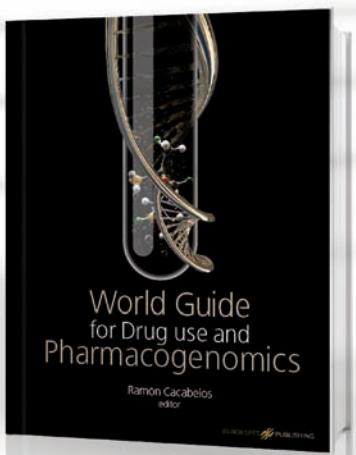
Gen-T

The EuroEspes Journal

Alzheimer's disease 2011 Where are we heading?

VACUNA CONTRA
EL ALZHEIMER
ALZHEIMER'S VACCINE



PRESENTACIÓN
DE LA PRIMERA GUÍA MUNDIAL
DE FARMACOGENÓMICA
INTRODUCTION TO THE FIRST
WORLD GUIDE OF PHARMACOGENOMICS

VI
Conferencia Anual
EuroEspes
III
Reunión de la Asociación Mundial de Medicina Genómica
III Meeting of the World Association of Genomic Medicine

ORGANIZADOR/ORGANIZER EuroEspes Group
PATROCINADOR/SPONSOR EuroEspes Foundation
DIRECCIÓN/CHAIRMAN Ramón Cacabelos

Grupo EuroEspes NEUROCIENCIA La Sabiduría de la Naturaleza

EUROESPES FOUNDATION

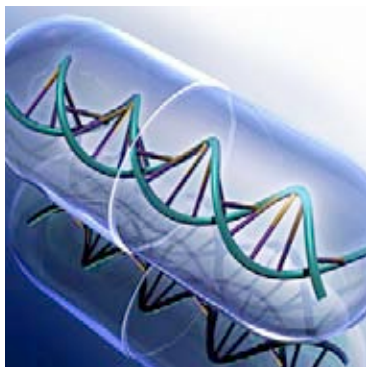
EuroEspes
Sistema Nervioso Central y Medicina Genómica

más de 12.000 pacientes ya conocen su genómica

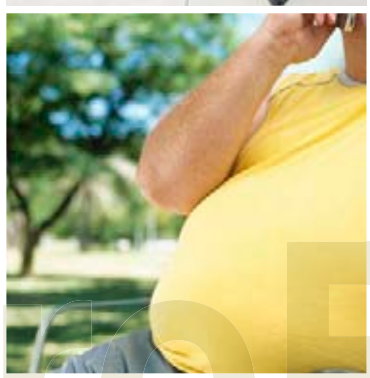
Plan de Prevención de la Demencia
Protocolo para el diagnóstico integral y el tratamiento multifactorial en pacientes con demencia (Alzheimer, vascular, carencial, metabólica).

Tarjeta Farmacogenética
La personalización de los tratamientos, dando el fármaco adecuado en la dosis óptima a cada persona, para mejorar su eficacia y evitar efectos adversos.

Plan de Prevención del Riesgo Cerebral para Directivos
Programa pionero en la prevención del riesgo cerebral en altos ejecutivos basado en los avances de la medicina genómica.



Plan PROFE
Plan para la identificación precoz, la prevención y tratamiento del fracaso escolar en niños, adolescentes y jóvenes.



Plan de Prevención Genética del Síndrome Metabólico y los Accidentes Cerebrovasculares
Primer protocolo de prevención genética y medicina personalizada para combatir el Síndrome Metabólico que afecta a más de un 20% de la población.

EuroEspes
1991-2011
XX Aniversario

Gracias por confiarnos su salud estos 20 años

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EDITORIAL

por Ramón Cacabelos
rcacabelos@gen-t.es

Historias de la Aldea, un poco de Paz Navideña

(…y Feliz Traspaso de Incompetencias)

ESTE globo pinchado se desinfla. Un agujero en Irak, otro en Afganistán, una raja en Egipto, un parche en Libia, un clavo en Irán, una chincheta en Corea del Norte, una mancha de chapapote en Cuba, una ralladura en Siria, una catástrofe en Japón, una fuga en Estados Unidos, un boquete ético-económico en Europa… y en la aldea de las 17 tribus, aunque casi todos pertenecen ahora a la misma parroquia, cada uno va a su bola socializando las mismas torpezas: desempleo, aumento de la pobreza, restricciones en bienestar social, secuelas del abuso de poder, el despilfarro, la negligencia, la irresponsabilidad… y un enorme derroche demagógico, cuando está feo alardear de derroche económico (porque ya no hay con qué). El esperpento tribal, aparte de pozo negro regional, se ha convertido en adalid de fractura, desigualdad, segregación y miseria. Por no poder, ni se puede enfermar fuera de los lindes del territorio propio, porque las tribus vecinas no reconocen a los enfermos de las otras tribus; ya no se le ocurre a nadie ir a pedir un clavo o un sacacorchos a la casa de al lado, cuanto más medianil peor. Son como extraños dentro de la misma aldea. Ante la necesidad no se busca la unión para generar fortaleza sino la destrucción del vecino (cuantos menos, toca más en el reparto, pero reparto ¿de qué? ya no queda nada que repartir; toca vender los recuerdos para seguir creyendo que se es algo; pero los compradores ni valoran las reliquias). Algunos que habían sido puestos al lado de la hucha para custodiarla, la rompieron y se llevaron las monedas (o repartieron el botín con sus cómplices). Unos cuantos jefuchos vaciaron las arcas colectivas, hicieron dispendio de poder y recursos, y dilapidaron la confianza de los suyos; pero nadie se atrevió a pedirles cuentas, porque todos tenían algo que perder, sobre todo sentar el precedente de que quien arruina una comunidad debe pagar con su vida (política y penal). La cobardía del mal precedente es el escudo de los necios cuando la justicia vive subyugada, porque la jurisprudencia es un reguero de pólvora que aterroriza a los impíos. Destruyeron los mercados de la aldea, atenazaron la independencia de los nativos con iniciativa y se entregaron en brazos de los extranjeros; y cuando los mercaderes foráneos descubrieron la calaña de aquellos mafiosillos de barro, les retiraron los préstamos y les estrujaron las vísceras con intereses de apisonadora, quedando la aldea como un páramo muerto, aplanada por la deuda, la ruina, la desconfianza y el desengaño. Ahora toca pedir limosna, excavar impuestos y soñar que un día la aldea resurja de sus cenizas; pero el pueblo está triste; los víveres escasean; nadie presta a nadie; todos desconfían; los jóvenes no tienen trabajo y se teme que aumente la delincuencia y el nomadismo maleante; los ancianos viven resignados a la espera del final. La gente sufre enfermedades de la carne y del espíritu; han perdido la fe en el chamán, especialmente desde que le retiraron los poderes y fue suplantado por el consejo de las 17 brujas.

En medio de esta desolación se ha elegido a un nuevo líder para la gran tribu. La mayoría absoluta no es un cheque en blanco ni una licencia para imponer el estado de sitio, restablecer la inquisición azul o reimplantar la censura anti-disidencia. La mayoría absolutista podría ser una necesidad desesperada, una concesión transitoria para medir hasta donde los hechos se corresponden con las promesas, o un mero ejercicio de penalización salvaje contra quien falseó la realidad y violó cobardemente el principio de lealtad y confianza. Cuando el pueblo otorga una mayoría está pidiendo cuentas. Los responsables del abuso y del desfalco no pueden quedar impunes; al brazo del poder absoluto le toca ejecutar a los profanadores del templo de la lealtad y el compromiso, limpiar los escombros y restaurar las ruinas. Los resultados permitirán considerar la reválida. El voto no es una declaración; es un préstamo.

Ante este panorama lúgubre, los que tienen el privilegio –todavía- de disfrutar de un puesto de trabajo y ejercen la libertad de manifestar lo que piensan –aunque algunos los preferirían mudos y enjaulados-, tienen la obligación moral y solidaria de mantener habitable la aldea, a pesar de los que orinan en cualquier esquina, defecan fuera de las letrinas, roban la verdura del huerto ajeno, ultrajan a los difuntos, se quedan con la limosna de la parroquia, trafican con voluntades, compran el silencio cómplice de los cobardes, contratan a plañideras para simular que lloran o pagan a los voceros para difundir calumnias. A pesar de esta camarilla de caciques tribales, los hombres y mujeres de la tribu forman un pueblo honrado, viejo y curtido, entrenado en el ejercicio de la autodestrucción, pero con capacidad para rehacer su historia legendaria. Ningún líder del pasado ha sobrevivido; todos, sin excepción, sin diferencia de color, han sido exterminados. Los historiadores no se ponen de acuerdo sobre los agentes del magnicidio psicológico y moral; hay dudas de si fueron sus súbditos o sus secuaces. Todo reyezuelo tiene un Judas en su guardia de corps. Algunos fueron aniquilados antes de empezar a gobernar; a otros los intoxicaron de poder y luego los arrojaron a la piara; y alguno cayó víctima de la traición a sí mismo y a los mismos que le degollaron.

> *"El voto no es una declaración; es un préstamo.*

Ahora la aldea está de fiesta. No hay nada que celebrar, pero la Navidad siempre arrastra, y papá Noel este año regala un gobierno.

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Referencias:
- Methods Find Exp Clin Pharmacol 2002; 24:573-8.
- Int Immunopharmacol 2005; 5:253-62.
- IPA Osaka. International Psychogeriatrics 2007; 19 (Suppl 1):416.

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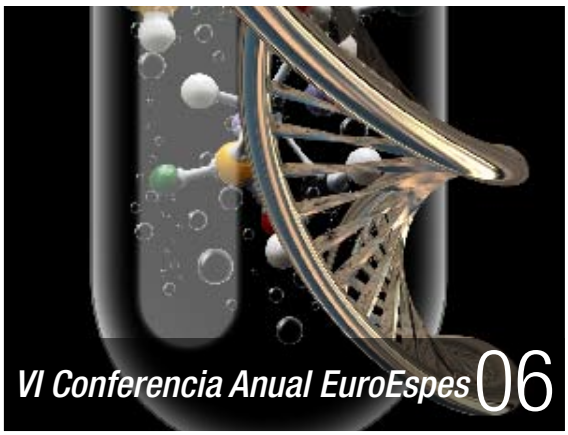
SUMARIO



Opinión

Ciencia

VI Conferencia Anual EuroEspes

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WELCOME HOME

VI Conferencia Anual EuroEspes

In spite of the crisis which has us in its grasp, and of the irremediable restrictions enforced by necessity, EuroEspes has once again kept its appointment with society at this VIth Annual Conference and welcomes you back home, both those within and outside the company.

This is a special year. We have always advocated that it is at the moments of scarcity when we most have to spur our inventiveness and, faithful to our philosophy, our wish was that in this International Year of Research into Alzheimer's Disease, which has passed almost unnoticed by society and the scientific community, EuroEspes should make its small contribution in its unending struggle –a struggle which has continued now for 20 years– against dementia. This year, the United States Patent Office has accepted the EuroEspes Vaccine against Alzheimer's disease, developed by the scientists of the EuroEspes Biomedical Research Center and of EuroEspes Biotechnology. Iván Carrera and Carmen Vigo will provide us with the first official data regarding the EuroEspes Vaccine, which are included in the EuroEspes Journal (Gen-T).

In the IIIrd EuroEspes Conference, in 2008, which was devoted to Genomic Medicine, and at which their Royal Majesties King Juan Carlos and Queen Sofía of Spain agreed to hold the Honorary Presidency, we counted on the support of the vast majority of the Rectors of Spanish Universities, of the Presidents of Scientific Societies and of the Official Physicians' and Pharmacists' Associations, and also of the most outstanding scientists in the world. There we founded the *World Association of Genomic Medicine* (WAGEM) and we promised to launch the first World Guide for Pharmacogenomics. This commitment has now become a reality in this VIth Conference, as we officially present the *World Guide for Drug Use and Pharmacogenomics* (WGPGx), the result of a heroic task (over 3,000 pages and 50,000 entries) performed by a number of people who, over 5 years, have managed to put in order and systematize the more than 100,000 scientific references published during the past 30 years concerning medical genomics and pharmacogenomics. At last we have at our disposal a practical tool to make pharmacogenomics leap from the laboratory to the clinic and to provide physicians with a guide that will enable us to optimize the use of drugs, in order to improve efficacy, minimize toxicity and reduce pharmaceutical costs.

We are also honored to be accompanied by Prof. Arturo Fernández-Cruz, who will give the opening lecture, and by Prof. Gjumrakch Aliev, who is the Speaker of today's closing lecture; it is also an honor and a pleasure to have with us our loyal friends Prof. José Miguel Sempere and Rector Rafael Cortés Elvira.

In this edition of the EuroEspes Annual Conference we will be joined by leading figures from the world of finance, and by stalwart associates; for example the President of the "Caja Rural" bank of Soria, Mr Carlos Martínez-Izquierdo; concerned intellectuals such as Fernando Sánchez Dragó; and authorities, such as the President of the Professional Medical Association, Dr. Juan José Rodríguez Sendín, known for his awareness of scientific progress and of the future of the medical profession.

We are living in a world which evolves towards new formulae for interprofessional coexistence and cooperation. Science must come out into the open so that the public may touch it, feel it, enjoy it and benefit from it. In order to achieve this, ideas must crystallize into tangible assets which provide benefit and added value; and the realization of ideas requires resources to be provided by society, in order that this benefit may be returned severalfold.

EuroEspes' vocation is to be there, at the cutting-edge of progress, at the service of our society. Turning material investment into formulae for health and well-being is the best tribute we can pay to those who have put their trust in us, and those who put their lives in our hands. This spirit of service is our guiding light.

Welcome, and thank you all for coming.

Ramón Cacabelos (*Chairman*)

BIENVENIDOS A CASA

VI Conferencia Anual EuroEspes

Apesar de la crisis que nos atenaza y de las irremediables restricciones que impone la necesidad, EuroEspes acude, un año más, puntual a su cita con la sociedad en su VI Conferencia Anual y os da la bienvenida a casa, a los de dentro y a los de fuera.

Este es un año especial. Nosotros siempre hemos defendido que es en los momentos de escasez cuando hay que estimular más el ingenio; y siendo fieles a nuestra filosofía hemos querido que en el Año Mundial del Alzheimer, que pasó sin pena ni gloria para la sociedad civil y para la comunidad científica, EuroEspes aportase su granito de arena en su continuada lucha –de más de 20 años de historia- contra la demencia. Este año, la Oficina de Patentes de Estados Unidos ha aceptado la Vacuna EuroEspes contra la enfermedad de Alzheimer que desarrollaron los científicos del Centro de Investigación Biomédica EuroEspes y de EuroEspes Biotecnología. Iván Carrera y Carmen Vigo nos brindarán en esta Conferencia los primeros datos oficiales de la Vacuna EuroEspes, cuyos contenidos se recogen en el EuroEspes Journal (Gen-T).

En la III Conferencia EuroEspes de 2008, dedicada a la Medicina Genómica, en la que tuvimos la fortuna de contar con la Presidencia de Honor de Sus Majestades los Reyes de España, D. Juan Carlos y Dña. Sofía, el respaldo de la inmensa mayoría de los Rectores de las Universidades Españolas y de los Presidentes de las Sociedades Científicas, los Presidentes de Colegios Médicos y Colegios Farmacéuticos, y los científicos más destacados del mundo, fundamos la *Asociación Mundial de Medicina Genómica (**World Association of Genomic Medicine, WAGEM**)* y prometimos sacar a la luz la primera guía mundial de farmacogenómica. Este compromiso lo hacemos realidad en esta VI Conferencia, presentando en sociedad la *World Guide for Drug Use and Pharmacogenomics* (WGPGx), un trabajo heroico (con más de 3.000 páginas y 50.000 entradas) de un amplio número de personas que a lo largo de 5 años hemos conseguido poner en orden y sistematizar las más de 100.000 referencias científicas publicadas en los últimos 30 años sobre genómica médica y farmacogenómica. Por fin disponemos ya de un instrumento práctico para que la farmacogenómica salte del laboratorio a la clínica y para que los médicos dispongamos de una guía que nos permita optimizar el uso de fármacos, con el fin de mejorar la eficacia, minimizar la toxicidad y reducir el gasto farmacéutico.

También nos sentimos muy honrados de contar con el Prof. Arturo Fernández-Cruz como ponente de la sesión inaugural, y del Prof. Gjumrakch Aliev como ponente de la sesión de clausura, así como tener con nosotros a amigos leales, como el Prof. José Miguel Sempere o el Rector Rafael Cortés Elvira.

En esta edición de la Conferencia Anual EuroEspes también nos acompañarán destacadas personalidades del mundo de las finanzas y socios fieles, como el Presidente de la Caja Rural de Soria, D. Carlos Martínez-Izquierdo; intelectuales comprometidos, como Fernando Sánchez Dragó; o autoridades, como el Presidente de la Organización Médica Colegial, el Dr. Juan José Rodríguez Sendín, sensible al progreso científico y al futuro de la profesión médica.

Vivimos en un mundo que evoluciona hacia nuevas fórmulas de convivencia y colaboración interprofesional. La ciencia debe salir a la calle para que el ciudadano la toque, la sienta, la disfrute y se beneficie de ella. Para conseguirlo, las ideas tienen que cristalizar en tangibles que aporten beneficio y valor añadido; y la realización de las ideas necesita recursos que la sociedad aporta para que luego ese beneficio retorne multiplicado a su origen.

La vocación de EuroEspes es estar ahí, en el filo del progreso, al servicio de nuestra sociedad. Convertir en fórmulas de salud y bienestar las inversiones materiales es el mejor tributo que podemos hacer a quienes confían en nosotros y a quienes ponen su vida en nuestras manos. Ese espíritu de servicio es el faro que nos guía.

Bienvenidos a todos y gracias por vuestra presencia.

Ramón Cacabelos (*Chairman*)

08:30 Recepción y entrega de material/Reception

09:00 Acto de Apertura/Opening Remarks

Prof. Ramón Cacabelos
PRESIDENTE WAGEM
Excmo Sr. D. Rafael Cortés Elvira
RECTOR MAGNÍFICO DE LA UNIVERSIDAD CAMILO JOSÉ CELA
D. Pablo Álvarez de Linera
GARRIGUES
D. Francisco González
ERNST & YOUNG

09:30 Conferencia Inaugural/Opening Lecture

Genómica de las enfermedades cardiovasculares
Genomics of Cardiovascular Disorders

Prof. Arturo Fernández-Cruz
UNIVERSIDAD COMPLUTENSE, MADRID

10:15 *Amiloidogénesis cerebral en la enfermedad de Alzheimer y opciones terapéuticas*
Pathogenic role of beta amyloid in the brain of Alzheimer's disease patients and therapeutic options

Dra. Carmen Vigo
PANORAMA RESEARCH INSTITUTE, SUNNYVALE, CALIFORNIA, USA

10:45 *Vacuna EuroEspes contra la enfermedad de Alzheimer*
EuroEspes vaccine against Alzheimer's disease

Dr. Iván Carrera
Dra. Lucía Fernández-Novoa, Dr. Valter Lombardi,
Dra. Carmen Vigo, Prof. Ramón Cacabelos
DEPARTAMENTO DE NEUROCIENCIAS, EUROESPES BIOTECNOLOGÍA, CORUÑA

11:15 Descanso/Break

11:45 *Perfil fenotípico cerebral en envejecimiento y demencia*
Phenotypic brain profile in aging and dementia

Dr. Leo Canuet e Iván Tellado
Prof. Ramón Cacabelos
DEPARTAMENTO DE DIAGNÓSTICO DIGITAL, CENTRO DE INVESTIGACIÓN BIOMÉDICA EUROESPES, CORUÑA

12:15 *Genómica y Farmacogenómica de la retinopatía diabética*
Genomics and Pharmacogenomics of diabetic retinopathy

Dr. Pablo Carnota
Prof. Ramón Cacabelos
UNIDAD DE NEUROOFTALMOLOGÍA, CENTRO DE INVESTIGACIÓN BIOMÉDICA EUROESPES, CORUÑA

12:45 *Propiedades biológicas de HepatoSar y Mineraxin*
Biological properties of HepatoSar and Mineraxin

Dra. Lola Corzo
Dr. Ramón Alejo, Dr. Valter Lombardi, Prof. Ramón Cacabelos
DEPARTAMENTO DE BIOQUÍMICA MÉDICA, CENTRO DE INVESTIGACIÓN BIOMÉDICA EUROESPES, CORUÑA

13:15 *Histamina en enfermedades cerebrales*
Histamine in brain disorders

Dra. Lucía Fernández-Novoa
Prof. Ramón Cacabelos
DEPARTAMENTO DE GENÓMICA, EUROESPES BIOTECNOLOGÍA, CORUÑA

13:45 *Histaminosis alimentaria no alérgica. Síndrome HANA.*
HANA syndrome. Non-allergic nutritional histaminosis
Dr. Félix López-Elorza
LABORATORIO LABSUR, SEVILLA

14:15 Comida/Lunch

15:30 *Genómica del Síndrome Metabólico*
Genomics of metabolic syndrome
Dr. Manuel Suárez-Tembra
DEPARTAMENTO DE MEDICINA INTERNA, CENTRO DE INVESTIGACIÓN BIOMÉDICA EUROESPES, CORUÑA

16:00 *Propiedades anti-inflamatorias de AntiGan en un modelo experimental de cáncer de colon*
Anti-inflammatory properties of AntiGan in an experimental model of colon cancer
Dr. Valter Lombardi
Dr. Ramón Alejo, Dr. Iván Carrera, Prof. Ramón Cacabelos
DIVISIÓN DE BIOTECNOLOGÍA DE LA SALUD, EUROESPES BIOTECNOLOGÍA, CORUÑA

16:30 *Inmunogenética*
Immunogenetics
Prof. J. Miguel Sempere
UNIVERSIDAD DE ALICANTE, ALICANTE

17:00 *Genómica cerebrovascular*
Cerebrovascular genomics
Dr. Juan C. Carril
Dra. Lucía Fernández-Novoa, Prof. Ramón Cacabelos
DEPARTAMENTO DE GENÓMICA, EUROESPES BIOTECNOLOGÍA, CORUÑA

17:30 Descanso/Break

18:00 *Presentación de la Guía Mundial de Farmacogenómica*
Introduction to the World Guide of Pharmacogenomics
Prof. Ramón Cacabelos
Javier Sánchez
WORLD ASSOCIATION OF GENOMIC MEDICINE AND
EUROESPES PUBLISHING

18:30 EuroPharmaGenics Website
Dr. Alfonso Lorenzo
COREMAIN, SANTIAGO DE COMPOSTELA

19:00 Conferencia de Clausura/Closing Lecture
Estrés oxidativo en neurodegeneración y cáncer
Oxidative stress in neurodegeneration and cancer
Prof. Gjumrakch Aliev
UNIVERSITY OF ATLANTA, USA

19:45 Acto de Clausura/Closing Remarks
Prof. Ramón Cacabelos
Excmo. Sr. D. Rafael Cortés Elvira
D. Carlos Martínez Izquierdo
PRESIDENTE DE LA CAJA RURAL DE SORIA
D. Fernando Sánchez Dragó
ERUDITO, ESCRITOR Y PENSADOR
Dr. Juan José Rodríguez Sendín
PRESIDENTE DE LA ORGANIZACIÓN MÉDICA COLEGIAL (OMC)
D. Roberto Pereira Costa
DECANO-PRESIDENTE, COLEGIO DE ECONOMISTAS DE A CORUÑA

Prof. Arturo Fernández-Cruz
UNIVERSIDAD COMPLUTENSE, MADRID

ABSTRACT

Genomics of cardiovascular disorders

Technological breakthroughs in medicine have achieved a significant increase in life expectancy. Reality, as seen by a clinician, is to assume that 50% of the conditions that determine health are genetic. Interaction of genes with the environment determines a new concept that has been identified as Geo-Medicine. This has a great impact on aging and disease development.

Our view is that we are already at the new paradigm of medical care, which is focused on treating healthy individuals. Indeed, contending against an advanced disease can be seen as the failure of our profession. Preventive medicine is the alternative, leading to the escalating success of our profession. The main tool is therefore, predictive medicine. With the help of genetic testing and telomeric signals, predictive medicine can reveal the pathway of our aging and the diseases to which we are predisposed. Furthermore, the concepts for personalized medicine have changed with the advances in the fields of pharmacogenomics and nutrigenomics. As a result, we are now deeply involved in redesigning our lifestyles and treatments "à la carte". Regarding CVD, a large number of candidate genes and polymorphisms have been identified. In clinical practice there are a number of genetic diseases that are expressed as congenital diseases which are already coded. Moreover, a number of clinical situations related to sudden death, hypertrophic cardiomyopathy, atrial fibrillation and those related to risk factors in the development of atherosclerosis are already coded and can be clearly identified by genetic testing.

On the other hand, the discovery of genes that control gene expression has allowed us to speculate about the rationale behind modifications in the lifestyle in the prevention of CVD. These are the so-called epigenetics that can recode our future. Finally, the application of genetic technology to stem cells opens another great chapter on organ and tissue regenerative medicine. Stem cells have been used to treat heart attack patients, with a clear improvement in cardiac function. We can conclude that genetic testing applied to CVD envisions a new paradigm on how to identify and treat heart patients.

Currriculum Vitae

Presidente Fundación Fernández-Cruz
Catedrático y Jefe de Servicio de Medicina Interna UCM
Director del Área de Prevención Cardiovascular del Hospital Clínico San Carlos de Madrid
Vicepresidente de la Sociedad Europea de Prevención Cardiovascular
Director de Revistas Médicas Españolas e Internacionales
Presidente-Fundador del Comité Científico de la Fundación Alcohol y Sociedad
Reconocido Experto Europeo en Prevención Cardiovascular
Ha sido y es miembro de diferentes comités Nacionales e Internacionales

FORMACIÓN ACADÉMICA

Licenciado en Medicina y Cirugía (Universidad de Barcelona, 1966); Doctor en Medicina y Cirugía (1969)
Especialista en Endocrinología, Universidad de Barcelona, 1970
Especialista en Hipertensión Internacional Society of Hipertensión, 2000
Profesor ayudante de clases prácticas desde 1968 al 70 por la Universidad de Barcelona
Profesor adjunto desde 1970-77 por la Universidad Complutense de Madrid
Profesor Agregado de la Universidad Complutense de Madrid, 1977
Catedrático y Chairman de Medicina de la Universidad de Cádiz, 1978
Catedrático y Jefe de Medicina Interna del Hospital Universitario San Carlos, en la Universidad Complutense de Madrid, desde 1981

PUESTOS HOSPITALARIOS

Médico asistente del Hospital Clínico de Barcelona desde 1964-66
Jefe de residentes del Hospital Clínico de Barcelona desde 1966-68
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ABSTRACT

Cerebral Amyloidopathy in Alzheimer's disease and Therapeutic Options

Alzheimer's disease (AD) is a chronic neurodegenerative disease characterized by brain atrophy and loss of neurons and synaptic function, secondary to beta amyloid (Aβ) plaques and neurofibrillary tangle (NFT) formation. AD is the most prevalent form of dementia, affecting an estimated 6 million people in North America and 20 million worldwide. At present, there is no effective disease-modifying therapy, and the cause of the disease is still unknown. AD constitutes a personal and societal tragedy of immense proportions, and its cost is estimated in $200 billion worldwide. Since 1960, research in laboratories and clinics has elucidated many features of this insidious and ultimately fatal syndrome, and this progress has led to initial human trials of potential disease-modifying agents. However, many of these agents have already failed. Gnawing controversies and important gaps in our knowledge of the genesis of this disease seem to cast additional doubt on the ability of the field to move forward effectively.

Beta amyloid, an amino peptide derived from a much larger protein, amyloid precursor protein (βAPP)[1], is considered by many both a cause and an effect of AD. Aβ is the main component of the "peculiar substance" first described by Alzheimer in 1907[2]. Aβ aggregates and forms soluble oligomers and compact plaques known as neuritic or senile plaques. Amyloid plaques and soluble Aβ oligomers cause synaptic loss (a strong morphological correlate of the degree of dementia in individuals with AD), impaired hippocampal synaptic plasticity, microgliosis, tau hyperphosphorylation, and NFT, the other pathology hallmark of AD. As AD begins, long before symptoms appear, there is a rise in Aβ monomer levels (resulting from partially identified factors such as mutations in APP or presenilins), a decrease in Aβ clearance by the apolipoprotein E4 variant, or perhaps increased β-secretase activity, promoting the formation of dimers and then larger oligomers. The dimers and other oligomers bind cell membranes and aggregate progressively to form protofibrils (~4 nm), fibrils (~8 nm), and plaques of fibrils, a process that markedly decreases their exposed surface area.

For the past decade, the amyloid cascade hypothesis has dominated the field of AD research. This hypothesis places the formation of early, toxic Aβ oligomers and the accumulation of Aβ aggregates at the center of AD pathogenesis. This hypothesis supports that over time, an imbalance in Aβ production and/or clearance leads to gradual accumulation, aggregation, and a deposit of Aβ in plaques in the brain, initiating a neurodegenerative cascade. Aβ deposition in senile plaques and its soluble oligomers induce a series of neuropathological changes through multiple mechanisms that include induction of oxidative stress, caspase activation, hyperphosphorylation of tau, and formation of NFT. Cognitive decline and subsequent dementia are, in turn, a consequence of these neuropathological events targeting the brain's cognitive circuitry[3-5]. According to this hypothesis, the deposit of Aβ is the first pathological change in AD and occurs in 100% of the cases. Supporting this hypothesis, *in vitro* and *in vivo* studies in animal models showed that oligomeric and fibrillar forms of Aβ cause long-term potentiation impairment and synaptic dysfunction[6-8]. The most convincing supporting evidence of this hypothesis came from the patients with familiar AD due to APP and presenilin mutations (both determine the length and quantity of the released Aβ), which in 100% of the cases results in the development of AD[9-13].

Support of the important role of Aβ in the brain of AD patients came also from our studies measuring the amyloid burden in the brain of AD patients compared with age-matched controls. Our studies showed that the amount of Aβ in the brain of patients who died with diagnosed AD versus age-matching controls without AD was 100-fold higher. We identified that the major component of Aβ in the neuritic plaques, parenchymal, and leptomeningeal cerebral blood vessels in the brain of the AD patients is $A\beta_{1-42}$, comprising 90, 75, and 70% respectively, the rest being $A\beta_{1-40}$. In the diffuse plaques, $A\beta_{17-42}$ represents 70% and the rest is $A\beta_{1-40}$[14].

Therapeutic approaches based in the amyloid hypothesis geared to reduce the Aβ burden in the brain would be expected to alleviate both the neuropathological alterations and dementia. Thus, drugs that inhibit the formation, aggregation, and deposit of Aβ were designed and tested. These include inhibitors of β and γ secretases, and activators of α secretases, proteolytic enzymes that cleave Aβ from its precursor APP. Other approaches consisted in clearing amyloid deposits using metal chelators, β sheet breakers, and Aβ passive and active immunizations. However, none of these therapeutic approaches have rendered a commercial drug; most of them already failed in clinical trials, often due to their inability to penetrate the blood brain barrier, formation of toxic soluble oligomers, meningoencephalitis-type reactions[15], microhemorrages, micro edema, and other unwanted side-effects. Despite all these failures, Aβ-focused therapies are still ongoing in the lab and in the clinic with over 20,000 patients enrolled in clinical trials worldwide.

No matter how important the amyloid pathology is in the progression of AD, it is likely that a therapy focused only in Aβ reduction might not be sufficient to prevent or cure this disease. A safe and effective

Aβ-focused therapy would in any case have its optimum effect if administered at the time of the formation of plaques, which can be decades before the clinical symptoms of this disease are manifested. Other therapeutic approaches under development addressing the pathological AD processes comprise anti-tau aggregation and phosphorylation inhibitors, neuronal growth factors, antiinflammatories, cholinesterase inhibitors, antioxidants, hormonal replacement therapy, lipid metabolism enhancers, antihistaminics, and others.

There are only five FDA approved drugs to treat AD. These are the acetyl cholinesterase inhibitors, tacrine, donepezil, rivastigmine, galantamine, and a partial antagonist of NMDA, memantine, which represent the actual standard treatment of AD. Systemic reviews on the effects of these drugs showed improvement on cognitive functions, activities of daily living, and global function for some patients with mild to moderate AD. However, the efficacy of these drugs in protecting subjects with mild cognitive impairment from converting into AD remains inconclusive and their side-effects often outweigh the benefits.

The great challenge for scientists and pharmaceutical companies is to systematically understand the metabolic and degenerative processes implicated in the genesis of AD, and focus on a therapeutic strategy by taking into consideration all the different aspects of this disease. A regime consisting in combinations of drugs focused in the most prominent pathological alterations of AD as well as in the maintenance of a normal brain metabolism and perfusion will probably emerge as the standard therapeutic regimen for the treatment of this disease. Rigorous preclinical validations of mechanism-based therapeutic agents, followed by meticulously designed trials that focus on the cardinal cognitive symptoms and their associated biomarkers in the mild or presymptomatic phases of AD are likely to lead to success, perhaps in the not-too-distant future.

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Currriculum Vitae

Carmen Vigo is a Ph.D. recipient in Biochemistry from the Royal College of Surgeons of England, University of London. She did postdoctoral work in lipid metabolism at the University of British Columbia, Canada, and was later a visiting professor at the University of South Florida, Medical School, where she taught the medical program and conducted research in cholesterol metabolism. For the last 20 years she worked in the biotech and pharmaceutical industry in northern and southern California. Half of these years were focused on Alzheimer's disease research in two of her research institutions, Athena Neuroscience, acquired by Elan Pharmaceuticals, and Neurocal, a company she founded and where she developed pioneer technology in neuronal stem cells in collaboration with the universities of Rockefeller and Stanford. At Athena Neurosciences she made key discoveries in the beta amyloid peptide field which were recognized worldwide and featured in local TV and CNN channels. From her research in Alzheimer's disease, she published over 35 papers in top peer-reviewed scientific journals and made presentations in numerous national and international meetings. She is the co-inventor of ten patents in Alzheimer's disease and another 10 in a broad area of the medical field. For her contributions in the Alzheimer's field she was awarded the Norage Pharmacia Award in recognition for her innovative research. She is the author of over 100 scientific papers and the Editor-in-chief and co-author of three CRC books on Membrane Lipid Oxidation used for academic teaching. She is the founder of two companies in the Bay area; the latest, Atlas Pharmaceuticals, which developed leading technology acquired from Daiichi Pharmaceuticals and a drug in phase II clinical trials, was recently acquired. After the acquisition of Atlas, she continued research on a vaccine for Alzheimer's disease in collaboration with EuroEspes, and in the discovery of new drugs and leading technology at Panorama Research Institute in Sunnyvale, California.

Dr. Iván Carrera

DEPARTAMENTO DE NEUROCIENCIAS, EUROESPES BIOTECNOLOGÍA, CORUÑA

ABSTRACT

Development of a novel vaccine to treat Alzheimer's disease

A novel vaccine addressing the major hallmarks of AD, Aβ neuritic plaques, neurofibrillary tangles, cognitive deficit, and neuroinflammation has been developed. This vaccine involves delivery of a novel immunogen-adjuvant designed to address the pitfalls of the Elan-Wyeth vaccine, AN 1793, which caused massive activation of T-cell-mediated autoimmune response, resulting in a meningoencephalitis-like reaction and death in 6% of the patients. We took a new approach to circumvent past failures withAβ vaccines by judiciously selecting an adjuvant consisting of a physiological matrix, liposomes composed of naturally-occurring phospholipids: phosphatidylcholine, phosphatidylglycerol, and cholesterol. To this phospholipid mixture, a biologically active sphingolipid, sphingosine-1-phosphate (S1P) was added. Aβ42 was incorporated in S1P-containing liposomes (EB101) and administered to double-transgenic mice that rapidly develop AD-like pathology, before and after the AD-like symptoms were established. Our findings show that treatment with EB101 results in a marked reduction of Aβ plaques, neurofibrillary tangle-like structures, and astrocytosis. In our model, EB101 reduces the basal immunological interaction between the T-cells in the affected hippocampal area in the brain of the treated mice and other immune activation markers, including glial fibrillary acidic protein and CD45, consistent with decreased amyloidosis-induced inflammation. EB101 treatment resulted in improved motor strength and coordination, as determined by certain motor coordination tasks. The present results indicate that immunization with the EB101 vaccine prevents and reverses AD neuropathology by halting disease progression and clearing the neuropathological hallmarks of AD(Aβ plaques, neurofibrillary tangles, and neuroinflammation) without producing brain atrophy or limb paralysis.

Currriculum Vitae

Doctor en Neurobiología del desarrollo
Director del departamento de Neurociencias
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FORMACIÓN ACADÉMICA

1998-2003 Licenciado en Biología, en la Facultad de Ciencias Biológicas, Universidad de Vigo, España 2003

2004-2005 Curso de Doctorado (Tercer ciclo) "Programa Interuniversitario de Neurociencia", en 9/09/2005, en la Univ. de Santiago de Compostela, España.

2005 Obtención del "Diploma de Estudios avanzados", en 9/09/2005, en la Univ. De Santiago de Compostela, España.

2005 Tesis de licenciatura titulada "Estudio comparado del desarrollo de los sistemas GABAérgico, dopaminérgico y serotoninérgico de la médula espinal de pintarroja", en 21/06/2005, en la Univ. of Santiago de Compostela, España

2008 Tesis Doctoral (PhD) titulada "Desarrollo de los sistemas GABAérgicos y aminérgicos en el sistema nervioso central de peces cartilaginosos", en 18/07/2008, en la Univ. de Santiago de Compostela, España

2009-2012 Beca de Investigacion de "Development Journal": Titled: 3D Brain Developmental Atlas of Sharks."

2009/2010 Tesis Doctoral en Biología premiada como Mejor del año 2008. University of Santiago de Compostela, Spain

PARTICIPACIÓN EN LOS SIGUIENTES PROYECTOS DE INVESTIGACIÓN

"Estudio de la morfogénesis y regionalización del sistema nervioso central de peces elasmobranquios", concedido por el Ministerio de Educación y Ciencia (BFU2004-03313), desde 13/12/2004 al 13/12/2007.

"Estudio da morfoxénese e rexionalición do sistema nervioso central en elasmobranquios" Incentivo Plan Nacional de la Xunta de Galicia (2005/PX009).

"Formación del patrón del encéfalo en condrictios (peces cartilaginosos)" Financiado por el Ministerio de Educación y Ciencia (BFU2007-61154), desde 14/12/07 al 13/12/08.

"Generating and characterizing induced pluripotent stem cells from skin cells of mice with Acute Intermittent Porphyria (AIP)", at the Mount Sinai School of Medicine, from 1/2/11-15/4/11.

PROYECTOS DE INVESTIGACIÓN EN DESARROLLO ACTUALMENTE

"3D Brain Developmental Atlas of Sharks" granted by Development Journal, a Journal travelling fellowship, from 15/1/2009 to 15/1/2012.

"Insights into the ancestral brain organization of Gnathostoms: Patterning, Migration, projection and asymmetries in the shark brain development", granted by the Spain Science and Innovation Ministry (BFU2010-15816), from 14/7/2010 to 13/7/2013.

"Targeted embryonic stem cells: Manipulating the mouse embryo", granted by EuroEspes Foundation, from 20/8/2010 to 19/8/2013.

Dr. Leo Canuet
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ABSTRACT

Phenotypic brain profile in aging and dementia

Aging is considered the major risk factor for Alzheimer´s disease (AD). Pathological hallmarks of AD include intracellular aggregates of tau protein filaments (neurofibrillary tangles) and extracellular deposits of beta amyloid peptides (amyloid plaques). This leads to prominent brain aging signs, namely loss of synaptic contacts and neuronal apoptosis, which provoke progressive cognitive decline. In addition to aging, genetic abnormalities have been identified as important risk factors for AD, especially the presence of ε*4* allele of the cholesterol transporter, apolipoprotein E. Interestingly, recent evidence indicates that elevated levels of serum LDL-cholesterol relate to amyloid plaque density, particularly in ε*4* carriers, as well as to increased risk for developing AD. A few neuroimaging studies revealed localized dysfunction in certain cortical regions associating with physiological and pathological aging, or with the presence of *APOE* ε*4*. Given that characterizing brain activity in terms of anatomically segregated responses is not sufficient to explain the complexity of AD symptomatology, a functional disintegration in distributed brain networks has been proposed as an important pathophysiological mechanism underlying AD. This study aims at assessing the effects of *APOE* genotype and serum LDL-cholesterol levels on brain oscillatory activity and functional connectivity, using a novel *lagged* connectivity index that is resistant to nonphysiological artifacts (i.e. volume conduction and low spatial resolution) that usually affect other connectivity measures. Resting EEG during the wakeful, eyes closed state was recorded in 125 patients (60 *APOE* ε*4* carriers; 65 non-carriers) with probable AD and 60 healthy elderly controls (12 *APOE* ε*4* carriers; 48 non-carriers). Twenty-five healthy young adults were also recruited to explore physiological aging effects. *APOE* genotype and serum levels of LDL were determined in all subjects. For data analysis, exact low-resolution brain electromagnetic tomography (eLORETA) was used. Functional images of spectral density and brain connectivity were calculated for each frequency band. A whole-brain Brodmann area approach was used for the ROI-based connectivity analysis. Our findings revealed a decrease in occipital alpha and increase in frontocentral slow oscillations with increasing age, and a specific physiological connectivity pattern involving the "default mode network" related to aging and *APOE* genotype. The slow activity sources correlated negatively with the MMSE scores. We also noted significant differences, mainly in parieto-occipital alpha oscillations, in patients with AD relative to elderly controls. Among AD patients, alpha1 activity in the left parieto-occipital region was particularly reduced in *APOE* ε*4* carriers compared with non-carriers. In addition, LDL effects on oscillatory activity differed in the patients depending on *APOE* genotype; the ε*4* carriers with high LDL levels showed reduced activation of the left temporo-parietal cortex relative to those with low levels. In the group of non-carriers we found an opposite effect of LDL levels involving the left parietal region. There was a significant disruption in interhemispheric theta functional connectivity, which was identified by both lagged-coherence and lagged-phase synchronization measures. Connections of left medial/inferior temporal regions were particularly compromised. This EEG phenotypic profile may represent a potential neurophysiological marker of AD.

Currriculum Vitae

Leo Canuet

Director del Departamento de Diagnóstico Digital
Centro de Investigación Biomédica EuroEspes

FORMACIÓN ACADÉMICA

Desarrolla sus estudios universitarios en el Instituto Superior de Ciencias Médicas de Santiago de Cuba en 1991, donde se desenvuelve como alumno ayudante e instructor de neurocirugía por 5 años. Se gradúa de médico en este instituto en 1997.

Se hace especialista de Neurología en el Instituto Nacional de Neurología y Neurocirugía de La Habana, Cuba (1998-2002).

Profesor Instructor de Medicina Interna y Neurología en el Hospital Provincial ¨Saturnino Lora¨ Santiago de Cuba. 2004-2005.

Recibe beca de la Federación Mundial de Neurología para asistir al III Congreso Iberoamericano de Epilepsia. Ciudad México 2004

Recibe beca del gobierno japonés en 2005 para desarrollar estudios de investigación sobre magnetoencefalografía, epilepsia y psicosis en el departamento de Psiquiatría de la Universidad de Osaka, Japón, recibiendo el título de Doctor en Neurociencias en 2010.

Continúa en la Universidad de Osaka realizando trabajo post-doctoral en el campo de la magnetoencefalografía clínica hasta mayo de 2011.

Recibe el premio de investigación Dr. Nishimura que otorga la universidad de Osaka por su tesis doctoral sobre "Marcadores magnetoencefalográficos de deterioro de la memoria de trabajo (working memory) en la psicosis epiléptica crónica interictal" en 2010.

