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DOTTORATO DI RICERCA IN

"*NEUROSCIENZE*"

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Neuroimaging studies in subjects at genetic risk of developing Alzheimer's disease: the role of neuroimaging to reveal the endophenotype.

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Introduction

Alzheimer's disease

Alzheimer's disease (AD) is one of the most common illnesses in later life and the most common type of dementia, accounting for 60% to 80% of cases. AD is clinically characterized by progressive memory loss and cognitive impairment: usually the disease begins with difficulty in remembering names and recent events as earliest symptoms, and later symptoms include impaired judgment, disorientation, confusion, behavior changes and trouble speaking, swallowing and walking [McKhann et al., 1984].

AD is one of the most pressing healthcare problems of the 21st century, with prevalence of dementia from 6% to 10% in adults aged 65 years and older [Alzheimer's Association 2008]; with the population of older adults increasing, the number of cases will continue to increase every year [Alzheimer's Association 2008]. Understanding the risk factors for AD is crucial to reduce disease occurrence or at least to slow its development [Brookmeyer et al., 1998].

At present, the clinical diagnosis of AD cannot be estabilished until the progressive cognitive deficits has affected patient's social and professional life [McKhann et al., 1984]; this clinical threshold varies greatly between individuals, depending on patient's premorbid cognitive and intellectual level and is to some extent arbitrary. This greatly limits the potential for early intervention and prevention research [Lansbury et al., 2004]. Furthermore, it is estimated that between 50% and 90% of dementia cases are left undiagnosed by standard clinical examinations [Hebert et al., 2003].

The definitive diagnosis of AD is possible only through post-mortem brain examination showing the presence of specific pathologic lesions: intracellular neurofibrillary tangles (NFT), amyloid beta (Aβ) deposition in the form of extracellular senile plaques and blood vessel deposits, associated with neuronal and synaptic loss, and atrophy in specific areas of the brain [Mirra et al., 1991]. These AD-related neuropathological changes begin years before the onset of clinical symptoms of dementia [Price et al., 1999].

During the preclinical phase, as plaque and tangle load increase, a selective synaptic and neuronal loss of vulnerable cortical pyramidal neurons occurs, leading to the first symptoms of dementia [Morrison et al., 2007]. The brain regions most vulnerable in AD

are the medial temporal lobes (including hippocampus, transentorhinal and entorhinal cortex, and subiculum) [Braak et al., 1991, 1996]. The pyramidal cells anatomically connected to the entorhinal cortex (EC) and the CA1 and subiculum regions of the hippocampus are particularly prone to NFT formation and degeneration, whereas primary sensory-motor and occipital areas and the cerebellum exhibit minimal neuronal loss [Braak et al., 1991, 1996; Delacourte et al., 1999]. Disruption of the pyramidal neurons in the perforant path is thought to disconnect the hippocampus from the rest of the cortex, strongly contributing to the decline in memory observed in early AD [Hyman et al., 1984]. There is an association between NFT staging and the severity of clinical status in AD [Delacourte et al., 1999]. NFTs originate in the medial temporal lobe, which plays a critical role in the neural control of memory functions, then begin to cluster in the adjacent inferior temporal and posterior cingulate cortex in mild AD, further disrupting episodic and autobiographic memory, and eventually disrupting the parieto-temporal and prefrontal association cortices, which are involved in the neural control of perception, attention, and language, in moderate and then severe dementia [Braak et al., 1996; Delacourte et al., 1999]. Despite an initial predilection for the neocortex, Aß depositions are also found in the medial temporal lobes at later stages of disease [Arriagada et al., 1992]. For many years, the aggregation of large Aß fibrils was considered the key event in AD pathogenesis and the main determinant of neuronal degeneration [Selkoe et al., 1997]. A recent reformulation of the amyloid cascade hypothesis states that Aß oligomers, not fibrillar Aß, confer greater neurotoxicity to neurons by disrupting nerve signaling pathways and subsequently causing neuronal cell death [Lambert et al., 1998].

