Pharmacogenomics in Alzheimer's Disease

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Abstract: Alzheimer's disease (AD) is a complex disorder associated with multiple genetic defects either mutational or of susceptibility. Information available on AD genetics does not explain in full the etiopathogenesis of AD, suggesting that environmental factors and/or epigenetic phenomena may also contribute to AD pathology and phenotypic expression of dementia. The genomics of AD is still in its infancy, but is helping to understand novel aspects of the disease including genetic epidemiology, multifactorial risk factors, pathogenic mechanisms associated with genetic networks and genetically-regulated metabolic cascades. AD genomics is also helping to develop new strategies in pharmacogenomic research and prevention. Functional genomics, proteomics, pharmacogenomics, high-throughput methods, combinatorial chemistry and modern bioinformatics will greatly contribute to accelerate drug development for AD and other complex disorders.

Main genes involved in AD include mutational loci (APP, PS1, PS2, TAU) and multiple susceptibility loci (APOE, A2M, AACT, LRP1, IL1A, TNF, ACE, BACE, BCHE, CST3, MTHFR, GSK3B, NOS) distributed across the human genome. Genomic associations integrate bigenic, trigenic, tetragenic or polygenic matrix models to investigate the genomic organization of AD in comparison to the control population. Similar genetic models are used in pharmacogenomics to elucidate genotype-specific responses of AD patients to a particular drug or combination of drugs. Using APOE-related monogenic models it has been demonstrated that the therapeutic response to drugs in AD is genotype-specific. A multifactorial therapy combining 3 different drugs yielded positive results during the 6-12 months in approximately 60% of the patients. With this therapeutic strategy, APOE-4/4 carriers were the worst responders, and patients with the APOE-3/4 genotype were the best responders. In bigenic and trigenic models it was possible to differentiate the influencial effect of PS1 and PS2 polymorphic variants on mental performance in response to multifactorial therapy.

The application of functional genomics to AD can be a suitable strategy for harmonization in molecular diagnosis and drug clinical trials. Furthermore, the pharmacogenomics of AD may contribute in the future to optimise drug development and therapeutics, increasing efficacy and safety, and reducing side-effects and unnecessary costs.

INTRODUCTION

Alzheimer's disease (AD) is the main cause of dementia and a major problem of health in developed countries, together with cardiovascular disorders, cancer, and stroke. More than 25 million people suffer from AD all over the world, and probably more than 75 million people are at risk of developing AD in the coming 20-25 years [1]. Age-specific prevalence for AD ranges from 1% at the age of 60-64 years to approximately 50% in people older than 90 years [1]. The average annual cost per person with AD ranges from $15,000 to $50,000, depending upon studies, disease stage, and country (USA, EU, Japan), with a lifetime cost per patient over $175,000 according to different estimations [2-6]. About 15-20% of the total costs in dementia are dedicated to pharmacological treatment, and it is expected that a successful treatment would cut-down global costs (direct + indirect costs) by 30-40% [1,6]. Unfortunately, no curative drugs have so far been developed for AD, and the available pharmacological treatments (e.g., cholinesterase inhibitors, neuroprotective agents, nootropics, antioxidants, anti-inflammatory agents, etc) are not effective enough, with cost-benefit and cost-effectiveness analyses showing questionable values at the present time [6].

Most therapeutic strategies in the past decades were oriented toward a substitutive therapy based on the assumption that a cholinergic deficit is responsible in part for AD. This wrong concept led to the development of cholinesterase inhibitors as the first generation of anti-dementia drugs [7-10]. In the past few years, novel therapeutic strategies have emerged with an etiopathogenic orientation aimed to develop new compounds able to regulate the pathogenic events underlying neurodegeneration in AD [11]. Despite the progress in understanding AD pathogenesis [12-15], few advances have been made in the therapeutic arena, though more than 200 compounds were tested during the past 10 years [11]. This failure can be attributed to several factors: (a) the complexity of AD, (b) economic restrictions, (c) high-risk investment, (d) misconceptions regarding AD etiology, (e) out-of-date protocols for new clinical trials, (f) the administration of outcome measures used with cholinergic enhancers to

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compounds devoid of cholinergic activity, (g) conflicts of interest in the industry, (h) deficient recruitment criteria and patients stratification in drug clinical trials, (i) poor selection of candidate drugs, and (j) neglecting AD heterogeneity based on genetic data [11].

During the past 20 years more than 5000 papers document the genetic basis of AD, with at least 10 genes associated with AD, and more than 50 genes potentially involved in dementia (www.ncbi.nlm.nih.gov). The multifactorial, polygenic features of AD confer this type of dementia the character of a 'complex' disorder where environmental, epigenetic and genetic factors (mutational, susceptibility) interact to accelerate neuronal death, this leading to the premature phenotypic expression of dementia, represented by its neuropathological hallmarks (amyloid deposition in senile plaques and brain vessels, neurofibrillary tangle (NFT) formation, synaptic loss, neuronal death) and clinical symptoms (memory deficit, behavioural changes, functional decline) [1,16,17]. Although in many other complex disorders with multiple genomic defects, such as hypertension, schizophrenia or Parkinson's disease, there are several drugs capable of reducing the phenotypic expression (clinical symptoms) of the disease (without a demonstrable, definitive cure), it is very unlikely that a single drug be effective enough to slow-down neurodegeneration precluding the possibility of the expression of AD onset and/or normalization of brain function in early-moderate stages of the disease, since premature neuronal death starts many years before the onset of the disease, probably at the end of the brain maturation period in the late 20's or early 30's [18,19]. By the same token, the multilocative genomic defects present in AD, either mutational loci or susceptibility loci, appear to suggest that such a complex neurodegenerative process would require multiple forms of pharmacological intervention [20]. With the advent of the initial sequencing and analysis of the human genome [21-23], the identification of more than one million single nucleotide polymorphisms (SNPs) in the human genome [24] and the implementation of DNA microarray technology in functional genomics and proteomics [25], probably many other genes will be associated with AD and neurodegeneration, since the present knowledge on AD genetics and most genomic loci associated with AD (Table 1) do not explain in full AD etiopathogenesis [1,26]. The availability of SNPs associated with AD [1,27-29] and point mutations directly linked to AD pathology [13,15,30] are of great help to design the first pharmacogenomic strategies for AD either at the preclinical level (e.g., biochips for primary screening [31-33], transgenic animals for basic studies [13,14,30], and at the clinical level to demonstrate whether or not the therapeutic response in AD is genotype-specific [11,34,35]. On the other hand, recent progress in understanding the molecular pathology of AD, together with advances in functional genomics, proteomics, high-throughput methods, combinatorial chemistry, and bioinformatics will contribute to design new procedures in pharmacogenomics to obtain better etiopathogenic drugs for AD.

Table 1. Selected Human Genes Associated with Dementia and Age-related Neurodegenerative Disorders

Locus	Symbol	Title/Gene	MIM	Disorder
21q21	AD1 APP AAA/CVAP	Amyloid beta (A4) precursor protein	104760	Alzheimer's disease-1 Dutch type cerebroarterial amyloidosis
19cen-q13.2	AD2	Alzheimer's disease-2	104310	Late-onset AD-2
14q24.3	AD3 PSEN1	Presenilin-1	104311	Alzheimer's disease-3
1q31-q42	AD4 PSEN2 STM2	Presenilin-2	600759	Alzheimer's disease-4
12p11.23-q13.12	AD5	Familial AD-5	602096	Alzheimer's disease-5
10q24	AD6	Alzheimer's disease-6	605526	Alzheimer's disease-6
10p13	AD7	Alzheimer's disease-7	606187	Alzheimer's disease-7
17q23	ACE/ACE1 DCP1	Angiotensin I converting enzyme Dipeptidyl carboxipeptidase-1	106180	Susceptibility to Alzheimer's disease
12p13.3-p12.3	A2M	Alpha-2-Macroglobulin	103950	Susceptibility to Alzheimer's disease
19q13.2	APOE	Apolipoprotein E	107741	Susceptibility to Alzheimer's disease Myocardial infarction Type III hyperlipoproteinemia
17q11.2	BLMH BMH	Bleomycin hydrolase	602403	Susceptibility to Alzheimer's disease
5460A/mtDNA	MTND1	Subunit 2 NADH dehydrogenase-ubiquinone oxidoreductase	516001	Susceptibility to Alzheimer's disease
4336G/mtDNA 3397V/mtDNA	MTND1	Subunit 1 NADH dehydrogenase-ubiquinone oxidoreductase	516000	Susceptibility to Alzheimer's disease
5904-7444 mtDNA	MTCO1	Cytochrome c oxidase subunit I Complex IV	516030	Susceptibility to Alzheimer's disease

(Table 1). contd.....

Locus	Symbol	Title/Gene	MIM	Disorder
7586-8294 mtDNA	MTCO2	Cytochrome c oxidase subunit II Complex IV	516040	Susceptibility to Alzheimer's disease
Chr.1	NCSTN	Nicastrin	605254	Alzheimer's disease
11q23.3	BACE1 BACE	Beta-site amyloid beta A4 precursor protein-cleaving enzyme Beta-secretase Memapsin-2	604252	Alzheimer's disease
21q22.3	BACE2 ALP56 DRAP	Beta-site amyloid beta A4 precursor protein-cleaving enzyme 2 Down syndrome-region aspartic protease	605668	Alzheimer's disease Down syndrome
12q13.1-q13.3	LRP1 A2MR	Low density lipoprotein-related protein-1 Alpha-2-macroglobulin receptor	107770	Alzheimer's disease
14q24.3	FOS	FBJ murine osteosarcoma viral (v-fos) oncogene homolog Oncogene Fos	164810	Alzheimer's disease
8p22	CTSB CPSB	Cathepsin B Amyloid precursor protein secretase	116810	Alzheimer's disease
2q14	IL1A	Interleukin-1-Alpha	147760	Alzheimer's disease
6p21.3	TNFA	Tumor necrosis factor-α Cachectin	191160	Alzheimer's disease
7q36	NOS3	Nitric Oxide Synthase-3	163729	Alzheimer's disease
3q26.1-q26.2	BCHE	Butyrylcholinesterase	177400	Alzheimer's disease
20p11.2	CST3	Cystatin 3	604312	Alzheimer's disease
1p36.3	MTHFR	Methylenetetrahydrofolate reductase	236253	Alzheimer's disease Homoystinuria
4q21	PARK1 SNCA NACP	Alpha-Synuclein	163890	Familial Parkinson's disease Alzheimer's disease
6q25.2-q27	PARK2 PRKN PDJ	Parkin	602544	Type 2 juvenile Parkinson's disease
2p13	PARK3	Parkinson's disease-3	602404	Familial Parkinson's disease
4p15	PARK4	Lewy body autosomal dominant Parkinson's disease	605543	Lewy body autosomal Parkinson's disease
4p14	PARK5 UCHL1	Ubiquitin C-terminal esterase L1	191342	Familial Parkinson's disease
1p36-p35	PARK6	Early-onset autosomal recessive Parkinson's disease-6	605909	Parkinson's disease
18p11.31-p11.2	NDUFV2	NADH dehydrogenase (ubiquinone) flavoprotein-2, 24 kD	600532	Susceptibility to Parkinson's disease
22q13.1	CYP2D P450C2D	Subfamily IID Cytochrome P450	124030	Susceptibility to Parkinson's disease Debrisoquine sensitivity
17q21	PPND	Parkinsonism-dementia with pallidopontonigral degeneration	168610	Parkinsonism-dementia with pallidopontonigral degeneration
17q21.1	MAPT MTBT1 DDPAC MST	Macrotubule-associated protein tau	157140	Frontotemporal dementia with parkinsonism Pick's disease
17q21-q22	GPSC	Familial progressive subcortical gliosis	221820	Familial progressive subcortical gliosis
3p11.1-q11.2	DMT1	Familial non-specific dementia	600795	Familial non-specific dementia
13q14	ITM2B BRI ABRI FBD	Integral membrane protein 2B (BRI gene)	603904	Familial British dementia Familial Danish dementia
9q21-q22	ALSFTD	Amyotrophic lateral sclerosis with frontotemporal dementia	105550	Amyotrophic lateral sclerosis with frontotemporal dementia

(Table 1). contd.....

