS

CIEN FOUNDATION

Scientific Director: Mr. Miguel Calero Lara

Managing Director: Ms. María Ángeles Pérez Muñoz

QUEEN SOFIA FOUNDATION

Mr. José Luis Nogueira Guastavino

DIRECTOR OF THE TECHNICAL CABINET OF THE SECRETARIAT FOR SCIENTIFIC POLICY COORDINATION

Ms. Petra Fernández Álvarez

ASSISTANT TO THE SECRETARIAT

Ms. Mª Dolores Donoso Mencía

The following members will will also form part of the Executive Committee:

- The General Director of Public Health, Quality and Innovation of the MSSI.

- The Deputy Director General of Evaluation and Promotion of Research of the Health Institute Carlos III.

- The General Director of Scientific and Technical Research of the Ministry of Science, Innovation and Universities.

- The Secretary will be the same as that of the Plenary.

Research Ethics Committee of the Carlos III Institute of Health

Since June 2010, the CIEN Foundation's research activity is supervised for clinical projects and for the activity of the Tissue Bank, by the Research Ethics and Animal Welfare Committee (CEIYBA, for its acronym in Spanish) of the ISCIII. The CEIYBA of the Carlos III Institute of Health is a collegiate body, according to what is described in Article 12 of Law 1412007 of July 3, on Research.

As of 2016, starting with RESOLUTION R-119/16 of Management, when the subcommittees that had been operating as CEIYBA were separated into the Research Ethics Committee and the Animal Welfare Ethics Committee.

From this moment on, the requests for reports will be processed to the corresponding committee.

With regard to the functions described in Law 14/2007 on Biomedical Research, the Committee provides services to the Centers and Units of the Carlos III Institute of Health, to the Foundations promoted by it and to mixed or associated Centers recognized by the Carlos III Institute of Health.

Thus, the CIEN Foundation requests a report on any study carried out with human beings, their samples, or their data through the Research Ethics Committee (CEI, for its acronym in Spanish).

1.4. Future outlook

In 2021, and in subsequent years, the CIEN Foundation will continue to promote the research work that has placed it as a reference in Spain in research on Alzheimer's and other dementias.

In addition, it will continue to promote its participation in research programs. The CIEN Foundation will carry on during 2021 with the management of CIBERNED (initials in Spanish for Center for Biomedical Research on Neurodegenerative Diseases Network). In this context of strengthening the CIEN Foundation-CIBERNED relationship, both institutions have postponed in

2020 due to the COVID-19 pandemic the Global Summit Neuro 2020, an event under the honorary presidency of Her Majesty Queen Sofia, which finally will be held in Salamanca in 2022, also coinciding with the VIII edition of the International Congress on Research and Innovation in Neurodegenerative Diseases, and the XV CIBERNED Scientific Forum.

During 2021 the launch of a new platform based on new ultrasensitive technologies for the detection of biomarkers in biological fluids useful in the early diagnosis of neurodegenerative diseases is expected. The acquisition of new equipment with this technology based on digital ELISA for single molecule detection will allow the establishment of the necessary procedures for its implementation during the first months of the year. In the context of the CIEN Foundation, the acquisition of a highly sensitive peripheral biomarker analysis platform is considered especially relevant for the enhancement and better use of the Vallecas Project, as well as other Foundation projects.

In addition to continuing the studies launched, there are several new lines of research around the Vallecas project that are considered to be of great interest in the near future. Likewise, the results of different ongoing studies (explained in detail in Section 4 of this report devoted to the Vallecas Project) are expected to be published in 2021.

Donations of brain tissue have been suspended since March 2020 for bio-safety reasons. During the year 2021, tissue extractions will be carried out in the hospital environment, while a reform of the CIEN Foundation autopsy facilities is designed, planned and executed to adapt them to the new biosafety requirements applicable to the pandemic and post-COVID period. In this new context, which includes strict inclusion criteria for carrying out extractions (guarantee that donors are not infectious for COVID-19), it is expected that around 50 extractions will be carried out during the year 2021. Consequently, one of the activities planned for 2021 will be the planning and execution of the necessary reforms in the BT-CIEN autopsy facilities, depending on the availability of financing for this.

In 2021 it is also intended to complete the redesign of the residence's patient registry. Given the large amount of data of various kinds that exists for each patient, and due to the increasing number of participants in this CAFRS Research Project, the need to redesign the current database to make it more efficient and manageable has been detected, and to facilitate the analysis of these data using different software such as MATLAB, R, or SPSS. Likewise, this improvement in the structure of the database will facilitate collaboration in joint research projects with other research centers at a national and international level, as well as the integration of the Alzheimer's Project data in international online platforms where the data from cohort studies, such as the European Medical Information Framework (EMIF) or the Global Alzheimer's Association Interactive Network (GAAIN).

Regarding the collaboration with the industry, the CIEN Foundation and Biocross SL collaborate in the deve-

lopment of an immunoturbidimetric kit for the qualitative determination of apoliprotein E4. For the year 2021, it is intended to maintain this collaboration through the development of a quantitative test for the detection of the E4 isoform of ApoE.

In addition, it will continue to promote the internationalization of its activity in the medium term. This commitment will be based on three fundamental pillars that have so far provided excellent results: the participation of the Foundation's professionals both in international research projects and in con-

gresses and scientific meetings where they can present the progress made in this field; the start-up of own initiatives or at the request of the Carlos III Health Institute outside our borders; and the participation of the CIEN Foundation together with CIBERNED in the Joint Programming on Neurodegenerative Diseases launched in the European Union, as well as the synergies that may derive from its incorporation into the CoEN network.

In terms of management, the medium-term evolution of the CIEN Foundation will be governed by the same
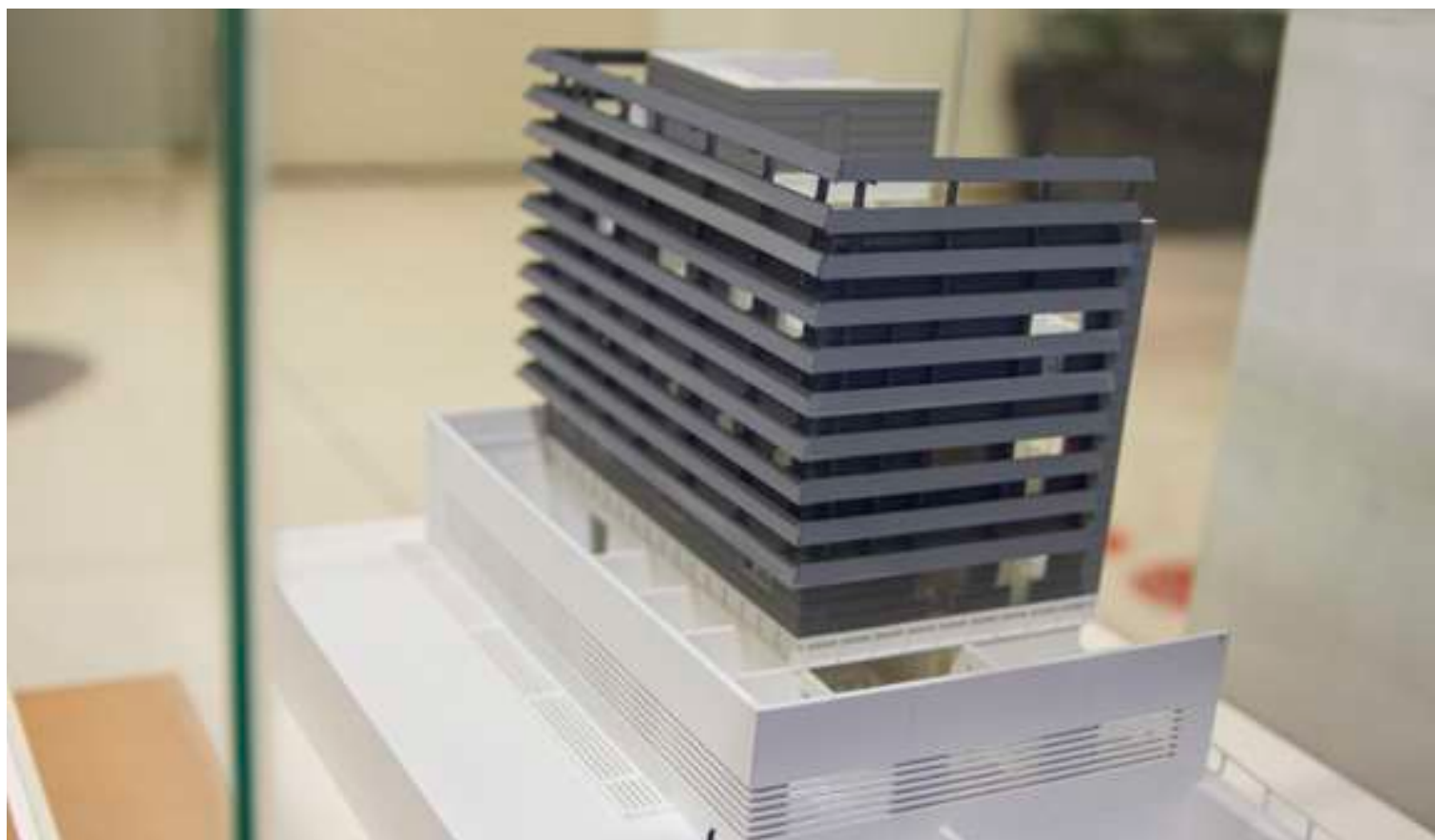
criteria applied since its constitution: sustainable management, betting on a model of public-private collaboration; the continuous training of its professionals, which guarantees a research model of excellence; and the information and awareness-raising work carried out by the Foundation to convey to society the importance of research to advance the knowledge of neurodegenerative diseases in general, and Alzheimer's disease in particular.

02.

MANAGEMENT REPORT

2. MANAGEMENT REPORT

2.1. General management

CIEN Foundation is a Statewide public sector Foundation supervised by the Carlos III Institute of Health under the Ministry of Economy, Industry and Competitiveness.

The CIEN Foundation is governed by its Statutes; by Law 50/2002, of December 26, on Foundations; by Royal Decree 1337/2005, of November 11, which approves the Regulations of the Foundations of State competence; by the provisions of the National Budget Bill, in everything that refers to the Foundations of the State Public Sector; by Royal Decree 384/1996, of March 1, on the Registry of Foundations of State Competence; by Law 49/2002, of December 23, on the Tax Status of Non-Profit Organizations and Tax Incentives to Patronage; and for the rest of the legal provisions of an administrative, civil, mercantile or labor nature that apply to it.

The purpose of the CIEN Foundation is to promote research in all fields of basic, clinical and epidemiological neurology, aspiring to become a Center of national and international reference on research in Alzheimer's disease and other dementias. The CIBERNED-CIEN Foundation consortium is the only Spanish institution recognized as a Center of Excellence in Neurodegeneration within the Centers of Excellence in Neurodegeneration Network (COEN), a complementary initiative to the JPND to establish a common approach to research in neurodegenerative diseases, promoting collaborative research between recognized national Centers of Excellence in neurodegeneration in order to accelerate progress in understanding the mechanisms of the disease, as well as the identification of new therapeutic approaches.

Specific aims include fostering scientific advances to have an impact on the healthcare system and the well-being of patients. To this end, an offer of services carried out by various Consultation Units and Research Groups of the CIEN Foundation has been articulated and made available to users outside the Center.

· Cerebral tissue sample request service

· Magnetic resonance Imaging acquisition service

· Diagnostic consultation service

2.2 Management of financial and economic resources

The CIEN Foundation is funded by specific subsidies granted by the State and other local or institutional public entities, as well as by income derived from European and national research projects, contracts for the provision of services and patronage activities.

Income statement for the year 2020

The annual accounts are prepared from the accounting records of the Entity, having applied the legal provisions in force in accounting matters in order to show the true image of the assets, the financial situation and the results of the CIEN Foundation.

INCOME STATEMENT	Notes	2020	2019
A) Surplus for the year			
1. Income from own activity		1.614.992,80	1.792.403,96
d) Subsidies, donations and legacies attributed to the surplus for the year	10.1	1.614.992,80	1.792.403,96
2. Sales and other income from commercial activity	10.1	124.289,43	150.155,95
3. Expenses for grants and others		-24.646,10	-45.242,70
a) Monetary aid	10.2	-24.646,10	-45.242,70
4. Variation in inventories of finished products and in manufacturing process		-1.181,19	-293,41
6. Supplies	10.2	-109.352,67	-175.725,59
7. Other income from the activity	10.2	871,86	1.298,46
8. Personnel expenses	10.2	-825.594,88	-835.432,52
9. Other expenses of the activity	10.2	-532.489,62	-592.332,23
10. Amortization of fixed assets	5.1-5.2	-519.532,08	-524.958,84
11. Subsidies, donations and bequests of capital transferred to the surplus for the year.	11	515.289,62	526.203,18
13. Other results		1.547,71	-5.318,89
A.1) SURPLUS OF THE ACTIVITY (1+2+3+4+5+6+7+8+9+10+11+12)		244.194,88	290.757,37
16. Financial income		121,63	233,66
19. Exchange differences		4311,94	0
A.2) SURPLUS FROM FINANCIAL OPERATIONS (16+19)		4.433,57	233,66
A.3) SURPLUS BEFORE TAXES(A.1+A.2)	3	248.628,45	290.991,03

A.4) Variation in equity recognized in the surplus for the year (A.3+18)		248.628,45	290.991,03
B) Income and expenses charged directly to equity			
1. Grants received	11	102.530,23	50.000,00
2. Donations and bequests received	11	290.199,55	505.403,96
B.1) Variation in equity due to income and expenses recognized directly in equity (1+2+3+4)		392.729,78	555.403,96
C) Reclassifications to the surplus for the year			
1. Grants received	11	-23.360,27	-35.617,90
2. Donations and bequests received	11	-781.922,15	-1.007.989,24
C.1) Variation in equity due to reclassifications to the surplus for the year (1+2+3+4)		-805.282,42	-1.043.607,14
D) Variations in equity due to income and expenses charged directly to equity (B.1+C.1)		-412.552,64	-488.203,18
F) Adjustments for errors		-	-
G) Variations in the initial endowment		-	-
H) Other variations		-	-
I) TOTAL RESULT, VARIATION IN EQUITY IN THE YEAR (A.4+-D+E+F+G+H)		-163.924,19	-197.212,15

Revenues

In 2020, the CIEN Foundation managed an income budget of over 2.2 million euros. The main source of income comes from the nominative assignment of the Carlos III Institute of Health, which amounts to € 1.325,000 (representing 58% of total income), aimed at achieving the specific goals and objectives that are materialized in the promotion of research in health sciences, and develop and offer scientific-technical services of the highest quality, aimed at the National Health System and the whole of society.

The breakdown of total revenues obtained in 2020 and 2019 has been as follows:

Description	2020	2019
Grants, donations and legacies charged to surplus for the year	1.614.992,80	1.792.403,96
Sales and other income from commercial activity	124.289,43	150.155,95
Other income	871,86	1.298,46
Grants, donations and legacies transferred to capital surplus for the year	515.289,62	526.203,18
Financial income	121,63	233,66
Exchange rate gains	4.327,75	-
Reversion of impairment loss from trade accounts receivables		6431,14
Provision surplus	1.547,71	-
Total	2.261.440,80	2.476.726,35

Revenues of the institution consist mainly of grants, donations and operating legacies and capital received from Public Administrations and other institutions, companies and individuals.

Grants, donations and legacies charged to surplus for the year	Importe
ISCIII 2018 NOMINATIVE ALLOCATION CURRENT EXPENSES	1.325.000,00
OTHER DONATIONS CHARGED TO SURPLUS	30.250,00
VALLECAS 2 PROJECT - QUEEN SOFIA FOUNDATION	200.000,00
QUEEN SOFIA FOUNDATION --MAPFRE FELLOWSHIPS	15.793,25
PT17-0015-0015/ ISCII	34.134,44
PEJ-2017-TL-BMD	3.536,11
RTI2018-098762-A-C32	6.279,00
	1.614.992,80

The Carlos III Institute of Health, in the exercise of its functions of planning, promotion and coordination of biomedical and health research and innovation, resolves to grant the CIEN Foundation a nominal allocation for current expenses of the year 2020 of 1,325,000 euros.

Resolution 191/20 (I), of April 22, 2020, of the Directorate of the O.A., M.P. Carlos III Institute of Health, which regulates the conditions for the concession to the Neurological Diseases Research Center Foundation of the nominative allocations provided in the Statement of Budget Expenditures of the Carlos III Institute of Health for the year 2020.

The Neurological Diseases Research Center Foundation is a foundation of the State public sector supervised by the ISCIII, whose specific aims and objectives can be summed up in the promotion of research in the neurological field and the creation and maintenance of the referred Center. For the fulfillment and development of these purposes, the Statement of Expenditure of the ISCIII Budget extended from 2019 to 2020 approves the granting of the following nominative contribution:

The financial aid regulated in this resolution constitutes the annual contribution of the ISCIII to the global financing of the Foundation's activity.

- The amount of 637,500 euros charged to the budget application 28.107.465A.445, (Resolution 191/20 (I), of April 22, 2020, registered allocation 50% 2020).

- The amount of 637,500 euros charged to the budget application 28.107.465A.445, (Resolution 191/20 (I), of September 15, 2020, remaining registered allocation 50% 2020).

On November 3, 2020, the Director of the Carlos III Institute of Health O.A., M.P., issued the Resolution, which regulates the concession conditions to the Foundation Center for Research of Neurological Diseases, of the nominative assignments provided for in the statement of expenses of the Carlos III Institute of Health budget.

In the General State Budget Bill for 2020, allocations to the CIEN Foundation were included in the budgetary applications 28.107.465A.445 "To the Research Center for Neurological Diseases Foundation. Current operations", of 1,275,000 euros and 28.107.465A.745 "To the Research Center for Neurological Diseases Foundation. Capital operations", of 50,000.00 euros. However, in the ISCIII budgets extended for 2020, there is a single allocation to the CIEN Foundation for current operations in application 28.107.465A.445 for an amount of 1,325,000.00 euros, therefore, there is no allocation in the aforementioned Foundation intended to finance capital operations, contemplating only amounts for operations of a current nature.

That is why, on June 23, 2020, a transfer of credit for current operations was approved, with a decrease in the budgetary application 28.107.465A.445 for the amount of 50,000.00 euros, in order to contribute to the financing necessary for the fulfillment of the foundational purposes.

On the other hand, the seventh section of the Agreement by the Council of Ministers of December 27, 2019, which establishes the criteria for the extension for 2020 of the General State Budget for the year 2019, establishes that from January 1, 2020 and until the approval of the General State Budget Bill for the year 2020, the bodies of the ministerial departments, their autonomous bodies and the competent social security management bodies and common services, as well as the rest of the entities with a limited budget,

may not initiate the processing of new expenditure files attributable to Chapters 4, 6, 7 and 8 of the corresponding budgets, with the exception of Transfers of Chapter 4 intended to finance pensions, passive classes and unemployment, neither approve nor commit new expenditure of files already initiated under the aforementioned chapters, when the sum of the amount of expenditure approved for the financial year 2020 and the amount subject to approval of the new file has reached 50% of the initial appropriation of the budget carried over to which the operation corresponds at the level of linkage and exceeds 50% of the initial amount of the chapter of the budget extended to that corresponds to the operation in each ministerial department, autonomous body and managing entity and common service of the Social Security and other entities with

a limited budget.

On October 25, 2020, the Ministry of Finance has authorized exceeding the previous limit in the processing of the expenditure file corresponding to ISCIII Contribution as support to the achievement of the purposes of the CIEN Foundation, with the amount of 50,000 euros charged to application 28.107.465A.745.

By virtue of this authorization issued for the purposes of the seventh paragraph of the Agreement by the Council of Ministers of December 27, 2019, the following is resolved:

FIRST: Allocate to the Foundation the following nominative contribution foreseen in the statement of expenses of the ISCIII extended budget for 2020:

- The amount of 50,000 euros charged to budget application 28.107.465A.745.

These contributions will be compatible with subsidies, grants, income, or resources for the same purpose, coming from any Administration or public or private, national, coming from the European Union or international organizations.

The contribution of the Queen Sofia Foundation in 2020 focuses on the funding of three main activities: i) collaboration in carrying out the Vallecas-2 Project, early detection of Alzheimer's disease. Risk factors and prevention;

ii) call for the Queen Sofia Foundation-MAPFRE Foundation fellowship.

On April 24, 2020, the Queen Sofia Foundation announces that the Board of Trustees of the entity agrees to contribute, during the year 2020, the amount of 300,000 euros, in accordance with the provisions of the second clause of the Collaboration Agreement for the execution of the Vallecas II project, signed with the CIEN Foundation on February 14, 2018. Subsequently, in October 2020 it is announced that the bodies of the Reina Sofia Foundation agree to allocate part of the amount granted, specifically 200,000 euros, to the acquisition of a platform that allows the quantification of biomarkers in plasma. Finally, and after the preparation and publication on the public sector procurement platform of the file for the award of the contract, it is agreed that 100,000 euros will be destined to the acquisition of the aforementioned equipment (amount of the base budget of the tender foreseen in the contracting file) and 200,000 euros will be destined to current expenses of the year.

The remaining amount corresponding to the subsidies, donations and legacies allocated to the surplus for the year is identified with the projects financed by the ISCIII (Biobank Platform-PT17), by the Region of Madrid (Youth Employment Program, PEJ2016/PEJ2017) and the RTI 2018 project.

The item of sales and other revenues from the activity correspond to the provision of research services, based on contracts signed with other public and private research centers.

Revenues from provision of services during 2019-2020

Description	2020	2019
Energy production	2.308,05	9.421,77
Revenues from performing MRIs and collaborative research projects	121.981,38	140.734,18
TOTAL	124.289,43	150.155,95

In the subsidies, donations and capital legacies item transferred to the surplus of the year (515,289.62 €), a contribution from the Queen Sofia Foundation of 491,929.35 € is recorded, corresponding to the rights of use of the building, equipment, and furniture of the CIEN Foundation. The remaining amount until reaching the total of this item corresponds to the official capital grants received from the ISCIII financing of capital expenditures, € 23,360.27.

Expenditure

The expenses are used for the development of the CIEN Foundation's own activities, which basically correspond to the research and management activities of the Foundation itself, which is part of the Queen Sofia Foundation Alzheimer Project Complex. The Queen Sofia Foundation and the CIEN Foundation formally signed in January 2006, renewed in January 2016, an agreement whereby the former cedes the use of the premises and their equipment, and the CIEN Foundation is committed to the maintenance of the dependencies and equipment, replacing, and repairing the one that is necessary. The use of facilities will be used as a priority for the research of Alzheimer's disease and other dementias, and in a complementary manner to other types of research on diseases that are part of the CIEN Foundation's mission.

Distribution of CIEN Foundation expenses

YEAR	2020	2019
Monetary aid and others	24.646,10	45.242,70
Variation of existences of merchandise	1.181,19	293,41
Supplies	109.352,67	175.725,59
Purchase of merchandise	39.306,66	108.071,46
Work performed by other companies	70.046,01	67.654,13
Staff costs	825.594,88	835.432,52
Wages, salaries and similar expenses	649.349,35	653.529,11
Social Security paid by employer	175.916,29	181.474,80
Other social welfare costs	329,24	428,61
Other operating expenses	532.489,62	592.332,23
Leases and fees	7.380,52	5.819,26
Repairs and preservation	300.902,95	280.518,43
Services of independent professionals	90.729,29	50.575,01
Transportation	2.707,82	3.249,82
Insurance premiums	18.720,94	22.355,77
Banking and similar services	126,89	125,42
Advertising and publicity	28.228,69	39.577,18
Supplies	46.660,84	54.885,84
Other services	36.651,04	131.468,02
Other taxes	380,64	3.757,48
Provisions for fixed assets amortization and depreciation	519.532,08	524.958,84
Amortization of intangible fixed assets	496.805,87	497.040,24
Amortization of tangible fixed assets	22.726,21	27.918,60
Impairment and gains on disposal fixed assets	0	5.318,89
Exchange rate losses	15,81	0
TOTAL EXPENDITURE	2.012.812,35	2.179.304,18

2.3. Research projects and grants

CIEN Foundation aims to support, promote, and coordinate research in neurological diseases. To do this, it focuses its efforts especially in neurodegenera tive diseases and in coordinating prominent Spanish research groups. Research projects managed by the Foundation seek to foster research and study in these fields, especially Alzheimer's disease and related disorders.

2.4.1 Research projects

The CIEN Foundation was created with the aim of promoting the foundation of a network center that supports, promotes, and coordinates research activities. The objectives of the CIEN Foundation are specified in six fundamental activities:

- The Vallecas project
- BT-CIEN Tissue Bank.
- Alzheimer Program
- Other research projects
- Service provisions
- Training

Vallecas Project: Research aimed at identifying individuals with a higher risk of developing Alzheimer's type dementia (AD), based on the combination of socio-demographic, clinical, neurological, neuropsychological, genetic, biochemical and neuroimaging data, within the framework of the project called "Vallecas-2, early detection of Alzheimer's disease. Risk and protection factors". The Queen Sofia Foundation, signatory of the agreement dated on February 14, 2018, undertakes to contribute during each of the 4 years of planned duration of the project, the successive amounts that are agreed annually by its Board of Trustees, corresponding to the first three years a total amount of € 300,000.00.

YEAR	2018	2019	2020
Queen Sofia Foundation Contribution	300.000,00	300.000,00	300.000,00

The Vallecas Project is one of the main research projects carried out by the CIEN Foundation, both in terms of resources used and its social impact. In parallel, the CIEN Foundation is engaged in other research projects on neurodegenerative diseases, among which the Alzheimer Center Project and the Biobank Platform stand out.

Tissue-Bank (BT-CIEN): Through the Department of Neuropathology of the CIEN Foundation, a biobank of neurological tissue is managed (CIEN Tissue Bank). Contributions to BT-CIEN come, in addition to those obtained in the CAFRS (Internal Donation Program), from donors from the Community of Madrid and other Regions (External Donation Program). In turn, the BT-CIEN, after processing, diagnosis and classification, maintains the biological material in long-term archive conditions and distributes it to researchers and institutions that require it for research projects, in accordance with the application and transfer of biobank

samples protocols.

Research Program of the Queen Sofia Foundation Alzheimer Center: Systematic evaluation, by means of a protocol adapted and agreed upon, of the patients institutionalized in the Queen Sofia Foundation Alzheimer Center (CAFRS, for its acronym in Spanish) and attendants to the Day Care Center, after signing a proper informed consent. This evaluation includes the following:

- Neurological evaluation
- Neuropsychological assessment
- Psychiatric evaluation
- Functional evaluation
- Magnetic resonance imaging
- Blood sampling and analysis of genetic risk factors
- Extraction and neuropathological study of brains donated by deceased patients

Other research projects granted in competitive competition in force in 2020:

- RTI2018-098762-A-C32. Call for projects with resolution date on August 26, 2019, from the Secretary of State for Universities, Research, Development and Innovation and the Presidency of the State Research Agency, approving the call for early processing for the year 2018 of the procedure

for granting support to R+D+i Projects "Research Challenges", corresponding to the State R+D+i Program aimed at Society Challenges, within the framework of the State Plan for Scientific and Technical Research and Innovation 2017-2020. Funded by the Ministry of Science, Innovation and Universities. Project title: Family history of Alzheimer's disease in older people: influence of subjective cognitive impairment. Principal Investigator: Dr. Miguel Ángel Fernández.
Duration: 01/01/2019 - 16/07/2020. On February 24, 2021, the Director of the State Research Agency signs and communicates the agreement to start the procedure for the early termination of the R+D+i research project due to the absence of link between the principal investigator 2 and the reference research project RTI2018-098762-A-C32, since July 16, 2020.

- PT17/0015/0014: Biobank Platform. Principal Investigator: Dr. Alberto Rábano Gutiérrez. Project funded by Resolution of the Director of the Carlos III Institute of Health, of December 4, 2017, by which grants are awarded for support research Platforms in health sciences and technologies of the 2017 call for Strategic Action in Health, with a total budget of 135.300 euros, distributed in 3 annuities of 45,100 € each. The Carlos III Institute of

Health authorized extending the project execution deadline until June 30, 2020.

- Collaboration agreement signed with King's College London, University of Pennsylvania and University College London, for carrying out the project entitled "Phases 2b-4 Field Validation of the MDS-NMS, the International Parkinson's and Movement Disorders Society Non-Motor Scale for Parkinson's disease" funded by The International Parkinson and Movement Disorders Society. Budget: 39,375€.

- Specific collaboration agreement Established between CIBER and CIBERNED for the execution of the project "Search for biomarkers for the early detection of Alzheimer's disease in the Vallecas project cohort", with the active participation of the Neuroimaging department and the CIEN Foundation Tissue Bank.