Actualmente se encuentra realizando proyecto de investigación sobre actividad bioeléctrica cerebral y genotipos específicos en el Centro de Investigación Biomédica EuroEspes con el objetivo de identificar marcadores neurofisiológicos del daño cognitivo y la demencia.

Currriculum Vitae

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Responsable Técnico del Departamento de Diagnóstico Digital. Áreas de Cartografía Cerebral y Topografía Óptica Digital, Centro de Investigación Biomédica EuroEspes.

FORMACIÓN ACADÉMICA

Licenciado en Biología por la Universidad de A Coruña (1998).

Desde el año 2003 trabaja en el Departamento de Diagnóstico Digital en el Centro de Investigación Biomédica EuroEspes y en estos años ha colaborado en tres ensayos clínicos sobre enfermedad de Alzheimer realizados por el Departamento de Neurofarmacología del Centro de Investigación Biomédica EuroEspes.

Además de estos ensayos también ha participado en la realización de estudios científicos en las áreas de Topografía Óptica Digital (Validación de la aplicación clínica de esta técnica y búsqueda de patrones de oxigenación según genotipo) y de Cartografía Cerebral (Estudio de la función bioeléctrica cerebral según patología o genotipo específica).

Dr. Pablo Carnota

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ABSTRACT

Genomics and pharmacogenomics of diabetic retinopathy

Diabetes mellitus is a group of metabolic diseases characterized by hyperglucemia as a consequence of a dysfunction in the secretion of insulin, in its action or both. It causes functional and structural alterations in almost all organs and tissues but there exist the so-called target organs in which such alterations are more severe: retina, central nervous system, heart and kidney. Diabetic retinopathy (DR) is the main cause of blindness at a working age in developed countries, and it is estimated to be responsible for 12% of new cases of blindness in people between 20 and 74 years of age in the United States.

We classify DR in two main groups: non-proliferative RD (NPRD) and proliferative RD (PRD). NPDR encompasses a wide clinical spectrum, from the appearance of microaneurysms, punctate hemorrhages and hard exudates (sometimes in asymptomatic patients) to venous tortuosity and intraretinal microvascular abnormalities (IRMA). RDP is characterized by the appearance of neovascularization both in the optic disc and peripheral retina. These new vessels can lead to recurrent bleeding and tractional retinal detachment. Moreover, regardless of the stage of DR, a macular edema may appear, which is the most common cause of vision loss in patients with RD.

Pathogenesis of RD involves molecular mechanisms and cellular factors. Among the molecular mechanisms we could include: the aldose reductase and polyol pathway, nonenzymatic glycation of proteins (resulting in the formation of advanced glycation end-products or AGE), the vascular endothelial growth factor (VEGF, associated with the formation of neovessels), activation of protein kinase C and oxidative stress. The main cellular factors are those related to breakdown of the blood-retinal barrier (disruption of tight junctions, death of pericytes) but there is also neuronal cell death and changes in glial cells. On the other hand, it is postulated that leukocytes and platelets may also play a role in the pathophysiology of diabetic retinopathy.

The main risk factors for the development of DR are, in order of importance, the time course of diabetes, poor control of blood glucose levels, pregnancy and the presence of other cardiovascular risk factors (smoking, hypertension, high cholesterol and obesity). However, in daily clinical practice it is found that there are patients who develop DR despite being well-controlled, while others are spared even in the case of poor metabolic regulation and / or carrying more than 25 years of disease progression. In addition, the onset, intensity and progression of diabetic complications show large interindividual differences. All this indicates a genetic predisposition to suffer certain diabetic complications such as DR. For this reason, there is a need to look for genetic risk markers for the development of DR, especially those related with the molecular and cellular factors linked to the pathogenesis of DR. To date, a multitude of candidate genes for DR have been studied, with promising but inconclusive outcomes. Among these genes are those encoding aldose reductase, VEGF, paraoxonase 1 (PON1), erythropoietin (EPO), the RAGE (encoding specific receptors of AGEs), angiotensin converting enzyme (ACE), the plasminogen activator inhibitor-1 (PAI-1), endothelin, nitric oxide synthase and the gene for hemochromatosis.

It is clear that good glycemic control is essential to prevent the onset or development of a DR. However, we have a wide variety of therapies for both PDR and diabetic macular edema. Several studies have demonstrated the utility of Argon laser for both PDR (in which we perform panretinal photocoagulation) and macular edema (focal photocoagulation), for which it remains the gold standard of treatment. We have also performed vitrectomy for couple of decades, a surgical technique that allows us to handle complicated cases of recurrent vitreous hemorrhage and tractional retinal detachments. However, in recent years, we have experienced the emergence and spread of intravitreal injections of anti-VEGF drugs. These drugs, bevacizumab and ranibizumab, are also used both to reduce macular edema and to cause regression of the neovascularization (the PDR marker). It is in this type of treatment where we should focus our attention nowadays in terms of pharmacogenomic issues, as there is a gap in this respect. By knowing the genes involved in the metabolism pathways of these drugs, we could predict in which patients injections will be effective and in which not. In addition, there are many lines of research into drugs that act on VEGF and other molecules, and there is no doubt that in the near future we will have many more therapeutic options. Knowing the patient's pharmacogenetic profile can facilitate the choice between one drug or another, thus making the dream of a personalized therapy come true.

Currriculum Vitae

Director de la unidad de Neuro-Oftalmología, Centro de Investigación Biomédica EuroEspes.

Licenciado en Medicina y Cirugía por la Universidad de Santiago de Compostela (1997-2003).

Beca de la Sociedad Española de Retina y Vítreo 2008. Estancia de 6 meses (en 2008) en la Clínica Zambrano (Buenos Aires – Argentina) con el Dr. Alberto Zambrano.

Realiza un fellowship en Retina Quirúrgica y desarrolla el trabajo "Retinotomía de relajación y aceite de silicona en el tratamiento del desprendimiento de retina regmatógeno complicado con PVR grado C" presentado en el XIII Congreso de la SERV en Madrid (marzo 2009).

Fellowship de Retina Médica en el Charleston Neuroscience Institute (Charleston – South Carolina – USA). Estancia de 7 meses (en 2009) con los Dres. Virgil D. Alfaro, John B. Kerrison y Eric P. Jablon.

Realiza un estudio epidemiológico sobre la prevalencia de la degeneración macular asociada a la edad (DMAE) en España (estudio patrocinado por la empresa Pfizer).

Dra. Lola Corzo

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ABSTRACT

Biological properties of Mineraxin and Hepatosar

Mineraxin is a nutraceutical made from a lyophilized lipoprotein extract of the blue mussel (*Mytilus galloprovincialis*) from the Atlantic coast. It is an important natural source of nutrients, with beneficial properties in different areas of health. It retains the innate properties of the raw material due to the non-denaturing manufacturing processes used to produce the final product. The product is 100% natural, with no preservatives and no known side-effects. Scientific references concerning the composition of mussels have confirmed its beneficial effect on bone and joint problems due to the high content in glucosamine (a precursor of collagen). Its glucosamine-associated anti-inflammatory effect, by inhibiting COX-1, COX-2 and leukotrienes, has been demonstrated in a recent study. Its content in vitamins, especially B-complex vitamins, minerals, iron and other substances such as selenium and vitamin E, has nutritional, antianemic and antioxidant properties. Our group conducted a study in 91 women in perimenopausal stage taking 750 mg/day Mineraxin for 3 months. Various biochemical markers were determined in the serum of the women selected: FSH (follicle stimulating hormone), LH (luteinizing hormone), Estradiol and Inhibin A to evaluate the hormonal response associated with menopause and its symptoms; GH (Hormone ultrasensitive growth) and IGF-1 (Insulin Growth Factor-1 or somatomedin C) for the assessment of the hypothalamic-pituitary-bone axis; bone alkaline phosphatase (BAP), calcium and β-CrossLaps, markers of bone formation and antiresorptive activity; TAS (total antioxidant status) to study the antioxidant capacity of Mineraxin; iron and ferritin to assess changes in body iron stores; cortisol, a stress-related hormone which is altered in perimenopausal women, and BMI (body mass index) to confirm Mineraxin as a low-calorie product. We assessed the perimenopausal symptoms after treatment and found that most patients had an overall improvement, especially noticeable in reducing hot flashes, mood swings and musculoskeletal pain. An increase in estradiol and inhibin A and a decrease in FSH and LH were observed, contrary to the usual profile in the perimenopausal stage. This pattern could indicate a delay or dampening of estrogen decrease, a cause of adverse symptoms of menopause. GH hormone, mediated by stimulating liver secretion of IGF-1, regulates bone and muscle growth through the hypothalamic-pituitary-bone axis. It has been shown that the hormonal function of this axis may be influenced by diet. Bone growth is regulated by a balance between formation and resorption called bone turnover. Estrogen and the GH/IGF-1 tandem stimulate osteoblastic activity while calcitonin and estrogens inhibit resorption. In the perimenopausal stage, characterized by a decrease in estrogenic activity, the degenerative process exceeds the formation one. This imbalance can lead to osteoporosis if it is not properly controlled. A significant increase of GH and IGF-1 serum levels was found post-Mineraxin treatment. To assess the rate of bone turnover, BAP, calcium and β-CrossLaps levels were analyzed. We found a decrease in the three biomarkers studied, it being most significant in the β-CrossLaps. Data showed a moderate increase in bone formation and a remarkable decrease in osteoblastic activity. These data classified Mineraxin as a natural product with beneficial effects on bone stability, the prevention of osteoporosis or as a complement to treatment in cases of advanced osteoporosis. Mineraxin presents a composition rich in antioxidants, with a high content of selenium, zinc, and vitamins C and E. A significant increase in serum TAS showed the important and rapid antioxidant power of the marine extract. Oxidative stress has been implicated in physiological situations such as aging, and in various pathological conditions such as ischemia, atherosclerosis, CNS degenerative processes, and DNA mutations with carcinogenic effects. Data also showed a reduction in cortisol serum levels, most prominent in those with high basal cortisol levels, corresponding to those women presenting high anxiety levels. When cortisol levels remain chronically high, they can lead to significant adverse effects on behavior or physical symptoms. A quantitative analysis of the composition of Mineraxin highlights its high iron content. Iron has important functions in our bodies, from oxygen transport, energy metabolism and antioxidant capacity, to DNA synthesis promoting ribonucleotide reductase activity. Iron is stored as ferritin in our bodies. The results show a significant increase in iron storage, especially in those patients with low basal ferritin values, while no changes were observed in most of the patients with high ferritin levels at baseline. Mineraxin has a clear antianemic effect, which is beneficial in iron-deficiency situations. The slight decrease in BMI showed the low calorific power of Mineraxin, it being recommendable in diets due to its high nutritional value. Analyzing all the results of the

study, we conclude that Mineraxin is a product with a wide range of beneficial effects on health: it slightly delays the perimenopausal estrogen decline, it supports bone stability, stimulates antioxidant capacity, significantly increases iron storage, prevents bone demineralization, strengthens joints and promotes growth and repair processes, and it has significant anti-inflammatory power. Therefore, we believe Mineraxin can be most beneficial in joint problems, arthritis, osteoporosis, growth period, oxidative stress-associated conditions, aging, degenerative CNS processes, iron-deficiency anemia, pregnancy, lupus, chronic inflammatory diseases, diets, or in perimenopausal women. Data associated with the hepatoprotective effect of HepatoSar will be presented during the lecture.

Currriculum Vitae

Directora del Dpto. de Bioquímica Médica, Centro de Investigación Biomédica EuroEspes.

Licenciada en Farmacia por la Universidad de Santiago de Compostela, España (1987-1991).

Título de Especialista en Análisis Clínicos por el Ministerio de Educación y Ciencia (1991-1994).

Título de Suficiencia Investigadora tras realizar los cursos de doctorado en el Departamento de Bioquímica de la Facultad de Medicina, Universidad de Santiago de Compostela (1995-1997).

Actualmente, y desde el año 1995, es directora del Departamento de Análisis Clínicos del Centro de Investigación Biomédica EuroEspes.

Durante estos 14 años ha participado en numeroso ensayos clínicos y proyectos de investigación nacionales e internacionales de diferentes instituciones públicas y privadas, fundamentalmente relacionados con la Enfermedad de Alzheimer.

Su actividad se desarrolla en el campo de la clínica y la investigación. Su labor investigadora se centra en el estudio de posibles biomarcadores en la Enfermedad de Alzheimer, siendo sus últimos proyectos relacionados con beta-amiloide sérico, estrés oxidativo y riesgo vascular en demencias.

Ha publicado 23 artículos en revistas y libros internacionales, colaborado como “reviewer” en revistas internacionales de importante impacto, y participado activamente en cursos post-grado y congresos a nivel mundial con numerosas comunicaciones.

Dra. Lucía Fernández-Novoa

DEPARTAMENTO DE GENÉTICA, EUROESPES BIOTECNOLOGÍA, CORUÑA

ABSTRACT

A Genomic approach to histamine function

Histamine is synthesized and released by different human cells, especially basophils, mast cells, platelets, histaminergic neurons, lymphocytes, and enterochromaffin cells. It is stored in vesicles or granules and is released on stimulation. HA exerts its effects on target cells through four different types of receptors: H1R, H2R, H3R and H4R. These receptors belong to the G protein-coupled receptor 1 family. In mammals, histamine is metabolized by two major pathways: N(tau)-methylation via histamine N-methyltransferase (HMT) and oxidative deamination via diamine oxidase. In the mammalian brain, the neurotransmitter activity of histamine is controlled by HMT, as diamine oxidase is not found in the central nervous system.

In the present study, we determined three genetic polymorphisms in the *HRH1*-17A>G (rs901865), *HRH2*-1018G>A (rs2067474) and in the *HNMT* Ile105Thr (rs11558538) genes in one hundred and ninety-five subjects, and we analyzed the relationship between histamine genotypes and blood histamine, IgA, IgG, IgM, IgE and PCR-us levels, as well as leukocyte, lymphocyte, neutrophil, monocyte, eosinophil and basophil counts. The rs2067474 in the *HRH2* gene is located in an enhancer element of the gene promoter and is common in all populations. The rs11558538 is a missense mutation in the *HNMT* gene and is considered a functional polymorphism; the enzyme containing isoleucine as residue 105 has been associated with decreased levels of HMT activity and immunoreactivity. The frequency of the T105I polymorphism is found increased in Caucasian patients with asthma.

The results of this study show that the genotype HRH2-1018GA is overrepresented in those subjects with PCR-us levels above 3 mg/dL. Those subjects with this genotype also have significant lower levels of monocytes compared to the -1018GG genotype. Significant differences were observed in the levels of IgG and monocytes in those subjects bearing the *HRH1*-17*A allele. The *HNMT**105T allele is significantly associated with and increase in eosinophil levels, and with a decrease in leucocyte levels. Those subjects with the levels of HA above the normal range (>90 ng/mL) have a significant increase in the levels of eosinophils and basophils; on the contrary, those subjects with HA levels below the normal range (<90 ng/mL) present significantly lower levels of IgM and neutrophils. No significant differences were found beteween HA levels and HA-related polymorphisms. In conclusion, the *HRH2*-1018GA genotype is associated with high levels of PCR-us and the *HNMT**105T allele is related to markers of allergy proccesses. The results of this study indicate that HA-related polymorphisms participate and modulate the immune-inflammatory response.

Currriculum Vitae

Directora del Dpto. de Genética, EuroEspes Biotecnología.

Licenciada en Medicina General y Cirugía en la Facultad de Medicina de la Universidad de Santiago de Compostela (1979-1987).

Más tarde obtuvo una beca post-doctoral en el Departamento de Bioquímica y Biofísica de la Facultad de Medicina de la Universidad de Pensilvania, Filadelfia, USA (1988-1991).

Investigadora Asociada, Departamento de Bioquímica y Biofísica, Universidad de Pensilvania, Filadelfia, USA (1991).

En ese mismo año, obtiene una beca de Reincorporación a España de Doctores y Tecnólogos del Ministerio de Educación y Ciencia, realizando su labor investigadora en el Departamento de Fisiología Humana de la Facultad de Medicina de la Universidad Complutense de Madrid. Allí realiza los cursos de doctorado y obtiene el grado de Suficiencia Investigadora (1991-1994).

Posteriormente se incorpora a la empresa EuroEspes y desde el año 2006 es la directora de la Unidad de Genética de la empresa de biotecnología Ebiotec, ubicada en Bergondo, A Coruña.

Ha participado en numerosos proyectos de investigación básicos y clínicos con empresas y/o administraciones, relacionados fundamentalmente con la enfermedad de Alzheimer.

Su labor científica durante estos años se ha centrado en el campo de la neurodegeneración, tanto desde un punto de vista genético como fisiopatológico.

Dr. Felix López Elorza

LABORATORIO LABSUR, SEVILLA

ABSTRACT

Non-allergic nutritional histaminosis - HANA Syndrome

The release of non-allergic histamine is a mechanism not mediated by IgE, in which the cell-cell interaction between mastocytes and lymphocytes results in a mechanism for the release of silent histamine, which is responsible for chronic clinical symptoms which usually progress throughout the lifespan of the sufferer.

The distribution of histamine throughout the tissues and the variety of receptors available explain the diversity of symptoms encountered.

Our observations are based on the use of *in vitro* histamine release, which we commenced in 1979 for the diagnosis of allergy-related problems. We saw that when we used nutritional proteins for stimulation in order to study cephalea, the results were highly dissonant with those obtained with specific IgE, but when we eliminated the histamine-positive food, this had a decisive influence on the patient's recovery. Initially, we interpreted this phenomenon as an allergic reaction, but subsequently rejected this idea on observing variations in other non-allergy-related symptoms which presented in patients with no clinical or analytical indication of allergies. Since 1987 we have been working with the hypothesis of a non-allergic process which was later described by Bachelet in 2006 and Weissle A in 2008. As described by Bachelet, the process explains perfectly the clinical and analytical behavior observed in these patients.

We have associated the following symptoms with this process: different types of cephaleas, all the symptoms evaluated in fibromyalgia, chronic muscular fatigue, intervertebral dehydration, recurrent muscle contractions, constipation/diarrhea, repeated abortions/infertility, abdominal distension, pain under pressure, secretory dysfunction, among others. Individually, all of these symptoms may be justified by other causes, but when several of them coincide in a single patient, we should deduce that we are faced by a non-allergic histaminosis. The variety of the accumulated symptoms enables a highly accurate clinical surmise.

The habitual story of these patients is that they visit the specialist related with the symptom about which they are most worried where, once examined, if there are no evident causes, as a general rule they are prescribed a symptomatic treatment which initially may improve the symptoms, but if the input of histamine continues, a different symptom arises, and so on. The patient will ultimately be taking a considerable quantity of symptomatic drugs which may aggravate the situation.

In our experience, the therapeutic diet is the best option, although it may demand a considerable effort from the patient and scrupulous clinical care.

We must also bear in mind other causes for the release of histamine, such as stress, likewise the histamine input from the diet in heavily medicated patients.

Currriculum Vitae

Especialista en Bioquímica Clínica, Hospital Virgen Macarena de Sevilla 1976-2010.

Socio fundador de la S.E. de Inmunología, S.E. de Bioquimica Clinica, miembro de la European Research S. (ERSH), Socio fundador y actual Presidente de la Sociedad Andaluza para el Estudio de Intolerancias Alimentarias (SAEIA) y otras Sociedades regionales.

Labor docente en el Dpto. de Bioquímica Clínica de la Facultad de Medicina de Sevilla.

Conferencias Nacionales e Internacionales: 86; trabajos publicados: 43.

Copropietario y Director del laboratorio LabSur de Sevilla.

Linea mantenida de investigación desde 1979 sobre la aplicación de la liberación de histamina en la clínica asistencial.

Dr. Manuel Suárez-Tembra

DEPARTAMENTO DE MEDICINA INTERNA, CENTRO DE INVESTIGACIÓN BIOMÉDICA EUROESPES, CORUÑA

ABSTRACT

Genomics of the Metabolic Syndrome

The grouping in a single individual of various prevalent cardiovascular risk factors, such as glucose intolerance, hypertension, hypertriglyceridemia, a reduction in high-density (HDL) cholesterol or atherogenic dyslipidemia is known as the Metabolic Syndrome, in which there are two underlying mechanisms: abdominal adiposity and insulin resistance. During recent years, other risk factors have been added, among these non-alcoholic hepatic steatosis, waist diameter, microalbuminuria and other biological markers, such as those related with adipose tissue (leptin/adiponectin ratio, apolipoproteins or size of the LDL particles), which have together formed a syndrome which has been given different names (Syndrome X, Raven's Syndrome, etc.) until recently, when the WHO proposed the name "Metabolic Syndrome". Although there exists certain skepticism in some medical circles regarding its existence, among other reasons because the cardiovascular risk associated with the metabolic syndrome is not always greater than that which would be obtained by adding the risk attributable to each of its components. However, the presence of the metabolic syndrome is an excellent predictor of the incidence of cardiovascular disease, and particularly of type 2 diabetes.

Its clinical importance is founded on its being the first step in the identification and correction of different pathologies, such as type 2 diabetes and cardiovascular disease, which enables a quick, early intervention regarding primary prevention, by means of the establishment of modifications in the lifestyle in order to reduce the level of risk and thus avoid the need for pharmaceutical intervention. It is also important to have at our disposal markers that provide a quick, trustworthy assessment of the evolution of the same. Likewise, regarding secondary prevention, pharmacological intervention may be used in order to resolve the different situations and to attempt to bring about a retrogression of the same. In this regard, over the past years, knowledge of the regulatory mechanisms and the biochemical pathways which play an important role in the different pathologies implicated in this syndrome has increased, and a plethora of signaling pathways has been determined regarding the development of diabetes, hypertension and dyslipidemia, many of these focusing on obesity and adipose tissue. There is evidence of an association between these pathologies and certain genes, some of which are particularly relevant, such as *PPARgamma*, *TCF7L2* (transcription factor) and *FTO* among others, which have a close relationship with adipose tissue. Thanks to recent studies, the number of loci associated with these pathologies has increased, and it is to be expected that the relationship between these and adipose tissue (obesity), which has been revealed as the most significant causal factor of this syndrome, will be studied ever further.

Research is currently underway regarding the role of adipose tissue in changes in glucose metabolism, insulin resistance and obesity-related inflammation. These dysfunctions bring about changes concerning other organs, and make possible the development of different diseases, among which are cardiovascular diseases and type 2 diabetes.

It is still to be seen whether all these discoveries may play an important part in the development of new therapeutic targets, thus enabling the design of different *a la carte* treatments which may enable us to improve the morbidity-mortality caused by the various pathologies associated with the metabolic syndrome.

Currriculum Vitae

Director del Dpto. de Medicina Interna, Centro de Investigación Biomédica EuroEspes.

FORMACIÓN ACADÉMICA

Médico Especialista en Medicina Interna.
Clínica Universitaria de Navarra. Facultad de Medicina,
Universidad de Navarra. Marzo 1988 - Marzo 1993.

Doctorado en Fisiopatología Clínica.
Suficiencia Investigadora con calificación de sobresaliente.
Tesis doctoral: *Glutation intralinfocitario y plasmático en pacientes afectos de hepatitis por VHC.* Calificación Apto Cum Laude. Facultad de Medicina. Universidad de Navarra. Pamplona,12 de Junio de 1993.

ACTIVIDAD PROFESIONAL RECIENTE

Médico Residente en el Departamento de Medicina Interna. Clínica Universitaria. Facultad de Medicina. Universidad de Navarra. Marzo 1988 - Marzo 1993.

Profesor Ayudante en la Facultad de Medicina de la Universidad de Navarra. Septiembre 1988- Marzo 1993.

Médico Adjunto de Medicina Interna. IMQ San Rafael, La Coruña. Desde Julio de 1993.

Médico asociado de la Clínica Universitaria de Navarra. Desde Marzo 2000.

Médico responsable de la Unidad de Lípidos del Hospital San Rafael. Desde 2002.

Dr. Valter Lombardi

DIVISIÓN DE BIOTECNOLOGÍA DE LA SALUD, EUROESPES BIOTECNOLOGÍA, CORUÑA

ABSTRACT

Anti-inflammatory properties of AntiGan in an experimental model of colon cancer

Colon cancer is, according to recent estimations, the third most frequent neoplasia worldwide, and accounts for approximately 15% of all new cancers. Its incidence is higher in Western countries and, in Europe, it is the third most frequent neoplasia, after lung and prostate cancer, in men, and the second most frequent neoplasia, after breast cancer, in women. In Spain, the global incidence is 1/10,000 persons. Regarding mortality, in our milieu it accounts for the second or third cause of cancer-related deaths, according to different records. A 50-year-old person presents a 5% risk of suffering a colon cancer, and a 2.5% risk of dying from it before the age of 80. This means that it is currently one of the most significant public health problems in oncology. Before the development of a colon cancer, certain pathological conditions may be observed, including inflammatory bowel disease (IBD). The term "IBD" refers to a recurrent, chronic, idiopathic inflammation of the gastrointestinal tract in which active periods of the disease and periods of remission of the same alternate. A chronic inflammatory process gives rise to tissue damage which leads to parenchyma damage, atrophy, fibrosis and loss of function. In addition to this, there is a greater risk of developing other malignancies, principally adenocarcinoma. The signs and symptoms depend on the extent, distribution and severity of the inflammatory process, and many of these are linked with the anatomical location of the disease. Recent studies have demonstrated the antitumoral properties of AntiGan, an extract obtained from *Conger conger*, both in *in vivo* animal models and in *in vitro* models using different human tumor lines. The aim of this study was to obtain further information regarding the anti-inflammatory properties of AntiGan, both in a chronic inflammation induction model using dextran sodium sulfate (DSS), and in intestinal epithelium cell lines [HTB-37™ ATCC® (Caco-2), HTB-38™ ATCC® (HT29), and CRL-1589™ ATCC® (IEC-18)]. Histological studies using hematoxylin-eosin staining showed less damage in the colon of animals receiving AntiGan, compared with the untreated colitic group (DSS). After five days of incubation with AntiGan, the results of the *in vivo* studies showed a significant reduction in the production of pro-inflammatory proteins and a reduction in lymphocyte activation. The result is a maximization of mucosal defense which, in the final analysis, leads to a reduced global inflammatory response.

Currriculum Vitae

Director del Dpto. de Biotecnología de la Salud, EuroEspes Biotecnología.

FORMACIÓN ACADÉMICA

En 1985 se licencia en Ciencias Biológicas en la Universidad "La Sapienza" de Roma.

En 1988 obtiene el título de Doctor en Ciencias y empieza su actividad científica en la Clínica Pediátrica de la facultad de Medicina de la Universidad "La Sapienza" de Roma.

Desde 1987 hasta finales de 1991 se traslada a Suecia y trabaja en el departamento de Inmunología del Instituto Karolinska de Estocolmo, dirigido por el Profesor Hans Wigzell. Durante estos cuatro años realiza varios proyectos de investigación relacionados con la transmisión del virus VIH.

En 1992 obtiene un Máster en Inmunología defendiendo la tesis: "Mecanismos de transmisión del virus VIH-1 durante el embarazo".

En 1992 obtiene una plaza de Investigador en la Universidad "Tor Vergata". Realiza proyectos de Investigación en el campo de la virología (VIH-1, VIH-2, HTLV-I, HTLV-II).

En 1996 se traslada a España y empieza a trabajar en el Centro de Investigación de Enfermedades del Sistema Nervioso Central EuroEspes de Bergondo, Coruña, ocupando la plaza de Director de la División de Biotecnología.

En estos trece años de actividad realiza numerosos estudios básicos y clínicos relacionados con la enfermedad de Alzheimer, siendo los aspectos inmunológicos involucrados en la progresión de esta enfermedad los principales campos de investigación.

Durante los 25 años de actividad ha publicado muchos artículos en revistas internacionales, ha participado en congresos internacionales en todos los continentes, ha colaborado activamente en la obtención de dos patentes en la empresa en la que actualmente trabaja, y sigue investigando en el área de la enfermedad de Alzheimer y en la identificación de nuevos principios activos a partir de organismos animales y vegetales con posible actividad anti-degenerativa y antitumoral.

En el año 2008 obtiene un Máster en Medicina Natural en la Universidad de Santiago de Compostela con el proyecto: "Propiedades biológicas antitumorales e inmunorreguladoras de extractos alcohólicos de plantas silvestres".

Prof. J Miguel Sempere
UNIVERSIDAD DE ALICANTE, ALICANTE

ABSTRACT

Immunogenetics

Immunogenetics is a branch of medical science in which Immunology and Genetics meet, in order to study the inheritance of the elements which constitute the immune system. In a broad sense, it may be defined as the scientific discipline which undertakes the study of the genetic factors implicated in the functioning and control of the immune response, of the analysis of how changes in some of these genetic factors may give rise to the appearance of different pathologies with a primary or secondary immunological basis, and of the development and fine-tuning of diagnostic and therapeutic techniques derived from this knowledge.

To find the origins of this discipline, we must go back to the year 1900, when Landsteiner described the different blood groups, although at that time blood groups were not yet associated with a genetic basis. The true development of this discipline arose when scientists first put forward the following questions: How does an organism produce an antibody? And how is an antibody able to neutralize a particular antigene in such a specific way? The answer to these two questions, together with the remarkable breakthroughs in Immunology and Genetics achieved during the second half of the 20th century, enabled, among other things, the discovery of how, with a limited number of genes, our organism is able to produce in the region of 1010 to 1012 antibodies with different specifities (the so-called gene shuffling mechanism of immunoglobulins). This information, together with the progress in the knowledge of the major histocompatibility system, the subsequent development of the so-called monoclonal antibodies and the progress in genetic engineering techniques, among which are recombinant DNA techniques, enabled the synthesis of different types of antibodies on an industrial scale, which nowadays form the basis of many immunological and molecular genetic techniques, in daily use in the different fields of Immunogenetics. The publication in 2003 of the detailed sequence of the 3 USbillion nucleotides of the human genome represented the definitive boost for this discipline. By comparative analysis with the genome of other species, it was possible to observe how the human being shares the same genes, not only with other mammalian species, but also with flies, worms and bacteria, thus discovering the genetic unity of living beings. The impact of these discoveries has been vital for the progress of biological sciences, and specifically for medicine and healthcare sciences. Nowadays, thanks to the drafting of complete genetic maps, it has been possible to locate the so-called “immunogenes” within the chromosomes, and this has made an enormous contribution to the detailed knowledge of the immune system. The term “immunogene” is applied to any gene which determines the synthesis of antibodies or of any other element involved in the immune response or in the regulation of said response, such as cytokines, complement system factors, cell membrane receptors or transcription factors.

The importance of Immunogenetics nowadays is founded not only on the role that said scientific discipline plays in the diagnosis and treatment of many immunologically-based diseases, such as primary/secondary immunodeficiencies and autoimmune diseases, but also on the fundamental role which it continues to play in the various typing protocols in individuals about to undergo transplantation, as it enables the determination of the degree of compatibility before the transplant, thus optimizing resources and results; or its proven importance in the development of the so-called “biological drugs” and their use in the treatment of certain autoimmune diseases or different types of cancer.

Its close relationship with other disciplines of relatively recent appearance, i.e. Pharmacogenetics or Pharmacogenomics, has likewise enabled the development of kits for the early diagnosis of diseases, or of kits capable of determining the degree of response or toxicity of an individual regarding a particular drug. In other words, immunogenetics enables predetermination of the clinical history of the individual, calculating the probability of his/her contracting a hereditary immunological disease in the near future, or revealing those drugs to which the individual will respond better, and will suffer less toxicity, when faced by a particular disease. All of these strengthen the pillars of the so-called “personalized medicine”, so much in vogue currently, which will without doubt govern healthcare in developed countries in the not-too-distant future. Its relationship with other, likewise recent, disciplines, such as Criminology, provides the exact knowledge, for example, of who are the parents of an individual, a practice often used by the Law for paternity testing or for the identification of bodies in the event of a catastrophe.

All things considered, we may state categorically that immunogenetics is nowadays a pivotal tool in the field of Biomedicine, closely related with other disciplines and whose development continues to progress.

Currriculum Vitae

Licenciado en Medicina y Cirugía por la Universidad de Alicante (1979-1985)
Grado de Licenciatura por la Universidad de Alicante, con la calificación de sobresaliente por unanimidad (1987).
Medicina Clínica en distintos ambulatorios, servicios de urgencia y hospitales de (1985-1989).
Médico Especialista en Inmunología por la via MIR. Hospital General Universitario Gregorio Marañón, Madrid (1989-1993).
Cursos de Doctorado y Suficiencia Investigadora en la Universidad Complutense de Madrid.
Becario FIS en el Laboratorio de Biología Celular y Molecular del Cancer del Hospital General Universitario de Elche (1994-1995)
Doctor en Medicina por la Universidad de Alicante, con la calificación de Apto Cum Laude por unanimidad (1996).
Director Científico del Grupo de Empresas ASAC Pharmaceutical International A.I.E, hasta finales del año 2002. Responsable directo de la puesta en marcha, Desarrollo y Gestión de diversos proyectos de investigación Empresa/Universidad, Empresa/Hospitales, Empresa/Centros Públicos de Investigación, tanto en España como en el extranjero, todos ellos financiados y/o subvencionados por el Ministerio de Ciencia y Tecnología Español, por el IMPIVA (Instituto de Investigación para la Mediana y Pequeña Empresa Valenciana), por el CDTI (Centro para el Desarrollo Tecnológico Industrial) o por fondos europeos (Programa Marco). Las lineas de investigación en la empresa se centraron en la busqueda de nuevos fármacos inmunomoduladores (fitofármacos), desarrollo y gestión de ensayos clínicos en fases II, III y IV para el tratamiento de enfermedades neurodegenerativas y autoinmunes, desarrollo de vacunas alergénicas hiposensibilizantes, y desarrollo y gestión de diagnósticos rápidos de un solo paso (diagnóstico PSA para el cancer de próstata).
Profesor Asociado (6 horas) de la Universidad de Alicante desde el año 1995.
Actualmente, Profesor Titular de Universidad en el Departamento de Biotecnología de la Universidad de Alicante, siendo el Responsable del Area de Conocimiento de Inmunología y Director del Grupo de Investigación de Inmunología de la Universidad de Alicante.
Investigador Principal de numerosos proyectos financiados por Organismos Públicos (Ministerio de Sanidad, Ministerio de Educación, Consellería de Sanidad, Consellería de Educación)y Privados, en el ámbito de la Inmunología, que se han traducido en diversas publicaciones y comunicaciones a Congresos nacionales e internacionales.
Ponente invitado en congresos nacionales e internacionales y Moderador de numerosas mesas redondas en el ámbito de la Inmunología y de la Industria.
Autor de varias patentes
Director de varias Tesis Doctorales
Director, coordinador y/o gestor de numerosos cursos docentes y diversas líneas dentro de Programas de Doctorado y Máster.

Dr. Juan C Carril

DEPARTAMENTO DE GENÓMICA, EUROESPES BIOTECNOLOGÍA, CORUÑA

ABSTRACT

Characterization of the cerebrovascular risk genetic profile

The cerebrovascular accident (CVA), ictus or cerebral infarct consists of a permanent or transitory disturbance of cerebral function which arises as a consequence of a circulatory disorder, either of the cerebral vessels or of hematic disturbances. As occurs in other complex diseases, its prevalence varies in different countries and is connected with genetic factors, the age of the population and other associated environmental factors. The incidence of new cases in Spain is in the region of 156 per 100,000 inhabitants, although it may be assumed that the true figure is nearer 200 cases per 100,000 inhabitants.

There are very few data regarding the prevalence of ictus in Spain, expressing frequencies that vary between 2.1% of the population over 20 years of age and 8.5% of the population over 65, depending on the study consulted. In Spain, ictus-related mortality varies between 10% and 34% in hospital statistics, being much higher in cases of cerebral hemorrhage.

The definition of the genetic cardiovascular risk panel entails undertaking the study of genes implicated in the different events which trigger the atherogenic process, these being lipid metabolism, endothelial function, immune response and atheroma plaque stability (atherothrombosis). Validation of the genetic panel, that is, the determination that the differences between affected individuals and controls are causal and not spurious, likewise the choice of the appropriate statistical model, are key for the obtaining of a useful predictive tool in medical practice.

A total of 20 polymorphic variants in 15 genes related with the atherogenic process were analyzed in a populational sample of 483 individuals over the age of 50, of whom 310 presented clinical conditions with associated vascular pathologies: vascular dementia (N=147), vascular encephalopathy (N=67), ictus (N=67), vascular migraine (N=18) and cerebrovascular insufficiency (N=11). The 173 individuals without associated vascular pathology comprised healthy controls (N=111) and Alzheimer patients (N=62).

Comparisons between the different groups of patients and the healthy controls highlighted a clear correlation of obesity and hypertension as risk factors for cerebrovascular complications, although we did not detect this association with cholesterol and triglyceride levels in the sample analyzed.

With regard to the usefulness of the genetic markers selected for the characterization of cerebrovascular risk, we established three levels of risk characterization: High risk, with Odds Ratio (OR) values of over 2; Moderate risk, with values in the range 1.2<OR<2; and Low risk, with OR<1.2. In this way, we may underscore the importance of APOE*2 alleles (OR=2.37), both homozygous and heterozygous, and IL6*-573G (OR=2.21) as risk factors.

If we group together the polymorphisms analyzed according to the atherogenic process in which they intervene, and we estimate the information capacity of the risk expressed as relative risk (RR), it may be concluded that the genetic panel which accumulates the greatest genetic risk load is that which encompasses the pro-inflammatory cytokines (immune response panel), with an accumulated RR of over 200%, followed by the lipid metabolism panel, with an accumulated risk of around 50%. Finally, the polymorphisms clustered in the endothelial function and thrombosis panels explain the negative genetic load, accumulating RR values of over 15-20%.

The use of genetic susceptibility panels is not only profitable due to the predictive capacity of the markers of which they are formed, but also due to their capacity of weighing up the specific burden of the different pathogenic processes which intervene in the appearance of the disease, thus contributing to the personalization of the treatment which must be initiated should the disease develop.

Currriculum Vitae

Director del Dpto. de Genómica, EuroEspes Biotecnología.

FORMACIÓN ACADÉMICA

En 1994 se licencia en Ciencias Biológicas (Especialidad Biología Fundamental) en la Universidad de Santiago de Compostela (USC).

En 1998 realiza una estancia como investigador pre-doctoral en el departamento de Genética de la Universidad de Leicester (Reino Unido) donde se centra en el estudio de la evolución y migraciones de poblaciones humanas europeas en el proyecto: "Analysis of Y-chromosome polymorphisms in European populations".

En 2000 obtiene el título de Doctor en Ciencias Biológicas "cum laude", en el departamento de Antropología de la USC, defendiendo la tesis: "Estructura y perfil genético de poblaciones de la Península Ibérica mediante la aplicación de marcadores (STRs y SNPs) del cromosoma Y humano".

Entre 1999 y 2002 es contratado como investigador post-doctoral por la USC y Pharmacia Biotech para desarrollar nuevas aplicaciones de biología molecular aplicadas a la genética de poblaciones en el proyecto "Optimización de técnicas fluorométricas de análisis de polimorfismos de DNA en multiplex: Aplicabilidad en sistemas automatizados ALFExpress II".

Entre 2001 y 2002 realiza una estancia post-doctoral en el Instituto de Medicina Legal de Oporto colaborando con la USC en el proyecto "Análisis de Polimorfismos de DNA en Poblaciones de Galicia y Norte de Portugal".

Entre 2002 y 2006 desarrolla su labor profesional en la empresa biotecnológica Genómica, S.A.U. en Madrid, donde se hace cargo de las áreas de Genética Forense y Transferencia Tecnológica. Como responsable del área de Genética Forense elabora más de 1000 informes periciales de Huella Genética y Relaciones de Parentesco, muchos de ellos como perito judicial, así como más de 10.000 análisis de Huella Genética y Parentesco en Genética Veterinaria. Como responsable del área de Transferencia Tecnológica se incorpora a la fase de capacitación de personal y últimas fases del proyecto de Laboratorio de ADN de la Policía Judicial de El Salvador; en el desarrollo del laboratorio de ADN para la Policía de Panamá; así como en la Asistencia Técnica en el Servicio de Biología de la Dirección General de la Guardia Civil.

Desde 2006 desarrolla su labor científica y profesional en EuroEspes Biotecnología, S.A., en Bergondo, A Coruña, como Director del Departamento de Genética de Identificación Humana. En este tiempo, además de los análisis de Huella Genética y Parentesco, es el responsable de los análisis de Genética Prenatal y Genética de Riesgo Cerebrovascular. En cuanto a su labor investigadora trabaja en la búsqueda de marcadores genéticos de riesgo de patologías cerebrovasculares.

En estos 15 años de actividad ha realizado más de 30 publicaciones científicas en los campos de la Genética de Poblaciones, Genética Forense y Epidemiología Genética, así como más de 30 comunicaciones a congresos nacionales e internacionales.

Además, es miembro de varias sociedades científicas entre las que cabe destacar: la International Society of Forensic Genetics (ISFG), el Grupo Español y Portugués de la ISFG (GEP-ISFG), la European Society of Human Genetics (ESHG), la Sociedad Española de Genética (SEG), la Asociación Española de Genética Humana (AEGH) y la World Association of Genomic Medicine (WAGEM).

Prof. Dr. **Ramón Cacabelos**

D. **Javier Sánchez**

WORLD ASSOCIATION OF GENOMIC MEDICINE AND EUROESPES PUBLISHING

ABSTRACT

Introduction to the World Guide of Pharmacogenomics

The World Guide for Drug Use and Pharmacogenomics (WGPGx) is a multidisciplinary, systematic exercise to put in order a myriad of data organized in selected databases and thousands of reports scattered throughout the international literature on genetics, genomics, pharmacology, drug metabolism, therapeutics, and pharmacogenomics, to provide the reader (physicians, geneticists, pharmacists, researchers, health professionals, regulators, etc.) with a body of practical information which is not available in other publications as a whole.

The Guide is divided into 5 main parts: (i) Drugs, (ii) Genes, (iii) References, (iv) Appendix, and (v) Index.

The section of Drugs includes 1,395 drugs classified in alphabetical order from A to Z. Each entry contains the following headings: Drug Name; Brand Names [in 27 European countries (Austria, Belgium, Bulgaria, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, UK), North America (Canada and USA), Latin America (Argentina, Brazil, Mexico), and Asia (Japan)]; Drug Combinations; Chemistry; Pharmacological Category; Mechanism of Action; Therapeutic Use; Unlabeled Use; Pregnancy and Lactation Implications; Contraindications; Warnings and Precautions; Adverse Reactions (cardiovascular, central nervous system, dermatologic, endocrine and metabolic, gastrointestinal, genitourinary, hematologic, hepatic, local, neuromuscular and skeletal, renal, respiratory, miscellaneous); Pharmacogenetics (genotypes of risk, genes involved in drug metabolism, condition of substrate, inhibitor or inducer, when appropriate); Drug Interactions; Nutrition/Nutraceutical Interactions; Dosage; Pharmacokinetics and Pharmacodynamics (absorption, distribution, protein binding, metabolism, bioavailability, half-life, time to peak, excretion); Special Considerations (diet, age, sex, monitoring parameters).

The section of Genes includes 447 genes of relevance in pharmacogenomics. Each entry contains the following headings: Gene Name; Alternative Names; Alternative Symbols; Locus; Codes (OMIM, PharmGKB); Gene Structure; RNA; Protein; Function; Related Diseases (list of diseases investigated; in **bold face** those diseases potentially associated); Related Drugs (list of drugs investigated; when appropriate, major substrates, minor substrates, strong inhibitors, moderate inhibitors, weak inhibitors, and inducers); Animal Models; Allelic Variants; Selected SNPs; Evolution; Genomics and Pharmacogenomics (description of relevant findings in genomics and pharmacogenomics according to the international literature); Drug-Gene Interactions.