Locus	Symbol	Title/Gene	MIM	Disorder
21q22.1	SOD1 ALS1	Soluble superoxide dismutase 1	147450	Amyotrophic lateral sclerosis due to SOD1 deficiency
22q12.2	NEFH	Heavy polypeptide neurofilament	162230	Susceptibility to amyotrophic lateral sclerosis
1q31-q32	PTPRC CD45 LCA	C Polypeptide receptor type Protein tyrosine phosphatase	151460	Susceptibility to multiple sclerosis
20pter-p12	PRNP PRIP	Prion protein (p27-30)	176640	Creutzfeldt-Jakob disease Gerstman-Straussler disease Fatal familial insomnia
4p16.3	HD ITIS	Huntingtin	143100	Huntington disease
20p	HLN1	Huntington-like neurodegenerative disorder	603218	Huntington-like neurodegenerative disorder
14q	BCH	Hereditary benign chorea	118700	Hereditary benign chorea
12p13.31	DRPLA	Atrophin	125370	Dentatorubro-pallidoluysian atrophy
21q22.3	DCR DSCR	Down syndrome chromosome region	190685	Down syndrome
4q35	CASP3 CPP32	Caspase-3 Apoptosis-related cystein protease	600636	Apoptosis
6p21.3	AGER RAGE	Advance glycosylation end product-specific receptor	600214	Oxidative stress Aging
14q32.1	SERPINA3 AACT ACT	Alpha-1-antichymotrypsin	107280	Occlusive cerebrovascular disease
19p13.2	NOTCH3 CADASIL CASIL	Drosophila Notch 3 homolog	600276	Cerebral arteriopathy with subcortical infarcts and leukoencephalopathy
20p11.2	CST3	Cystatin C	604312	Cerebral amyloid angiopathy
18q11.2-q12.1	TTR PALB	Transthyretin Prealbumin	176300	Senil systemic amyloidosis Familial amyloid angiopathy
9q34	GSN	Gelsolin	137350	Finnish type amyloidosis
15q21-q22	B2M	Beta-2-microglobulin	109700	Hemodialysis-related amyloidosis
11q23	APOA1	Apolipoprotein A 1	107680	Amyloidosis Hypertriglyceridemia
19q13.1	TYROBP PLOSL	TYRO protein tyrosine kinase-binding protein	604142	Polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy
3q26	SERPINI1 PI12	Portease inhibitor 12	602445	Familial encephalopathy with neuroserpin inclusión bodies
20p13-p12.3	NBIA1	Neurodegeneration with brain iron accumulation	234200	Hallervorden-Spatz syndrome

Source: http://www.ncbi.nlm.nih.gov/OMIM

GENETICS OF ALZHEIMER DISEASE

Our present knowledge on AD genetics derives from population studies, family studies, twin studies, adoption studies, and molecular biology studies carried out during the past 50 years [1,36]. After the pioneering work of Schooky, Lowenberg, Waggoner and MacManemey in the 1930's, Sjögren in the 1950's and Heston and associates in the 1960's and 1970's [1], complex seggregation analysis in the early 1990's led to the conclusion that AD is determined, in part, by a major autosomal dominant allele with an additional multifactorial component [1]. Furthermore, epidemiological studies also suggested that most cases of AD (>80%) are familial [1]. Advances in molecular genetics during the past two decades allowed the identification of several genetic loci associated with AD (Table 1) and the genetic classification of AD (AD1 to ADn) as depicted in the OMIM database (www.ncbi.nlm.nih.gov/) [15,38] (Table 1). AD-related genes can be classified into genes with demonstrated mutations following a mendelian inheritance pattern (mutational genetics) (e.g., APP, PS1, PS2), susceptibility genes or polymorphic loci potentially contributing to AD predisposition (susceptibility genetics) (APOE, A2M, LRP1, IL1, ACE), and defective genes linked

to mitochondrial DNA (mtDNA) (COI/COII) with heteroplasmic transmission [1] (Table 1).

Amyloid Precursor Protein (APP) Gene

The APP gene (21q21.2-q21) (Table 1) encodes the amyloid precursor protein (APP), a type-I integral membrane glycoprotein containing the β-amyloid protein (BAP) region (4 kD) extending 28 amino acids of the ectodomain and 11-14 aminoacids of the adjacent transmembrane domain [39]. APP has at least 10 isoforms generated by alternative splicing of a 19-exon gene with 3 predominant transcripts (APP695, APP751, APP770) of which APP695 is preferentially expressed in neurons [40]. Exons 16 and 17 encode the BAP domain of APP [41]. APP is processed by several different proteases called secretases. β-Secretase generates the NH_2-terminus of BAP, producing a soluble fragment of APP (β-APPs) and a 99-residue COOH-terminal fragment (C99) bound to the membrane. α-Secretasecleaves APP at the BAP region to produce α-APPs and an 83-residue COOH-terminal fragment (C83). γ-Secretase acts on the C99 and C83 substrates at the transmembrane domain to produce C-99-derived 4-kD BAP and C83-derived 3-kD p3 peptide. γ-Secretase-related proteolysis is heterogeneous yilding an abundant 40-residue peptide (BAP40) and small amounts of a 42-residue COOH-terminal variant (BAP42) whose hydrophobic properties facilitate amyloidogenic fibril formation [42]. Several missense mutations have been identified in APP that potentially result in early-onset AD (EOAD), including separate mutations in codon 717 of the APP transcript found in familial AD (fAD) (V717I, V717F, V717G) [43-47] referred to as the London APP717 mutation; the Swedish APP670/671 double mutation (Lys670Asn/Met671Leu) [48]; and the Florida APP716 mutation (Ile716Val) [49]. These mutations involve codons near the β-secretase and γ-secretase cleavage sites, while the Flemish APP692 mutation (C692G transversion, A692G), the Dutch APP693 mutation (Glu22Gln), the Arctic APP693 mutation (Glu22Gly), and the Italian APP693 mutation (Glu22Lys) in the APP gene are located within BAP near the α-secretase cleavage site [50,51]. All these mutations are grouped into the genetic classification of type 1 familial AD (Table 1).

β-Amyloid formation in senile plaques and brain vessels (amyloid angiopathy) is a major neuropathological hallmark in AD due to mutations in the APP gene, alterations in APP metabolism and/or processing or secretase-related dysfunction. β-Amyloid Protein (BAP) formation as a proteolytic byproduct of a degradation process leading to brain amyloidogenesis can result from: (a) point mutations in the APP gene, (b) excess amounts of APP, (c) expression of aberrant APP isoforms, (d) structural misfolding, and (e) abnormalities in post-translational modifications [52]. Disease-linked mutations in the APP and presenilins (PS1, PS2) genes result in increased production of the BAP42 form, predominant in AD senile plaques. BAP (4 kD) occurs in the above mentioned two predominant forms (BAP40 and BAP42), and overproduction of BAP42 was suggested as a common cause of fAD. BAP generation depends on proteolytic cleavage of the APP by the proteases α-, β-, and γ-secretases (Fig. **1**). Normal APP cleaved is produced by α-secretase precluding BAP formation into the amyloidogenic pathway (Fig. **1**). The metalloproteases ADAM-10 and TACE (a putative α-secretase) are involved in APP α-cleavage, suggesting that α-secretase might be a zinc-dependent metalloprotease [42]. A transmembrane aspartic protease (β-secretase), termed BACE (Beta-site APP-cleaving enzyme) was recently cloned and characterized by Vassar *et al.* [53]. The gene for BACE is located on chromosome 11 (11q23.2-q23.3) and so far no apparent AD-related mutations have been demonstrated on it [54]. BACE2 maps on chromosome 21, and it is suggested that this protease may contribute to AD associated with Down syndrome [42]. γ-Secretase appears to be a multiprotein complex, and presenilins might be the catalytic component of γ-secretase [43]. Neprisylin deficiency results in both the defects i.e., the degradation of exogenous BAP and in the metabolic suppression of endogenous BAP, suggesting that partial down-regulation of this candidate BAP-degrading peptidase may contribute to AD pathology [55]. BAP accumulation is toxic for neurons and can induce apoptosis by a mechanism that requires c-Jun N-terminal kinase activation [56]. One potential target of neurotoxic BAP may be a novel BAP-binding protein (BBP) containing a G protein-coupling module which regulates caspase-dependent vulnerability to BAP toxicity [57]. Many studies suggest that neuronal death in AD is the result of an apoptotic mechanism [58]; but, the stereotypical profile of the terminal phases of apoptosis (chromatin condensation, apoptotic bodies, blebbing) are not seen in AD. Caspase-6, the protease that cleaves APP and presenilin, is localized in senile plaques. In a recent, provocative paper, Raina *et al.* [59] demonstrated that in AD there is a lack of effectived apoptotic signal propagation to downstream caspase efectors, suggesting that this novel phenomenon of apoptotic avoidance, termed abortive apoptosis or abortosis, may represent an exit from the caspase-induced apoptotic program leading to neuronal survival in AD [59]. However, AD lymphocytes show a clear apoptotic behavior which might reflect the peripheral expression of similar mechanisms occurring at the central level in neurons and microglia [31-33].

It is very likely that a therapeutic approach to slow-down BAP formation and/or inhibiting amyloidogenesis, as well as BAP scavenging, would help to neutralize in part or reduce neuronal damage and neurodegeneration in AD [52]. APP proteases are prime therapeutic targets to control APP metabolism and future development of BACE inhibitors may be beneficial for AD [42,53]. In addition, Schenk *et al.* [60] have demonstrated that BAP immunization of PDAPP transgenic mice overexpressing mutant human APP (P717V) prevented the development of BAP plaque formation, neuritic dystrophy and astrogliosis, suggesting that immunization with BAP may be effective in preventing and treating AD; however, it is unlikely that similar mechanisms be effective in humans since BAP deposition is but a factor among many other pathogenic events underlying AD neuropathology [11,61,62], though functional γ-secretase inhibitors have shown to be effective in reducing the levels of BAP in brain [63]. Furthermore, BAP neurotoxicity can be inhibited in part by tachykinins, some calcium-channel blockers, neurotrophic factors, NMDA receptor blockers, inhibitors of free radical formation and lipid peroxidation,

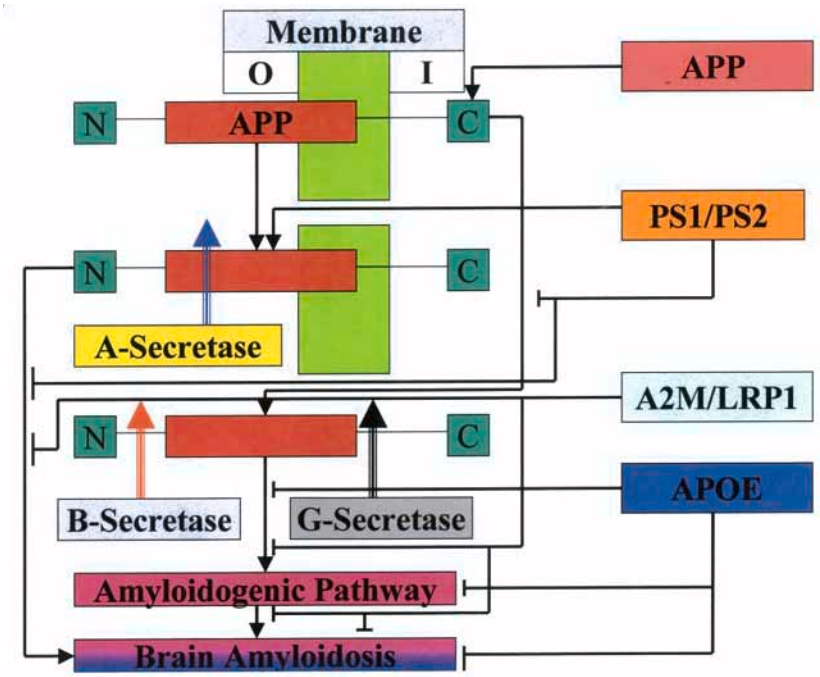

Fig. (1). Genetic regulation of the amyloidogenic cascade.

estrogen replacement therapy, and β-sheet breaker peptide fragments (iAβ11/LPFFD) analogous to the BAP sequence [64-66]. Disregulations in matrix metalloproteinases might also account for alterations in APP metabolism and BAP accumulation [67]. Novel compounds able to help proteolytic enzymes involved in the remodelling of the extracellular matrix might also be useful to preserve brain microstructural changes due to abnormal accumulation of protein aggregates, including BAP deposition and other degradation products [11].

