Alzheimer's disease 2011

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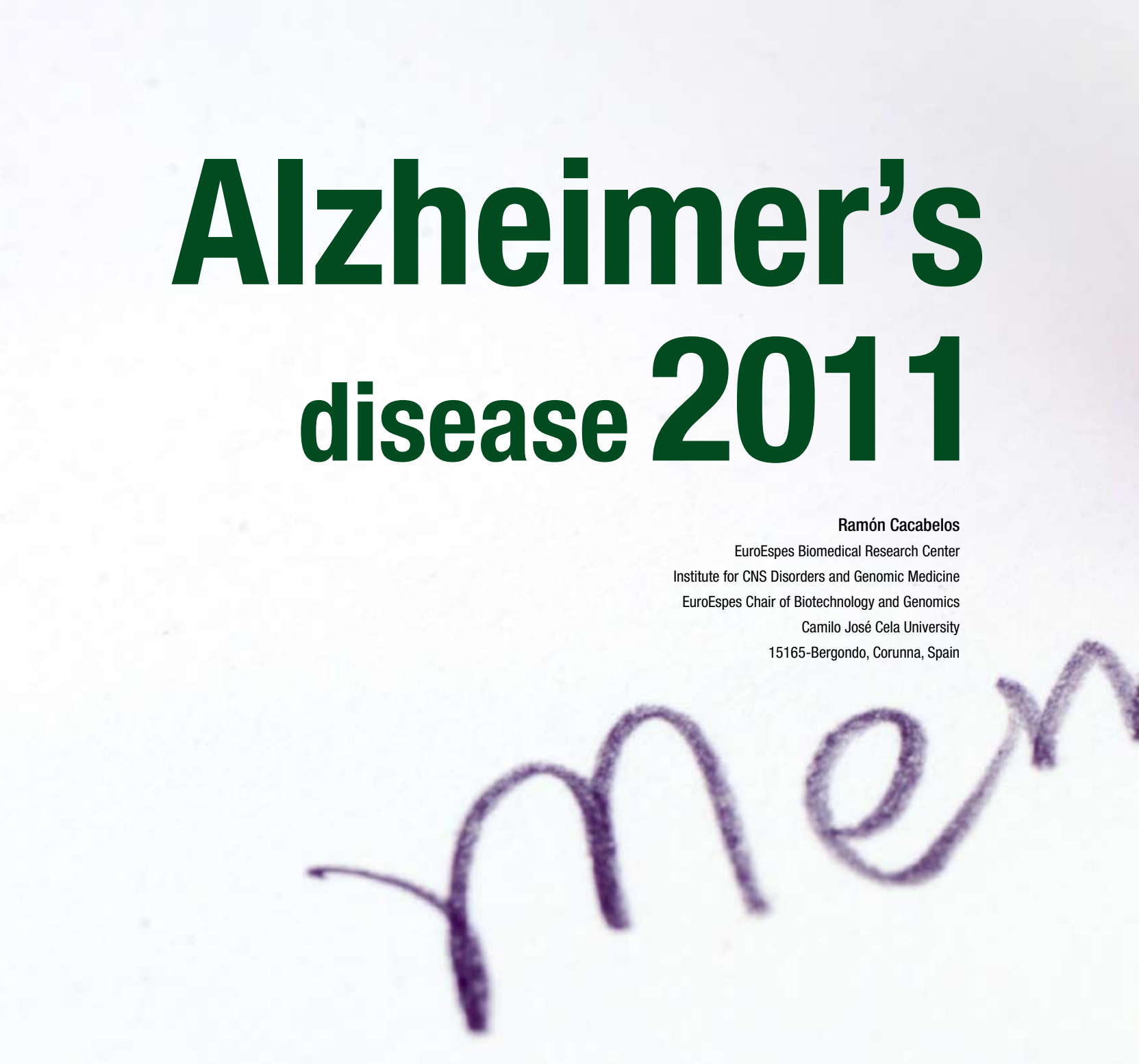


Summary

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.