The section of References includes two categories: (i) Websites and International Databases, and (ii) 17,947 References alphabetically classified by author and by gene. The List of References is included in the attached CD-ROM. Due to the length of said list of references, it has been omitted in the book. The Guide's authors have respected the data and opinions of the referenced original authors where contradictory results on drugs or genes are reported. Data included in the WGPGx have been strictly extracted from the referenced authors, avoiding personal interpretations. Approximately 100,000 references have been reviewed, of which 17,947 were finally selected for this edition.

The Appendix includes 4 sub-sections: (i) Classification of Drugs, (ii) Genes-Diseases (a list of genes associated with specific diseases), (iii) Diseases-Genes (a list of diseases associated with specific genes), and (iv) Pharmacogenomic synopsis (a list of internationally-used drugs with those genes potentially involved in the metabolism of each drug).

The Index contains approximately 52,000 entries divided into 5 sections: (i) Drugs (approx. 7,750 entries), (ii) Brand names (approx. 31,750 entries), (iii) Pharmacological categories (1,891 entries, (iv) Genes (approx. 4,450 entries), and (v) Diseases (approx. 9,200 entries).

The ***World Guide for Drug Use and Pharmacogenomics***, is presented in 3 formats: Book (over 3,000 pages), CD-ROM, and the EuroPharmaGenics Database for worldwide distribution and accessibility

Currriculum Vitae

Prof. Dr. Ramón Cacabelos

Licenciado en Medicina y Cirugía, Facultad de Medicina, Universidad de Oviedo (1980).

Doctor en Medicina y Cirugía, Facultad de Medicina, Universidad de Santiago de Compostela (1985).

Doctor en Ciencias Médicas/Medicina Interna/Psiquiatría. Especialista en Psiquiatría, Facultad de Medicina, Universidad de Osaka, Japón (1987).

Instructor Científico de Postgrado, Osaka University Medical School, Osaka, Japón (1985-1987). Jefe del Laboratorio de Psiconeuroendocrinología y Neurocibernética, Departamento de Psiquiatría, Facultad de Medicina, Universidad de Osaka (1983-1987).

Profesor Titular Asociado B-1, Departamento de Psiquiatría, Facultad de Medicina, Universidad de Santiago de Compostela (1987-1989).

Profesor Visitante, Departamento de Psiquiatría, Universidad de Navarra (1990).

Profesor Visitante, Departamento de Patología, New York University Medical Center, New York, EEUU (1991).

Profesor Titular Numerario, Departamento de Fisiología Humana, Facultad de Medicina, Universidad Complutense, Madrid (1989-1995).

Máster Internacional en Alta Dirección Hospitalaria, Escuela Internacional de Alta Dirección Hospitalaria, Madrid (1996).

Presidente de EuroEspes. Director del Instituto para Enfermedades del Sistema Nervioso Central, La Coruña (1991-).

Presidente de la Fundación EuroEspes (1992-2004).

Presidente de la Asociación Española de Neurogerontología y Neurogeriatría (1994-2005).

Presidente de Sociedad Española de Medicina Genómica (2005-).

Presidente de la World Association of Genomic Medicine (2008-).

Director General del Centro de Investigación Biomédica EuroEspes (CIBE) (1995-).

Presidente de Ebiotec (EuroEspes Biotecnología, S.A.) (2001-).

Director de la Cátedra EuroEspes de Biotecnología y Genómica, Universidad Camilo José Cela, Madrid (2003-).

Currriculum Vitae

Javier Sánchez

Diplomado en C.C. Empresariales (2001)

Licenciado en Administración y Dirección de Empresas (2003)

MBA (Máster en Administración y Dirección de Empresas) (2004)

Postgrado en Control de Gestión y Planificación Estratégica (2009)

En el año 2004 trabaja como Adjunto a Gerencia de Hipermercado en una Empresa de Distribución Minorista. En enero de 2005 pasa a ser Gerente de Hipermercado.

Entre febrero de 2006 y junio de 2007 es Director Gerente y Financiero de una Industria Alimentaria.

De junio de 2007 a julio de 2008 trabaja como Director del Negocio de Distribución de un Grupo Holding.

Entre julio de 2008 y septiembre de 2009 es Director Financiero y de Organización de una Empresa de Telecomunicaciones.

En octubre de 2009 se incorpora en el Grupo EuroEspes con el cargo de Director Financiero. Es responsable de las Finanzas Corporativas del Grupo. Asimismo es responsable de la preparación de la Compañía para su salida a Bolsa.

Prof. Gjumrakch Aliev
UNIVERSITY OF ATLANTA, USA

ABSTRACT

Oxidative Stress in Neurodegeneration and Cancer

Free radical-induced oxidative damage of the organ and/or tissues, especially mitochondrial dysfunctions, have been implicated in the pathogenesis of several diseases, including neurodegeneration, such as Alzheimer disease (AD), tumor growth, and metastasis. A decline in mitochondrial function may lead to cellular energy defects, which will compromise vital cellular components and regulators. Neurodegeneration, tumor growth, and metastases characterize tissue oxygen deficiency. Overexpression of enzymes such as NOS induces the production of an unwanted large amount of free radicals which cause oxidative stress, cellular changes and, particularly, concomitant mitochondrial lesions and decline in normal organ function. The proposed study used animal models that mimic human neurodegeneration and human colorectal carcinoid cancer or malignant brain cancer to determine if an intimate, i.e. causal, relationship between oxidative stress, mitochondrial damage and/or vascular lesions occurs before the development of human AD.

In situ hybridization and ultrastructural analysis of the mitochondria (mitochondria with electron-dense matrix, mitochondrial-derived lysosomes) showed that abnormal mitochondria and lipofuscin appear to be features of damaged hippocampal neurons in human AD, aged Tg (+) mice, and malignant (primary and metastatic) cancer. The abnormal mitochondria appeared to be a permanent feature in all cellular compartments; *in situ* hybridization analysis with mouse and human mtDNA probes found a large amount of deleted mtDNA in human AD and in all models that mimic human AD (mice, rats etc.) hippocampus and cancer tissues, compared to aged controls. The majority of these mtDNA deletions were found in mitochondrial-derived lysosomes in regions closely associated with lipofuscin and/or tumor growth regions, and suggest that proliferation, deletion, and duplication of mtDNA occurs in mitochondria, many of which have been fused with lysosomes in human AD, Tg(+) mice, and malignant tumors. Moreover, the biopsy samples from AD and cancer patients were dominated by abnormal mitochondria as compared to a control group. *In situ* hybridization with a chimeric cDNA probe for the 5kb common deletion indicated that the 5kb mtDNA is increased at least 3- and 4-fold respectively in AD and malignant tumor cases as compared to controls. In quantitative analysis of the mtDNA deletion and 8OHG in the same cases, we found a strong positive correlation (r=0.934). Only hippocampal and cortical vulnerable neurons, as well as malignant cancer tissues, showed immunopositive staining for 8-OHG, NOSs, and all oxidative stress markers. This observation indicates that the oxidative stress marker seen in the AD brain and malignant cancer selectively affects the population of vulnerable neurons, vascular EC, and perivascular cells, suggesting that oxidative stress induced mitochondrial DNA overproliferation and/or deletion plays a key role in the pathogenesis of AD and cancer. The mitochondrial DNA overproliferation and deletion detected by using cytological techniques suggests that successful dysregulation of the cell cycle is also the hallmark of neoplasm; early mitochondrial-dependent cell-cycle pathophysiology in AD may recruit oncogenic signal transduction mechanisms and hence, can be viewed as an abortive neoplastic transformation. Common features of mitochondrial abnormality were seen in the brain during tumorigenesis and AD, indicating that mitochondrial DNA overproliferation and/or deletion are the key initiating factors for development, maturation, and progression of neurodegeneration as well as tumor growth and/or metastases. Our study, for the first time, demonstrated the pattern of oxidative stress marker activity during the development of human AD, in animals that mimic human AD, colorectal cancer in liver metastasis, malignant brain cancers, and the features of mitochondrial DNA overproliferation and/or deletion as well as mitochondrial enzyme activities in these conditions. Therefore, mitochondrial lesions, especially mitochondrial DNA abnormalities, are responsible for cell viability, and can be used as new diagnostic tools and/or criteria for the earlier detection of diseases, and will open new windows for a better understanding and treatment strategies in these conditions.

Acknowledgements: I am very grateful to Mr. Russell Pool and Ms. Galina Alieva for their critiques and editorial work throughout the preparation of this paper. This study was supported by "GALLY" International Biomedical Research Institute Inc., San Antonio, TX, USA.

Currriculum Vitae

Gjumrakch Aliev, M.D, Ph.D., the internationally recognized founder of Gally International Biomedical Research Institute Inc., has more than a decade of research in the area of gerontology. He is internationally recognized for his expertise in vascular and mitochondrial factors in the pathogenesis of gerontology research fields, especially in atherosclerosis, ischemia/reperfusion, stroke, AD and in the models representing these conditions.

In 1982 Gjumrakch Aliev graduated summa cum laude from the Azerbaijan Medical Institute in Baku, Azerbaijan, receiving an M.D. degree in the fields of general medicine and health sciences. In 1989, he received his PhD (Summa Cum Laude) in the field of Cardiovascular Biology and Pathology from the Ivanovo Medical Institute, Department of Electron Microscopy and Cell Pathology of the Institute of Human Morphology, Moscow State University, and Russian Cardiology Research Center, Moscow, Russia.

He received postdoctoral training under the prestigious British Heart Foundation Grant Program at University College, London (advisor Professor Geoffrey Burnstock, FRS) in the specialty of Neurosciences, Cardiology and Medicine. Professor Aliev has developed research and educational programs in neuroscience, neurodegeneration, mitochondrial, cardiovascular and cerebrovascular pathology, anatomy, histology, cancer, electron microscopy and other areas. Additionally, he has extensive experience managing dynamic academic research and teaching environments.

Dr. Aliev has developed unique technologies and treatment protocols for age-associated diseases. In addition, Dr. Aliev was the first to propose the consideration of energy crisis as a main driving force for the acceleration of aging. He has authored more than 180 peer-reviewed published journal articles and book chapters and more than 170 scientific abstracts of conference presentations in the fields of neurodegenerative disease research (Alzheimer's disease), as well as cardio- and cerebrovascular disease, cancer, and electron microscopy. He also holds several patents and rationalizations. Currently, Dr. Aliev is the Project Director and lead investigator of an international scientific project concerning the study of the role of cellular hypoperfusion-induced oxidative stress and mitochondrial failure in the pathogenesis of cerebral athero- and arteriosclerosis and AD, which shows promise in offering not only a deeper understanding of the underlying mechanisms of AD, Parkinson's disease, stroke and other brain diseases, but also new and more effective treatment for these devastating diseases.

Dr. Aliev has extensive expertise in all of the various aspects of microscopic analysis such as experimental design, data analysis, and manuscript/grant writing for both biological and non-biological studies. With many years of experience in LM, EM, 2-photon microscopy, Atomic Force and Confocal Microscopy, he has produced pioneering work in different areas of EM, including new methods of EM techniques. These techniques include cytological *in situ* hybridization at the light and electron microscopic levels using non-isotopic colloidal gold probes, peroxidase-anti-peroxidase (PAP), and pre- and post-embedding single-, double- and triple-immunogold cytochemistry and quantification.

Dr. Aliev serves as an editor or an editorial board member for many journals. He has served and is currently serving as a grant review board member and reviewer for international granting agencies and foundations.

Desarrollo de la Vacuna EuroEspes EB101

Introducción

La enfermedad de Alzheimer (EA) es la forma más frecuente de demencia en los países desarrollados, con una prevalencia de alrededor del 1% a la edad de 65 años y más del 25% en personas mayores de 85 años. Clínicamente, se caracteriza por un progresivo deterioro cognitivo, trastornos conductuales y deterioro funcional. La EA es un trastorno poligénico-complejo en el que podrían participar cientos de genes, distribuidos por el genoma humano, en estrecha cooperación con inductores ambientales, disfunción cerebrovascular y fenómenos epigenéticos [1]. Los principales sellos neuropatológicos de EA incluyen acumulación de péptido amiloide-β (Aβ); ovillos neurofibrilares (NFT) compuestos principalmente por filamentos helicoidales aparejados con proteínas tau hiperfosforiladas; pérdida neuronal y sináptica [2]; reducción del volumen cerebral total, con daños específicos de la corteza entorrinal del hipocampo [3]; neuroinflamación; gliosis; formación de radicales libres [4]; déficit de neurotransmisor y factores neurotróficos; deterioro metabólico [5]; y desregulación de proteosomas y chaperonas [6]. En la última década, numerosos estudios inmunoterapéuticos han demostrado que algunas

contra la enfermedad de Alzheimer

Iván Carrera[1], Ignacio Etcheverría[1], Lucía Fernández-Novoa[1], Valter Lombardi[1], Carmen Vigo[3], Ramón Cacabelos[1,2]

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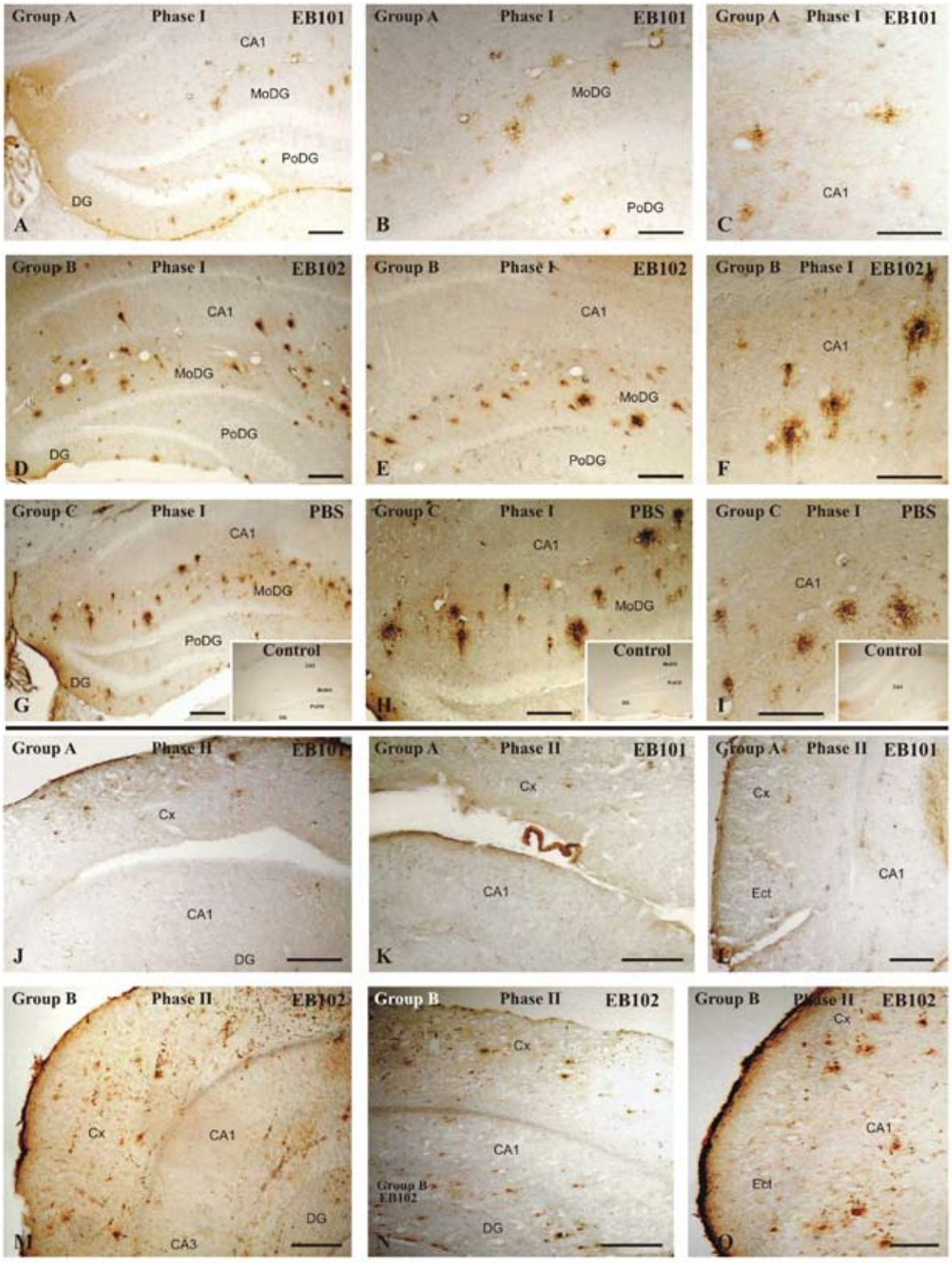
formas de péptidos Aβ agregados desempeñan un papel importante en la patogenia de esta enfermedad neurodegenerativa [7-9]. Estos resultados llevaron a estrategias de inmunización terapéutica experimental y más tarde a los ensayos clínicos orientados a reducir el exceso perjudicial de Aβ cerebral. Ratones transgénicos que expresan las formas mutadas del gen de la proteína precursora amiloidea humana (hAPP) muestran un prematuro incremento en los niveles de proteínas Aβ y su acúmulo en forma de depósitos en la corteza cerebral y en el hipocampo [10-12], desarrollando características neuropatológicas similares a las observadas en el cerebro de los pacientes con la enfermedad de Alzheimer [13]. Ratones transgénicos con la mutación en el gen de la presenilina-1 (PS1) también muestran un aumento en la generación de péptidos $A\beta_{42}$, potenciando su depósito en el cerebro a los 6 meses de edad [14]. En nuestros estudios, hemos utilizado ratones transgénicos con doble mutación, derivados de la co-expresión de los genes mutados de APP y PS1, ya que demuestran una aceleración en el proceso de acumulación de depósitos Aβ en el cerebro en comparación con los modelos animales con mutación simple [15-19]. Estos ratones transgénicos dobles se han utilizado »

Figura 1. Efecto de la vacuna EB101 en los depósitos beta-amiloide (Aβ) en cerebro de ratones B6C3F1/J. Imágenes comparativas de la inmunoreactividad frente a Aβ en el hipocampo (A-K,M,N) y regiones corticales (L,O) del cerebro de ratones transgénicos inmunizados con la vacuna EB101 (Grupo A). Fase preventiva (Figuras A-I), antes de la formación de las placas de Aβ, y fase terapéutica (Figuras J-O), después de la neurodegeneración amiloidea. Se observa la notable reducción de placas Aβ en las secciones cerebrales del grupo A en comparación a los demás grupos experimentales. Barra de calibrado: 100 µm.



en numerosos estudios para investigar terapias emergentes con el fin de prevenir o tratar las características neuropatológicas de la enfermedad de Alzheimer.

La inmunoterapia basada en Aβ ha demostrado ser el más prometedor enfoque terapéutico [20-25]. Hay más de 10.000 pacientes actualmente implicados en tratamientos de inmunoterapia Aβ activa o pasiva, que hasta ahora muestran un cierto grado de éxito en la disminución de beta amiloide [26-30] y de déficits de memoria [9,31,32]. Sin embargo, el primer ensayo clínico en 2001 por Elan y Wyeth con inmunización activa, consistente en agregados peptídica sintéticos de $A\beta_{42}$ con el adyuvante QS21, resultó en la muerte del 6% de los pacientes debido a una reacción meningoencefalítica [26,33], probablemente provocada por una extrema respuesta autoinmune mediada por células-T [34,35]. Sorprendentemente, los pacientes con un tratamiento abreviado generaron niveles satisfactorios de anticuerpos anti-Aβ, una leve reducción de los niveles de proteína tau cerebroespinal y un declive cognitivo más lento [36,37]. Apoyándonos en todos estos estudios hemos desarrollado un novedoso inmunógeno diseñado para reducir la deposición de Aβ evitando la activación masiva de respuesta inmune medida por células T, que potencialmente causaron los efectos adversos observados en la vacuna de Wyeth/Elan. Nuestra vacuna pretende tener un efecto de larga duración, dirigido principalmente a la reducción de la carga Aβ en núcleos hipocámpicos, generando una producción de anticuerpos anti-Aβ robusta, previniendo o frenando el deterioro cognitivo y reduciendo la formación de los ovillos neurofibrilares asociados. Esta nueva vacuna también favorece la regeneración de las neuronas dañadas y estimula una respuesta inmune de tipo Th2 antiinflamatoria. Todos estos requisitos se han logrado en esta vacuna mediante el diseño de un adyuvante compuesto por fosfolípidos naturales, probando ser seguro y eficaz en otro tipo de vacunas como la gripe, y con un fosfolípido añadido, S1P, biológicamente activo, fundamental para estimular una reacción antiinflamatoria y actuar como agente de regeneración neuronal. La inclusión del componente S1P fue crucial para el éxito de los resultados obtenidos, ya que

Figura 2. **Cuantificación de placas Aβ en cerebro y efecto psicomotriz de ratones B6C3F1/J.**

Gráfico A: Estimación media de placas Aβ en el hipocampo y regiones corticales presente en los tres grupos de tratamiento (EB101, EB102 y PBS). Véase la notable reducción de placas Aβ en el grupo A (EB101) en comparación con los demás grupos experimentales, grupo B (EB102) y grupo C (PBS), tanto en fase preventiva como terapéutica.

Gráfico B: Análisis cuantitativo del área ocupada por las placas Aβ en el hipocampo y regiones corticales de ratones transgénicos tratados con EB101, EB102 o PBS, siendo dichas áreas representadas por el número de pixeles en cada placa Aβ.

Gráfico C: Representación de los resultados psicomotores y comportamentales obtenidos del test Rota-rod en las fases preventiva y terapéutica. Se observa un índice de coordinación motora normal en los modelos vacunados con EB101 a diferencia de los demás grupos experimentales.

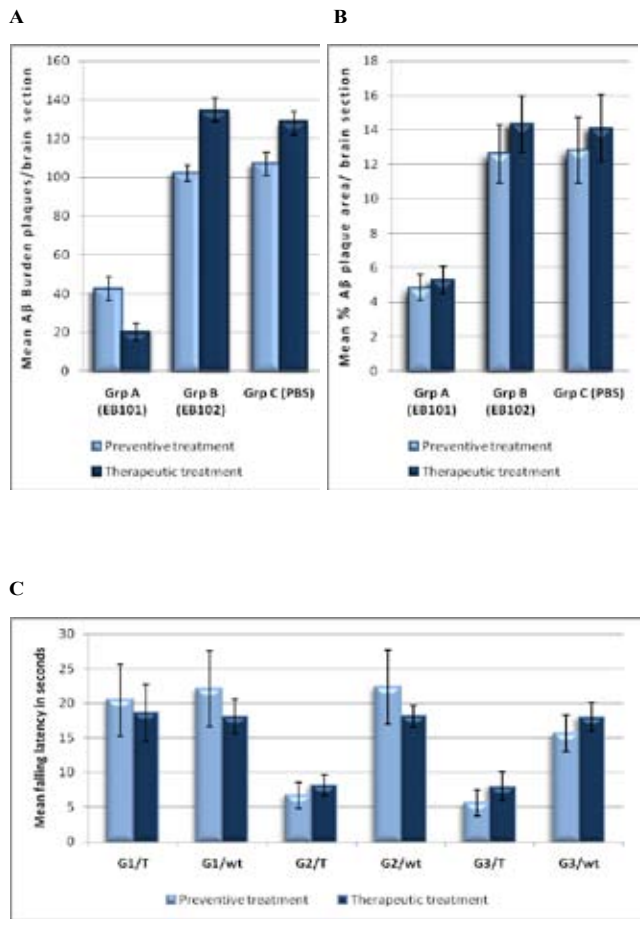
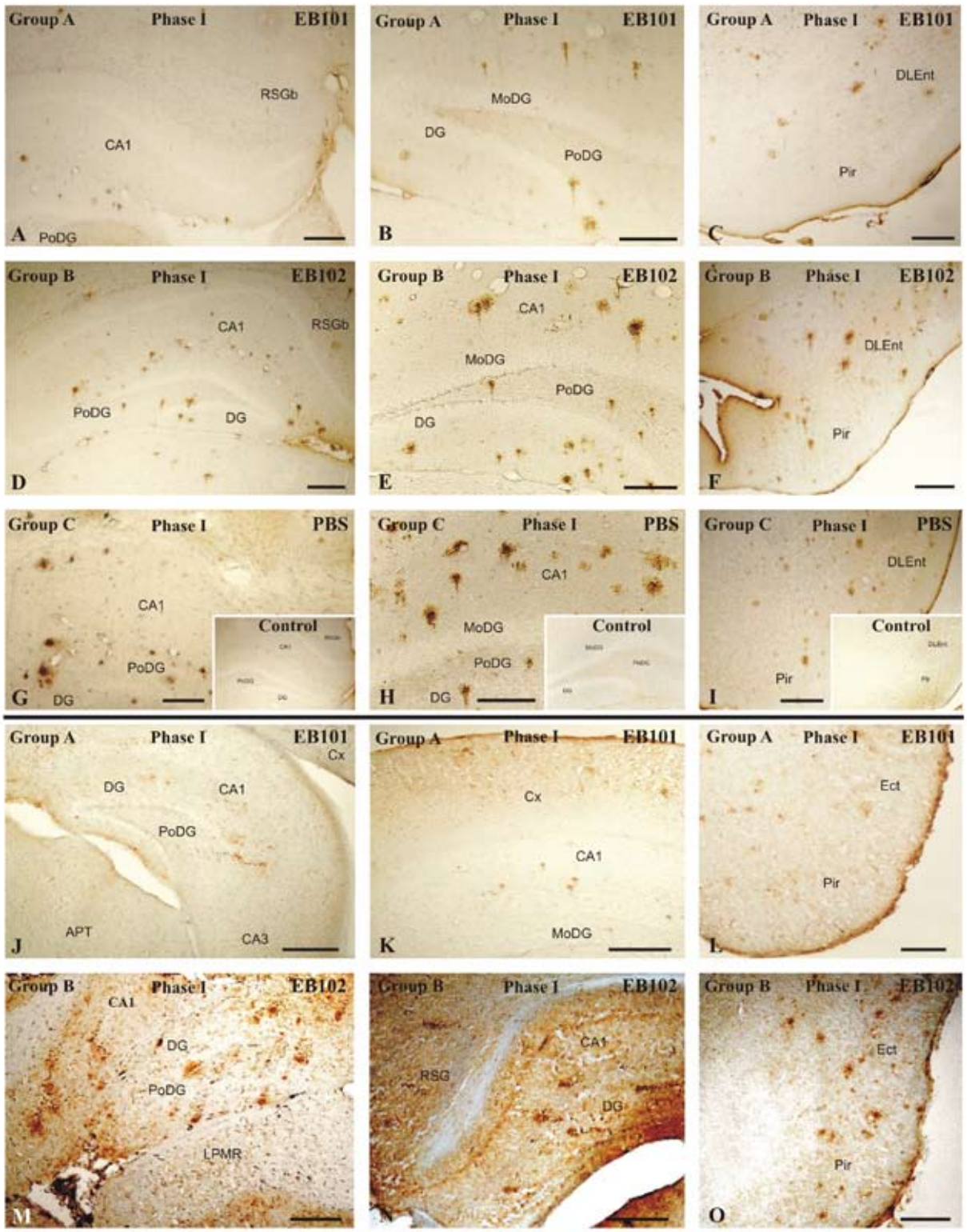


Figura 3. Efecto de la vacuna EB101 en los ovillos neurofibrilares (NFT) presentes en el cerebro de ratones B6C3F1/J. Imágenes comparativas de la inmunoreactividad frente a NFT en el hipocampo (A,B,D,E,G,H,J,K,M,N) y regiones corticales (C,F,I,L,O) del cerebro de ratones transgénicos durante la fase preventiva (Figuras A-I) y terapéutica (Figuras J-O). Véase la notable reducción de ovillos neurofibrilares en el grupo A, tratados con la vacuna EB101 durante las dos fases.
Barra de calibrado: 100 µm.



está presente en el plasma sanguíneo y es uno de los factores de crecimiento celular más potentes con propiedades pro-angiogénicas [38]. En diversos estudios el S1P ha demostrado actuar como un mensajero intracelular y extracelular en el sistema nervioso [39] para controlar la proliferación, supervivencia, diferenciación y la prevención de la apoptosis en células neuronales, regulando así las señales nerviosas y su función [40,41]. S1P también controla la migración de las células madre neuronales hacia el sitio de la lesión en la médula espinal lesionada, sugiriendo con ello que el S1P tiene un enorme potencial terapéutico como agente regenerativo en el sistema nervioso central [42].

Diseño Experimental

En este estudio se utilizaron ratones transgénicos con doble mutación, B6C3F1/J del Jackson Laboratory de EEUU, expresando una proteína amiloide precursora quimérica ratón/humanas (Mo/HuAPP695swe) y presenilina humana 1 (PS1-dE9), ambas dirigidas a las neuronas del sistema nervioso central. Se realizaron dos estudios experimentales; un estudio del efecto profiláctico de la vacuna (antes del comienzo de la patología en ratones, tratamiento preventivo) y un segundo estudio paliativo de los signos de la enfermedad tras su aparición en ratones de 35 semanas de edad (una vez desarrolladas las patologías neuronales, tratamiento terapéutico). En cada estudio los ratones B6C3-Tg (APPswe, PSEN1dE9) 85Dbo/J fueron divididos en tres grupos de tratamiento: Grupo A formado por ratones inmunizados por vía intraperitoneal con un cóctel sintético humano Aβ42/S1P/liposoma (EB101); Grupo B, formado por ratones inmunizados sólo con liposomas (EB102); y el grupo C, formado por ratones inoculados con PBS (suero salino). El mismo procedimiento experimental se aplicó posteriormente al tratamiento terapéutico. Los ratones fueron inmunizados con inyecciones durante siete meses, inoculando un volumen de 100 µL por inyección que contienen un total de 100 g de Aβ y 1mg de

fosfolípidos. Al final del tratamiento preventivo los ratones tenían 11 meses de edad y 18 meses de edad al final del tratamiento terapéutico. Todos los procedimientos experimentales están en conformidad a las directrices establecidas por la Directiva del Consejo de las Comunidades Europeas (86/609/CEE) y por el Real Decreto 1201/2005 para la experimentación con animales.

Resultados

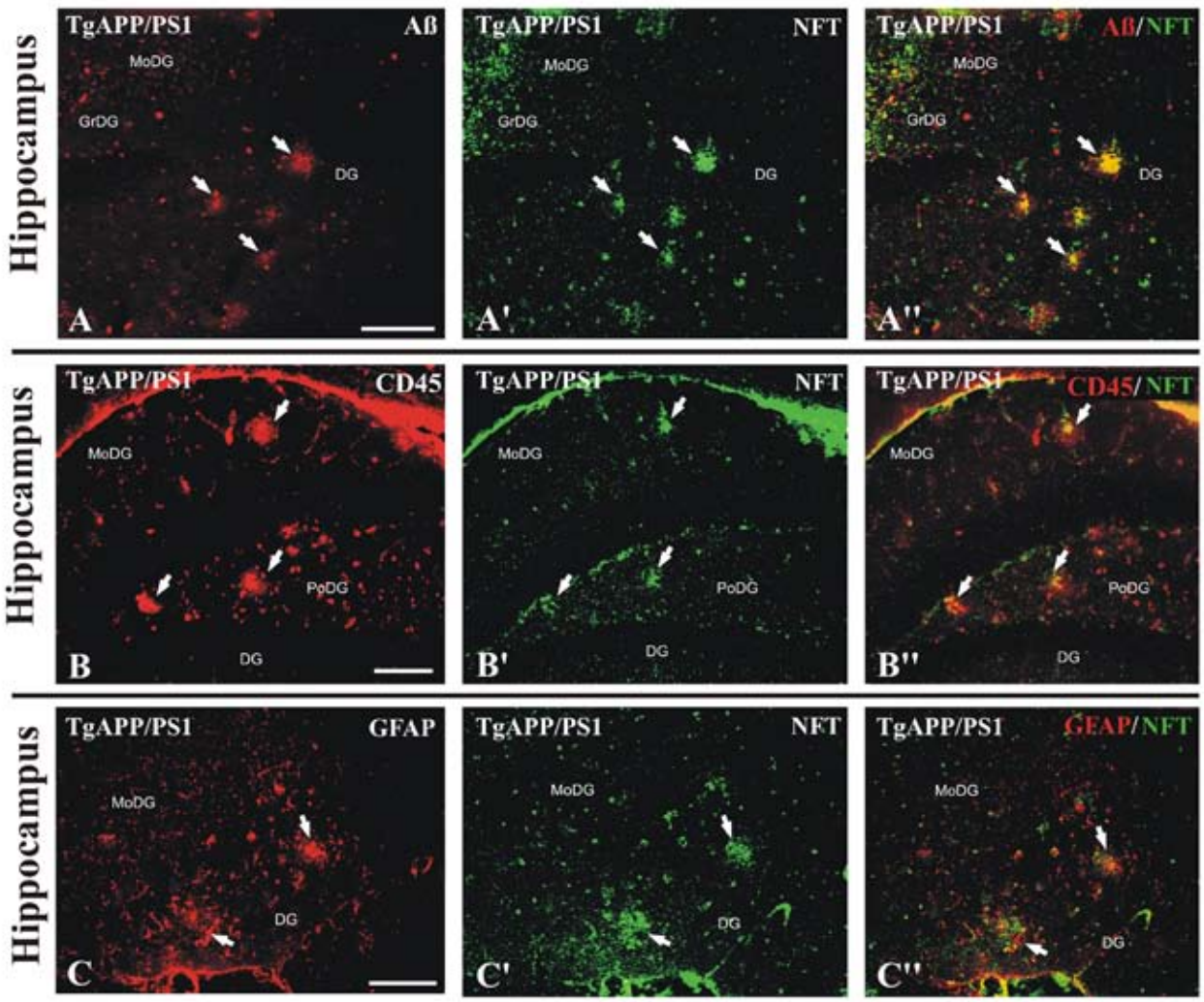
Efectos profilácticos de la vacuna EB101 sobre la patología Alzheimer

El análisis de imágenes de las secciones del cerebro de los ratones transgénicos (Figuras 1A-O), indica una notable reducción del valor promedio de depósitos Aβ en el hipocampo y capas corticales de ratones transgénicos tratados con la vacuna EB101 (grupo A; 42,5 Aβ placas/cerebro) cuando son comparados con los

Tabla

Antibody	Antigen	Type	Source	Dilution	Ref.
β-Amyloid	$A\beta_{1-42}$ (mouse)	Mouse monoclonal	Millipore	1:1000	21
Neurofibrillary tangles	NT (rabbit)	Rabbit polyclonal	Millipore	1:300	19
Glial fibrillary acidic p	GFAP (mouse)	Mouse monoclonal	Sigma	1:400	27,29,45
CD45RA	B-cells (mouse)	Mouse monoclonal	Dako	1:100	28,30
CD3	T-cells (rabbit)	Rabbit polyclonal	Dako	1:100	30

Figura 4. Relación de los ovillos neurofibrilares con los diversos elementos neuropatológicos en el cerebro de ratones transgénicos B6C3F1/J.
Imágenes de secciones transversales del hipocampo de ratones transgénicos en los que se observa una co-distribución en la inmunoreactividad frente a ovillos neurofibrilares, placas Aβ y células gliales marcadas con GFAP.
Barra de calibrado: 100 µm.



ratones tratados sin la vacuna, grupo B (102.2 Aβ placas/cerebro) y grupo C (106.9 Aβ placas/cerebro), (Figura 2A). Las escasas placas Aβ que aparecen en ratones transgénicos tratados con la vacuna se localizan principalmente en las capas externas de la región encefálica del giro dentado (Figuras 1A-C), y que al ser de pequeño tamaño, dispersas y con núcleo difuso, representan los estadios iniciales de su desarrollo (tipo 2a). El análisis histológico de las secciones del cerebro de los ratones del estudio revelaron cuatro tipos diferentes de placas Aβ: tipo 1, 2a, b y c, basado en la caracterización morfológica descrita por Bussière *et al* [45], como se observa en la figura 1A-I. Estas placas β-amiloide estuvieron presentes principalmente en el hipocampo del cerebro del ratón transgénico de los grupos B y C (Figura 1-I), ubicadas principalmente en el giro dentado (capa granular), seguido por las regiones neocorticales como las zonas de la corteza retrosplenial, ectorrinal y capas piriformes. Analizando el área ocupada por los depósitos Aβ en el hipocampo y la corteza cerebral de secciones de cerebro de ratones transgénicos (Figuras 1A-C) después de tratamiento preventivo con la vacuna, se constata una reducción notable (4.83%, $p<0,05$; Figura 2B) y difiere significativamente del área de depósitos Aβ observada en las secciones de los ratones tratados sin la vacuna, grupo B (12,6%, $p<0,05$; Figura 2B) y grupo C (12.81%, $p< 0,05$; Figura 2B). Los ratones control no mostraron depósitos Aβ en ninguna región del cerebro (véase área cuadrado en figuras 1G-I).

El efecto en la psicomotricidad en los ratones del estudio se evaluó al final del tratamiento (7 meses) mediante una prueba estandarizada de rota-rod. Los resultados de habilidades motoras mostraron un mejor índice de coordinación motora en los ratones tratados con la vacuna EB101 en comparación con los dos otros controles, que

Figure 5. Efecto de la vacuna EB101 en la incidencia de astrogliosis y activación inmunitaria. Imágenes comparativas de la inmunoreactividad frente a marcadores gliales (GFAP), células B (CD45RA) y células T (CD3) en el hipocampo (Figuras A-I,L,O,R) y regiones corticales (Figuras J,K,M,N,P,Q) durante la fase preventiva (Figuras A-I) y terapéutica (Figuras J-R). Se observa una notable ausencia de procesos neuroinflamatorios en forma de astrogliosis en el giro dentado (A), contrastando con los astrocitos reactivos densamente distróficos, típicos de una reacción inflamatoria, observada en las secciones de cerebro de ratón correspondientes a los grupos B (ratones tratados con EB102) y grupo C (PBS-tratados). Barra de calibrado: 100 µm.

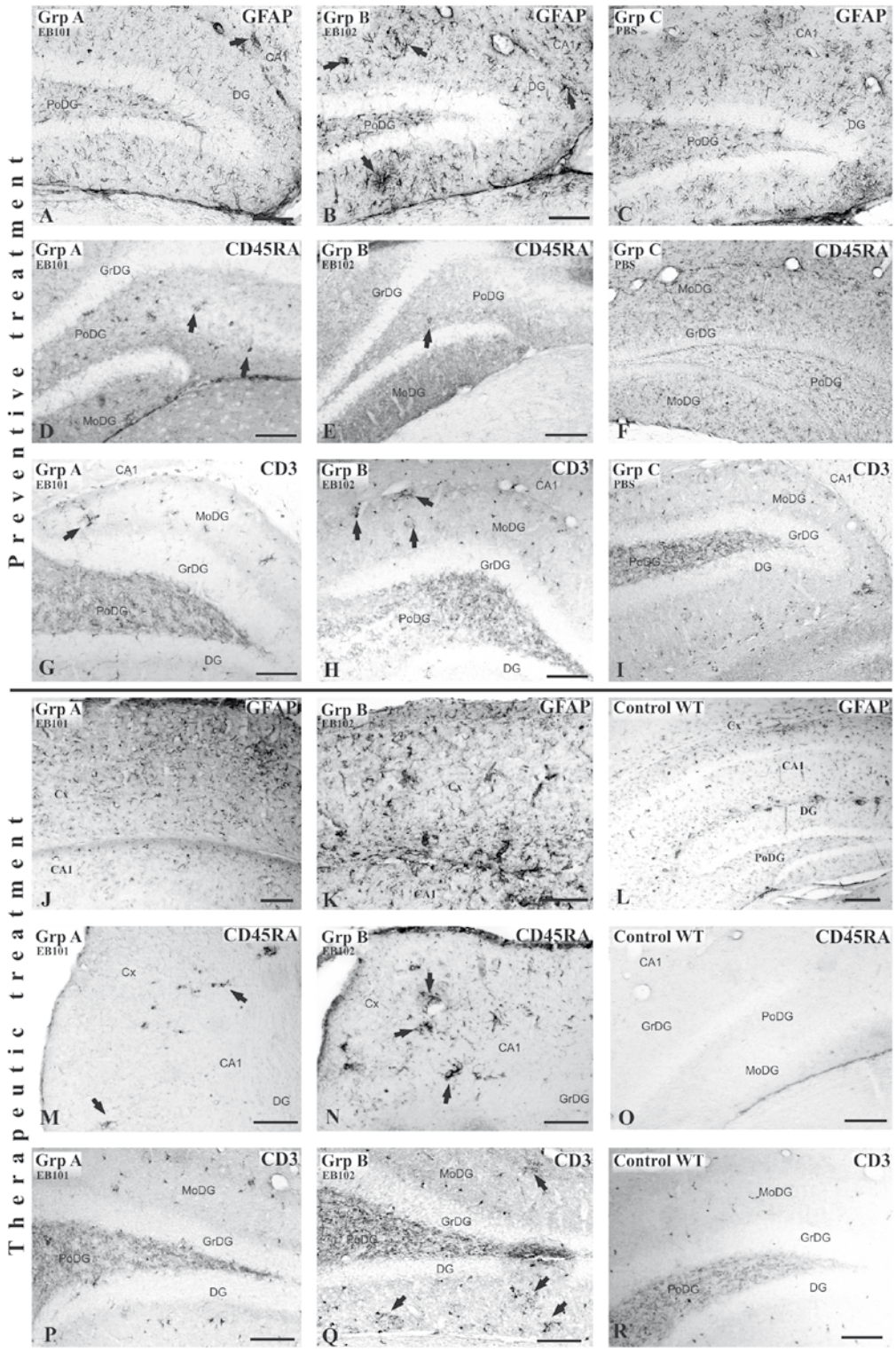
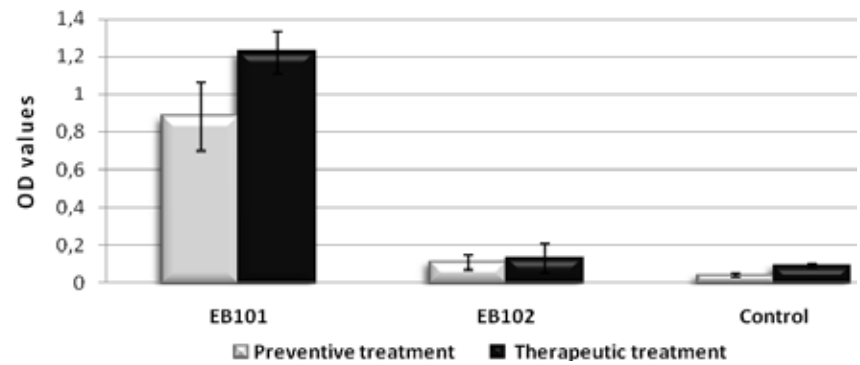
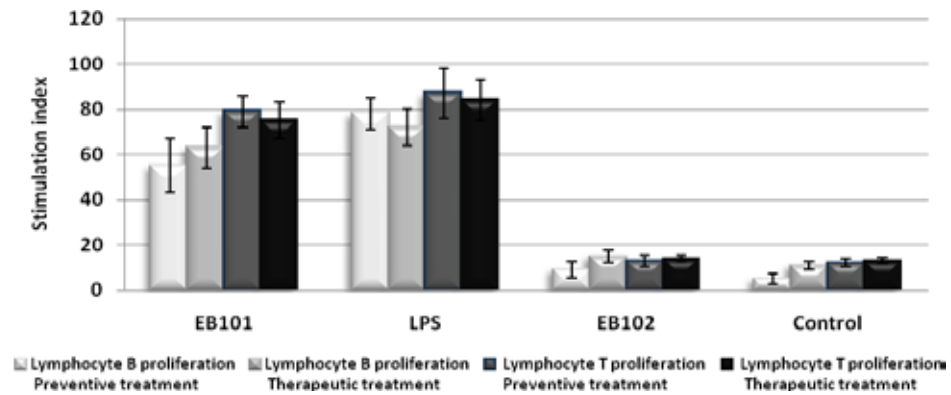


Figura 6. Cuantificación de anticuerpos IgG anti β-amiloide y citoquinas Th1/Th2.
A. Gráfico de la presencia de anticuerpos antiβ-amiloide en el suero de ratones transgénicos después del tratamiento preventivo y terapéutico. Véase el efecto de la vacuna EB101 con una notable producción de anticuerpos IgG. Cada barra representa el valor medio OD significativo ± desviación típica en cada grupo experimental.
B. Respuesta proliferativa de las células del bazo de ratones tratados con EB101 y controles tras el tratamiento preventivo y terapéutico. Los resultados mostraron que EB101 y LPS habían inducido respuestas proliferativa de linfocitos T yB.
C. Detección de citoquinas Th1 y Th2 en ratones transgénicos después del tratamiento terapéutico. Análisis de varianza (ANOVA) para IL-5, -6, -10 y -13 con diferencias significativas entre vacunados con EB101 y grupos de control. Resultados en pg/mL ± SEM

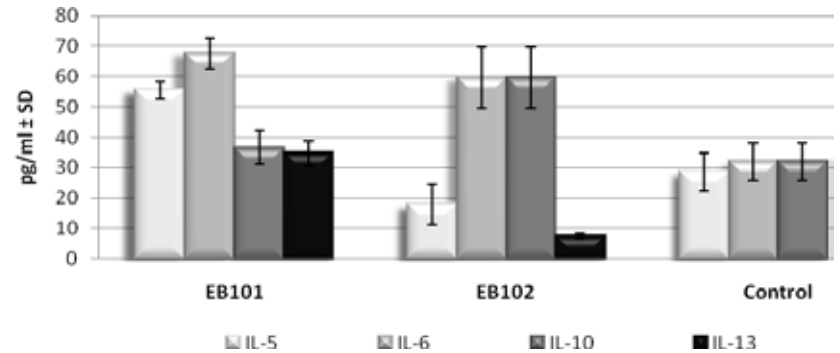
A



B



C



mostraban un moderado-severo deterioro en la coordinación motriz (Figura 2).