Alzheimer's disease genetics

AD, as many other neurodegenerative disorders, is a genetically complex disease associated with multiple pathogenetic factors. Although largely influenced by genetic factors, the pathogenesis of AD is by far more elaborate, involving aging mechanisms and other age-related pathological conditions (i.e., cerebrovasular deterioration), epigenetic phenomena and environmental factors (i.e. nutrition).
The genetic dysfunction in AD includes several mutational loci and susceptibility loci, distributed across the human genome, probably converging in a common pathogenic mechanism that leads to premature neuronal death, in which mitochondrial DNA mutations may contribute, too.
A feature of AD, which is common to many neurodegenerative disorders, is the presence of both familial (rare and usually characterized by an earlier onset of disease symptoms) and non-familial (more common and with later onset of disease symptoms) forms. Although the latter form is frequently described as "sporadic", there is increasing evidence suggesting that a large proportion of these cases are actually influenced by genetic factors. Recent estimates showed a heritability (measure of the proportion by which phenotypic variation is determined by genetic variation) of AD between 60% and 80% [Gatz et al., 2006, Pedersen et al., 2001], indicating that genetic factors play a crucial role in determining the risk of late-onset AD (LOAD).

The research of the heritable component of AD so far has found four established AD genes (APP, PSEN1, PSEN2, APOE) and many potential susceptibility loci, none of which however has been proven to significantly modify disease risk. In the following chapters, we will discuss both the established and potential genetic factors involved in the pathogenesis of AD.

Early onset familial Alzheimer's disease

Rare AD cases (<5%) occur with a Mendelian pattern of inheritance, and usually show an early (<65 years), or sometimes very early (<50 years), age of onset; this form of AD, because of its typical familial accumulation and autosommal-dominant inheritance pattern, is known as "early onset familial AD" (EOFAD), and is caused by mutations in three genes, all of which alter the production of A**β**, one of the neuropathological hallmarks of AD [Tanzi et al., 2005]. The mutated genes leading to EOFAD are located in the amyloid precursor protein (APP) gene [Goate et al., 1991], or in the genes encoding the proteins that forms the active site of the γ-secretase complex: presenilin 1 (PSEN1) [Sherrington et al. 1995] and presenilin 2 (PSEN2) [Rogaev et al., 1995] (Table 1).

Even if EOFAD accounts for a little fraction of all AD cases, the knowledge of its genetic factors has been crucial in the development and support of the "amyloid hypothesis", according to which a dysregulation of the production and function of Aβ is the initializing event in the pathogenic cascade leading to neurodegeneration and dementia [Hardy et al., 2002]. Moreover, these genes have provided encouragement for the discovery of amyloid-modifying medications and immunization therapies.

Table 1. Genetic findings for EOFAD.

Gene	Location	Relevance to AD pathogenesis
APP [Goate et al., 1991]	21q21.3	Increase in Aβ production or Aβ 42/Aβ 40 ratio; mutations in the Aβ sequence or close to the band γ-secretase site of APP; locus duplications
PSEN1 [Sherrington et al., 1995]	14q24.3	Increase in Aβ 42/Aβ 40 ratio; mutations throughout molecule; enzymatic role in γ-secretase complex
PSEN2 [Rogaev et al., 1995]	1q31–42	Increase in Aβ 42/Aβ 40 ratio; mutations throughout molecule; enzymatic role in γ -secretase complex

Late onset Alzheimer's disease

Late onset Alzheimer's disease (LOAD) accounts for the vast majority of AD cases; it appears later in life (usually > 60 years) and, despite its high heritability [Gatz et al., 2006, Pedersen et al., 2001], it shows a less evident genetic association than EOFAD. This may be due to a combination of factors, including the complexity of the disease, its slow progression, patients' old age, the concomitance of other age-related medical illnesses, and the lack of established standard criteria for incipient AD.
Although all these difficulties, several different genes thought to have a substantial impact on AD predisposition have been identified, including the well-known Apolipoprotein E (ApoE) gene [Gatz et al., 2006]; most of them affect various pathways likely to be involved in the production, aggregation and removal of Aβ (Figure 1). However, the non-ApoE-related LOAD genes show a modest risk effect, with odds-ratio (OR) of 1.2, as compared to the OR of 3-4 for the ApoE gene.