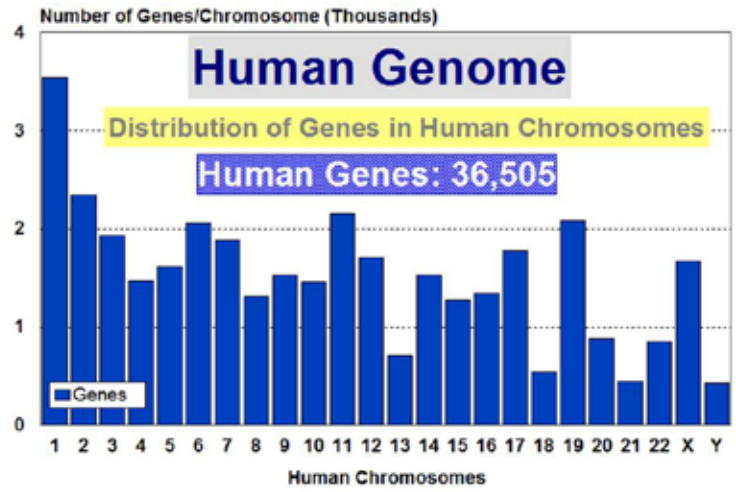


Fig. 1a

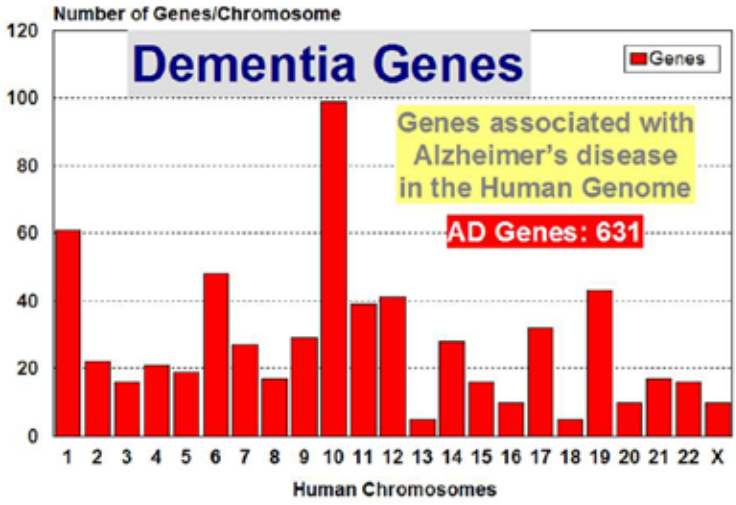


Fig. 1b

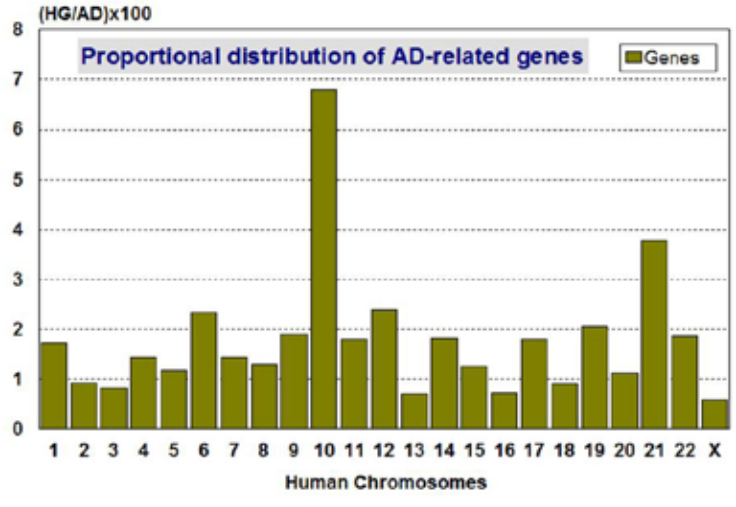


Fig. 1c

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].

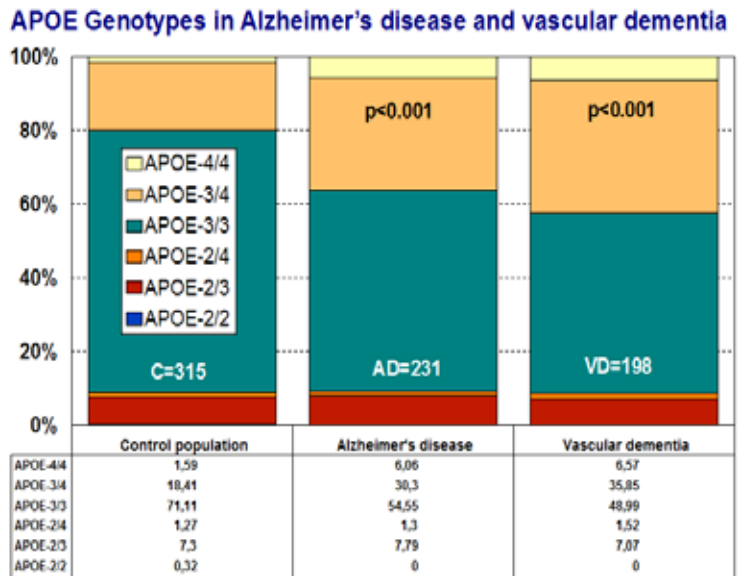
Abuse, misuse, self-prescription, and uncontrolled medical prescription of CNS drugs are becoming major problems with unpredictable consequences for brain health. The pharmacological management of dementia is an issue of special concern due to the polymedication required to modulate the symptomatic complexity 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.

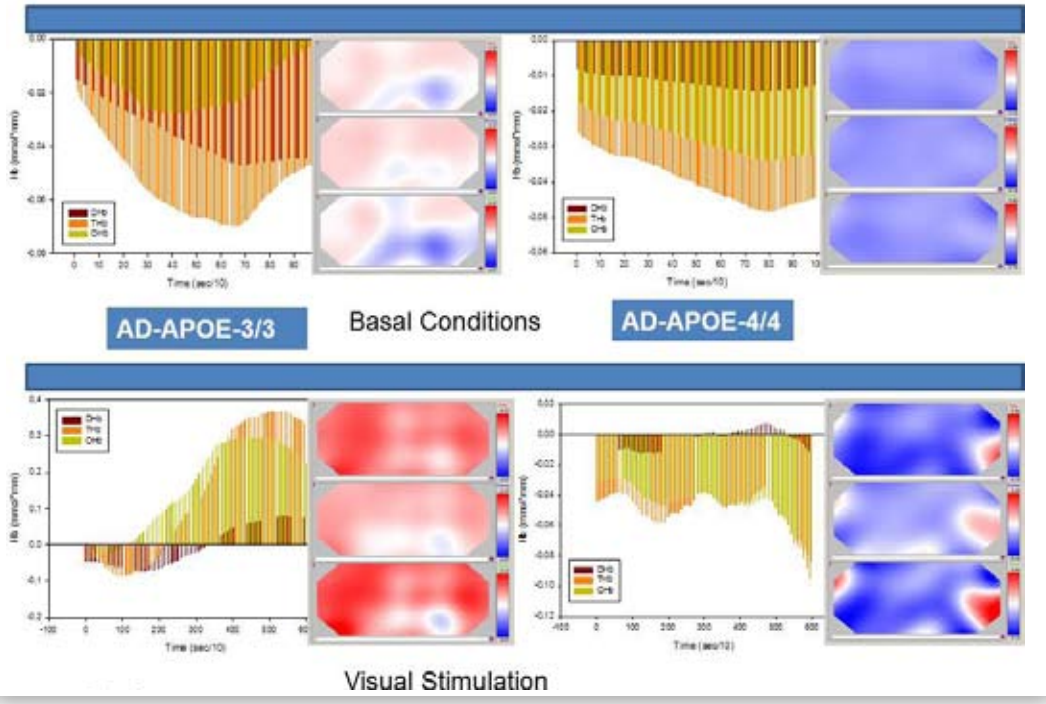


10^{11} neurons in the brain having around 10^3-10^4 synapses with a complex multiprotein structure integrated by 10^3 different proteins) requires a very powerful technology for gene expression profiling, which is still in its very early stages and is not devoid of technical obstacles and limitations [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