También se estudió el efecto de la vacuna EB101 en los ovillos neurofibrilares utilizando anticuerpos contra NFT. Estas placas de desarrollo degenerativo fueron observados en los ratones transgénicos (Figuras 3A-I), aunque su densidad fue sustancialmente mayor en cerebros de ratón del grupo B (Figuras 3D-F) y C (Figuras 3G-I) en comparación con los ratones tratados con la vacuna EB101, grupo A. Estas estructuras immunopositivas están situadas principalmente en las regiones del hipocampo del cerebro de los ratones tratados sin vacuna (EB102 y PBS) y están compuestas por agregados hiperfosforilados de la proteína tau, situada a lo largo de los filamentos helicoidales neuronales, presentando un núcleo inmunorreactivo similar y una extensión variable de dendrita apical (a menudo con una apariencia en forma de llama como se muestra en la figura 3). Las regiones del cerebro de ratones transgénicos tratados con la vacuna EB101 (grupo A) está en su mayor parte desprovista de NFT (Figuras 3A-C), y sólo se observaron de forma espaciada en el giro dentado (Figuras 3B) y en capas de la corteza entorrinal (Figura 3). Sin embargo, las mismas regiones del grupo B (Figuras 3D-F), notablemente similares a las observados en los ratones transgénicos del grupo C (Figuras 3G-I), mostraron una amplia densidad de ovillos neurofibrilares ocupando todas las regiones del hipocampo, área retrosplenial, ectorrinal y corteza piriforme. La intensidad del marcaje de los ovillos neurofibrilares observados en los grupos B y C, incluyendo la densidad y el área ocupada, contrasta con la escasez y la casi ausencia de estas características neuropatológicas en ratones tratados con la vacuna EB101.

Los ratones control no mostraron placas en ninguna región del cerebro (véase área del cuadrado en figuras 3G-I). La citoarquitectura y distribución neuronal del marcador presináptico, sinaptofisina, no muestra variación de densidad ni diferencias significativas entre los cerebros de ratón de los tres grupos estudiados.

Efecto terapéutico de la vacuna EB101 en placas Aβ, NFT y neuroglía de ratones B6C3F1/J

El efecto terapéutico de la vacuna EB101 en ratones B6C3F1/J se inició a las 35 semanas de edad después de la aparición de la neuropatología asociada al Alzheimer. Los ratones fueron tratados y se analizaron utilizando el mismo protocolo que en el tratamiento preventivo. Para determinar si la vacuna EB101 invierte el desarrollo masivo de placas β-amiloide, NFT y glía reactiva en cerebro, secciones de todos los ratones experimentales (transgénicos y de control) fueron analizados con los anticuerpos específicos para identificar estos sellos patológicos.

Los resultados obtenidos tras el tratamiento terapéutico mostraron que los depósitos Aβ estaban casi completamente ausentes en las secciones del cerebro de ratones tratados con la vacuna EB101 (20,3 placas Aβ/cerebro; Figuras 1J-L, 2A), diametralmente diferente de los niveles de placas Aβ observadas en los demás grupos de ratones, grupo B (134.3 Aβ placas/cerebro; Figuras 1 M-O, 2A) y grupo C (128.6 Aβ placas/cerebro; Figura 2A). Las pocas placas Aβ observadas en ratones vacunados presentan un pequeño núcleo central rodeado de escaso material fibrilar (tipo 2a), con una menor área de depósito Aβ (5,27%, $p<0,05$; Figura 2B), ubicadas principalmente en las capas corticales externas (Figuras 1J-L) y casi totalmente ausente en las regiones del hipocampo. En la sección del cerebro de las mismas regiones transversales, ratones del grupo B presentan una extensa área de placas Aβ (14.32%, $p<0,05$; Figuras 1 M-O, 2B), mostrando una distribución dispersa a lo largo de las capas del hipocampo y corticales, similares a la observada en el grupo C (14,07%, $p<0,05$; Figuras 2B).

La prueba de psicomotricidad en el rota-rod, para medir la coordinación motora durante el tratamiento terapéutico, no mostró ningún efecto negativo en los ratones tratados con la vacuna EB101 (Figura 2), pero indica un moderado/severo deterioro en la capacidad motora de los demás ratones tratados (Figura 2). El análisis histológico del grupo A de ratones después del tratamiento terapéutico mostraba

un patrón de distribución menos denso de ovillos neurofibrilares (Figuras 3J-L) que el observado en el tratamiento preventivo. Después del tratamiento terapéutico (Figuras 3 M-O), los ratones de grupo B presentaron una densidad elevada de placas neuríticas similar a la observada en el tratamiento preventivo. El análisis de la plasticidad sináptica revela que estos grupos B y C presentan niveles de densidad diferentes pero no significativamente menores de sinaptofisina, principalmente en las regiones del hipocampo, en comparación con el grupo A.

Marcadores neuropatológicos. Detección por doble inmunofluorescencia

La detección por doble inmunofluorescencia se aplicó en el análisis de colocalización de placas placas Aβ y ovillos neurofibrilares en los cerebros de ratones transgénicos (Figuras 4A-A"). Observamos algunas placas dispersas en el hipocampo y en la región entorrinal de ratones B6C3F1/J, colocalizadas principalmente en la capa molecular del giro dentado (Figuras 4A-A") y en la corteza entorrinal. Inmunofluorescencia a CD45RA (linfocitos B) y a los ovillos neurofibrilares también fue analizada por microscopía confocal, mostrando que los linfocitos B rodean los ovillos neurofibrilares principalmente en las capas moleculares del giro dentado (Figuras 4B-B"). Doble inmunofluorescencia GFAP y a ovillos neurofibrilares mostraron que las células gliales activas se localizan alrededor de los ovillos neurofibrilares en el giro dentado (Figuras 4 C-C") y en la corteza entorrinal, aunque no se observó ninguna colocalización.

Efecto de la vacuna EB101 en la respuesta immune

Con el fin de evaluar el efecto de la vacunación en la reactividad microglial y neuroinflamación, analizamos la distribución de proteína ácida fibrilar glial (GFAP), de células B (CD45RA) y células T (CD3) de marcadores de superficie en la sección transversal de los cerebros de ratón (Figuras 5A-R). Después del tratamiento preventivo, se observó neuroglia reactiva, astroglía, formando agregados de astrocitos remplazando neuronas muertas, alrededor de placas Aβ y ovillos neurofibrilares en la región cortical e hipocámpica del cerebro de ratones transgénicos de los grupos B (Figura 5B) y C (Figura 5C). Las mismas regiones del cerebro de ratones transgénicos tratados con la vacuna están casi desprovistas de neuroglía reactiva (Figura 5A). Las secciones del cerebro de ratones transgénicos vacunados con EB101 mostraban algunos linfocitos B inmunorreactivos en la región del hipocampo (Figura 5D), con mucho menor densidad en los grupos B (EB102; Figura 5E) y C (PBS; Figura 5F). No hay indicios de astrocitosis ni de linfocitos B en ningún ratón control (Figuras 5L,O).

En el tratamiento preventivo, las células T inmunorreactivas fueron encontradas en todos los cerebros de ratones transgénicos, formando conspicuos agregados en el hipocampo (Figuras 5G-I) y regiones corticales, aunque estos grupos microgliales se encontraron en menor densidad en los ratones transgénicos tratados con la vacuna EB101 (Figura 5). La distribución glial en cerebros de ratón transgénico tratados con la vacuna EB101 (Figura 5J) tenía un patrón similar a los ratones control, con ningún acúmulo reactivo glial en todo el cerebro excepto en zonas corticales específicas (Figura 5J). Por lo tanto, el patrón de distribución de células inmunorreactivas a GFAP mostró una ausencia generalizada de astrocitosis en ratones con vacuna EB101, contrastando con los grupos B (Figura 5K) y C, donde procesos de astrocitosis se observaron en las regiones del hipocampo y corticales. También hemos apreciado que la densidad de células B en los ratones tratados con la vacuna EB101 (Figura 5M) después del tratamiento terapéutico era similar a la densidad del régimen preventivo. Cabe destacar que los agregados de células T no se observaron en ninguno de los grupos control (Figura 5R). Sin embargo, los ratones transgénicos de los grupos B (Figura 5N) y C mostraron un pronunciado incremento en los marcadores de CD45RA en las zonas corticales cerebrales con densas placas amiloides. Los grupos B (Figura 5P) y C mostraron gran densidad de microglía en el hipocampo y el neocórtex, formada por células inmunorreactivas a CD3 en áreas ricas en placas amiloides (Figura 5Q; véase doble inmunoreactividad en la figura 4). La ausencia de linfocitos T en ratones transgénicos tratados con la vacuna EB101 (Figura 5P) contrasta con los resultados observados en los demás grupos experimentales.

Inmunogénesis de anticuerpos Aβ en ratones transgénicos

La concentración de anticuerpos Aβ en ratones transgénicos fueron determinadas por ELISA utilizando anticuerpos específicos anti-Aβ. El tratamiento preventivo con la vacuna EB101 dio lugar a un marcado aumento de la producción de anticuerpos IgG $A\beta_{42}$ con valores de 1:1400-1:2000 hasta 1:6000 en algunos animales. Los ratones transgénicos de los demás grupos tratados con liposomas vacíos (EB102) o con PBS, mostraron niveles bajos de anticuerpos anti-$A\beta_{42}$. Después del tratamiento terapéutico, todos los ratones tratados con la vacuna EB101 produjeron anticuerpos IgG (Figura 6A). Las concentraciones de anticuerpos eran similares a las detectadas después del tratamiento preventivo. En los modelos preventivos y terapéuticos, los resultados mostraron que

la vacunación por EB101 induce una fuerte respuesta proliferativa linfocitaria de células B y un consecuente incremento en células T ($P<0.01$; Figura 6B), mientras que en los casos de los grupos tratados con EB102 y PBS no mostraron ninguna respuesta inmune destacable.

La detección de la respuesta de citoquinas Th1 y Th2 se llevó a cabo en sueros de cada ratón estudiado (Figura 6). Los resultados obtenidos de dicha detección indican diferencias significativas entre los ratones vacunados y los demás grupos control en cuanto a citoquinas IL-5, 6, 10 y 13, lo que sugiere que la inmunidad mediada por anticuerpos observada en el presente estudio induce a un tipo de reacción inmunológica esperable de células T Th2.

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Conclusiones

En estos últimos años hemos enfocando nuestros esfuerzos en el desarrollo de una vacuna contra la patología cerebral del Alzheimer, dirigida no sólo a la reducción de placas Aβ mediante la génesis endógena de anticuerpos anti-Aβ, sino también en la reducción de la formación de los ovillos neurofibrilares, prevención de deterioro cognitivo y regeneración de las neuronas dañadas. Impulsados por el gran potencial de una vacuna eficaz en la prevención y tratamiento de EA y la selectiva eficacia de la primera vacuna activa para reducir los niveles de Aβ, hemos optado por un nuevo enfoque para eludir el fracaso de intentos anteriores (AN1793), seleccionando experimentalmente todos los componentes que aseguran una eficacia tanto preventiva como terapéutica frente a los obstáculos empíricos de pruebas anteriores. Los componentes de la vacuna presentan una eficacia comprobada por numerosos estudios, entre los que destacan el adyuvante de matriz fisiológica, liposomas compuestos por fosfolípidos naturales: fosfatidilcolina, fosfatidilglicerol y el colesterol, utilizados también en otros tipos de vacunas, como en la vacuna de la gripe. Además se añadió un esfingolípido biológicamente activo, el S1P, y que actúa como un mediador importante de lípidos tanto dentro como fuera de las neuronas [46,47]. Se sabe que el S1P facilita la secreción de glutamato en las neuronas del hipocampo y participa en los mecanismos subyacentes de la regulación de la transmisión sináptica. Extracelularmente, S1P se une a los miembros de la proteína GTP (proteína G), provocando diversos efectos celulares, incluida la angiogénesis, desarrollo cardíaco, inmunidad, motilidad celular y extensión de desarrollo [37,38], actuando en una red autocrina y paracrina [48-50]. Intracelularmente, el S1P ha demostrado actuar en la movilización de calcio celular, crecimiento celular y supresión de la apoptosis [51-54]. Estas acciones inducidas por S1P pueden facilitar la formación de un ciclo de activación positiva en neuronas excitatorias ya que el S1P intracelular aumenta la excitabilidad inducida por el factor de crecimiento nervioso en las neuronas sensoriales [39]. Por último, el S1P también controla la migración de las células madre neuronales hacia el sitio de la lesión en la médula espinal, sugiriendo con ello que el S1P tiene un potencial terapéutico como agente regenerativo en el sistema nervioso central [42].

Aprovechando estas propiedades del S1P, hemos incorporado S1P con la mezcla de fosfolípidos para formar una matriz liposomal utilizada como coadyuvante para entregar el antígeno activo, $A\beta_{42}$ [56]. Esta combinación añadió un componente regenerativo y antiinflamatorio a la vacuna, elementos clave para aumentar la actividad neuronal y evitar la posible inflamación en el cerebro de los pacientes de Alzheimer.

Las principales conclusiones de este estudio indican que: las principales señas de identidad de la enfermedad de Alzheimer como (i) placas de beta amiloide y los ovillos neurofibrilares, se evitan de forma notable en los ratones transgénicos experimentales; (ii) la activación de la respuesta de microglía y consecuente neuroinflamación, que espontáneamente se manifiestan en estos ratones transgénicos como patología asociada a la enfermedad de Alzheimer, se reducen por tratamiento con la vacuna EB101; (iii) una fuerte respuesta inmunitaria se observó tras el tratamiento con la vacuna EB101, según lo determinado por las concentraciones de anticuerpos Aβ detectados en sueros; (iv) nuestra vacuna EB101 también dio lugar a una mejora significativa en la psicomotricidad de los ratones transgénicos tratados; y (v) no provoca ningún efecto de atrofia o parálisis en las extremidades de los ratones tratados con la vacuna EB101.

Esta nueva vacuna EB101 superó la patológica respuesta autoinmune que se detectó con la vacuna AN1793 (Elan y Wyeth, 2001): un error debido a la incorporación de un coadyuvante, QS-21 y el detergente polisorbato 80, se cree que pudo haber inducido la respuesta pro-inflamatoria de tipo Th1 [33]. En este trabajo hemos demostrado que la formulación y composición de nuestra vacuna EB101 es efectiva e inócua, cubriendo la necesidad que hay en nuestra sociedad desde hace mucho tiempo de una vacuna segura y eficaz para prevenir o mejorar la neuropatología de la enfermedad de Alzheimer. La vacuna EB101 cumple íntegramente los requisitos para la puesta en marcha de la primera fase de ensayos clínicos: inducción de una fuerte respuesta inmune del tipo Th2 para la reducción de las neuropatologías características, placas beta amiloide y ovillos neurofibrilares, reduciendo así la neuroinflamación, mediada por la activación de la microglía. Estos resultados respaldados por el tratamiento preventivo y terapéutico, sugieren que la vacuna EB101 es eficaz no sólo en la prevención de la neuropatología asociada al Alzheimer sino también en su reducción terapéutica.

Los resultados obtenidos confirman que en los ratones no vacunados con EB101 la tinción anti-Aβ se acumula en placas amiloides no fibrilares en el periodo inicial del proceso patológico. Estos resultados se intensifican en etapas posteriores, como era de esperar, con el avance de la enfermedad, en las que las placas fibrilares difusas son cada vez más numerosas en estas regiones del cerebro

[13]. Sin embargo, los resultados obtenidos después de la vacunación preventiva con EB101 en ratones transgénicos demuestran un índice significativamente bajo en la densidad de placas Aβ presentes en las mismas regiones cerebrales de ratón, así como una notable reducción del áreas de placas en comparación con los ratones no vacunados con EB101 (Grupo B y C). El hecho de que el análisis de imagen inmunohistoquímica y de fluorescencia confirmara esta eficaz reducción de los primeros depósitos Aβ (4,83%) en el cerebro de ratones por la vacuna EB101 sugiere que dicha vacuna funciona como un tratamiento preventivo en la aparición de las placas amiloide de la enfermedad de Alzheimer en ratones transgénicos. Durante este periodo temprano del desarrollo de la enfermedad, la inmunización con la vacuna EB101 también impidió significativamente el desarrollo masivo de otras señales patológicas como los ovillos neurofibrilares, la deficiencia en la coordinación motora y la reactivación microglial. Esta activación microglial en respuesta a los primeros depósitos Aβ [17,57,58-60] se ha visto reducida por nuestra vacuna, mostrando una marcada ausencia de astrocitosis relacionada con acúmulos Aβ en ratones inmunizados con EB101. Mediante el uso de técnicas de doble tinción inmunohistoquímica en cerebros de ratón, se comprobó dicha interacción patológica, puesto que un gran número de astrositos inmunopositivos al marcador celular GFAP se identificaron alrededor de los ovillos neurofibrilares, estrechamente relacionados con las placas amiloides y con los marcadores de neuroinflamación [61]. Por lo tanto, la vacuna EB101 parece prevenir significativamente la acumulación, en el período inicial de la enfermedad degenerativa, de placas amiloides no fibrilares así como procesos de inflamación asociados a las regiones cerebrales de la corteza y el hipocampo en ratones transgénicos APPswe/PS1dE9.

En cuanto a los resultados obtenidos después del tratamiento terapéutico con la vacuna EB101, se observó una clara disminución de los principales sellos patológicos asociados a la enfermedad de Alzheimer. Estos resultados obtenidos mediante el análisis inmunohistoquímico y ELISA demostraron un fuerte efecto del tratamiento EB101 en la eliminación de la densidad de placas Aβ, incremento de los niveles de anticuerpos anti-Aβ, ausencia de neuroinflamación y un índice normal en las tareas de coordinación motriz en comparación con los ratones tratados con EB102 y PBS. Estos efectos, observados en los tratamientos preventivos y terapéuticos, demuestran una vez más la eficacia de la vacuna EB101 para prevenir y reducir la carga β-amiloide y, en consecuencia, la neurodegeneración resultante. Los análisis de inflamación y respuesta inmune, realizados al finalizar el tratamiento terapéutico, no mostraron ninguna reacción inflamatoria importante en el cerebro de ratones vacunados con EB101, al contrario de lo que se observó anteriormente con otros tipos de vacunas realizadas en estudios con ratones tg APP [62] y en pacientes vacunados con Aβ [33]. No se han detectado signos de neuroinflamación, medido por citoquinas pro-inflamatorias [63] o por un aumento de la GFAP, marcador de astrogliosis [64], en los ratones inmunizados de EB101, en contraste con los resultados observados en los grupos de ratones transgénicos vacunados con EB102 y PBS. Niveles bajos de células T reactivas se detectaron en cerebros de ratones inmunizados con EB101, al contrario de lo que se observó en los pacientes vacunados y con lo que se relaciona directamente los procesos de meningoencefalitis que parecen haber sido la causa probable de los efectos adversos de la vacuna de Elan [9,65].

En estudios previos, mediante inmunoterapia Aβ en ratones APP-tg, se han observado diferentes niveles de anticuerpos Aβ en suero, dependiendo del modelo de ratón utilizado, metodología de inmunización y tipo de coadyuvante en la formulación de la vacuna. Nuestra formulación de la vacuna liposomal (EB101) difiere notablemente de las demás, principalmente por el agente coadyuvante utilizado, presentando mejores resultados inmunogénicos que los utilizados en estudios anteriores como el Freund [20,29] o el Quil-A[66]. El adyuvante utilizado en esta nueva vacuna tienen un impacto significativo en la respuesta inmune suscitada [67], potenciando así el efecto positivo de la inmunización (EB101) observada incluso en ratones después del establecimiento de depósitos encefálicos de Aβ y posteriores cambios neuropatológicos, indicando claramente que esta vacuna tiene un gran potencial como agente terapéutico para prevenir y revertir la patología cerebral de la enfermedad de Alzheimer [31,44,68,69]. Aunque falta por descifrar el mecanismo por el cual los procesos de inmunización disminuyen los efectos neuropatológicos de la EA y previenen o disminuyen el déficit cognitivo, se cree que varios aspectos metabólicos podrían ser responsables de la degradación de las placas Aβ, tales como el tráfico de anticuerpos específicos anti-Aβ al cerebro [70], el transporte de Aβ soluble en el plasma con una posterior degradación mediada por anticuerpos (hipótesis de receptores periféricos) [71] y los sistemas de transporte de IgG [43]. El concepto de que la completa especificidad de la respuesta inmune frente al antígeno Aβ disminuye la probabilidad de activar la respuesta inflamatoria ha sido respaldada por muchos autores [43,72] y parece confirmarse con nuestra vacuna.

La evidente reducción de los marcadores neuropatológicos producida por la administración de la vacuna EB101 en ratones transgénicos para la enfermedad de Alzheimer abre paso a nuevas etapas de investigaciones preclínicas y clínicas. Parece previsible que la respuesta terapéutica a la inmunización Aβ sea positiva y variable dependiendo del perfil genotípico específico de cada paciente [73,74]. En el modelo transgénico utilizado en nuestra plataforma experimental de vacunación, los resultados demuestran inequívocamente que la vacuna EB101 no sólo puede detener las diferentes neuropatologías relacionada con la enfermedad de Alzheimer y déficits motores, sino también llegar a revertir el proceso neurodegenerativo, conduciendo a una sólida respuesta inmunológica y a un efecto antiinflamatorio que conduce a la estabilización de los patrones neuronales afectados. Con este trabajo de varios años, nuestro grupo de investigación cree haber aportado un rayo de esperanza en el largo y penoso camino hacia la prevención de la enfermedad de Alzheimer. ■

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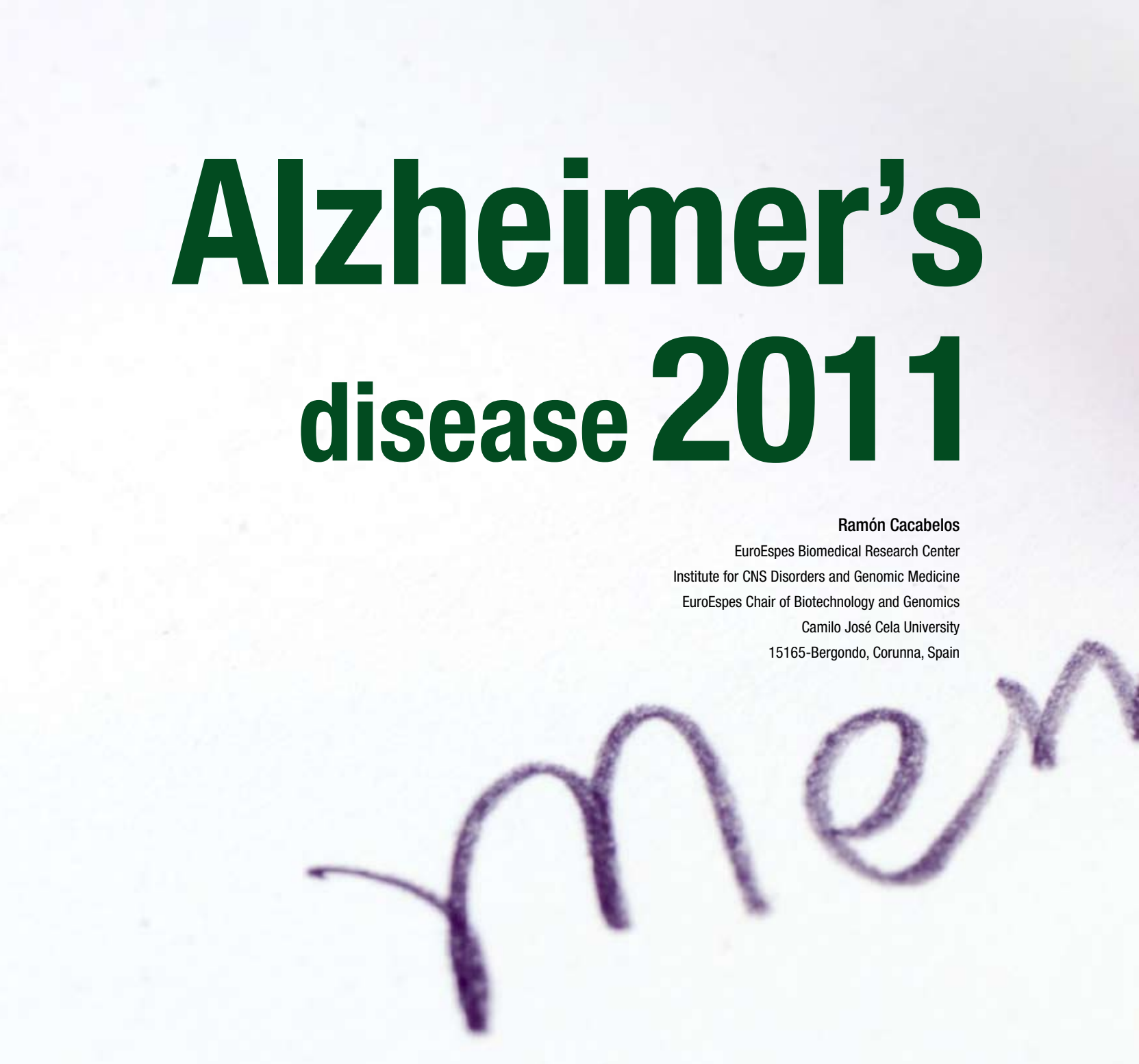


Summary

Alzheimer's disease (AD) is a major problem of health and disability, with a relevant economic impact in our society (€177 billion in Europe). Despite important advances in pathogenesis, diagnosis and treatment, its primary causes still remain elusive, accurate biomarkers are not well characterized, and the available pharmacological treatments are not cost-effective. As a complex disorder, AD is a polygenic and multifactorial clinical entity in which hundreds of defective genes distributed across the human genome may contribute to its pathogenesis (with the participation of diverse environmental factors, cerebrovascular dysfunction, and epigenetic phenomena), leading to amyloid deposition, neurofibrillary tangle formation and premature neuronal death. Future perspectives for the global management of AD predict that structural and functional genomics and proteomics may help to search for reliable biomarkers, and that pharmacogenomics may be an option to optimize drug development and therapeutics.

Where are we heading?

Keywords

Alzheimer's disease, *APOE*, Biomarkers, CYPs, Genetics, Genomics, Pathogenesis, Pharmacogenomics, Treatment.

Introduction

Since the identification of its pathogenic features by Alois Alzheimer in 1906 and its characterization as a clinical entity by Emile Kraepelin in 1912, over 78,000 papers have been published on Alzheimer's disease (AD) to date (2.5 million references on cancer since 1818; 1.6 million references on cardiovascular disorders since 1927; 1.01 million on central nervous system disorders since 1893). The number of people affected by dementia is becoming a public and socioeconomic concern in many countries all over the world, independently of the economic condition of the society in question. The growth of the elderly population is a common phenomenon in both developed and developing countries, bringing about future challenges in terms of health policy and »

Figure 1. Distribution of genes in the human chromosomes. (a) Distribution of genes of the human genome in the haploid set of autosomes and sex chromosomes; (b) Distribution of AD-related genes in the human chromosomes; (c) Proportional distribution of AD-related genes in the human chromosomes, as a function of the total number of genes mapped in each chromosome.

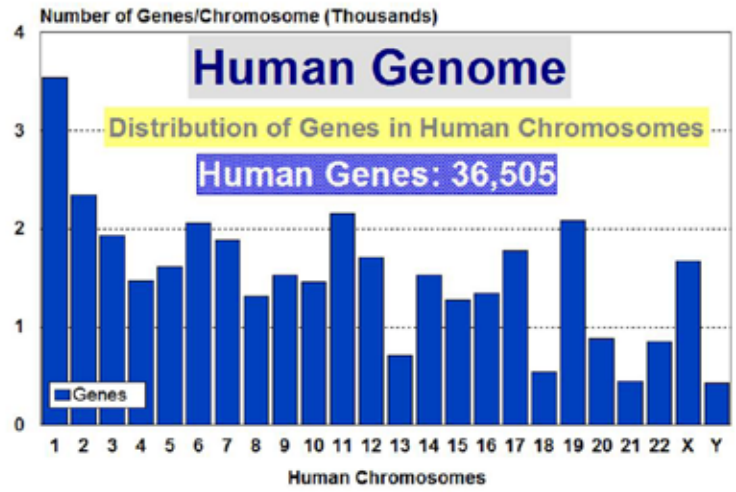


Fig. 1a

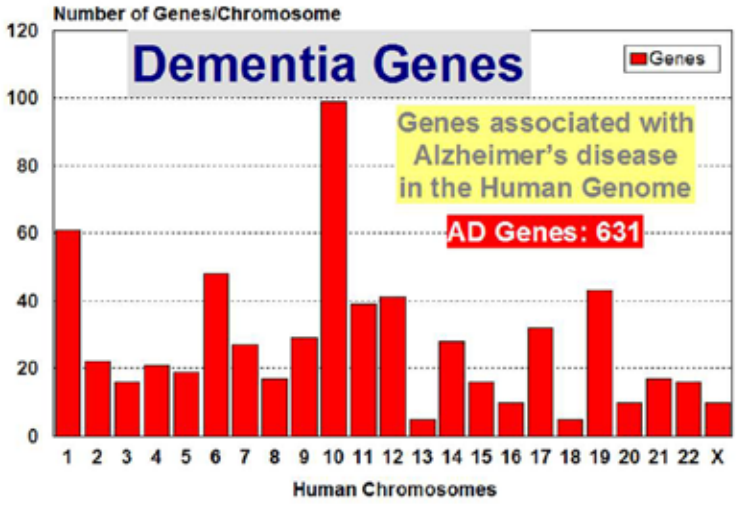


Fig. 1b

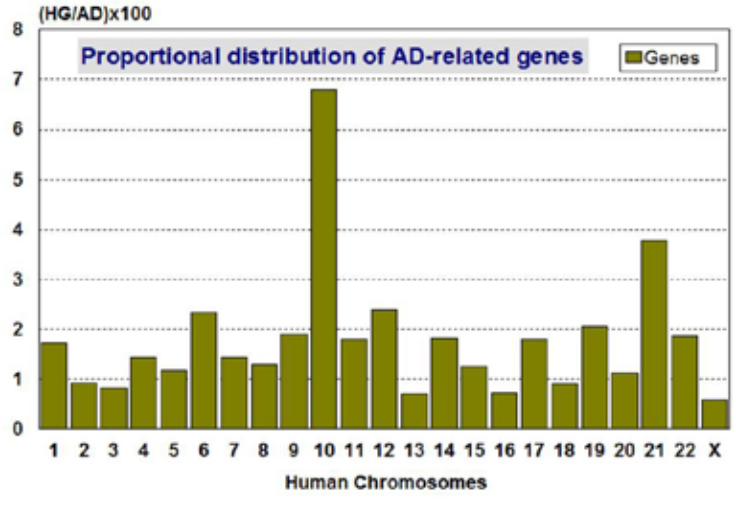


Fig. 1c

disability rates. In the U.S.A., death rates for the leading causes of death are heart disease (200.2 x 100,000), cancer (180.7 x 100,000), and stroke (43.6 x 100,000), with AD, as the fifth leading cause of death in people older than 65 years of age, representing 71,600 deaths/year. Disability caused by senility and dementia affects 9.2 x 1,000 in the population aged 65-74 years, 33.5 x 1,000 in those within the 75-84 range, and 83.4 x 1,000 in the population over 85 years of age [1]. In countries with low and middle income, dementia makes the largest contribution to disability, with a median population-attributable prevalence fraction of 25.1%, followed by stroke (11.4%), limb impairment (10.5%), arthritis (9.9%), depression (8.3%), eyesight problems (6.8%), and gastrointestinal impairments (6.5%) [2]. In Western countries, AD is the most prevalent form of dementia (45-60%), followed by vascular dementia (30-40%), and mixed dementia (10-20%), which in people older than 85 years of age may account for over 80% of the cases. Geographic and temporal variation in occurrence of dementia has received little attention. Gillum *et al* [3] analyzed the US multiple cause of death files for 2005-2006 and 1999-2000, in search of US deaths with AD and other dementias coded as underlying or contributing cause of death based on the death certificate. In 2005-2006 combined, 555,904 total deaths occurred with any dementia type (212,386 for AD). Among the states, age-adjusted rates per 100,000 per year varied by two-fold, ranging from 458 in New York to 921 in Oregon. However, between 1999-2000 and 2005-2006 the US death rate for all dementia increased only from 559 to 695 (24%) while that for AD doubled from 135 to 266. There are an estimated 25-30 million cases of AD in the world, with numbers approaching 70 million cases in 20 years.

The different forms of dementia pose several challenges to our society and the scientific community: (i) they represent an epidemiological problem, and a socio-economic, psychological and family burden; (ii) most of them have an obscure/complex pathogenesis; (iii) their diagnosis is not easy and lacks specific biomarkers; and (iv) their treatment is difficult and inefficient.

In terms of economic burden, approximately 10-20% of direct costs are associated with the pharmacological treatment, with a gradual increase in parallel with the severity of the disease. In a Canadian study, Herrmann and coworkers [4] showed that the mean total cost to treat patients with very mild AD was $367 per month, compared with $4063 per month for patients with severe or very severe AD. Only 20-30% of patients with dementia respond appropriately to conventional drugs, and the onset of adverse drug reactions imposes the additional administration of other drugs to neutralize side-effects, this multiplying the initial cost of the pharmacological treatment and health risk for the patients [5]. Wimo *et al* [6] studied the economic impact of dementia in

Europe within the EU-funded Eurocode project and found that the total cost of dementia in the EU27 in 2008 was estimated to be €160 billion (€22,000 per demented patient per year), of which 56% were costs of informal care. The corresponding costs for the whole of Europe were €177 billion.

In addition (and related) to the problem of direct and indirect costs for the management of dementia, there is an alarming abuse of inappropriate psychotropic drug consumption worldwide. Almost half (49.1%) of participants in the cross-sectional study using the 2004 National Nursing Home Survey (NNHS) in Canada had dementia, and 30.0% of those with dementia were receiving cholinesterase inhibitors (ChEIs). Donepezil accounted for 71% of all ChEI prescriptions [7]. Antipsychotic medications were taken by 32.88% of elderly patients with dementia. More elderly residents received atypical agents (31.63%) than typical agents (1.75%) [8]. In one study involving analysis of household and prescription files of the Medical Expenditure Panel Survey (MEPS) data from 1996 to 2004 in Houston, an average of 0.62 million elderly patients received antipsychotic agents annually during the study period. The majority of the elderly using antipsychotic agents were female (70%), white (86%), non-Hispanic (95%), and living in metropolitan areas (79%). Frequently reported diagnoses among the elderly taking antipsychotic agents were dementia (26.12%), anxiety (20.42%), and schizophrenia (6.62%). Of the elderly receiving antipsychotic agents, 50.39% received atypical agents and 51.88% received typical agents. The most frequently used atypical agents were risperidone, olanzapine, and quetiapine [9]. Conventional antipsychotics are associated with a higher risk of all-cause mortality than atypical agents among nursing home residents. After adjusting for potential confounders relative to users of atypicals, the rate of death is increased for users of conventional antipsychotics. Relative to risperidone, a higher rate of death was documented for haloperidol, phenothiazines and other conventional medications [10]. In Australia, the prevalence of antidepressant prescribing among care home residents is 33.0%. Antidepressants are more likely to be prescribed in people treated for dementia with mood disorder, depression, and Parkinson's disease [11].

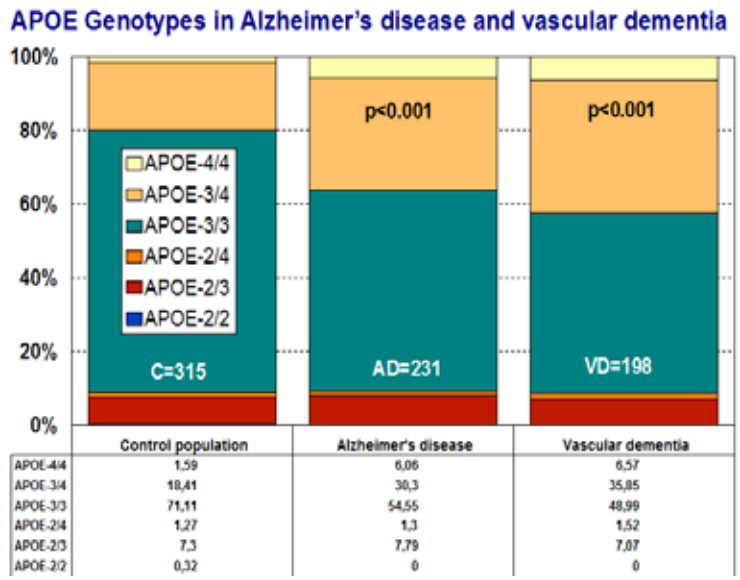
Abuse, misuse, self-prescription, and uncontrolled medical prescription of CNS drugs are becoming major problems with unpredictable consequences for brain health. The pharmacological management of dementia is an issue of special concern due to the polymedication required to modulate the symptomatic complexity of dementia where cognitive decline, behavioral changes and psychomotor deterioration coexist. In parallel, a growing body of fresh knowledge on the pathogenesis of dementia, together with data on neurogenomics and pharmacogenomics of CNS disorders is emerging in recent times. The incorporation of this new armamentarium of molecular pathology and genomic medicine into daily medical practice, together with educational programs for the correct use of drugs, must help to: (i) understand AD pathogenesis, (ii) establish an early diagnosis, and (iii) optimize therapeutics either as a preventive strategy or as a formal symptomatic treatment [5,12].

Towards a personalized medicine of dementia and CNS disorders

Common features in CNS disorders include the following: (i) polygenic/complex disorders in which genetic, epigenetic and environmental factors are involved; (ii) deterioration of higher activities of the CNS; (iii) multifactorial dysfunctions in several brain circuits; and (iv) accumulation of toxic proteins in the nervous tissue in cases of neurodegeneration. For instance, the neuropathological hallmarks of AD (amyloid deposition in senile plaques, neurofibrillary tangle formation, and neuronal loss) are but the phenotypic expression of a pathogenic process in which different gene clusters and their products are potentially involved [5,12].

It is very likely that over 80% of the genes which form the structural architecture of the human genome are expressed in the brain in a time-dependent manner along the lifespan. The cellular complexity of the CNS (with 10^3 different cell types) and synapses (with each of the

Figure 2. Distribution and frequency of *APOE* genotypes in CNS disorders and dementia.
Source: R. Cacabelos. CIBE Database. EuroEspes Biomedical Research Center, Institute for CNS Disorders and Genomic Medicine, Corunna, Spain.

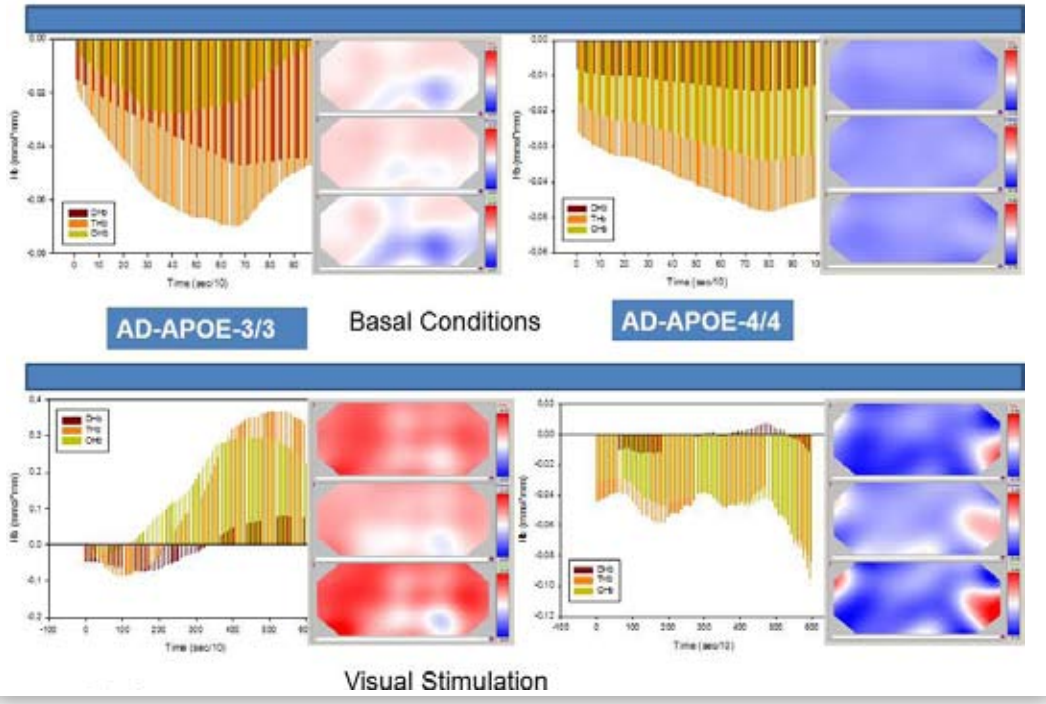


10^{11} neurons in the brain having around 10^3-10^4 synapses with a complex multiprotein structure integrated by 10^3 different proteins) requires a very powerful technology for gene expression profiling, which is still in its very early stages and is not devoid of technical obstacles and limitations [13]. Transcripts of 16,896 genes have been measured in different CNS regions. Each region possesses its own unique transcriptome fingerprint which is independent of age, gender and energy intake. Less than 10% of genes are affected by age, diet or gender, with most of these changes occurring between middle and old age. Gender and energy restriction have robust influences on the hippocampal transcriptome of middle-aged animals. Prominent functional groups of age- and energy-sensitive genes are those encoding proteins involved in DNA damage responses, mitochondrial and proteasome functions, cell fate determination and synaptic vesicle trafficking. The systematic transcriptome dataset provides a window into mechanisms of neuropathogenesis and CNS vulnerability [14].