Table 2 summarizes the most significant data on genetics of LOAD, including the Top Results Gene list of the AlzGene database, a publicly available database (http://www.alzgene.org), which systematically collects, summarizes and meta-analyzes all genetic association studies published in the field of AD [Bertram et al., 2008]. This database includes LOAD genes identified both with candidate-gene approach (studies focusing on certain genes based on some prior hypothesis regarding their potential involvement in the disease process) and with genome-wide association approach, a large-scale genotyping technology that enables more comprehensive and unbiased analyses [Bertram et al., 2009].
The functional relevance of all these potential AD susceptibility loci may not only lead to a better understanding of the pathogenic processes driving to neurodegeneration in AD, but may also help to pinpoint novel treatments for AD.

Table 2. Genetics findings for LOAD

Gene	Polymorphism	location	OR	Relevance to AD pathogenesis
ApoE [Kim et al., 2009]	ε2,ε3,ε4	19q13.2	3,81	• increased AD risk and decreased age at onset in ε4 carriers; • increased accumulation of Aβ42 and binding to tau protein
CHRNB2 [Kawamata et al., 2002, Cook et al., 2004]	rs4845378	1q21.3	0,67	• reduced AD risk in T-allele carriers; • nicotinic cholinergic receptors could contribute to cognitive performance during aging [Zoli et al., 1999], with an age-dependent decrease of receptor subunits in the cortex, particularly the hippocampus [Tohgi et al., 1998]; • evidence in LOAD of loss of cholinergic neurons and reduction of nicotinic cholinergic receptors [Oddo et al., 2006]
ACE [Li et al., 2008, Thornton-Wells et al., 2008]	I/D, rs1800764, rs4291 and rs4343	17q23	0,87	• ACE-1 protein can degrade secreted Aβ in vitro [Hu et al., 2001, Hemming et al., 2005]; • ACE levels have been reported to be increased in AD brains in proportion to the parenchymal Aβ [Miners et al., 2008]; • Role in blood pressure regulation (vascular hypothesis suggested for AD pathogenesis [Takeda et al., 2008, Ohrui et al., 2004]).
CH25H [Reiman et al., 2007, Morgan et al., 2007, Li et al., 2008b]	rs13500	10q23	1,44	• part of lipid metabolism, as well as ApoE; • the risk-associated CH25H haplotype leads to increased CSF concentration of lathosterol, a metabolic precursor of cholesterol, to higher brain Aβ load and to lower Aβ 1-42 CSF levels in normal elderly subjects [Papassotiropoulos et al., 2005].

Gene	Polymorphism	location	OR	Relevance to AD pathogenesis
CST3 [Sun et al., 2008, Sundelof et al., 2008]	rs1064039	20p11.2	1,28	• A mutation in CST3 gene has been associated also with amyloid angiopathy [Schnittger et al., 2003]; • cystatin c binds to Aβ [Vinters et al., 1990] and inhibits Aβ fibril formation in vitro and in vivo [Sastre et al., 2004, Kaeser et al., 2007].
GAB2 [Reiman et al., 2007, Sleegers et al., 2009]	rs10793294	11q13.4 –q13.5	0,69	• GRB-associated binding protein 2 is a member of a family of scaffolding/adapter proteins that are involved in multiple pathway, in particular in the transduction of cytokines and growth receptor signalling [Sarmay et al., 2006]. • increased Tau phosphorylation and formation of neurofibrillary tangles [Reiman et al., 2007].
SORL1 [Rogaeva et al., 2007, Lee et al., 2008]	SORL1-18ex26	11q24	0,7	• SORL1 gene encodes for a protein that modulates subcellular trafficking of APP; • Reduced expression of SORL1 leads to releasing of APP into endosomal pathways, where it is subjected to beta- and gamma-secretase cleavage with subsequent production of amyloid-beta.; • SORL1 protein also belongs to a superfamily of low-density lipoprotein receptors (SorLA/LR11) that bind ApoE and are implicated in cholesterol metabolism and atherogenesis; • There is evidence for reduced SORL1 expression in the brains of patients with AD [Dodson et al., 2006], associated to increased amyloid-beta production [Kolsch et al., 2008].