Presenilin Genes

Familial AD3 and fAD4 are caused by mutations in the presenilin-1 (PS1) and presenilin-2 (PS2) genes located on chromosomes 14 (14q24.3) and 1 (1q31-q42), respectively [11,68-70] (Table 1). PS1 and PS2 encode very similar integral membrane proteins with multiple transmembrane domains [71]. The open reading frame of PS1 is encoded in 10-13 exons expanning at least 60 kb [72,73]. More than 70 different mutations have been detected in the PS1 gene and at least 3 mutations are present in the PS2 gene [1,70,74-76]. Presenilin (PS) mutations at the PS1 and PS2 loci on chromosomes 14 and 1, respectively, PS1 exon 9 deletions, as well as changes in intronic polymorphisms at the PS loci account for a growing number of AD cases either early- (EOAD) or late-onset AD (LOAD) [1,77]. PS1 (463 amino acids) and PS2 (448 amino acids) are 46- to 49-kD proteins that share 67-80% amino acid identity. PSs are serpentine integral membrane proteins with 8 transmembrane domains localized in the endoplasmic reticulum and the Golgi subcellular compartments of neurons and other cells throughout the animal kingdom. PSs are rapidly cleaved by proteolysis to yield a 30-kD N-terminal and a 20-kD C-terminal fragments, and can accumulate in the aggresomes, a cytoplasmic structure reflecting cell stress and overloading of the proteasome compartment [78]. The subcellular localization of PSs in the endoplasmic reticulum and early Golgi overlaps with the intracellular sites of amyloidogenic BAP-42 with which they co-precipitate. The main biological functions of PSs may include: (a) APP processing (Fig. **1**), (b) protein sorting/trafficking, (c) Notch signaling, (d) chromosome organization and segregation, and (e) apoptosis [79-82]. Proteins interacting with PSs include APP, nicastrin, β-catenin, calsenilin, filamin/Fh1, and Sel-10 [81,83]. Nicastrin is a type 1 transmembrane glycoprotein coded on chromosome 1 that interacts with both PS1 and PS2 regulating PS-mediated APP processing. Nicastrin also binds C-terminal derivatives of B-APP and modulates BAP production from these derivatives [83]. Calsenilin is a substrate for caspase-3 that interacts with the fAD-associated C-terminal fragment of PS2 [84]. Calsenilin increases BAP42 production in cells expressing the APP Swedish mutation; this effect is potentiated by PS2, suggesting a role for apoptosis-associated BAP42 production of calsenilin/DREAM/KChIP3 [85]. Apparently presenilin mutations do not affect the intrinsic physiological functions of presenilins or cause lethal effects but they (a) increase their toxic functions, (b) alter APP processing leading to BAP deposition and accumulation by inducing functional changes in γ-secretase proteolytic activity, (c) increase neuronal sensitivity to apoptosis, (d) perturb calcium homeostasis activating excitotoxic phenomena, (e) promote mitochondrial dysfunction, and (f) disrupt cholinergic signaling and responses to NGF [80]. New findings show that PSs affect APP processing acting on γ-secretase, and are involved in the cleavage of the Notch receptor regulating γ-secretase activity or serving as protease enzymes [78,82]. Amyloid production and deposition is increased in AD

patients with PS mutations, in transgenic mice with PS mutations, and in mutant APP and PS1 yeast artificial chromosome transgenic mice [86]. Transgenic mice with AD-related PS1 mutations show accelerated neurodegeneration with intracellular BAP deposition and without amyloid plaque formation, suggesting that PS1 mutation is upstream of the amyloid cascade in AD [87]. The expression of wild type (wt)-PS2 in human HEK293 cells increases the production of α-scretase-derived product APPα, and APPα production is drastically reduced in cells expressing the N141I-PS2 mutation. The PS-associated APP-α-secretase non-amyloidogenic pathway is under the catalytic control of proteasome enzymes [88].

PSs also regulate the Notch signaling pathway, involved in axon pathfinding, neurite outgrowth, neuronal stem cell differentiation and maturation [89]. Missense mutations in the Notch3 gene cause CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy) (Table 1). Notch is a type I integral membrane protein proteolytically processed in its extracellular domain by furin and the metalloproteinase kuzbanian. The signal transduction cascade is activated by Notch receptor binding to members of the DSL ligand family (Delta, Serrate, Lag-2), then the Notch cytoplasmic domain is cleaved, released, and translocated to the nucleus. The cleavage of Notch resembles the γ-secretase-mediated APP cleavage, and the Notch cleavage may be facilitated by PSs [78,82]. Presenilin fragments are assembled into a biologically active complex with other proteins, and PS molecules that are not incorporated to the complex are degraded by proteasome caspases and calpain-like enzymes. Two aspartate residues in the TM6 (Asp-257) and TM7 (Asp-385) domains in the amino- and carboxyl-terminal fragments are reqired for PS endoproteolysis and γ-secretase activity [90]. Mutations in the two conserved transmembrane aspartate residues in PS1 increase the production of the APP carboxy-terminal fragments which are the substrates for γ-secretase [90]. When PS mutations occur, the aspartate residues are replaced by other amino acids, the PS variant does not undergo endoproteolytic cleavage, and the full-length protein accumulates replacing the endogenous PS [78]. Since aspartyl protease inhibitors block BAP production, it has been proposed that PS1 might be an unusual aspartyl protease or a γ-secretase [82,90-91]. In this regard, β-secretases and γ-secretases associated with PS function might be the potential targets for AD treatment. However, presenilin-like γ-secretase inhibitors might have deleterious effects acting on the physiological functions linked to Notch receptors in neurons. Notch signaling regulates the capacity of neurons to extend and elaborate neurites; and up-regulation of Notch activity is concomitant with an increase in the number of interneuronal contacts and cessation of neurite outgrowth. The interesting study of Sestan *et al.* [92] indicates that the formation of neuronal contacts results in activation of Notch receptors, leading to restriction of neuronal growth and arrest in adult brains. PS is required for the normal proteolytic production of the carboxy-terminal Notch fragments necessary for maturation and signaling [93-94]. Loss of PS function leads to Notch/lin-12-like mutant phenotypes in *Caenorhabditis elegans,* and to reduced Notch expression of the paraaxial mesoderm in mice. The PS-regulated Notch signaling might also be associated with BAP production in AD [93]. Since, ws-PS2, N141I-PS2, and the PS2 C-terminal maturation fragment are degraded by proteasome multicatalytic complex, selective proteasome inhibitors (Z-IE(Ot-Bu)A-Leucinal, Lactacystin) potentiate APPα secretion and decreased APPα production in N141I-PS2 mutation carriers. However, secretase inhibitors acting on Notch and APP-BAP production might have unwanted effects on interneuronal communication, neurite outgrowth, hematopoietic systems, and immune function [95,96]. Since the PS1 mutation-related pathogenic events seem to be an upstream of the amyloid cascade [87], a preventive therapeutic intervention on PS function might preclude BAP formation and further neuronal degeneration [11].

Some authors have reported that PS1 may be a susceptibility gene for LOAD [97-99]. An A-to-C single nucleotide polymorphism located in intron 8 revealed evidence of disequilibrium between the most common allele PS1-1 and LOAD. However, these results could not be replicated in other studies [100]. In our casuistics the 3 PS1 polymorphic genotypes in controls (PS1-1/1: 29.6%; PS1-1/2: 55.2%; PS1-2/2: 15.2%) and AD patients (PS1-1/1: 29.05%; PS1-1/2: 52.32%; PS1-2/2: 18.62%) are practically identical (Fig. **2**). A novel mutation (V148I) in the predicted TM2 domain of the PS2 gene has been identified in LOAD [74,75]; and a polymorphic variant in PS2-exon 5 (PS2E5+/PS2+) appears in 40-50% of AD patients showing accelerated cognitive decline and poorer therapeutic outcomes [1,11,34] (Fig. **2**).

Apolipoprotein E (APOE) Gene

Polymorphic variants in the APOE gene are associated with the risk (APOE-4 allele) or protection (APOE-2 allele) for AD [1,101-103]. The 3 major isoforms of human ApoE (ApoE-2, ApoE-3, ApoE-4) are coded by the ε2, ε3, and ε4 alleles. Differences in the amino acid sequence at sites A (residue 112) and B (residue 158) of the ApoE molecule distinguish the ApoE-2 (Cys/Cys), ApoE-3 (Cys/Arg), and ApoE-4 (Arg/Arg) isoforms [104,105]. ApoE-3 is the most frequent isoform (wildtype), and ApoE-4 differs from ApoE-3 in a Cys-to-Arg change at position 112 (ApoE-4/Cys112Arg). ApoE-2 (Arg158Cys) is the more common isoform of the 4 different mutations at the E2 position with isoelectric focusing. The other 3 ApoE-2 isoforms are E2(Lys146Gln), E2(Arg145Cys), and E2(Arg136Ser) [106]. APOE polymorphic variants are involved in the pathogenesis of type III hyperlipoproteinemia, dysbetalipoproteinemia, familial hyperbeta- and prebetalipoproteinemia, familial hypercholesterolemia with hyperlipemia, cardiovascular disease, cerebrovascular disorders, vascular dementia, hypertension, diabetes, and AD [1,2,107].

Many studies have confirmed the early findings of Saunders and Corder and their co-workers[108-110] reporting an increased frequency of the APOE-4 allele in AD and the association of the APOE-4 allele with late-onset AD (LOAD) and sporadic forms of AD [1,102,111,112]. A protective effect of APOE-2 for LOAD has also been proposed [113]. APOE-4 promotes artheriosclerosis and is

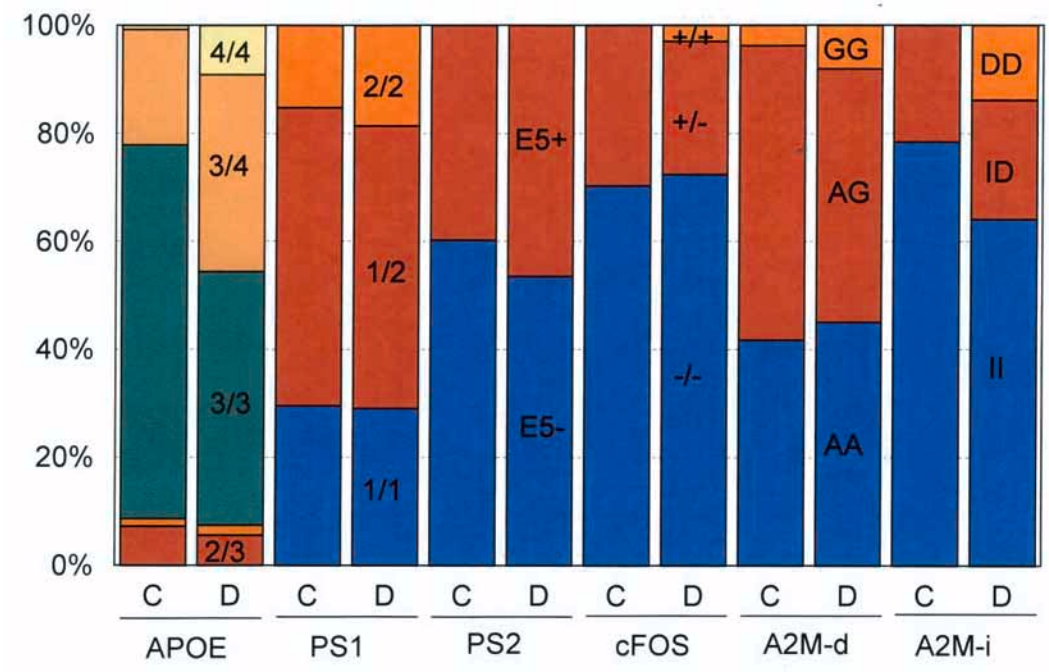

Fig. (2). Genotype distribution of AD-related genes in healthy subjects (C) with no family history of dementia and age-matched Alzheimer's disease patients (D) in the Spanish population.

less frequent in centenarians than in controls, and APOE-2, which was associated with type III and type IV hyperlipemia, is more frequent in people with higher longevity rates [114]. The risk for AD increases from 20% to 90% and mean age at onset decreases from 84 to 68 years with increasing number of APOE-4 alleles [110], this confirming the dosage effect of the APOE-4 allele which in APOE-4/4 homozygotes anticipates the age at the onset in their 60's [1,115]. In the Spanish population the frequency distribution of the APOE genotypes in healthy control subjects with no family history of dementia and in AD (C/AD) is the following (Fig. **2**): APOE-2/2 <1/<1%; APOE-2/3: 7.24/5.58%; APOE-2/4: 1.45/1.86%; APOE-3/3: 69.2/46.97%; APOE-3/4: 21.37%/36.51%; and APOE-4/4: 0.72/9.06% [1,111,112] (Fig. **2**). APOE-3/4 and APOE-4/4 genotypes tend to be more frequent in females than in males in AD patients [1,116], and women with APOE-4/4 have higher risk for AD than those without APOE-4 [117].