The introduction of novel procedures into an integral genomic medicine protocol for CNS disorders and dementia is an imperative requirement in drug development and in clinical practice in order to improve diagnostic accuracy and to optimize therapeutics. This kind of protocol should integrate the following components: (i) clinical history, (ii) laboratory tests, (iii) neuropsychological assessment, (iv) cardiovascular evaluation, (v) conventional X-ray technology, (vi) structural neuroimaging, (vii) functional neuroimaging, (viii) computerized brain electrophysiology, (ix) cerebrovascular evaluation, (x) structural genomics, (xi) functional genomics, (xii) pharmacogenomics, (xiii) nutrigenomics, (xiv) bioinformatics for data management, and (xv) artificial intelligence procedures for diagnostic assignments and probabilistic therapeutic options [5]. All these procedures, under personalized strategies adapted to the complexity of each case, are essential in order to depict a clinical profile based on specific biomarkers correlating with individual genomic profiles [15].

Our understanding of the pathophysiology of CNS disorders and dementia has advanced dramatically during the last 30 years, especially in terms of their molecular pathogenesis and genetics. The drug treatment of CNS disorders has also made remarkable strides, with the introduction of many new drugs for the treatment of schizophrenia, depression, anxiety, epilepsy, Parkinson's disease, and AD, among many other quantitatively and qualitatively important neuropsychiatric disorders. Improvement in terms of clinical outcome, however, has fallen short of expectations, with up to one third of the patients continuing to experience clinical relapse or unacceptable medication-related side-effects in spite of efforts to identify optimal treatment regimes with one or more drugs. Potential reasons to explain this historical setback might be that: (i) the molecular pathology of most CNS disorders is still poorly understood; (ii) drug targets are inappropriate, not fitting into the real etiology of the disease; (iii) most treatments are symptomatic, but not anti-pathogenic; (iv) the

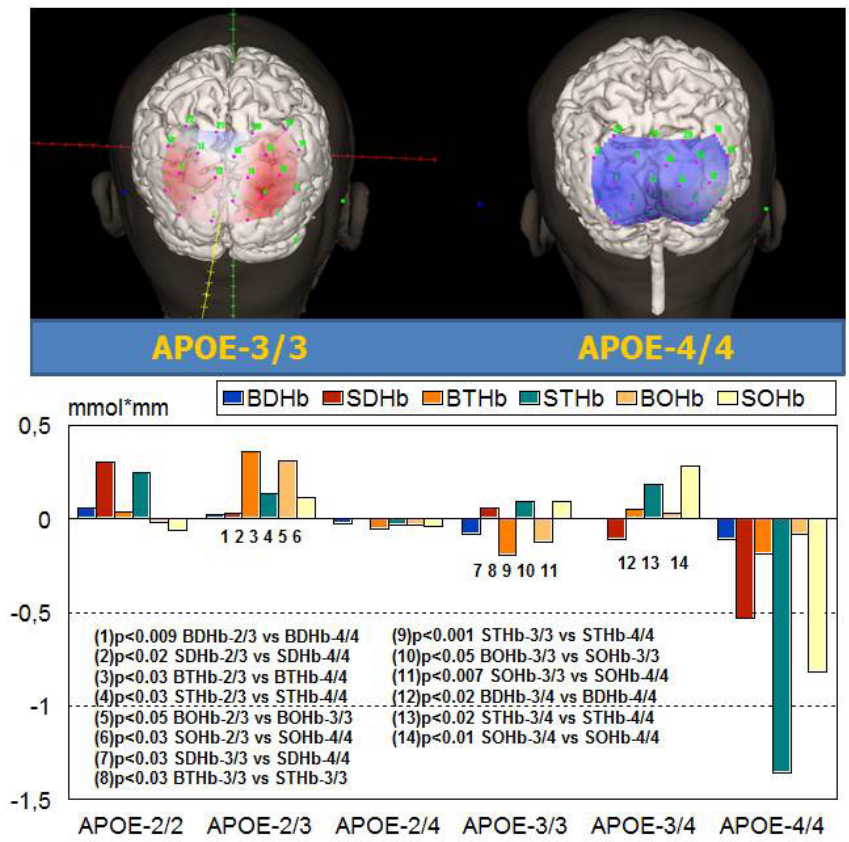
Figure 3. APOE-related cortical Hemoglobin changes in the occipital region in basal conditions and after visual stimulation in Alzheimer's disease as assessed by brain optical topography analysis.
DHb: Deoxyhemoglobin; THb: Total Hemoglobin; OHb: Oxyhemoglobin.



genetic component of most CNS disorders is poorly defined; and (v) the understanding of genome-drug interactions is very limited [5,12].

The optimization of CNS therapeutics requires the establishment of new postulates regarding (i) the costs of medicines, (ii) the assessment of protocols for multifactorial treatment in chronic disorders, (iii) the implementation of novel therapeutics addressing causative factors, and (iv) the setting-up of pharmacogenomic strategies for drug development [12]. Personalized therapeutics based on individual genomic profiles implies the characterization of 5 types of gene clusters: (i) genes associated with disease pathogenesis; (ii) genes associated with the mechanism of action of drugs; (iii) genes associated with drug metabolism (phase I and II reactions); (iv) genes associated with drug transporters; and (v) pleiotropic genes involved in multifaceted cascades and metabolic reactions.

Figure 4. APOE-related cortical hemoglobin changes in the occipital region of patients with Alzheimer's disease.
BDHb: Basal Deoxyhemoglobin; SDHb: Stimulated Deoxyhemoglobin;
BTHb: Basal Total Hemoglobin; STHb: Stimulated Total Hemoglobin;
BOHb: Basal Oxyhemoglobin; SOHb: Stimulated Oxyhemoglobin.



Genomics of Alzheimer's disease

Over 3,000 genes distributed across the human genome have been screened for association with AD during the past 30 years [16]. In the Alzgene database there are 631 genes potentially associated with AD, of which the top ten are (in decreasing order of importance): *APOE* (19q13.2), *BIN1* (2q14), *CLU* (8p21-p12), *ABCA7* (19p13.3), *CR1* (1q32), *PICALM* (11q14), *MS4A6A* (11q12.1), *CD33* (19q13.3), *MS4A4E* (11q12.2), and *CD2AP* (6p12). Potentially defective genes associated with AD represent about 1.73% of the human genome, which is integrated by 36,505 genes (Figure 1a). The highest number (>5%) of AD genes concentrate on chromosomes 10 (15.69%), 1 (9.67%), 6 (7.61%), 19 (6.81%), 12 (6.50%), 11 (6.18%), and 17 (5.07%) (Figure 1b), with the highest proportion (related to the total number of genes mapped on a single chromosome) located on chromosome 10 and the lowest on chromosome X (Figure 1c).

The genetic and epigenetic defects identified in AD can be classified into 4 major categories: Mendelian mutations, susceptibility SNPs, mtDNA mutations, and epigenetic changes. Mendelian mutations affect genes directly linked to AD, including 32 mutations in the amyloid beta (Aβ) (ABP) precursor protein (*APP*) gene (21q21) (AD1); 165 mutations in the presenilin 1 (*PSEN1*) gene (14q24.3) (AD3); and 12 mutations in the presenilin 2 (*PSEN2*) gene (1q31-q42) (AD4) [16-21]. *PSEN1* and *PSEN2* are important determinants of γ-secretase activity responsible for proteolytic cleavage of APP and NOTCH receptor proteins. Mendelian mutations are very rare in AD (1:1000). Mutations in exons 16 and 17 of the *APP* gene appear with a frequency of 0.30% and 0.78%, respectively, in AD patients. Likewise, *PSEN1*, *PSEN2*, and microtubule-associated protein Tau (*MAPT*) (17q21.1) mutations are present in less than 2% of the cases. Mutations in these genes confer specific phenotypic profiles to patients with dementia: amyloidogeneic pathology associated with *APP*, *PSEN1* and *PSEN2* mutations; and tauopathy associated with *MAPT* mutations, representing the two major pathogenic hypotheses for AD [16-21,301].

Multiple polymorphic risk variants can increase neuronal vulnerability to premature death. Among these susceptibility genes, the apolipoprotein E (*APOE*) gene (19q13.2) (*AD2*) is the most prevalent as a risk factor for AD, especially in those subjects harboring the *APOE-4* allele, whereas carriers

Figure 5. ***APOE****-related brain optical topography mapping in AD patients.*

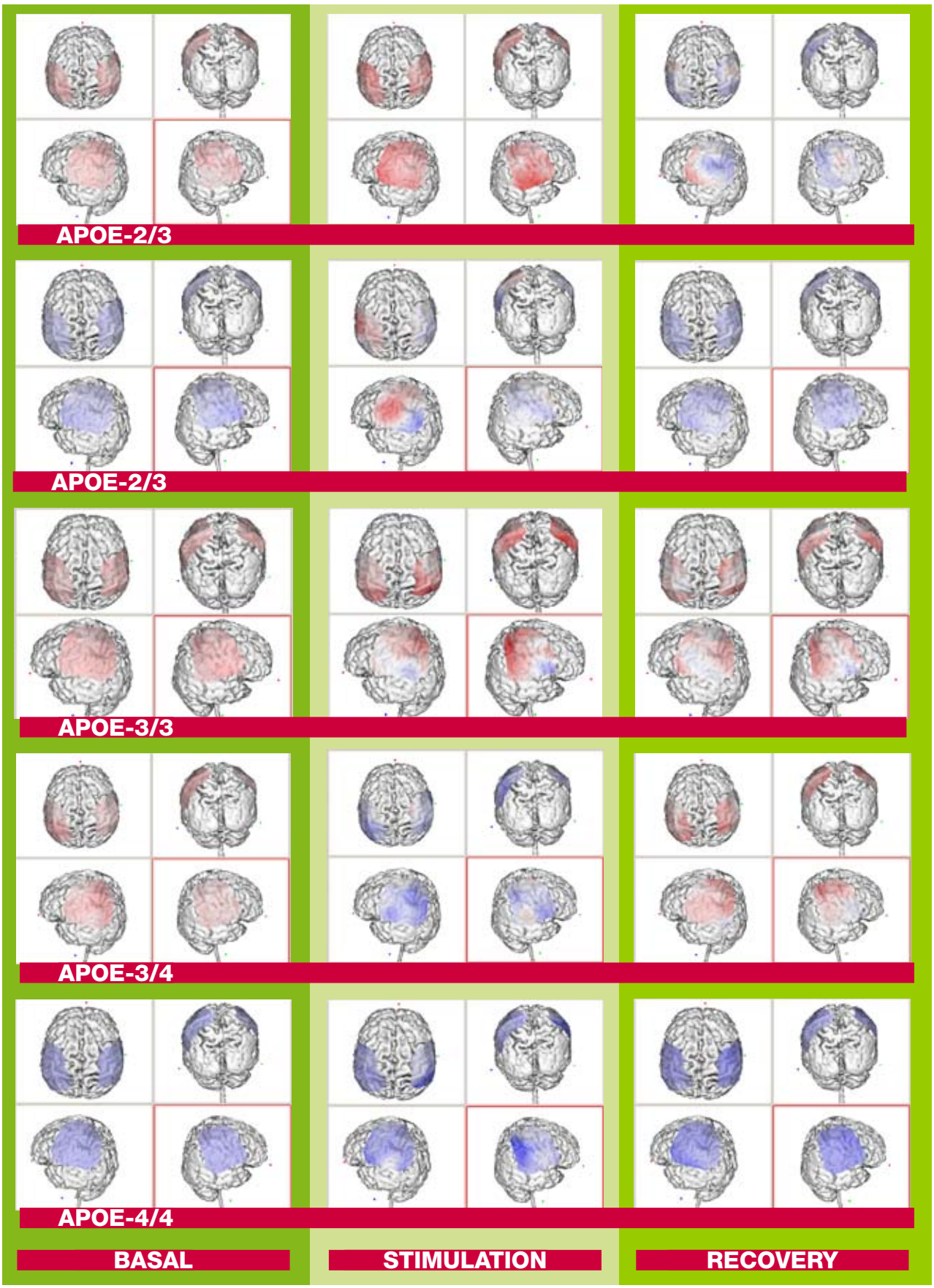
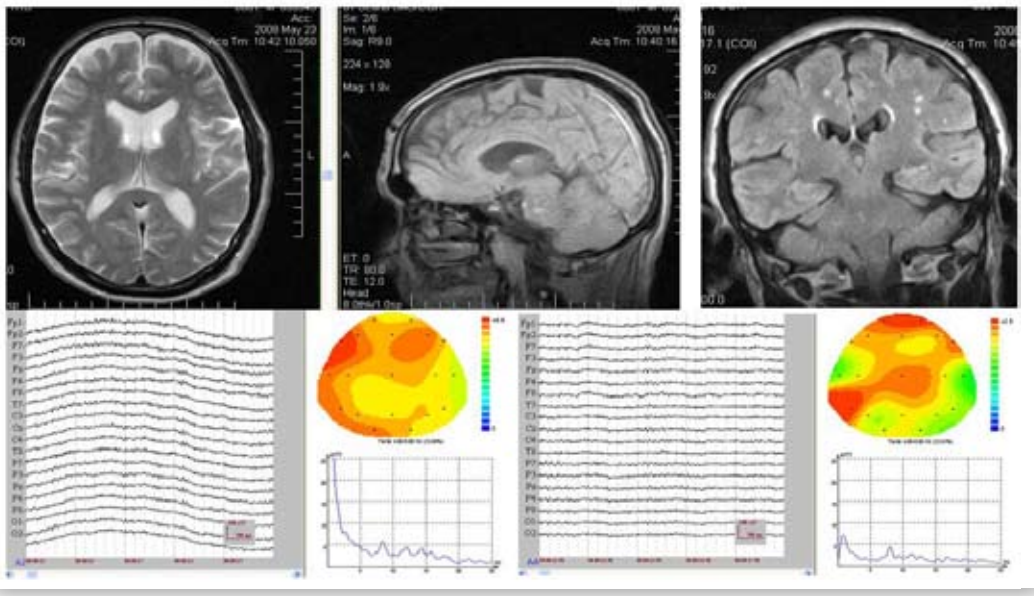


Figure 6. MRI and brain mapping activity (pre- *vs* post-treatment with a multifactorial therapy) in an AD-*APOE-3/3* carrier.



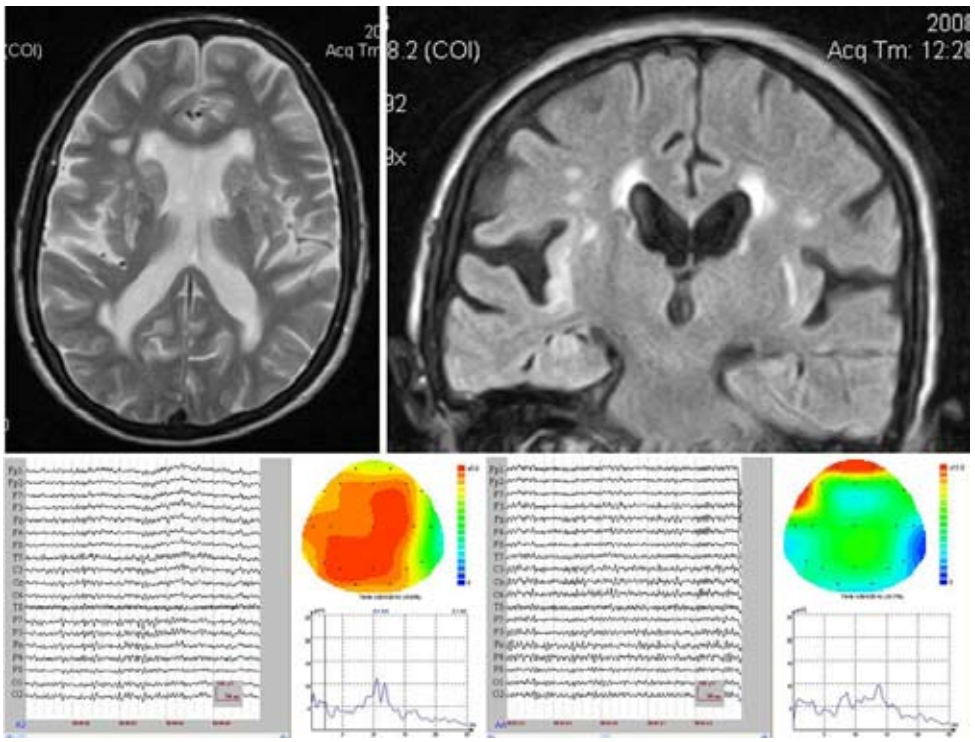
of the *APOE-2* allele might be protected against dementia. *APOE*-related pathogenic mechanisms are also associated with brain aging and with the neuropathological hallmarks of AD [16].

Pleiotropic activity of *APOE* in Dementia

APOE is the prototypical paradigm of a pleiotropic gene with multifaceted activities in physiological and pathological conditions [16,22]. ApoE is consistently associated with the amyloid plaque marker for AD. APOE-4 may influence AD pathology interacting with APP metabolism and Aβ accumulation, enhancing hyperphosphorylation of tau protein and NFT formation, reducing choline acetyltransferase activity, increasing oxidative processes, modifying inflammation-related neuroimmunotrophic activity and glial activation, altering lipid metabolism, lipid transport and membrane biosynthesis in sprouting and synaptic remodeling, and inducing neuronal apoptosis [16,23-25]. To address the complex misfolding and aggregation that initiates the toxic cascade resulting in AD, Petrlova *et al* [26] developed a 2,2,6,6-tetramethylpiperidine-1-oxyl-4-amino-4-carboxylic acid spin-labeled amyloid-β (Aβ) peptide to observe its isoform-dependent interaction with the ApoE protein. Oligomer binding involves the C-terminal domain of ApoE, with ApoE3 reporting a much greater response through this conformational marker. ApoE3 displays a higher affinity and capacity for the toxic Aβ oligomer. ApoE polymorphism and AD risk can largely be attributed to the reduced ability of ApoE4 to function as a clearance vehicle for the toxic form of Aβ. MAPT and APOE are involved in the pathogenic mechanisms of AD, and both *MAPT H1/H1* genotype and *APOE ε4* allele lead to a more rapid progression to dementia among MCI subjects, probably mediating an increased rate of amyloid-β and tau brain deposition [27].

The distribution of *APOE* genotypes in the Iberian peninsula is as follows: *APOE-2/2* 0.32%, *APOE-2/3* 7.3%, *APOE-2/4* 1.27%, *APOE-3/3* 71.11%, *APOE-3/4* 18.41%, and *APOE-4/4* 1.59% (Figure 2). These frequencies are very similar in Europe and in other Western societies. There is a clear accumulation of *APOE-4* carriers among patients with AD (*APOE-3/4* 30.30%; *APOE-4/4* 6.06%) and vascular dementia (*APOE-3/4* 35.85%, *APOE-4/4* 6.57%) as compared to controls (Figure 2). The distribution and frequencies of *APOE* genotypes in AD also differ from those of patients with anxiety, depression, psychosis, migraine, vascular encephalopathy, and post-traumatic brain injury syndrome. Different *APOE* genotypes confer specific phenotypic profiles to AD patients and in certain cases a risk factor for various CNS disorders [16,22]. Some of these profiles may add risk or benefit when the patients are treated with conventional drugs, and in many instances the clinical phenotype demands the administration of additional drugs which increase the complexity of therapeutic protocols. From studies designed to define *APOE*-related AD phenotypes [5,12,16,20,23-25,30-36], several conclusions can be drawn: (i) the age-at-onset is 5-10 years earlier in approximately 80% of AD cases harboring the *APOE-4/4* genotype; (ii) the serum levels of ApoE are lowest in *APOE-4/4*, intermediate in *APOE-3/3* and *APOE-3/4*, and highest in *APOE-2/3* and *APOE-2/4*; (iii) serum cholesterol levels are higher in *APOE-4/4* than in the other genotypes; (iv) HDL-cholesterol levels tend to be lower in *APOE-3* homozygotes than in *APOE-4* allele carriers; (v) LDL-cholesterol levels are systematically higher in *APOE-4/4* than in any other genotype; (vi)

Figure 7. MRI and brain mapping activity (pre- *vs* post-treatment with a multifactorial therapy) in an *APOE-3/4* carrier with mixed dementia (AD + Binswanger's disease).



triglyceride levels are significantly lower in *APOE-4/4*; (vii) nitric oxide levels are slightly lower in *APOE-4/4*; (viii) serum and cerebrospinal fluid Aβ levels tend to differ between *APOE-4/4* and the other most frequent genotypes (*APOE-3/3*, *APOE-3/4*); (ix) blood histamine levels are dramatically reduced in *APOE-4/4* as compared with the other genotypes; (x) brain atrophy is markedly increased in *APOE-4/4>APOE-3/4>APOE-3/3*; (xi) brain mapping activity shows a significant increase in slow wave activity in *APOE-4/4* from early stages of the disease; (xii) brain hemodynamics, as reflected by reduced brain blood flow velocity and increased pulsatility and resistance indices, is significantly worse in *APOE-4/4* (and in *APOE-4* carriers in general, as compared with *APOE-3* carriers); brain hypoperfusion and neocortical oxygenation is also more deficient in *APOE-4* carriers; (xiii) lymphocyte apoptosis is markedly enhanced in *APOE-4* carriers; (xiv) cognitive deterioration is faster in *APOE-4/4* patients than in carriers of any other *APOE* genotype; (xv) in approximately 3-8% of the AD cases, the presence of some dementia-related metabolic dysfunctions accumulates more in *APOE-4* carriers than in *APOE-3* carriers; (xvi) some behavioral disturbances, alterations in circadian rhythm patterns, and mood disorders are slightly more frequent in *APOE-4* carriers; (xvii) aortic and systemic atherosclerosis is also more frequent in *APOE-4* carriers; (xviii) liver metabolism and transaminase activity also differ in *APOE-4/4* with respect to other genotypes; (xix) hypertension and other cardiovascular risk factors also accumulate in *APOE-4*; and (xx) *APOE-4/4* carriers are the poorest responders to conventional drugs. These 20 major phenotypic features clearly illustrate the biological disadvantage of *APOE-4* homozygotes and the potential consequences that these patients may experience when they receive pharmacological treatment for AD and/or concomitant pathologies [5,12,16,20,23-25,28-36].

Pathogenic events

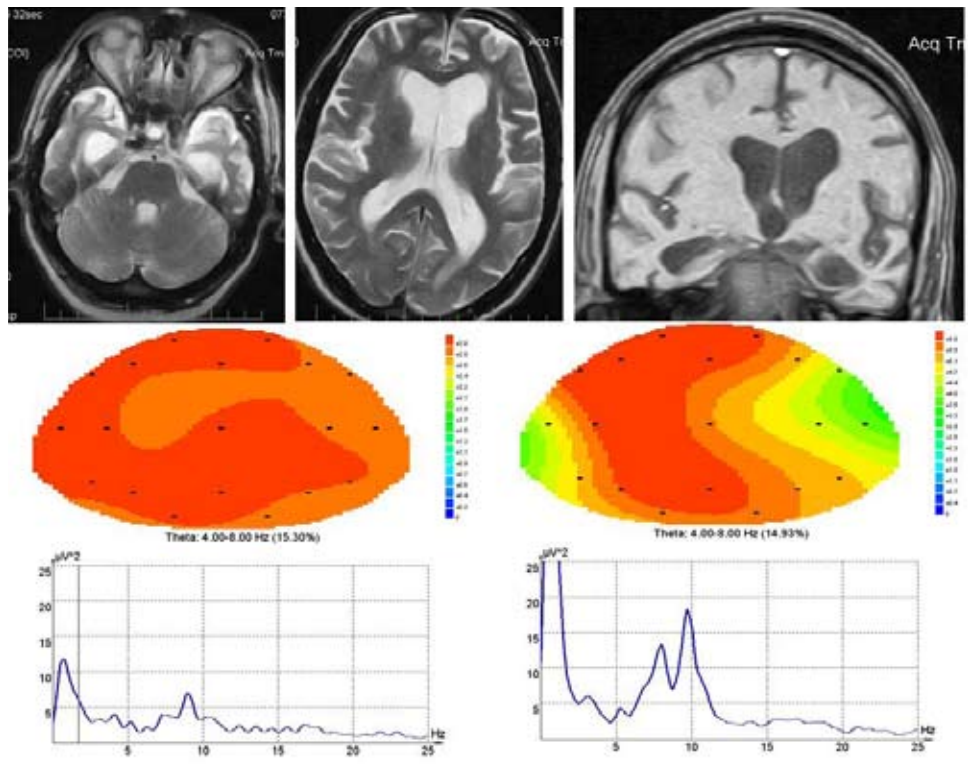
The dual amyloidogenic-tauopathic theory of AD has dominated the pathogenic universe of AD-related neurodegeneration (and divided the research community, as well) for the past 50 years, nourished by the presence of *APP*, *PSEN1*, *PSEN2* and *MAPT* mutations in a very small number of cases with early-onset AD; however, this theory does not explain in full AD pathogenesis, and consequently novel (or complementary) theories have been emerging during the past decades and in recent times. A summary of the pathogenic events in AD include the following:

Genomic defects: As a complex polygenic/multifactorial disorder, in which hundreds of polymorphic variants of risk might be involved, AD fulfils the "golden rule" of complex disorders, according to which the larger the number of genetic defects distributed in the human genome, the earlier the onset of the disease and the poorer its therapeutic response to conventional treatments; and the smaller the number of pathogenic SNPs, the later the onset of the disease, and the better the therapeutic response to different pharmacological interventions [12,16,20,23,24,29]. Genetic variation associated with different diseases interferes with microRNA-mediated regulation by creating, destroying, or modifying microRNA (miRNA) binding sites. miRNA-target variability is a ubiquitous phenomenon in the adult human brain, which may influence gene expression in physiological and pathological conditions. AD-related SNPs interfere with miRNA gene regulation and affect AD susceptibility. The significant interactions include target SNPs present in seven genes -related to AD prognosis with the miRNAs- miR-214, -23a & -23b, -486-3p, -30e*, -143, -128, -27a &-27b, -324-5p and -422a. The dysregulated miRNA network contributes to the aberrant gene expression in AD [37-39].

Epigenetic phenomena: Epigenetic factors have emerged as important mediators of development and aging, gene-gene and gene-environmental interactions, and the pathophysiology of complex disorders. Major epigenetic mechanisms (DNA methylation, histone modifications and chromatin remodeling, and non-coding RNA regulation) may contribute to AD pathology [38,39].

Cerebrovascular dysfunction: Vascular and metabolic dysfunctions are key components in the AD pathology throughout the course of disease. Although common denominators between vascular and metabolic dysfunction are oxidative stress and Aβ [40], genetic factors and cardiovascular risk factors may also account for the cerebrovascular damage present in AD [41]. Inherited polymorphisms of the vascular susceptibility gene Ninjurin2 (*NINJ2*) are associated with AD risk [42]. Endothelial dysfunction has been implicated as a crucial event in the development of AD. Breakdown of the blood-brain barrier (BBB) as a result of disruption of tight junctions and transporters, leads to increased leukocyte transmigration and is an early event in the pathology of many CNS disorders. BBB breakdown leads to neuroinflammation and oxidative stress, with mitochondrial dysfunction. The high concentration of mitochondria in cerebrovascular endothelial cells might account for the sensitivity of the BBB to oxidant stressors [43]. Chronic brain hypoperfusion may be sufficient to induce premature neuronal death and dementia in vulnerable subjects [16,23-25,34,44,45]. APOE-related changes in cortical oxygenation and hemoglobin consumption are evident, as revealed by brain optical topography analysis, reflecting that *APOE-4* carriers exhibit deficient brain hemodynamics and a poorer panneocortical oxygenation than *APOE-3* or *APOE-2* carriers (Figures 3-5). In older persons, extreme changes in hemoglobin levels may be associated with an increased hazard for developing AD and more rapid cognitive decline [46]. Hypoperfusion in frontal, parietal, and temporal regions is a common finding in AD. White matter hyperintensities (WMH) correlate with age and with disease severity [47]. Cerebral amyloid angiopathy (CAA) accounts for the majority of primary lobal intracerebral hemorrhages (ICH) among the elderly and represents the cause of 20% of spontaneous ICHs in patients over 70 years of age. The basis for this disease process is the deposition and formation of eventually destructive amyloid plaques in the walls of brain vessels, predominantly arterial but not excluding venules and capillaries. CAA and CAA-associated microhemorrhages may also participate in the pathogenesis of AD [48]. *APOE* ε*2* and ε*4* are independent risk factors for lobar intracerebral hemorrhage (ICH), consistent with their known associations with amyloid biology. Alleles ε*2* and ε*4* were associated with lobar ICH and ε4 was also associated with increased risk for deep ICH [49]. Incidental cerebral microhemorrhage is frequently found in older individuals scanned with susceptibility-weighted MRI (SWI) or gradient-recalled echo MRI. MH has been linked with β-amyloid (Aβ) deposition using ^{11}C-Pittsburgh compound B (PiB) PET in AD and CAA. Aβ deposition in asymptomatic elderly individuals is associated with lobar MH (LMH). LMH is present in 30.8% of AD, 35.7% of MCI, and 19.1% of controls) [50]. Neurovascular dysfunction in AD leads to reduced clearance across the BBB and accumulation of neurotoxic Aβ peptides in the brain. The ABC transport protein P-glycoprotein (P-gp, ABCB1) is involved in the export of Aβ from the brain into the blood. *P-gp*, *LRP1*, and *RAGE* mRNA expression is reduced in mice treated with $A\beta_{1\text{-}42}$. In addition to the age-related decrease in P-gp expression, $A\beta_{1\text{-}42}$

Figure 8. MRI and brain mapping activity (pre- *vs* post-treatment with a multifactorial therapy) in an AD-*APOE-4/4* carrier.



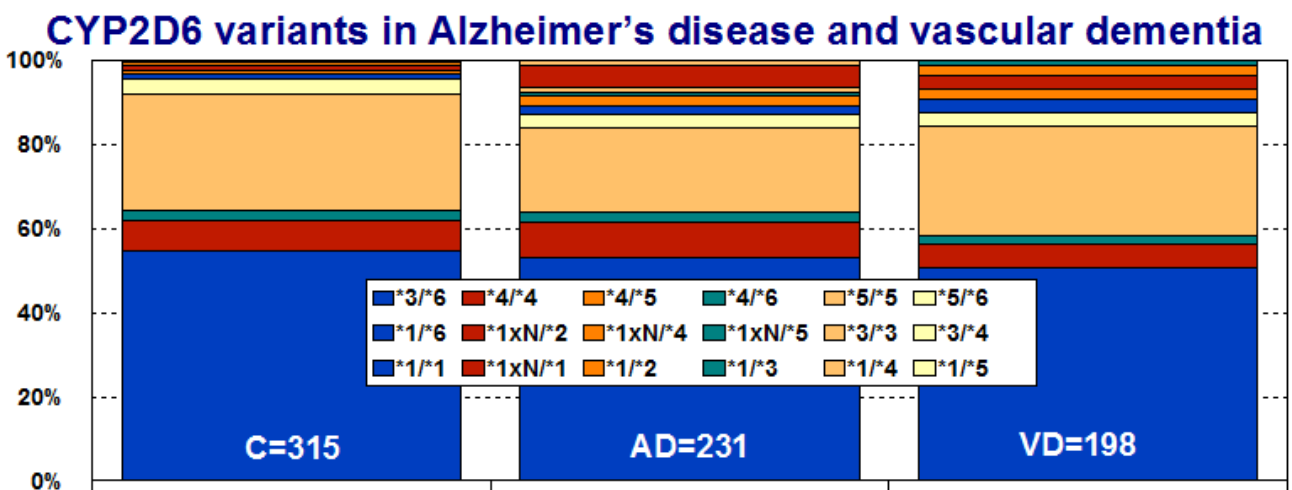
itself downregulates the expression of P-gp and other Aβ-transporters, which could exacerbate the intracerebral accumulation of Aβ and thereby accelerate neurodegeneration in AD and cerebral β-amyloid angiopathy [51].

Phenotypic expression of amyloid deposits and neurofibrillary tangles (NFT): β-Amyloid deposits in senile and neuritic plaques and hyperphosphorylated tau proteins in NFT are extracellular and intracellular expressions, respectively, of the AD neuropathological phenotype, together with selective neuronal loss in hippocampal and neocortical regions. Aβ plaque in the brain is the primary (post mortem) diagnostic criterion of AD. The main component of senile plaques is Aβ, a 39 to 43 amino acid peptide, generated by the proteolytic cleavage of amyloid precursor protein (APP) by the action of beta- and gamma-secretases. Aβ is neurotoxic and the neurotoxicity of Aβ is related to its aggregation state. A new family of fluorescent markers containing an amino naphthalenyl-2-cyano-acrylate (ANCA) motif has been synthesized and evaluated for its capability to associate with aggregated Aβ peptides [52]. Atrophy of the medial temporal lobe, especially the hippocampus and the parahippocampal gyrus, is considered to be the most predictive structural brain biomarker for AD. The medial and posterior parts of the parietal lobe seem to be preferentially affected, compared to the other parietal lobe parts. A new model proposed that myelin breakdown is a beginning of the chain of pathological events leading to AD pathology and an AD diagnosis [53]. Twin studies revealed that cognitively preserved monozygotic cotwins of cognitively impaired probands had increased cortical ^{11}C-PiB uptake (117%-121% of control mean) in their temporal and parietal cortices and the posterior cingulate. Cognitively preserved dizygotic subjects did not differ from the controls [54].

Neuronal apoptosis: Neuronal loss is a pathognomonic finding in AD and the final common path of multiple pathogenic mechanisms leading to neurodegeneration in dementia.

White matter changes: Alterations in white matter, either primary or secondary to vascular events, are frequent findings in AD. White matter damage begins in the core memory network of the temporal lobe, cingulum and prefrontal regions, and spreads beyond these regions in later stages [55].

Figure 9. Distribution and frequency of *CYP2D6* genotypes in dementia.
Source: R. Cacabelos. CIBE Database. EuroEspes Biomedical Research Center, Institute for CNS Disorders and Genomic Medicine, Corunna, Spain.

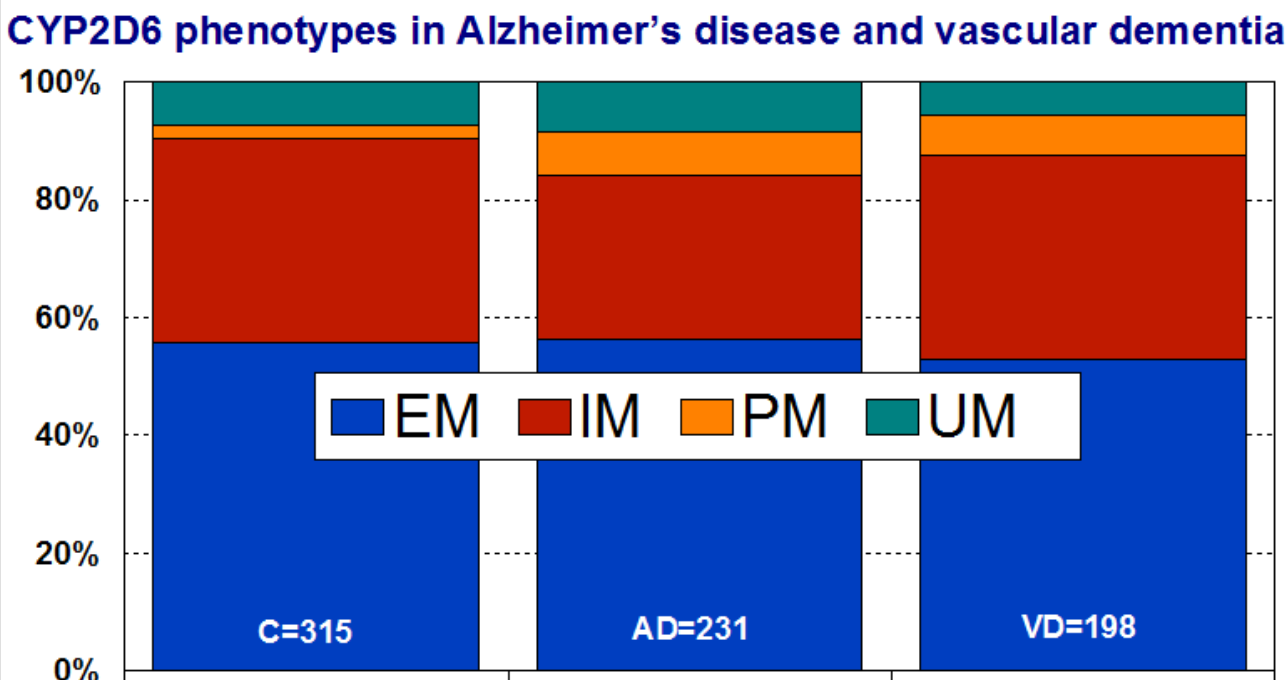


	Control population	Alzheimer's disease	Vascular dementia
*5/*6	0,46	0	0
*5/*5	0	1,06	0
*4/*6	0	0	1,12
*4/*5	0,46	0	2,25
*4/*4	1,37	5,32	3,37
*3/*6	0	0	0
*3/*4	0	0	0
*3/*3	0	1,06	0
*1xN/*5	0	1,06	0
*1xN/*4	0,91	2,12	2,25
*1xN/*2	0	0	0
*1/*6	0,91	2,13	3,37
*1/*5	3,65	3,19	3,37
*1/*4	27,85	20,21	25,84
*1/*3	2,28	2,13	2,25
*1/*2	0	0	0
*1xN/*1	7,31	8,51	5,62
*1/*1	54,79	53,19	50,56

Neurotransmitter deficits: An imbalance of different neurotransmitters (glutamate, acetylcholine, noradrenaline, dopamine, serotonin, some neuropeptides) has been proposed as the neurobiological basis of behavioral symptoms in AD. Altered reuptake of neurotransmitters by vesicular glutamate transporters (VGLUTs), excitatory amino acid transporters (EAATs), the vesicular acetylcholine transporter (VAChT), the serotonin reuptake transporter (SERT), or the dopamine reuptake transporter (DAT) are involved in the neurotransmission imbalance in AD. Protein and mRNA levels of VGLUTs, EAAT1-3, VAChT, and SERT are reduced in AD [56].

Oxidative stress: Oxidative damage is a classic pathogenic mechanism of neurodegeneration. Oxidative damage is greater in brain tissue from patients with AD than age-matched controls. Tayler *et al* [57] studied the timing of this damage in relation to other pathogenic processes in AD. Antioxidant capacity is elevated in AD and directly related to disease severity as indicated by Braak tangle stage and the amount of insoluble Aβ. *APOE ε4* is associated with increased antioxidant capacity in AD. Antioxidant capacity in AD is closely related to the level of insoluble Aβ and increases with pathological progression of the disease. Increased β-secretase activity associated with oxidative stress is likely to contribute to the accumulation of Aβ and this, in turn, to induce antioxidant capacity. Accumulation of Aβ has been shown in brain mitochondria of AD patients and of AD transgenic mouse models. The presence of Aβ in mitochondria leads to free radical generation and neuronal stress. A novel mitochondrial Aβ-degrading enzyme, presequence protease (Pre), has been identified in the mitochondrial matrix. hPreP activity is decreased in AD brains and in the mitochondrial matrix of AD transgenic mouse brains (Tg mAβPP and Tg mAβPP/ABAD). Mitochondrial fractions isolated from AD brains and Tg mAβPP mice have higher levels of 4-hydroxynonenal, an oxidative product. Activity of cytochrome c oxidase is significantly reduced in the AD mitochondria. Decreased PreP proteolytic activity, possibly due to enhanced ROS production, may contribute to Aβ accumulation in mitochondria leading to the mitochondrial toxicity and neuronal death in AD [58]. There is an age-dependent increase in oxidative stress markers, loss of lipid asymmetry, and Aβ production and amyloid deposition in the brain of *APP/PS1* mice. Proteomic analysis of $APP^{NLh}/APP^{NLh}\times PS\text{-}1^{P246L}/PS\text{-}1^{P246L}$ human double mutant knock-in *APP/PS-1* mice revealed specific targets of brain protein carbonylation in an age-dependent manner [59].

Figure 10. Distribution and frequency of *CYP2D6* phenotypes in dementia.
Source: R. Cacabelos. CIBE Database. EuroEspes Biomedical Research Center, Institute for CNS Disorders and Genomic Medicine, Corunna, Spain.



	Control population	Alzheimer's disease	Vascular dementia
UM	7,31	8,51	5,62
PM	2,28	7,45	6,74
IM	34,7	27,66	34,83
EM	55,71	56,38	52,81

Cholesterol and lipid metabolism dysfunction: Cholesterol seems to be intimately linked with the generation of amyloid plaques, which is central to the pathogenesis of AD. APOE variants are determinant in cholesterol metabolism and diverse forms of dyslipoproteinemia [60]. Cholesterol protects the Aβ-induced neuronal membrane disruption and inhibits beta-sheet formation of Aβ on the lipid bilayer [61]. Jones *et al* [62] found a significant overrepresentation of association signals in pathways related to cholesterol metabolism and the immune response in both of the two largest genome-wide association studies for LOAD. Intracellular lipid metabolism is perturbed in cardiovascular and neurodegenerative diseases with genetic and lifestyle components. Neural membranes contain several classes of glycerophospholipids (GPs), that not only constitute their backbone but also provide the membrane with a suitable environment, fluidity, and ion permeability. GP and GP-derived lipid mediators may be involved in AD pathology. Degradation of GPs by phospholipase A_2 can release two important brain polyunsaturated fatty acids (PUFAs), arachidonic acid and docosahexaenoic acid. Non-enzymatic and enzymatic oxidation of these PUFAs produces several lipid mediators, all closely associated with neuronal pathways involved in AD neurobiology [63].

Neuroinflammation and immunopathology: Several genes associated with immune regulation and inflammation show polymorphic variants of risk in AD, and abnormal leves of diverse cytokins have been reported in the brain, CSF and plasma of patients with AD [16,23]. The activation of inflammatory cascades has been consistently demonstrated in the pathophysiology of AD. Reactive microglia are associated with Aβ deposits and clearance in AD. Resident microglia fail to trigger an effective phagocytic response to clear Aβ deposits although they mainly exist in an "activated" state. Oligomeric Aβ (oAbβ) can induce more potent neurotoxicity when compared with fibrillar Aβ (fAβ). $Aβ_{1-42}$ fibrils, not $Aβ_{1-42}$ oligomers, increased the microglial phagocytosis. Pan *et al* [64] found that the pretreatment of microglia with $oAβ_{1-42}$ not only attenuated $fAβ_{1-42}$-triggered classical phagocytic response to fluorescent microspheres but also significantly inhibited phagocytosis of fluorescent labeled $fAβ_{1-42}$. Compared with the $fAβ_{1-42}$ treatment, the $oAβ_{1-42}$ treatment resulted in a rapid and transient increase in interleukin 1β (IL-1β) level and produced higher levels of tumor necrosis factor-alpha (TNF-α), nitric oxide (NO), prostaglandin E_2 (PGE2) and intracellular superoxide anion. Microglial phagocytosis was negatively correlated with inflammatory mediators in this process and the capacity of phagocytosis in $fAβ_{1-42}$-induced microglia was decreased by IL-1β lipolysaccharide and tert-butyl hydroperoxide. The decreased phagocytosis could be relieved by pyrrolidone dithiocarbamate, a nuclear factor-kappaB inhibitor, and N-acetyl-L-cysteine, a free radical scavenger, indicating that the oAβ-impaired phagocytosis was mediated through inflammation and oxidative stress-mediated mechanism in microglial cells. Aβ oligomers induce a potent inflammatory response and subsequently disturb microglial phagocytosis and clearance of Aβ fibrils, thereby contributing to a neurodegenerative cascade. Among several putative neuroinflammatory mechanisms, the TNF-α signaling system has a central role in this process. TNF-α levels are altered in serum and CSF in AD. The abnormal production of inflammatory factors may accompany the progression from mild cognitive impairment (MCI) to dementia. Abnormal activation of TNF-α signaling system, represented by increased expression of sTNFR1, is associated with a higher risk of progression from MCI to AD [65].