Gene	Polymorphism	location	OR	Relevance to AD pathogenesis
TF [van Rensburg et al., 1993]	rs1049296	3q21	1,18	• Iron dysregulation promotes neurodegeneration via the generation of reactive oxygen species [Brewer et al., 2007]; • Increased iron levels in AD brains [Loeffler et al., 1995], associated with plaques and NFTs [Smith et al., 1997]; • Iron might also modulate the aggregation of hyperphosphorylated tau into insoluble paired helical filaments, the core component of NFTs [Yamamoto et al., 2002].
MAPT [Li et al., 2008]	rs2471738	17q21	1,24	• MAPT gene encodes for tau protein; • MAPT gene mutations have been associated with several neurodegenerative disorders such as AD, Pick's disease, frontotemporal dementia, cortico-basal degeneration and progressive supranuclear palsy; • Discordant results about its role in AD risk [Mukherjee et al., 2007];
PRNP [Casadei et al., 2001]	rs1799990	20p13	0,88	• Prion protein is the major determinant of familial and sporadic prion diseases (such as Creutzfeldt–Jakob disease), characterized by rapidly progressing neurodegeneration with spongiosis and amyloid plaques; • Aβ-positive plaques in AD brains often contain prion protein deposits; • In APP-PRNP double-transgenic mice prion protein might promote plaque formation in AD by increasing Aβ aggregation [Schwarze-Eicker et al., 2005]