ApoE may affect NFT and BAP deposition in AD [118]. ApoE-4-related proteins may interfere with binding of tau to microtubules, altering tau glycation and phosphorylation [119]. The presence of APOE-4 increases the odds ratio for cerebral amyloid angiopathy; and APOE-4 is strongly associated with increased neuritic plaques and BAP deposition in AD [120-122]. The oxidized form of purified ApoE-4 shows a higher affinity binding to synthetic BAP and MAP2 than the ApoE-3 isoform, and propably ApoE may affect microtubule function and BAP accumulation in AD [118-123]. Carriers of APOE-2 and APOE-4 alleles are also more proned to recurrent cerebral amyloid angiopathy than APOE-3/3 carriers [124]. The frequency of APOE-4 was also found increased in patients with BAP deposition following head injury [125]; and the neurologic recovery after brain trauma is poorer in APOE-4 carriers than in subjects without that allele. AD APOE-4 carriers show reduced glucose metabolism in selected brain regions [126]. There is also an APOE-related cognitive decline in AD patients which is more accelerated in subjects with the APOE-4/4 genotypes. These patients are also the worst responders to different treatments [11,34,35].

APOE-4 may influence AD pathology interacting with APP metabolism and BAP 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 remodelling, and inducing neuronal apoptosis [1,31-33,118,119,127-131]. BAP deposition enhanced by APOE-4 precedes NFT formation in the frontal cortex [132]. However, despite abundant information associating APOE-4 with AD [1], some studies conclude that the APOE locus is neither necessary nor sufficient to cause AD [133].

Microtubule-Associated Protein Tau Gene (MAPT)

It has been convincingly demonstrated that tau protein mutations and tau protein pathology can cause neurodegeneration and are associated with a diverse group of diseases currently called tauopathies [15,134-138]. Diseases with abundant tau-positive filamentous lesions, currently known as tauopathies include the following: Alzheimer's disease, corticobasal degeneration, dementia pugilistica, dementia with tangles only, dementia with tangles and calcification, Down syndrome, frontotemporal dementias and parkinsonism linked to chromosome 17 mutations, myotonic dystrophy, Niemann-Pick disease type C,

Parkinsonism-dementia complex of Guam, Pick's disease, postencephalitic parkinsonism, prion diseases with tangles, progressive supranuclear palsy, and subacute sclerosing panencaphalitis [137]. Tau-positive neurofibrillary lesions, representing cytoskeletal changes in AD neurons, constitute a well recognized neuropathological feature of AD. Intracellular neurofibrillary lesions appear in the neocortex, hippocampus, and some subcortical nuclei of AD, correlating with the presence of dementia. These lesions are found in nerve cell bodies and apical dendrites as neurofibrillary tangles (NFTs), in distal dendrites as neuropil threads and in the abnormal neurites associated with senile plaques [136,137]. These neurofibrillary lesions are integrated by paired helical filaments (PHFs) and straigh filaments made of microtubule-associated protein tau in a hyperphosphorylated state. There are 6 isoforms of tau in brain involved in microtubule assembly and stabilization. The tau isoforms are produced by alternative splicing of mRNA from a sigle gene located on the long arm of chromosome 17 (17q21.1) [139,140] whose missense mutation and splicing defects can lead to frontotemporal dementia and familial progressive subcortical gliosis [137,141-143]. Over 10 exonic and intronic mutations in the tau gene have been identified in 20 frontotemporal dementia families. Different FTDP-17 missense mutations might be responsible for disease pathogenesis by reducing the ability of tau to bind microtubules and promote microtubule assembly. Tau mutations are divided in three groups according to their locations in the intron after exon 10, in exon 10 or in the remaining tau-coding region, causing the different phenotypic expression of heterogeneous, atypical dementias [141,142]. Some FTDP-17 mutations alter the MT-binding properties of tau, and others alter the ratio of 4R/3R tau isoforms. The missense mutations P301L, V337M, and R406W alter the biochemical properties of tau. Hyperphosphorylation and abnormal phospholyation are major biochemical abnormalities of PHF-tau and early events in NFT formation due to the incapacity of tau to bind microtubules. Tau pathology in AD is circumscribed to neurons, while in other tauopathies, such as corticobasal degeneration, progressive supranuclear palsy and familial multiple system tauopathy with presenile dementia, both nerve cells and glial cells are affected [137].

Other AD-Related Genes

Since mutations in APP, PS1, PS2 and TAU genes account for less than 10% of AD cases and the APOE locus is neither necessary nor sufficient to cause AD [133], it seems plausible that other genetic factors may be involved in AD in combination or not with environmental factors and/or epigenetic phenomena [1,144]. Some candidate genes with polymorphic loci include AACT, A2M, ACE, FOS, IL1, NOS, and others. A common polymorphism in the signal peptide of the α-1-antichymotrypsin (AACT) gene (14q24.3-q32.1) encoding the plasma protease inhibitor AACT has been associated with increased risk for AD, and the combination of the AACT-A/A genotype with APOE-4/4 genotype has been proposed as a potential susceptibility marker for AD [145]. AACT participates in acute-phase inflammation and accumulates in amyloid plaques and plasma of AD patients [146].

A polymorphism in the butyrylcholinesterase (BCHE) gene (3q26.1-q26.2) (BCHE-K) appears as a susceptibility factor for AD, and is associated with LOAD [147], enhancing the AD risk from APOE-4 in an age-dependent manner [148].

α-2-Macroglobulin (A2M), the A2M receptor (LRP1), 2 low density lipoprotein-related protein (LRP) ligands, APOE, APP, BACE, BLMH, PS1, and PS2 are probably genetically linked in the regulation of brain amyloidogenesis (Fig. **1**). A2M is a carrier protein for BAP decreasing fibril formation and influencing BAP neurotoxicity [149]. The sibship disequilibrium test revealed a significant association between A2M (12p13.3.-p12.3) and AD; and the inheritance of a deletion in the A2M gene at the 5'-splice site of exon II of the bait region (exon 18) (A2M-2) confers increased risk for AD [150], but these findings could not be replicated by others [151-153]. The A2M-V1000I polymorphism was also found associated with AD [154], but some authors did not find association between the A2M polymorphisms (intronic 5-bp deletion, Ile1000Val) and AD [155,156]. In a recent study, no change in A2M mRNA, protein, or protein expression could be found in AD [156] and finally it appears that A2M is not genetically associated with LOAD [157]. In our casuistics, the A2M-G/G genotype is more frequent in AD (8.1%) than in controls (3.79%), and the homozygous A2M deletion (A2M-D/D) is absent in controls and appears in 5.21% of AD patients [158] (Fig. **2**).

The LRP is identical to the A2M receptor (A2MR) of the serum panprotease inhibitor A2M. The multifunctional LRP receptor is located in the soma regions and proximal processes of neurons, and acts as a receptor for the uptake of ApoE-containing lipoprotein particles by neurons. LRP is involved in the internalisation and degradation of A2M/BAP complexes [159]. An association between AD and the 87-bp allele of a tetranucleotide repeat polymorphism located 5' to the LRP gene (12q13.1-q13.3) was found [160]. Five coding polymorphisms in the LRP1 gene (A217V, A775P, D2080N, G4379S, D2632E) were discovered by sequencing the LRP1 89 exons resulting in the sequence contig of 33567 nucleotides [161]. The LRP-T allele is less frequent in AD than in controls, and the LRP-C/C genotype accumulates in younger patients [162]. About 10 cM proximal to LRP1 there is a gene (1211.23-q13.12) linked to type 5 AD [163]. Bleomycin hydrolase (BMH) was suspected of being a β-secretase regulating the secretion of APP [164]. In the BMH gene (17q11.1.-q11.2) a 1450A-G polymorphism results in an I443V conserved substitution in the carboxy-terminus of the protein. Of the 3 BMG genotypes (BMG-A/A, BMH-A/G, BMH-G/G), the distribution of the G/G homozygote genotype is more frequent in AD (12.7%) than in controls (6.6%) preferentially in the non-APOE-4 groups (15.9% vs 4.7%) [165,166].

The FOS gene (14q24.3) maps in the AD3 region (PS1 gene) and is linked to 3 genes (dihydrolipoamide succinyltransferase, S31iii125, S20i15) also present in the Fugu rubripes genome (400 Mb), an approximately 7-8-fold smaller genome to that of humans (3,000 Mb), with a similar complement of genes [167]. Fos is a major component of the activator protein-1 (AP-1) transcription

factor complex. Although the FOS open reading frame was excluded as the site of 14-linked type 3 AD [168], other authors found an accumulation of FOS-B allele in AD [112]. The homozygous polymorphic variant of FOS-B (FOS-B/B) is present in 2.5% AD patients and absent in controls [112].

The interleukin-1 (IL1) gene cluster (2q14) has been recently associated with AD [169-172]. The acidic (IL1-α) (pI5) and neutral (IL1-β) (pI7) forms of IL1 (17-kD) are coded by separate genes assigned to 2q13-q21 (Table 1). The IL1A gene comprises 10,206 bp with 7 axons and 6 introns, and a variable number of 46-bp sequence repeats within intron 6. This gene is highly polymorphic, with at least 6 different alleles ranging from 5 to 18 repeats containing 3 potential binding sites for transcription factors. Since inflammation can contribute to AD pathology, as recently demonstrated [169,173,174], from the early 1990's it was observed that IL-1 was abnormally expressed in AD glial cells, and high levels of IL1 have been detected in brain tissue and blood of AD patients [175,176]. Recently, several authors demonstrated that IL1 polymorphisms increased the risk for AD [170-172]. The IL1A-2/2 genotype was associated with an increased risk for AD and with earlier age of onset [177]. There is also evidence supporting the association between a polymorphism (C850T) in the regulatory region of the TNF-α gene (6p21.3) and AD [178]. Three TNF polymorphisms comprising the –308 TNF promoter polymorphism, the –238 TNF promoter polymorphism, and microsatellite TNFa conform the TNF haplotype 2-1-2, respectively, which was significantly associated with AD using the sibling disequilibrium test [179]. A functional dissociation between IL1 and TNF in the brain and blood of AD patients have been reported, with high levels of IL1 [176,180] and low levels of TNF [181].

The nitric oxide synthase 3 (NOS3) gene (7q36) contains approximately 26 exons spanning approximately 21 kb of genomic DNA and encodes a mRNA of 4,051 nucleotides which is translated into a 1.203 amino acids protein with about 60% identity with the rat brain NOS isoform. The NOS3 Glu298Asp polymorphism has been associated with AD. The homozygous NOS3-E/E genotype is over-represented in AD patients [182]. Other candidate genes potentially associated with AD include the angiotensin I converting enzyme gene (ACE) (17q23) [183,184]; the insulin-degrading enzyme gene (IDE) (10q24) associated with type 6 AD (AD6) [185-187]; the AD2 gene (19cen-q13.2) associated with type 2 AD (AD2) [188]; the AD5 gene (12p11.23-q13.12) associated with type 5 AD (AD5) [163]; the beta-site amyloid beta-A4 precursor protein-cleaving enzyme gene (BACE) (11q23.3) (BACE1, BACE2, β-secretase, memapsin-2) [54,189]; the glycogen synthase kinase 3 beta gene (GSK3B) [190]; the α-synuclein gene (SNCA) (4q21) [191,192]; the cystatin C gene (CST3) (20p11.2) [193]; the methylenetetrahydrofolate reductase gene (MTHFR) (1p36.3) [194]; and specific mtDNA point mutations at position 5460 in codon 331 of ND2 (complex I of the respiratory chain, subunit 2 of NADH dehydrogenase-ubiquinone oxidoreductase) and other mtDNA point mutations such as mitochondrial aldehyde dehydrogenase gene mutations [195-198]. APOE/mtDNA interactions may influence susceptibility to AD, since some mtDNA haplogroups (K and U) seem to neutralize the harmful effect of APOE-4 in AD [199]. In addition, point mutations in mtDNA of cytochrome c oxidase coexist with normal mtDNA in AD, reflecting complexity and heteroplasmy [200]. It is very plausible that many of these genes interact with each other to regulate specific metabolic pathways either confluent with or different from the amyloid cascade (Fig. **1**). For instance, ACE and BCHE interact with APOE-4 as risk factors in AD and Parkinson's disease with coexisting Alzheimer's pathology [201], and APOE-4 and BCHE-K display a synergistic association in AD [148]. From a genetic epidemiology perspective, it seems clear that the genetic dosage effect influence the age at onset, the higher the number of genes involved in AD the earlier the disease onset [74,75]. Other genes may exert a protective effect against AD, as APOE-2 [113,114,202] or the human DIMINUTO/DWARF1 homolog seladin-1 that confers resistance to AD-associated neurodegeneration and oxidative stress [203].

Over 40 genes have been tested as AD candidate genes, and none of them has been clearly established as a primary risk factor for most cases of AD, clearly indicating the complex/polygenic/multifactorial nature of this disease [204]. The future characterization and classification of complex disorders will require: (a) identification of etiological genetic factors, either mutational or susceptibility factors; (b) identification of polymorphisms reflecting normal variations in the population and/or susceptibility SNPs; (c) characterization of interacting environmental factors; (d) characterization of epigenetic phenomena associated with dementia heterogeneity and pathogenesis; and (e) identification of phenocopies and interacting medical conditions which influence, aggravate or accelerate the phenotypic expression of dementia. Furthermore, the characterization of phenotypic-genotypic associations in a particular disease is necessary to organize databases for early diagnostic procedures, genomic studies, preventive programmes, and pharmacogenomic intervention [11,16,34,35].