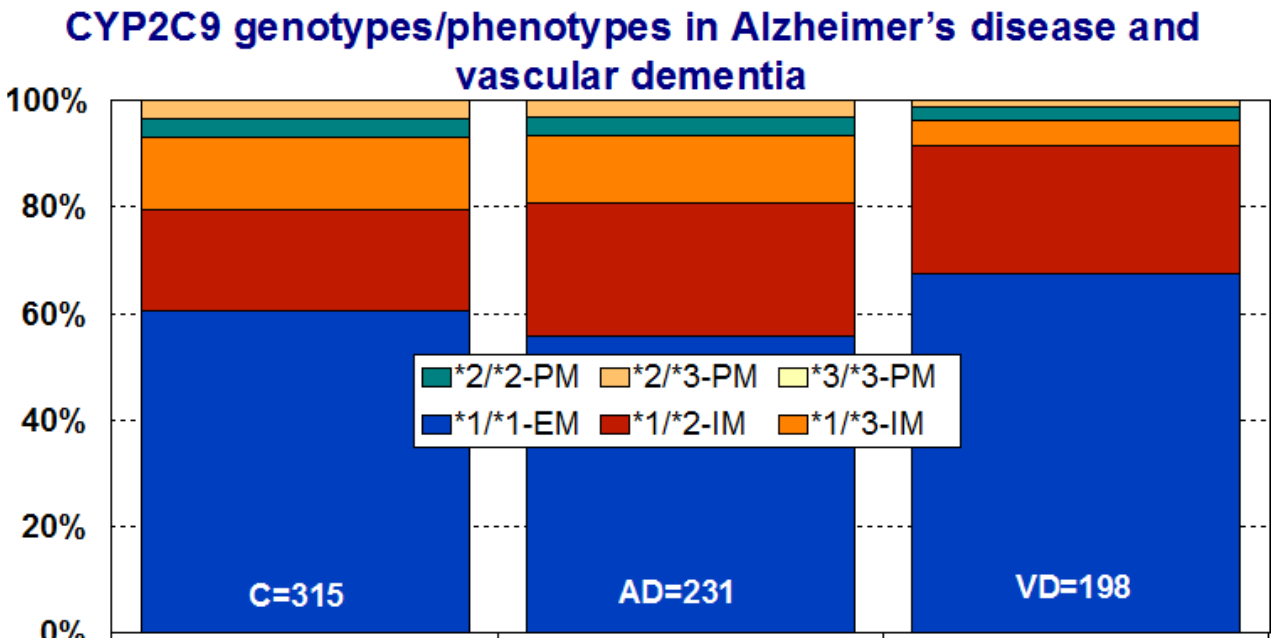
Neurotoxic factors: Old and new theories suggest that different toxic agents, from metals (i.e. aluminium, copper, zinc, iron) to biotoxins and pesticides, might contribute to neurodegeneration. Dysfunctional homeostasis of transition metals is believed to play a role in the pathogenesis of AD [66].

Other players: Many novel pathogenic mechanisms potentially involved in AD neurodegeneration have been proposed in recent times and the revival of some old hypotheses has also occurred. Examples of other pathogenic players in AD include the Ca^{2+} hypothesis [68], insulin resistance [69], NGF imbalance [70], glycogen synthase kinase-3 (GSK-3), advanced glycation end products (AGEs) and their receptors (RAGE), the efflux transporter P-glycoprotein (P-gp), c-Abl tyrosine kinase [71], post-transcriptional protein alterations, compromising the proteasome system and the chaperon machinery (HSPB8-BAG3) [16,23,72], autophagy as novel Aβ-generating pathway, hypocretin (orexin), cathepsin B [73], Nogo receptor proteins [74], adipocytokines and CD34+ progenitor cells [75], CD147 [76], impairment of synaptic plasticity (PSD-95) [77], anomalies in neuronal cell division and apoptosis [78], stem cell factor (SCF), telomere shortening [79], deficiency in repair of nuclear and mitochondrial DNA damage, and microRNAs [80].

Biomarkers and Comorbidity

The phenotypic features of the disease represent the biomarkers to be used as diagnostic predictors and the expression of pathogenic events to be modified with an effective therapeutic intervention. Important differences have been found in the AD population as compared with healthy subjects in different biological parameters, including blood pressure, glucose, cholesterol and triglyceride levels, transaminase activity, hematological parameters, metabolic factors, thyroid function, brain hemodynamic parameters, and brain mapping activity

Figure 11. Distribution and frequency of *CYP2C9* genotypes in dementia.
Source: R. Cacabelos. CIBE Database. EuroEspes Biomedical Research Center, Institute for CNS Disorders and Genomic Medicine, Corunna, Spain.



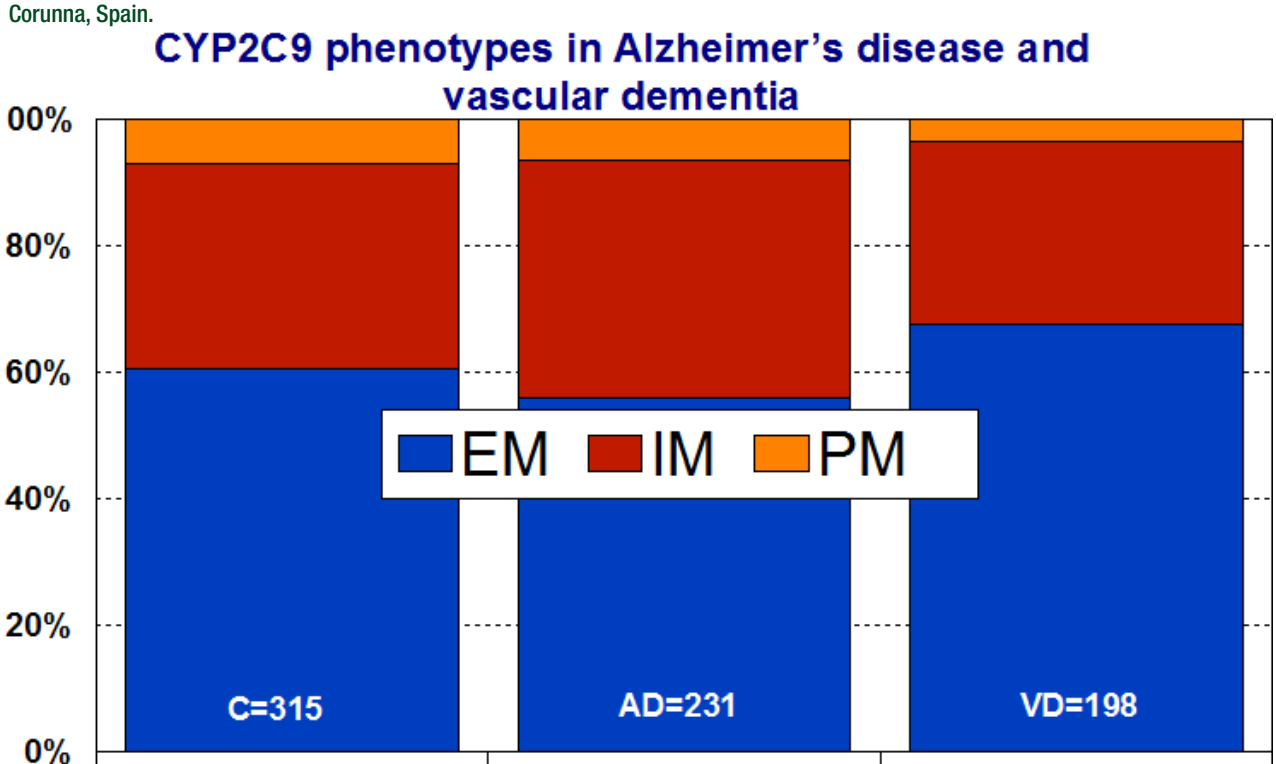
	Control population	Alzheimer's disease	Vascular dementia
*3/*3-PM	0	0	0
*2/*3-PM	3,28	3,23	1,2
*2/*2-PM	3,76	3,23	2,41
*1/*3-IM	13,62	12,9	4,82
*1/*2-IM	18,78	24,73	24,1
*1/*1-EM	60,56	55,91	67,47

[5,20,23-25,30-32]. These clinical differences indicate clear signs of comorbidity rather than typical features of AD. Blood pressure values, glucose levels and cholesterol levels are higher in AD than in healthy elderly subjects. Approximately 20% of AD patients are hypertensive, 25% are diabetic, 50% are hypercholesterolemic, and 23% are hypertriglyceridemic. Over 25% of the patients exhibit high GGT activity, 5-10% show anemic conditions, 30-50% show an abnormal cerebrovascular function characterized by poor brain perfusion, and over 60% have an abnormal electroencephalographic pattern, especially in frontal, temporal, and parietal regions, as revealed by quantitative EEG (qEEG) or computerized mapping [5,12,23]. Significant differences are currently seen between females and males, indicating the effect of gender on the phenotypic expression of the disease. In fact, the prevalence of dementia is 10-15% higher in females than in males from 65 to 85 years of age. All these parameters are highly relevant when treating AD patients because some of them reflect a concomitant pathology which also needs therapeutic consideration.

AD biomarkers can be differentiated within several categories: (i) neuropathological markers, (ii) structural and functional neuroimaging markers, (iii) neurophysiological markers (EEG, qEEG, brain mapping), (iv) biochemical markers in body fluids (blood, urine, saliva, CSF), and (iv) genomic markers (structural and functional genomics, proteomics, metabolomics).

Neuropathology: Plaques and tangles in the hippocampus and cortex are still considered the seminal findings in AD neuropathology, and conventional features to establish the boundary between amyloidopathies and tauopathies; however, both phenotypic markers are also present in normal brains [81], in over 60% of cases with traumatic brain injury [82], and in many other brain disorders. Steroid-responsive encephalopathies can be considered vasculitic or non-vasculitic. Clinical features are suggestive of Creutzfeld-Jakob disease (CJD), dementia with Lewy bodies (DLB), and parkinsonism, but pathological examination revealed only AD-related findings without evidence of Lewy bodies or prion disease in most cases. AD is not diagnosed in life due to the atypical clinical features, lack of hippocampal atrophy on brain imaging, and a dramatic symptomatic response to steroids [83]. Some cases of new-variant CJD or the variably protease-sensitive prionopathy (VPSPr) may also be misdiagnosed as AD. The dentate gyrus is a major site of neuropathology in

Figure 12. Distribution and frequency of *CYP2C9* phenotypes in dementia.
Source: R. Cacabelos. CIBE Database. EuroEspes Biomedical Research Center, Institute for CNS Disorders and Genomic Medicine, Corunna, Spain.



	Control population	Alzheimer's disease	Vascular dementia
PM	7,04	6,45	3,61
IM	32,39	37,64	28,92
EM	60,56	55,91	67,47

FTLD-TDP (frontotemporal lobar degeneration with transactive response DNA-binding protein of 43 kDa proteinopathy), and most laminae of the cerebral cortex are affected. GRN mutation cases are quantitatively different from sporadic cases while cases with associated hippocampal sclerosis and AD have increased densities of dystrophic neurites and abnormally enlarged neurons, respectively. There is little correlation between the subjective assessment of subtypes and the more objective quantitative data [84]. Atrophy of the corpus callosum in AD is independent of white matter lesions and may be associated with cognitive deterioration [85]. Medial temporal lobe atrophy is a recognized marker of AD; however, it can be prominent in frontotemporal lobar degeneration (FTLD). Posterior atrophy (PA) is important in AD and may aid the differentiation of AD from FTLD. About 30% of AD patients show PA in the absence of MTA.

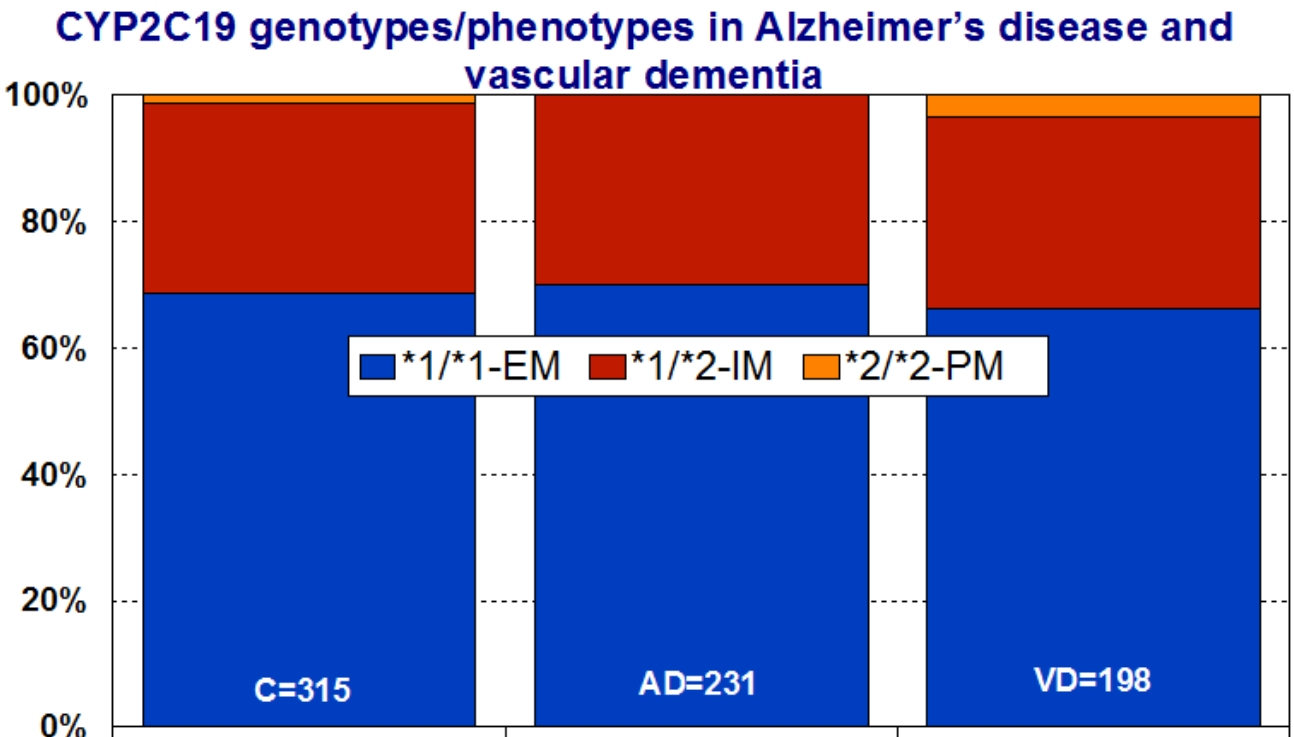
Structural and functional neuroimaging: Structural and functional neuroimaging techniques (MRI, fMRI, PET, SPECT) are essential diagnostic tools in dementia, though the specificity of the visual observations in degenerative forms of dementia is of doubtful value; however, these procedures are irreplaceable for differential diagnosis (Figures 6-8). There is a characteristic regional impairment in AD that involves mainly the temporo-parietal association cortices, mesial temporal structures and, to a more variable degree, also the frontal association cortex. This pattern of functional impairment can provide a biomarker for diagnosis of AD and other neurodegenerative dementias at the clinical stage of mild cognitive impairment, and for monitoring of progression. Lu *et al* [86] used Tensor-based morphometry (TBM), a novel computational approach for visualizing longitudinal progression of brain atrophy, to determine whether cognitively intact elderly participants with the ε4 allele demonstrate greater volume reduction than those with the ε2 allele, and found that possession of the *ε4* allele is associated with greater temporal and hippocampal volume reduction well before the onset of cognitive deficits. Healthy young *APOE ε4* carriers have smaller hippocampal volumes than *APOE ε2* carriers. The difference in hippocampal morphology is cognitively/clinically silent in young adulthood, but could render *APOE ε4* carriers more prone to the later development of AD possibly due to lower reserve cognitive capacity. LOAD patients have a selective parahippocampal white matter (WM) loss, while EOAD patients experience a more widespread pattern of posterior WM atrophy. The distinct

regional distribution of WM atrophy reflects the topography of gray matter (GM) loss. *ApoE ε4* status is associated with a greater parahippocampal WM loss in AD. The greater WM atrophy in EOAD than LOAD fits with the evidence that EOAD is a more aggressive form of the disease [87]. Elderly normal *APOE E2* (*APOE2*) carriers exhibit slower rates of hippocampal atrophy and memory decline compared to *APOE3/3* carriers, and *APOE2* carriers have less Alzheimer pathology as reflected by CSF biomarkers [88]. FDG-PET is quantitatively more accurate than perfusion SPECT. Regional metabolic and blood flow changes are closely related to clinical symptoms, and most areas involved in these changes will also develop significant cortical atrophy. FDG-PET is complementary to amyloid PET, which targets a molecular marker that does not have a close relation to current symptoms. FDG-PET is expected to play an increasing role in diagnosing patients at an early stage of AD and in clinical trials of drugs aimed at preventing or delaying the onset of dementia [89]. Functional neuroimaging biomarkers are becoming popular with the introduction of novel tracers for brain amyloid deposits. Amyloid deposition causes severe damage to neurons many years before onset of dementia via a cascade of several downstream effects. Positron emission tomography (PET) tracers for amyloid plaque are desirable for early diagnosis of AD, particularly to enable preventative treatment once effective therapeutics are available. The amyloid imaging tracers flutemetamol, florbetapir, and florbetaben labeled with ^{18}F have been developed for PET; they can be produced commercially at central cyclotron sites and subsequently delivered to clinical PET scanning facilities. These tracers are currently undergoing formal clinical trials to establish whether they can be used to accurately image fibrillary amyloid and to distinguish patients with AD from normal controls and those with other diseases that cause dementia [89]. Changes in the level of plaque burden, as quantified by an amyloid plaque PET tracer, may provide valuable insights into the effectiveness of amyloid-targeted therapeutics. [^{18}F]MK-3328 was identified as a promising PET tracer for *in vivo* quantification of amyloid plaques [90]. Fleisher *et al* [91] characterized quantitative florbetapir-PET measurements of fibrillar Aβ burden in a large clinical cohort of participants with probable AD or mild cognitive impairment (MCI) and older healthy controls (OHCs) who differed in mean cortical florbetapir standard uptake value ratios (SUVRs), in percentage meeting levels of amyloid associated with AD by SUVR criteria (80.9%, 40.0%, and 20.7%, respectively), and in percentage meeting SUVR criteria for the presence of any identifiable Aβ (85.3%, 46.6%, and 28.1%, respectively). Among OHCs, the percentage of florbetapir positivity increased linearly by age decile. *APOE ε4* carriers had a higher mean cortical SUVR than did noncarriers. Wolk *et al* [92] determined the correspondence of *in vivo* quantitative estimates of brain uptake of fluorine 18-labeled flutemetamol with immunohistochemical estimates of amyloid levels in patients who underwent previous biopsy.

Neurophysiology: There is a renewed interest for the use of computerized brain mapping as a diagnostic aid and as a monitoring tool in AD [93]. Electroencephalography (EEG) studies in AD show an attenuation of average power within the alpha band (7.5-13 Hz) and an increase of power in the theta band (4-7 Hz) [94] (Figures 6-8). Thalamocortical circuitry underpins the generation and modulation of alpha and theta rhythms [95]. *APOE* genotypes influence brain bioelectrical activity in AD. In general, *APOE-4* carriers tend to exhibit a slower EEG pattern from early stages [16]; however, it has also been reported that early onset AD and *APOE ε4* negative AD patients present with more severe EEG abnormalities than late onset and *APOE ε4* positive AD patients [96].

Biochemistry of body fluids: Other biomarkers of potential interest include cerebrospinal fluid (CSF) and peripheral levels of $A\beta_{42}$, protein tau, histamine, interleukins, and some other novel candidate markers such as chitinase 3-like 1 (CHI3L1) protein [5,16,25,97-101]. The concentration of the 42 amino acid form of Aβ ($A\beta_{1-42}$) is reduced in the CSF from AD patients, which is believed to reflect the AD pathology with plaques in the brain acting as sinks. Novel C-truncated forms of Aβ ($A\beta_{1-14}$, $A\beta_{1-15}$, and $A\beta_{1-16}$) were identified in human CSF. The presence of these small peptides is consistent with a catabolic amyloid precursor protein cleavage pathway by β- followed by α-secretase. $A\beta_{1-14}$, $A\beta_{1-15}$, and $A\beta_{1-16}$ increase dose-dependently in response to γ-secretase inhibitor treatment while $A\beta_{1-42}$ levels are unchanged [102]. Kester *et al* [103] investigated change over time in CSF levels of amyloid-beta 40 and 42 ($A\beta_{40}$ and $A\beta_{42}$), total tau (tau), tau phosphorylated at threonine 181 (ptau-181), isoprostane, neurofilaments heavy (NfH) and light (NfL). $A\beta_{42}$, tau, and tau phosphorylated at threonine 181, differentiated between diagnosis groups, whereas isoprostane, neurofilaments heavy, and NfL did not. In contrast, effects of follow-up time were only found for nonspecific CSF biomarkers: levels of NfL decreased, and levels of isoprostane, $A\beta_{40}$, and tau increased over time. An increase in isoprostane was associated with progression of mild cognitive impairment to AD, and with cognitive decline. Contrary to AD-specific markers, nonspecific CSF biomarkers show change over time which might be potentially used to monitor disease progression in AD. Soluble amyloid precursor proteins (sAPP) in CSF might also help to improve the identification of patients with incipient AD among patients with MCI [104]. Weight changes are common in aging and AD and postmortem findings suggest a relation between lower body mass index

Figure 13. Distribution and frequency of *CYP2C19* pheno-genotypes in dementia.
Source: R. Cacabelos. CIBE Database. EuroEspes Biomedical Research Center, Institute for CNS Disorders and Genomic Medicine, Corunna, Spain.



	Control population	Alzheimer's disease	Vascular dementia
*2/*2-PM	1,41	0	3,61
*1/*2-IM	30,05	30,11	30,12
*1/*1-EM	68,54	69,89	66,27

(BMI) and increased AD brain pathology. BMI is associated with higher core AD brain pathology as assessed by CSF-based biological markers of AD. Lower BMI is indicative of AD pathology [105]. Furthermore, diet may be a powerful environmental factor that modulates AD risk through its effects on CNS concentrations of $A\beta_{42}$, lipoproteins, oxidative stress, and insulin [106]. Lo *et al* [107] delineated the trajectories of $A\beta_{42}$ level in CSF, fludeoxyglucose F18 (FDG) uptake using PET, and hippocampal volume using MRI and their relative associations with cognitive change at different stages in aging and AD. $A\beta_{42}$ level in CSF, FDG uptake, and hippocampal volume vary across different cognitive stages. The longitudinal patterns support a hypothetical sequence of AD pathology in which amyloid deposition is an early event before hypometabolism or hippocampal atrophy, suggesting that biomarker prediction for cognitive change is stage-dependent.

Genomics and proteomics: Structural markers are represented by SNPs in genes associated with AD, polygenic cluster analysis, and genome-wide studies (GWS). Functional markers attempt to correlate genetic defects with specific phenotypes (genotype-phenotype correlations). In proteomic studies, several candidate CSF protein biomarkers have been assessed in neuropathologically confirmed AD, non-demented (ND) elderly controls and non-AD dementias (NADD). Markers selected included apolipoprotein A-1 (ApoA1), hemopexin (HPX), transthyretin (TTR), pigment epithelium-derived factor (PEDF), $A\beta_{1-40}$, $A\beta_{1-42}$, total tau, phosphorylated tau, α-1 acid glycoprotein (A1GP), haptoglobin, zinc α-2 glycoprotein (Z2GP) and apolipoprotein E (ApoE). The concentrations of $A\beta_{1-42}$, ApoA1, A1GP, ApoE, HPX and Z2GP differed significantly among AD, ND and NADD subjects. The CSF concentrations of these three markers distinguished AD from ND subjects with 84% sensitivity and 72% specificity, with 78% of subjects correctly classified. By comparison, using $A\beta_{1-42}$ alone gave 79% sensitivity and 61% specificity, with 68% of subjects correctly classified. For the diagnostic discrimination of AD from NADD, only the concentration of $A\beta_{1-42}$ was significantly related to diagnosis, with a sensitivity of 58% and a specificity of 86% [108]. Carrying the *APOEε4* allele was associated with a significant decrease in the CSF $A\beta_{1-42}$ concentrations in middle-aged and older subjects. In AD, the $A\beta_{1-42}$ levels are significantly lower in the *APOEε4* carriers compared to the non-carriers. These findings demonstrate significant age effects on the CSF $A\beta_{1-42}$ and pTau181 across lifespan, and also suggest that the decrease in $A\beta_{1-42}$, but not the increase in

pTau181 CSF levels, is accelerated by the *APOE ε4* genotype in middle-aged and older adults with normal cognition [109]. Han *et al* [110] carried out a genome-wide association study (GWAS) in order to better define the genetic backgrounds of normal cognition, mild cognitive impairment (MCI) and AD in terms of changes in CSF levels of Aβ1-42, T-tau, and P-tau181P. CSF $A\beta_{1-42}$ levels decreased with *APOE* gene dose for each subject group. T-tau levels tended to be higher among AD cases than among normal subjects. *CYP19A1* 'aromatase' (rs2899472), *NCAM2*, and multiple SNPs located on chromosome 10 near the *ARL5B* gene demonstrated the strongest associations with $A\beta_{1-42}$ in normal subjects. Two genes found to be near the top SNPs, *CYP19A1* (rs2899472) and *NCAM2* (rs1022442) have been reported as genetic factors related to the progression of AD. In AD subjects, *APOE ε2/ε3* and *ε2/ε4* genotypes were associated with elevated T-tau levels, and the *ε4/ε4* genotype was associated with elevated T-tau and P-tau181P levels. Blood-based markers reflecting core pathological features of AD in pre-symptomatic individuals are likely to accelerate the development of disease-modifying treatments. Thambisetty *et al* [111] performed a proteomic analysis to discover plasma proteins associated with brain Aβ burden in non-demented older individuals. A panel of 18 2DGE plasma protein spots effectively discriminated between individuals with high and low brain Aβ. Mass spectrometry identified these proteins, many of which have established roles in Aβ clearance, including a strong signal from ApoE. A strong association was observed between plasma ApoE concentration and Aβ burden in the medial temporal lobe. Targeted voxel-based analysis localized this association to the hippocampus and entorhinal cortex. *APOE ε4* carriers also showed greater Aβ levels in several brain regions relative to *ε4* non-carriers. Both peripheral concentration of ApoE protein and *APOE* genotype may be related to early neuropathological changes in brain regions vulnerable to AD pathology even in the non-demented elderly. Transcriptome analysis of leukocytes from patients of mild cognitive impairment MCI, AD, and controls by oligonucleotide microarray identified 8 genes significantly associated with purine metabolism and the ABC transporters. The *ABCB1* gene exhibited significantly positive correlation with MMSE scores [112].

Therapeutic Strategies

Modern therapeutic strategies in AD are addressed to interfering with the main pathogenic mechanisms potentially involved in AD [5,12,15,20,23,24,28,29,30-36]. Since the early 1980s, the neuropharmacology of AD was dominated by the acetylcholinesterase inhibitors, represented by tacrine, donepezil, rivastigmine,

and galantamine [113-115]. Memantine, a partial NMDA antagonist, was introduced in the 2000s for the treatment of severe dementia [116]; and the first clinical trials with immunotherapy, to reduce amyloid burden in senile plaques, were withdrawn due to severe ADRs [117,118]. During the past few years no relevant drug candidates have been postulated for the treatment of AD, despite the initial promises of β- and γ-secretase inhibitors [119,120]. However, assuming that the best treatment for AD is neuronal death prevention prior to the onset of the disease, novel therapeutic options and future candidate drugs for AD might be a new generation of anti-amyloid vaccines, such as DNA $A\beta_{42}$ trimer immunization [121] or vaccines developed with new immunogenic procedures [122], heterocyclic indazole derivatives [inhibitors of the serum- and glucocorticoid-inducible-kinase 1 (SGK1)] [123], NSAID-like compounds [124], neostatins [125], IgG-single chain Fv fusion proteins [126], Hsp90 inhibitors and HSP inducers [127], inhibitors of class I histone deacetylases [128], some phenolic compounds [129], agonists of the peroxisome proliferator activated receptor gamma (PPARgamma) [130], microRNAs [131,132], and gene silencing (RNAi) [133]. Current drug development for the treatment of AD is principally based on the amyloid cascade theory, and aims to reduce the levels of Aβ amyloid peptide in the brain. Some novel therapeutic options and candidate drugs postulated up to 2011 include: (i) new cholinesterase inhibitors, cholinergic receptor agonists, and monoamine regulators [134-137]; (ii) diverse natural compounds derived from vegetal sources (alkaloids from the calabar bean (*Physostigma venenosum*); huperzine A from *Huperzia serrata*; galantamine from the snowdrop *Galanthus woronowii*; cannabinoids (cannabidiol from *Cannabis sativa*); saffron (*Crocus sativus*); ginseng (Panax species); sage (Salvia species); lemon balm (*Melissa officinalis*); *Polygala tenuifolia*; nicotine from Nicotiana species [138]; grape seed polyphenolic extracts; Fuzhisan, a Chinese herbal medicine [139]; resveratrol [140]; xanthoceraside [141]; garlic (*Allium sativum*) [142]; linarin from *Mentha arvensis* and *Buddleja davidii* [143]; carotenoids such as retinoic acid, all trans retinoic acid, lycopene and β-carotene [144]; curcumin from the rhizome of *Curcuma longa* [145]; plants of different origin such as Yizhi Jiannao, *Moringa oleifera* (Drumstick tree), *Ginkgo biloba* (Ginkgo/Maidenhair tree), *Cassia obtisufolia* (Sicklepod), *Desmodium gangeticum* (Sal Leaved Desmodium), *Melissa officinalis* (Lemon Balm), and *Salvia officinalis* (Garden sage, common sage) [146]; decursinol from the roots of *Angelica gigas* [147]; *Bacopa monniera* Linn (Syn. Brahmi); olive oil; phytoestrogens [148]; walnut extract [149]; *Erigeron annuus* leaf extracts; Epigallocatechin-3-gallate and luteolin [150]; the brown algae *Ecklonia cava* [151]; Gami-Chunghyuldan, a standardized multi-herbal medicinal formula [152]; Salvia species [153]; *Punica granatum* extracts [154]); (iii) immunotherapy and treatment options for tauopathies (tau kinase inhibitors, 2-aminothiazoles, phosphoprotein phosphatase 2A (PP2A) inhibitors, c-Jun N-terminal kinase (JNKs) inhibitors, p38 MAP kinase inhibitors (CNI-1493), the β-carboline alkaloid Harmine) [155-158]; (iv) immunotherapy and Aβ breakers for AD-related amyloidopathy [159-163]; (v) secretase inhibitors (β- and γ-secretase inhibitors) [164]; (vi) statins [125]; (vii) neurosperoids; (viii) phosphodiesterase inhibitors [165]; (ix) protein phosphatase methylesterase-1 inhibitors [166]; (x) Histone deacetylase inhibitors [167]; (xi) mTOR inhibitors [168]; (xii) peroxisome proliferator-activated receptor agonists; (xiii) P-glycoprotein regulators [169]; (xiv) nuclear receptor agonists [170]; (xv) glycogen synthase kinase-3β (GSK-3β) regulators [171]; (xvi) histamine H_3 receptor inverse agonists [172]; (xvii) estrogens [173]; (xviii) kynurenine 3-monooxygenase inhibitors [174]; (ixx) chaperones (small heat shock proteins, sHSPs) [175-177]; (xx) a series of miscellaneous strategies (sodium fullerenolate [178], glucagon-like peptide-1 (GLP-1) [179], chemokines, macrophage inflammatory protein-2 (MIP-2) and stromal cell-derived factor-1α (SDF-1α) [180], cyclooxygenase-1 and cyclooxygenase-2 inhibitors [181], bone morphogenetic protein 9 (BMP-9) [182], granulocyte colony stimulating factor (G-CSF)/AMD3100 (CXCR4 antagonist) and stromal cell-derived factor-1α (SDF-1α), vitamin D, vitamin C, retinoids, ω-3 polyunsaturated fatty acids (n-3 PUFAs), docosahexaenoic acid (DHA, C22:6 n-3), sphingosylphosphorylcholine [183], citidine-5-diphosphocholine or citicoline (CDP-choline) [12,15,23,32,34], cathepsin B inhibitors, pituitary adenylate cyclase activating polypeptide, NAP (davunetide), transcription factor specificity protein 1 (Sp1) inhibitors (tolfenamic acid), TNF inhibitors (2-(2, 6-dioxopiperidin-3-yl)phthalimidine EM-12 dithiocarbamates, N-substituted 3-(phthalimidin-2-yl)-2,6-dioxopiperidines, 3-substituted 2,6-dioxopiperidines) [184], pyrrolo[3,2-e][1,2,4] triazolo[1,5-a]pyrimidine (SEN1176) [185], latrepirdine, leucettines, dihydropyridines (inhibitors of L-type calcium channels), brain-penetrating angiotensin-converting enzyme (ACE) inhibitors (perindopril), NADPH oxidase inhibitors (apocynin) [186]); and (xxi) microRNAs (miRNAs) [132,187].

Pharmacogenomics

AD patients may take 6-12 different drugs/day for the treatment of dementia-related symptoms, including memory decline (conventional anti-dementia drugs, neuroprotectants), behavioral changes (antidepressants, neuroleptics, sedatives, hypnotics), and functional decline, or for the treatment of concomitant pathology (epilepsy, cardiovascular and cerebrovascular disorders,

parkinsonism, hypertension, dyslipidemia, anemia, arthrosis, etc). The co-administration of several drugs may cause side-effects and adverse drug reactions (ADRs) in over 60% of AD patients, who in 2-10% of the cases require hospitalization. In over 20% of the patients, behavioral deterioration and psychomotor function can be severely altered by polypharmacy. The principal causes of these iatrogenic effects are (i) the inappropriate combination of drugs, and (ii) the genomic background of the patient, responsible for his/her pharmacogenomic outcome. Pharmacogenomics account for 30-90% variability in pharmacokinetics and pharmacodynamics. The genes involved in the pharmacogenomic response to drugs in AD fall into five major categories: (i) genes associated with AD pathogenesis and neurodegeneration (*APP*, *PSEN1*, *PSEN2*, *MAPT*, *PRNP*, *APOE* and others); (ii) genes associated with the mechanism of action of drugs (enzymes, receptors, transmitters, messengers); (iii) genes associated with drug metabolism [phase I (*CYPs*) and phase II reactions (*UGTs*, *NATs*)]; (iv) genes associated with drug transporters (*ABCs*, *SLCs*); and (v) pleiotropic genes involved in multifaceted cascades and metabolic reactions (*APOs*, *ILs*, *MTHFR*, *ACE*, *AGT*, *NOS*, etc).

In over 100 clinical trials for dementia, *APOE* has been used as the only gene of reference for the pharmacogenomics of AD [5,12,15,16,20,22-25,28,29,30-36]. Several studies indicate that the presence of the *APOE-4* allele differentially affects the quality and extent of drug responsiveness in AD patients treated with cholinergic enhancers (tacrine, donepezil, galantamine, rivastigmine), neuroprotective compounds (nootropics), endogenous nucleotides (CDP-choline), immunotrophins (anapsos), neurotrophic factors (cerebrolysin), rosiglitazone or combination therapies [188-190]; however, controversial results are frequently found due to methodological problems, study design, and patient recruitment in clinical trials. The major conclusion in most studies is that *APOE-4* carriers are the worst responders to conventional treatments [5,12,15,16,20,22-25,28,29,30-36].

When *APOE* and *CYP2D6* genotypes are integrated in bigenic clusters and the *APOE*+*CYP2D6*-related therapeutic response to a combination therapy is analyzed in AD patients, it becomes clear that the presence of the *APOE-4/4* genotype is able to convert pure *CYP2D6*1/*1* extensive metabolizers into full poor responders to conventional treatments, indicating the existence of a powerful

Table 1. Pharmacogenomic profile of selected anti-dementia drugs.
Source: World Guide for Drug Use and Pharmacogenomics [191]

Donepezil Pharmacogenetics

Caution and personalized dose adjustment in patients with the following genotypes:
APOE: *APOE4/4*
CHAT: rs733722
CYP2D6: *CYP2D6*3*, *CYP2D6*4*, *CYP2D6*5*, *CYP2D6*6*, *CYP2D6*7*, *CYP2D6*8*, *CYP2D6*10*, *CYP2D6*17*, *CYP2D6*1xN*
CYP3A4 and *CYP3A5*: *CYP3A4*1*, *CYP3A4*1B*, *CYP3A4*2*, *CYP3A4*3*, *CYP3A4*4*, *CYP3A4*5*, *CYP3A4*6*, *CYP3A4*8*, *CYP3A4*11*, *CYP3A4*12*, *CYP3A4*13*, *CYP3A4*15*, *CYP3A4*17*, *CYP3A4*18*, *CYP3A4*19*, *CYP3A5*3*
Substrate of: CYP2D6 (major); CYP3A4 (major); UGTs
Inhibits: ACHE; BCHE

Galantamine Pharmacogenetics

Caution and personalized dose adjustment in patients with the following genotypes:
APOE: *APOE-2*, *APOE-3* and *APOE-4* (SNPs at codons 112 and 158)
CYP2D6: *CYP2D6*3*, *CYP2D6*4*, *CYP2D6*5*, *CYP2D6*6*, *CYP2D6*7*, *CYP2D6*8*, *CYP2D6*10*, *CYP2D6*17*, *CYP2D6*1xN*
CYP3A4 and *CYP3A5*: *CYP3A4*1*, *CYP3A4*1B*, *CYP3A4*2*, *CYP3A4*3*, *CYP3A4*4*, *CYP3A4*5*, *CYP3A4*6*, *CYP3A4*8*, *CYP3A4*11*, *CYP3A4*12*, *CYP3A4*13*, *CYP3A4*15*, *CYP3A4*17*, *CYP3A4*18*, *CYP3A4*19*, *CYP3A5*3*
Other genes that may be involved: *APP*; *BCHE*; *CHRNA4*; *CHRNA7*; *CHRNB2*
Substrate of: CYP2D6 (major); CYP3A4 (major); UGT1A1
Inhibits: ACHE

Memantine Pharmacogenetics

Caution and personalized dose adjustment in patients with the following genotypes:
APOE: *APOE-2*, *APOE-3*, *APOE-4* (SNPs at codons 112 and 158)
Other genes that may be involved: *GRINA*; *MT-TK*; *PSEN1*
Inhibits: CYP1A2 (weak); CYP2A6 (weak); CYP2B6 (strong); CYP2C9 (weak); CYP2C19 (weak); CYP2D6 (strong); CYP2E1 (weak); CYP3A4 (weak)

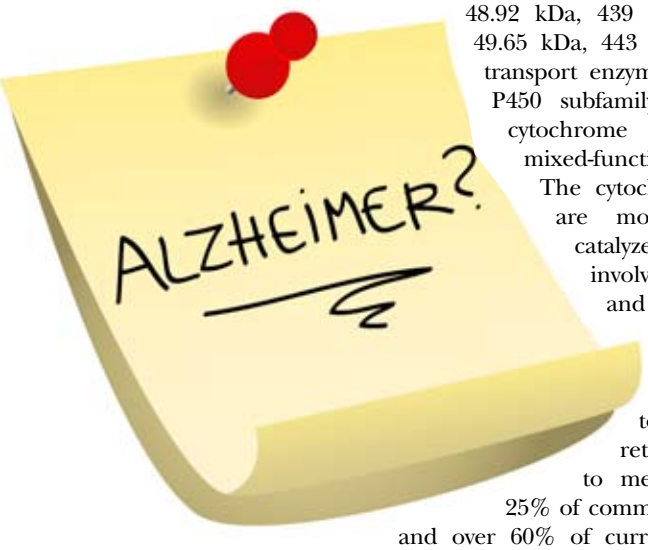
Rivastigmine Pharmacogenetics

Caution and personalized dose adjustment in patients with the following genotypes:
APOE: *APOE-2*, *APOE-3* (wild-type), *APOE-4*. The 2 variant alleles derive from a diplotype of these 2 polymorphisms, such that *APOE-2* is defined by 334T/472T and *APOE-4* is defined by 334C/472C, with combination of 334T/472C characterizing the wild genotype.
Other genes that may be involved: *CHRNA4*; *CHRNB2; MAPT*
Inhibits: ACHE; BCHE

influence of the *APOE-4* homozygous genotype on the drug-metabolizing capacity of pure *CYP2D6* extensive metabolizers. In addition, a clear accumulation of *APOE-4/4* genotypes is observed among *CYP2D6* poor and ultra-rapid metabolizers [12].

In dementia, as in any other CNS disorder, CYP genomics is a very important issue since in practice over 90% of patients with dementia are daily consumers of psychotropics. Furthermore, some acetylcholinesterase inhibitors (the most prescribed anti-dementia drugs worldwide) are metabolized via CYP enzymes (Table 1). Most CYP enzymes display highly significant ethnic differences, indicating that the enzymatic capacity of these proteins varies depending upon the polymorphic variants present in their coding CYP genes. The practical consequence of this genetic variation is that the same drug can be differentially metabolized according to the genetic profile of each subject, and that knowing the pharmacogenomic profile of an individual, his/her pharmacodynamic response is potentially predictable. This is the cornerstone of pharmacogenetics. In this regard, the *CYP2D6*, *CYP2C19*, *CYP2C9* and *CYP3A4/5* genes and their respective protein products deserve special consideration.