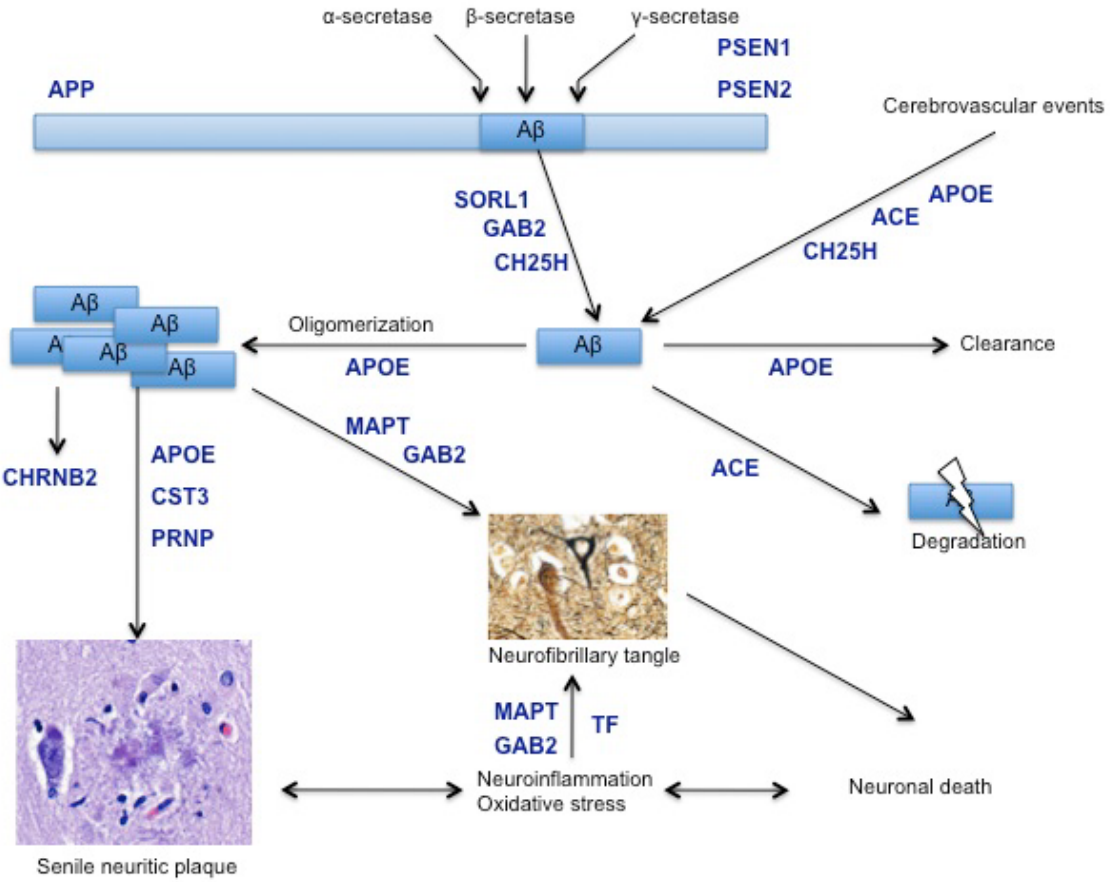
Gene	Polymorphism	location	OR	Relevance to AD pathogenesis
MTHFR [Seripa et al., 2003, Anello et al., 2004]	rs1801131	1p36.3	1,13	• MTHFR gene codes for 5,10-methylenetetrahydofolate reductase, which converts 5,10 methylenetetrahydrofolate to 5-methyltetrahydrofolate. • Despite contrasting results were reported, the systematic meta-analysis of the AlzGene database reported an association with LOAD.
IL1B [Deniz-Naranjo et al., 2008]	rs1143634	2q14	1,2	• IL1B gene encodes a protein member of the IL1 cytokine family, an important mediator of the inflammatory response. • Despite the role of inflammation in LOAD, the role of IL1B appears to be marginal.
CLU [Harold et al., 2009]	rs11136000	8p21.1	0,85	• CLU gene encodes for clusterin, which has a role in protecting the brain from damage, markedly influencing Aβ structure and its neuritic toxicity
PICALM [Harold et al., 2009]	rs541458, rs3851179	11q14.2	0,87	• PICALM (phosphatidylinositol-binding clathrin assembly) protein is involved in clathrin-mediated endocytosis, an essential step in the intracellular trafficking of proteins and lipids such as nutrients, growth factors and neurotransmitters. • changes in PICALM function result in perturbations at the synapse, possibly through synaptic vesicle cycling, increasing risk for AD. • PICALM could also influence AD risk through APP processing via endocytic pathways, resulting in changes in Aβ levels.

Gene	Polymorphism	location	OR	Relevance to AD pathogenesis
KIBRA [Papassotiropoulos et al., 2006]	rs17070145	5q	n.a.	• Associated with memory performance; • KIBRA T allele carriers show better episodic memory performance as compared with non carriers [Papassotiropoulos et al., 2006]; • KIBRA CC carriers had moderate but significantly increased risk of LOAD as compared to T-carriers [Corneveaux et al., 2008, Rodriguez-Rodriguez et al., 2009]
TNK1 [Figgins et al., 2009]	rs1554948	17p13.1	0,86	• TNK1 encodes a nonreceptor tyrosine kinase involved in negative regulation of cell growth. • It has been reported that the SNP rs1554948 may be protective against LOAD.
TFAM [Alvarez et al., 2008]	rs2306604	10q21	0,82	• TFAM encodes for mitochondrial transcription factor A that is a key activator of mitochondrial transcription as well as a participant in mitochondrial genome replication. • It can be considered a minor risk factor for LOAD.
CR1 [Lambert et al., 2009]	rs6656401	1q32	1,19	• CR1 encodes for a complement receptor 1, which could have roles in protecting the brain from damage. • Perhaps the SNP removes this protection.
SORCS1 [Grupe et al., 2006]	rs600879	10q23–q25	1,24	• This gene encodes one member of vacuolar protein sorting 10 domain-containing receptor proteins family. • These genes are strongly expressed in the CNS, but only one GWA studies suggested an involvement of this gene in LOAD.