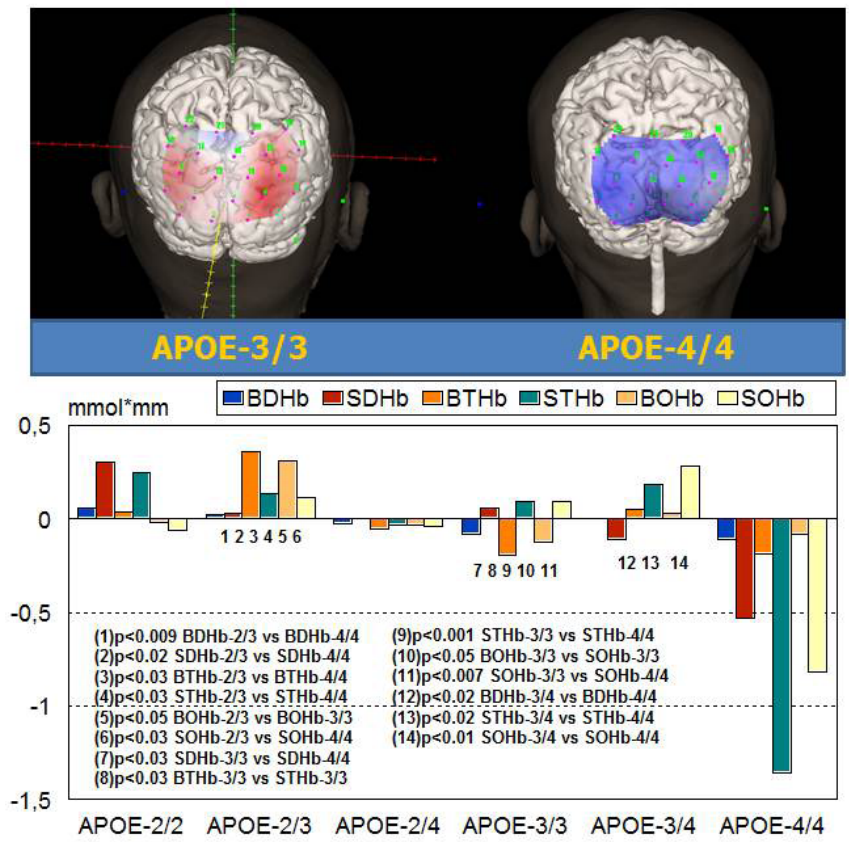
Figure 3. APOE-related cortical Hemoglobin changes in the occipital region in basal conditions and after visual stimulation in Alzheimer's disease as assessed by brain optical topography analysis.
DHb: Deoxyhemoglobin; THb: Total Hemoglobin; OHb: Oxyhemoglobin.