CYP2D6: *CYP2D6* is a 4.38 kb gene with 9 exons mapped on 22q13.2. Four RNA transcripts of 1190-1684 bp are expressed in the brain, liver, spleen and reproductive system where 4 major proteins are identified: CYP2D6-001, 55.73 kDa, 497 aa; CYP2D6-002, 50.02 kDa, 446 aa; CYP2D6-004, 55.19 kDa, 494 aa; CYP2D6-201, 48.92 kDa, 493 aa; CYP2D6-202, 48.92 kDa, 439 aa; and CYP2D6-203, 49.65 kDa, 443 aa. This protein is a transport enzyme of the cytochrome P450 subfamily IID or multigenic cytochrome P450 superfamily of mixed-function monooxygenases. The cytochrome P450 proteins are monooxygenases which catalyze many reactions involved in drug metabolism and synthesis of cholesterol, steroids and other lipids. This protein localizes to the endoplasmic reticulum and is known to metabolize as many as 25% of commonly prescribed drugs and over 60% of current psychotropics. Its substrates include debrisoquine, an adrenergic-blocking drug; sparteine and propafenone, both anti-arrhythmic drugs; and amitryptiline, an anti-depressant. The gene is highly polymorphic in the population. There are 141 *CYP2D6* allelic variants of which -100C>T, -1023C>T, -1659G>A, -1707delT, -1846G>A, -2549delA, -2613-2615delAGA, -2850C>T, -2988G>A, and -3183G>A represent the 10 most important variants [191,305,306]. Different alleles result in the extensive, intermediate, poor, and ultra-rapid metabolizer phenotypes, characterized by normal, intermediate, decreased, and multiplied ability to metabolize the enzyme's substrates, respectively. The hepatic cytochrome P450 system is responsible for the first phase in the metabolism and elimination of numerous endogenous and exogenous molecules and ingested chemicals. P450 enzymes convert these substances into electrophilic intermediates which are then conjugated by phase II enzymes (e.g. UDP glucuronosyltransferases, N-acetyltransferases) to hydrophilic derivatives that can be excreted. According to the database of the World Guide for Drug Use and Pharmacogenomics [191], 982 drugs are *CYP2D6*-related: 371 drugs are substrates, over 300 drugs are inhibitors, and 18 drugs are CYP2D6 inducers. In a study to investigate the elimination routes for the 200 drugs most often sold by prescription count in the United States, the majority (78%) of the hepatically cleared drugs were found to be subject to oxidative metabolism via cytochromes P450 of the families 1, 2 and 3, with major contributions from *CYP3A4/5* (37% of drugs) followed by *CYP2C9* (17%), *CYP2D6* (15%), *CYP2C19* (10%), *CYP1A2* (9%), *CYP2C8* (6%), and *CYP2B6* (4%). Clinically well-established polymorphic CYPs (i.e. *CYP2C9*, *CYP2C19*, and *CYP2D6*) were involved in the metabolism of approximately half of those drugs, including (in particular) NSAIDs metabolized mainly by *CYP2C9*, proton-pump inhibitors metabolized by *CYP2C19*, and β-blockers and several antipsychotics and antidepressants metabolized by *CYP2D6* [192].



The distribution and frequency of *CYP2D6* genotypes (Figure 9) and phenotypes (Figure 10) were investigated in 315 Spanish controls with no family history of neuropsychiatric disorders and in patients with Alzheimer's disease or vascular dementia (Figures 9-10). In healthy subjects, extensive metabolizers (EMs) accounted for 55.71% of the population, whereas intermediate metabolizers (IMs) were 34.7%, poor metabolizers (PMs) 2.28%, and ultra-rapid metabolizers (UMs) 7.31% (Figure 10). There is a European gradient South-North with a decreasing number of PMs from 7-10% to 2-3%, proportional to the distance from Africa where *Homo sapiens* emerged. These geno-phenotypic profiles might be important in the pathogenesis of some CNS disorders and in the therapeutic response to conventional psychotropic drugs as well.

CYP2D6 data in patients with dementia allow to conclude the following: (i) The most frequent *CYP2D6* variants in the Southern European population (Iberian peninsula) are the **1/*1* (57.84%), **1/*4* (22.78%), **1xN/*1* (6.10%), **4/*4* (2.56%), and **1/*3* (2.01%) genotypes, accounting for more than 80% of the population; (ii) the frequency of EMs, IMs, PMs, and UMs is about 59.51%, 29,78%, 4.46%, and 6.23%,

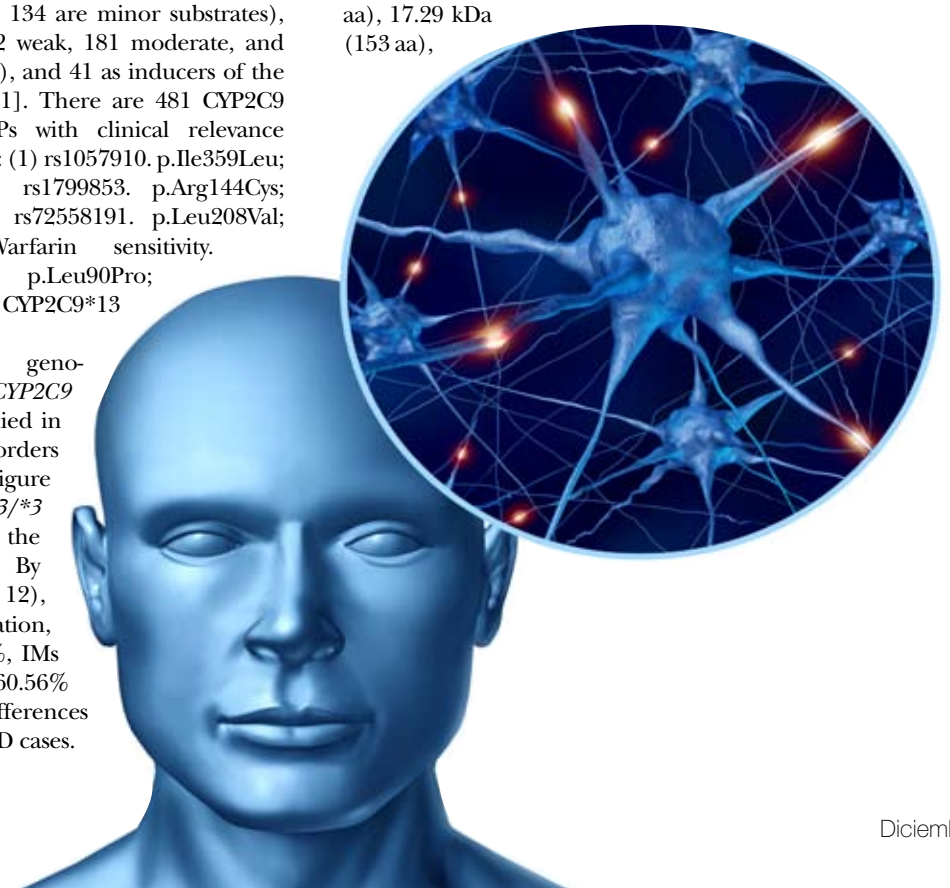
respectively, in the general population, and 57.76, 31.05%, 5.27%, and 5.90%, respectively in AD cases; (iii) EMs are more prevalent in GP (59.51%) than in AD (57.76%); IMs are more frequent in AD (31.05%) than in GP (29.78%); the frequency of PMs is slightly higher in AD (5.27%%) than in GP (4.46%); and UMs are more frequent in GP (6.23%) than in AD (5.90%); (iv) there are differences between females and males in the distribution and frequency of *CYP2D6* genotypes which might be of relevance in therapeutic terms and risk of ADRs; (v) there is an accumulation of AD-related genes of risk in PMs and UMs; (vi) PMs and UMs tend to show higher transaminase activities than EMs and IMs; (vii) EMs and IMs are the best responders, and PMs and UMs are the worst responders to a combination therapy with cholinesterase inhibitors, neuroprotectants, and vasoactive substances; and (viii) the pharmacogenetic response in AD appears to be dependent upon the networking activity of genes involved in drug metabolism and genes involved in AD pathogenesis [5,12,15,23,28,29,30-34,36].

CYP2C9: *CYP2C9* is a gene (50.71 kb) with 9 exons mapped on 10q24. An RNA transcript of 1860 bp is mainly expressed in hepatocytes where a protein of 55.63 kDa (490 aa) can be identified. This protein is a transport enzyme of the cytochrome P450 subfamily IIC, a multigenic cytochrome P450 superfamily of mixed-function monooxygenases, involved in an NADPH-dependent electron transport pathway which oxidizes a variety of structurally unrelated compounds including steroids, fatty acids, and xenobiotics, such as phenytoin, S-warfarin, tolbutamide, and psychotropics. Over 600 drugs are *CYP2C9*-related, 311 acting as substrates (177 are major substrates, 134 are minor substrates), 375 as inhibitors (92 weak, 181 moderate, and 102 strong inhibitors), and 41 as inducers of the CYP2C9 enzyme [191]. There are 481 CYP2C9 SNPs. Selected SNPs with clinical relevance include the following: (1) rs1057910. p.Ile359Leu; g.15489579A>C. (2) rs1799853. p.Arg144Cys; g.15450573C>T. (3) rs72558191. p.Leu208Val; g.15456202T>G. Warfarin sensitivity. (4) rs72558187. p.Leu90Pro; g.47506179T>C. CYP2C9*13 [191].

As with CYP2D6, geno-phenotypes of the *CYP2C9* gene have been studied in different CNS disorders and dementia (Figure 11). No *CYP2C9-*3/*3* cases were found in the control population. By phenotypes (Figure 12), in the control population, PMs represent 7.04%, IMs 32.39%, and EMs 60.56% with no significant differences when compared to AD cases.

CYP2C19: *CYP2C19* is a gene (90.21 kb) with 9 exons mapped on 10q24.1q24.3. RNA transcripts of 1901 bp, 2395 bp, and 1417 bp are expressed in liver cells where a protein of 55.93 kDa (490 aa) is identified. This protein is a transport enzyme of the cytochrome P450 subfamily IIC, a multigenic cytochrome P450 superfamily of mixed-function monooxygenases, which hydroxylates mephenytoin and other xenobiotics, such as omeprazole and other proton pump inhibitors (PPIs), benzodiazepines (e.g. diazepam), and many psychotropics. Nearly 500 drugs are *CYP2C19*-related, 281 acting as substrates (151 are major substrates, 130 are minor substrates), 263 as inhibitors (72 weak, 127 moderate, and 64 strong inhibitors), and 23 as inducers of the CYP2C19 enzyme [191]. About 541 SNPs have been detected in the *CYP2C19* gene. Some of these variants have clinical relevance, including: (1) rs4244285. g.47346080G>C. *CYP2C19*2*. (2) rs4986893. p.Trp212X; g.15288936G>A. *CYP2C19*3*. (3) rs28399504. p.Met1Val; g.15270989A>G. *CYP2C19*4*. (4) rs56337013. p.Arg433Trp; g.15361021C>T. *CYP2C19*5*. (5) rs11188072 (C-3402T) and rs12248560 (C-806T): *CYP2C19*17*. (6) rs58973490. p.Arg150His; g.47339728G>A. *CYP2C19*11*. (7) rs6413438. p.Pro227Leu; g.47346079C>T. *CYP2C19*10*. [191]. The frequencies of the 3 major *CYP2C19* geno-phenotypes in the control population are *CYP2C19-*1/*1*-EMs 68.54%, *CYP2C19-*1/*2*-IMs 30.05%, and *CYP2C19-*2/*2*-PMs 1.41%.(Figure 13).

CYP3A4/5: *CYP3A4* is a gene (27.2 kb) with 13 exons mapped on 7q21.1. RNA transcripts of 2153 bp, 651 bp, 564 bp, 2318 bp and 2519 bp are expressed in intestine, liver, prostate and other tissues where 4 protein variants of 57.34 kDa (503 aa), 17.29 kDa (153 aa),



40.39 kDa (353 aa), and 47.99 kDa (420 aa) are identified. The human *CYP3A* locus contains the three *CYP3A* genes (CYP3A4, *CYP3A5* and *CYP3A7*), three pseudogenes as well as a novel *CYP3A* gene termed *CYP3A43*. The gene encodes a putative protein with between 71.5% and 75.8% identity to the other CYP3A proteins. The predominant hepatic form is CYP3A4, but CYP3A5 contributes significantly to the total liver CYP3A activity. This protein is a transport enzyme of the cytochrome P450 subfamily IIIA, a multigenic cytochrome P450 superfamily of mixed-function monooxygenases, which metabolizes over 1900 drugs, 1033 acting as substrates (897 are major substrates, 136 are minor substrates), 696 as inhibitors (118 weak, 437 moderate, and 141 strong inhibitors), and 241 as inducers of the CYP3A4 enzyme [191].

About 347 SNPs have been identified in the *CYP3A4* gene (*CYP3A4*1A*: Wild-type), 25 of which are of clinical relevance. Concerning *CYP3A4/5* polymorphisms in AD, 82.75% of the cases are EMs (*CYP3A5*3/*3*), 15.88% are IMs (*CYP3A5*1/*3*), and 1.37% are UMs (*CYP3A5*1/*1*). Unlike other human P450s (*CYP2D6*, *CYP2C19*) there is no evidence of a 'null' allele for *CYP3A4*. Generally, variants in the coding regions of *CYP3A4* occur at allele frequencies <5% and appear as heterozygous with the wild-type allele. These coding variants may contribute to, but are not likely to be the major cause of, inter-individual differences in CYP3A-dependent clearance, because of the low allele frequencies and limited alterations in enzyme expression or catalytic function. The most common variant, *CYP3A4*1B*, is an A-392G transition in the 5'-flanking region with an allele frequency ranging from 0% (Chinese and Japanese) to 45% (African-Americans). Studies have not linked *CYP3A4*1B* with alterations in CYP3A substrate metabolism. In contrast, there are several reports about its association with various disease states including prostate cancer, secondary leukemias, and early puberty. Linkage disequilibrium between *CYP3A4*1B* and another *CYP3A* allele (*CYP3A5*1*) may be the true cause of the clinical phenotype. *CYP3A5* is polymorphically expressed in adults with readily detectable expression in about 10-20% in Caucasians, 33% in Japanese and 55% in African-Americans. The primary causal mutation for its polymorphic expression (*CYP3A5*3*) confers low CYP3A5 protein expression as a result of improper mRNA splicing and reduced translation of a functional protein. The *CYP3A5*3* allele frequency varies from approximately 50% in African-Americans to 90% in Caucasians. Functionally, microsomes from a *CYP3A5*3/*3* liver contain very low CYP3A5 protein and display on average reduced catalytic activity towards midazolam. Additional intronic or exonic mutations (*CYP3A5*5*, **6*, and **7*) may alter splicing and result in premature stop codons or exon deletion. Several *CYP3A5* coding variants have been described, but occur at relatively low allelic frequencies and their functional significance has not been established. As CYP3A5 is the primary extrahepatic CYP3A isoform, its polymorphic expression may be implicated in disease risk and the metabolism of endogenous steroids or xenobiotics in these tissues (e.g. lung, kidney, prostate, breast, leukocytes). CYP3A7 is considered to be the major fetal liver CYP3A enzyme. Although hepatic CYP3A7 expression appears to be significantly down-regulated after birth, protein and mRNA have been detected in adults. Increased *CYP3A7* mRNA expression has been associated with the replacement of a 60-bp segment of the *CYP3A7* promoter with a homologous segment in the *CYP3A4* promoter (*CYP3A7*1C* allele). This mutational swap confers increased gene transcription due to an enhanced interaction between activated PXR:RXRalpha complex and its cognate response element (ER-6). The genetic basis for polymorphic expression of CYP3A5 and CYP3A7 has now been established. The substrate specificity and product regioselectivity of these isoforms can differ from that of CYP3A4, such that the impact of CYP3A5 and CYP3A7 polymorphic expression on drug disposition will be drug-dependent. In addition to genetic variation, other factors that may also affect CYP3A expression include: tissue-specific splicing (as reported for prostate CYP3A5), variable control of gene transcription by endogenous molecules (circulating hormones) and exogenous molecules (diet or environment), and genetic variations in proteins that may regulate constitutive and inducible CYP3A expression (nuclear hormone receptors). Thus, the complex regulatory pathways, environmentally susceptible milieu of the CYP3A enzymes, and as yet undetermined genetic haplotypes, may confound evaluation of the effect of individual CYP3A genetic variations on drug disposition, efficacy and safety, as reported by Lamba *et al* [193].

CYP Clustering: The construction of a genetic map integrating the most prevalent *CYP2D6+CYP2C19+CYP2C9* polymorphic variants in a trigenic cluster yields 82 different haplotype-like profiles. The most frequent trigenic genotypes in the AD population are **1*1-*1*1-*1*1* (25.70%), **1*1-*1*2-*1*2* (10.66%), **1*1-*1*1-*1*1* (10.45%), **1*4-*1*1-*1*1* (8.09%), **1*4-*1*2-*1*1* (4.91%), **1*4-*1*1-*1*2* (4.65%), and **1*1-*1*3-*1*3* (4.33%). These 82 trigenic genotypes represent 36 different pharmacogenetic phenotypes. According to these trigenic clusters, only 26.51% of the patients show a pure 3EM phenotype, 15.29% are 2EM1IM, 2.04% are pure 3IM, 0% are pure 3PM, and 0% are 1UM2PM (the worst possible phenotype). This implies that only one-quarter of the population normally processes the drugs which are metabolized via CYP2D6, CYP2C9 and CYP2C19 (approximately 60% of the drugs of current use) [12]. Taking

into consideration the data available, it might be inferred that at least 20-30% of the AD population may exhibit an abnormal metabolism of cholinesterase inhibitors and/or other drugs which undergo oxidation via *CYP2D6*-related enzymes. Approximately 50% of this population cluster would show an ultrarapid metabolism, requiring higher doses of cholinesterase inhibitors in order to reach a therapeutic threshold, whereas the other 50% of the cluster would exhibit a poor metabolism, displaying potential adverse events at low doses. If we take into account that approximately 60-70% of therapeutic outcomes depend upon pharmacogenomic criteria (e.g. pathogenic mechanisms associated with AD-related genes), it can be postulated that pharmacogenetic and pharmacogenomic factors are responsible for 75-85% of the therapeutic response (efficacy) in AD patients treated with conventional drugs [12,23,29,30-32,34,36].

By knowing the pharmacogenomic profiles of patients who require treatments with anti-dementia drugs and/or psychotropic drugs of current use (Table 1), it might be possible to obtain some of the following benefits related to efficacy and safety issues: (i) to identify candidate patients with the ideal genomic profile to receive a particular drug; (ii) to adapt the dose in over 90% of the cases according to the condition of EM, IM, PM or UM (diminishing the occurrence of direct side-effects in 30-50% of the cases); (iii) to reduce drug interactions by 30-50% (avoiding the administration of inhibitors or inducers able to modify the normal enzymatic activity on a particular substrate); (iv) to enhance efficacy; and (v) to eliminate unnecessary costs (>30% of pharmaceutical direct costs) derived from the consequences of an inappropriate drug selection and the overmedication administered to mitigate ADRs.

Conclusions

AD is a major problem of health, with a high cost for our society. As a clinical entity, AD is a polygenic/complex disorder in which many different gene clusters may be involved. Most genes screened to date belong to different proteomic and metabolomic pathways potentially affecting AD pathogenesis, represented by accumulation of Aβ deposits in senile plaques, intracellular NFTs with hyperphosphorylated tau, and neuronal loss. The presence of the APOE-4 allele of the apolipoprotein E gene seems to be a major risk factor for both degenerative and vascular dementia, and APOE variants are directly involved in AD pathogenesis at multiple levels. Specific biomarkers (structural and functional genomic markers, proteomic markers in body fluids, neuroimaging markers) are needed for an accurate diagnosis of AD. The present pharmacological treatment of AD with cholinesterase inhibitors (donepezil, rivastigmine, galantamine) and memantine is not cost-effective, and there is an overuse of psychotropic drugs in patients with dementia (which contribute to deteriorate cognitive and psychomotor functions). Old treatments addressed memory impairment; however, new treatments are oriented to halt disease progression by interfering with Aβ accumulation, NFT formation, oxidative stress, neuroinflammation, and cerebrovascular damage. Over the past few years diverse candidate drugs have been investigated in AD models but not one has reached the market. Since only 25-30% of the population is extensive metabolizer for drugs which are metabolized via CYP2D6, CYP2C9, and CYP2C19 enzymes, it seems reasonable to incorporate pharmacogenomic procedures to optimize AD therapeutics, reducing ADRs and unnecessary costs. The therapeutic response to conventional drugs in patients with AD is genotype-specific, with *CYP2D6*-PMs, *CYP2D6*-UMs, and *APOE-4/4* carriers acting as the worst responders. *APOE* and *CYP2D6* may cooperate, as pleiotropic genes, in the metabolism of drugs and hepatic function. ■

Future perspective

To make AD a global health priority in the coming years, conceptual and procedural changes are needed on several grounds, such as (i) political, administrative, economic, legal, ethical, industrial, regulatory and educational issues; (ii) the implantation of novel biomarkers (genomics, proteomics, molecular neuroimaging) as diagnostic aids; (iii) the introduction of innovative therapeutics; (iv) the implementation of pharmacogenomics in the clinical practice in order to optimize therapeutics; and (v) the promotion of selective preventive plans for the population at risk.

There is a disharmony in the world concerning the interest of the public and governments toward dementia and its social, medical, and economic implications. The diagnosis and management of dementia is dissimilar in Europe, North America, Iberoamerica, Asia, Africa, and Oceania. The economic/cultural status of each country (developed *vs* developing), the particular epidemiology of aging and dementia in each latitude, national standards of education, health priorities (infectious diseases *vs* degenerative diseases) and the quality and efficiency of the medical services are conditioning factors for investing (or not) national resources in dementia as a health priority. Within the same country, general practitioners, geriatricians, neurologists and psychiatrists face AD from different perspectives. In about 80% of the cases, general practitioners are the first who have a medical contact with the patients, under the initiative of

their relatives; less than 20% of the cases are seen by a specialist; and probably over 30% of dementia patients are underdiagnosed or misdiagnosed. The available diagnostic criteria of dementia for physicians (NINCDS-ADRDA, ICD-10, DSM-IV) are insufficient and require permanent updating [194,195]. The concept and diagnostic criteria of mild cognitive impairment [196] still needs further substantiation as a pre-dementia condition [197]. Daily information in the lay press is sometimes confusing and contradictory, enhancing with it the public disinformation. Therefore, educational programs, international guidelines, and consensus protocols for the management of dementia are necessary for a global harmonization of the subject, to speak the same conceptual language among societies and among professionals, and to improve cost-effectiveness ratios [198,199]. There are many legal (i.e. informed consent, lawsuit, testament, tutorship) and ethical issues (i.e. clinical trials, use of genetic information, institutionalization) which deserve more attention to humanize the end of life in the very frail conditions under which demented patients survive.

The updating of regulatory issues is also a matter of deep concern. Regulatory aspects of drug development are not universal, with notable peculiarities in the EU (EMEA), USA (FDA) and Japan (Koseisho). Within the EU, drugs approved in one country are not necessarily approved (or available) in another member state, with the consequent multiplication of unnecessary trials and costs which later on will have repercussions on the price of drugs. In some European countries the average cost of medicines for the monthly treatment of a patient with AD ranges from 300 to 600€ (3,600-7,200€/year); however, in Spain, for instance, the lowest wage that an employer was allowed to pay in 2010 was 633.3€/month (gross earnings, 21,500€/year; half of that in Germany, The Netherlands or UK, estimated at 40,000€; and 20% lower than the European average) (gross earnings in Hungary, Slovakia, Rumania, and Bulgaria, 10,000€/year), and the gross remuneration of the 5,209,427 Spanish pensioners was 908.49€/year [193]. The numbers are self-explanatory for anyone who wishes to understand. Consequently, the costs of dementia cannot be fully assumed by over 60% of the European population; therefore, the European authorities must take into

account this circumstance when the new Health Reform is implemented in the coming years.

Genomics, transcriptomics, proteomics, and metabolomics will revolutionize medicine in the next decades. Genetic testing is gaining acceptance among physicians and patients in different countries [199-202], although African Americans and Whites in the USA, Europeans, and Japanese differ notably in their knowledge, beliefs, and attitudes regarding genetic testing for AD [199,202,203]. In a Boston study, Kopits *et al* [201] reported that 71% of Americans would ask for genetic testing from their doctor if it were covered by health insurance, and 60% would ask for it even if it required self-pay. Forty-one percent were willing to pay more than $100 for testing, and more than half would have been willing to pay for the test out of pocket. Single gene analysis is of poor value as a diagnostic aid or as a prognostic marker; instead, polygenic analysis is more informative for diagnosis and therapeutics. Genomic screening, contemplating a highly specific gene cluster analysis of AD-related genes or clusters of genes associated with other dementias, would be of a great help for the identification of risk and for the early diagnosis of dementia. Genome-wide family-based association studies, using single SNPs or haplotypes, will help to identify associations with genome-wide significance [204-207]; similarly, genome-wide expression analysis will be useful for the discovery of new drug targets. Some studies will try to elucidate the weight of genome-environment interactions in the pathogenesis and clinical course of CNS disorders, and also the emerging role of epigenetics. The validation of protocols for genomic screening will contribute to introducing structural genomics, functional genomics, and proteomics as diagnostic aids and therapeutic targets [208].

An accurate diagnosis of AD demands the urgent introduction of reliable biomarkers into routine protocols at a reasonable price [98]. The proteomic analysis of levels of specific secreted cellular signaling proteins in cerebrospinal fluid or plasma correlate with pathological changes in the AD brain and can thus be used as a biomarker procedure [209]. It is likely that the best biomarkers result from the combination of genomic, transcriptomic and proteomic analyses of body fluids. The measurement of these biomarkers would correlate with brain imaging markers and cognitive performance [109-112]. New initiatives for the prevention of dementia (global *vs* selective prevention) will also emerge [210], together with new insights into the role of nutrition and nutrigenomics in brain function and neurodegeneration [211]. In terms of prevention, it must be taken into consideration that neuronal death and Aβ accumulation starts many years before the onset of the disease, and that preventive strategies should be selective to protect to the population at risk. For this purpose, accurate biomarkers are essential; and surrogate markers are needed to facilitate primary prevention.

Without doubt, the maximum priority for the coming decade will be an intense search for novel therapeutic options in the form of both symptomatic treatments and preventive strategies. Past failures must be learned by researchers and the pharmaceutical industry in order to avoid unnecessary expenses in redundant trials which lead nowhere. Combination treatments require further evaluation and more sophisticated strategies than dual combinations [212,213]. The administration of psychotropic drugs to demented patients should be reduced and predicted with pharmacogenetic markers to minimize side-effects and cognitive deterioration. Nanomedicine will also contribute to enhance the quality and brain accessibility of novel products and vaccines. According to the Derjaguin-Landau-Verwey-Overbeek theory, the immuno-nanovehicles have a much lower propensity to aggregate than the control nanovehicles. Immuno-nanovehicles show enhanced uptake at the BBB and better targeting of the Aβ proteins deposited in the cerebral amyloid angiopathy (CAA) model *in vitro* compared to the control nanovehicles. For example, chitosan enhanced aqueous dispersibility and increased the stability of immuno-nanovehicles during lyophilization, thus transforming them into ideal vehicles for delivering therapeutic/diagnostic agents to the cerebral vasculature ridden with vascular amyloid [214].

Priority areas for pharmacogenetic research are the prediction of serious adverse reactions (ADRs) and the establishment of variation in efficacy [215]. Both requirements are necessary in CNS disorders and dementia, to cope with efficacy and safety issues associated with current psychotropics and anti-dementia drugs, and new CNS drugs as well. Since drug response is a complex trait, genome-wide approaches (oligonucleotide microarrays, proteomic profiling) may provide new insights into drug metabolism and drug response. Of paramount importance is the identification of polymorphisms affecting gene regulation and mRNA processing in genes encoding cytochrome P450s and other drug-metabolizing enzymes, drug transporters, and drug targets and receptors, with broad implication in pharmacogenetics, since functional polymorphisms which alter gene expression and mRNA processing appear to play a critical role in shaping human phenotypic variability [216]. It is also most relevant, from a practical point of view, to understand the pharmacogenomics of drug transporters, especially *ABCB1* (P-glycoprotein/*MDR1*) variants, and other ABCs, due to the pleiotropic activity of these genes on a large number of drugs [191,217]. There are over 170 human solute carrier transporters which transport a variety of

substrates, including amino acids, lipids, inorganic ions, peptides, saccharides, metals, drugs, toxic xenobiotics, chemical compounds, and proteins [218]. Clearance of Aβ from the brain occurs via active transport at the blood-brain barrier (BBB) and blood-cerebrospinal fluid barrier (BCSFB). With increasing age, the expression of the Aβ efflux transporters is decreased, and the Aβ influx transporter expression is increased at the BBB, adding to the amyloid burden in the brain. Changes in expression of the Aβ transporters, the low density lipoprotein receptor-related protein-1 (LRP-1), P-glycoprotein (P-gp), LRP-2 (megalin) and the receptor for advanced glycation end-products (RAGE) have been reported at the BCSFB [219]. There is an increase in the transcription of the Aβ efflux transporters, *LRP-1* and *P-gp*, no change in *RAGE* expression and a decrease in *LRP-2*, the choroid plexus epithelium influx transporter, at the BCSFB with aging. Decreased $A\beta_{42}$ concentration in the choroid plexus may be associated with these Aβ transporter alterations [219]. The active form of vitamin D, $1,25(OH)_2D_3$, appears to enhance brain-to-blood $A\beta_{1-40}$ efflux transport at the BBB through both genomic and non-genomic actions. Compounds activating these pathways may be candidate agents for modulating $A\beta_{1-40}$ elimination at the BBB [220].

Another important issue in the pathogenesis and therapeutics of CNS disorders is the role of microRNAs (miRNAs), RNA interference (RNAi) and gene silencing. RNAi is being considered as an important tool for functional genomics and for gene-specific therapeutic activities that target the mRNAs of disease-related genes [221-223]. Nearly 97% of the human genome is non-coding DNA, and introns occupy most of it around the gene-coding regions. Numerous intronic sequences have been found to encode microRNAs, which are responsible for RNA-mediated gene silencing through RNA interference (RNAi)-like pathways. microRNAs (miRNAs), small single-stranded regulatory RNAs capable of interfering with intracellular messenger RNAs (mRNAs) that contain either complete or partial complementarity, are useful for the design of new therapies. RNAi has led in recent years to powerful approaches to silencing targeted genes in a sequence-specific manner with potential therapeutic applications in neurodegenerative diseases. RNAi procedures for gene-selective inhibition must improve (a) cytoplasmic delivery of short sdRNA oligonucleotides (siRNA), which mimics an active intermediate of an endogenous RNAi mechanism, and (b) nuclear delivery of gene expression cassettes which express a short hairpin RNA (shRNA), which mimics the micro interfering RNA (miRNA) active intermediate of a different endogenous RNAi mechanism. These technologies, complemented by non-viral gene delivery systems and ligand-targeted plasmid-based nanoparticles for RNAi agents, will bring new hopes for the treatment of different complex disorders [224-226], but we must be sure that gene silence in CNS disorders does not affect proteomic and/or metabolomic networks, which are fundamental for correct brain function [39,227].

Adult neurogenesis and stem cells represent another area of interest in CNS disorders [228,229]. Neural stem cells (NSCs) reside along the ventricular zone neuroepithelium during the development of the cortical plate. These early progenitors ultimately give rise to intermediate progenitors and later, the various neuronal and glial cell subtypes that form the cerebral cortex. The capacity to generate and expand human NSCs (neurospheres) from discarded normal fetal tissue provides a means with which to study directly the functional aspects of normal human NSC development. This approach can also be directed toward the generation of NSCs from known neurological disorders, thereby affording the opportunity to identify disease processes that alter progenitor proliferation, migration and differentiation [228]. The availability of human neuronal progenitors (hNPs) in high purity would greatly facilitate neuronal drug discovery and developmental studies, as well as cell replacement strategies for neurodegenerative diseases [229]. Stem cell therapy has been suggested as a possible strategy for replacing damaged circuitry and restoring learning and memory abilities in patients with AD and other neurodegenerative disorders; however, there is a long path ahead from the promising investigations which are raising hopes, and the challenges behind translating underlying stem cell biology into an effective therapy for CNS disorders and dementia [230]. Stem cell therapy requires a direct assessment of stem cell survival, interaction with the host and impact on neuronal functions after transplantation, and requires advanced *in vivo* imaging techniques. PET might be a potential molecular imaging modality in the future to evaluate the viability and function of transplanted tissue or stem cells in CNS [231].

With regard to the future of pharmacogenomics as a practical discipline to efficiently optimize therapeutics,

several issues should be addressed: (i) the education of physicians in medical genomics and pharmacogenomics is fundamental (less than 2% of the members of the medical community are familiar with genomic science); (ii) genomic screening of gene clusters involved in pharmacogenomic outcomes must become a clinical routine (whithout genetic testing there is no pharmacogenetics); (iii) each patient must be a carrier of a pharmacogenetic card [232] indicating what kind of drugs he/she can take and which medications he/she should avoid; (iv) Regulatory Agencies should request pharmacogenetic data to the pharmaceutical industry when applying for drug approval; (v) pharmacogenetic data must be incorporated to the patient information leaflet and the pharmaceutical vade mecum; and (vi) new guidelines for daily praxis, such as that of the first World Guide for Drug Use and Pharmacogenomics [191], will facilitate the understanding of the relationship between drugs and genes (and vice versa) to make drug prescription a real personalized procedure.

Finally, to foresee AD as a true health priority, it would be necessary for our society first to consider the possibility that demented patients should be managed in centers of medical excellence instead of in custody, in residual nursing homes.

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P_1

Caracterización del perfil genético de riesgo cerebrovascular

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El accidente cerebrovascular (ACV), ictus o infarto cerebral consiste en la alteración permanente o transitoria de la función cerebral que aparece como consecuencia de un trastorno circulatorio, bien de los vasos cerebrales o bien de alteraciones hemáticas. Del mismo modo que ocurre con otras enfermedades complejas, la prevalencia varia en diferentes países y tiene relación con factores genéticos, edad de la población y factores ambientales asociados. La incidencia de nuevos casos en España se sitúa alrededor de 156 por 100.000 habitantes, aunque es presumible que la cifra real esté más cerca de los 200 casos por 100.000 habitantes.

Existen muy pocos datos sobre la prevalencia de ictus en España, con frecuencias que oscilan entre el 2.1% en la población mayor de 20 años hasta el 8.5% en la población mayor de 65 años, según el estudio consultado. La mortalidad por ictus en España oscila entre un 10% y un 34% en las estadísticas hospitalarias, siendo mucho más elevada en los casos de hemorragia cerebral.

La definición del panel de riesgo genético cerebrovascular pasa por abordar el estudio de genes implicados en los diferentes eventos que desencadenan el proceso aterogénico, es decir, metabolismo lipídico, función endotelial, respuesta inmunitaria y estabilidad de la placa de ateroma (aterotrombosis). La validación del panel genético o la determinación de que las diferencias entre individuos enfermos y controles sanos son causales y no espurias, así como la elección del modelo estadístico adecuado, son claves para obtener una herramienta predictiva útil en la práctica médica.

Se han analizado un total de 20 variantes polimórficas en 15 genes relacionados con el proceso aterogénico, en una muestra poblacional de 483 individuos mayores de 50 años, de los cuales 310 presentaban cuadros clínicos con patologías vasculares asociadas: demencia vascular (N=147), encefalopatía vascular (N=67), ictus (N=67), migraña vascular (N=18) e insuficiencia cerebrovascular (N=11). Los 173 individuos sin patología vascular asociada estaban compuestos por: controles sanos (N=111) y enfermos de Alzheimer (N=62).

Las comparaciones entre los diferentes grupos de enfermos con los controles sanos evidencian una clara correlación de la obesidad y la hipertensión como factores de riesgo de complicaciones cerebrovasculares, aunque no hemos detectado dicha asociación con los niveles de colesterol y triglicéridos en la muestra analizada.

En cuanto a la utilidad de los marcadores genéticos seleccionados en la caracterización del riesgo cerebrovascular, hemos establecido tres niveles de caracterización del riesgo: Riesgo alto, con valores de Odds Ratio (OR) superiores a 2; Riesgo moderado, con valores en el rango 1,2<OR<2; y, Riesgo bajo, con OR<1,2. De este modo, cabe destacar la importancia como factores de riesgo de los alelos APOE*2 (OR=2,37), tanto en homocigosis como en heterocigosis, e IL6*-573G (OR=2,21).

Si agrupamos los diferentes polimorfismos analizados en función del proceso aterogénico en que intervienen, y valoramos la capacidad informativa del riesgo expresada como riesgo relativo (RR), se concluye que el panel genético que acumula mayor carga genética de riesgo es el que incorpora las citoquinas pro-inflamatorias (panel de respuesta inmunitaria), con un RR acumulado superior al 200%, seguido de panel de metabolismo lipídico, con un riesgo acumulado en torno al 50%. Finalmente, los polimorfismos agrupados en los paneles de función endotelial y trombosis, explican la carga genética negativa acumulando valores de RR superiores al 15-20%.

La utilización de paneles de susceptibilidad genética no sólo son útiles por la capacidad predictiva de los marcadores que los conforman, sino también por la capacidad de ponderar el peso específico de los distintos procesos patogénicos que intervienen en el debut de la enfermedad, contribuyendo de este modo a la personalización del tratamiento que se deba iniciar en el caso de aparecer la enfermedad.

P_2

Citoquinas proinflamatorias polimórficas como marcadores genéticos de aterosclerosis

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La aterosclerosis puede considerarse una forma de inflamación crónica resultado de la interacción de lipoproteínas modificadas, macrófagos derivados de monocitos, linfocitos T, y elementos celulares normales de la pared arterial. El proceso inflamatorio puede desembocar finalmente en el desarrollo de

lesiones complejas o placas de ateroma, que aparecen en el lumen arterial. La ruptura de la placa da lugar a complicaciones clínicas agudas, como son el infarto de miocardio y el ictus.

Se han identificado diversos factores de riesgo tanto ambientales como genéticos en numerosos estudios de asociación. Los marcadores de inflamación presentes en el torrente sanguíneo se asocian con riesgo incrementado de aterosclerosis, infarto de miocardio, ictus y progresión de enfermedades autoinmunes, aunque las razones de estas asociaciones aun no están bien definidas.

Hoy en día está ampliamente aceptado que la aterosclerosis es un ejemplo específico de respuesta inflamatoria crónica principalmente frente a la dislipemia y otros factores de riesgo. Las células espumosas y endoteliales activadas producen citoquinas pro-inflamatorias como la interleuquina 1B (IL- 1B), la interleuquina 6 (IL-6), el receptor de IL-6 (IL-6R) y el factor de necrosis tumoral (TNF-alfa), las cuales precipitan el desarrollo de la respuesta inflamatoria.

Se describe la relación de 5 variantes polimórficas en genes que codifican para citoquinas proinflamatorias (*IL1B**3954C, *IL6**-174G, *IL6**-573G, *IL6R**1510A, *TNFA**-308A) en una muestra de 292 individuos, de los cuales 148 presentaban complicaciones de tipo cerebrovascular: 69 pacientes con demencia vascular, 28 con encefalopatía vascular, 32 con ictus, 11 con migraña y 8 con insuficiencia cerebrovascular.

De los 5 polimorfismos analizados, 3 presentan una clara asociación con el fenotipo de riesgo elevado de patología vascular: *IL6**-573G (OR=2,21), IL6R*1510A (1,54), *TNFA**-308A (OR=1,33). 3 de los 5 SNPs se encuentran en las regiones promotoras de los genes, estrechamente relacionadas con los niveles de expresión y, por lo tanto, con los niveles finales cuantificables de citoquinas circulantes en sangre.

Los resultados encontrados en este estudio preliminar muestran la relación existente entre los niveles plasmáticos de citoquinas y la mayor incidencia de accidentes cerebrovasculares. Los niveles incrementados de IL-6 y de su receptor IL-6R aumentan las tasas de reclutamiento de monocitos y macrófagos en los lugares próximos a la lesión vascular, contribuyendo a la formación de la placa de ateroma.

P_3

Perfil fenotípico EEG de conectividad funcional y actividad oscilatoria en la enfermedad de Alzheimer

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Introducción: En los últimos años se han producido importantes avances en la fisiopatología y neurogenética de la enfermedad de Alzheimer (EA). Se ha demostrado que la presencia del alelo ε4 del gen de la apolipoproteína E (APOE) está relacionada con un aumento significativo del riesgo de desarrollar la enfermedad. Estudios recientes indican la existencia de áreas de disfunción cerebral en pacientes con EA, e incluso en individuos sanos con alto riesgo. Teniendo en cuenta que alteraciones circunscritas a determinadas áreas de la corteza cerebral no explican la complejidad de los síntomas de la EA, se ha propuesto además la existencia de una desintegración funcional de redes neuronales relacionada con procesos patológicos como la pérdida de sinapsis y la apoptosis neuronal. El objetivo de este trabajo es determinar alteraciones en las oscilaciones corticales y la conectividad funcional en la EA, así como su asociación con el genotipo APOE, usando un método novedoso de análisis de conectividad cerebral puramente fisiológica.

Método: Se realizaron registros de encefalograma (EEG) a 125 pacientes con EA probable (60 portadores del ε4 y 65 no-portadores) y también a 60 ancianos sanos (12 portadores del ε4 y 48 no-portadores) que actuaron como controles. Para el análisis de datos se utilizó la actividad EEG en reposo con ojos cerrados, y se aplicó un índice novedoso de conectividad implementado en el programa estadístico "exact low-resolution brain electromagnetic tomography" (eLORETA) que calcula "*lagged*-coherencia" y "*lagged*-sincronía de fase" entre pares de señales encefalográficas, como medida de conectividad linear y no linear, respectivamente, con corrección para comparaciones múltiples. Este índice es resistente a artefactos no fisiológicos, (e.g., efectos de conducción de volumen y baja resolución espacial), que afectan a la mayoría de los métodos de conectividad funcional. Tanto la localización de actividad oscilatoria como la conectividad funcional se analizaron en las bandas de frecuencias *delta* (2-4 Hz), *theta* (4-7 Hz), *alpha1* (8-10 Hz), *alpha2* (10-13 Hz), *beta1* (13-18 Hz) y *beta* 2 (18-25 Hz). En todos los participantes se hizo determinación del genotipo APOE.

Resultados: Se observó una reducción significativa de las oscilaciones alfa1 en la región parieto-occipital (p=0.037) con una tendencia al aumento de la actividad delta en regiones fronto-centrales en pacientes con EA comparado con los controles. La actividad alfa1 en región parieto-occipital izquierda se encontró significativamente reducida en los pacientes portadores del alelo ε4 en relación a los no portadores. El análisis de conectividad funcional mostró un aumento de la conectividad interhemisférica en la banda theta en pacientes con EA. Estos hallazgos afectaron tanto la coherencia (p=0.0002) como la sincronía de fase (p=0.0006) entre regiones temporales mediales e inferiores izquierdas y un área extensa de la corteza parietal posterior y temporo-occipital, lo cual fue independiente del genotipo APOE.

Conclusiones: Los hallazgos de este estudio demuestran que en la EA, además de las alteraciones en la actividad alfa como signo de disfunción cortical especialmente en portadores de la APOE ε4, existe un deterioro de la conectividad funcional que involucra principalmente a conexiones del lóbulo temporal izquierdo. Estas alteraciones pueden representar potenciales marcadores neurofisiológicos de la EA.

P_4

Relación del genotipo APOE y niveles séricos de LDL-colesterol con la actividad bioeléctrica cerebral en pacientes con enfermedad de Alzheimer

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Introducción: La enfermedad de Alzheimer (EA) es una enfermedad multifactorial compleja que involucra varios genes de susceptibilidad, sobre todo el alelo ε4 del gen de la apolipoproteína E, la cual está involucrada en el transporte de colesterol en el cerebro. Se sugiere que un evento clave que conduce a la EA es la formación y agregación cerebral del péptido β-amiloide, que es un derivado de la proteína precursora amiloide (APP), estando el colesterol involucrado en la modulación de este proceso. Además, se ha reportado que los niveles séricos de LDL-colesterol están claramente relacionados con la densidad de las placas neuríticas, que es una de las manifestaciones neuropatológicas típicas de la enfermedad. Por otra parte, los niveles altos de colesterol se asocian con mayor riesgo de la EA, y los pacientes que llevan tratamiento con agentes reductores del colesterol suelen tener una menor prevalencia de la enfermedad. Todo esto ha llevado a que el colesterol esté recibiendo una gran atención como factor potencialmente importante en la etiología de la EA. Nuestro estudio tiene como propósito determinar si la combinación del genotipo APOE y niveles séricos de LDL se asocia a algún patrón de actividad electroencefalográfica (EEG) en pacientes con EA.