Actions funded within the framework of Law 18/2014, of October 15, of approval of urgent measures for growth, competitiveness, and efficiency and of the Youth Employment Operative Program:

- PEJ-2017-TL-BMD. Order 4606/2017 of December 14 for which grants are given for the execution of contracts for laboratory technicians corresponding to the 2017 call. Granting of support for the employment of a laboratory technician during 2018. The CIEN Foundation, as the grant beneficiary, formalized the corresponding contract on 03/08/2018, with a duration of two years, until 03/07/2020

2.3.2 Fellowships and grants

During 2020 the CIEN Foundation has awarded/carried out the following fellowships and grants:

- MAPFRE-Queen Sofia Foundation Fellowship 2020. Research program focused on the identification of functional brain networks in cognitively healthy individuals who subsequently develop mild cognitive impairment, as well as individuals over the age of 80 who show extraordinary memory skills ("Superagers") identified within the Vallecas project. Duration of six months, extendable to an additional 6 months (maximum, 12 months). The awardee started the grant on October 1, 2020, at the University Hospital of Cologne, Germany.
  Host laboratories:

  » Department of Neuroimaging, Queen Sofia Foundation Alzheimer's Center, Madrid, Spain.

  » Department of Neurology.

University Hospital of Cologne, Germany.

- MAPFRE-Queen Sofia Foundation Fellowship 2018-2019. Research program focused on the identification of individuals at high risk of imminent conversion to cognitive impairment. Six months, extendable for another 6 additional months (maximum, 12 months). The winner of this scholarship made her last stay at the CIEN Foundation until February 29, 2020.
  Host laboratories:

  » Department of Neuroimaging, Queen Sofia Foundation Alzheimer's Center, Madrid, Spain.

  » Biomagnetic Center, Brain Structural Mapping Group, Department of Neurology, Jena University Hospital, Germany (period of stay is negotiable, up to a maximum of 6 months).

2.4. Management of Human Resources

2020 has been an atypical year due to the impact suffered, both professionally and personally, derived from the COVID-19 pandemic. This has made us modify our work habits and we have all had to adapt to a new normal, which no one was prepared for.

From the CIEN Foundation, always fo-

llowing the guidelines and recommendations of the Ministry of Health, the Ministry of Territorial Policy and Public Function and the Carlos III Institute of Health, a series of actions have been carried out to try to mitigate the impact of the pandemic on the development of the activity.

After the state of emergency was established nationwide on March 14, 2020, the organization had to adapt to the new scenario and for this the following measures were carried out:

- Drafting of an action and reincorporation plan against COVID-19.

A plan was drawn up so that all staff from the CIEN Foundation knew the health and prevention measures that should be complied with, as well as the procedures to be followed in case of showing any symptoms.

- Promoting remote working from home.
  Following the recommendations of the competent authorities, the habits have been modified so that the staff who can carry out their work from home could do so, being able to combine it with the face-to-face activity. In this way, the activity of the entity has been maintained, avoiding possible contagions in the workplace, and reducing mobility. During this period, the activity carried out has been monitored

through activity reports. Workers were enrolled in an occupational risk prevention course, for the correct use of data visualization screens in a working from home situation.

- Organization of shifts.
  In positions where working from home it is not possible, or in positions in which part of the day must be attended in person at the center, work shifts have been reorganized to avoid the accumulation of people. This measure facilitates interpersonal distancing, avoiding possible risks of infection.

- Conciliation.
  Several steps have been taken to facilitate the balance of family and work, either through remote working or through flexibility in schedules.

- New clock in system.
  In compliance with Royal Decree-Law 8/2019, of March 8, the clock in system has been updated. In June, a new tool was implemented through the intranet, so that all workers register their day in an simple and effective way, being able to do it both in the remote working and in the in-person modalities.

- COVID-19 Training
  Given the great lack of knowledge about the virus at the beginning of the pandemic, workers were enrolled in online training on the

following topics:

- » COVID-19 general knowledge
- » COVID-19 prevention service
- » COVID-19 health indications and preventive measures

2.4.1. Job announcements

The publication of the hiring calls planned along 2020 has been greatly affected by the COVID-19 pandemic.

The CIEN Foundation is located within the Queen Sofia Foundation Alzheimer's Center and shares some spaces with the residence. Residents are very vulnerable to the virus, so since the beginning of the pandemic extreme precautions have been taken to help avoid any risk in the center.

Once the worst part of the pandemic was over, the planned incoming hirings

calls were published, with the new personnel additions occurring over the last 3 months of the year.

It should be noted that by virtue of the replacement rate assigned to the entity and the process of stabilization of job positions, both included in the General State Budget Bill for 2018, 11 indefinite jobs have been called, something very positive, since it allows to generate stability in structural positions in the CIEN Foundation, reducing the temporality of contracts in the entity from 70% to 10%.

2.4.2. CIEN Foundation Organization Structure

The different departments in which the human resources that make up the CIEN Foundation are located and where our professionals have developed their work are the following:

- Department of Management and Administration
- Department of Neuroimaging
- Department of Neuropathology and Tissue Bank
- Department of Molecular Genetics
- Clinical Department

The following table shows a list of CIEN Foundation research staff in 2020, and its distribution by sex:
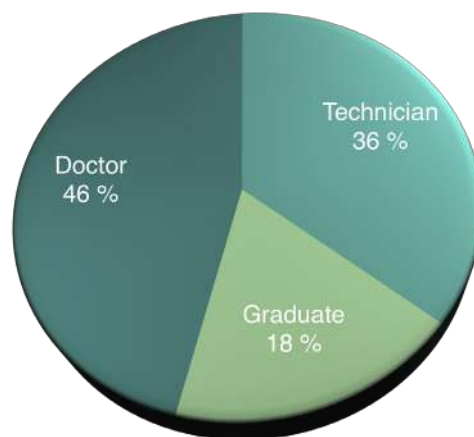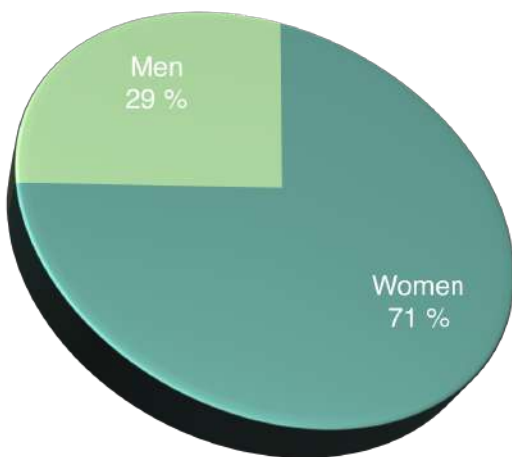
2.4.3. Equal Opportunities Plan

The CIEN Foundation seeks to ensure that the management is in line with the needs and demands of society, and therefore has assumed the commitment to the preparation of the I Equal Opportunities Between Women and Men Plan, following the guidelines set out in the legislation on the matter, as set out in Organic Law 3/2007, for the effective equality of women and men, as well as the current regulations indicated in the urgent measures to guarantee gender equality of Royal Decree-Law 6/2019, of March 1. With the implementation of this Equality Plan we intend to continue advancing in the development of a favorable framework of labor relations based on equal opportunities, non-discrimination, and respect for diversity, promoting a safe and healthy environment; as well as an improvement of our management system contributing to the progress towards a society in which equality is real and effective.

2.4.4. Psychosocial evaluation

The evaluation of psychosocial factors is a tool whose main objective is to provide information that allows the psychosocial diagnosis of an entity or partial areas of it, in order to establish improvement actions appropriate to the risks detected and the environment in which they must be carried out. It measures factors such as working time, autonomy, workload, psychological demands, variety/content, participation/supervision, interest in

TRAINING COURSES AND ACTIONS CARRIED OUT BY OUR STAFF

TRAINING ACTIVITY	CENTER/INSTITUTION
28th Annual Computational Neuroscience Meeting	CNS
Biobank accreditation ISO 20387 standards	Official College of Chemists of Madrid
Alzheimer's & Parkinson's Diseases Congress	Alzheimer's & Parkinson's Diseases Congress
CONY 2019: the 13th World Congress on Controvesies in Neurology	World Congress on Controvesies in Neurology
Immunohistochemistry Course	Zamora's Association of Pathology and Cytology Technicians
Microcopy and applications Course	Optics Institute
Course Multiple aspects of your activity in pathological anatomy services	IFAPES
World ICTUS Day	Spanish Society of Neurology (SEN), Stop Ictus, GEECV
FCIEN-PurinesDX Symposium on Advances in clinical techniques and challenges faced in the treatment of neurological disorders	CIEN Foundation and Marie Sklodowska-Curie Innovative Training Network
I Meeting of CNIE Researchers	CNIE
Artificial Intelligence in Radiology, opportunity or threat	Radiodiagnosis Service of the University Hospital of La Zarzuela
First aid	QUIRÓN PREVENCIÓN
Automatic microscopic image processing	Castilla-La Mancha University
Responsible Innovation in smart homes and smart health virtual summit	Living Innovation
Meeting of the Spanish Society of Neurology in Seville	Spanish Society of Neurology
World Congress on Controversies in Neurology	COMTECMED
X National Biobanks Congress	National Network of Biobanks, Center for Biomedical Online Research in Respiratory Diseases, Generalitat Valenciana, Valencian Network of Biobanks
VII International Congress of Research and Innovation in Neurodegenerative Diseases	CIEN Foundation, CIBERNED and Queen Sofia Foundation

the worker, role performance and relationships and social support.

2.4.5. Training program

Continuous training and updating of professional knowledge and skills have been configured as a training system intended to accompany workers in their personal development and professional promotion capacity. It constitutes a fundamental support to the competitive and innovative capacity of organizations based on quality human resources.

The main objective of the CIEN Foundation through the training program is to provide adequate means for the development of professional skills, in order to effectively manage the challenges that each job entails. Training is an integral part of our culture as an organization, and we are aware of the need to permanently improve the knowledge and skills of our professionals.

In 2020 the CIEN Foundation has offered or participated in the following training activities:

TRAINING ACTIVITY

END OF DEGREE INTERNSHIPS, MASTER'S FINAL PROJECTS

- Tutoring of students in the Clinical Area:

- 1 student from URJC in curricular intership.

- Tutoring of students in the field of Neuropathology:

- 1 student from the Autonomic University of Madrid in extracurricular internship

- 1 Medical Intern Resident from the University Hospital Virgen de las Nieves.

- 1 Medical Intern Resident from University Hospital 12 Octubre.

- 1 Medical Intern Resident from the Integrated Attention Management from Albacete.

FELOWSHIPS

- MAPFRE-Queen Sofia Foundation Fellowship 2020

- MAPFRE-Queen Sofia Foundation Fellowship 2018-2019

2.4.6. Prevention of Occupational Hazards

To guarantee the protection of occupational health and safety, CIEN Foundation has carried out, in coordination with the Prevention Service, various preventive activities during the year 2020, among which the following stand out:

- Emergency and evacuation drill.

- Online emergency response training.

- Carrying out specific training on prevention with the External Prevention Service for new

employees on the payroll.

- Information for newly incorporated staff members of the CIEN Foundation.

- Study of the possibility of carrying out measurements of chemical pollutants in the brain cutting room and in the laboratory. Due to the extraordinary situation of a global pandemic, measurements have been postponed.

- Beginning of the risk assessment of psychosocial factors.

- Individual health surveillance has been carried out offering it to the entire staff. The medical examinations carried out have been subjected to specific protocols based on the risks to which the workers are exposed. Serological tests have been performed on all personnel for the detection of IgG and IgM.

As a result of the state of emergency due to the SARS-CoV-2 pandemic, different actions have been implemented:

- Online training for all staff on the following topics:

- COVID-19 general knowledge

- COVID-19 prevention service

- COVID-19 health indications and preventive measures

- Risk prevention when working with data visualization screens in a teleworking situation.

- Informing the entire staff about the risk of exposure to COVID-19, and its preventive measures.

- Sending the questionnaire for the development of teleworking at home in temporary and extraordinary situations.

- Creation of the reinstatement plan and communication to all staff.

- Coordination of business activities on the preventive measures implemented with all external companies that access the CIEN Foundation facilities.

- Study and search for personal protection equipment (PPE).

- Study by the Health Surveillance of especially sensitive workers according to the prevention protocol published by the Labor Ministry.

- Preparation of the complementary evaluation of occupational risks for the personnel who temporarily carry out remote working.

- Regarding health surveillance, during the annuity contracted with the External Prevention Service in the period between January 2020 and January 2021, a total of 27 medical examinations have been carried out. The health examinations have included a work history that includes a detailed job description, the time spent

in it, the risks detected in the analysis of the working conditions and the prevention measures adopted, anamnesis data, clinical examination, biological control and complementary studies, directed and chosen according to the risks inherent to the work performed.

- Finally, the objectives of improving the health and safety conditions of workers and reducing the accident rates taken as a reference by the Mutual Association for Work Accidents and Occupational Diseases have been met, keeping these rates at zero.

2.5. Quality Policy

During 2020, the follow-up audit of the Quality Management System has been carried out. In this audit, and as in previous years, the suitability of the System implemented in the CIEN

Foundation was demonstrated, and its commitment to continuous improvement. In this period, we have worked on various aspects to achieve the objectives of the management system, signing collaboration agreements with other third parties, improvement of laboratory equipment and databases of records of the Tissue Bank.

In addition, this year the necessary adjustments have been made to the Quality Management System, to adapt to the pandemic situation and minimize as much as possible the potential effects of lockdown and mobility restrictions. The improvements made to the communications system have made it possible to carry out meetings and audits remotely allowing the maintenance activities of the System to continue.

As every year, in the 2020 Management Review Report a summary of all aspects related to the Management

System is made, in this report the information related to these topics is reviewed:

- Changes in both the internal and external environment.

- The risks and opportunities related to the activity of the CIEN Foundation are analyzed.

- Objectives, quality indicators and information on customer satisfaction are reviewed.

- The results of internal and external audits are reviewed.

- Possible non-conformities and opportunities for improvement are analyzed.

- Reflect on strengths and areas for improvement.

## 2.6. Data Protection and Digital Rights Guarantee Law (LOPDG-DD)

In compliance with the RGPD 2016/679, the Center for Research of Neurological Diseases Foundation (hereinafter, the CIEN Foundation) with NIF G83671826 and address at Calle de Valderrebollo, 5, 28031 Madrid, treats personal data in order to comply with all the competences that are established in article 5 of the Foundation Statutes, being the basis that legitimizes said treatments the fulfillment of a legal obligation.

Said data will only be communicated to third party providers of strictly necessary services, and will not be transferred to third parties, except legal obligation. The CIEN Foundation will keep personal data during the legal period in which some type of responsibility may be demanded of it. The data used is strictly necessary for the correct achievement of the purpose, so that, if said data is not provided, the CIEN Foundation will not be able to fulfill such purposes.

In any case, the Interested Party may exercise their rights of access, rectification, deletion, opposition, and limitation by means of a written request sent to lopd@fundacioncien.es. The Interested Party may contact the CIEN Foundation Data Protection Officer (DPO) at the email address lopd@fundacioncien.es.

The CIEN Foundation is committed to fulfilling its obligation to ensure the confidentiality of personal data and its duty to keep them and to adopt the measures deemed necessary to avoid their alteration, loss, treatment or unauthorized access, taking into account at all times the state-of-the-art of technologies, guaranteeing compliance with the LOPDGDD at all times.
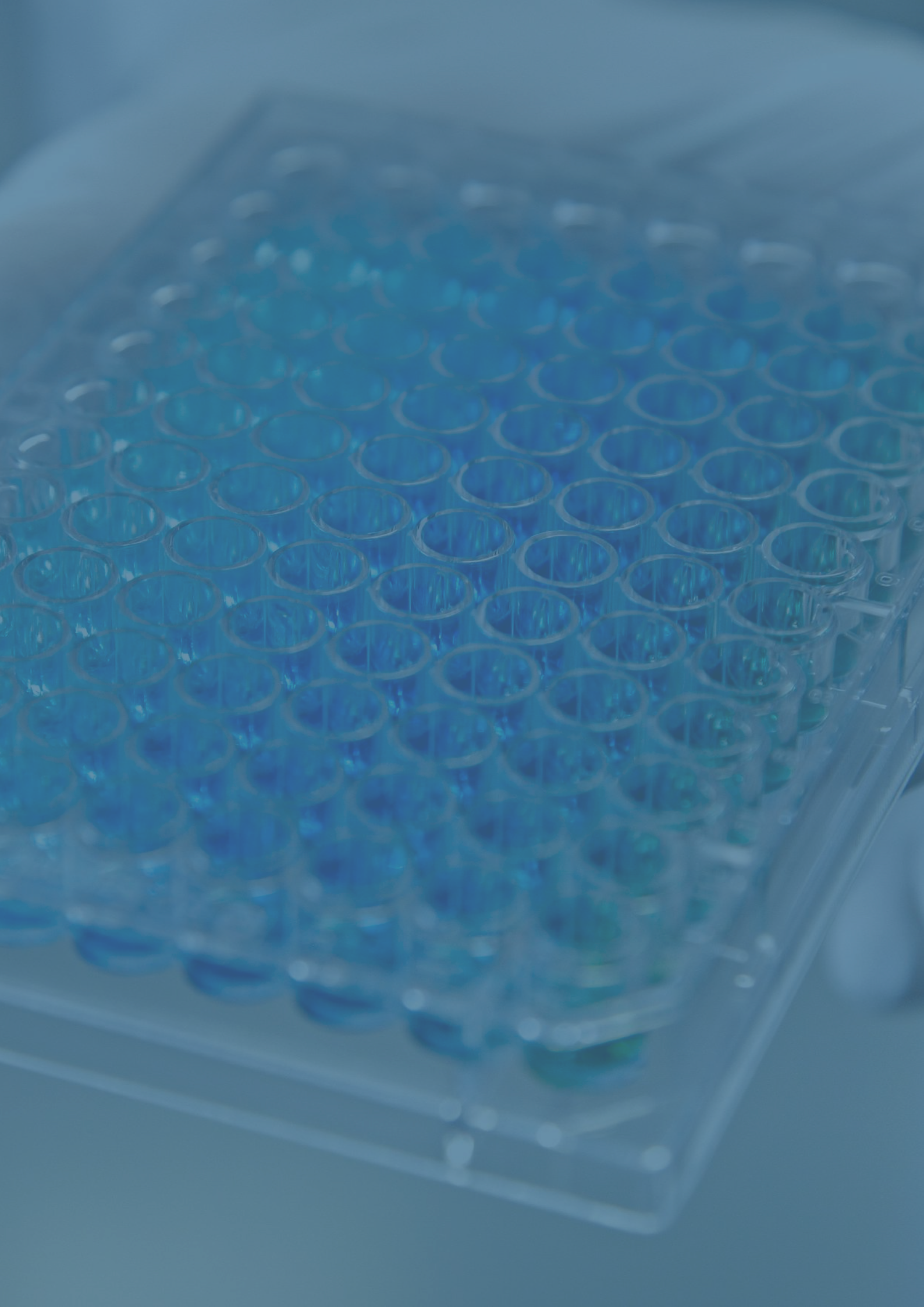
03.

Scientific activity

2.1. Overview

On January 18, 2006, the CIEN Foundation signed an agreement with the Queen Sofia Foundation, under which the former assumes the management of the Alzheimer Project Research Unit (UIPA, for its acronym in Spanish). The context in which the UIPA is framed is the Alzheimer Project, promoted by the Queen Sofia Foundation. The main exponent of this project is the Queen Sofia Foundation Alzheimer Center (Alzheimer Complex), located in the Vallecas neighborhood (Madrid), which in 2017 has celebrated its 10th Anniversary. One of the most outstanding aspects of the Alzheimer Complex is that there is a Healthcare Center (formed by a Residence and a Day Center) for Alzheimer's patients and related diseases as well as a Training Center, in addition to the UIPA. This Unit began its activity in April 2007, while the healthcare activity began at full capacity in the second half of that year.

Currently, the UIPA consists of five departments with a clear multidisciplinary approach and with specific objectives, among which are the following: the monitoring and periodic evaluation of patients at the Alzheimer Center; the development of clinical, epidemiological, genetic, research projects as well as on biomarkers in biological samples or neuroimaging, in the field of neurodegenerative diseases, with special
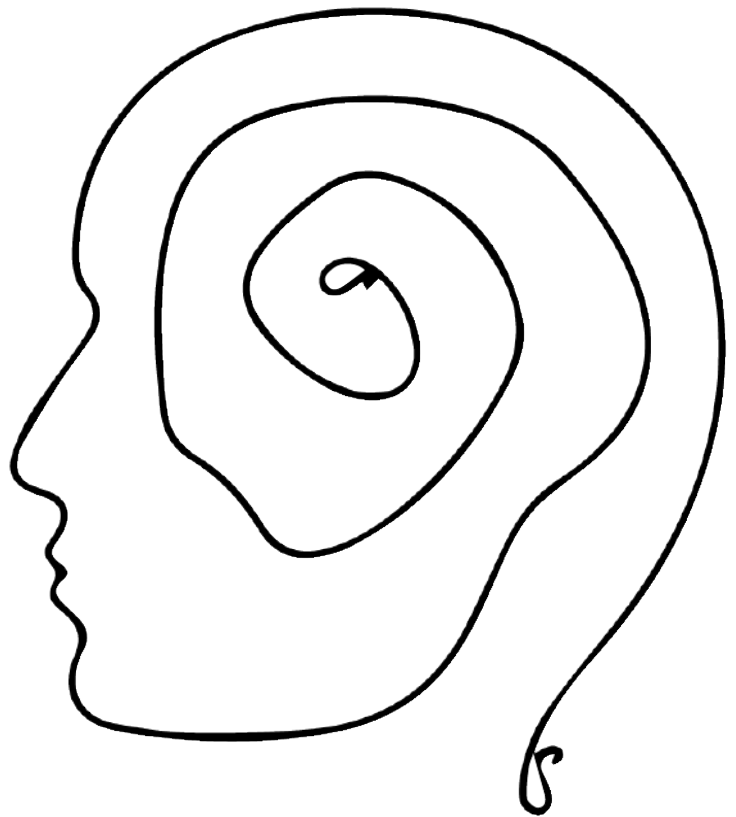
focus in Alzheimer's disease and related dementias.

Through this series of studies, new knowledge in genetics and molecular biology that have different applications is generated, that teach the researchers about the pathogenic mechanisms of the disease which can be in turn implemented into the field of diagnosis and, desirably, can result in the development of better treatments.

However, far from promising a simple solution to the problem of neurodegenerative dementias, these advances anticipate an increasingly complex

picture, in which the remedies will be achieved through small goals, and only by the complementary and synergistic work of many research groups. This complexity is the main feature of neurodegenerative diseases since they affect both the biological aspect as well as the clinical and personal level. Thus, the psychological and social aspects involved in dementia need to be taken into account and be aware that ethical and legal issues such as the right to information and participation in medical decisions are increasingly gaining prominence every day.

3.2. Department Structure

The scientific activity of UIPA is structured around four complementary research areas:

- Clinical Department
- Department of Neuroimaging
- Department of Neuropathology
- Department of Biochemistry and Molecular Genetics

CIEN Foundation counts with a group of researchers from the Clinical Departments who are in daily contact with patients who come to the Queen Sofia Foundation Alzheimer Center (CAFRS,

for its acronym in Spanish) and with the staff at the healthcare Residence, as well as with the cohort of volunteers from the Vallecas Project (see section 4), playing a role of mediation between basic researchers, relatives and caregivers. This role is critical in making patients, relatives and caregivers aware of the research purpose of the CIEN Foundation, give consent and collaborate with the research projects. One of the research activities of these departments consists in carrying out a clinical, syndromic and etiological diagnosis of the patients from the CAFRS, either in the resident regime (Units of Life) or in daycare (Day Center). In addition, a set of clinical data is obtained that will be very useful for the other CIEN Foundation scientific areas studies.

In addition to the clinical departments, the CIEN Foundation has a translational perspective in its original project, made up of the departments of Biochemistry and Molecular Genetics, Neuropathology and Neuroimaging. These three areas bring together the most promising fields of research on the biological processes underlying dementia.

The CIEN Foundation is characterized by its marked multidisciplinary

approach, so that both clinical and basic aspects are in continuous contact, through those five departmental areas, elaborating and contrasting hypotheses, and jointly carrying out various research projects. On the other hand, it is from reinforcing these two perspectives, how concepts such as translational research in Medicine have been developed. The scientific activity of the CIEN Foundation is based on this idea: to transfer to the clinical field the advances obtained with basic research.

3.2.1. Clinical Department

Patients with dementia require specific attention, consisting of an accurate and early diagnosis, an assessment of the affected cognitive areas and its severity, as well as applying and monitoring the treatment. It is essential that various medical disciplines be involved, due to the need to follow the progression, the specific treatment, the overseeing of complications, the application of measures to neutralize them, and the corresponding practice of social health resources.

The Clinical Department has been set up a translational vocation to investigate the progression of clinical knowledge in dementias. It is established as an intermediary between basic sciences and the fields of clinical and social sciences related to health, to promote knowledge about neurodegenerative dementias and its application. A team of specialists in Neurology and Neuropsychology make up the Department, which is associated with geriatricians, occupational therapists, physiotherapists and social workers from the Cen-

ter's healthcare area. The evaluations and assessments carried out in these areas composed the clinical and sociological database that, in addition to its intrinsic interest for research purposes, supports the biological samples and neuroimaging data obtained systematically in the Center.

In the Clinical department, further advancing in the knowledge of neurodegenerative diseases, especially Alzheimer's disease, is established as a priority. The main purpose of the department is to progress in the study of degenerative disorders that cause dementia in order to ultimately obtain better treatment for those who, directly or indirectly, suffer from these pathologies.

Every six months the professionals of the Clinical Department and the Residency of the Queen Sofia Foundation Alzheimer's Center carry out reviews based on a rigorous protocol that enables continuous monitoring of each patient, by assessing their quality of life, neurological status as well as their mental, affective and functional behavior.

The objective of this process is to establish and collect variables that allow a subsequent correlation and analysis with respect to other analytical, genetic, histopathological and neuroimaging variables.Lastly, the professionals of the Clinical department play a mediating role between fundamental scientists, family members and caregivers of the patients. This role is essential so that patients, family members and caregivers know the CIEN Foundation's research aims, authorize, and collaborate with the research lines, both internally and externally.

The Clinical Department develops the following activities:

- General medical, neurological, and neuropsychological assessment, and clinical and cognitive diagnosis of the Vallecas Project participants

- Detection and management of possible clinical complications of the Vallecas Project participants.

- Preparation of clinical reports

- Evaluation and diagnosis of patients attending the Center

- Neurological monitoring of patients admitted to the Residence

- Management and curation of databases

- Statistical analysis and preparation of scientific reports

- Teaching and mentoring of Psychology last course students

from the Autonomous University of Madrid, Rey Juan Carlos University, and Complutense University as well as PhD students who collaborate in some research projects

- ·Communication in scientific forums of research work carried out in the department

- Development of research projects on neurodegenerative diseases, especially focused on Alzheimer's disease.

- Dissemination to society of the progress of the investigation.

Main lines of research

The activities of this department focus on the following main areas:

The Vallecas Project for early detection of Alzheimer's disease. Risk factors and prevention (due to its size, a complete section in this Report is dedicated to this study, see section 4 of this Report). In this project, it is carried out an annual systematic clinical and neurological evaluation of the more than one thousand volunteers participating in the study cohort. This information, together with the data from the neuropsychological assessment, allows establishing the evolutionary diagnosis of each subject and is stored in a large database for carrying out various research projects.

Queen Sofia Foundation Alzheimer Center Research Program (CAFRS):

it consists on the systematic clinical evaluation, every six months, of the patients who are in the CAFRS, both in internment regime (Units of Life) and in day care (Day Center). This evaluation, carried out together with the rest of the staff from the Center and healthcare professionals leads to the syndromic and etiological diagnosis and to the protocoled collection of neurological, psychiatric, neuropsychological, demographic, analytical, therapeutic and neuroimaging data. This systematic gathering of information, from the moment the patient enters the study until it is transferred to another Day Center or passes away, allows for the generation of a large database of clinical data that can be exploited in itself or in relation to the neuroimaging and/ or neuropathological data.

The COVID-19 pandemic forced the closure of the CAFRS residence and has prevented the routine evaluation of these patients during 2020. Prior to the lockdown of this atypical year, 4 new admissions were registered in the Day Center and in the Residence, all of which signed the informed consent to participate in the periodic multidisciplinary evaluations. Along with the 5 baseline evaluations, a total of 399 clinical evaluations (twice a year), 10 brain MRI studies (annually) and 27 laboratory tests were performed.

Periodic multidisciplinary assessments during 2020

Admissions in Day Centre and Residence	4
Informed Consents	4
Baseline Assessments	5
Baseline Assessments	399
Brain MRI Studies	10
Blood testing	27

Other ongoing research projects.

The Clinical Department, with its own resources or, occasionally, in collaboration with other CAFRS or external groups, is carrying out several other research projects. Most of them are mainly based on information o from the Vallecas Project or the Queen Sofia Foundation Alzheimer Center Research Program databases. In other instances, information provided by various collaborators is used.