GENOMICS OF CNS DISORDERS

The impact of the sequencing and analysis of the human genome on geriatric medicine and CNS disorders will be very important in several areas: epidemiology, etiopathogenesis, diagnosis, and treatment. The main conclusions obtained from the initial data released from the International Human Genome Sequencing Consortium and Celera Genomics can be summarized in the following items: (a) the human genome contains approximately 30,000-40,000 protein-coding genes; (b) the human proteome is more complex than those of invertebrates; (c) many human genes are likely to be the result of a horizontal transfer from bacteria, and other genes derived from transposable elements; (d) in the hominid lineage there is a marked decline in the overall activity of transposable elements, with DNA transposons and long-terminal repeat retrotransposons completely inactive; (e) the pericentromeric and subtelomeric regions of chromosomes are filled with segmental duplications of sequence from other genomic regions; (f) Alu elements abound in GC-rich regions and appear to benefit their human hosts; (g) the mutation rate is higher in males than in females meiosis; (h) GC-poor regions correlate with

dark G-bands in karyotypes; (i) recombinantion rates are higher in distal regions of chromosomes (20 Mb) and on shorter chromosome arms; (j) in the human genome there are about 1.42-2.1 million single nucleotide polymorphisms (SNPs); (k) the apoptotic molecular machinery is much more complex in vertebrates than in other species; and (l) there are at least 130 known human DNA repair genes [21,23].

The 1.42 million SNPs are distributed throughout the human genome with an average density of one SNP every 1.9 kb. The International SNP Map Working Group [24] estimates that 60,000 SNPs fall within exon, and 85% of exons are within 5 kb of the nearest SNP. Chromosomes 1, 2 and 5 contain the highest number of SNPs, with 129,931, 103,664, and 117,882 SNPs, respectively. There is a great heterogeneity in the level of polymorphisms across the genome, and less than 1% of all polymorphisms results in variations in proteins [21]. SNPs are single-based differences in the DNA sequence that can be observed between individuals in the population. DNA sequence polymorphisms result from mutation. This DNA sequence variation may or may not have functional consequences. Mutations in a single gene alter function to induce a monogenic disease, but many common diseases are polygenic, resulting from complex interactions of multiple genes. In these polygenic/multifactorial/complex disorders, the alteration of a single gene may not be detrimental, but in combination with different variants of other genes located in different positions of the human genome, may contribute to a disease phenotype. Under some conditions, the variant genes might be sufficient to induce the phenotypic expression of a given disease, whereas in other circumstances some environmental factors appear necessary to express a disease phenotype after interaction with the genes affected [205]. A polymorphism has been defined as the least common allele occurring in 1% or greater of the population, whereas mutations are differences which occur in less than 1% of the population. SNPs are associated with many diseases [22,27,28]. Major types of SNPs include restriction fragment length polymorphisms (RFLPs), minisatellites with a variable number of tandem repeats (VNTR), and microsatellites with dinucleotide repeats [205]. SNP mapping has been incorporated as a tool for personalized genetic profiling with high value in diagnosis and pharmacogenomics [22,27,28,206]. SNPs can be used to characterize the genomic profile of many complex disorders including dementia.

Epigenetic factors could also be important in understanding the origins of complex diseases [207-210]. DNA methylation acts as a major determinant for the eventual partitioning of the genome in active and inactive compartments, in such a way that genomic segments whose activity is not required in a cell or tissue are methylated and inactivated to reduce transcriptional background noise [209,210]. An important consequence of CpG methylation is the local silencing of the gene expression [211]. Small RNAs derived from cleavage of double-stranded RNA can trigger epigenetic gene silencing at the genome level and in the cytoplasm guiding transcriptional degradation of complementary mRNAs and transcriptional gene silencing by DNA methylation [211]. Specific gene silencing induced by double-stranded RNA is a process referred to as RNA interference (RNAi) or post-transcriptional gene silencing (PTGS). RNAi is mediated by a sequence-specific RNA-induced silencing complex (RISC) integrated by multicomponent nucleases that destroy mRNAs homologous to the silencing trigger [212]. Conceptually, epigenetics refers to modifications in gene expression that are controlled by heritable but potentially reversible changes in DNA methylation and/or chromatin structure [207,209,210]. Both, SNPs and epigenetics might be at the basis of many complex disorders of the CNS and the use of both genetic markers could represent useful strategies for the molecular diagnosis and pharmacogenomics of dementia syndromes, tough evidence on epigenetic mechanisms in AD is lacking as yet. In some instances, epigenetic misregulation of genes is more consistent with the neuropathological features and phenotypic expression of complex disorders than is DNA sequence variation [207]. The limitations of mendelian genetics in complex disorders include (a) discordance of monozygotic twins, (b) age at disease onset, (c) sex effects, (d) parent-of-origin effects, (e) clinical fluctuation, and (f) heterogeneity [207]. Some epigenetic phenomena might clarify the mechanisms of age-related genetic switch on-off contributing to the phenotypic expression of neurodegenerative processes in later life, and the role of intergenerational transmission of epimutations in sporadic cases [207].

On the other hand, proteomics, as the large-scale analysis of proteins, will contribute to understand gene function in CNS disorders [213]. One of the major goals in CNS research is to characterize protein function, biochemical pathways and networks. The application of proteomics tools combined with database mining is an optimal option to achieve this objective in neuroscience [214]. Experimental genomics and sequence information will revolutionize basic and clinical neuroscience, and high-density DNA microarrays will be gradually introduced to measure levels of gene expression (mRNA) for thousands of genes simultaneously in different areas of medicine and research [215,216]. According to Peltonen and McKusick, a paradigm shift will happen in biomedical research [218]. The scientific interest and the availability of new research tools will induce a shift from structural genomics to functional genomics, from genomics to proteomics, from map-based gene discovery to sequence-based gene discovery, from monogenic disorders to multifactorial disorders, from specific DNA diagnosis to monitoring of susceptibility, from analysis of one gene to analysis of multiple genes, from gene action to gene regulation, and from etiology (specific mutations) to pathogenesis (mechanisms).

Approximately 1500 genetic disorders with identified mutations are listed in the OMIM [38] (http://www.ncbi.nlm.nih.gov/Omim), and 110 genes with at least one disease-related mutation have been reported [217]. Since 1986 positional cloning became the leading procedure for elucidating the molecular basis of genetic disorders. In the coming future the availability of the human genome sequence [21,23] will accelerate the identification of new disease-related genes, and sequence-based gene discovery will eventually replace map-based gene discovery.

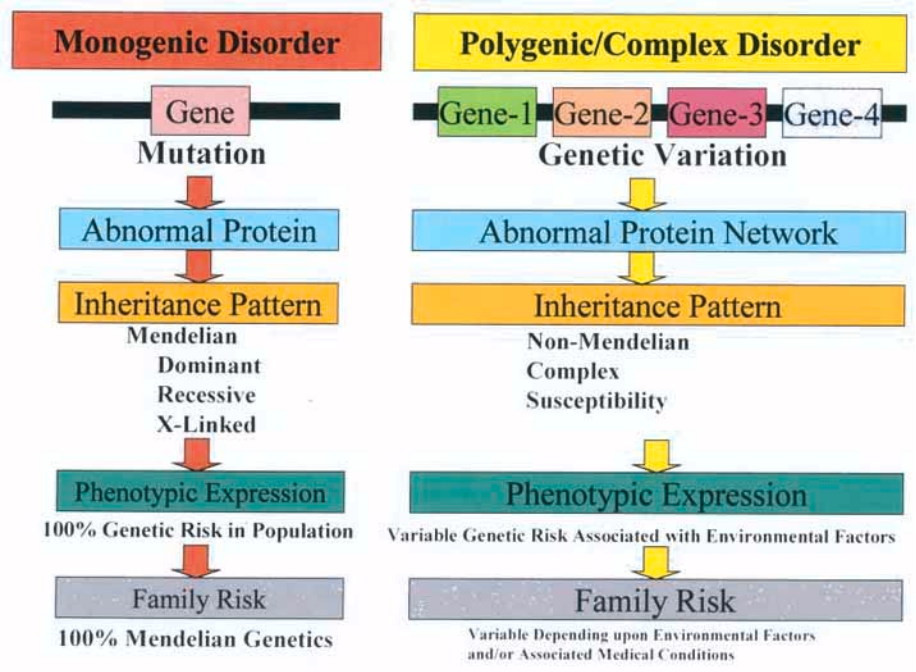

Fig. (3). Inheritance of monogenic and polygenic/multifactorial/complex disorders.

On a broad basis, inherited diseases can be classified into two major types: (a) monogenic disorders, and (b) polygenic/multifactorial disorders. Both types of genetic disorders are studied at different levels: (a) gene mutation and/or genetic variants, (b) abnormal proteins, (c) inheritance pattern, (d) phenotype expression, and (e) family risk [1,218] (Fig. **3**). Most types of behaviors have not a clear-cut pattern and depend on interplay between environmental factors and multiple genes (Fig. **3**). Genes in such multiple-gene systems are called quantitative trait loci (QTLs), because they are likely to result in continuous (quatitative) distributions of phenotypes that underlie susceptibility to common disorders [218]. Genetic variations make substantial contribution to phenotypic variation for behavioural domains, and most behaviors show moderate to high heritability. In contrast, as pointed out by McGuffin *et al*. [218], environmental factors make people different from, rather than similar to, their relatives.

From the list of selected human genes associated with dementia and age-related disorders shown in Table 1, we can draw some conclusions: (a) one disease can be linked to many genes; in other words, genetic defects in multiple loci of the human genome can give rise to an apparently common phenotype (clinical symptoms, nosological entity); (b) most genetically-linked CNS disorders are associated with either mendelian genetics or susceptibility genetics or both; (c) most CNS disorders are linked to many candidate genes, indicating the complex/polygenic/multifactorial character of the vast majority of this kind of diseases; (d) mutations in different parts of the same gene can cause different diseases in different tissues; (e) apparently, mutations in mitochondrial DNA and in nuclear DNA can generate similar phenotypes; (f) susceptibility polymorphic loci and specific point mutations may provide similar genetic vulnerability to develop a disease; and (g) most diseases of later life tend to be linked to more genetic defects (multifactorial disease) than diseases whose onset occurs early in life (monogenic disease) (Table 1; Fig. **3**). The final clarification on whether or not different defective genes can induce a similar phenotype will be achieved when proteomic studies demonstrate networking dysregulation in the proteins set regulating a metabolic pathway. AD can be a practical example in several ways. From the genetics of AD we can infer that alterations in some of the genes regulating brain amyloid deposition (Fig. **1**) may lead to the phenotypic expression of the disease. Moreover, the fact that CNS disorders of different origin and onset, such as AD or schizophrenia, are linked to multiple genes in the human genome might indicate that major disorders affecting higher activities of the CNS are the result of accumulation of multiple genetic defects across the genome during the recent evolution of Homo sapiens. Late- and early-onset AD might also represent different forms of the same disease directly related to the genetic load carried by the patients. It has been demonstrated that families who carry more than two genetic risk factors for AD develop the disease earlier than those families with less genetic load [1,74,75]. Something similar might apply for schizophrenia and depression [219,220] or for cerebrovascular disorders [221].

Genomic Characterization of Alzheimer's Disease

According to the information collected during the past 30 years regarding genetic factors in dementia, AD might be the result of a multistep process of mutations in regulatory genes associated with genomic susceptibility factors and epigenetic alterations that induce a loss of balanced polygenic expression in the CNS. Linkage analysis and association analysis are the two main strategies currently used to identify genetic changes in AD [26]. Linkage

analysis investigate chromosomal polymorphic sequences segregating with disease stage as a mutational surrogate, whereas association analysis focuses on allelic sets (genotypes) or haplotypes associated with AD patients as compared with healthy control subjects. Linkage analysis is very useful for the identification of specific mutations leading to a particular phenotype using family materials; in contrast, linkage studies are less successful in complex disorders where polygenic and epigenetic factors interact with environmental factors (Fig. **3**). In such complex disorders, association studies based on SNPs have become a fashion, but methodological issues (e.g., clinical material, target genes, genomic screening, bioinformatics and statistical analysis) and technical problems (false-positive type-I error, publication bias, population stratification, heterogeneity, multifactorial complexity, conservative multiple-test correction) make them poorly reliable, confusing and rather inaccurate [26]. Linkage-disequilibrium mapping in population samples has increased power to detect susceptibility loci contributing to AD, but this method is constrained by the amount of allelic heterogeneity, and if used independently it can be more restrictive than linkage-based methods [222]. Consequently, improved methodologies for the assessment and validation of the allelic spectrum associated with complex disorders (e.g., AD, schizophrenia, major depression) are needed [220,223,224], as well as technical guidelines for a better interpretation of the meaning of allelic variants and SNPs associated with particular disease, assuming that a SNP is not the same as a disease-predisposing allele [225].