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.*

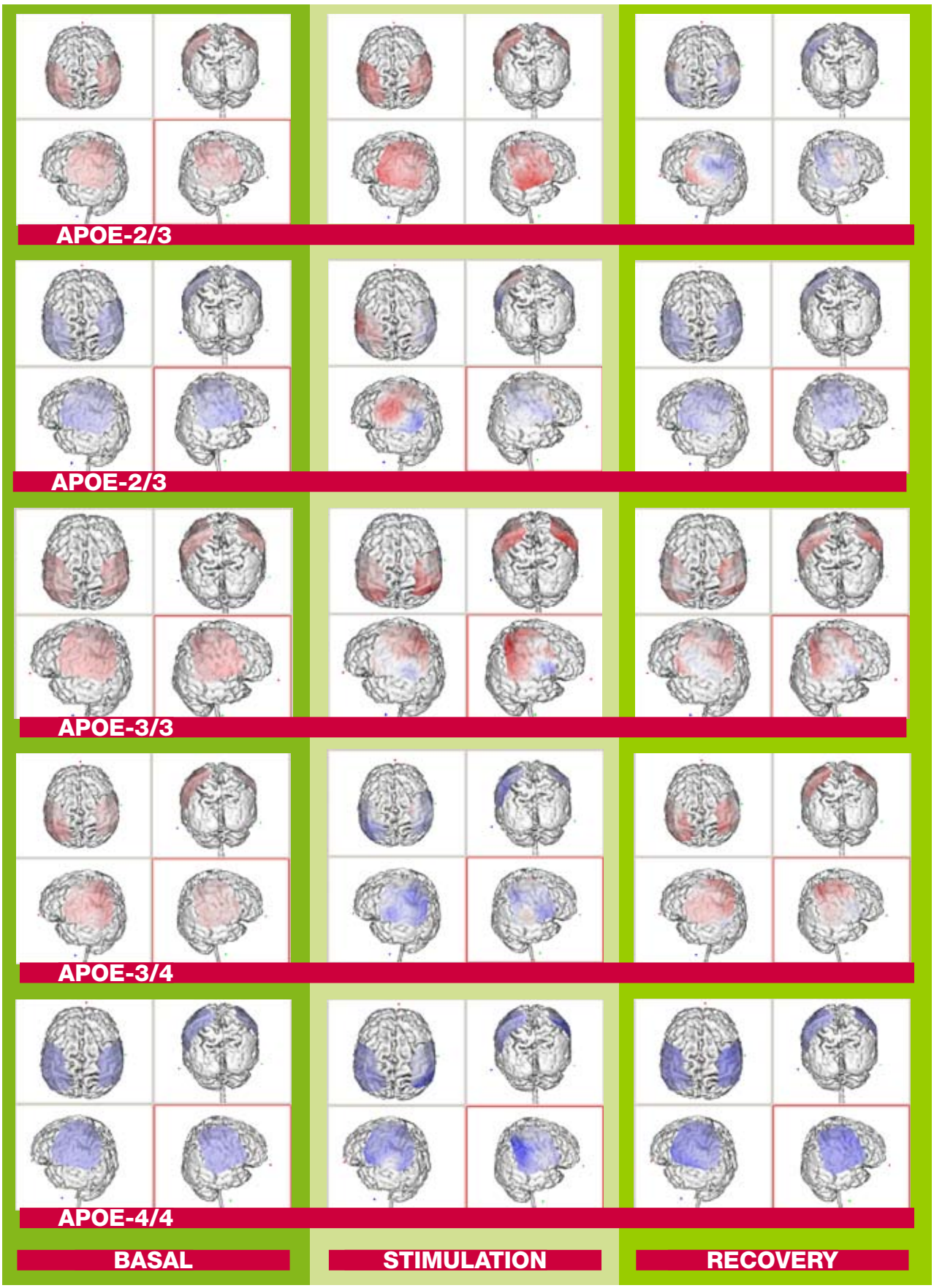
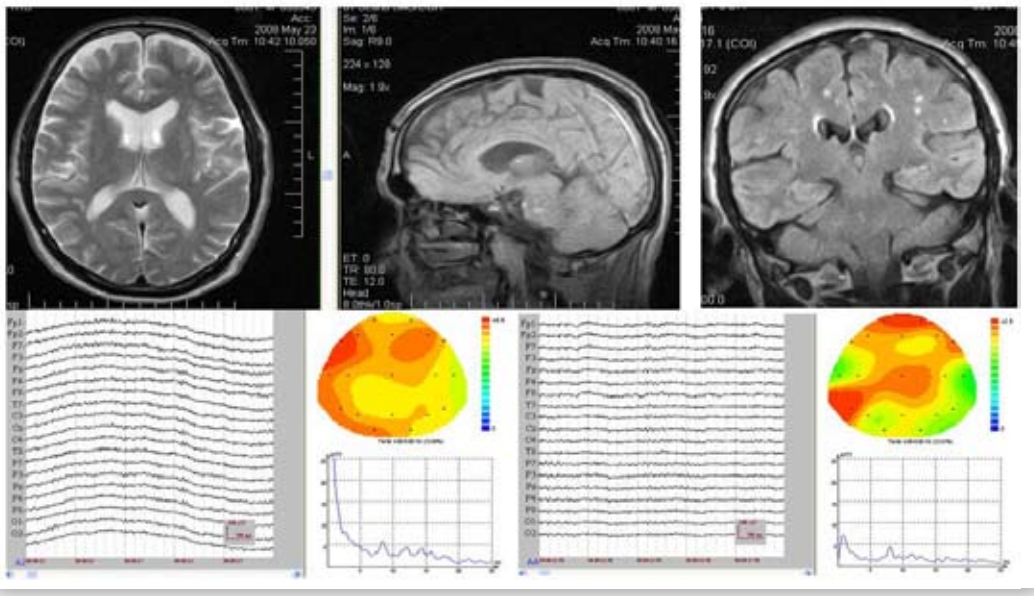


Figure 6. MRI and brain mapping activity (pre- *vs* post-treatment with a multifactorial therapy) in an AD-*APOE-3/3* carrier.



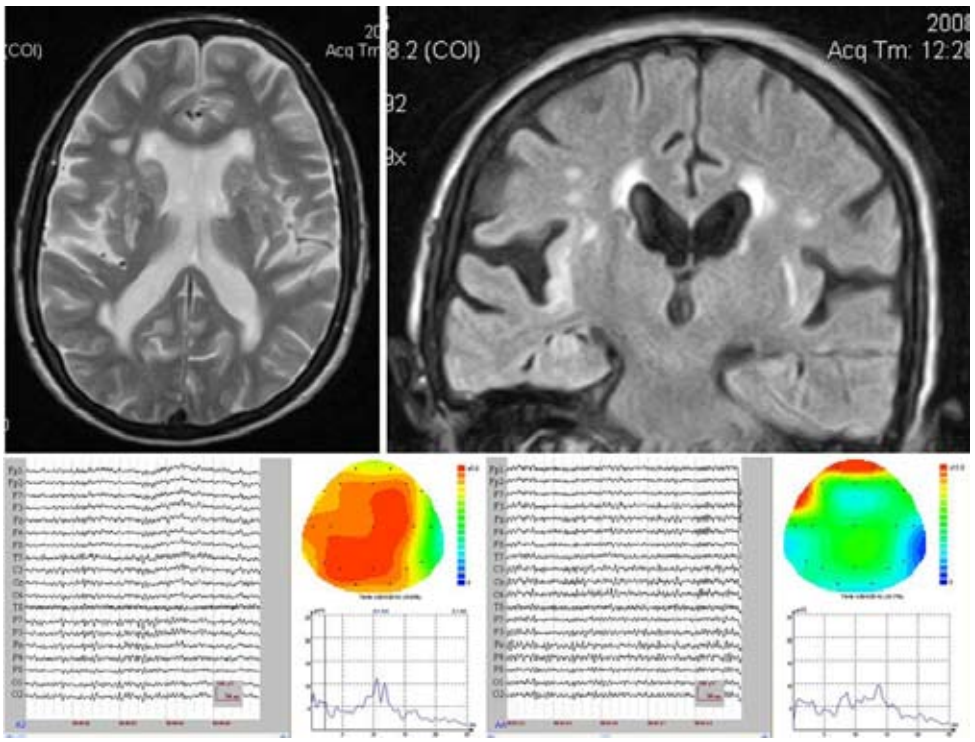
of the *APOE-2* allele might be protected against dementia. *APOE*-related pathogenic mechanisms are also associated with brain aging and with the neuropathological hallmarks of AD [16].