Clinicopathological study of patients with early Alzheimer's disease. Alzheimer's disease is a degenerative process with a very long evolution, with an asymptomatic preclinical phase, which can last more than a decade, and with clinical manifestations that can begin from the fifth or sixth decade of life, although they generally appear after the 70 years. It is known that the familial forms of Alzheimer's disease usually begin their clinical manifestations at a fairly early age, and it is also known that the disease is less aggressive the later its onset. The clinical study of cases of sporadic Alzheimer's disease with early onset can be used to identify the factors that determine its greater or lesser aggressiveness, and the pathological study to explore its pathophysiology.

Usefulness of subjective cognitive complaints as an early marker. This is perhaps one of the most consolidated lines of research in the department.

Subjective Cognitive Impairment (SCI) appears in preclinical stages of Alzheimer's disease, even a person can often experience this type of SCI despite showing a cognitive performance in a formal neuropsychological assessment within normal parameters. For this reason, there has been renewed scientific interest in recent years for the study of subjective cognitive complaints as a possible marker of future objective cognitive impairment.

The neuropsychologists from the Clinical Department have carried out different studies to analyze the role of cognitive complaints as a predictor of cognitive impairment in a sample of elderly people from the general population. Specifically, the focus is placed on studying which specific aspects and what type of cognitive complaints show a greater relationship with the development of cognitive impairment. In general, the following conclusions can be drawn from all the research work carried out:

• The SCI rate in the Vallecas Project cohort (~ 70%) is slightly higher than that obtained in other research studies. This can be explained by the fact that our cohort has a higher mean age.

• Our results are consistent with the accumulated evidence: SCI is more strongly associated with subjective variables (i.e., depression, anxiety, quality of life, etc.) than with
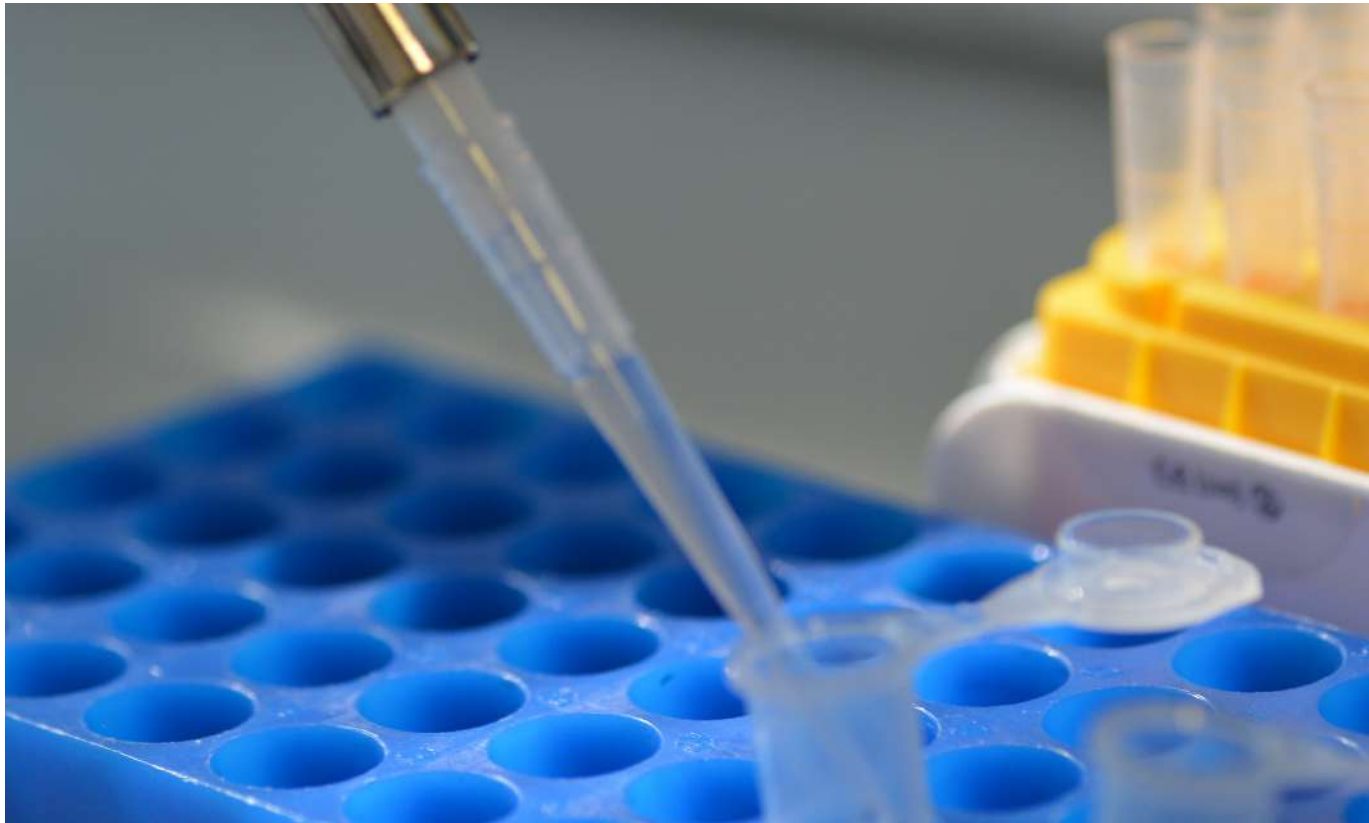
objective cognitive performance in neuropsychological tests.

- Not all cognitive complaints have the same specific weight when differentiating between cognitively healthy people and patients with Mild Cognitive Impairment (MCI). More specifically, complaints related to forgetting recent events, with things that we must remember in the future or with the ability to manage ourselves effectively on a day-to-day basis, allow us to differentiate between those two groups.

- Using the criteria of the International SCI Working Group, it is possible to classify individuals in preclinical stages of AD into three groups: non-SCI, SCI and SCI -Plus. This last group is considered the one with the highest risk of conversion based on a series of evidence-based parameters. Our results support the clinical interest of the classification proposed by the International Working Group: SCI -Plus has a 4 times higher risk of developing MCI in just one year compared to non- SCI. Although there are no significant differences between non- SCI and SCI, there is a higher conversion rate in SCI. Perhaps the one-year follow-up is not enough to show greater

differences.

- A comprehensive protocol proposal for collecting SCI -related information has been described and published. An operational definition has also been provided to classify individuals into the above three groups, namely non- SCI, SCI and SCI Plus.

- An opinion paper has been published related to the need to demonstrate that the SCI classification is stable over time. This is a prerequisite to be able to consider SCI as a variable of interest in the field of early detection of MCI.

- Our results have shown that when an assessment protocol is used systematically and rigorous operational criteria are employed, the SCI classification is sufficiently stable.

- After analyzing the temporal dynamics of the DCS groups during 3 years, the following sequential transition is observed that fits perfectly with the hypothesis of the different stages of AD: non-SCI SCI SCI-Plus MCI. The risk of developing MCI therefore increases progressively in the SCI and SCI-Plus groups respectively.

- Our future interest is to combine the available cognitive data with fMRI data. The objective is to determine if there are significant differences between control subjects and individuals with SCI-Plus with respect to the pattern of brain connectivity (a greater hyperconnectivity associated with SCI-Plus would be expected, as the literature has already shown in later phases of mild cognitive impairment).

Usefulness of various cognitive parameters as early markers. Numerous studies have shown that there are certain cognitive variables that allow the identification of subjects at higher risk of developing Alzheimer's disease years before their diagnosis. With this idea in mind, we investigate whether certain parameters of the neuropsychological protocol, in the context of a longitudinal research project such as the Vallecas Project, are useful to identify individuals at risk of cognitive impairment. These parameters are analyzed in an evolutionary way together with the rest of clinical and genetic information of each volunteer to obtain a classification algorithm that could eventually be generalized to daily clinical practice.

In addition, the Clinical Department is interested in developing new assessment tools that allow examining the role of other cognitive markers not studied in standard neuropsychological evaluations. It is expected that the identification of these markers increases sensitivity and specificity when detecting individuals at risk of dementia. Among the most important results

obtained over the years in this line of research, the following are noteworthy:

- Cognitive, tests and in particular, the Free and Cued Selective Reminding Test (FCSRT) verbal episodic memory test, are the most sensitive tools for identifying subjects at risk of conversion to MCI. Its discriminating capacity is above other variables of interest such as APOE genotyping.

- The predictive capacity of the FCSRT has been shown to be high even in a 4-year follow-up. Especially useful is the parameter of immediate free recall, in which an individual's score below 18 is considered risk for conversion in the next 4 years.

- Processing speed (i.e., mental agility) is another cognitive parameter that has been revealed as an early marker of interest for MCI although we still need to carry out additional analysis in this regard.

- The analysis of the possible effect of drugs on cognitive performance in the Vallecas Project cohort has been examined. The two-year cross-sectional and longitudinal results have not identified any effects of interest and, when any association has been found, it is inseparable from the underlying pathology of the drug itself.

- · A pilot study has been carried out with an attentional blinking task among the subjects of the Vallecas Project. The attentional blink task is a classic paradigm in Experimental Psychology whose use has not been extended to clinical samples. It allows obtaining first-order cognitive parameters

not explored in conventional evaluations that may be of interest as early markers. Our preliminary results show a high correlation between performance in the attentional blink task and classical cognitive parameters. It is pending to investigate whether differences at the fMRI level exist.

- Given that the experience in applying the State-Trait Anxiety Inventory (STAI) test during the Vallecas Project baseline visit raised serious doubts about its suitability for our reference population, a psychometric study was carried out and published to reduce test items and categories of response. As a result, a much more useful reduced scale was obtained, but just as precise as the original one, which

we have incorporated into the Vallecas Project as of the evaluation visit 6. The same was also done with the inventory of cognitive symptoms (MFE-11).

The relationship between cognitive reserve and socioeconomic status with the onset of cognitive impairment. The cognitive reserve is a theoretical construct that formulated several decades ago to explain the lack of correspondence between the degree of brain damage and clinical symptoms sometimes observed among patients. It is presumed as an individual capacity that develops throughout the life cycle fundamentally through formal education, the type of professional activity performed, and the intellectual activity developed. Numerous studies have supported the protective role played

by the cognitive reserve in modifying the course of different neurodegenerative pathologies, including Alzheimer's disease. Likewise, the socioeconomic status associated with a person's area of residence can be understood as an environmental factor that determines to some extent the probability of developing a possible cognitive impairment.

The interest of the Department for this construct focuses on analyzing different variables that apparently have the capacity to confer cognitive reserve and therefore could play a protective role against cognitive impairment. Within the Vallecas Project, this set of variables is being studied individually and as groups, with special emphasis on the activities of daily life performed during mid-life. Likewise, different variables of lifestyle are examined to classify them as risk or protective factors against the appearance of dementia. The results have shown that both the variables associated with the individual socioeconomic level and those associated with the neighborhood of residence play a complementary role in the conversion to cognitive impairment.

Spain-Portugal Longevity Research Program (PILEP + 90). PILEP + 90 is a research project designed to examine the role of biological and environmental determinants associated with longevity and, more specifically, dementia-free longevity. For this, the study analyzes different variables associated with cognition, health, lifestyles, and brain structure in two cohorts of people over 90 from Madrid and Braga.

This research program is led by the CIEN Foundation and it includes researchers from four different institutions in Spain (CIEN Foundation, Madrid Health Autonomous Organization, Complutense University of Madrid, and Biomedical Technology Center), Portugal (University of Minho), and Denmark (University of Southern Denmark).

The results of PILEP+90 will allow obtaining reliable data on the prevalence of cognitively healthy and cognitively impaired long-lived people, as well as validating a battery of clinical tests to study the elderly. In addition, thanks to the combination and exploitation through machine learning techniques of demographic, lifestyle, clinical, cognitive and neuroimaging data collected in the study, PILEP+90 is expected to help delay the effects of cognitive aging, to reduce the risk of developing dementia, promote the level of functional independence and increase the quality of life of all older people

Study of non-pharmacological therapies for the prevention and modification of the course of Alzheimer's disease. Non-pharmacological interventions have usually three specific objectives: to stimulate cognitive processes, promote the level of autonomy and, ultimately, increase the quality of life of

individuals. These interventions can be carried out both in healthy elderly people, in order to prevent the onset of cognitive impairment, as well as in patients with cognitive impairment, to try to modify the course of the disease. Among the most used non-pharmacological interventions, changes in lifestyle, especially regarding diet, physical exercise, and cognitive training stand out.

Although scientific evidence has shown that non-pharmacological therapies have a protective effect against cognitive impairment, there are still some unresolved issues related to this topic. For example, determine what type of intervention is most beneficial or what clinical variables are associated with a better prognosis of the therapy. Taking advantage of the knowledge accumulated throughout the Vallecas Project, the objective of the Department of Neuropsychology is to study the impact that these non-pharmacological interventions have on subjects at risk of developing mild cognitive impairment. Thus, the department is associated to a consortium made up of various research groups from the Complutense University of Madrid (UCM, for its acronym in Spanish), the technical University of Madrid (UPM, for its acronym in Spanish) and the San Carlos Clinical University Hospital of Madrid through the joint research project "Study of the functional connectome modulations in young and elderly

relatives of AD patients: assessment of the influence of an intervention and SCI" (RTI2018-098762-B-C31) funded by the Ministry of Science, Innovation and Universities through the Research Challenges call 2018 of the R+D+i National Plan. Specifically, within this project the department of neuropsychology leads the subproject entitled "Elderly Relatives of Alzheimer Disease (ERAD): influence of the Subjective Cognitive Decline".

Spatial analysis of changes in retinal layer thickness in Alzheimer's disease and other neurodegenerative disorders. In collaboration with the Institute of Knowledge Technology of the Complutense University of Madrid and the ACE Foundation, a series of studies on the retina, the most accessible part of the central nervous system, have been initiated using the optical coherence tomography technique and the application of advanced data analysis tools. It is expected to obtain early and specific retinal markers of Alzheimer's disease that may be useful for its diagnosis and follow-up with a technique that is easy to apply and very low cost.

Registry of cases with Mild Cognitive Impairment with biological samples (blood and CSF). In collaboration with the Service of Neurology from the Infanta Leonor Hospital, a registry of cases with mild cognitive impairment will be created in which clinical data and biological samples will be systematically collected to facilitate their future

study and participation in therapeutic trials.

Perceived health and risk of dementia. In collaboration with the University 12 de Octubre Hospital Research Institute, an analysis of the data from the extensive epidemiological study NE-DICES is being carried out to examine to what extent the perception that the elderly individual has of their state of health is predictive of the future development of deterioration cognitive and dementia in later years.

Future lines of research

Using the available data from the Vallecas Project, at least eight studies are proposed some of which have already produced preliminary results:

- Study of the variables associated with the reversion of the MCI state to that of cognitive normality. The objective of this work is to identify those lifestyle variables that could be playing an essential role in the reversion and, later, propose possible interventions on them in another sample of subjects.

- Analysis of discrepancies and unreliability in the collection of educational level within the Vallecas Project. Fortuitously, the self-reported data regarding the educational level of the subjects has been collected during three consecutive visits to the Vallecas Project. The results show a completely unexpected finding: the reliability in the collection of this variable, which is highly relevant in this field, is highly questionable since 1 in 3 subjects do not report consistently on their actual educational level.

- Longitudinal normative data and reliable change index in a selection of cognitive tests from the Vallecas Project. To carry out this work, we will collaborate with partners of the consortium that emerged under
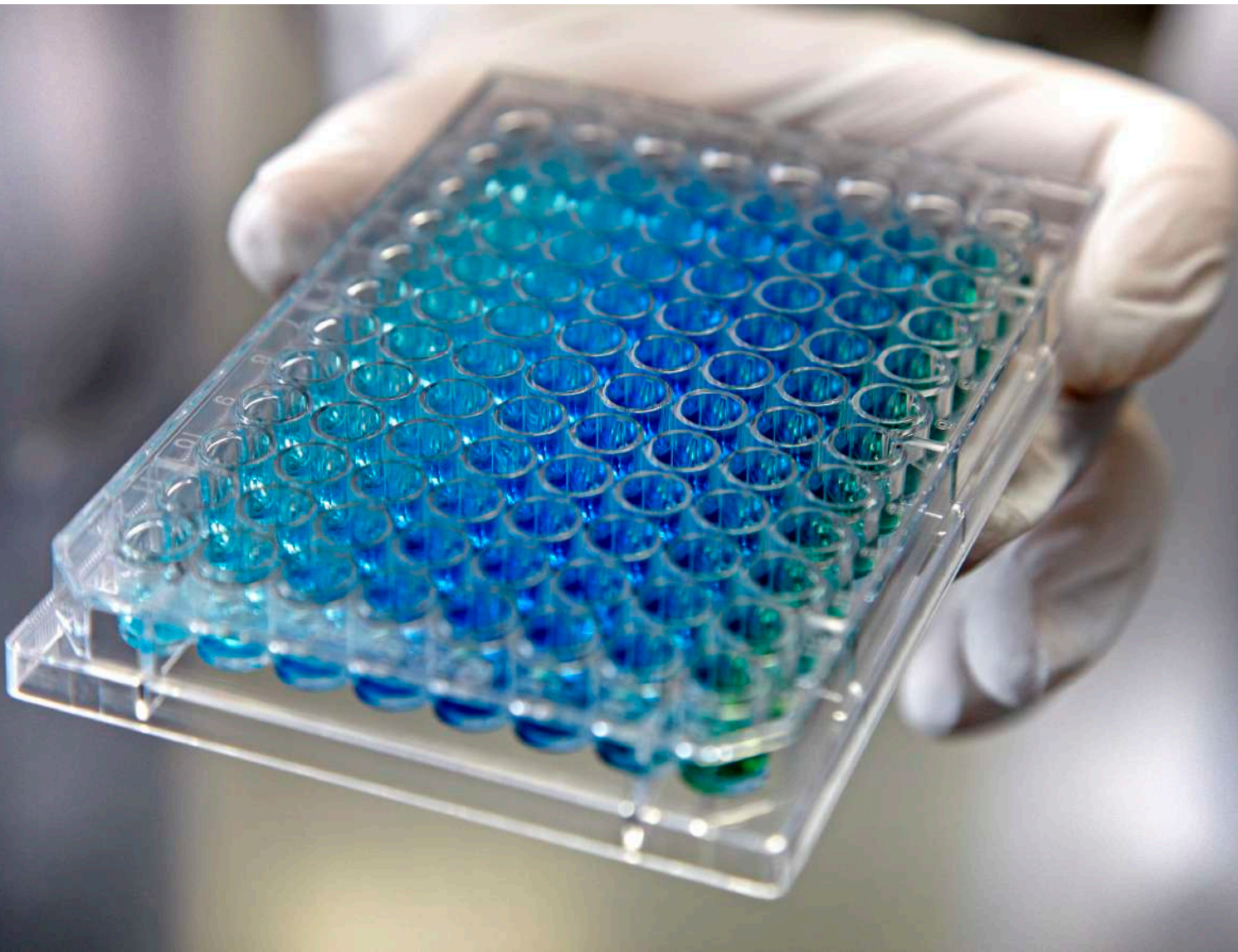
the project financed within the 2018 call of the National Plan.

» Mild functional impairment and risk of dementia. In collaboration with the 12 de Octubre University Hospital Research Institute, the extensive epidemiological study NEDICES will study the presence of functional impairment and to what extent the onset of this mild impairment predicts the future development of cognitive impairment.

» Adapted diet in advanced stage of dementia. Feeding patients in the advanced stages of dementia is difficult and poses practical problems in the administration of care, as well as the risk of aspiration and development of bronchopneumonic processes. Easier and safer feeding methods must be implemented and tested for these patients and some of them will be examined in a systematic and controlled way in our center.

» Analysis of the clock test drawings using advanced

image analysis and artificial intelligence techniques. The aim is to develop an automatic and powerful method of analysis for this test, which can be extended to other graphic materials, and can be applied to the detection and predicion of cognitive impairment.

Team

- Teodoro del Ser Quijano, Dr. Medicine, Neurology. Head of Department

- Meritxell Valentí Soler, Dr. Medicine, Neurology

- María Ascensión Zea Sevilla, Dr. Medicine, Neurology

- Miguel Ángel Fernández Blázquez, Dr. Psychology, Neuropsychology

- Marina Ávila Villanueva, Dr. Psychology, Neuropsychology

- Belén Frades Payo, Grad. Psychology, Neuropsychology

- Francisca Martínez Lois, Administrative Assistant

- Beatriz Salado Martínez, Administrative Assistant

3.2.2. Department of Neuroimaging

Knowledge of the morphological variations occurring in brain structure throughout life is essential to assess the corresponding pathological changes that occur in neurodegenerative diseases. Currently, neuroimaging in any form, and combined, is one of the areas of greatest progress in the understanding of various aspects of Alzheimer's disease and other neurodegenerative diseases: etiology, early diagnosis and differential functioning of brain areas, metabolism, neurotransmission.

In this regard, neuroimaging techniques such as magnetic resonance imaging (MRI) have led to significant progress in understanding brain changes associated with age. MRI is a noninvasive tool that allows the study of normal aging individuals at different times of his life. However, conventional MRI techniques are unable to detect and quantify microstructural changes dependent on age who have been described in post-mortem studies of brain tissue.

For this reason, the Department of Neuroimaging has a state-of-

Members of the neuroimaging department

the-art 3 Tesla (T) MRI equipment as well as a collaboration agreement for research with the supplier: General Electric.

The main objectives Department of Neuroimaging are:

- Promotion and development of neuroimaging research projects in the field of neurodegenerative diseases with special interest in AD and related dementias

- Acquisition and postprocessing of MR images for UIPA ongoing researc projects

- Dissemination of knowledge on neuroimaging techniques related to neurodegenerative diseases

- Personnel training related to obtaining, postprocessing or interpretation of advanced neuroimaging techniques

	0	13	25	38	50
fMRI_QA					
CONNECT-AD					
PVALLECAS8					
PSYSCAN					
LABORATORIO					
PVALLECAS7					
NAC					
RESIDENTES					
MADRID+90					
PURPOSE					
UDDC					
PVALLECAS9					
UOD					
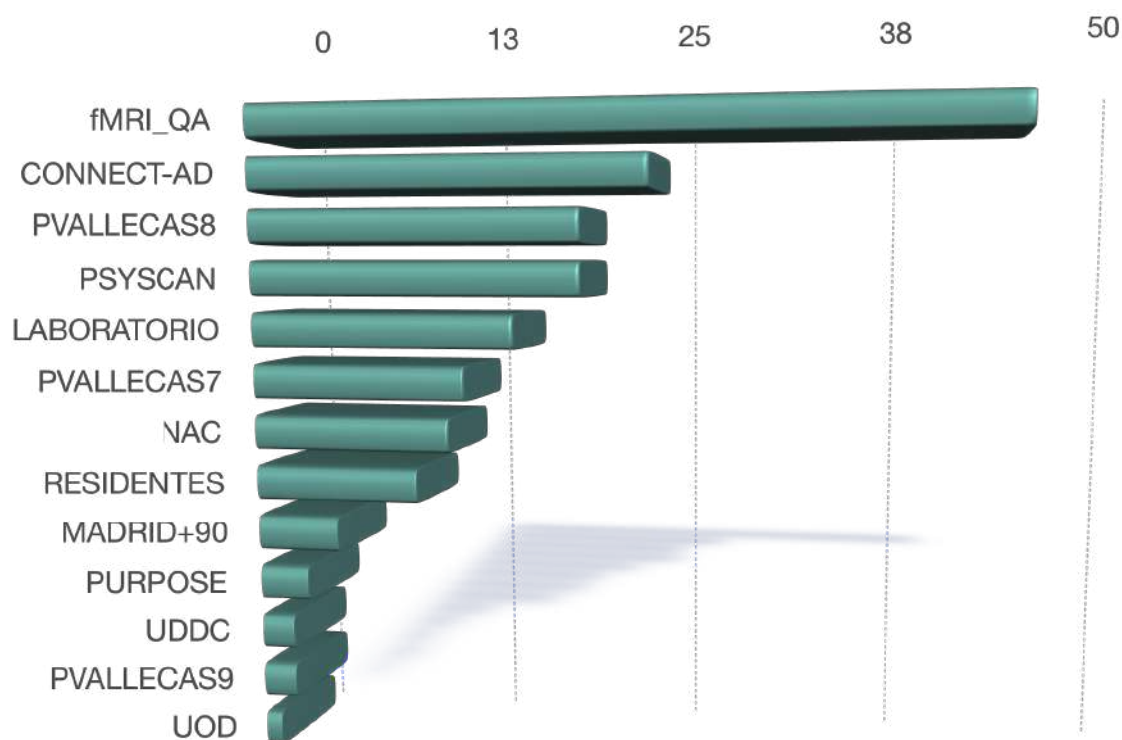
Department activities

CIEN Foundation's Department of Neuroimaging primarily deals with the acquisition of MR data (and, where appropriate, the performance of other imaging techniques such as PET or CT through external collaborations) and post-processing and analysis of the data obtained. All studies are monitored and reported by a neuroradiologist.

In addition, the Department provides technical assistance to both the rest of the scientific areas of the CIEN Foundation and external research groups. It also searches for new resources and promotes the CIEN Foundation research projects and the post-processing of images service among other research groups.

This activity complements the internal seminars and external courses, both nationals and internationals, on specific neuroimaging techniques.

During 2020, the Department of Neuroimaging has participated in MRI studies in the following projects:

- Vallecas Project: Early detection of Alzheimer's disease. CIEN Foundation. PI: Dr. Miguel Calero.

- Alzheimer Project Research Unit. Longitudinal study, acquisition and yearly MRI analysis of patients and residents from the Queen Sofia Foundation Day Center. CIEN Foundation. PI: Dr. Alberto Rábano.

- TEMPACOR. "Temporal-parietal cortex dysfunction as endophenotype of depression"

PI: Dr. Stephan Moratti, Basic Pasuchology I Professor.

- PURPOSE "Placebo-controlled trial in subjects at ultra-high Risk for Psychosis with Omega-3 fatty acids in Europe". PI: Dr. Covadonga Martínez Díaz-Caneja.

- "NAC II": "Combination of N-Acetyl Cysteine (NAC) Treatment and Cognitive Training to Promote Changes in Behavioral and Neural Activation Patterns Underlying Cognitive Deficit in Adolescent Psychosis" (Exp/n. FAK182018). Dr. Marta Rapado Castro. Child and Adolescent Psychiatry Service, Gregorio Marañón University General Hospital, Faculty of Medicine, Complutense University, CIBERSAM

- "Effect of 48-Week N-Acetylcysteine Treatment on Gray Substance Loss and Oxidative Metabolism in Patients with Early-Onset First Psychotic

Episodes: Randomized, Double-Blind, Placebo-Controlled Clinical Trial" (Exp/n. SAM18PI02/2019). Dr. Celso Arango López. Child and Adolescent Psychiatry Service, Department of Psychiatry. Gregorio Marañón University General Hospital.

- "Study of the Ethiophysiopathological Mechanisms Involved in the Progressive Loss of Brain Gray Matter in Children and Adolescents with a First Psychotic Episode, Adolescents at Risk and Healthy Controls by the Alonso Family Foundation" (Exp/n° FIBHGM_CCA004-2018). PI: Dra. Marta Rapado Castro. Child and Adolescent Psychiatry Service, Gregorio Marañón General University Hospital, Faculty of Medicine, Complutense University, CIBERSAM.