Point mutations in APP and PS genes are directly attributed as causal factors for AD [15]. However, mutational genetics (APP, PS1, PS2 mutations) accounts for less than 10% of AD cases, and many AD patients (>80%) do not show any clear linkage to AD-related mutational genes. In contrast, when susceptibility genetics is considered, approximately 50% of AD cases fall within the APOE-4 risk category whereas the other 50% fall into the APOE-3/3 group where PS1-, PS2-, and other genes-related risk factors might accumulate. In total, about 95% of AD patients screened for genetic risk factors show some link with APOE, PS1 and PS2 genotypes of potential risk. Assuming that the accumulation of genetic defects and/or SNPs of risk for dementia anticipates the onset of AD as demonstrated in several studies [1,74,75], it is very likely that the association of multiple genetic risk factors (e.g., susceptibility factors + mutational factors) and probably non-conventional epigenetic phenomena as well can contribute to the clinical expression of AD. Excluding APP mutations whose frequency is lower than 1% in the AD population (<1 x 1000 in the Spanish population), the most important genetic risk factors for AD identified to date are the APOE-4 and IL-1 polymorphisms, PS1 and PS2 mutations, and potential allelic associations among these genes, although some other genes (e.g., A2M, cFOS, etc) (Table 1) also show differential profiles when AD genotypes are compared with controls in a randomly selected sample of AD patients and healthy control subjects without family history of dementia (Fig. **2**). Using a trigenic matrix model in selected cases genotyped for APOE + PS1 + PS2 including 6 APOE genotypes (APOE-2/2, APOE-2/3, APOE-2/4, APOE-3/3, APOE-3/4, APOE-4/4), 3 PS1 genotypes (PS1-1/1, PS1-1/2, PS1-2/2), and 2 PS2 genotypes differentiating cases devoid of a frequent allelic variation in PS2 exon 5 (PS2-) (56.32%) from cases positive for that genetic defect (PS2+) (43.68%), we can distinguish 36 different AD genotypes (Table 2; Fig. **4**), with a clear different distribution in AD and controls (Table 2; Fig. **4**). With a tetragenic matrix model (APOE + PS1 + PS2 +cFos) we can identify 108 different genotypes, and with a pentagenic matrix model (APOE + PS1 + PS2 + cFos + A2M) the number of genomic combinations yields 972 different genotypes in the population. The most frequent genotypes (>5%) in the AD trigenic model are Codes-21 (E33P112P2- = 33122-; 14.208%), 22 (E33P112P2+, 13.669%), 27 (E34P112P2-, 10.251%), 19 (E33P111P2-, 8.633%), 28 (E34P112P2+, 7.014%), 20 (E33P111P2+, 6.834%), 25 (E34P111P2-, 6.115%), and 23 (E33P122P2-, 5.215%), whereas in control subjects the most frequent genotypes (>5%) are Codes-21 (E33P122P2-, 20.454%), 22 (E33P112P2+, 15.34%), 19 (E33P111P2-, 13.068%), 20 (E33P111P2+, 11.931%), 28 (E34P112P2+, 6.818%), 27 (E34P112P2-, 6.25%), and 24 (E33P122P2+, 6.25%) (Table 2; Fig. **4**). Codes 1-6 associated with APOE-2/2 are very rare in both AD and controls, with a frequency of 0.179% in AD, and Codes 31-36 associated with APOE-4/4 are practically absent in controls, with the 3 most frequent genotypes present in AD patients (E44P112P2-, 2.877%; and E44P111P2- and E44P112P2+, 1.079%), thus confirming APOE-4/4 as a major risk factor associated with AD. AD trigenic genotypes with a frequency higher that 1% (SNPs criteria) accounts for 93.34% of the AD sample and for 97.15% in the control group. PS1-related allelic variation appears not to affect genotype variability between AD and controls, as reported in other studies [100] while PS2+ seems to confer a mild higher risk in AD (43.68%) vs controls (39.76%), PS2- being more frequent in controls (60.25%) than in AD (56.32%). The most relevant differences between AD and controls are in Codes 19-24 associated with APOE-3/3 (AD: 69.2%; C: 46.97%), and in Codes 31-36 associated with APOE-4/4 (AD: 9.06%; C:0.72%). These 2 major genotype clusters clearly separate AD patients (78.26%) from healthy subjects (47.69%) in approximately 30% differential genotypes, and this percentage fits very well with the prevalence of AD from 60 years (1%) to >80 years of age (>30%) [1]. However, since approximately 50% of AD patients belong to the APOE-3/4- and APOE-4/4-related clusters (22% in the normal population), and more than 45% of AD cases fall into the APOE-3/3-related cluster (70% of the normal population), it is very likely that genetic risk factors associated with PS1 (P111: 17%, P112: 29%, P122: 9%) and/or PS2 genes (P2+: 25%, P2-: 28%), as well as other potential genetic factors, accumulate in APOE-3/3 patients in a dose similar to or higher than that seen in AD patients homozygous or heterozygous for APOE-4 (P111: 12%, P112: 34%, P122: 12%, P2+: 17%, P2-: 24%).

This preliminary data indicate that the genomic characterization of AD patients can be useful for molecular diagnosis and pharmacogenomics [11,34]. Moreover, conclusions derived from genomic data also suggest that many inconsistencies in AD genetics and etiopathogenesis

Table 2. Genomic Distribution of AD Genotypes Associated with APOE+PS1+PS2 Integrated in a Matrix Model

Code	Genotype	AD (N)	fAD(%)	C (N)	fN(%)
1	22112-	0	0	0	0
2	22112+	1	0.179	0	0
3	22122-	0	0	0	0
4	22122+	0	0	0	0
5	22222-	0	0	0	0
6	22222+	0	0	0	0
7	23112-	5	0.899	1	0.568
8	23112+	3	0.539	1	0.568
9	23122-	9	1.618	3	1.704
10	23122+	6	1.079	2	1.136
11	23222-	3	0.539	0	0
12	23222+	0	0	0	0
13	24112-	1	0.179	0	0
14	24112+	3	0.539	0	0
15	24122-	1	0.179	0	0
16	24122+	3	0.539	3	1.704
17	24222-	2	0.359	0	0
18	24222+	3	0.539	0	0
19	33112-	48	8.633	23	13.068
20	33112+	38	6.834	21	11.931
21	33122-	79	14.208	36	20.454
22	33122+	76	13.669	27	15.340
23	33222-	29	5.215	6	3.409
24	33222+	20	3.597	11	6.250
25	34112-	34	6.115	4	2.272
26	34112+	20	3.597	6	3.409
27	34122-	57	10.251	11	6.250
28	34122+	39	7.014	12	6.818
29	34222-	19	3.417	0	0
30	34222+	17	3.057	6	3.409
31	44112-	6	1.079	0	0
32	44112+	5	0.899	1	0.568
33	44122-	16	2.877	0	0
34	44122+	6	1.079	0	0
35	44222-	3	0.539	0	0
36	44222+	4	0.719	1	0.568

require further clarification. For instance, SNPs-related susceptibility genetics seems to be more relevant for AD than mutational genetics; however, the main focus of AD pathology is concentrated on the bipolar amyloid-tau hypotheses relying on mutational factors [138,226]. Although epigenetics may explain better than mendelian genetics many features of AD, no information is available concerning epigenetic phenomena in AD [208]. Epistatic interactions among different genes potentially involved in AD etiology are unknown, with the exception of the role played by PS1, PS2, A2M, TAU and APOE genes on amyloid production as revealed by transgenic models [13,14,42,138,227-229]. Additional data regarding the influence of environmental factors and/or associated medical conditions (e.g., hypertension, hypotension, heart disease, cerebrovascular disorders, dyslipemia, diabetes,

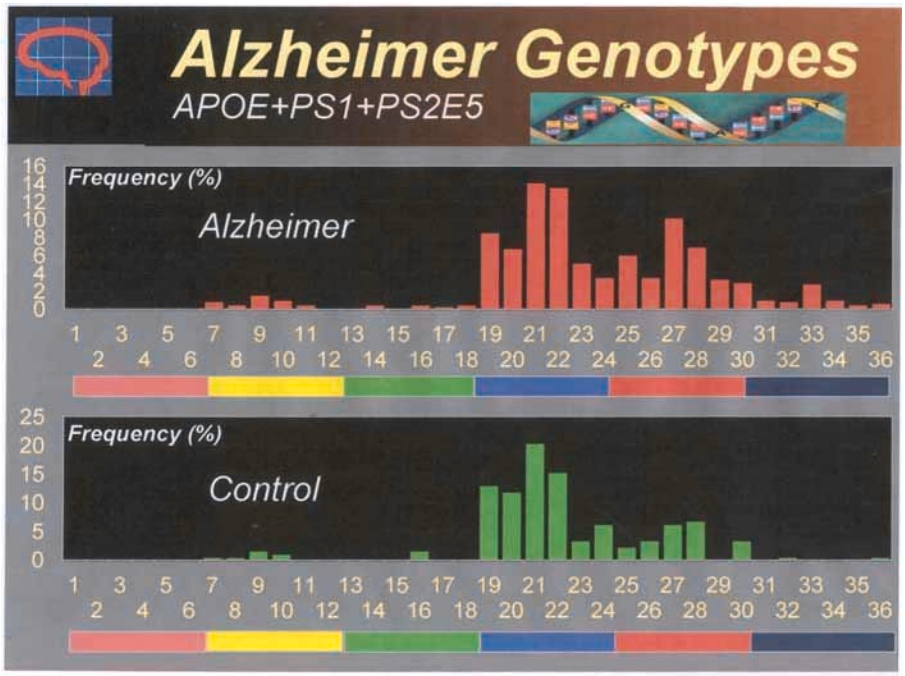

Fig. (4). Genomic distribution of 36 genotypes derived from a trigenic matrix model integrating APOE + PS1 + PS2 genotypes.

neuroendocrine dysfunction) on AD pathology are very necessary for prevention and treatment [2,107]. Since most concomitant diseases of the elderly are also polygenic/multifactorial in nature, functional genomics and proteomics studies will be very valuable for understanding their pathogenic interactions with AD.

From a practical perspective, AD genomics would serve to: (a) define more precisely the molecular mechanisms underlying dementia syndromes in general and AD in particular; (b) subdivide dementia syndromes that are clinically indistinguishable into molecularly-distinct clinical entities to facilitate early molecular diagnosis and to choose rational treatments or preventive measures; (c) identify genotypic markers and other biomolecular markers that predict therapeutic response; (d) identify new therapeutic targets; (e) reveal pathogenic mechanisms; and (f) fractionate the population into individuals at more or less risk of AD, allowing preventive measures or costly surveillance procedures to be used judiciously [230].