As explained before, AD is a really genetically complex disorder, in which numerous genetic and environmental factors contributes with an individual small effect, resulting in the clinical expression of the disease only when their combined effects cross a “threshold of liability”. This complexity represents a great challenge for genetic studies. In addition to this genetic complexity, there is the fact that the brain is the most complex organ in the human body. Indeed, brain cells are different from each other, not only morphologically and functionally, but also in the connections and interactions between neurons and glia. Besides, the brain is also influenced by interactions between individuals and experiences. So, the clinical phenotypic expression of AD is subject to all these interactions, and it is not simply a sum of them.

Figure 1. Summary of the AD genes role (EOFAD and LOAD).

APP gives rise to Aβ through serial cleavage by β-secretase and γ-secretase. γ-secretase is a complex of proteins in which the enzymatic core is composed by PSEN1 and PSEN2.
SORL1 and CH25H might both affect APP processing and Aβ generation, the first by affecting APP trafficking and the latter by the production of 25-hydroxycholesterol.
GAB2 binds GRB2, which binds APP and presenilins.
ApoE and CH25H genes, affecting cholesterol metabolism, with ACE gene, affecting blood pressure, might modulate the effects of cerebrovascular events on Aβ production. ApoE can also transport Aβ out of the brain and influence Aβ aggregation.
Nicotinic cholinergic receptor subunit b2 (encoded by CHRNB2) might be affected by Aβ. Moreover, Aβ fibrillization can be affected by ApoE, Cystatin 3 (encoded by CST3) and prion protein (encoded by PRNP).
Transferrin (encoded by TF gene) regulates the metabolism of iron, involved in oxidative stress, and associated with abnormal tau phosphorylation and aggregation, and with the formation of neurofibrillary tangles.
The principal component of neurofibrillary tangles is tau, which is encoded by MAPT gene. GAB2 also has been reported to affect tau phosphorylation and NFT formation.



Endophenotypes in Alzheimer's disease

Despite the improvement of genome data analysis techniques, the research of genetic variants associated with LOAD is still limited. Why has the pursuit of susceptibility LOAD genes been so difficult and elusive?

The first greatest problem that affects genetic studies of AD, as well as other psychiatric disorders in general, is that various types of phenotypic misclassification could occur, thus reducing the power of the analyses. Labeling affected family members as 'unaffected' reduces the apparent penetrance, so larger sample sizes are required to obtain significant results. Conversely, labeling unaffected family members as 'affected' masks the presence of linkage, making detection of linkage more difficult and specific localization of susceptibility genes almost impossible.

Besides, there is heterogeneity both in the clinical presentation of AD and in the genetic background of the subjects. Finally, sample sizes in most genetic association studies may be too small to have adequate statistical power in this complex disease in which multiple genes with small effect each contribute to the different traits associated with the disease, such as memory performance, amyloid/tau pathology or hippocampal atrophy.