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

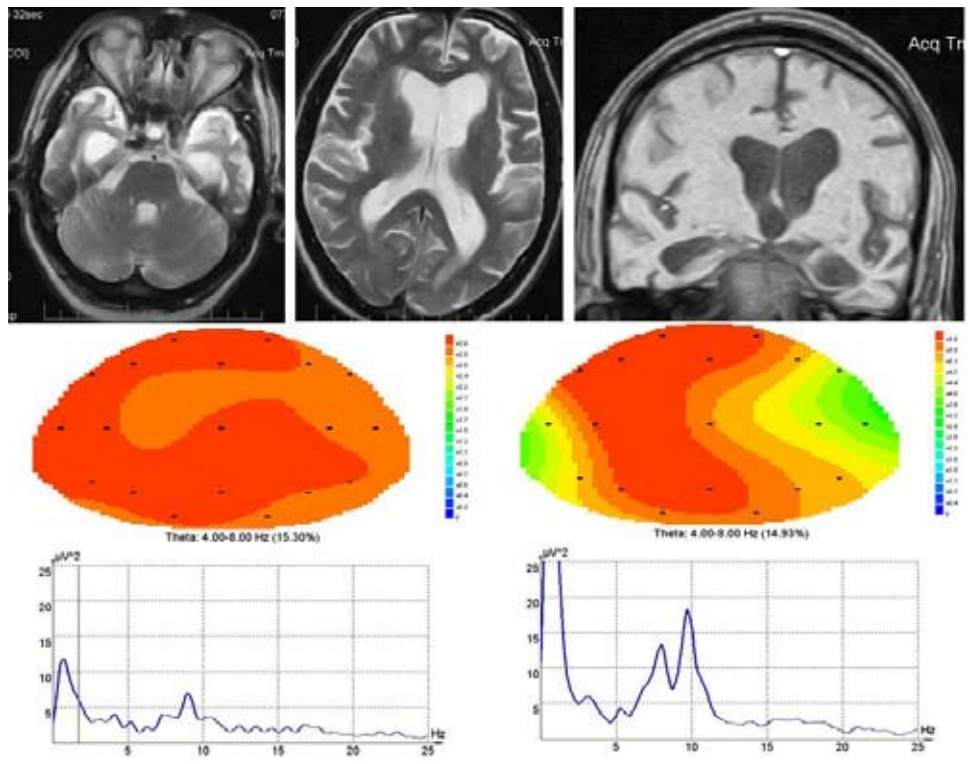
The dual amyloidogenic-tauopathic theory of AD has dominated the pathogenic universe of AD-related neurodegeneration (and divided the research community, as well) for the past 50 years, nourished by the presence of *APP*, *PSEN1*, *PSEN2* and *MAPT* mutations in a very small number of cases with early-onset 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.



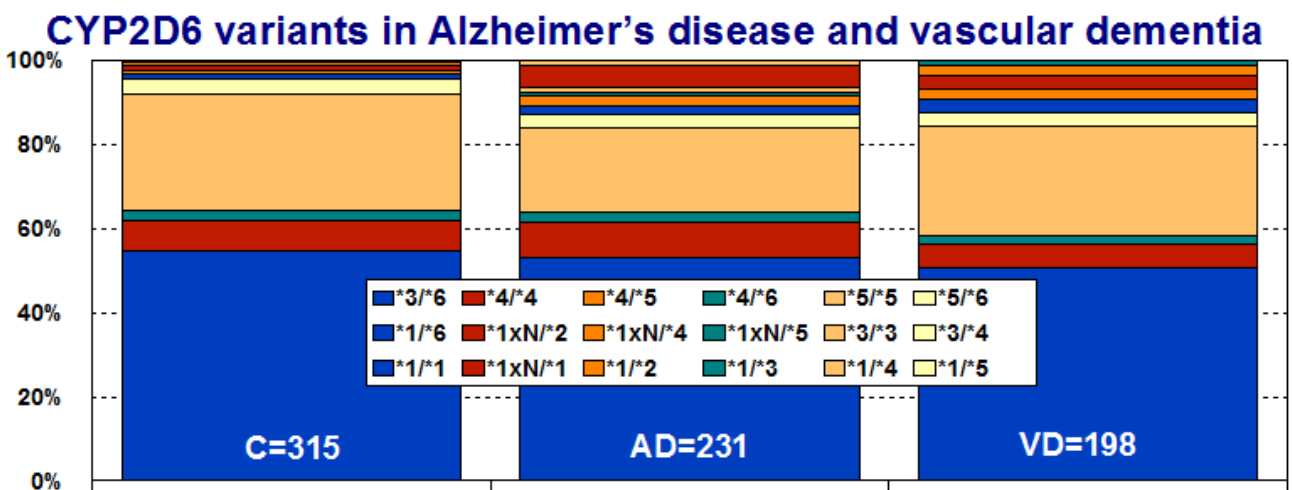
itself downregulates the expression of P-gp and other Aβ-transporters, which could exacerbate the intracerebral accumulation of Aβ and thereby accelerate neurodegeneration in AD and cerebral β-amyloid angiopathy [51].

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.