PHARMACOGENOMICS

Pharmacogenomics is a novel science that refers to the genomic conditions by which different genes determine the behavior and sensitivity of drugs on a specific organism or genotype. A reductionist view entitles pharmacogenomics as the practice of designing drugs according to individual genotypes to enhance safety and/or efficacy [231]. In contrast, pharmacogenetics is currently used to define the spectrum of inherited differences in drug metabolism and disposition [232]or the pharmacological responses and their modification by hereditary influences [231]. Both definitions at present are complementary, since modern pharmacogenomics covers all the genetic factors regulating the potential multifactorial, polygenic nature of drug response on the unitary basis [233] (Fig. **5**). The conceptual framework of pharmacogenomics would enclose all the steps and procedures for individual and/or disease-specific pharmacological tailoring including (a) drug discovery (natural products, chemical synthesis, combinatorial chemistry, proteomics), (b) drug design: (i) pharmacokinetics (absorption, distribution, metabolism, elimination); (ii) primary pharmacokinetic parameters: absorption rate constant, hepatic clearance, renal clearance, volume of distribution; (iii) secondary pharmacokinetic parameters: half-life, elimination rate constant, unchanged fraction excretion, area under the curve, steady state concentration, average plateau concentration; and (iii) target-oriented pharmacodynamics (cells, tissues, organs); (c) genes regulating phase I and phase II reactions of specific drug metabolism (cytochrome P450-CYP, NAT1, NAT2, GSTA, GSTM1, GSTP, GSTT1, MDR1, DPD, TPMT, MTHFR, UGT); (d) genes regulating the behavior of target cells; (e) genes regulating the activity of cell membrane receptors on which the active drug interacts; (f) genes regulating signal transduction; (g) SNPs on nuclear DNA responsible for phenotype/disease-related genetic variability; (h) effector genes; (i) transcription factors; (j) translation factors; (k) mRNAs and tRNAs; (l) expression profile of final products (proteins, enzymes); (m) cell, tissue, and organ responses; (n) global efficacy profiles; and (o) safety conditions (Fig. **5**) [2,234]. SNPs in genes coding for drug-metabolising enzymes, drug transporters, and ion channels can influence an individual's risk of having an adverse drug reaction, and can modify the efficacy of drug treatment. Mutant alleles at a single gene locus coding for drug-metabolising enzymes

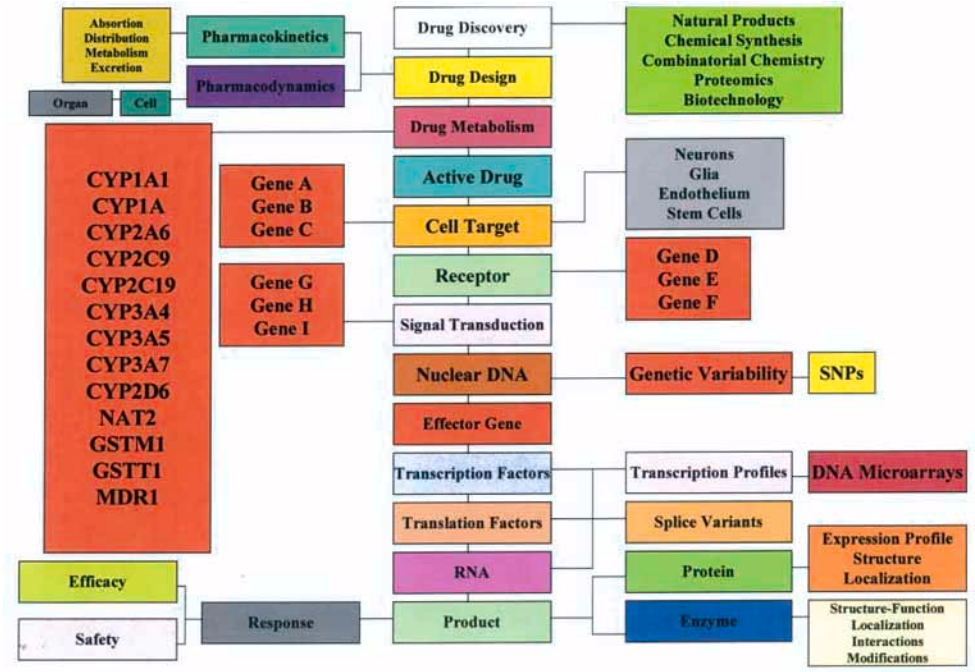

Fig. (5). Pharmacogenomics-related drug evaluation.

produce the phenotypes of "poor metabolisers", "normal metabolisers" or "ultrarapid metabolisers" of many different drugs [235,236]. Genetic factors can determine individual susceptibility to both dose-dependent and dose-independent adverse drug reactions [237]. The clinical significance of SNPs may be related to substrate, metabolite, and major elimination pathways. Genotyping tests and pharmacogenomic techniques allow the efficient analysis of these risk factors for adverse drug reactions and provide a powerful tool to optimise drug therapy in numerous diseases [236]. The application of clinical pharmacogenomics promises to enhance the discovery of drug response biomarkers, reduce the size and expense of clinical trials, and provide a new tool for addressing regulatory approval issues [238]. However, the use of SNP maps in pharmacogenomics is not an easy task, since practical issues, such as patient sample size, SNP density and genome coverage, and data interpretation in part limit the applicability of pharmacogenomic information to medical practice [239].

Developmental steps in pharmacogenomics applied to CNS disorders include the following: (a) mapping of genes for specific diseases; (b) identification of genetic polymorphisms responsible for drug metabolism and disposition; (c) identification of genetic polymorphisms associated with drug transporters; (d) identification of genetic polymorphisms linked to drug targets; (e) characterization of polymorphic variations in population clusters to be treated for a specific disease; (f) primary screening of novel and/or conventional drugs in biochips for specific targets; (g) preclinical assessment of drugs for a given disorder, acting on specific targets, by using biochips and/or DNA microarray technology; (h) clinical trials with polygenic evaluation for efficacy and safety; (i) development of new technologies in functional genomics, proteomics, high-throughput screening methods, DNA microarrays, and biochips for drug evaluation; and (j) development of powerful databases and bioinformatic tools to speed-up clinical trials, improving patient stratification based on polygenic genotyping, reducing costs and potential side-effects, and optimising therapeutic outcomes [27,28,232,240-243].

In performing pharmacogenomics studies, we can use different approaches including cell or animal models genetically manipulated with one or more genes of interest when dealing with mutational genetics (e.g., transfected cells, biomacrochips, knockout animals, transgenic animals), multiple genetic markers of susceptibility (e.g., SNPs), and combined procedures. Gene-targeted mice (APP knockout mice, PS1 knockout mice), APP-, tau-, APOE-, and PS1-transgenic mice have been developed for AD research and therapeutics [13,14,87,229,244]. APP transgenic mice express wtAPP, fAD-linked APP variants, APP C-terminal fragments, and BAP, developing amyloid plaques in the neocortex and hippocampus similar to those seen in AD [245,246]. Several promoters (PDGF, PrP, Thy-1), different constructs (APP minigen, APP695 cDNA, APP751 cDNA) and some mutations (V717I, V717F, K670N/M671L) can induce an age-dependent increase in the concentration of BAP together with amyloid deposition in dense-core plaques and astrogliosis [30]. A prominent cerebral amyloid angiopathy is apparent in transgenic mice expressing the London APP mutation in neurons [247]. Different PS1 transgenic mice express some of the most frequent PS1 mutations associated with fAD; and PS1-APP transgenic mice express genetic mutations present in both APP and PS1 genes [13]. Some PS1 transgenic mice facilitate neuronal apoptosis and neurodegeneration, but fail to produce brain amyloidogenesis, suggesting that the

pathogenic role of PS1 mutations is upstream of the amyloid cascade [87]. JNPL3 transgenic mice expressing a mutant tau protein crossed with Tg2576 transgenic mice expressing APP yielded a double mutant tau/APP progeny with enhanced neurofibrillary degeneration in the limbic system and olfactory cortex [227]. The administration of BAP42 fibrils into the brains of P301L mutant tau transgenic mice induced a fivefold increase in the concentration of intracellular NFTs in the amygdala, indicating that BAP accelerates NFT formation [228]. Transgenic mice carrying the APOE-4(C112R) and APOE-4(L28P;C112R) variants were generated and revealed changes in behavior and in GFAP production [244].

The transgenic technology is the best option for investigating AD etiopathogenesis and AD-related pharmacodynamics, as well [34,248]. The application of gene targeting techniques to different types of cells is very useful for the same purpose in neuroscience and neuropharmacology [249-251]. The incorporation of AD-associated APP/PS1 mutations into transgenic mice is replacing in part the conventional animal models to study the effects of aging on cognition [252] and the animal models to mimic mnemonic impairment in AD [253]. Transgenic knockouts can be used as part of high-throughput, evidence-based target selection and validation strategies [254]. For the investigation of non-cognitive symptoms in AD (e.g., psychotic symptoms), genetic animal models of schizophrenia [255] or a combination of SZD+AD mutants might also be suitable. This is a very critical point in the pharmacological treatment of AD, where the vast majority of conventional psychotropic drugs exert a deleterious effect on cognition, with the additional fact that cognitive enhancers not always improve non-cognitive functions or even aggravate abnormal behaviors in AD patients [11,256]. In this regard, the availability of combined mutants for neurodegeneration and behavioural dysfunction could be helpful in drug development for dementia. However, AD transgenic models are expensive and do not reflect the complete polygenic dysfunction in AD, considering that transgenic mice only represent the mutational perspective of AD, present in less than 10% of the AD population. A novel pharmacogenomic approach to AD is the development of biomacrochips [1,11], incorporating normal cells or gene targeted clones of cells kept on structural supports in culture for high-throughput primary screening. With this procedure it has been possible to demonstrate the influence of AD-related gene mutations on apoptosis and premature neuronal death in culture. Another strategy was to use biomacrochips integrating neuronal, glial and endothelial cells in culture to demonstrate patient-specific microglial activation, IL-1, TNF, and NGF production, BAP-induced cell toxicity, apoptosis, histamine-induced vascular endothelial regulation, and flow cytometry analysis of class I and class II major histocompatibility complex (MHC) antigen expression on microglia [31-33,257,258]. The sera of AD patients contain genotype-specific factors that influence neuronal survival and microglial function in culture [31-33,259,260]. One of these factors is a 19/20 kDa low molecular weight protein with potential biochemical prognostic value in different types of dementia [261]. Using genotyped sera from AD patients in biomacrochip models it is possible to test drug activity on specific cells (neurons, microglia, astrocytes, brain vascular endothelial cells, lymphocytes) and also to predict specific therapeutic responses with novel drugs in individual patients prior to clinical administration of the biochip-tested drug [31,257,259]. The biomacrochip technology is very useful and efficient in drug primary screening, in eliciting valuable information for decision-making when transferring a drug from the preclinical stage to clinical studies, in testing safety and toxicity on specific cells, and also in predicting, on an individual basis, whether or not a particular patient will biologically respond to a specific compound.

It is very well known for many years the heterogeneity of AD and how apparently identical phenotypes assessed with international clinical criteria (NINCDS-ADRDA, DSM-IV, ICD-10) do not always respond to the same drugs [11]. In fact, the therapeutic response of AD patients to conventional cholinesterase inhibitors is partially effective in only 10-20% of cases, with side-effects, intolerance and non-compliance in more than 60% of the patients due to different reasons (e.g., efficacy, safety) [7,11,260]. Therefore, the individualization of therapy or pharmacological tailorization in AD and other CNS disorders is just a step forward of the longstanding goal of molecular pharmacogenomics [230,234.261] taking advantage from the information and procedures provided by the sequencing of the entire genomes of free-living organisms [22].

Preliminary Pharmacogenomics of Alzheimer's Disease: Genotype-specific Therapeutic Responses in Clinical Trials

Several studies indicate that the presence of the APOE-4 allele differentially affects the quality and size of drug responsiveness in AD patients treated with cholinergic enhancers (tacrine, donepezil) [128]. For example, APOE-4 carriers show a less significant therapeutic response to tacrine (60%) than patients with no APOE-4 [127]. In contrast, other studies do not support the hypothesis that APOE and gender are predictors of the therapeutic response of AD patients to tacrine [262]. An APOE-related differential response has also been observed in patients treated with other compounds devoid of acetylcholinesterase inhibiting activity (CDP-choline, anapsos) [263,264], suggesting that APOE-associated factors may influence drug activity in the brain either directly acting on neural mechanisms (choline acetyltransferase activity, nicotinic-receptor binding, neurotransmission modulation, amyloid deposition, tau degradation or phosphorylation) or indirectly influencing diverse metabolic pathways (cholesterol internalisation, apoE/LDL receptor regulation, neuronal membrane phospholipid homeostasis) [128,265]. Since APOE, PS1 and PS2 genes participate in AD etiopathogenesis regulating neuronal function and brain amyloidogenesis (Fig. **1**), in an attempt to envision the potential influence of major AD-associated genes on the therapeutic response in AD patients, we have performed the first pharmacogenomic study in AD using a genetic matrix model to identify the response of a multifactorial therapy in different AD genotypes combining allelic associations of APOE+PS1+PS2 genes [11,34]. AD-related genotypes integrating APOE+PS2+PS1 in a trigenic matrix model yield the 36 predicted genotypes. The 10 most

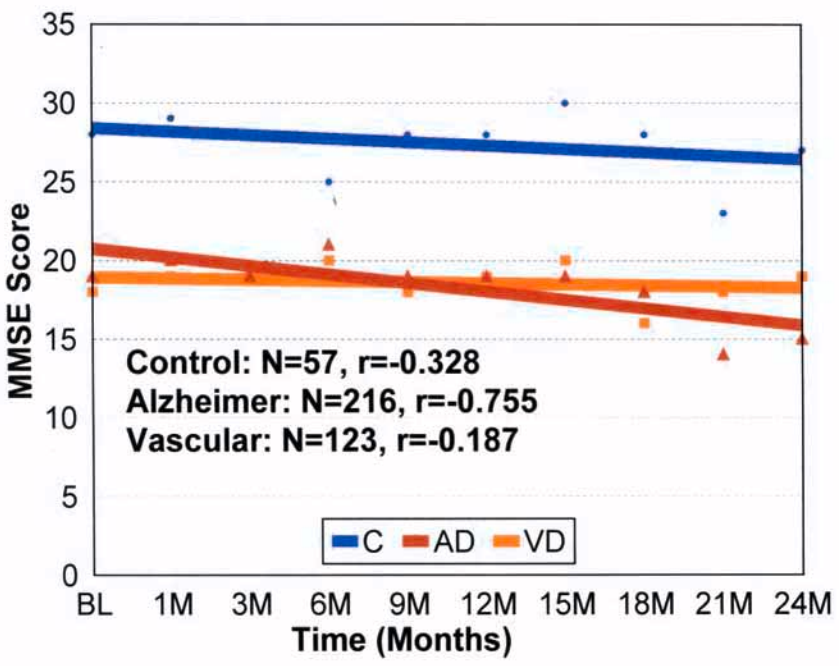

Fig. (6). Cognitive response to a multifactorial therapy in patients with Alzheimer's disease, vascular dementia and healthy subjects with family history of dementia.