- "Observational study in young people with high-risk mental
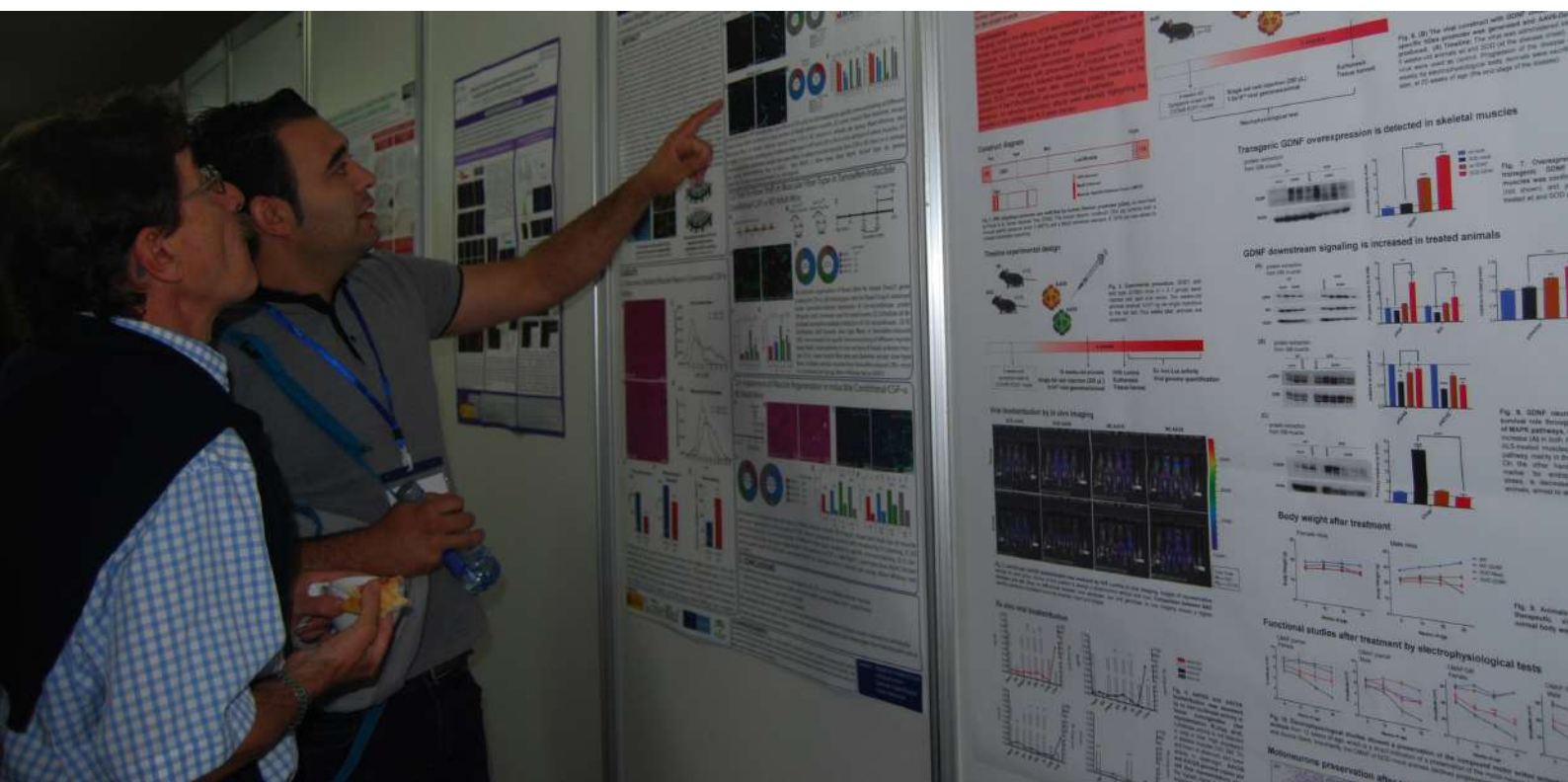
states. Differential profiles and influence of traumatic experiences and biological variables in the development of psychosis" (Exp/n. PI17-00481). Dr. María Mayoral Aragón. Child and Adolescent Psychiatry Service, Department of Psychiatry. Gregorio Marañón University General Hospital.

- "Longitudinal Study of Oxidative and Neuroinflammatory Markers of Cognitive Function in First Episodes of Adolescent Psychosis" (Exp / n° PI18/00753). Dr. Marta Rapado Castro. Child and Adolescent Psychiatry Service, Gregorio Marañón General University Hospital, Faculty of Medicine, Complutense University, CIBERSAM

- "Effect of 48-Week N-Acetylcysteine Treatment on Gray Substance Loss and Oxidative Metabolism in Patients with Early-Onset First Psychotic Episodes: Randomized, Double-Blind, Placebo-Controlled Clinical Trial" (Exp/n° SAM18PI02/2019). Dr. Celso Arango López. Foundation for Biomedical Research of the Gregorio Marañón Hospital.

- "First Early-Onset Psychotic Episodes: Randomized, Double-Blind, Placebo-Controlled Clinical Trial" (Exp/n° SAM18PI02/2019). Dr. Celso Arango López. Foundation for Biomedical Research of the Gregorio Marañón Hospital

- PSYSCAN. "Translating neuroimaging findings from research into clinical practice". PI: Dr. Celso Arango.

- EMAR-GM. "Follow-up study of young people with high-risk mental states". EMAR- FIS Mª Mayoral 2017 (PI17/00997) PI: Dr. Celso Arango, Dra. María Mayoral Aragón.

During 2020 the acquisition of MR images from 221 subjects has been completed. Overall, 1248 MRI studies have been performed distributed among the different research projects. Despite the exceptional circumstances that we have experienced in 2020, the department has maintained minimal activity after the lockdown. Since May 2020, a total of 93 acquisitions have been made to periodic quality control phantoms and project volunteers, who have undergone a PCR test, all of them with a negative result, before visiting the Center.

59,595 MRI sequences have been conducted since the creation of the department, distributed by year and type of sequence, as it is shown in the next graph:
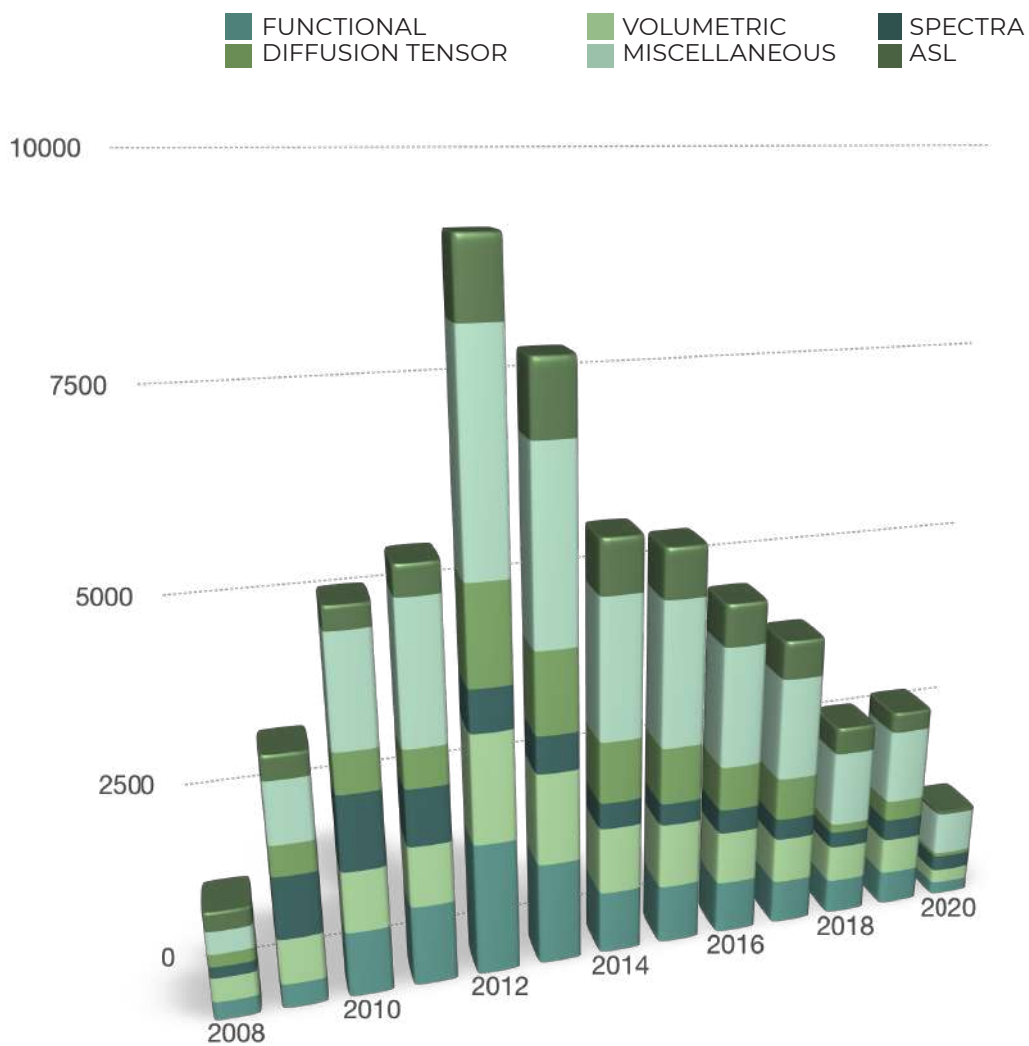
	FUNCTIONAL	VOLUMETRIC	SPECTRA	DIFFUSION TENSOR	MISCELLANEOUS	ASL
2008	255	339	156	178	312	259
2009	337	638	866	423	845	326
2010	910	852	1042	556	1506	305
2011	1124	845	786	528	1904	398
2012	1858	1512	583	1330	2938	949
2013	1436	1276	516	1116	2544	950
2014	880	937	367	847	1920	729
2015	828	912	309	783	1985	669
2016	739	749	341	618	1661	572
2017	614	648	299	594	1415	523
2018	487	519	226	124	1051	367
2019	473	513	306	285	1033	278
2020	161	163	206	53	514	46

Provision of services

The Department of Neuroimaging has a 3T MR scanner (GEHC, HDxt) system equipped with dual gradient system of up to 50mt/m, 3 antennas for brain studies (transmitter/receiver quadrature antenna, receiving 8 channels antenna and 16 channels receiving antenna) and small antennas for rats and mice. Data is stored in PACS with direct recovery capacity for five years of work.

For Functional MRI studies, the Department has an audio/video system compatible with 3T MRI.

A variety of software packages is used, mainly SPM12, FSL and Freesurfer.

Sequences

Image acquisition of 3D isotropic studies with T1 sequences for VBM.

Image acquisition of T2 sequences, DWI, ASL, BOLD and spectroscopy.

The service provision data are publicly available on the CIEN Foundation website: (https://www.fundacioncien.es/documentos/Tarifas-resonancia-magnetica_FCIEN.pdf)

Team

The Department of Neuroimaging team, led by Dr. Bryan Strange (MD, PhD, Clinical Neuroscience), has a highly multidisciplinary nature and consists of the following personnel:

Research fellows

Linda Zhang (PhD Radiological Imaging. Graduate in Psychology).

Marta Garo (Graduate in Biomedical Sciences, Master in Neuroscience, UAM). Queen Sofia Foundation-MAPFRE Scholarship until July 2020. PhD student from the UAM Neuroscience PhD program. Collaborator.

Alba Peris-Yagüe. PhD student from the UAM Neuroscience PhD program. Collaborator.

Darya Frank. Dr. Cognitive Neuroscience (UPM Agreement). Collaborator.

Radiodiagnostics

Mabel Torres Llacsa (MD, Radiodiagnostics)

Image Acquisition

Eva Alfayate Sáez (Technical Coordinator. Technician in Radiodiagnostics)

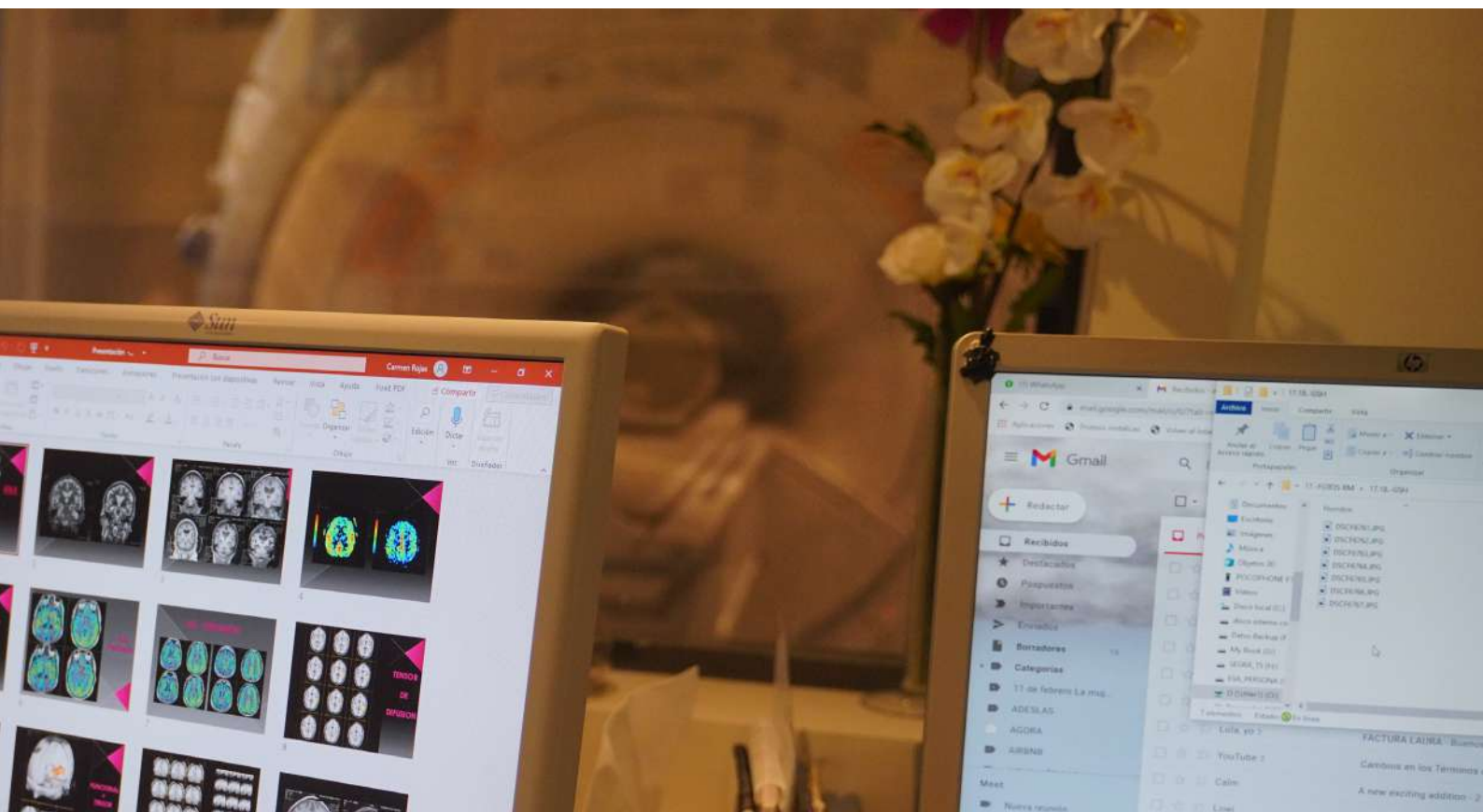Felipe García Fernández (Advanced Technician in Diagnostic Imaging)

Carmen Rojas Obregón (Technician in Radiodiagnostics)

Administration

Arantza Narciso (Administrative Assistant). Until October 2020

Corina Ghinea (Administrative Assistant). Until May 2020.

Gema Sánchez González (Administrative Assistant). Since November 2020.

3.2.3. Departament of Neuropathology

Neuropathology of neurodegenerative disorders is a specialty in continuous progress with capacity for contrasting clinical judgment and performance of any diagnostic test, including the most recent biomarkers, with the final diagnosis that is still neuropathology ("gold standard"). However, in the field of basic research, neuropathology plays an additional role, providing critical information about the molecular components of the characteristic lesions of each disease, the pathogenic mechanisms, and the potential associated biomarkers.

The post-mortem neuropathological findings observed in donations of brain tissue from patients with neurodegenerative diseases, especially in the case of dementias, have allowed to know in recent decades the epidemiological reality of these diseases in the population, and among other evidence, have revealed the high prevalence of combined pathology (mainly Alzheimer's pathology, Vascular pathology, Lewy pathology, TDP-43 pathology, various tauopathies linked to advanced age).

Members of the department of neuropathology

The neuropathology of dementia landscape has dramatically changed in recent years. The incorporation to the neuropathological diagnosis of new antibodies for immunostaining and new molecular techniques has helped establishing the boundaries and internal heterogeneity of entities such as dementia with Lewy bodies and frontotemporal dementia and has also led to the discovery of new entities in this area (DFT-TDP. DFT-FUS, etc.). The neuropathological study of an increasing number of patients with neurodegenerative pathology has allowed identifying new genetic causes of these diseases, thus helping to define new molecular targets for possible therapeutic approaches. In addition, the definition of diagnostic criteria from large series of brains (in biobanks of neurological tissue or brain banks) has allowed us to address the problem of combined and mixed pathology, specifically regarding Alzheimer's disease. The evolution of the diagnostic criteria themselves (e.g., the new criteria for the diagnostic classification of Alzheimer's disease proposed by the National Institute of Aging - Alzheimer's Association, 2012) and molecular techniques are converting the histological diagnosis into an fundamental element in the process of classifying dementias, definitive or quasi-definitive in some cases, but partial or probabilistic in many others.

new entity with TDP pathology, LATE (Limbic-Predominant age-related TDP-43 encephalopathy), all of which occur frequently in combination with the most prevalent pathologies that produce dementia in elderly subjects.

A need for dementia research is the availability of well-diagnosed, classified and long-term preserved brain tissue. Brain banks (biobanks of neurological samples) respond to this need, and the CIEN Foundation has one of the main brain banks in the country, the CIEN Tissue Bank (BT-CIEN, for its acronym in Spanish).

Neuropathology also provides significant support for studies based on animal models of neurological diseases, both for the histological evaluation of transgenic animals and for the search for natural models of the disease. The Department of Neuropathology of the CIEN Foundation participates regularly in the histological evaluation of animal models of different neurodegenerative diseases developed by CIBERNED researchers, as well as in the neuropathological study of elderly mammals of the Madrid Zoo.

As repeatedly demonstrated in clinical-pathological sessions, made possible in most cases thanks to the donation of brain tissue by patients or their families and the close collaboration of clinicians, the final classification of a case requires the integration of all clinical, neuroradiological, neuropathological and molecular information, when available.

Thanks to the joint work of neuropathologists from various continents, all of them associated with brain tissue donation programs, in recent years the precise characterization of histological brain lesions that until now had not been interpreted as defined pathological entities has been achieved. This has led to the description of new tauopathies, such as PART (Primary Age Related Tauopathy) and ARTAG (Aging-Related Tau Astrogliopathy) and of a

Department activities

The core activity of the UIPA's Department of Neuropathology corresponds to the BT-CIEN, both to its organizational and logistical components as well as the neuropathological diagnostic work and the management of biological samples.

The Department also participates in numerous collaborations in external research projects, mainly with CIBER-NED research groups, and carries out its own internal projects, mainly based on series of cases from postmortem donation. Among the active lines of research in the Department are the following:

- Neuropathological and molecular study of tauopathies, including Alzheimer's disease. Pathogenic significance and spread of associated cellular lesions. Argyrophilic grains disease and other recently described tauopathies (PART, ARTAG) as models of tauopathy with predominant involvement of the medial temporal lobe. Lewy pathology limited to the amygdala. Hippocampal sclerosis and associated TDP-43pathology (LATE).

- Clinicopathological profiles in advanced dementia. Characterization of the combined and mixed pathology and its

impact on the clinical trajectories of the patients, with special attention to disease progression rate and survival time (project based mainly on the patient cohort from the residency of the Queen Sofia Foundation Alzheimer's Center Research Program).

- Pathological role of fungal colonization and the polymicrobial infection of the Central Nervous System in Alzheimer-type pathology. Risk factors and clinical impact. Impact of neuroinflammation associated with polymicrobial infection on the origin and progression of neurodegenerative pathology.

- Distinctive features of Alzheimer-type pathology in nonagenarians and centenarians. Neuropathological findings in subjects without cognitive disorder above 90 years of age.

- Advance age-associated changes in Central Nervous System and cerebral pathology in mammals and non-human primates. Search for natural models of Alzheimer in primates and other mammalian groups.

Provision of services

The range of activities undertaken by the department derives from the ability of its members to collect, process, evaluate and diagnose brain tissue sample from human or animal origin. All the services listed below provided by the BT-CIEN are accessible to donors and researchers through the website.

- Performing neuropathological autopsies in brain tissue donors, both in the Region of Madrid and neighboring Regions, as well as in other Regions that do not have a brain bank.

- Management of a biobank of neurological samples. Transfer of samples to researchers according to the BT-CIEN standard operating protocols together with biobank external committees.

- Prospective collection of special biological samples for research projects, at the request of the researchers, once approved in compliance with BT-CIEN protocols (and by the scientific and ethical external committees).

- Diagnostic consultations of neuropathological cases. Among the external consultations, those carried out as support of other biobanks of neurological samples with which BT-CIEN maintains a regular collaboration (Murcia, Salamanca, Leon and Cordoba) stand out. Consultations are also received from different public hospitals and from the Anatomical Forensic Institute of Madrid

- Performing neurohistological and immunohistochemical techniques in neurological samples of human and experimental origin.

- Incorporation in the biobank of samples collections (usually, biological fluids, blood, and CSF) from clinical research projects.

- Organization of informative lectures and visits to the biobank for the dissemination and promotion of brain tissue donation among patients and relatives, health professionals and the general population.

- Participation in formal training programs for Pathological Anatomy Technicians, Laboratory Technicians, and Pathological Anatomy and Neurology resident physician interns.

CIEN Foundation Tissue Bank (BT-CIEN)

Since its opening in May 2010, the CIEN Foundation Tissue Bank (BT-CIEN) has traveled a path of growth and consolidation in the field of Spanish Neuroscience, supporting national and international research groups and maintaining close contact with neuro-

logical disease patients and relatives' associations.

The number of registered donors in the BT-CIEN registry has continued to grow every year, as has the tissue donations made at our Center within our Internal Donation Program, which involves residents of the Queen Sofia Foundation Alzheimer Center (CAFRS), and the External Donation Program, that involves donors from the Region of Madrid and other Regions.

There is also an increasing number of research groups applying for biological samples from BT-CIEN, especially groups from the Center for Networked Research in Neurodegenerative Disea-ses (CIBERNED).

One of the missions of BT-CIEN is to promote the creation of new neurological samples biobanks whenever donors and researchers demand them. The Region of Murcia Brain Bank (BCRM), the Neurological Tissue Bank from the Institute of Neuroscience of Castilla y León (BTN-CyL) and the of Queen Sofia University Hospital Biobank from Cordoba are active examples of this commitment. At the present time, support is being provided for the creation of a new NTB in Ciudad Real, as a reference biobank for the Community of Castilla-La Mancha, and a NTB in Zaragoza.

In 2013, the BT-CIEN has been accredited by the Council of Health of the Region of Madrid, according to what is established in the Royal Decree 1716/2011 on Biobanks and registered in the National Registry of Biobanks of the Carlos III Institute of Health.

In January 2014 the Biobanks National Network Platform (PRNBB, for its acronym in Spanish), promoted and funded by the Carlos III Institute of Health (2014-2017) was constituted, with participation of the main biobanks in the country, both hospital and non-hospital, including BT-CIEN. PRNBB mission is to create a stable organizational structure that allows the coordinated activity of participating biobanks in the collection, management, and transfer of biological samples of human origin. Before concluding its period of activity in December 2017, the PRNBB renewed its commitment to the ISCIII for a new funding period, 2018-2020. The BT-CIEN has played a prominent role in the R&D&I Program of the PRNBB and in the new phase of the platform, initiated in January 2018, has assumed the coordination tasks of the R+D+i program as a whole. Moreover, since 2012 the BT-CIEN has the ISO 9001/2008 quality certification, renewed in 2016, and that was also renewed in 2018 under the ISO 9001/2015 standard.

Throughout 2020, the BT-CIEN has continued to develop its tasks within the Neurological Tissue Banks Working Group (GT-BTN, for its acronym in Spanish) of the ISCIII National Biobank Network Platform (RNBB, for its acronym in Spanish). Among these tasks, the preparation, with the leadership of BT-CIEN, of the Guide of the Neurological Tissue Banks Working Group in relation to the COVID-19 pandemic stands out. Recommendations for the action of BTN during and after the pandemic

31 new donors were registered during 2020, taking the total number to 739 by December 31, 2020.

The Neuropathology laboratory processed 39 cases during 2020, with the following distribution depending on the origin:

- 13 donations from the External Program (depicted in blue in the graph)
- 0 donations from the Internal Program (depicted in red in the graph)
- 26 consultation cases (not included in the graph)
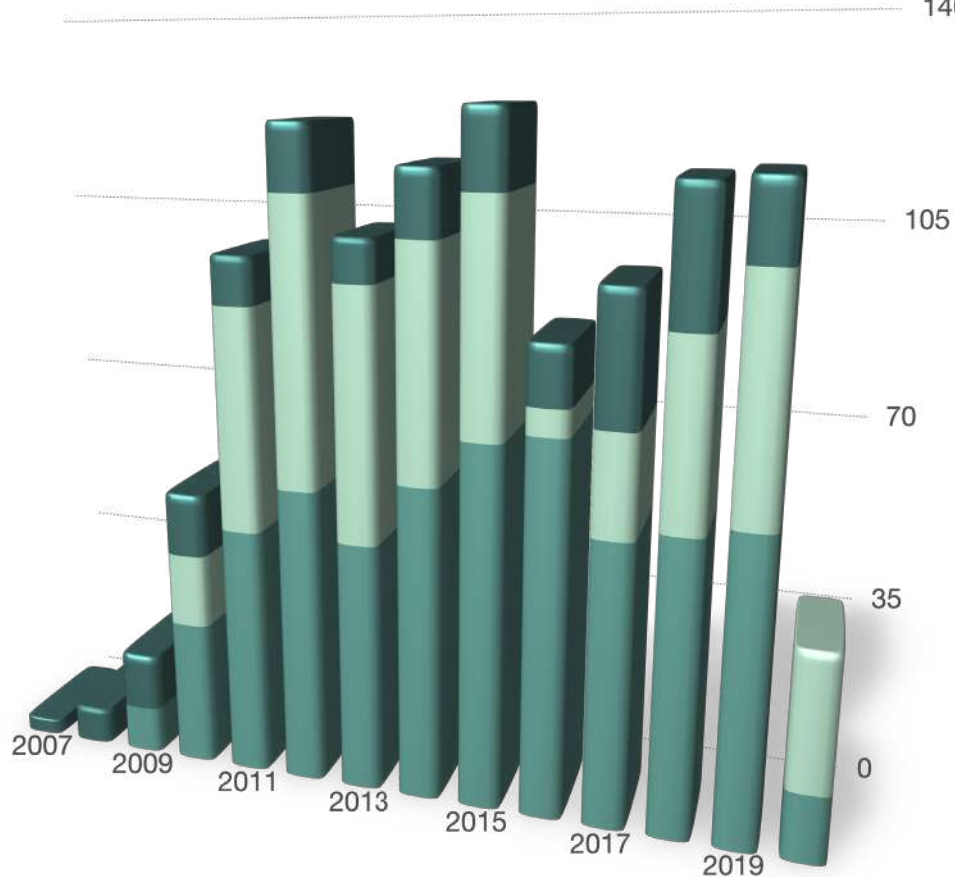
Year	External donations	Consultations	Internal donations
2007			3
2008			7
2009	9		11
2010	28	14	12
2011	48	42	9
2012	57	53	12
2013	48	47	8
2014	60	43	12
2015	69	42	14
2016	71	5	11
2017	54	19	24
2018	56	34	24
2019	58	43	14
2020	13	26	0

According to these data, the number of donation cases extracted and processed entirely in the CIEN Foundation in 2020 amounted to 13.

Regarding the cases accumulated since the beginning of BT-CIEN activity, the distribution by main neuropathological diagnosis is the one shown in the following figure, with approximately 50% of Alzheimer's cases, and extensive representation of the rest of neurodegenerative diseases.

Legend:
- Sporadic Alzheimer's disease
- Cerebrovascular disease
- Lewy bodies disease
- Frontotemporal lobar degenetarion – TDP
- Frontotemporal lobar degenetarion - Pick
- Frontotemporal lobar degenetarion - Tau
- Frontotemporal lobar degenetarion - FUS
- Parkinson's disease
- Progressive supranuclear palsy
- Multisistemic atrophy
- Huntington's disease
- Control
- Amyotrophic lateral sclerosis
- Other tauopathies
- Ataxias
- CADASIL
- Genetic Alzheimer's disease
- Other diagnostics

The research centers that have received samples from BT-CIEN during 2020 have been:

- Institute for Biomedical Research "Alberto Sols", IIBm (CSIC-UAM)

- Hertie Institute for Clinical Brain Research, University Clinic Tübingen, Germany

- Cajal Institute, CSIC.

- Doce de Octubre University Hospital in Madrid.

- Carlos III National Center for University of Madrid (UAM).

- Dementia Genetics Spanish Consortium (DEGESCO).

Research projects

During 2020, the Department of Neu-

ropathology has been involved in the following research projects:

- National Biobank Network (RNBB, for its acronym in Spanish), Platform funded by ISCIII, 2018-2020, which has the BT-CIEN as one of its nodes. Alberto Rábano is co-coordinator of Program 3 (R+D+i) while Laura Saiz performs tasks to support the Coordination. Within the Program 3research lines, Ana Belén Pastor participates in Working Group 3.2 dedicated to the study of microRNAs as new types of biological sample, and Valentina González collaborates in line 2.1 dedicated to the study of quality markers in tissue samples (OPTIMARK multi-center research project, funded by FIS, ISCIII).