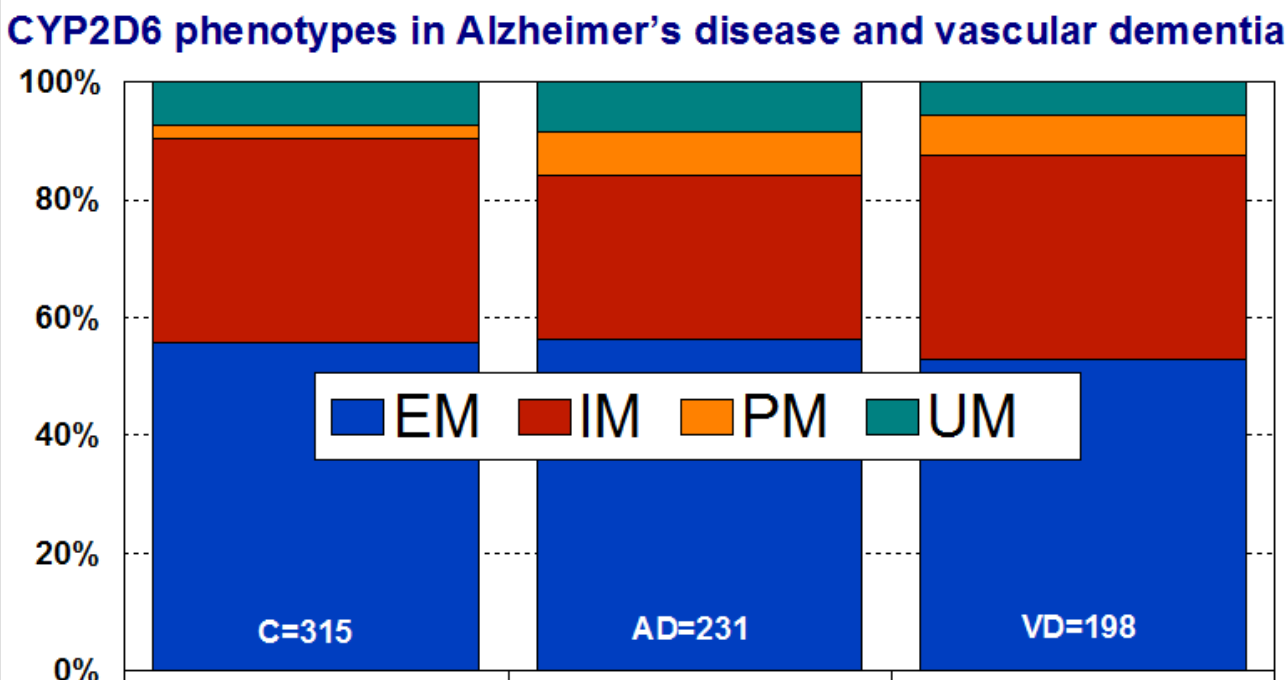


	Control population	Alzheimer's disease	Vascular dementia
*5/*6	0,46	0	0
*5/*5	0	1,06	0
*4/*6	0	0	1,12
*4/*5	0,46	0	2,25
*4/*4	1,37	5,32	3,37
*3/*6	0	0	0
*3/*4	0	0	0
*3/*3	0	1,06	0
*1xN/*5	0	1,06	0
*1xN/*4	0,91	2,12	2,25
*1xN/*2	0	0	0
*1/*6	0,91	2,13	3,37
*1/*5	3,65	3,19	3,37
*1/*4	27,85	20,21	25,84
*1/*3	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].

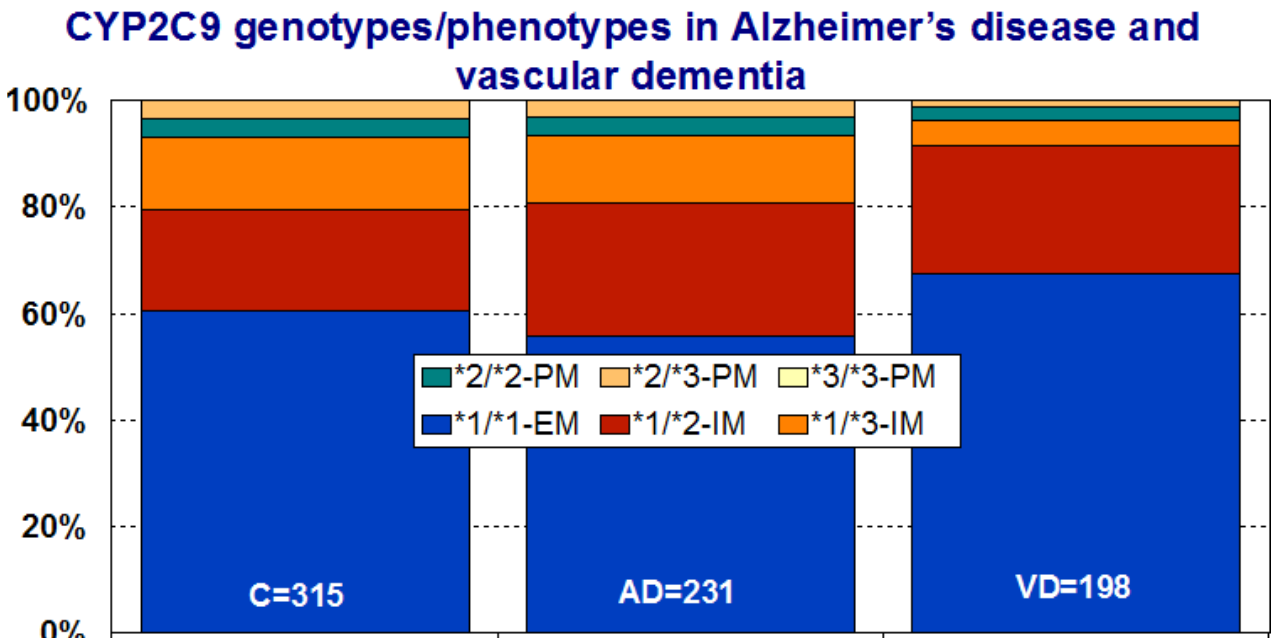
Neurotoxic factors: Old and new theories suggest that different toxic agents, from metals (i.e. aluminium, copper, zinc, iron) to biotoxins and pesticides, might contribute to neurodegeneration. Dysfunctional homeostasis of transition metals is believed to play a role in the pathogenesis of AD [66].