Método: Se realizaron registros de encefalograma (EEG) a 125 pacientes con EA (60 portadores del ε4 y 65 no-portadores). Para el análisis de datos se utilizó la actividad EEG en reposo con ojos cerrados. La localización de la fuente de actividad oscilatoria cerebral y su comparación entre grupos según el tipo de APOE y niveles de LDL se realizó mediante el uso del programa "exact low-resolution brain electromagnetic tomography" (eLORETA). Las imágenes funcionales de densidad espectral de eLORETA se analizaron en las bandas de frecuencias *delta* (2-4 Hz), *theta* (4-7 Hz), *alpha*1 (8-10 Hz), *alpha*2 (10-13 Hz), *beta*1 (13-20 Hz) y *beta*2 (20-25 Hz).

Resultados: Se observó que 20 pacientes en el grupo de portadores del ε4, y 23 en el grupo de no-portadores tenían niveles elevados de LDL-colesterol. Los portadores del ε4 con niveles altos de LDL sérico mostraron una disminución significativa de la actividad *beta*1 en la región temporo-parietal izquierda ($p<0.05$) comparado con los portadores (N=40) que tenían niveles séricos normales. En el grupo de no-portadores del ε4, los que presentaban LDL sérico elevado mostraron un aumento de la actividad *alpha*1 en la región parietal inferior izquierda ($p<0.05$) comparado con los que presentan niveles normales de LDL-colesterol.

Conclusiones: La presencia de niveles elevados de LDL sérico en pacientes con EA portadores de la APOE ε4 muestran manifestaciones electroencefalográficas que sugieren hipofunción cortical parieto-temporal izquierda que contrasta con un aumento de la actividad alfa local en no portadores. Los hallazgos apoyan la noción de que la combinación del genotipo APOE ε4 y colesterol elevado ejerce un efecto negativo en la actividad cerebral en pacientes con demencia de tipo Alzheimer.

P_5

Methodological quality in Pharmacogenetic studies: a review in binary assessment of treatment response

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Objective: To evaluate the reporting of critical design issues and methods of statistical analysis in pharmacogenetic studies published in the medical literature over the last 15 years. The main results showed that there is considerable room for improvement in the current standards of design, analysis, and reporting of pharmacogenetic research.

Introduction: The number of pharmacogenetic studies has increased in recent years (a PubMed search of 'pharmacogenetics' returned 299 entries in 2000, 898 entries in 2009 and 9370 in 2011, and in all likelihood it will continue to do so. However, the low replicability of results from genetic association studies is a cause of concern. Several potential explanations have been proposed (such as population stratification, misclassification of outcome, and allelic heterogeneity); some investigators argue that

the most likely causes of low replicability are poor study design, multiplicity of statistical testing, and misinterpretation of 'negative' results. The quality of research has been repeatedly reviewed in the area of clinical trials, and the results of these reviews suggest that the information supplied in publications is very often insufficient or inaccurate, and that certain methodological problems are recurrent and as a consequence, efforts have been made to improve the quality of design, analysis, and reporting of clinical trials, with encouraging results. To our knowledge, the quality of pharmacogenetic studies has not been systematically reviewed. Given the potential importance of pharmacogenetic research, the low replicability problem and the concerns that have been raised regarding design and analysis issues, we decided to undertake a systematic review of pharmacogenetic studies to quantify (i) the reporting of critical design issues, (ii) the prevalence of analysis methods, and (iii) the prevalence of multiple statistical testing and the measures adopted to deal with it.

Methods: We conducted a PubMed search for studies that investigated an association of at least one single nucleotide polymorphism (SNP) and which examined the patient's response to the drug treatment, in whom this response could be a measure of safety or efficacy. The search was executed in the MEDLINE database in May 2010 and 179 articles were returned. The preselected articles were fully inspected to determine their eligibility according to the following predefined criteria:

(i) *independence of the study design;*
(ii) *studies should be of a confirmatory nature* and should follow the 'candidate gene' approach
(iii) *the treatment response should be dichotomous;* and
(iv) *genotype or allele frequencies should be documented for 'responders' and 'nonresponders'.*

Double data entry was then checked for discrepancies that were resolved by consensus of two reviewers. The data items extracted from each paper were predefined according to concerns and recommendations available in the literature, and were as follows:

- *Design characterization.*
- *Planned sample size and sample size determination.*
- *Number of patients treated and number of patients analyzed.*
- *Number of associations assessed.*
- *Statistical analysis.*
- *Multiple testing and methods used to deal with Multiplicity.*

Conclusions: This review shows that there is considerable room for improvement in the current standards of pharmacogenetic research and reporting.

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P_6

Bioproperties and clinical effects of Juritrofin (DefenVid®)

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Juritrofin (E-JUR-94013®) is a food supplement based on lipoproteins extracted from Atlantic fish (*T. trachurus*). This product is 100% natural, prepared by biotransformation and lyophilization processes, which preserve the original properties of the species. Lipoproteins extracted from the muscle of *T. trachurus* have demonstrated immunomodulatory effects in both *in vitro* and *in vivo* studies. After complex analysis it was concluded that the isolated fraction with immunomodulatory activity corresponded to the amino acid spinacine. The first Juritrofin study in rats showed a short-term stimulatory effect in immune cell counts. In a second trial, 300 piglets were treated with 5 diets based on fish extracts including Juritrofin and a commercial food supplement for 42 days. The final analysis data confirmed an increase in immune cells (cellular immunity) as well as an active response in the levels of serum immunoglobulins (humoral immunity). To confirm in humans the immunostimulatory effect of E-JUR-94013® observed in animals, an *in vitro* study was designed in human lymphocytes cultured with Juritrofin for 2 days. The analysis of different lymphocyte activation markers by flow cytometry showed significant immunoactivation compared with the control group and with other known lymphocytic inducers. A significant reduction in the percentage of apoptotic cells reflected an increase in extract-associated cell viability. In a sample of 56 subjects supplemented with 750 mg/day of DefenVid® for 6 months we observed an increase in all leukocyte subpopulations, with a significant increase in the number of neutrophils and eosinophils. We also found an increase in serum immunoglobulins A, G and M, while IgE concentrations decreased, IgE being an allergy-related protein. Previous studies linked the oily fish supplements with a preventive effect on childhood allergies. With regard to previous results, we proposed a study in patients with immunologic dysfunction, in the hope that Defenvid® may be a useful complement to enhance their defenses. 205

patients aged 50 years were grouped according to high or low leukocyte cell count (total or subclasses) relative to reference ranges. They were treated with a daily dose of 750 mg for 3 months. The results showed an interesting immunomodulatory effect not previously encountered, presenting an increase in cell counts from patients with immunodeficiency at baseline and a decrease in the cell percentages from patients with high basal cell counts, reaching normal values in both situations. Serum immunoglobulin levels were also positively affected, noting an increase in IgA, G and M and a decrease in IgE levels. These results confirm our hypothesis and demonstrated DefenVid® to be a nutritional supplement with a rapid response in the recovery of immune status. Among all the human studies performed, we emphasize an analysis in a sample of 1500 randomly selected subjects aged 1-98 years and treated with a multifactorial therapy in which the only common denominator was Defenvid®. A descriptive analysis of data showed the regulatory effect of Defenvid® on the white cell patterns. A modulation of the extreme cases, high and low, to normal values was found, confirming previous data. With respect to immunoglobulins, the decline of the high values of IgE levels after treatment was the most interesting change. We determined the serum levels of high sensitive C-reactive protein (hs-CRP), a common monitoring biomarker in chronic inflammatory diseases. After 6 months of treatment, we observed a strong decrease in the higher hs-CRP values, indicating a possible anti-inflammatory effect of Defenvid®. In order to discover whether DefenVid® response was affected by the genotype of inflammatory factors, *IL1B-147720, IL6-147620, IL6R-147880* and *TNF-191160* genotypes were evaluated, observing a genotype-dependent response.

P_7

Biological properties of Mineraxin

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Mineraxin is a nutraceutical made from a lyophilized lipoprotein extract of blue mussel (*Mytilus galloprovincialis*) from the Atlantic coast. It is an important natural source of nutrients with beneficial properties in different areas of health. It retains the innate properties of the raw material due to the non-denaturing manufacturing processes used. The product is 100% natural with no preservatives and no known side-effects. Scientific data have confirmed its beneficial effect on bone and joint problems due to its high content in glucosamine (a precursor of collagen). Its glucosamine-associated anti-inflammatory effect has also been demonstrated in a recent study. Its vitamin content, especially B-complex vitamins, minerals, iron and other substances such as selenium and vitamin E, has nutritional, antianemic and antioxidant properties. Our group conducted a study in 91 women in perimenopausal stage taking 750 mg/day Mineraxin for 3 months. Various biochemical markers were determined in the serum of the women selected: FSH (follicle stimulating hormone), LH (luteinizing hormone), Estradiol and Inhibin A to evaluate the hormonal response associated with menopause and its symptoms; GH (Hormone ultrasensitive growth) and IGF-1 (Insulin Growth Factor-1 or somatomedin C) for the assessment of the hypothalamic-pituitary-bone axis; bone alkaline phosphatase (BAP), calcium and β-CrossLaps, markers of bone formation and antiresorptive activity; TAS (total antioxidant status) to study the antioxidant capacity of Mineraxin; iron and ferritin to assess changes in body iron stores; cortisol, a stress-related hormone which is altered in perimenopausal women, and BMI (body mass index) to confirm its low-calorific power. We found an overall improvement in perimenopausal symptoms, especially noticeable in reducing hot flashes, mood swings and musculoskeletal pain. An increase in estradiol and inhibin A and a decrease in FSH and LH were observed, contrary to the usual profile in the perimenopausal stage. This pattern could indicate a delay or dampening of estrogen decrease, a cause of adverse symptoms of menopause. A significant increase in serum GH and IGF-1 levels and a decrease in BAP, calcium and β-CrossLaps concentrations were found post-Mineraxin treatment. Data showed a moderate increase in bone formation and a remarkable decrease in osteoblastic activity. These data classified Mineraxin as a natural product with beneficial effects on bone stability or prevention of osteoporosis. A significant increase in serum TAS demonstrated the important and rapid antioxidant power of the marine extract. Oxidative stress has been implicated in physiological situations such as aging, and in various pathological conditions. Data showed a reduction in cortisol serum levels, most prominent in those with high basal cortisol, associated with anxiety. A quantitative analysis of the composition of Mineraxin highlights its high iron content. Iron is stored as ferritin in our bodies. The results show a significant increase in iron storage, especially in those patients with low basal ferritin values, while no changes were observed in patients presenting high levels. The slight decrease in BMI showed the low caloric power of Mineraxin, it being recommendable in diets due to its high nutritional value. Analyzing all the results of the study, we conclude that Mineraxin is a product with a wide range of beneficial effects on health: it slightly delays the perimenopausal estrogen decline, it supports bone stability, stimulates antioxidant capacity, increases iron storage, prevents bone demineralization, strengthens joints and promotes growth and repair processes, and it has significant anti-inflammatory power. Therefore, we believe it can be most beneficial in joint problems, osteoporosis, arthritis, oxidative stress-associated conditions, aging, degenerative CNS processes, iron-deficiency anemia, pregnancy, lupus, chronic inflammatory diseases, diets, or in perimenopausal women.

P_8

The number of arterial branches of the human coronary tree is influenced by the hif1a pro582ser single nucleotide polymorphism

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Objectives: Hypoxia is required for the development of the cardiovascular system. Tissue adaptation to low oxygen is mediated through hypoxia-inducible factor 1. Hypoxia-driven gradients of vascular endothelial growth factor within the heart drive vessel tip sprouting and the angiogenic phase of vasculogenesis. We hypothesized that functional variants of *HIF1A* Pro582Ser single nucleotide polymorphism may be associated with the number of coronary artery branches in humans.

Methods: The branching of coronary arteries of 88 individuals was assessed by dynamic counting of arterial branches seen in coronary angiograms. Values were classified according to the branches emerging from the right and from the left coronary arteries. *HIF1A* Pro582Ser genotypes were determined using TaqMan-based assays. A generalized linear model was used to measure the effect of each SNP on the response variables. Multiple regression analysis was performed adjusting for co-variables that may influence total coronary branching. Statistical analyses were performed with Statistical Software STATA version 10 (StataCorp, College Station, Tex., USA) and SNPstat.

Results and Conclusions: Individuals carrying the T allele (Ser) of *HIF1A* Pro582Ser SNP (CT and TT genotypes) showed a significantly lower number of coronary arterial branches. This result was confirmed both by considering the total number of ramifications of the coronary arteries (81.03±1.79 branches for CC individuals *versus* 74.09±2.48 for T-carrying ones, p=0.042) and by only including branches arising from the left coronary artery (60.12±1.59 for CC *versus* 53.68±2.31 for CT and TT individuals, p=0.034). Clinical-epidemiological co-variables did not significantly affect the association between *HIF1A* Pro582Ser SNP and the number of branches of coronary arteries.

The Pro582Ser substitution in *HIF1A* gene alters the amino acid sequence within the carboxyl-terminal domain of HIF-1α that regulates protein stability and transcriptional activity, suggesting that they may have functional consequences. Our observations provide a novel and interesting insight that *HIF1A* Pro582Ser SNP may account for the inter-individual differences in the number of ramifications of the human coronary tree, suggesting that this SNP may be a genetic marker that determines inter-individual differences in human coronary arteries pattern. The ability to develop coronary artery branches may be of clinical interest, in that it gives the heart a greater capacity to develop a well-extended vascular network in the myocardium in response to hypoxia.

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P_9

A Genomic approach to histamine function

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Histamine is synthesized and released by different human cells, especially basophils, mast cells, platelets, histaminergic neurons, lymphocytes, and enterochromaffin cells. It is stored in vesicles or granules and is released on stimulation. HA exerts its effects on target cells through four different types of receptors: H1R, H2R, H3R and H4R. These receptors belong to the G protein-coupled receptor 1 family. In mammals, histamine is metabolized by two major pathways: N(tau)-methylation via histamine N-methyltransferase (HMT) and oxidative deamination via diamine oxidase. In the mammalian brain, the neurotransmitter activity of histamine is controlled by HMT, as diamine oxidase is not found in the central nervous system.

In the present study, we determined three genetic polymorphisms in the *HRH1*-17A>G (rs901865), *HRH2*-1018G>A (rs2067474) and in the *HNMT* Ile105Thr (rs11558538) genes in one hundred and ninety-five subjects, and we analyzed the relationship between histamine genotypes and blood histamine, IgA, IgG, IgM, IgE and PCR-us levels, as well as leukocyte, lymphocyte, neutrophil, monocyte, eosinophil and basophil counts. The rs2067474 in the *HRH2* gene is located in an enhancer element of the gene promoter and is

common in all populations. The rs11558538 is a missense mutation in the *HNMT* gene and is considered a functional polymorphism; the enzyme containing isoleucine as residue 105 has been associated with decreased levels of HMT activity and immunoreactivity. The frequency of the T105I polymorphism is found increased in Caucasian patients with asthma.

The results of this study show that the genotype *HRH2*-1018GA is overrepresented in those subjects with PCR-us levels above 3 mg/dL. Those subjects with this genotype also have significant lower levels of monocytes compared to the -1018GG genotype. Significant differences were observed in the levels of IgG and monocytes in those subjects bearing the *HRH1*-17*A allele. The *HNMT**105T allele is significantly associated with and increase in eosinophil levels, and with a decrease in leucocyte levels. Those subjects with the levels of HA above the normal range (>90 ng/mL) have a significant increase in the levels of eosinophils and basophils; on the contrary, those subjects with HA levels below the normal range (<90 ng/mL) present significantly lower levels of IgM and neutrophils. No significant differences were found between HA levels and HA-related polymorphisms. In conclusion, the *HRH2*-1018GA genotype is associated with high levels of PCR-us and the *HNMT**105T allele is related to markers of allergy proccesses. The results of this study indicate that HA-related polymorphisms participate and modulate the immune-inflammatory response.

P_10

Histamine function in brain disorders

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In 1974, the decrease in histidine decarboxylase activity found in many rat brain areas after lesions of the lateral hypothalamus was the first evidence for the existence of an ascending histamine (HA) neuronal pathway with widespread projections to almost all regions of the mammalian brain. In all the animal species studied, the histaminergic neurons were found to be confined to the tuberal region of the posterior hypothalamus, in an area called the tuberomammillary nucleus (TM) The histaminergic system has been implicated in the regulation of basic body functions, including the sleep-waking cycle, energy and endocrine homeostasis, synaptic plasticity and learning. There are at least three non-neuronal pools for HA in the brain: mast cells, glial cells and vascular endothelial cells. Four HA receptors have now been cloned, and three of them are widely distributed in the mammalian brain.

The role of HA in central nervous system (CNS) disorders is not clearly defined and contradictory results have been reported. Concerning a neurodegenerative pathology such as Alzheimer's disease (AD), findings in both directions, both increased and decreased levels of HA in brain, have been observed. These apparently contradictory results may indicate that HA responds to damage depending on the disease stage. In this sense, using blood HA levels from AD patients, an increase in HA levels is observed according to disease severity. This probably indicates an up-regulation of the histaminergic system as the disease progresses. Vascular dementia (VD) is another neurodegenerative disorder and is the second commonest dementia after AD. Both diseases behave in an opposite manner when we confront blood HA content and the patient's total functional capacity, using the global deterioration scale (GDS). While in VD patients a mild impairment in functional capacity correlates with high HA levels, in AD patients at the same functional level, HA values are lower, and this difference is statistically significant. Conflicting results have also been reported concerning HA in schizophrenia (SCH). High levels of HA metabolites in brain and altered number of HA receptors have been reported. Our studies found decreased HA levels in blood and serum from SCH patients compared to control healthy subjects. Furthermore, we found a low-resistance perfusion brain pattern in SCH patients that correlates in a positive manner with blood and serum HA levels.

The histaminergic system seems to be involved in brain pathology, although no disease entity has been directly linked to brain HA dysfunction. Thus, HA dysfunction may be a precipitating factor for disease susceptibility, severity, and progression.

P_11

Prevención de colitis experimental crónica inducida por dextran sulfato sódico (DSS) en ratones tratados con FR-91

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Uno de los principales tratamientos actualmente utilizados en humanos para combatir el cáncer es la quimioterapia. Un gran número de compuestos con actividad antitumoral están presentes en la naturaleza, y muchos de sus derivados son producidos por microorganismos. Sin embargo, debido fundamentalmente a la toxicidad de los fármacos y a la resistencia a muchos agentes quimioterápicos que se observa durante el tratamiento, la búsqueda de nuevos medicamentos aún representa uno de los objetivos principales

de la terapia antitumoral. En modelos animales, la administración oral de dextran sulfato sódico (DSS) durante un período relativamente corto determina colitis, con características similares a los daños clínicos e histopatológicos que se observan en la colitis ulcerosa (UC). Los factores patogenéticos responsables de la colitis inducida por el DSS y sucesivo desarrollo del cáncer de colon aún no han sido identificados. Hemos investigado los efectos del compuesto FR-91, un lisado estandarizado de células microbianas que pertenecen al género Bacillus, que en anteriores estudios ha demostrado una significativa actividad inmunomoduladora, en la prevención de la carcinogénesis colorrectal pre-maligna. La colitis ha sido inducida en ratones durante un período de cinco semanas mediante administración oral de una solución al 2% de DSS. Los cambios morfológicos en la mucosa del colon fueron evaluados mediante tinción con hematoxilina-eosina y mediante métodos inmunohistoquímicos. Se ha demostrado, en células crípticas y adenocarcinomatosas del epitelio displásico intestinal, la expresión de catenina-β, MLH-1, APC y p53, junto con un aumento en la expresión de IFN-γ. En este modelo, la mejor dosis-respuesta observada ha sido la concentración del 20% del FR-91, en la que no se han observado alteraciones histológicas o solo modestas lesiones inducidas por el DSS. Estos resultados sugieren que el FR-91 posee unas importantes propiedades antiinflamatorias en el modelo de inducción con DSS, y que puede actuar como agente quimiopreventivo frente a procesos de carcinogénesis de cáncer de colon.

P_12

Testaje experimental de las propiedades de bioproductos en preparados de Alimentos Naturales, S.A.

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La asociación entre alimentación y salud ha ocupado la base del pensamiento y la práctica médica incluso antes de la aparición de la medicina científica, de la farmacología y de los avances tecnológicos en diagnóstico médico. Actualmente existe un interés creciente por el estudio de la correlación entre nutrición y prevención de enfermedades crónicas y en el tratamiento de diferentes cuadros patológicos. Numerosas evidencias científicas apoyan la existencia de una asociación de factores alimentarios y nutricionales con diversas patologías, tales como las enfermedades cardiovasculares, la hipertensión, diferentes tipos de cáncer, la diabetes, la obesidad o la osteoporosis. La dieta es por lo tanto un factor esencial de la salud, aunque la contribución exacta de una dieta adecuada para promover la salud y prevenir la enfermedad es difícil de cuantificar. Con el fin de contribuir a un mejor conocimiento de los efectos biológicos de distintas clases de legumbres suplementadas con extractos naturales de origen marino E-SAR-94010, E-CAB-94011 y E-JUR-94013 con demostradas propiedades sobre el organismo humano, se han evaluado distintos parámetros en varios grupos de sujetos normales que han recibido raciones diarias de diferentes legumbres con un determinado extracto, según las pautas establecidas en el diseño experimental del estudio. En todos los participantes se han determinado parámetros antropométricos, bioquímicos, hematológicos e inmunológicos con el fin de evaluar tanto el impacto sobre el estado nutricional como los efectos a nivel orgánico de la suplementación con los extractos estudiados. En el ensayo con E-JUR 94013 dadas las características de los efectos derivados de este suplemento se han medido además parámetros inmunológicos específicos para evaluar la respuesta mediada por células: subclases de linfocitos, factores de activación de linfocitos, regulación de la capacidad fagocítica de granulocitos y monocitos. Los resultados demuestran que los ácidos grasos poliinsaturados (PUFAs) y las lipoproteínas presentes en E-SAR-94010, E-CAB-94011 y E-JUR-94013 tienen la propiedad de actuar sobre la fluidez, la permeabilidad, la función receptora, la actividad enzimática y la producción de mediadores lipídicos y proteicos, que a su vez regulan las interacciones entre distintos tipos celulares y muchas funciones de importancia vital. La posibilidad de alterar de forma activa la absorción de grasas saturadas mediante una dieta a base de legumbres y lipoproteínas marinas abre la puerta a nuevas estrategias de prevención de riesgos de enfermedades relacionadas con disfunciones del metabolismo lipídico.

P_13

Cambios en la actividad bioeléctrica cerebral durante el envejecimiento y el deterioro cognitivo

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Diversos estudios han relacionado el envejecimiento fisiológico cerebral con apoptosis neuronal y con la pérdida de conexiones corticales. Trabajos recientes usando EEG han mostrado diversas anormalidades en el cerebro de ancianos sanos sin deterioro cognitivo. Los hallazgos más frecuentes son enlentecimiento del ritmo *alpha* occipital y aparición de ondas lentas bilaterales en la región temporal. Estas alteraciones presentes en individuos sanos aparecen de forma más evidente en individuos con deterioro cognitivo. Con

el objetivo de investigar cómo se altera la actividad bioeléctrica cerebral con la edad y con el deterioro cognitivo, hemos realizado dos estudios de regresión. En el primero de ellos hemos enfrentado la actividad bioeléctrica cerebral contra la edad en 85 sujetos sanos de entre 19 y 91 años. En el segundo estudio de regresión se enfrentó la actividad bioeléctrica contra las puntaciones del test mini-mental (MMSE) en 125 ancianos con diverso grado de deterioro cognitivo (puntación MMSE entre 10 y 24).

Para la realización de estas regresiones se usó el software de análisis eLORETA (*low resolution brain electromagnetic tomography*) sobre épocas de 30 segundos libres de artefactos seleccionadas a partir de un registro EEG realizado a cada sujeto en condiciones de reposo y con ojos cerrados, en 19 electrodos siguiendo el sistema internacional 10-20, donde se estudiaron las siguientes bandas de frecuencia: *delta* (1.5-3.5 Hz), *theta* (4-7.5 Hz), *alpha* 1 (8-10 Hz), *alpha* 2 (10-13 Hz), *beta* 1 (13.5-18 Hz) y *beta* 2 (18.5-25 Hz).

Los resultados obtenidos muestran una tendencia ($p=0.09$) hacia el descenso de la actividad *alpha* 2 con la edad en la región occipital en sujetos sanos y una correlación estadísticamente muy significativa ($p<0.004$) entre la actividad bioeléctrica cerebral y la puntación en el MMSE, de tal manera que a mayor puntación en el MMSE (mejor estado cognitivo) menor actividad *delta* en región parieto-temporal bilateral y menor actividad *theta* fronto-central.

El software de análisis eLORETA ha demostrado ser una potente herramienta de evaluación de la función cerebral. Los resultados de este estudio apoyan las observaciones de investigaciones anteriores e indican que el envejecimiento y el deterioro cognitivo provocan la aparición de enlentecimiento de la actividad bioeléctrica en regiones cerebrales específicas, que se relaciona positivamente con un descenso en la puntación en pruebas psicométricas que evalúan el rendimiento cognitivo.

P_14

Influencia del alelo APOE ε4 sobre la función cerebral en ancianos sanos

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El gen ApoE ha sido identificado como factor de riesgo para la demencia y además está implicado en inmunoregulación, regeneración neural y crecimiento y regeneración de neuritas. Recientes estudios de genómica funcional han revelado la asociación existente entre este gen y la expresión fenotípica de diferentes rasgos biológicos como atrofia cerebral, deterioro cognitivo, deposición de beta-amiloide, disfunciones del metabolismo lipídico, etc. Existen tres alelos principales (ε2, ε3, ε4) asociados con riesgo (ε4) o protección (ε2) para la enfermedad de Alzheimer y otras enfermedades del Sistema Nervioso Central. Estudios mediante PET y fRMN realizados por Reitman *et al.* muestran que los portadores del alelo ε4 presentan menor actividad en el córtex cingulado posterior, parieto-temporal y frontal. Machulda *et al.* encuentran alteraciones en la conectividad entre regiones implicadas en el llamado "*default mode network*" (DMN), relacionado con la función cerebral en reposo y regiones subcorticales. En contraposición a estos hallazgos, recientes investigaciones encuentran un aumento de conectividad entre regiones del DMN e hipocampo y mejores rendimientos cognitivos en portadores sanos del alelo ε4. Otros autores definen a este alelo como ejemplo de antagonismo pleiotrópico, teniendo inicialmente un efecto beneficioso para la función cerebral para luego asociarse a la demencia en edades avanzadas.

Pocos estudios han evaluado el efecto del alelo ε4 sobre la función cerebral utilizando la actividad bioeléctrica. Por ello y a la vista de los resultados controvertidos hemos estudiado y comparado la actividad oscilatoria EEG y la conectividad (lineal y no lineal) en 40 ancianos sanos sin deterioro cognitivo divididos en función de su genotipo ApoE (12 ε4 positivos y 28 ε4 negativos). Se realizó a cada sujeto un registro EEG en condiciones de reposo con ojos cerrados, en 19 electrodos (sistema 10-20). Se estudiaron las siguientes bandas de frecuencia: *delta* (1.5-3.5 Hz), *theta* (4-7.5 Hz), *alpha* 1 (8-10 Hz), *alpha* 2 (10-13 Hz), *beta* 1 (13.5-18 Hz) y *beta* 2 (18.5-25 Hz). Para el análisis de fuentes EEG y de conectividad se utilizó el software de análisis eLORETA sobre épocas de 30 segundos libres de artefactos. El análisis de conectividad se llevó a cabo mediante un novedoso método llamado Índice de Lagged Conectividad Fisiológica usando la posición de los electrodos como regiones de interés.

Los resultados obtenidos muestran un aumento estadísticamente significativo ($p<0.03$) de la actividad *alpha* 1 en región temporal derecha y occipital en los ε4 positivos.

La conectividad linear aumentó significativamente ($p<0.05$) entre la región temporal izquierda y regiones implicadas en el DMN (córtex parietal lateral izquierdo), y la no linear refleja una tendencia ($p=0.08$) de aumento de conectividad entre la región temporal posterior derecha y la región parietal lateral izquierda, también relacionada con el DMN, en los sujetos portadores del ApoE ε4.

Estos resultados muestran que este alelo presenta un impacto sobre la actividad cerebral implicando a regiones corticales relacionadas con el DMN, dando lugar a diferencias fenotípicas en el patrón bioeléctrico en ancianos sanos, en función de su genotipo ApoE.

Ebiotec y Neovital organizaron un congreso sobre Nutracéutica Médica en Barcelona con participación del Presidente de EuroEspes, que disertó sobre la importancia de la Medicina Genómica

El pasado 22 de octubre, el *Dr. Ramón Cacabelos*, Presidente de EuroEspes, fue el encargado de inaugurar el Primer Congreso de Nutracéutica Médica, que se celebró en el salón de actos de la Universidad Pompeu Fabra de Barcelona. La organización del Congreso corrió a cargo de EuroEspes Biotecnología y Neovital, distribuidor de los productos de Ebiotec en Cataluña.

Al acto acudieron cerca de 200 profesionales médicos de distintas especialidades. En la conferencia inaugural, el Dr. Cacabelos presentó los avances llevados a cabo por EuroEspes en los últimos años en materia de Medicina Genómica, en la personalización del tratamiento farmacológico y en la aplicación de la Nutracéutica Médica.

Otros ponentes invitados fueron el Dr. Valter Lombardi, Jefe del Departamento de Biotecnología de la Salud de Ebiotec, que presentó datos sobre las propiedades nutracéuticas y biológicas del AntiGan y la aplicación de los test FIS de intolerancia alimentaria; la Dra. Lola Corzo, Directora del Departamento de Bioquímica Médica y Análisis Clínicos del Centro Médico EuroEspes, que mostró interesantes resultados clínicos obtenidos con los bioproductos Mineraxin y DefenVid; y Ramón Alejo, Director Técnico de EuroEspes Biotecnología, que habló sobre la Nutrigenómica y estrategias prospectivas en nutracéutica médica.

EuroEspes Publishing prepara el lanzamiento internacional de su megaproyecto EuroPharmaGenics (EPG)

Después de cinco años de trabajo multidisciplinar, el Grupo EuroEspes, a través de su filial EuroEspes Publishing, prepara el lanzamiento de la primera guía mundial de farmacogenónica en tres versiones, book, CD-ROM y plataforma informática on-line. EPG es un proyecto pionero a nivel mundial, a través del cual la comunidad científica y médica internacional puede tener acceso directo a información actualizada sobre fármacos y genes que les permitan aplicar de forma eficiente los conocimientos de la farmacogenómica para personalizar el tratamiento farmacológico. La versión original de EPG sale en inglés para todo el mundo. Los potenciales beneficiarios de EPG son médicos, farmacéuticos, científicos e investigadores, genetistas, bioinformáticos y personal sanitario. A nivel institucional, EPG es de referencia obligada en bibliotecas, hospitales y departamentos universitarios.

El Dr. Ramón Cacabelos impartió una conferencia sobre la aplicación de la Medicina personalizada en el entorno laboral, en una jornada organizada por el Dr. Javier Sanz en la sede de Garrigues

El pasado mes de octubre el Dr. Cacabelos, Presidente de EuroEspes, habló de "Nuevas perspectivas en medicina genómica en el entorno laboral", dentro del marco de una jornada organizada por el Dr. Javier Sanz y la *Sociedad Española de Medicina del Trabajo* en la sede de Garrigues en Madrid, ante una alta concurrencia de profesionales de la medicina del trabajo vinculados a las grandes empresas de nuestro país.

Este tipo de actividad formativa, en colaboración con diferentes sociedades médicas españolas y extranjeras, se integra dentro del programa educacional de la Fundación EuroEspes y del Centro Médico EuroEspes para difundir conocimientos sobre la importancia que la medicina genómica está teniendo ya en la medicina actual y en el impacto futuro sobre la salud de la población en términos de diagnóstico precoz, medicina predictiva y tratamiento personalizado.

EuroEspes registra en la Oficina de Patentes de Estados Unidos una nueva vacuna contra la enfermedad de Alzheimer

En su larga carrera de más de 20 años de lucha contra la enfermedad de Alzheimer, el Centro de Investigación Biomédica EuroEspes y su filial biotecnológica, EuroEspes Biotecnología, bajo la dirección del Dr. Ramón Cacabelos, acaban de dar un paso trascendental con un nuevo modelo de vacuna que previene la enfermedad y reduce eficientemente las lesiones cerebrales en aquellos casos en los que el Alzheimer ya se ha manifestado. La documentación oficial de la nueva vacuna EE-AD-S1P de EuroEspes acaba de ser hecha pública por la oficina de patentes de Estados Unidos.

Esta vacuna de carácter preventivo y terapéutico, completamente innovadora, se caracteriza por la introducción de un nuevo inmunógeno-adyuvante diseñado para generar anticuerpos contra las placas neuríticas donde se acumula la proteína beta-amiloide (Aβ) que daña el cerebro de los pacientes con Alzheimer. La otra característica distintiva de esta vacuna es que va encapsulada en liposomas ricos en esfingosina-1-fosfato (S1P), que contribuyen a la regeneración neuronal.

La vacuna se experimentó en animales transgénicos portadores de las principales mutaciones genéticas responsables de la enfermedad en seres humanos. En el modelo preventivo de la vacuna se vio que los animales inmunizados no desarrollaban la enfermedad a lo largo de la vida; y en el modelo terapéutico se comprobó que en los animales que manifestaban signos de degeneración cerebral, la administración de la vacuna reducía de forma espectacular los rasgos patogénicos genuinos que caracterizan a la enfermedad de Alzheimer en el cerebro: los depósitos de beta-amiloide, los ovillos neurofibrilares y las reacciones neuroinflamatorias mediadas por las células gliales. Con esta modalidad de vacuna se evitaban las reacciones meningoencefalíticas letales que hicieron fracasar a otros modelos previos de vacunas anti-Alzheimer.

El equipo de científicos que participaron en el proyecto, dirigidos por el Dr. Ramón Cacabelos, y coordinados por la Dra. Carmen Vigo, investigadora asociada a EuroEspes Biotecnología en California, lo integraban el Dr. Iván Carrera, del departamento de Neurociencias Básicas, la Dra. Lucía Fernández-Novoa, del departamento de Genómica Médica, y el Dr. Valter Lombardi, del departamento de Biotecnología de la Salud.

La enfermedad de Alzheimer es la principal forma de demencia, junto a la demencia vascular; es el primer problema de discapacidad, y representa la quinta causa de muerte en mayores de 65 años en Estados Unidos, con 71.600 muertes/año. Las 3 principales causas de muerte son las enfermedades cardiovasculares (200.2 x 100.000), el cáncer (180.7 x 100.000) y el ictus cerebral (43.6 x 100.000).

En la Unión Europea, los costes por demencia suponen a los contribuyentes unos 160.000 millones de euros, con un coste medio por paciente al año de 22.000€. Un 10% de los costes directos del Alzheimer son gasto farmacéutico. La posibilidad de implantar programas preventivos capaces de reducir la prevalencia de la enfermedad, que afecta a más de un 15% de mayores de 65 años, permitiría mejorar notablemente las condiciones de vida de la población a riesgo y reducir el gasto sanitario en Alzheimer entre un 20% y un 30% en 5 años.

El presidente de la Asociación Mundial de Medicina Genómica, Ramón Cacabelos, promueve en Australia el uso de la farmacogenómica para reducir efectos secundarios y gasto farmacéutico

En el curso del Tercer Congreso Mundial de Psiquiatría Asiática, celebrado en Melbourne, Australia, del 31 de julio al 4 de agosto, se constató el importante papel que va a desempeñar la genómica en el futuro de la medicina y muy especialmente en las enfermedades del cerebro. Una de las sesiones científicas que despertó más interés fue la dirigida por los doctores Ramón Cacabelos, presidente de la Asociación Mundial de Medicina Genómica, y Masatoshi Takeda, presidente de la Sociedad Japonesa de Psiquiatría Biológica, que reunieron a importantes científicos para analizar el impacto de la genómica y la farmacogenómica en las enfermedades psiquiátricas. En torno al problema de la esquizofrenia se obtuvieron importantes conclusiones. Esta enfermedad, que afecta a un 1% de la población, con claro componente genético, sigue siendo un grave problema de salud por la discapacidad que genera en gente joven, agravada por la influencia que el abuso de drogas puede tener en el desarrollo de psicosis tóxicas. Aproximadamente un 1-2% de la estructura del genoma humano, integrado por 35.000 genes, se halla alterada en la esquizofrenia; sólo un 20% de los pacientes responden adecuadamente a los tratamientos convencionales con neurolépticos; y los efectos secundarios en este colectivo son muy relevantes. Según el Dr. Cacabelos, director del Instituto para Enfermedades del Sistema Nervioso Central y Medicina Genómica del Centro Médico EuroEspes de La Coruña, un 20% de los pacientes tratados con psicofármacos desarrollan obesidad; y de un estudio realizado en España en más de 3000 pacientes se pudo comprobar que sólo un 26% son capaces de metabolizar adecuadamente los medicamentos que reciben; el 74% restante de la población española muestra defectos genéticos en los genes *CYP2D6*, *CYP2C19* y *CYP2C9*, responsables del metabolismo de un 60% de los fármacos de uso común. Ante esta situación, la utilización del perfil farmacogenético de estos pacientes permitiría optimizar los recursos terapéuticos disponibles, sabiendo el tipo de medicamento que pueden tomar y los fármacos que deben evitar; con ello se reduciría en más de un 50% de los casos la emergencia de efectos secundarios, y se reduciría en un 20-30% el gasto farmacéutico corriente, puesto que entre un 40% y un 60% de los tratamientos que se administran rutinariamente o no son efectivos o causan daños colaterales que contribuyen a multiplicar el gasto farmacéutico.

El Centro Médico EuroEspes y el Centro de Ojos de Coruña colaboran en una jornada sobre Genética del Glaucoma Pseudoexfoliativo y Degeneración Macular Asociada a la Edad (DMAE) organizada por los doctores Pablo Carnota y José Antonio Saavedra

El pasado jueves 24 de noviembre se celebró en Coruña una jornada científica, organizada por los Drs. Pablo Carnota y José Antonio Saavedra, sobre la genética del glaucoma pseudoexfoliativo y la degeneración macular asociada a la edad (DMAE). Fruto de la colaboración entre el Centro Médico EuroEspes y el Centro de Ojos de La Coruña, se han elaborado unos paneles de riesgo genético para dos de las patologías oftalmológicas más frecuentes en nuestro entorno: el glaucoma pseudoexfoliativo, un tipo especialmente agresivo de glaucoma muy prevalente en la población gallega, y la DMAE, patología muy habitual en personas de más de 60 años. En esta reunión se presentaron estos paneles de riesgo genético a especialistas en oftalmología de la comunidad autónoma de Galicia con el objetivo de que puedan ir incorporando estos avances tecnológicos y científicos en el campo de la genética a su práctica clínica diaria. Los test genéticos no sustituyen el papel del oftalmólogo en el diagnóstico y tratamiento de estas enfermedades sino que son una herramienta de apoyo con la que el especialista puede contar para optimizar su labor médica, hacer una oftalmología predictiva y personalizar sus estrategias terapéuticas. Durante la reunión se analizó en qué consisten estos paneles de riesgo genético para después abrir un debate sobre su uso, principalmente saber a qué tipo de pacientes está dirigido. En la reunión intervinieron el Dr. Ramón Cacabelos, el Dr. José Antonio Saavedra, el Dr. Pablo Carnota Méndez y el Dr. Juan Carlos Carril.

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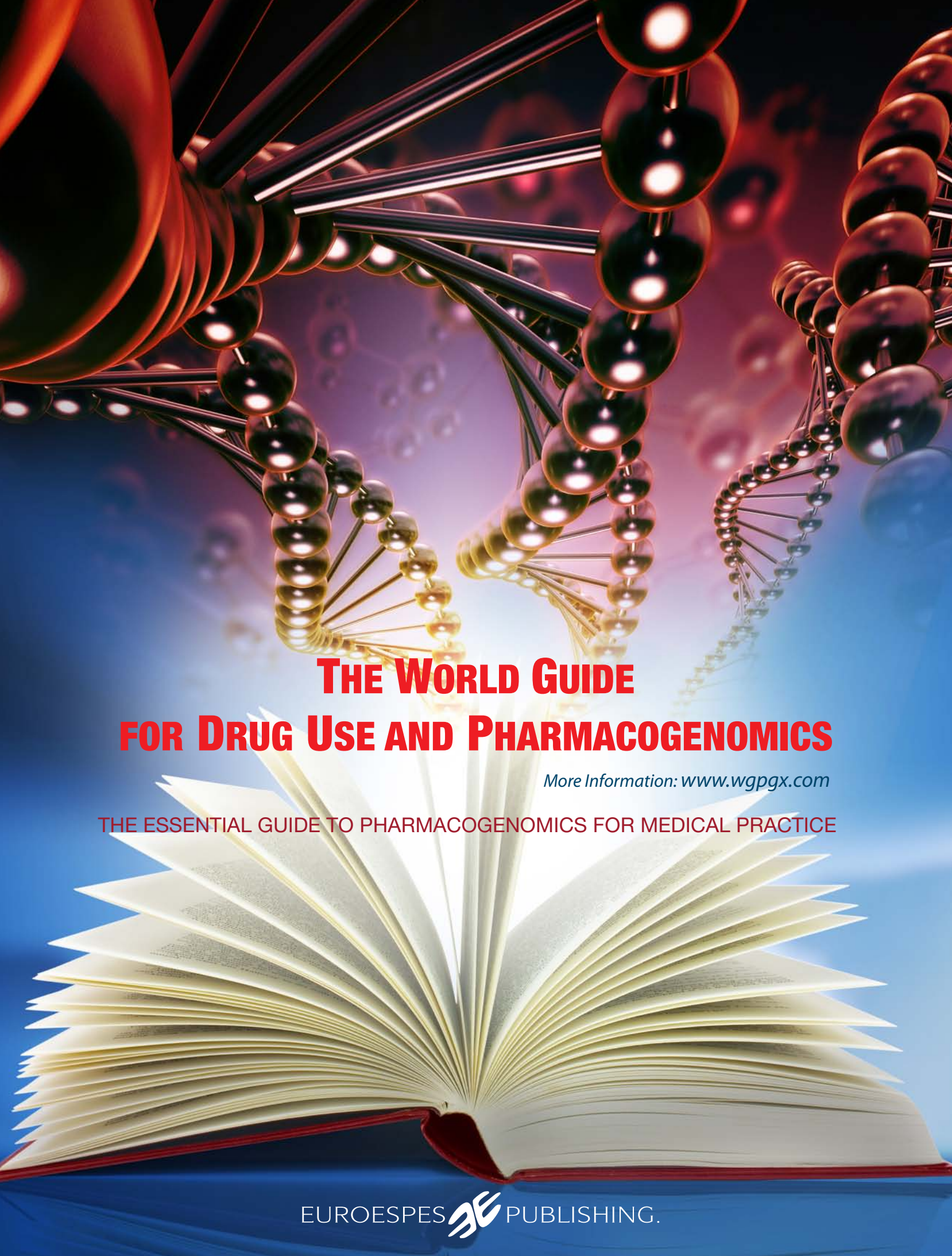

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