Progressive Supranuclear Palsy. Identification of susceptibility loci involved cellularity and molecular pathways for drug development. Funding entity: CIBERNED (Cooperative projects). Participating entities: ACE Foundation, H. U. Marqués de Valdecilla, Severo Ochoa Molecular Biology Center (CSIC), CIEN Foundation

10 years of BT CIEN activity

On April 21, it was the 10th anniversary since the CIEN Foundation's Tissue Bank (BT-CIEN) was inaugurated. Until that moment (April 21, 2010), the Alzheimer Project biobank (of the Queen Sofia Foundation and the CIEN Foundation), had focused its activity on the donations of brain tissue from the Queen Sofia Foundation Alzheimer's Center in Vallecas. Thus, since its inception, the BT-CIEN has had an internal donation program, aimed at the cohort of patients followed in CAFRS, and an external donation program, aimed at the general population of the Region of Madrid, and other Regions that at that time did not have a Neurological Tissue Bank. The external program gathered at that time the experience and tradition of the Tissue Bank for Neurological Research, which had begun its activity in 1996 at the Faculty of Medicine of the Complutense University of Madrid.

Within the framework of the regulations created in our country on biomedical research (Biomedical Research Law, 2007 and Royal Decree of Biobanks, 2011), in 2013 the BT-CIEN received the Accreditation of the Ministry of Health and Social Welfare of the Community of Madrid and was registered in the Biobank Registry of the Carlos III Institute of Health. Likewise, in terms of Quality Management, in 2012 BT-CIEN received the quality certification ISO:

9001 2008, and the updated standard ISO: 9001 2015 in 2017.

During these years, the BT-CIEN's own activity has experienced sustained growth, both in registering donors and obtaining donated brain tissue, as well as in the transfer of samples to researchers. The BT-CIEN registry currently includes more than 700 active donors. The BT-CIEN sample files on the 10th anniversary of BT-CIEN counted with brain tissue from a total of 742 donations, 165 of which from patients from the cohort of the Queen Sofia Foundation Alzheimer´s Center (CAFRS). It should be noted that the latter have abundant associated clinical data, serial blood samples obtained during the follow-up of the patients, and numerous magnetic resonance studies carried out in the center pre- and postmortem. Slightly more than 50% of donations have a primary final diagnosis of Alzheimer's disease, while the remainder include the most prevalent neurodegenerative disorders and the rare and less frequent neurological diseases, as well as brain tumors and vascular and inflammatory diseases of the nervous system. BT-CIEN also stores about 8,000 samples (about 200,000 aliquots) of blood from the CAFRS and Vallecas Project cohorts, as well as about 300 saliva samples and about 600 postmortem cerebrospinal fluid samples. BT-CIEN has provided thousands of samples to some 50

different national and foreign research centers, many of them part of the CIBERNED research consortium. The results obtained in these samples have so far resulted in more than 100 scientific publications, many of them with a high impact factor.

The trust of clinicians, patient, and family associations and, naturally, donors and their families, has been essential for BT-CIEN to be today among the first banks of neurological tissues in our country, and for allowing us to continue looking forward to the future, despite the current difficulties.

As the only neurological tissue bank within a national public body, BT-CIEN has promoted and contributed to the development of other brain banks in our country, such as those currently operating in Murcia, Salamanca, León, and Córdoba. BT-CIEN also participates in the National Network of Biobanks, promoted by the Carlos III Institute of Health, and has contributed to creating the Neurological Tissue Banks Working Group (GT-BTN), a cooperative structure that for the first-time groups together the brain banks in Spain.

As in so many other social and scientific aspects, the current COVID-19 pandemic represents possibly the greatest challenge that neurological tissue banks have had to face in their history. This disease is being particularly cruel to the population group to which most

of BT-CIEN donors belong to, the elderly, and in particular the most fragile and vulnerable of them. The BT-CIEN, like the rest of the neurological tissue banks, will have to overcome this crisis hand to hand with its potential and current donors, and our health and social health systems. The resumption of brain tissue donation programs, now temporarily suspended, will mean that the pandemic has been overcome in our country.

Team

During 2020, the Department of Neuropathology staff has been made up by the following personnel:

- Dr. Alberto Rábano (neuropatholgist), Head of Neuropathology and BT-CIEN Scientific Director

- Laura Sáiz Auz (sample management technician)

- Eugenia Hitt Rech (Pathology Technician).

- Collaborators (24 h autopsy team):

- Luis Javier Martín Lentijo (Neuropathology Technician)

- Mª Cruz Santiago San Marcos (Neuropathology Technician).

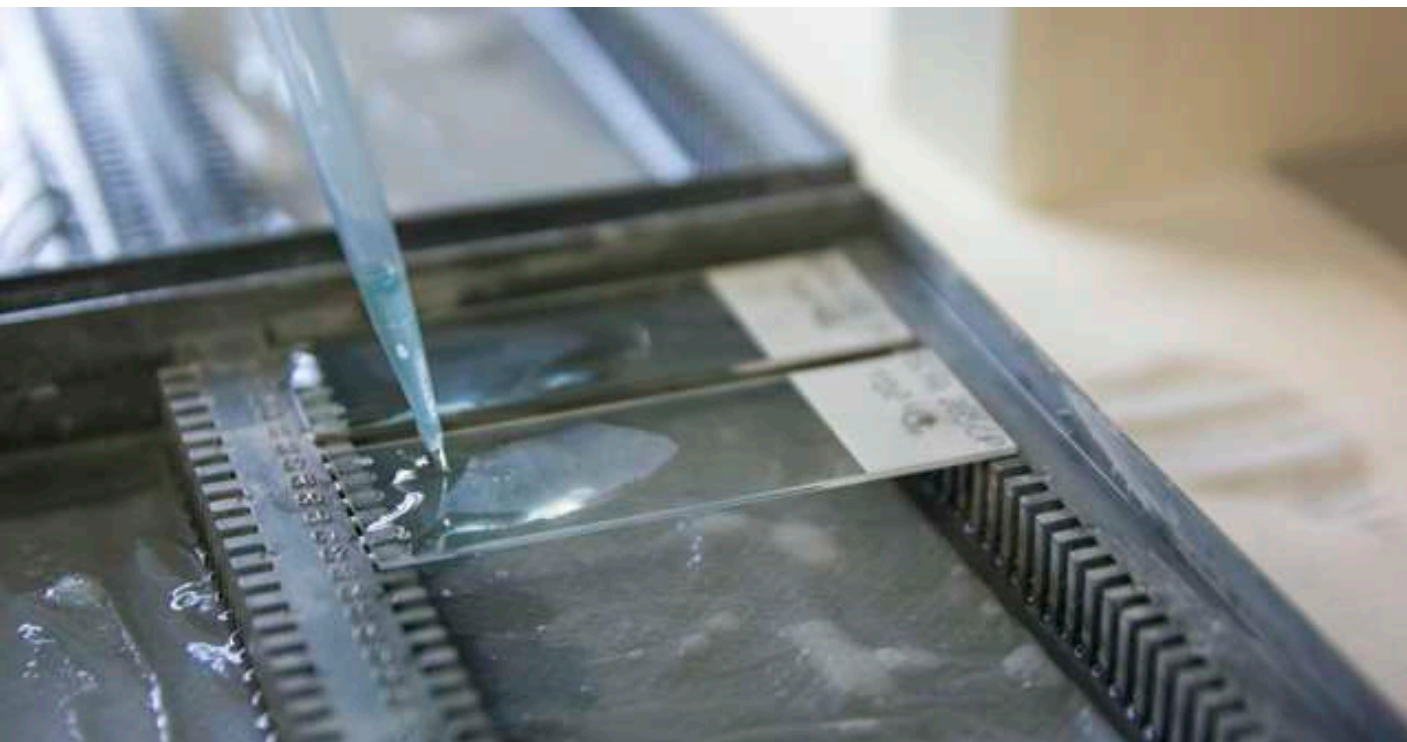### 3.2.5. Department of Biochemistry and Molecular Genetics

The aging of the population and the growing epidemic of Alzheimer's disease (AD) highlight the importance of research in the molecular mechanisms of pathology, as well as in the development of methods for the early detection of the disease to carry out an adequate evaluation of risk and to be able to implement early and effective therapies. Currently, it is widely accepted that changes at the cellular level associated with AD, including the formation of neurofibrillary plaques and tangles, begin many years before clinical symptoms are evident or the existence significant cell death in the brain. Therefore, the development of biomarkers that allow the identification of patients with incipient AD or asymptomatic people at risk is of great importance, so that treatments aimed at stopping neurodegeneration can be initiated before it becomes irreversible.

The most extensively studied biochemical markers are the tau protein (total levels and different phosphorylated isoforms) and the amyloid ß peptide in cerebrospinal fluid (CSF), that are both directly related to neurofibrillary and amyloid pathology, respectively. However, the drawbacks derived from obtaining CSF, together with

Members of the Department of Biochemistry and Molecular Genetics

a limited precision of these assays in early phases, highlight the need to identify new markers, in particular in more accessible biological fluids such as blood. Currently, many researchers believe that both the development of neurofibrillary and amyloid pathologies in AD represent relatively late events in the evolution of the disease, which may or may not reflect the fundamental biochemical-molecular dysfunctions that give rise to the disease. The clinical manifestations of AD are preceded by an asymptomatic preclinical phase, after which the first symptoms appear in the prodromal phase of the disease characterized by mild cognitive impairment (MCI). In this sense, AD can be understood as a continuous process that evolves from the asymptomatic phases to the dementia phase. This evolution is largely determined by genetic risk variants and is associated with biochemical changes that can ideally serve as early markers of the disease.

Department activities

The activity of the Biochemistry and Molecular Genetics Department focuses mainly on the search for biomarkers of early diagnosis of Alzheimer's disease and the study of genetic susceptibility factors of AD and other neurodegenerative disorders. This activity has the following objectives: to deepen the molecular basis of the disease and develop predictive algorithms that combine information on genetic and biochemical markers with diagnostic, prognostic, or response value to modifying therapies.

For this purpose, multidisciplinary research with the rest of the CIEN Foundation departments, together with the CIEN Tissue Bank (BT-CIEN),

are decisive for working on the two main projects of the CIEN Foundation-Queen Sofia Foundation: the Vallecas Project for the early detection of Alzheimer's disease and the Alzheimer's Center of the Queen Sofia Foundation Research Program.

• The Vallecas Project

It is currently known that the pathological processes that determine Alzheimer begin many years before the disease leads to the first noticeable symptoms in patients. Years before that future drug treatments preventing or slowing down disease progression could be applied to the "population at risk" who has developed these subclinical lesions or has a higher risk of developing it than the rest of the

population. In this context it is framed the Vallecas Project, which is constituted as a 5-year longitudinal study specifically aimed at discovering the factors that would allow us to detect this "population at risk" in a phase of potentially treatable pathology.

During 2020, 91 volunteers were studied, with the following distribution: 2 volunteers from the fifth follow-up visit of the study, 33 volunteers from the sixth visit, and 13 from the seventh visit.

Of all volunteers recruited in the study and having an informed consent, a blood sample is collected and immediately transferred to the laboratory for fractionation into aliquots following the so-called Vienna Institute of Neurology protocol, which allow different types of analysis, as well as classification and storage (see Figure below). Additionally, one blood tube (BD-CPT citrate Vacutainer) for the isolation of mononuclear leukocytes, together with another tube lacking anticoagulant to obtain serum are processed. Furthermore, saliva samples from 43 volunteers have been collected as well.

Vienna Institute of Neurology Protocol for blood processing in various fractions for the search for biomarkers and susceptibility genes.

EVALUATION	1ª	2ª	3ª	4ª	5ª	6ª	7ª	8ª	9ª	TOTAL
N° SAMPLES	1169	767	755	699	663	447	314	131	0	4645
Samples 2020	0	0	0	0	0	0	13	33	2	48
Total	1169	767	755	699	663	447	327	164	2	4993
N° Aliquots	16366	10738	10570	9786	9282	6258	4578	2296	28	69902

Within the Molecular Genetics department, the activity of the Vallecas Project is shown in the table on the following page:

The type of primary aliquots that are obtained in duplicate are the following:

- Whole blood (ST, for its acronym in Spanish)

- Platelets-rich plasma (PRP)

- Platelets-free plasma (PFP)

- Buffy Coat (BC)

- Red blood cells (RBC)

- Mononucleate leukocytes

- Serum

Genomic DNA was extracted from whole blood of all participants who have signed informed consent to it and the APOE gene, an important marker of genetic risk for Alzheimer's disease, was analyzed. The comparison of the frequency of APOE allele 4 carriers between CAFRS patients and Vallecas Project volunteers confirms the risk to suffer from Alzheimer's disease with an OR = 3.53 (p <0.001). In addition, in order to define different subpopulations of genetic risk, other possible genetic susceptibility genes have also been analyzed in a subset of participants (see below).

APOE GENOTYPES	n
e2/e2	5
e2/e3	118
e2/e4	10
e3/e3	848
e3/e4	195
e4/e4	12

Distribution of genotypes of APOE gene in the population of volunteers from the Vallecas Project.

It is also important to emphasize that the samples obtained from Vallecas Project volunteers aged between 70 and 85 years (at the baseline evaluation) that include a comprehensive assessment of cognitive, sociological and neuroimaging state are optimal for its use as a control population in various projects related to neurodegenerative diseases, especially Alzheimer's disease. The monitoring for a period of 5 years will allow us to detect early, even before clinical symptoms manifestation, susceptibility factors and biomarkers associated with Alzheimer's disease.

- Queen Sofia Foundation Alzheimer Center Research Program

The Queen Sofia Foundation Alzheimer Center Research Program focuses on regular and protocol-based monitoring of a cohort of CAFRS patients with dementia, ether as residents at the Center or attendants at the Day Center, with the main objective of investigating the final stages of Alzheimer's disease. A family member or guardian recruits patients into the monitoring program after signing an Informed Consent. The Alzheimer Project program consists of i) a biannual clinical and neuropsychological assessment by the Multidisciplinary Support Unit (UMA. for its acronym in Spanish), ii) a biannual blood sampling, coincident with the usual one taken at the residence, iii) conducting an annual cranial MRI if the patient's condition allows it, and iv) donation of brain tissue after patient's death.

The CAFRS takes care of 156 patients in residence, and 40 patients in the Day Centre. The Alzheimer project-monitoring program includes obtaining a blood sample biannually coinciding with the one routinely performed at the Center for conventional analytics. Thus, performing a venipuncture in the patient for research purposes only is avoided. After extraction, each blood sample is processed at once, resulting in 14 aliquots comprising various hematologic derivatives (whole blood, plasma, serum, etc.), including

extraction of DNA for genetic studies.

Aliquots obtained from blood samples are incorporated into the CIEN Tissue Bank (BT-CIEN, for its acronym in Spanish) collection according to the protocols of the biobank. The total number of samples incorporated to the BT-CIEN so far, corresponding to the Alzheimer project monitoring program, adds up to 3,412 (15.2% patients corresponding to the Day Centre), which have resulted in a total of 47,768 aliquots of different fractions derived from blood samples.
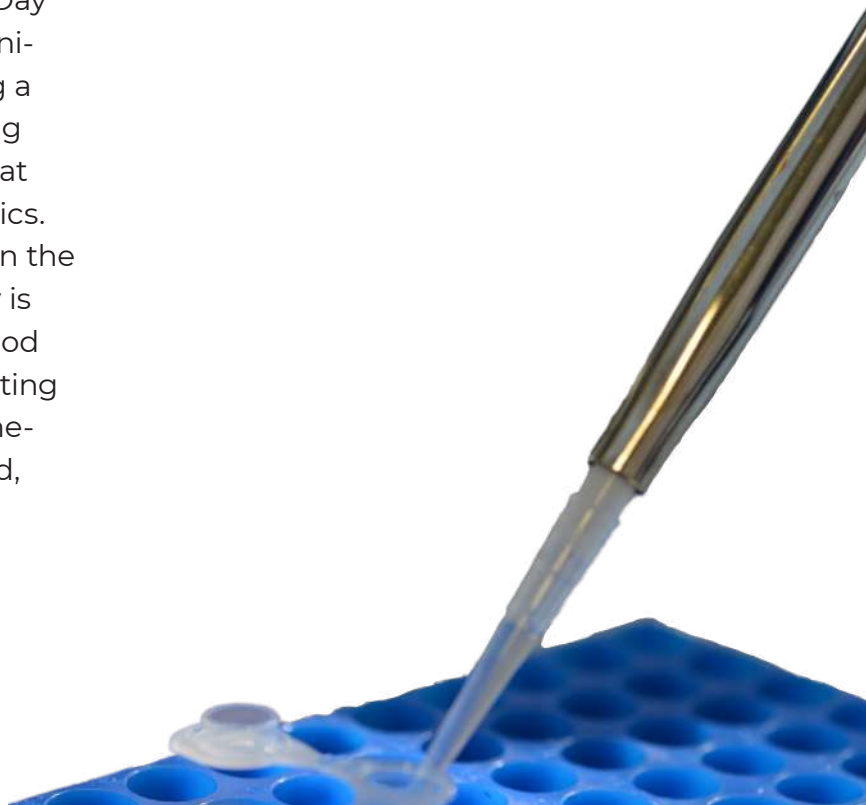
FOLLOW UP VISIT	1ª	2ª	3ª	4ª	5ª	6ª	7ª	8ª	9ª	10ª	11ª	12ª	13ª	14ª	15ª	16ª	17ª	18ª	TOTAL
Residence	0	4	2	7	5	5	3	4	0	3	2	1	2	0	0	0	0	0	184
Day center	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		26

Cumulative 2020

FOLLOW UP VISIT	1ª	2ª	3ª	4ª	5ª	6ª	7ª	8ª	9ª	10ª	11ª	12ª	13ª	14ª	15ª	16ª	17ª	18ª	19ª
Residence	349	324	283	241	229	183	157	146	141	131	99	89	74	68	45	39	35	27	202
Day center	133	102	77	58	44	29	15	10	6	4	4	4	3	1	1	1	0	0	26

Samples obtained up to December 31, 2020 according to the number of semiannual evaluations. Above: samples obtained in 2020. Below: samples accumulated since the beginning of the CAFRS Research program

Consistent with other studies, the analysis of the APOE gene polymorphism in CAFRS patients revealed a high presence of allele ε4, which in this population appears to be more prevalent in men. In addition, the phenomenon of advancement of the age of onset of Alzheimer's disease associated with the ε4 allele observed in other cohorts, is noted, as it is the reverse phenomenon of delayed age of onset associated with the ε2 allele.

APOE GENOTYPES	n
e2/e2	0
e2/e3	19
e2/e4	5
e3/e3	214
e3/e4	168
e4/e4	35

Distribution of APOE genotypes in the CAFRS patients cohort

- **Other projects**

Currently, several lines of study are being followed up based on the combined use of biochemical markers and genetic data.

- **MicroRNAs and lipid metabolism markers as potential links between vascular dysfunction and Alzheimer's physiopathology**

This project, funded by MINECO (RE-TOS call for projects) and which has had Drs. Miguel Medina and Miguel Calero as Principal Investigators, ended on December 31, 2020. During this period, the possible role of miRNAs and lipid metabolism markers as possible links between peripheral vascular dysfunction and the pathophysiology of AD has been explored, having identified some miRNAs, and developed advanced techniques for determining its blood levels, in particular miR206. In addition, we have developed diagnostic algorithms that allow us to advance in the identification of cognitively healthy individuals, but with a higher risk of developing cognitive impairment and AD in the future. The results of the project have led to numerous publications in international scientific journals and have also been presented at various national and international conferences. Finally, two patents directly related to the results of the project have been registered, one of which has been licensed to a Spanish biotechnology company.

- **Molecular mechanisms associated to risk factors and resilience in neurodegeneration. Lipid metabolism and membrane dynamics (PID2019-110401RB-100)**

This project has been funded by the MICINN and has Drs. Miguel Medina and Miguel Calero as Principal In-vestigators. Its general objective is to explore the molecular and cellular mechanisms that confer susceptibility or protection/resistance to the onset and progression of Alzheimer's disease (AD) and related disorders, focusing on the interaction of lipid metabolism and membrane dynamics with the transce-llular spread of pathognomonic pro-teins in the brain. For this purpose, we will analyze physiological and patholo-gical brain aging, focusing specifically on apolipoprotein-mediated modula-tion in the spread of neurofibrillary tau pathology and amyloid pathology. Our working hypothesis is based on the idea that lipid metabolism and mem-brane dynamics (and more specifically ApoE as a key regulator of cholesterol homeostasis) are common elements that regulate the onset and progres-sion of different neurodegenerative diseases. Therefore, our objective is to study the risk and protective/resi-lience factors that contribute to the development of the disease or brain resilience in advanced ages. To do this, we propose three interconnected and complementary objectives in order to address the study of potential mole-cular pathways that underlie the me-

chanisms of susceptibility and protection, as well as their potential role in the spread of the pathology. The first objective builds on results from the previous project (SAF2016-78603-R) and also exploits genetic data from the ongoing GWAS with samples from the Vallecas project volunteers.

The first objective will identify new risk factors related to the duration of the disease, the rate of progression and the age of onset. For this purpose, with the available genetic data, we will perform association analysis with special emphasis on the potential interaction with APOE haplotypes. We will also validate and functionally characterize some miRNAs previously identified as biomarkers of mild cognitive impairment and AD. The second objective focuses on the identification of the resilience mechanism in cognitively intact people over 80 years of age. For this purpose, we will study genetic factors, hallmarks of aging, neurodegeneration markers, and structural determinants of resilience in our AD case-control cohorts. The last objective addresses the role of specific risk/protection pathways in cell-to-cell spread of tau pathology using cell and animal models. In addition, we will analyze the expression and localization of proteins that modulate tau pathology in brain tissue.

This project follows a multidisciplinary approach, integrated into a long-standing research strategy focused on understanding neurodegeneration, and specifically AD. The project will provide crucial information on the functional relationship between the development of tau pathology, lipid metabolism and membrane dynamics. The described experimental strategy could eventually be easily extended to other studies and risk factors/protection from AD and other tauopathies. Identifying the biological effects of these key factors in AD in preclinical studies is essential for the development of evidence-based therapies.

- **Dementia Genetics Spanish Consortium (DEGESCO).**

Likewise, the CIEN Foundation continues its active participation in the Dementia Genetics Spanish Consortium (DEGESCO) and in this context, research activities aimed at defining new genetic risk factors and the participation of the national consortium in international proposals continue to be carried out. Samples from the Vallecas Project and the CAFRS Research Program have been used in genome-wide genetic association studies (GWAS). These association studies, in addition to serving as a replica in a Spanish population of studies carried out in other populations, will allow us to determine the most important genetic factors in the development of cognitive dysfunction in our Vallecas Project population,

as well as to define endophenotypes based on genetic variations and specific and measurable characteristics of patients and controls based on clinical neuroimaging, biochemical or pathological measures (see figure).

YEAR	CSF (n)
2008	3
2009	11
2010	32
2011	38
2012	54
2013	38
2014	57
2015	61
2016	58
2017	58
2018	53
2019	47
2020	9
TOTAL	519

Samples of cerebrospinal fluid (CSF) obtained post-mortem since 2008.

In this regard, we have participated in a large European genome-wide genetic association study that pooled available case-control data sets from more than 450,000 subjects. The study identified six new genetic variants associated with the risk of Alzheimer's disease (close to APP, CHRNE, PRKD3/NDUFAF7, PLCG2, and two exon variants in the SHARPIN gene). The polygenic risk assessment and stratification by APOE reveal a difference of 4 to 5.5 years in the median age of onset of patients with Alzheimer's disease in subjects with APOE ε4. This study allows us to refine the study of the molecular mechanisms underlying the disease, while the polygenic risk score provides a tool to select individuals at high risk of suffering from Alzheimer's disease..

- **A European DNA bank for deciphering the missing heritability of Alzheimer's disease - EADB (European AD DNA Bank) and GR@ACE project (Genomic Research at Fundació ACE)**

Deciphering the genetic landscape of Alzheimer's disease (AD) is essential to define the pathophysiological pathways involved and to successfully translate genomics into potential tailored healthcare. To generate the most complete knowledge of AD genetics, a European Alzheimer's Disease genetic data biobank (EADB) has been created. This project is an international collaboration initiative that is carried out through the DEGESCO consortium and that aims to significantly increase the generation of data based on GWAS. In this study, more than 30,000 AD cases and 40,000 controls from 11 countries can be analyzed. GWAS and complementary statistical studies (based on genotype and imputation data) have been carried out in order to identify new genetic factors and identify pathophysiological mechanisms

of the disease. Thus, the number of EA samples available has multiplied by more than 4 in Europe and by almost 2 worldwide. Carrying out this project has made it possible to identify 31 new loci associated with AD risk, in addition to the 34 already known loci. The analysis clearly indicated the involvement of sets of genes related to amyloid and Tau, but also highlighted microglia, in which increased gene expression corresponds to a more significant risk of AD. Furthermore, nine of the new candidate genes are expressed primarily in microglia. Finally, it was observed that a polygenic risk score generated from this new genetic landscape is strongly associated with the risk of progression from mild cognitive impairment (MCI) to dementia, regardless of age and APOE e4 allele.

Additionally, in the context of the DEGESCO consortium, collaboration has begun with the GR@ACE project, led by the ACE Foundation, which will be carried out in three years, and whose objective is the application of high-resolution genomic technologies to the identification of a new generation of genes that provide data in the design of new treatments to deal with Alzheimer's disease.

A project has also been developed in collaboration with the laboratory of Dr. Carlos Dotti (Center for Molecular Biology "Severo Ochoa") with the aim of determining the possible functional relationship between the hemopro-

tein neuroglobin and amyloid peptide levels. Our analysis showed that plasma Ngb levels in older people are decreased in those individuals whose cognitive abilities worsened during a longitudinal 5-year follow-up period. These results have been published at the end of 2020 (de Vidaña et al, 2020 Front. Neurosci. 14: 562581).

With regards to the study of biomarkers and in the collaborative context with the company Biocross SL and various Spanish hospitals, we continue with the plasma metabolomic studies of people with Alzheimer's disease, mild cognitive impairment or without cognitive dysfunction. In addition, we also continue with the development of a non-genetic test adapted to the hospital diagnostic routine for measuring blood ApoE4 as a marker for determining the AD risk and that has currently obtained the CE label.

Other collaborations

- Assessment of lactoferrin levels in saliva as a marker of Alzheimer's disease in collaboration with Drs. Eva Carro and Félix Bermejo from the 12 de Octubre Hospital

- Study of the role of lipofuscin in neurodegeneration in collaboration with Dr. A Kun from the University of the Republic, Uruguay

- Determination of plasma levels of miRNAs using new devices based

on microfluidic techniques. In collaboration with Drs. Tobias Engel (RCSI, Dublin) and Robert Forster (Dublin City University).

Contribution to BT-CIEN

The Biochemistry and Molecular Genetics department also contributes to the BT-CIEN with processing of various samples and collaborates on several external projects focused on Alzheimer's disease and other neurodegenerative diseases.

In the context of research focused on the study of biomarkers and genetic susceptibility factors, the UIPA Laboratory department is responsible for collecting, processing, and storing biological samples for research related to various projects or for its deposit in the BT CIEN, whose ultimate purpose is to use in different research areas on neurodegenerative diseases.

Currently, the department contributes to BT-CIEN with various biological samples including 519 CSF samples from donor's brain, 456 skin samples, and 2319 saliva samples.

Team

During 2020, the team of the Biochemistry and Molecular Genetics Department was composed of the following personnel:

Miguel Calero Lara (PhD, Chemistry),

Head of Department. Until April 2020

Miguel Medina Padilla (PhD, Biology),
Head of Department. From April 2020

Ana Belén Pastor López (Laboratory
Technician)

Alicia Ruiz González (MSc, Biology).
Since December 2020

Collaborators

Olga Calero Rueda (PhD, Biology)

Andrés Rodríguez Martín (Laboratory
Technician, CIEN Foundation-Biocross
agreement)

Sergio Veiga Herrero (PhD, Biology,
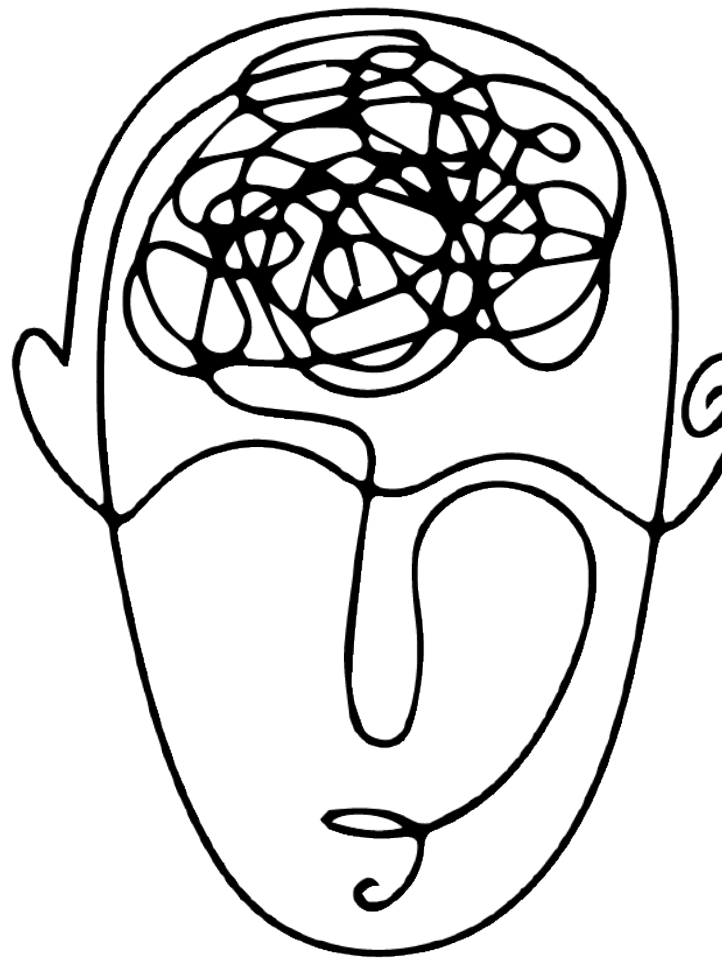CIEN Foundation-Biocross agreement)

04.