frequent polymorphic genotypes in this AD sample were the following: (1) E33P112P2+ (17.75%), (2) E33P112P2- (15.55%), (3) E33P111P2+ (10.85%), (4) E34P112P2+ (9.60%), (5) E34P122P2+ (7.56%), (6) E33P111P2- (7.10%), (7) E34P111P2+ (4.80%), (8) E33P122P2+ (4.38%), (9) E34P111P2- (4.18%), and (10) E34P122P2+ (3.55%), constituting approximately 85% of the AD population [34]. Of these 10 major genotypes, 55% are APOE-3/3-, 45% APOE-3/4-, 79% PS1-1/1-, 42% PS1-1/2-, 13% PS1-2/2-, 50% PS2(+)-, and 35% PS2(-)-related [34]. APOE-4/4-related genotypes represent less than 3% of the AD population in the following order: E44P112P2+ > E44P111P2+ = E44P111P2- > E44P112P2+ > E44P122P2+ = E44P122P2-. APOE-4/4 patients are younger than patients with other genotypes [34]. From these series, AD patients were divided into 3 different groups: (a) APOE (N=440; 273 females, 167 males; age: 72.76±6.84 years; basal MMSE score: 20.47±8.03); (b) PS1 (N=347; 209 females, 138 males; age: 72.05±0.05 years; basal MMSE score: 20.42±7.63); and (c) PS2 (N=296; 177 females, 119 males; age: 71.25±5.35 years; basal MMSE score: 20.65±7.96). AD patients received (open trial) for 2 years 3 different drugs in combination: (a) CDP-choline (1000 mg/day, p.o.), an andogenous nucleotide [11,20,175,176,180,181,263,266]; (b) piracetam (2400 mg/day, p.o.), a nootropic agent [268]; and (c) anapsos/calagualine (360 mg/day, p.o.), a neuroimmunotrophic agent [263,264,266]. These 3 compounds have been previously tested as useful neuroprotectants and cognition enhancers in individual trials for AD [11], and are currently used in memory disorders and dementia in several countries [176,181]. Furthermore, this multifactorial therapeutic strategy has been successfully proven in previous studies in AD, vascular dementia, and as a preventive strategy in people at risk with genetically confirmed family history of dementia [11,20] (Fig. **6**). Mental performance in APOE-2/3 patients improved from baseline (MMSE score: 19.71±8.20) to 23.6±2.52 (6 months) and decreased thereafter to 8.5±3.6 (24 months) (r=-0.62). APOE-2/4 patients also improved during the first 12 months (from 19.85±9.37 to 20.66±8.5) and then deteriorated progressively (r=-0.75). APOE-3/3 patients improved from baseline (21.41±7.57) up to 6 months (22.3±5.77, $p<0.003$) and were stable until the 15th month (20.03±5.48) to decline thereafter (r=-0.68). APOE-3/4 were the best responders, showing a progressive improvement during the first 18 months and a positive regression line along the study (r=+0.013); in contrast, APOE-4/4 patients were the worst responders (r=-0.93), although a clear improvement was observed from baseline (21.93±7.35) to the 3rd month (26.17±1.54) (Fig. **7**). Patients with different PS1 genotypes showed a very similar therapeutic response (Fig. **7**). All patients exhibited a mild cognitive improvement during the first 6 months and then progressively declined with minor differences. PS1-1/1 patients were the worst responders (r=-0.718), while PS1-1/2 (r=-0.48) and PS1-2/2 patients (r=-0.46) showed a very similar cognitive deterioration (Fig. **7**). PS2 patients exhibited a clearly different therapeutic response according to the two PS2 exon 5 variants considered in the study (Fig. 7). PS2+ patients performed significantly worse (r=-0.727) than PS2- (r=-0.292), indicating that, with regard to PS-related genetic factors, mutational genetics is more influential than allelic variation in AD [34] (Fig. **7**).

When considering bigenic (APOE+PS1, APOE+PS2) or trigenic (APOE+PS1+PS2) associations, the therapeutic responses are very variable probably due to two major methodological factors, such as the reduced number of patients per group, and the difficulty of getting sufficient patients in a similar disease stage. For instance, patients in the E33PS1 group behave in a similar manner (E33PS111,

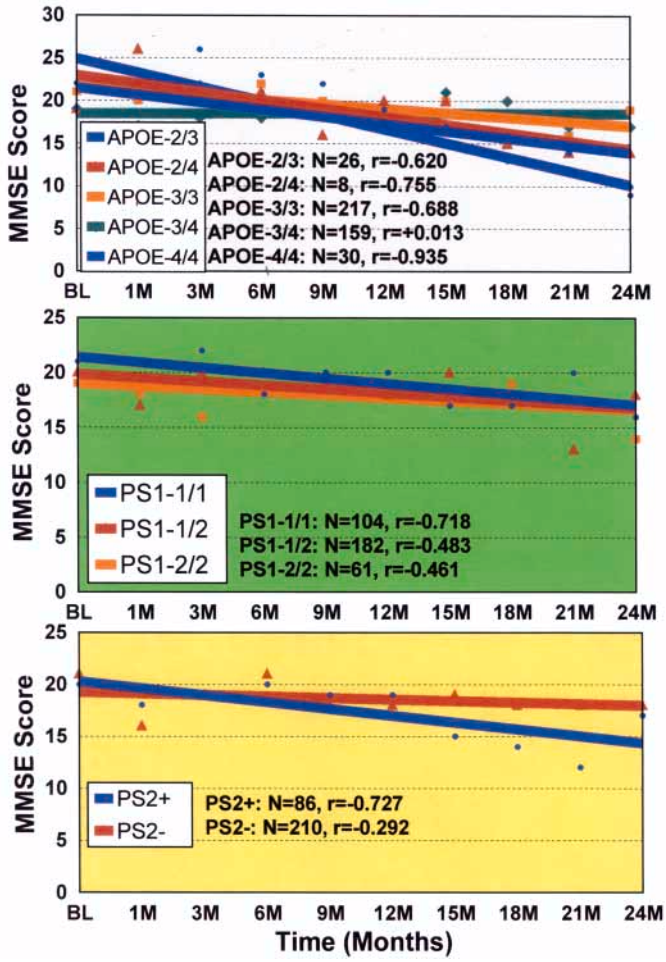

Fig. (7). Genotype-dependent cognitive response to a multifactorial therapy in Alzheimer's disease.

r=-0.869; E33PS112, r=-0.780; E33PS122, r=-0.820); however, in the E34PS1 group significant differences appear, with E34PS122 (r=0.000) responding much better than E34PS111 (r=-0.201) > E34PS112 (r=-0.464) [34]. In the E33PS2, E34PS2, and E44PS2 series, patients with the PS2+ genotype always showed a worse performance than the others. E34PS2- patients kept a stable clinical condition (r=+0.0004), and E34PS2+ patients showed a very mild decline (r=-0.114). In the E44PS1 series the worst responders were patients with the E44PS112 genotype (r=-0.837), and the best responders were those with the E44PS111 genotype who improved during the first year of treatment from 22.4±4.8 to 23.3±5.0 (r=+0.390). In the E44PS2 series, the E44PS2+ genotypes (r=-0.986) performed much worse than the E44PS2- genotypes (r=-0.289). In the trigenic analysis the worst responders were patients with the E44P112PS2+ genotype [34]. CDP-choline [263,266]and anapsos [264] in independent double-blind placebo-controlled clinical trials also showed genotype-specific effects in AD [11].

Since genetic alterations and/or genetic variability in major AD genes (APP, APOE, PS1, PS2, A2M, TAU) seem to play a differential role in AD pathology associated with or independent from abnormal APP metabolism and BAP accumulation, it is highly recommended that novel therapeutics for AD be tested on an independent basis for each gene involved at the preclinical level, and also on a polygenic substrate to assess the influence of a particular drug on the genetic interactions potentially involved in AD neuropathology [11,34,35]. In this context, future etiopathogenic treatments in AD would require a better understanding about the mechanisms of action of a drug on a gene-related metabolic cascade (e.g., APP metabolism) as well as an overall testing of the effects of the novel treatment on other genomic loci and their metabolic pathways potentially associated with AD. This is the niche to be covered by pharmacogenomics in AD basic pharmacology by using transgenic models and biomacrochips [2,11]. At the clinical level, the pharmacogenomics of AD is much more complex due to several reasons: (a) AD patients older than 70-75 years usually show an important vascular component aggravating AD [107,267]; (b) more than 60% of dementia patients are taking many drugs simultaneously to treat concomitant pathologies [1]; (c) nutritional factors in the elderly can interfere with drug metabolism; (d) CYP genes family-dependent drug metabolism is not well studied in elderly demented people [268,269]; (e) the direct influence of mutational genetics and susceptibility genetics and their interactions to elicit AD pathology is not yet clear [26,30,239]; and (f) the involvement of epigenetic phenomena and environmental factors in AD is practically unknown [209,210]. In consequence, the preliminary approach to the pharmacogenomics of AD is very limited,

but substantially informative when both susceptibility factors (SNPs) and mutational factors are integrated in a polygenic screening applied to evaluate conventional therapeutic outcomes (e.g., cognitive improvement, biological parameters) in AD clinical trials [11,34]. However, many issues surrounding the technical, regulatory, legal, and ethical framework of pharmacogenomics remain unanswered, and educational programmes for the public and healthcare professionals are needed before this new discipline can be widely accepted [4,74,241,270-272].

CONCLUSIONS

AD is a genetically-based complex disorder potentially associated with multiple genetic defects across the human genome. Information available on mutational genetics and/or susceptibility genetics does not explain in full the etiopathogenesis of AD [1,26,30], suggesting that environmental factors and epigenetic phenomena can also contribute to AD pathology and phenotypic expression of dementia in later life [2,207]. The genomics of AD is still in its infancy, but is helping to understand novel aspects of the disease including genetic epidemiology, multifactorial risk factors, pathogenic mechanisms associated with genetic networks and metabolic cascades, and is also helping to develop new strategies in pharmacogenomic research [11,34,35]. Functional genomics, proteomics, pharmacogenomics, high-throughput methods, combinatorial chemistry and modern bioinformatics will greatly contribute to accelerate drug development for AD and other complex disorders [27,28,273]. At the present time, based on preliminary studies using a pharmacogenomic approach to AD, we can conclude the following: (1) the combination of 6 APOE genotypes with 3 PS1 genotypes and 2 PS gene-related variants generates the 36 most frequent genotypes in the AD population; (2) the accumulation of genetic defects in AD anticipates the onset of the disease and influence the therapeutic response of patients to conventional drugs; (3) 10 of the major AD genotypes represent approximately 85% of AD cases, of which the 3 most frequent genotypes are E33P112P2+ (17.75%), E33P112P2- (15.55%), and E33P111P2+ (10.85%); (3) APOE-4/4-related AD genotypes account for less than 10% in the AD population, and appear in the youngest group of AD cases; (4) the multifactorial therapy with CDP-choline + piracetam + anapsos, as a novel strategy, seems to be useful as a palliative, neuroprotective therapy, with more than 60% of the patients improving cognitive performance during the first year of treatment; (5) the therapeutic response in AD using cholinesterase inhibitors and/or non-cholinergic compounds is genotype-specific; (6) APOE-3/4 patients are the best responders (r=+0.013) and APOE-4/4 patients are the worst responders (r=-0.93); (7) patients classified according to PS1 genotypes respond in a similar manner with no major differences among the 3 PS1-related genotypes; (8) patients with a defective PS2 gene exon 5 (PS2+) always show a worse therapeutic response than PS2- patients; (9) the application of functional genomics seems to be a suitable strategy for AD homogenisation and harmonization criteria in molecular diagnosis and clinical trials; and (10) the pharmacogenomics of AD can contribute in the future to optimise drug development and therapeutics, increasing efficacy and safety, and reducing toxicology, adverse events, and unnecessary costs for the pharmaceutical industry and the public budgetary resources.

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