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.



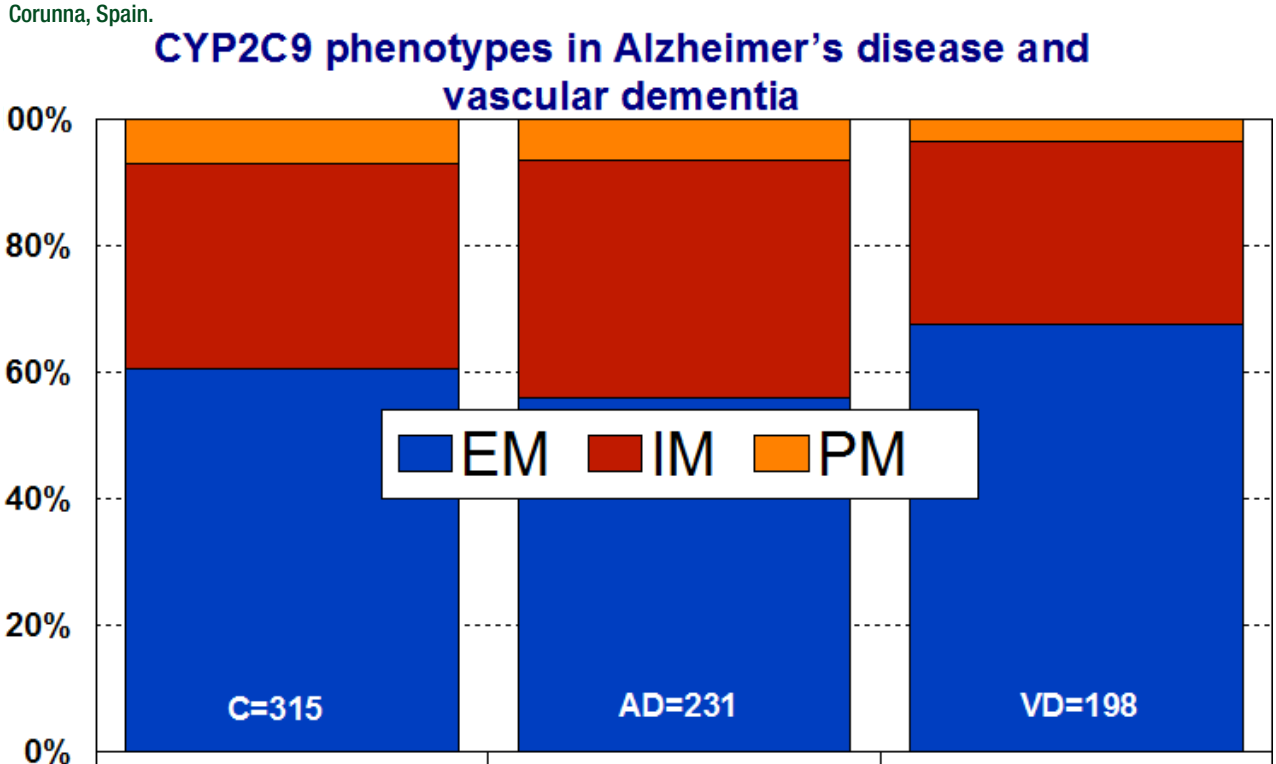
	Control population	Alzheimer's disease	Vascular dementia
*3/*3-PM	0	0	0
*2/*3-PM	3,28	3,23	1,2
*2/*2-PM	3,76	3,23	2,41
*1/*3-IM	13,62	12,9	4,82
*1/*2-IM	18,78	24,73	24,1
*1/*1-EM	60,56	55,91	67,47

[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.

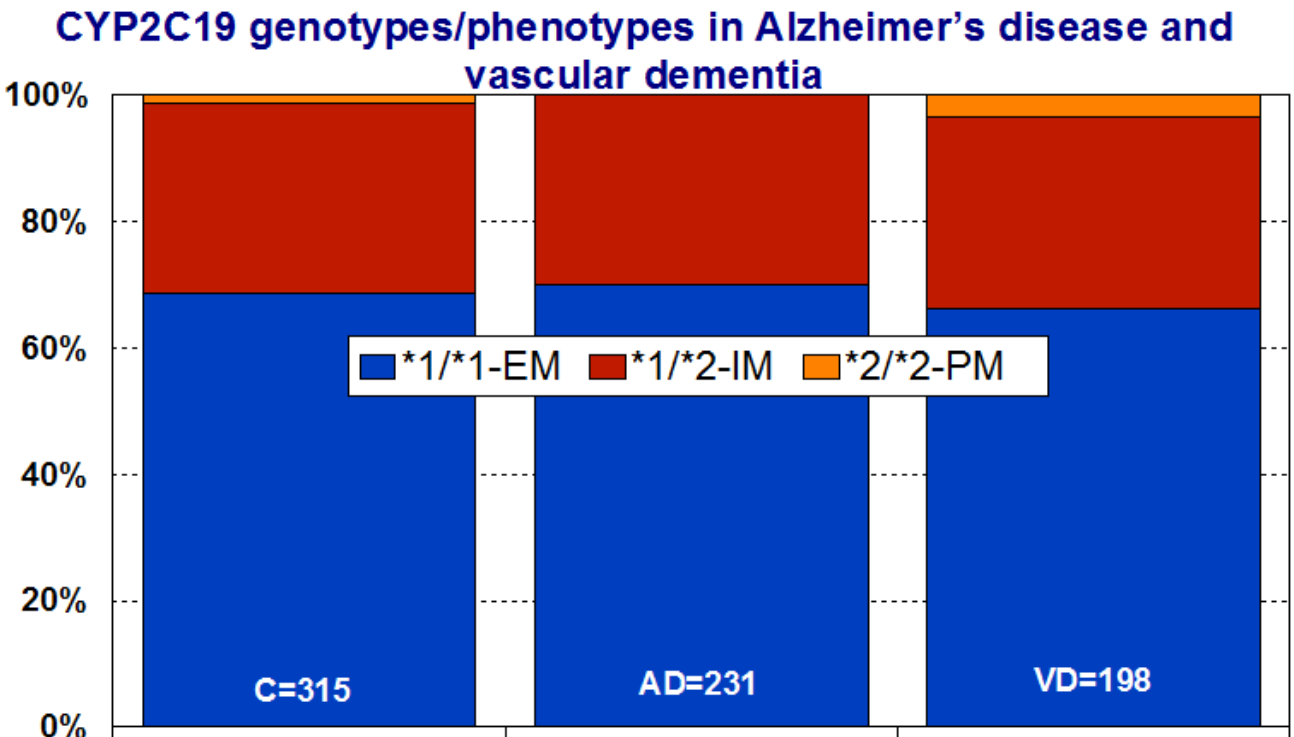
Structural and functional neuroimaging: Structural and functional neuroimaging techniques (MRI, fMRI, PET, SPECT) are essential diagnostic tools in dementia, though the specificity of the visual observations in degenerative forms of dementia is of doubtful value; however, these procedures are irreplaceable 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)