The Vallecas project

4. The Vallecas Project

4.1. Introduction

Aging is one of the major risk factors for some of the most prevalent diseases such as cancer, cardiovascular disorders, or neurodegenerative diseases, but while the number of cases of the first two tends to stabilize, the number of patients with neurodegenerative pathologies, particularly dementia, continues to grow exponentially. According to the demographic estimates of the National Institute of Statistics, in 2019 there are 8.9 million people in Spain aged 65 or over (19.4% of the population) and it is estimated that in 2050 about 15 million Spaniards (approximately one third of our population) will be over 65 years old. In fact, the combination of one of the highest life expectancies in the world and one of the lowest birth rates make the Spanish population the oldest in Europe and one of the oldest in the world after South Korea and Japan.

Dementia is a clinical syndrome characterized by a progressive cognitive impairment severe enough to affect personal and social functioning of an individual. Alzheimer's disease (AD) is the leading cause of dementia in our environment. According to the National Epidemiology Center, 7.3% of the population over 65 years could suffer from this disease nowadays. In total, AD constitutes about 75% of the etiology of dementias, either alone or in combination with cerebrovascular disease. Because of increased life expectancy and the progressive aging of the population in Western countries, dementia represents a huge challenge for public health systems. In our country, it is estimated that approximately one million Spaniards could have dementia by 2050.

According to the Survey of Disability, Personal Autonomy and Dependency Situations developed by the National Institute of Statistics, the rate of disability stands at ninety dementia cases per thousand inhabitants. However, the impact of dementia is not only produced directly on the patient, but also has a great influence his/her fami-

ly and social environment concerning affective, organizational, and economic aspects. In this sense, dementia should be understood as a social problem that must be approached in a comprehensive manner.

The transition from a cognitively healthy stage to an AD-type dementia is a continuum in which some intermediate stages, preclinical and prodromal can be recognized. These stages are characterized by the presence of an incipient cognitive impairment that increases the probability of conversion to dementia in the future. An effective therapeutic intervention in these phases prior to AD could eventually slow the progression of deterioration and thus reduce the prevalence of the disease. For this reason, one of the challenges currently faced by research is the development of useful tools that allow early diagnosis of AD.

Currently, there is no reliable method to predict safely which individuals in these preclinical stages have an increased risk of converting to dementia. The emergence in the last decade of various diagnostic neuroimaging techniques (eg, brain PET amyloid) has led to considerable progress in research, although its use in regular clinical practice is not feasible due to its high cost.

The most recent descriptive epidemiological research on dementias is allowing us to investigate how prevalence and incidence are changing over time. To establish clear trends, these comparisons should be based on studies that use similar diagnostic and research methods consistently over time. It is important to bear in mind that the main non-genetic risk factors for the development of dementia are related to lifestyle, which makes it particularly important to carry out studies in the Spanish population. Important changes in our society and improvements in living conditions and education in recent decades can have a decisive influence not only on physical well-being, but also mental and cognitive health and therefore on the incidence of dementia in the elderly population. Thus, the analysis of risk factors related to the symptoms of dementia must be accompanied by a comprehensive phenotypic characterization in order to provide a better understanding of the underlying neurobiological mechanisms.

The concept of population studies - that is, the recruitment of participants from community environments to ensure good representations of the entire population - must be incorporated in future neurobiological and neuropathological investigations in dementia. The results of small clinical-based samples, which include only patients from memory clinics or other medical services, have inherently limited generalization and the considerable potential for bias due to highly selec-

tive recruitment. In particular, people who are socially disadvantaged are less likely to participate in that research. The integration of neuroscience with population studies and neuroscientific epidemiological approaches is of vital importance and provides the opportunity to integrate the understanding of brain health, neurobiology, and neuropathology within the general population to support better prevention, care and cure of dementia.

The main objective of the population-based study "Vallecas Project" for Early Detection of Alzheimer's Disease, is to elucidate, through tracking of progression, the best combination of clinical parameters and complementary tests (imaging and laboratory) that allow deciphering at medium- and long-term features that distinguish those who will develop memory impairment (MCI and dementia) from those who will not. Thus, it intends to identify various markers to determine eventually the potential risk that each individual could have to develop the disease in the future.

4.2 Background: Pilot project

A pilot study was conducted between June 2010 and February 2011, prior to the final project, whose first preliminary results are presented in this report. The aims of this study were:

- To verify the feasibility of

the working procedure, the cooperation of the target population and the adequacy of screening protocols to the study objectives.

- To obtain early and sufficient information on the characteristics of the recruited volunteers and those that could not be recruited, as well as the limitations of the actual sampling compared to the intended one.

- To get experience in the implementation of the different elements of the protocol and to estimate the burden of the evaluator and the evaluated.

- To promote the Project to achieve the participation of volunteers and attracting enough funds to carry out the Vallecas Project.

- 175 volunteers were involved in this phase of the project, of which:

- 95 people were able to participate in the project.

- 80 people were unable to participate because they met at least one exclusion criterion.

4.3. The Vallecas Project

Following the completion and analysis of the pilot study the protocol was amended based on the experience gained and a volunteer recruitment strategy was established (social awareness campaign in the media, visits to centers for seniors, contact pensioner's organizations, etc.). In September 2011, after the "Global Summit on Alzheimer Disease Research" held in Madrid and with the financial support of the Queen Sofia Foundation.

The Vallecas Project is being carried out in the Queen Sofia Foundation Alzheimer Center Research Unit by researchers from the CIEN Foundation (Carlos III Institute of Health). It aims to develop a probabilistic algorithm to identify individuals at risk for AD-type dementia over the course of a few years. Such an algorithm will be based
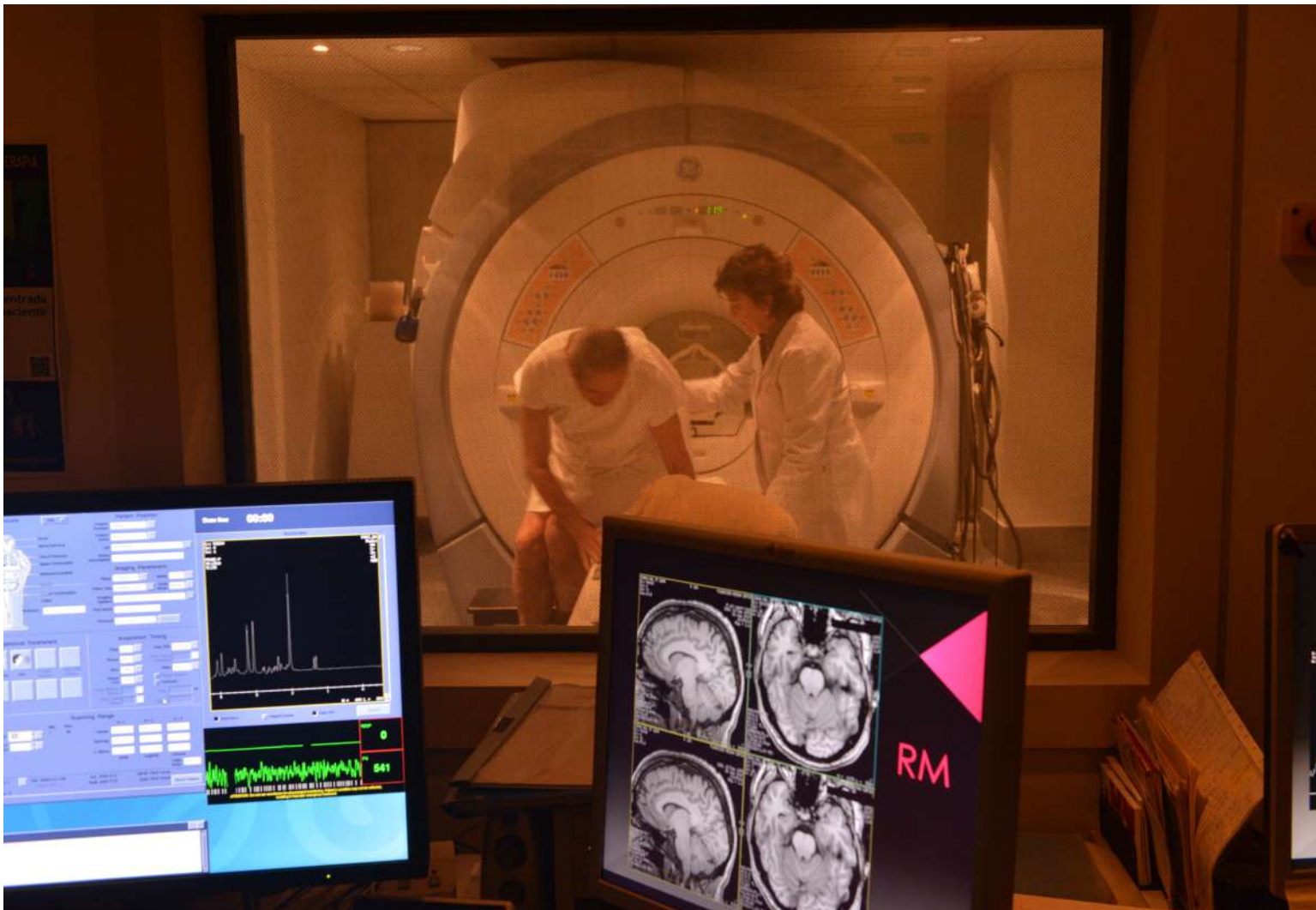
on a combination of socio-demographic, historical, clinical, neurological and neuropsychological, biological (from blood tests) and neuroimaging (various forms of 3T MRI).

The participant recruitment phase for the Vallecas project lasted from October 2011 to December 2013. By then, 1,213 individuals of both genders, aged 70-85 years were recruited and evaluated at baseline. Once included in the study, the project conducts an annual follow-up for five years to assess the evolution profile of all participants, specifically identifying those that develop cognitive impairment and/or dementia. At the beginning of 2018, an agreement was signed between the CIEN Foundation and the Queen Sofia Foundation to extend the duration of the Vallecas Project beyond the five years of follow-up originally planned. This second phase covered by the new agreement will allow the cohort to be followed for an additional four-year period.

At the beginning of 2020, the sixth follow up visit for the whole cohort had been completed, were approaching the end of the seventh, mid-way through the eighth and just starting the ninth one. Regular monitoring of the cohort was sharply affected from March 2020 onwards by the outbreak of the SARS-Cov-2 virus, the expansion of the COVID-19 pandemic, with especially marked morbidity and mortality in the elderly population, and the
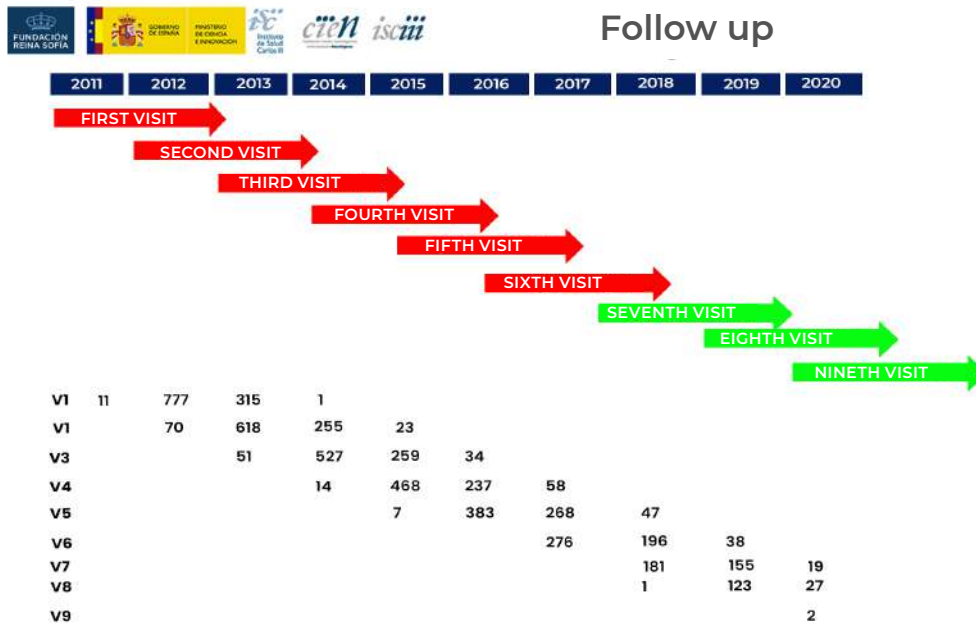
serious outbreaks of the infection in nursing homes, such as the one located in the CAFRS, all of which forced the lockdown of the population for several months. The study of the Vallecas Project volunteers had to be suspended for safety reasons, especially of the participating volunteers.

In these circumstances, during the month of May 2020, a telephone survey was designed and carried out in which most of the Vallecas Project volunteers were contacted (913 volunteers, 90%) and the incidence, severity, mortality, clinical symptoms and risk factors of COVID-19 in this cohort of elderly were established, and their relationship with the type of residence and cognitive function. This telephone survey was also useful to reestablish contact with some of the volunteers who had left the project and to gather information on their current cognitive and functional status.

4.3.1. Baseline evaluation

Before entering the study, volunteers interested in participating in it were subjected to an initial assessment to determine whether they meet the criteria for inclusion and/or whether an exclusion criterion exists. Overall, all volunteers were required to meet four inclusion criteria to be considered for entering the study:

- Signing an informed consent.

Follow up

At 31 December, 2020

	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020
V1	11	777	315	1						
V1		70	618	255	23					
V3			51	527	259	34				
V4				14	468	237	58			
V5					7	383	268	47		
V6							276	196	38	
V7								181	155	19
V8								1	123	27
V9										2

- Be aged between 70 and 85 years old.
- Availability and ability to reach the Alzheimer Centre for visits.
- Visual and hearing abilities that allow conducting the study tests.

In addition, several exclusion criteria were established, including the following: i) suspected or diagnosed dementia; ii) inability to perform neuroimaging studies; iii) alcohol abuse; iv) mental retardation; or v) history of certain psychiatric or neurological diseases (e.g., schizophrenia, stroke, severe head trauma, Central nervous system infections, uncorrected vitamin deficiencies, etc.).

In the table below some global data from the cohort of approximately 1,213 individuals evaluated to date are indicated.

THE VALLECAS PROJECT IN FIGURES

Recruited sample	1.213
Excluded at baseline	47 (3,87%)
Age	
Sample mean	74,46 años
Age group 69-74	671 (55,32%)
Age group 75-79	379 (31,24%)
Age group > 80	163 (13,44%)
Gender	
Females	780 (64,30%)
Males	433 (35,70%)
Education	
Sample mean	10,35 años
Illiteracy	4 (0,34%)
Read/Write	60 (5,11%)
Minimum studies (numeracy skills)	154 (13,11%)
Primary education	389 (33,11%)
Senior High School / Proffesional Training	282 (23,99%)
University Education	286 (24,34%)

4.3.2. Sociodemographic profile

The following variables are collected through semi-structured interview: gender, date of birth, marital status, number of children, type and amount of income, primary occupation and education level, hobbies, and leisure activities, etc.

In addition, each year volunteers also must complete a scale of quality of life and subjective well-being (mobility, personal care, daily activities, pain/discomfort, anxiety/depression, and perceived health status) as well as various questionnaires to gather information related to lifestyle.

4.3.3. Clinical evaluation

At each visit relevant information is collected from each volunteer by applying a semi-structured clinical interview:

- Vascular risk factors: blood pressure, diabetes mellitus, smoking, heart disease, stroke

- Neurological history: mental retardation, head injuries, etc.

- Consumption and/or toxic addiction: alcoholism/level of regular alcohol intake, addiction/ consumption of other psychotropic substances.

- Psychiatric pathology: depression, dysthymia, bipolar disorder, psychotic disorders, anxiety syndromes.

- Other relevant systemic diseases: hepatic failure, renal failure, Obstructive Sleep Apnea Syndrome (OSA)...

- Family history with special attention to the history of dementia or movement disorders, developmental delay, or psychiatric disorders.

- Regular drug treatment during the last 5 years.

- From 207 to 2019, all volunteers who consent to carry an accelerometer device (Actigraph®), similar to a wristwatch, are being provided with it for the recording

of physical activity and sleep for a week. During that period, the following records have been registered

- » 2017: 251 records
- » 2018: 375 records
- » 2019: 181 records

Distributed as follows:

- » Volunteers with only 1 record: 249
- » Volunteers with 2 records: 262
- » Volunteers with 3 records: 12

Hence, we have a sample size of 523 volunteers with at least one record already registered, and 274 volunteers with a second record after one year follow up.

This information must be processed to obtain several variables related to light, moderate or intense activity, as well as indicators of sleep duration and pattern.

4.3.4. General examination

All subjects undergo a general and neurological standard examination: cranial nerves, muscle balance, coordination, extrapyramidal system, gait, osteotendinous reflexes, midline release reflexes, etc. The following parameters are analyzed in a special way:

- Gait disturbance

- Handwriting
- Instrumental activities of daily living

4.3.5. Neuropsychological Examination

The neuropsychological evaluation allows to explore in a holistic way the various cognitive domains (gnosias, attention, memory, language, praxis, and executive functions), as well as a series of variables related to affectivity, behavior, and level of autonomy in the daily life of an individual. To do this, the neuropsychologist can use different assessment instruments, including clinical interviews, behavioral observation, cognitive tests and different questionnaires that allow for collecting information from both the individual and a reliable informant.

The Vallecas Project neuropsychological examination protocol has been designed to comprehensively assess neuropsychological functioning of study participants, as well as their progression during the longitudinal follow-up. Specifically, although the neuropsychological battery focuses especially on the evaluation of memory processes, attention and executive functions as potential early markers of Alzheimer's disease, the neuropsychological profile is completed by getting information related to other cognitive domains such as language, visuospatial ability and visuoconstruction. All these data allow identifying the strengths and weaknesses in the cognitive profile and characterizing, if necessary, the type of cognitive impairment that an individual presents.

Table below lists the different tests that make up the neuropsychological battery of the Vallecas Project, as well as the visit number in which they have been applied to all study participants:

		VISITS								
		1	2	3	4	5	6	7	8	9
COGNITIVE PERFORMANCE	Reading test to evaluate premorbid intelligence	X								
	Mini Mental State Examination (MMSE)	X	X	X	X	X	X	X	X	X
	Clock Drawing Test	X	X	X	X	X	X	X	X	X
	Free And Cued Selective Reminding Test (FCSRT)	X	X	X	X	X	X	X	X	X
	Semantic Lexical Evocation (P, M, R)	X	X	X	X	X	X	X	X	X
	Semantic Verbal Fluency (Animals, Fruits and Vegetables, Kitchen Utensils)	X	X	X	X	X	X	X	X	X
	Number Key (WAIS-III)	X	X	X	X	X	X	X	X	X
	Rey-Osterreith Complex Figure Test	X		X	X	X	X	X	X	X
	Forward and reverse digits (WAIS-III)		X	X	X	X	X	X	X	X
	Symbolic gesture and Imitation of bilateral postures (TBR)		X	X	X	X	X	X	X	X
	Rules Change (BADS)		X	X	X	X	X	X	X	X
	Test of the five points		X	X	X	X				
	Boston Naming Test (BNT-15)		X	X	X	X	X	X	X	X
COGNITIVE COMPLAINTS	Interview for the assessment of cognitive complaints	X	X	X	X	X	X	X	X	X
	Memory Failures in Everyday (MFE)		X	X	X	X	X	X	X	X
DEPRESSION AND ANXIETY	Geriatric Depression Scale (GDS-15)	X	X	X	X	X	X	X	X	X
	State-Trait Anxiety Inventory (STAI)	X	X	X	X	X	X	X	X	X
FUNCTIONAL SCALES	Functional Activities Questionnaire (FAQ)	X	X	X	X	X	X	X	X	X
	Clinical Dementia Rating (CDR)	X	X	X	X	X	X	X	X	X

4.3.6. Determination of biomarkers

It is currently widely accepted that the molecular changes associated with AD, including the formation of amyloid plaques and neurofibrillary tangles begin many years before the appearance of clinical symptoms. In recent years, the need to define and develop new early biomarkers of AD that allow us to assess the risk and early diagnosis of the disease has become clear. Thus, blood samples will be collected within the Vallecas Project for the study of genetic and biochemical markers. Samples are obtained according to the protocol "Collection and Processing of Human Blood Samples in the Vallecas Project" and processed to obtain the various fractions indicated in the protocol, which are stored at -80°C. On one hand, DNA is extracted from blood cells to determine, by PCR and sequencing techniques, genetic markers associated with the various polymorphisms in the following genes: APOE, CR1, BIN1, CLU, PICALM, ABCA7, SORL1, PRNP, GRM8, and BACE1. These genes are studied using DNA obtained from the extraction of samples from the first visit.

Also, in the context of the project funded by the MINECO (Projects RETOS) entitled "miRNA and lipid metabolism markers as potential links between vascular dysfunction and Alzheimer's pathophysiology", and whose main researchers are Drs. Miguel Medina and Miguel Calero, in collaboration with the group of Dr. Tobias Engel (Royal College of Surgeons, Dublin, Ireland) plasma-derived microRNAs are being analyzed as potential biomarkers, as well as molecules related to vascular

dysfunction, lipid metabolism and inflammation: Adiponectin/Acrp30, P-Selectin, ICAM-1, IL-6, MMP-9, Serpin E1/PAI-1, TNF-alpha, VCAM-1, CCL2/MCP-1, IL-1 beta, CXCL8/IL-8, E-Selectin, MMP-3, and CRP. The usefulness of these biomarkers is complementary with the information derived from the study of genetic risk markers already mentioned and can define risk factors already revealed in previous studies.

Samples collected and processed to date are summarized in the table below:

EVALUATION	Total
1st Visit	1169
2nd Visit	767
3rd Visit	755
4th Visit	699
5th Visit	663
6th Visit	447
7th Visit	327
8th Visit	164
9th Visit	2
TOTAL	4993

4.3.7 Neuroimaging Studies

Knowing the morphological variations occurring in brain structure throughout life is essential to assess the corresponding pathological changes that occur in neurodegenerative diseases. In this context, neuroimaging techniques such as magnetic resonance imaging (MRI) have led to significant progress in understanding brain changes associated with age.

MRI is a noninvasive tool that allows the study of normal aging individuals at different moments of his life. However, conventional MRI techniques are unable to detect and quantify age-dependent microstructural changes who have been described in post-mortem studies of brain tissue. Accordingly, the project aims to conduct a series of studies based on various MRI modern techniques that can provide volumetric quantitative indexes of the morphological changes.

In this regard VBM (voxel-based morphometry techniques), based on creating statistical comparisons of gray and white matter patterns are the method of choice in research. The discriminatory power of volumetry in degenerative pathologies such as Alzheimer's disease (volumetric reduction in amygdala, hippocampus, entorhinal cortex, etc.) decreases if age-dependent morphological changes are not well established in control samples, so that it is critical to have large, well quantified

samples.

- **Structural Study (3D volumetry, T2 and FLAIR)**

Determining the progressive loss of brain volume during aging, especially in white matter provides volumetric quantitative indexes of the morphological aging-associated changes. In this sense, the VBM (Voxel-Based Morphometry) techniques, based on creating statistical comparisons of gray and white matter patterns constitute the method of choice, and allows us to determine the volume reduction of the amygdala, hippocampus, entorhinal cortex, etc.

- **Diffusion Study (b: 800)**

White matter, partly due to Wallerian degeneration and partly to reduced connectivity by decreased cortical activity, presents ultrastructural changes that can be detected with diffusion techniques (DTI).

- **Brain Perfusion Study**

Cerebral perfusion related to cortical activity may be assessed -without needing to inject contrast-through MR sequences (Arterial Spin Labelling, ASL) and therefore hypofunctioning areas will present decreased perfusion.

Throughout 2015 all Neuroradiology reports from every subject and each of the visits from the 'Vallecas Project' have been incorporated in the single project database. Text reports have been encoded, incorporating each item to the database, as well as attaching the report of each visit in pdf format, enabling viewing, and downloading to all researchers who have access to the database.

On the other hand, we have organized MRI data corresponding to the 'Vallecas Project' and Queen Sofia Foundation Alzheimer Center subjects, converting the data obtained directly from MRI equipment into the appropriate format for analysis.

A collaborative project with CESVIMA (Supercomputing and Visualization Center of Madrid), a center from the UPM (Technical University of Madrid) has also been established.

As a result, a VBM analysis of T1 sequences from visits 1 and 2 of the subjects 'Vallecas Project' has been performed. The results of this analysis form the basis of a new project awarded to Dr. Bryan Strange by the Alzheimer's Association ("The healthy elderly brain: MRI predictors for developing MCI"), which has allowed the hiring of a new team member, Dr. Linda Zhang. Dr. Zhang, expert in analysis of structural MR images, has examined the white matter in visit 1 of the subjects of the Vallecas Project.

In 2020, Dr. Linda Zhang continued working on the predictive algorithm in Dr. Jussi Tohka's laboratory at the

University of Eastern Finland in Kuopio, Finland, as part of her Queen Sofia Foundation-MAPFRE scholarship. Dr. Tohka is specialized in machine learning and assisted in the validation of the predictive algorithm, both through validation in an internal sample (subjects of the Vallecas Project with mild cognitive impairment-MCI beyond visit 2), as well as in an external sample (subjects with DCL from the ADNI cohort).

Dr. Zhang has also focused on investigating the effects that the ε4 allele of the APOE gene has on the hippocampal volume of cognitively healthy elderly from the Vallecas Project, as this is a genetic risk factor for Alzheimer's disease. These effects were investigated both transversally and longitudinally from visit 1 to visit 5. In addition, she identified subjects carrying the ε4 allele who experienced MCI during the Vallecas Project follow-up and performed a longitudinal analysis between these subjects and a control group with an allelic combination ε3/ε3 of the APOE gene and who had also developed MCI.

Overall, she has completed more than 4,000 hippocampal segmentations in 788 cross-sectional individuals and 430 longitudinal individuals. Her results were presented as a virtual poster at the Alzheimer's Association International Conference (AAIC) 2020.

Dr. Zhang also performed diffusion imaging analysis to investigate differences in white matter microstructure between controls and MCI converters at 1 and 4 years prior to conversion. She continues to study these changes longitudinally, and the abstract detailing her results has been accepted for AAIC 2021.

Lastly, Dr. Zhang has been working with the radiology team to complete quality control of all Vallecas neuroimaging data to date, to ensure a clean database with no duplicates or bad scans. Additionally, she has pre-processed the T1 images of subjects and visits in preparation for future VBM studies.

In 2020, Marta Garo Pascual collaborated with the study of diffusion tensor images in the population of superagers and controls identified in the Vallecas Project cohort. Superagers respond to people over 80 with an episodic memory at least as good as that of a healthy person between the ages of 50 and 60.

The study of the diffusion tensor images was performed using the TBSS (Tract-Based Spatial Statistics) protocol. And both a cross-sectional and longitudinal study of these parameters that account for the microstructure of the cerebral white matter was carried out.

On the other hand, a collaboration was established with Dr. Jussi Tohka from the University of Eastern Finland and

expert in machine learning. Machine learning tools were applied to demographic, lifestyle, and clinical variables in order to find the factors that help to classify superagers against their controls.

In 2020, Qumar Behfar was a recipient of the Queen Sofia Foundation-MAPFRE Fellowship, which is developed in collaboration with Dr. Özgur Onur Department of Neurology, University Hospital of Cologne, Germany. The objective of the scholarship is to continue in the study of the population of superagers. His work will focus on functional MRI, performing connectivity and network analysis to compare superagers and controls.

In 2020, Dr. Strange was awarded the Researcher Excellence Incorporation award from the Polytechnic Univer-

sity of Madrid. As part of his 'Consolidator' project in neuroscience of the European Research Council (ERC) he has refined new functional resonance acquisition sequences to study memory formation in humans and has implemented a sequence sensitive to neuromelanin.