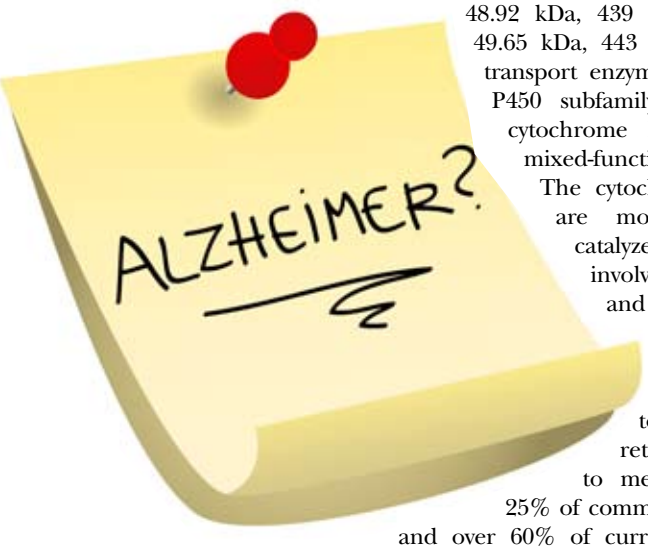
Rivastigmine Pharmacogenetics

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%,

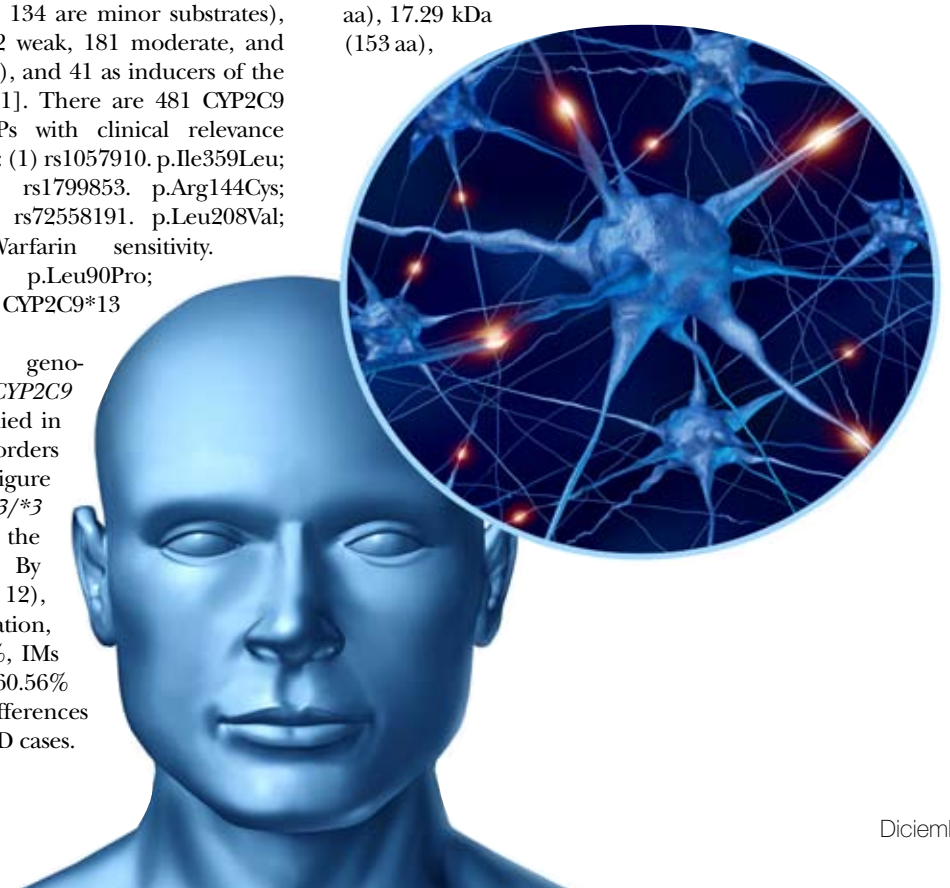
respectively, in the general population, and 57.76, 31.05%, 5.27%, and 5.90%, respectively in AD cases; (iii) EMs are more prevalent in GP (59.51%) than in AD (57.76%); IMs are more frequent in AD (31.05%) than in GP (29.78%); the frequency of PMs is slightly higher in AD (5.27%%) than in GP (4.46%); 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.