4.3.8. Current Status and Future Outlook

The Vallecas Project is the main research project conducted at CIEN Foundation, both in terms of resources and social impact. In late 2013, the project completed the recruitment phase and the baseline first visits of volunteers. During 2020, parts of the seventh and eighth follow-up visits of the project volunteers have been carried out simultaneously.

VALLECAS PROJECT ACTIVITIES DURING 2020

Number of seventh visit assessments	19
Number of eighth visit assessments	27
Number of ninth visit assessments	2

The following table shows the status of clinical evaluations conducted to date:

VALLECAS PROJECT CLINICAL EVALUATIONS

First visit	1.176
Excluded at baseline	47
Second visit	967
Third visit	870
Fourth visit	778
Fifth visit	703
Sixth visit	511
Seventh visit	355
Eighth visit	151
Ninth visit	2
Drop outs	862
Do not comply with inclusion criteria	35
Deceased	89
Diagnosis of neurological disease	52
Volunteer withdrawal	686

In addition to continuing the studies already launched, there are several new lines of research around the Vallecas project that are considered to be of great interest in the near future. During the year 2021, the end of the seventh year of follow-up (eighth evaluation) of the entire Vallecas Project cohort is expected. In addition, during the COVID-19 outbreak that forced the center's facilities to close and the Vallecas Project's routine clinical evaluations to be suspended, a telephone survey was conducted with almost all its volunteers (913 responses were obtained, 90% of the survivors) and the interest of many of those who had left the follow-up to rejoin the study was registered. The Vallecas Project has provided an abundance of rich and

varied information to examine the initial phases of cognitive decline in this cohort and to support new projects based on the data, biological samples and images accumulated in an extensive database whose curation and exploitation is a continuous task. The analysis of the data obtained so far and systematically collected in the project's database has led to several research studies, some of which have already been published and others are pending of publication or being concluded. In particular, the effect of various drugs on cognitive impairment, subjective cognitive complaints and prior cognitive reserve has been examined, and the effect of some pathologies, such as diabetes or sleep disorders, is being examined. These studies will also consider the concomitant effect of some of the biomarkers (genetic, biochemical and neuroimaging) that have been collected to characterize the population at higher risk of

developing cognitive impairment. The course of cognitive performance observed in these subjects over 4-5 years will also be studied to identify subgroups with different evolution and prognostic relevance. Likewise, with the data obtained in the neuropsychological evaluations of the subjects who have not shown any cognitive impairment during the entire follow-up, normality standards will be established that will be of great use in clinical practice for the diagnosis of cognitive impairment in the Spanish population. During the first months of 2021, the project entitled "MicroRNAs and lipid metabolism markers as a potential link between vascular dysfunction and the pathophysiology of Alzheimer's disease" will be completed, the main objective of which is to explore the possible role of miRNAs and metabolism markers of lipids as possible links between peripheral vascular dysfunction and AD pathophysiology. Likewise, a pro-

ject that continues this line of research will begin, entitled "Molecular mechanisms associated with risk factors and resilience in neurodegeneration. Lipid metabolism and membrane dynamics (NeuroRisk)". The general objective of this proposal is to explore the molecular and cellular mechanisms that confer susceptibility or resilience to the appearance and progression of AD and related disorders, focusing on the interaction of lipid metabolism and membrane dynamics with the transcellular spread of pathognomonic proteins such as tau, in the brain. This project, like the previous one, benefits from a design conceived with a double approach with complementary objectives related to the cohorts of the Vallecas Project, and the residence of the Queen Sofia Foundation Alzheimer's Center. Following an interdisciplinary approach, the analysis of the data obtained in this project will benefit from the availability of other data generated within the Vallecas Project (sociodemographic data, medical history, annual neurological evaluation, neuropsychological assessment, structural and functional brain MRI, genotyping , etc.) in order to identify molecular (biomarker, genetic), clinical, or neuroimaging signals that can serve to define populations at higher risk of developing dementia later in life. Likewise, we hope to be able to publish in 2021 the results of the "Vallecas Brain" that has been created. To enhance the usefulness of the Vallecas

Project cohort, we have collaborated with various researchers and the contributions of doctorate and master's students has been collected. Likewise, the integration of data from the Vallecas Project with those of other national and international cohorts has been facilitated. The most outstanding collaboration of this type has been the participation in a large genetic study in collaboration with the Dementia Genetics Consortium (DEGESCO) and the European DNA Bank, which has led to results of high impact and scientific and clinical interest on the genes involved in the pathophysiology of sporadic Alzheimer's disease. These collaborations have facilitated the genetic characterization of all volunteers included in the Vallecas Project, which may be of great value in future studies. These collaborations will continue to be promoted during 2021, the year in which collaborative works of these consortia are expected to be published in high-impact journals. Collaboration has been agreed with a group from the IMDEA Research Institute Food & Health Sciences Center in two research projects for which funding has been requested. If this is achieved, the pathophysiological relevance and diagnostic utility of the characterization of extracellular vesicles that can be isolated in brain, plasma, and other biological media and that show certain specificity in patients with Alzheimer's disease will be studied. The clinical data and samples collected in the

Vallecas Project will be very useful in both projects. The data obtained in the survey on COVID-19 affectation carried out in two waves during the first months of 2020, accepted for publication in the journal Gerontology, is being examined for possible integration with those of the ACE Foundation of Barcelona and the exploration of genetic polymorphisms as risk factors for this disease. Finally, it should be noted that a project has been submitted to La Caixa's call to study in the Vallecas Project cohort the late neurological sequelae of SARS-CoV-2 infection and, more specifically, its eventual effect on the neurodegenerative process of Alzheimer's disease. If approved, work would begin in the third quarter of 2021. During 2021, the launch of a new platform based on new ultrasensitive technologies for the detection of biomarkers in biological fluids useful in the early diagnosis of neurodegenerative diseases is expected. The acquisition of new equipment with this technology based on digital ELISA for the detection of single molecule will allow the establishment of the necessary procedures for its implementation during the first months of the year. In the context of the CIEN Foundation, the acquisition of a highly sensitive peripheral biomarker analysis platform is considered especially relevant for the enhancement and better use of the Vallecas Project, as well as other Foundation projects. The Vallecas Project, due to its own exploratory nature in

healthy people, lacks data that allow confirming the disease as CSF or PET image markers. Therefore, the incorporation of informative biomarkers in blood such as Aß40, Aß42, tau or Nf-L will be of vital importance to strengthen the Vallecas project, allowing us to increase the validity and impact of the research results. Using data from volunteers who have converted to MCI between visit 1 and visit 2, the Department of Neuroimaging has developed an algorithm capable of predicting the development of Mild Cognitive Impairment (MCI) in previously cognitively healthy elderly. This algorithm based solely on the verbal memory score and the density of gray matter in the entorhinal cortex has made it possible to predict the conversion to MCI with high precision. The application of this algorithm to the analysis of people who convert to MCI on subsequent visits has also shown high precision in a group of individuals different from the discovery group, thus allowing its applicability to be validated. These results have been sent for publication to the journal Science Advances, and following the comments of the reviewers, during 2021, we will complement these findings with data on Alzheimer's disease pathology confirmed by plasma biomarkers in the converter groups (currently in process). In addition to examining memory decline (in MCI converters), the Department of Neuroimaging is studying Vallecas participants with extraordi-

nary memory capacity. These "superagers", are defined as people over the age of 80, with the scores in psychological tests of memory of people of 50 years. Currently, we have identified an extensive cohort of 64 "superagers" in the Vallecas project. In 2021 we will publish the first results of this study, showing which areas of the brain are preserved in these extraordinary individuals, and which demographic, clinical and lifestyle factors are associated with the resilience to aging displayed by this exceptional group of volunteers. Currently, the MAPFRE 2020 Scholarship recipient is working on functional MRI connectivity in these subjects in collaboration with researchers from Cologne, Germany, participating directly in a research project application (> 1 million euros) by the Cologne group to the German Research Foundation (DFG; https://www.dfg.de). Finally, several members of the Vallecas project group participate in the study of telomere length in blood cells and its correlation with resilience to aging, with results scheduled for publication in 2021.

05.

International relations

International scientific collaboration increases more and more, not only because of the availability of international funding and the drive of modern communication technologies, but also because science itself has become a truly international collaborative activity. In particular, the scope and scale of the problem of neurodegenerative diseases in today's society require a global response to confront this great challenge and thus has been recognized by various international institutions such as the European Union (EU), the Organization for Economic Cooperation and Development (OECD), the World Health Organization (WHO), etc., and the industrialized countries that constitute the G8. This global concern has led to the creation of the World Dementia Council (WDC) with the aim of collectively spur action against dementia worldwide in the areas of research, clinical care and social awareness.

The leaders of governments, businesses and academia also recognize the need for a coordinated strategy to address this major global challenge for health systems. There is consensus among all stakeholders on the need to build capacities, infrastructures and R&D resources in the field of neurodegenerative diseases. As a

result, WHO has decided to establish a global observatory on dementia to monitor the prevalence of the condition and resources to care for patients in Member States as well as to track the establishment of national plans and policies against dementia.

There is also a pressing need for global participation and a commitment to a significant increase in investment in skills and resources to reduce the duration of these chronic brain pathologies and/or the number of people at risk. This budgetary effort should be accompanied by sound policies and legislative initiatives to encourage public-private partnerships. History has shown that collaboration between academic
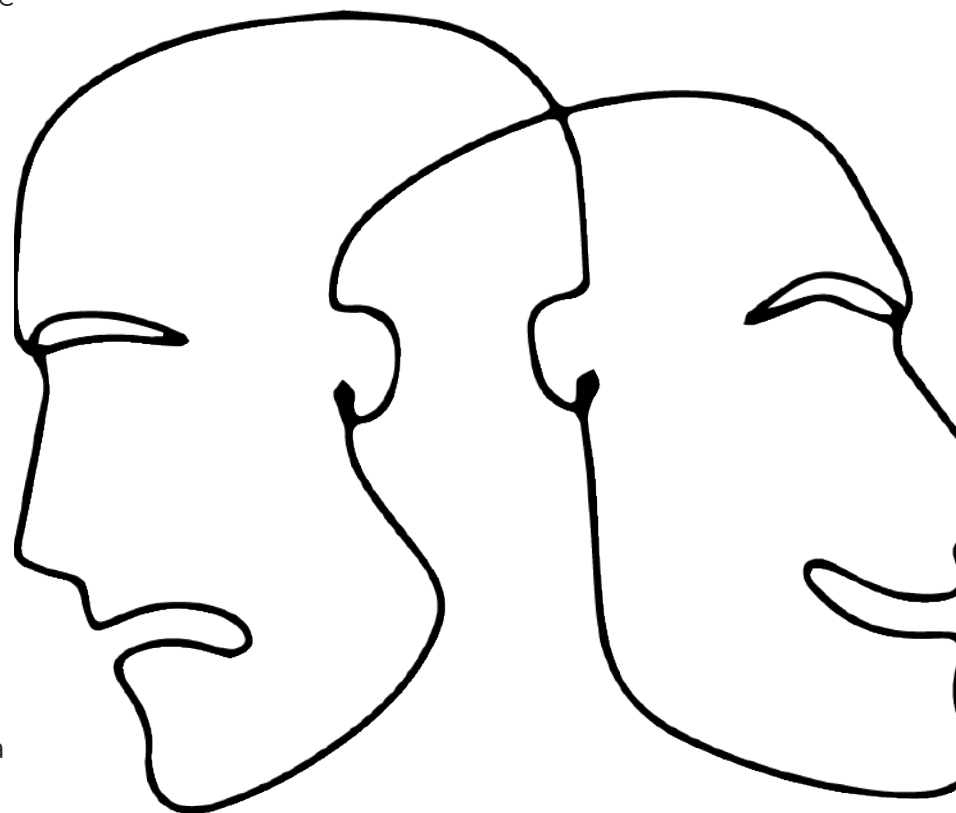
researchers, government agencies and pharmaceutical and biotechnology companies is an essential ingredient in promoting this type of ambitious initiatives, especially when resources are limited.

In this context, in recent years CIEN Foundation together with the Network Center for Biomedical Research in Neurodegenerative Diseases (CIBER-NED, for its acronym in Spanish) has given a boost to its relations with international organizations in the area of research in neurodegenerative diseases such as the EU Joint Programme for Research in Neurodegenerative Diseases (JPND) and the Network of Centers of Excellence in Neurodegeneration (COEN), among other initiatives.

Although 2020 has not been very productive in terms of international collaborations, these and other internationalization activities carried out during 2020 by the CIEN Foundation are detailed below.

EUROPEAN UNION JOINT PROGRAMMING ON NEURODEGENERATIVE DISEASE RESEARCH (JPND)

The EU Joint Programming for Research in Neurodegenerative Diseases (JPND) is an innovative collaborative research initiative created to address the growing challenges posed by these disorders. The JPND is a pioneering example of joint programming for the promotion of research within the European Union aimed at scientific challenges requiring a response that exceeds the capacity of a single country, based on the alignment of national research programs devoted to these challenges. Its objective is to enhance the impact of research by aligning existing national research programs and the identification of common objectives whose scope would benefit from joint action. The JPND Scientific Advisory Committee has significant participation of two CIBERNED researchers, Drs. Jesús Avila and Jesús de Pedro, as well as Dr. Angel Cedazo-Mínguez, from the Karolinska Institute in Stockholm and member of the CIBERNED Scientific External Advisory Committee.

The Research Strategy designed by JPND provides a framework for future investments and shows that the research effort within the European Union can be leveraged to improve care on prevention, diagnosis and treatment of patients suffering from these diseases.

To achieve impact there is a need to encourage novel as well as multidisciplinary approaches, and to strengthen and extend existing capabilities across the full spectrum of basic, clinical, health and social care, and translational research. To that end, a number of priority areas for future research have been identified: The origins of neurodegenerative diseases; Disease mechanisms and models; Disease definition and diagnosis; Treatment and prevention; Health and social care.

This Research Strategy also provides a framework of opportunities for countries involved in JPND and willing to participate in joint actions, which will be implemented through co-operative activities that realign or link national investments to achieve increased impact, and the provision of new funding. A guiding principle for its delivery will be that the research to be supported is of the highest scientific quality.

In this regard, during 2011 took place the first call for European research projects JPND. Under the theme "Optimization of biomarkers and harmonization of their use in the clinic", four transnational projects were awarded for the period 2012-2015, with participation of the CIEN Foundation in in one of them, the DEMTEST Project: Biomarker based diagnosis of rapid progressive dementias-optimization of diagnostic protocols. Subsequently, at the end of 2012, a new call was launched with the topic "Identification

of genetic and epigenetic protective and risk factors in neurodegenerative diseases", resolved with the approval of five projects for the period 2014-2018. During 2020 there have been no CIEN Foundation research groups involved in active projects within the joint European Union program for research in neurodegenerative diseases (JPND).

CENTERS OF EXCELLENCE IN NEURODEGENERATION (CoEN)

A major obstacle to the advancement of research on neurodegenerative diseases is the relative lack of common standards and mechanisms for validation of potentially relevant results in preclinical studies, and clinical studies based on population. One approach to deal with these challenges on a large scale is through a more effective use of large centers and institutes, where there is already the necessary critical mass of resources and expertise. Increased collaboration between national centers of excellence should also provide the opportunity to accelerate progress in understanding the basic mechanisms of disease, and the identification of new therapeutic approaches.

To this end, on June 10, 2010, the Canadian Institutes of Health Research (CIHR), the German Centre for Neurodegenerative Diseases (DZNE, Germany) and the Medical Research Council (MRC, UK) launched a funding initiative to establish a collaborative

approach to research in neurodegenerative diseases, called "Centers of Excellence in Neurodegeneration (COEN)". These founding members were later joined by other European institutions and thus, in December 2011 the COEN membership application by CIBER-NED-CIEN Foundation was approved, recognizing the scientific excellence in both basic and clinical science of the institution which became part of the COEN Oversight Group.

In 2012, CIBERNED and CIEN Foundation joined this Committee to participate actively in the design of the future COEN scientific strategy. Both institutions are represented by Dr. Miguel Medina, CIBERNED Deputy Scientific Director and member of the CIEN Foundation Scientific Advisory Board.

During 2015 the French Agence Nationale de la Recherche (ANR) has also been acknowledged as a new COEN member.

Current COEN members are:

- Canadian Institutes of Health Research (CIHR)
- Deutsche Zentrum für Neurodegenerative Erkrankungen (DZNE, Germany)
- Medical Research Council (MRC, United Kingdom)
- Flanders Institute of Biotechnology (VIB Flanders, Belgium)
- Health Research Board (HRB) / Science Foundation Ireland (SFI), Ireland
- Ministero della Salute (MDS, Italy)

Centre of Excellence for Brain Research (MESRS), Slovakia

- CIBERNED-Fundación CIEN, Spain

- Agence Nationale de la Recherche (ANR), France

The overlapping of the members of the COEN group with those of the JPND ensures that their complementary objectives are developed in close cooperation with the rest. This has been carried out through a two-phase process: first conducting expert workshops to determine the scope of the needs, followed by a call for proposals to establish collaborative teams among the PIs assigned to the participating National Centers of Excellence.

The first phase of the COEN initiative began at the end of 2010 and was intended to establish common resources and methodological approaches to support future studies. Some of the key issues addressed have been: the refinement and validation of cellular and animal disease models; the development of new measures to define patient subgroups for clinical trials; the identification of new biomarkers to support translational research; the development and harmonization of cognitive test batteries for diagnosis and follow-up of disease progression; and the establishment of common computer platforms to improve data analysis and exchange.

Phase II of the initiative was launched during the year 2013, with the launch of the 1st call for projects called "Pathfinder." In the years 2015 and 2017, the subsequent 2nd and 3rd calls were launched, whereas the 4th call was launched during 2019.

These calls for "Pathfinder" research projects aim to catalyze collaborative research between the different centers with a critical mass of resources and knowledge to drive a radical change in research in the field of neurodegeneration. Pathfinder project calls aim to encourage the scientific community to think outside the pre-established frameworks, to stimulate new unconventional approaches and thus reach creative solutions to the challenges of neurodegeneration research, carrying out high-risk/high-reward projects and welcoming unconventional and innovative applications.

The scientific scope of the "Pathfinder" projects is very broad and applications may include studies to improve our understanding of neurodegenerative mechanisms or create technological advances to support new diagnostic or therapeutic approaches. Joint nominations of researchers from identified Centers of Excellence are invited, and projects must include researchers from two or more countries. The projects address issues that would not be easily funded through the standard grant mechanisms of COEN partners, and it is expected that, in addition to

collaborations between Centers of Excellence, the projects will also serve to provide a platform for future collaboration with the industry

OTHER INTERNATIONAL ACTIVITIES

H2020 MARIE SKŁODOWSKA-CURIE ACTIONS: INNOVATIVE TRAINING NETWORKS

The Innovative Training Networks (ITN) are actions created by the European Union (within the Horizon 2020 program framework) to support research in the European Research Area and are aimed to form, through an international network of public and private centers, a new generation of creative and innovative researchers, capable of transforming knowledge and ideas into products and services for the economic and social benefit of the European Union. The CIEN Foundation actively participates as Associated Partner in the following ITN:

Targeting the purinergic P2X7 receptor to treat Alzheimer's disease

Brain disorders affect some 180 million people and their families in Europe alone, with an annual cost to the taxpayer estimated at 800 billion €, an economic burden greater than cardiovascular disease and cancer combined. Despite diverse etiologies, the overlap in clinical symptoms and comorbidities among brain disorders suggests shared pathogenic mechanisms. In particular, hyperexcitable states driven by glial activation and neuroinflammation appear almost ubiquitous in these disorders. With advances in understanding the function of the purinergic ionotropic P2X7 receptor (P2X7R), the highly promising effects demonstrated by its antagonists in models of brain disease, and the large investment in P2X7R-related drug development programs, now is the perfect time to pool resources. Studying these mechanisms offers the potential to improve symptoms and reverse disease progression in a wide range of brain disorders. Through its role in regulating neuroinflammation and the mechanistic link between neuronal hyperexcitability and glial activation, P2X7R with ATP is one of the most promising molecular targets for pharmacological intervention in the neuroinflammation-hyperexcitability pathway. PurinesDX brings together world leaders in translational research on purinergic signaling, leading European clinical specialists in a wide range of neurological diseases and industry partners specialized in drug and biomarker development. The ability to share unique genetic tools, newly developed diagnostic devices and novel, selective and stable P2X7R antagonists in the brain, along with the synergy facilitated by the PurinesDX consortium will be extended to the training of a new generation of highly skilled, innovative scientists, creative

and entrepreneurs that are urgently needed. Along with the provision of this interdisciplinary, international and cross-sector environment, original and high-level training in cutting-edge neuroscience will be provided, nurturing a highly competitive cohort of researchers with the potential to drive a new era of neuroscience research.

For more information, please check the PurinesDX program website: http://purinesdx.eu/.

06.

Scientific Productivity

6. SCIENTIFIC PRODUCTIVITY

6.1. BIBLIOMETRIC ANALYSIS

The CIEN Foundation maintains a strong commitment to the development of research, as well as to generate and promote scientific knowledge in improving the diagnosis and treatment of neurodegenerative diseases both within and outside our borders. Translating to society and the scientific community the progress made in the knowledge about neurological diseases in general, and more specifically for Alzheimer's disease, is a fundamental task. The scientific production of the CIEN Foundation researchers during 2020 has reached a total of 24 publications, all of them published in nationally and internationally recognized scientific journals (18 original articles, three reviews, and three editorial pieces). The analysis of these publications has allowed studying, through a series of quantitative indicators, both the CIEN Foundation scientific activity as

the production, subject, and degree of collaboration and impact of scientific publications. Through this analysis it is shown, for instance, that the average impact factor of the 2020 original articles within the first and second quartiles, which comprise 80% of the total publications, has been 6.937. In addition, during this year the CIEN Foundation has continued its national and international collaborations, so that 40% of the articles corresponded to studies carried out in collaboration with international institutions, 80% with Spanish ones, and the remaining 10% were performed exclusively by CIEN Foundation researchers. Also noteworthy is the high proportion of collaborative publications with other CIBERS and research networks in the first and second quartiles (50%).

11 %

33 %

56 %

- Collaborative publications, only nationals
- Collaborative publications, mixed nationals and internationals
- Collaborative publications, only internationals
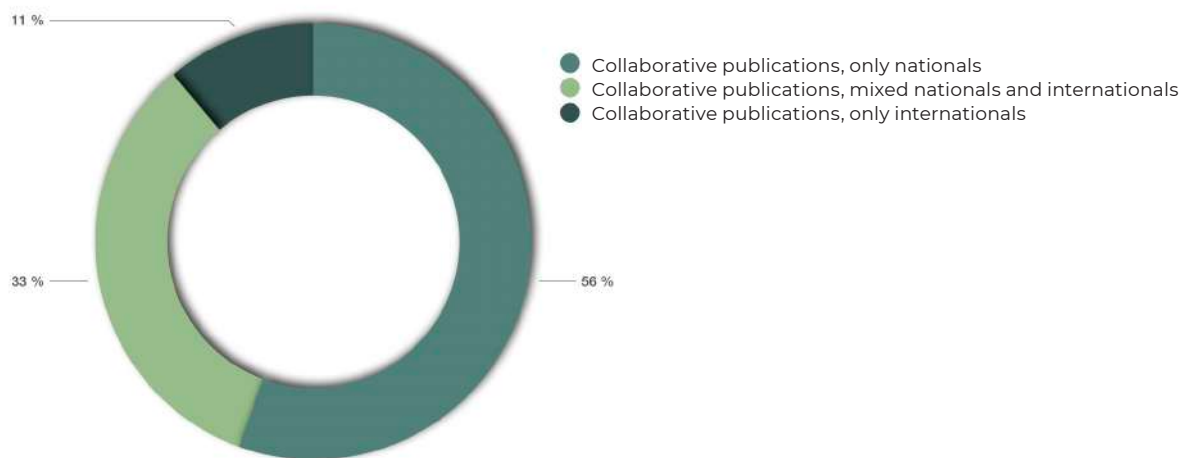
The following table shows output indicators of production (number of publications), quality (publications in journals ranked within the first and second quartile of their subject category), impact (determined by the accumulated and average impact factor of the journals in which it has been published) and degree of collaboration at national and international level.

2020 indicators

Total number of publications	24
Number of publications in scientific journals	24
Total number of publications in the ISI citation index within the first and second quartile	20
Cumulative impact factor of publications within the first and second quartile	138,735
Average impact factor of the publications of the first and second quartile	6,937
Number of collaborative publications of all kinds (CIBERNED, other national groups, international groups) within the first and second quartile	18
Number of international collaborative publications within the first and second quartile	8
Number of national collaborative publications within the first and second quartile	16
Number of collaborative publications with other CIBERs and networks within the first and second quartile	10

In summary, during 2020 the CIEN Foundation researchers have published 24 scientific papers, of which 22 (91.6%) have been in journals included in the Journal Citation Report (JCR), accessible through the Web of Science portal (WoS, Clarivate Analytics) and 20 (83.3%) have been published in journals ranked within the first and second quartile in their category. Considering the type of document, 40% of the publications in scientific journals (18) correspond to original articles. Moreover, according to their scientific subject category 40% of the publications within the first and second quartiles have focused on the following categories: Multidisciplinary Sciences and Neuroscience.

With regards to scientific dissemination activities in meetings and national and international events during the year 2020,

Regarding scientific dissemination activities in national and international

meetings and forums, during 2020 the activity has been quite affected. Most of the scientific events have been suspended or postponed and those that have continued, have mostly opted for a virtual format. In the CIEN Foundation a total of 15 participations have been registered this year at scientific conferences, 8 of which correspond to lectures and oral presentations, and 7 correspond to written poster communications. These communications have been presented at national (7) and international scientific conferences and meetings (8).

6.2. Publications

References of the 24 scientific publications from CIEN Foundation scientists are listed below according to type of publication: all of them in scientific journals (16 original articles, three reviews, and three editorial pieces).

6.2.1. Journal articles

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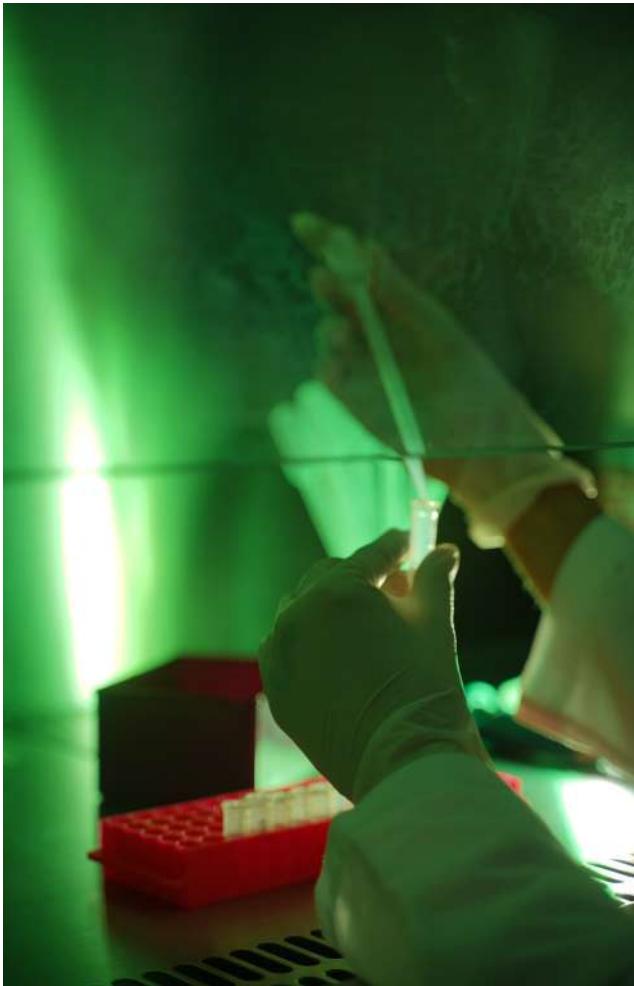
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Number of publications by subject category in 2020

Multidisciplinary Sciences	5
Neurosciences	3
Clinical Neurology	2
Geriatrics & Gerontology	2
Chemical Neurology	2
Gerontology	2
Chemistry, Analytical	2
Biochemistry & Molecular Biology	1
Biochemical Research Methods	1

## 6.3. Communications to Conferences

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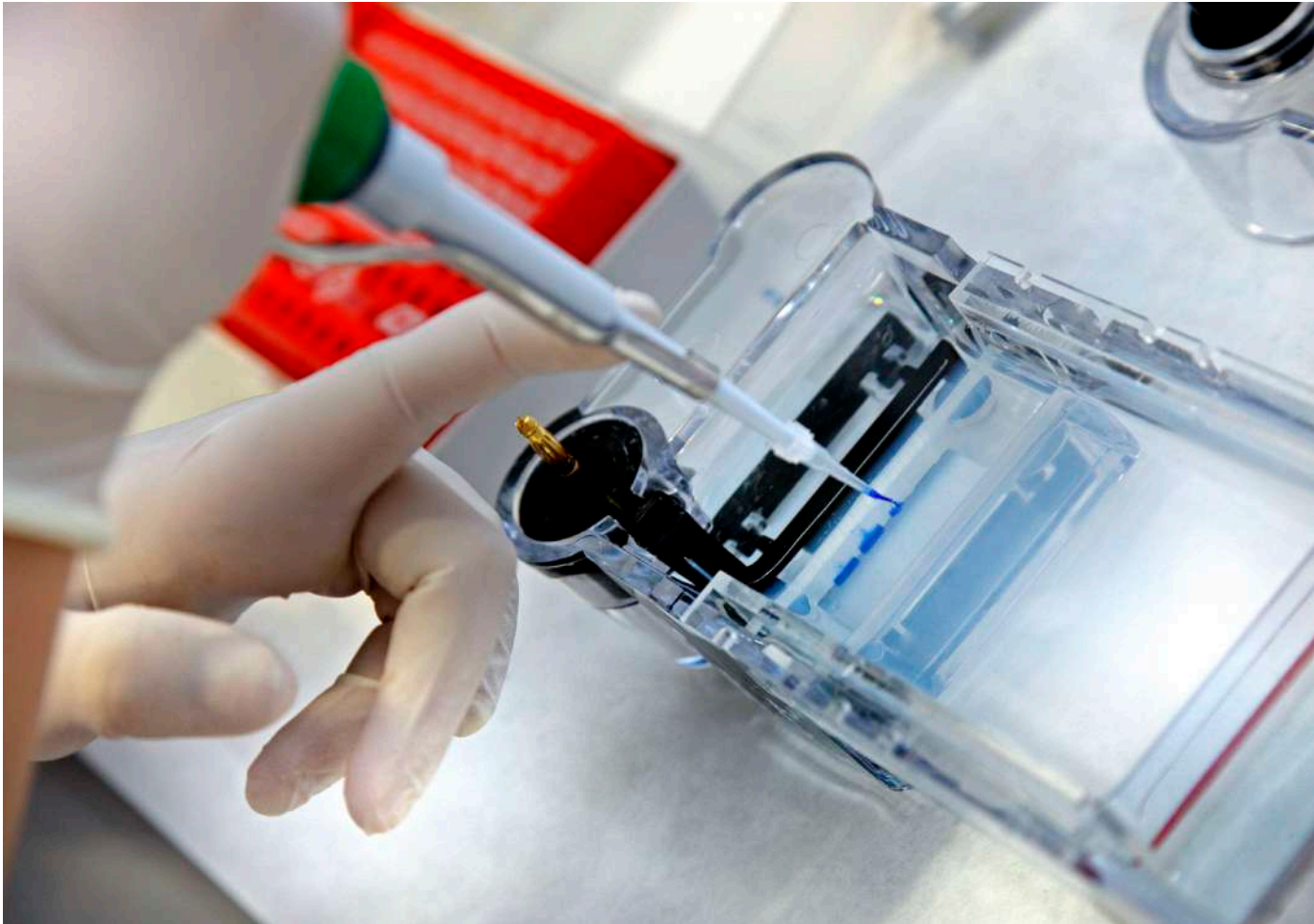
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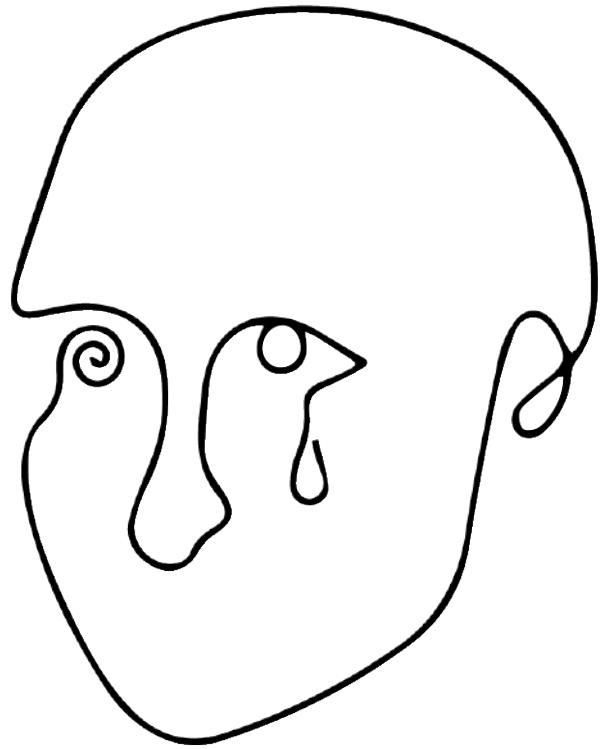
Rare Diseases. European Joint Programme on Rare Diseases. ISCIII, Madrid. 29/10/2020. Oral communication (online).

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- Valentí M, González B, Rodríguez C, Zea MA, del Ser T. Análisis prospectivo de la afectación por Covid-19 en una residencia pública de la Comunidad de Madrid. LXXII

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6.4. Funded projects

During 2020 the CIEN Foundation researchers have participated in five scientific research projects granted through various national and international competitive calls and funded by different institutions as well as in two research service provisions.

Funded research projects are cited below:

- **Code: Vallecas 2**

Principal Investigator: Dr. Miguel Medina

Title: Vallecas 2 – Early detection of Alzheimer's disease. Risk and protection factors

Funding agency: Queen Sofia Foundation

Duration: 2018-2021

Budget 2018-2020: 900,000 € (The Queen Sofia Foundation undertakes to contribute during each of the 4 years of planned duration of the project, the successive amounts that are agreed annually by its Board of Trustees, corresponding to the first three years a total amount of nine hundred thousand euros (300,000€ in 2018, 300.000€ in 2019 and 300.000€ in 2020).

In February 2018, a new collaboration agreement was signed with the Queen Sofia Foundation, with an expected duration of 4 years. This agreement governs the framework of cooperation to carry out the research oriented to the identification of individuals with greater risk of developing Alzheimer's type dementia (AD) within the project called "Vallecas 2, early detection of Al-

zheimer's disease. Risk and protection factors ".

- **Code: RTI2018-098762-A-C32**

Principal Investigator: Dr. Miguel Ángel Fernández

Title: Antecedentes familiares de enfermedad de Alzheimer en personas mayores: influencia del deterioro cognitivo subjetivo. (CONNECT-AD)

Funding agency: Ministerio de Ciencia, Innovación y Universidades

Duration: 2019-2022

Total budget: 108.900 €

2018 budget: 52.272,00€

2019 budget: 10.890,00 €

2020 budget: 45.738,00 €

CALL: Resolution of the Secretary of State for Universities, Research, Development and Innovation and the Presidency of the State Research Agency, approving the call for early processing for the year 2018 of the procedure for allocating grants to R&D&i Projects on «Research Challenges» corresponding to the State Program of R&D&i Oriented to the Challenges of Society, within the framework of the State Plan for Scientific and Technical Research and Innovation 2017-2020.

- **Code: PT17/0014/0015**

Principal Investigator: Dr. Alberto Rábano

Title: Biobanks Platform

Funding agency: Carlos III Institute of Health

Duration: 2018-2020

Total budget: 135,300 €

2018 budget: 45,100 €

2019 budget: 45,100 €

Call for the year 2017 of grants within the Strategic Health Action 2013-2016. These grants are co-funded by the European Regional Development Fund, Intelligent Growth Operational Program 2014-2020. The Carlos III Health Institute granted an extension for the execution of the project without additional funding, extending the deadline until June 20, 2021.

- **PEJ16/MED/AI-1963**

Principal Investigator: Dr. Alberto Rábano

Title: Grants for hiring research assistants and laboratory technicians.

Funding agency: Council of Education, Youth and Sports, Region of Madrid

Duration: 2017-2019

Total budget: 45,000 €

Call for grants to hire research assistants and laboratory technicians throu-

gh the Youth Employment Operational Program and the Youth Employment Initiative (YEI) (2016). These grants will be co-funded by the European Social Fund (FSE, for its acronym in Spanish) through the Youth Employment Operational Program. In February 2018, the execution of the 2nd annuity of the awarded grant within the framework of the 2016 call for the execution of contracts for research assistants and laboratory technicians begins, which will be valid until July 2019.

- **PEJ17-TL-BMD**

Principal Investigator: Dr. Alberto Rábano

Title: Grants for hiring research assistants and laboratory technicians.

Funding agency: Council of Education, Youth and Sports, Region of Madrid

Duration: 2018-2020

Total budget: 38,000 €

2018 budget: 19,000 €

2019 budget: 19,000 €

Call for grants to hire research assistants and laboratory technicians throu-gh the Youth Employment Operational Program and the Youth Employment Initiative (YEI) (2017). These grants will be co-funded by the European Social Fund (FSE, for its acronym in Spanish) through the Youth Employment Operational Program. The grant was termi-

nated in March 2020, once the term of the formalized contract ended.

Service Provisions / Research Services

- **Project Code 0348_CIE_6_E**

Funding agency: University of Salamanca General Foundation (FGUSAL)

Call: International Center on Aging Project (CENIE)

Code: PILEP +90

Title: Factors associated with healthy and pathological aging in the sample of people over 90 years of the city of Madrid "

Principal Investigator: Miguel Ángel Fernández-Blázquez

Starting date January 1, 2019

End date: November, 2020

Budget: 39,875 € (VAT excluded)

- **Project Code 0348_CIE_6_E**

Funding agency: University of Salamanca General Foundation (FGUSAL)

Call: International Center on Aging Project (CENIE)

Code: ImageH

Title: Application of data mining techniques for the identification of factors of healthy and pathological aging

(ImageH)

Principal Investigator: Jaime D. Gómez Ramírez

Starting date January 1, 2019

End date: November 30, 2020

Budget: 33,000 € (VAT excluded)

As for these two contracts, the parties signed different extensions postponing the term of the contract until November 30, 2020 (originally it expired on June 30, 2020), motivated by the entry into force of Royal Decree 463 / 2020 of March 14, which gave effect to the State of Emergency motivated by the COVID-19pandemic, and the impact that the pandemic had on the normal development of research activity.

6.5. Patents

During 2020 a new patent has been applied for and a co-ownership agreement has been signed with the participation of CIBER, CIBERNED, the UPV and the CIEN Foundation:

- Inventors: María Carmen Martínez Bisbal, Ramón Martínez Máñez, Miguel Medina, Miguel Calero, Ana Pastor.
- Title: Method for the diagnosis, prognosis and/or monitoring

of Alzheimer's disease using metabolomic techniques.

- Application No: P20203030235
- Priority date: 23/03/2020.
- Type: National.

07.

Outreach

7.1 Outreach activities

The 2020 exercise has presented us with an unprecedented challenge that, of course, has also had a full impact on the outreach activities of the CIEN Foundation, being one of its founding objectives to bring to society in the most accessible way the advances obtained in the research of neurodegenerative diseases.

The COVID-19 pandemic has supposed a global disruption. However, the confinement has not meant the paralysis of the outreach activities of the CIEN Foundation during the year 2020, although it has meant some limitations and adaptations due to the new circumstances. Since mid-March 2020, several events organized by the CIEN Foundation had to be postponed, the most significant being the Global Summit NEURO2020, scheduled for September 2020 and which has finally been postponed to 2022. Some of our more beloved events, such as the Volunteer's Day or the Tree of Memory of the CIEN Foundation, had to be canceled (hoping to be able to resume them in future editions) and other events have been maintained with an adapted format, such as the Memorable Shorts Film Festival, which in its second edition was moved to the virtual plane. It should be noted that our outreach work has been maintained through publications on our website www.fundacioncien.es, our quarterly newsletter (CIEN Foundation Newsletter) and our social networks. Below, we highlight some of the most important dissemination activities carried out in 2020:

NEURO2020: "Neurodegenerative Diseases 2020. International Year of Research and Innovation"

This year the meetings of the Governing Commission of the event of exceptional public interest "Neurodegenerative Diseases 2020. International Year of Research and Innovation" have continued. The event was consolidated in March 2019, in order to comply with the provisions of the ninety-seventh additional provision of Law 6/2018, of July 3, on the General State Budget for the year 2018 with the heading: Tax benefits applicable to "Neurodegenerative Diseases 2020. International year of Research and Innovation".

The EN 2020 event, promoted by the Reina Sofía Foundation and the Neurological Diseases Research Center Foundation (CIEN Foundation) and promoted by the Ministries of Finance; Health, Consumption and Social Welfare; Science and Innovation, and the Carlos III Health Institute, is aimed at: (a) raising awareness among citizens of the importance of promoting research on neurodegenerative diseases to advance the knowledge of the origin of their causes, the only way to reduce the number of affected population and to stop the advance of these diseases; (b) promoting the acquisition of economic resources that will be used for the scientific and social purposes of both foundations and as a platform for the generation of ideas and the exchange of experiences between experts and scientists in the field of neuroscience from around the world.

Among the activities that will be developed around this Event, the NEURO 2020 Global Summit stands out, initially scheduled to pass September 2020, but which, due to the emergency situation experienced worldwide, will be held in 2022. This forum, of international ambition, will contribute to Spain being among the leading countries in global scientific research on neurodegenerative diseases, collaborating in the consolidation of the European program against these diseases.

The main objectives of this initiative are:

- To contribute to Spain being among the leading countries in international scientific research on neurodegenerative diseases.

- Collaborate in the consolidation of the European program against these diseases.

- To attract resources of private and philanthropic origin destined to the social and research purposes of the Reina Sofía Foundation and the CIEN Foundation.

The International Year of Research and Innovation on Neurodegenerative Diseases, on the ONCE coupon

The International Year of Research and Innovation on Neurodegenerative Diseases (Neuro2020/22), starred in the ONCE (for its acronym in Spanish) lottery ticket on Monday, September 21 coinciding with World Alzheimer's Day. Five and a half million lottery tickets spread this initiative, which is linked to the celebration of the special edition of an annual congress, this year the World Summit, which due to the Covid-19 pandemic, has been postponed to 2022.

The first edition of the International Congress of Research and Innovation in Neurodegenerative Diseases (CiiiEN, for its acronym in Spanish), was held on September 21, 2013, coinciding with World Alzheimer's Day, organized by the CIEN Foundation and CIBERNED, in collaboration with the Reina Sofía Foundation.

The objective was and continues to be, to have a meeting forum in which to share the latest advances and information of interest on neurodegenerative diseases

among the international scientific community.

This key meeting on Neurodegenerative Diseases, already strongly consolidated, offers a forum to discuss the basic and clinical aspects of research on these diseases.

Friends of the CIEN Foundation

On the occasion of World Alzheimer's Day, in September 2018, we launched the Friends of the CIEN Foundation initiative, with which we can all help the research, diagnosis and treatment of neurodegenerative diseases, especially Alzheimer's and other dementias.

Friends of the CIEN Foundation is also a way to support and make visible the tireless work of scientists, families, and caregivers, and contribute to improving the quality of life of those who suffer from a disease for which there is still no cure.

Through the form that can be found on our website, www.fundacioncien.es, those interested can fill in the corresponding fields and choose a way to collaborate: Collaborator Friend, Protective Friend or Benefactor Friend.

"Together we can do it" was the slogan chosen for this campaign that, at the end of 2020, already had 700 members. We would like to take this opportunity to thank our donor community as well as all followers for their encouragement and support in such a tough year. After more than two years of the Friends of the CIEN Foundation initiative, we feel responsible for the trust they have placed in our institution, and we are responding in the best way we know: through Alzheimer's research.

II Memorables Film Festival

The fight against Alzheimer's can inspire both scientific research and artistic creativity. In this case, the Reina Sofía Foundation and the CIEN Foundation once again summoned filmmakers from all over Spain to portray the social reality of Alzheimer's. The Memorables Film Festival projected stories on the big screen to help the public understand the impact of this disease on specific people: from the patients and their families to researchers or professional caregivers. In this second edition and considering the current social and health context generated by COVID-19, which has uniquely affected the elderly, those short films with allusion to the impact of the pandemic on people with Alzheimer's had a greater meaning.

Mª Ángeles Pérez, manager of the CIEN Foundation, wanted to highlight in her welcoming words that "this Festival is consolidating itself as an unbeatable opportunity to bring the social reality of Alzheimer's closer to society through the screen, always with an eye on the importance of care and scientific research". In addition, she wanted to recognize "the effort of all the people" who are behind this disease: relatives, medical staff, scientists, and volunteers, and that the shorts presented "reflect with veracity, emotion and talent the reality of this disease".

For his part, Dr. Miguel Calero, scientific director of the CIEN Foundation, welcomed the fact that this reality, that of a disease that "so many people suffer and that affects hundreds of thousands of families in Spain", is increasingly "more present in the mind and imagination of creators and artists who contribute decisively to raise awareness".

The difficulties encountered by a mother and her child while caring for a father with advanced Alzheimer's are Alimezher's main theme. The short film, directed by Liteo Pedregal, from the Spanish city of Ceuta, won the Golden Neuronita for the best short film at the II Edition of the Memorables Film Festival, held on December 17 at the Artistic Metropol cinema in Madrid, with reduced capacity and broadcasted by streaming to comply with the sanitary measures against COVID-19. The well-known actors Manuel Zarzo and María José Alfonso won, respectively, the Neuronita for best actor and best secondary performance for the same short film.

In official section of the contest another twelve shorts competed, in which other well-known faces have not been missing, such as María Galiana or Adriana Ozores, who has won the Neuronita for best actress for the short film Te quiero, madre (directed by Charo Anaya), for her interpretation of a daughter who must abandon her mother, María Galiana, due to a police problem.

Additionally, the second prize (Silver Neuronita) was won by the short film Olvidé no quererte, by Carine Nonnast Fornieles, a documentary about a couple who, after more than 60 years together, face the cruelty of Alzheimer's. He suffers it and

she clothes and cares for him despite the toll that the years have taken on her. The third prize (Bronze Neuronita) went to Adiós hija, by Olga de Lorian, which tells the story of Sara, a young woman who returns home after spending a few years abroad but will encounter a reality that she would have never imagined. A letter from her mother will change everything.

The jury, chaired by the deputy director of networks and cooperative research centers of the Carlos III Health Institute, Ms. Margarita Blázquez, has valued "the high quality and professionalism of all the proposals, as well as the diversity of approaches when addressing a topic as complex as Alzheimer's disease". During the contest, a colloquium in which the actress and member of the Film Academy María Luisa San José, the film director Alfonso Albacete, and the director and actress Cindy Fuentes, winner of the Golden Neuronita for the best short film in the First edition of the festival, talked about the relationship between cinema and Alzheimer's also took place.

The CIEN Foundation at the Spanish Brain Council

The Spanish Brain Council (CEC, for its acronym in Spanish), was founded in 2009 as a non-governmental organization that brings together scientific societies and patient and industry associations, with the fundamental objective of promoting brain research in Spain and thus expanding knowledge of the functioning of the healthy brain, its diseases and their social impact. The CIEN Foundation is one of its founding members and has participated as secretary in its Board of Directors since November 2013. The CEC carries out these tasks in permanent contact with the European Brain Council (EBC), participating in turn as an observer member in the Academy of the European Brain Council and as a deputy member of representation of the Academy in the General Assembly of the EBC.

In 2020 the CEC has participated in several activities, in which we highlight the following:

1. Organization of activities:

Brain Research Information Day. The Spanish Brain Council, the Spanish Parkinson's Federation and the CIEN Foundation organized an information day about brain research. The event was scheduled for March 18, 2020 and was part of the Dana Foundation's Brain Awareness Week. Patients and researchers were to meet to discuss the importance of clinical and neuropsychological evaluation, neuroimaging studies, and brain tissue donation. In the end this event was canceled due to the pandemic.

Erasmus + Care4Autism Project. The application for the Care4Autism project is submitted to the Erasmus+ call with several European partners, including several National Brain Councils (NBCs). The Council for the first time leads a European project

as coordinator and participant. The aid of 87,000€ is granted, but 66,000€ are requested in advance as guarantee. The CEC could not fulfill this request, so the grant and the opportunity to carry out the project were lost.

2. Participation in activities of the European Brain Council (EBC):

Participation in the Horizon Europe public consultation (first strategic plan 2021-2024) requesting investment in basic and applied research on the brain and its diseases. Translation of the text to Spanish and publication on the https://www.consejocerebro.es/ website.

Sending correspondence to ministers and representatives in Europe. The Council joined the EBC, National Councils and other European organizations in calling for Horizon Europe to maintain a strong and ambitious focus on programs about brain health, research, and disorders (July 2020). A response was requested to the letters sent to the ministers.

Participation in activities organized by other member institutions:

- Erasmus + Share 4 Brain Project. The Council has been actively involved in the development of material for the guide of good practices. Several mem bers of the Council have provided the information of the events organized by their respective institutions to the secretary who, with this information,
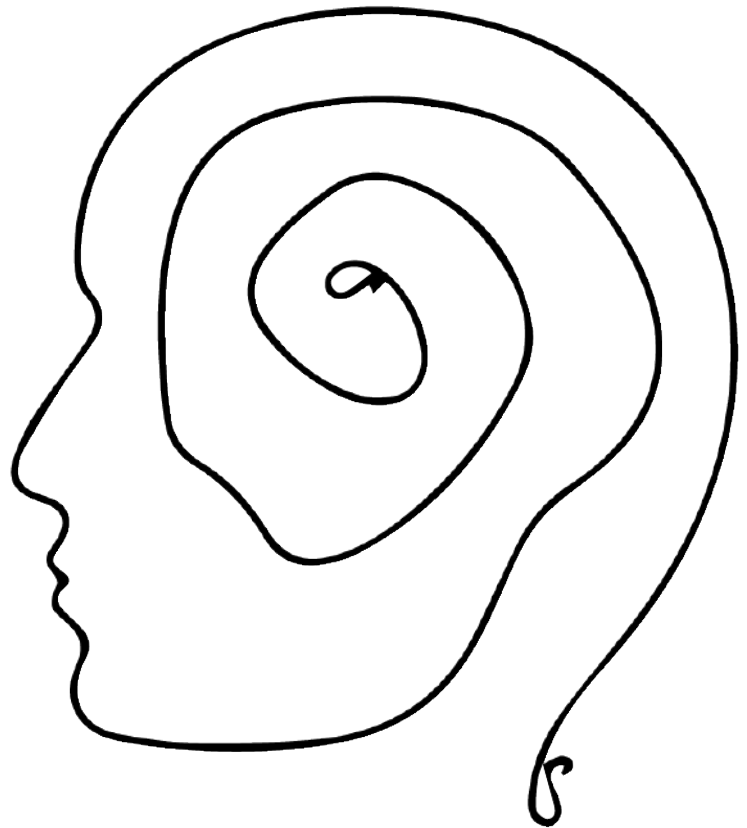
has completed the online survey of the project. It has been requested on several occasions the inclusion of this information on the project website, which at the moment only includes the event organized by the Council in 2019.

- NEURO 2020, International Year of Research and Innovation in Spain. Research on Alzheimer's disease was declared an Event of Exceptional Public Interest for the year 2020 in Spain by the Congress. The event "Neurodegenerative Diseases 2020. International Year of Research and Innovation NEURO 2020" aimed to promote research in neurodegenerative diseases and raise public awareness of the social

consequences of these pathologies whose prevalence increases in parallel with the aging of the population. The NEURO2020 event was promoted by the Reina Sofía Foundation and the Neurological Diseases Research Center Foundation (CIEN Foundation) and was supported by the Carlos III Health Institute and several ministries. This event was postponed due to the pandemic to 2022.

· Parkinson's Congress. The Spanish Parkinson's Federation organized the Parkinson's Congress "Reality of the disease and future challenges", which was scheduled to be held in October in Granada. This event was also cancelled due to the health situation.

· World Brain Day. The Council participates in the re-broadcast of the activities of 22 July 2020, this year dedicated to raising awareness of Parkinson's disease.

The Council was going to participate in other events with associations and media that had to be canceled due to the pandemic.

3. Attendance at meetings:

Share4Brain Project meetings. The project kick-off meeting was held in March 2020 virtually. Several virtual meetings of the project have been held throughout the year. At the September meeting, a seminar on depression was held in which the president of the EBC, Dr. Mónica di Luca, participated.

Meetings of the European Brain Council. The Council is renewed as an observer member of the EBC at the virtual meeting held in June 2020. The Council attends all the virtual meetings scheduled by the EBC and the National Councils:

General Assembly of the EBC (December 9, 2020) held by teleconference where we presented our list of activities and the Care4Autism project. The Share4Brain project was also presented.

EBC's NBC Advocacy Meeting (December 19, 2020), held via teleconference, with the presence of its Chair. The National Councils requested that the EBC request a meeting with the European Commissioners and that the EBC was more involved with the National Councils. A list of the European parliamentarians to be contacted was also provided.

4. Endorsement

The Board supports the candidacy of Lars Edvinsson (Lundt University) for the BBVA Frontiers of Knowledge Award in Biology and Biomedicine, for his work in migraine and headaches. This endorsement is signed by all the relevant European societies in this field, including the SEN.

5. Outreach activities

Website and social networks (Twitter).

7.2. Presence in the media

Throughout 2020, the Communication Area of the CIEN Foundation developed a communication plan, which was altered due to the outbreak of the COVID-19 pandemic and adapted to the new circumstances and needs. However, and following the usual mechanics of the communication work of the CIEN Foundation, constant communications and periodic meetings have been maintained with the CIEN Foundation Management about media appearances, press releases, reports, scheduled initiatives, organization, etc.

For the formulation and achievement of the objectives of the year 2020, different adjustments were made in the programmed communication actions, among which was the annual CiiiEN congress, which in 2020 was included within the Event of Exceptional Public Interest NEURO2020 and which, each year, is part of the main axis of the conception and strategy of the communication plan of the CIEN Foundation. As described above, this and other regular events of the CIEN Foundation were not able to be carried out face-to-face due to the health situation, such as the Volunteer's Day or the Tree of Memory. Fortunately, and making the necessary adaptations, it was possible to celebrate the second edition of the Memorables Film Festival, which brought together a multitude of spectators in a hybrid format: in person, in the Artistic Metropol cinema in Madrid for a small number of attendees, for reasons of reduced capacity

and following all health regulations, and online with live broadcast.

In addition, other communication actions and initiatives were carried out in 2020. A constant flow of press releases and contact with the media has been maintained, with the aim of reflecting the updated information about the CIEN Foundation and the "Vallecas Project", its most important project. During 2020, the notable impact in the press, radio, television, and online media has been maintained, both in terms of dissemination and extension of the network of contacts with researchers in national and international neurodegenerative diseases. Appearances have

been achieved in the press and online and audiovisual media that include extensive reports in media such as La Razón, El Mundo, La Vanguardia, Diario Médico, ConSalud, Acta Sanitaria, in addition to regular outreach through the main news agencies of our country.

In the press interventions, the control protocol and prior informative consent introduced in 2017 has been followed in 2020, by which journalists are informed in detail of the affiliation of the CIEN Foundation to the Carlos III Health Institute and the Ministry of Science and Innovation.

The number of media impacts during 2020, expressed as reach as the estimated number of readers/people reached, amounted to more than 75 million readers, which expressed as ROI was 726,462€.

Since 2017, quarterly newsletters are prepared with the latest developments in the activity of the CIEN Foundation: research, seminars, presentations, publications, etc. In addition, in 2020 the graphic line and design of the newsletters has been renewed and updated, making them more visual and attractive to readers.

7.3. Presence on social networks

The CIEN Foundation has continued to implement its promotion strategy, which has been rewarded both in the results obtained in social networks and in the recognition and prestige it has nationally and internationally as an organization.

Throughout 2020, the community has had the support and advice of numerous experts who have resolved their doubts and queries in real time through social networks.

There are several campaigns that have been promoted from the official accounts of the CIEN Foundation, highlighting above the rest the one that aimed to increase the visibility of Alzheimer's disease to the population through the contribution of data and new discoveries (#Alzheimer), the one related to the campaign of "Friends of the CIEN Foundation" (#Amigosdela-FundacionCIEN) or the "Vallecas Project" (#ProyectoVallecas).

Sporadic campaigns and events have also been covered and disseminated. Among these events, the coverage of the II edition of the Memorables Film Festival (#MemorablesFilmFestival) stands out.

Analyzing the status of the official accounts of the CIEN Foundation we can observe the evolution they have experienced during 2020:

· Facebook: 2020 ended with 5,360 followers, which have been increasing progressively and whose growth continues constantly. This figure represents an increase of 2% compared to the previous year.

· Twitter: at the end of 2020 the profile had 14,088 followers; with whom we constantly interact, receiving numerous comments, retweets and likes. Compared to last year, followers have

experienced an increase of 2.31%.

- LinkedIn: activity on LinkedIn has increased throughout 2020, selecting content according to the needs of the platform. Compared to 2019, followers on the platform have increased by more than 45% reaching more than 350 followers. That is significant increase in the number of followers, taking into account that the Linkedin profile was created in 2019.

www.fundacioncien.es