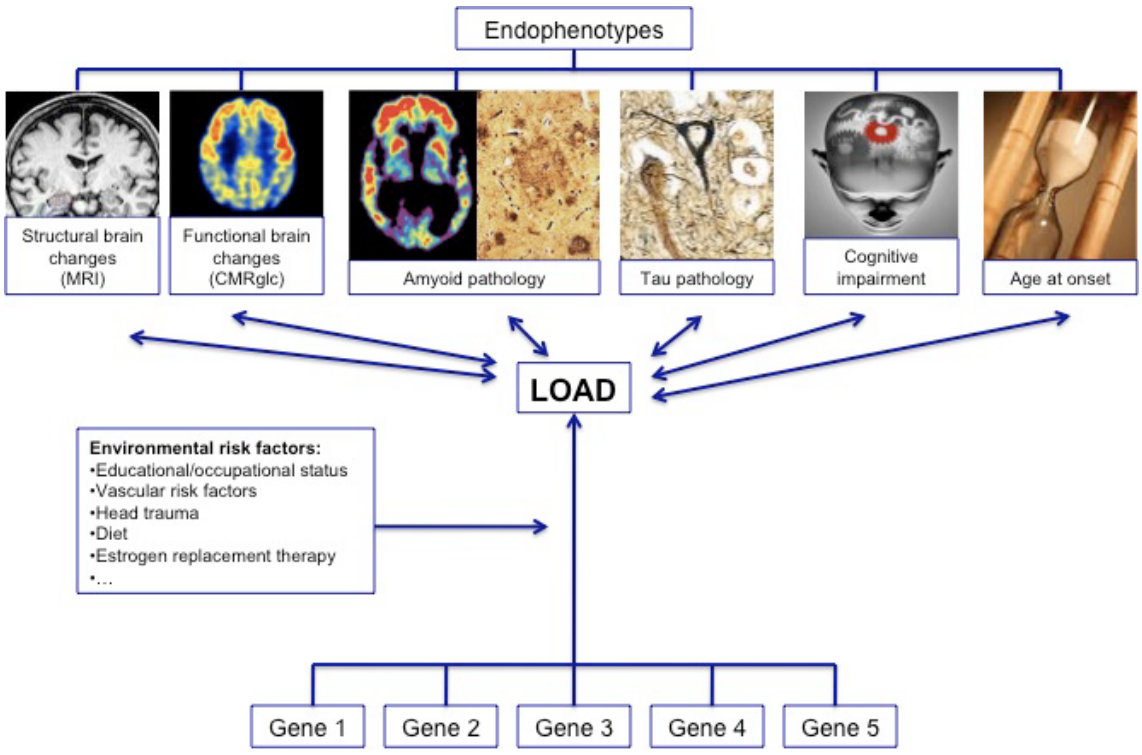
The genetic analysis in AD might be improved by the identification of more basic phenotypes than the full phenotypic expression of AD, for which a more homogeneous etiology might be expected. These AD intermediate phenotypes, or endophenotypes, are generally closer to the action of the gene than the affection status, and thus exhibit higher genetic signal-to-noise ratio (Figure 2) [Gottesman et al., 2003, Reitz et al., 2009].

In AD the identification of the endophentypes could be achieved with two strategies: the description of AD affected patients and the identification of vulnerability traits in non-affected AD relatives. This second approach is particularly interesting in AD, indeed the subclinical endophenotypes might be useful for identifying common alleles with non-specific and moderate effects on diseases risk [Matthysse et al., 1992, Leboyer et al., 1998]. The first approach, instead, could be described as the "candidate symptom approach" [Gottesman et al., 2003]. The description and analysis of affected subjects could help in identifying AD subgroups. For example, in AD, the mutations in genes encoding for APP, PSEN1 and PSEN2 were identified only after the selection of early-onset familial cases of AD showing an autosomal pattern of inheritance.

For all these reasons, only through the identification of endophenotypes in AD we can increase the power to localize and identify AD related susceptibility genes, more easily than using the affection status alone [Blangero et al., 2003].

Possible AD endophenotypes, which may be more proximal to its genetic variations, could be particular profiles of clinical onset of the disease, or typical characteristics of cognitive impairment, or the presence or distribution of the typical AD pathological alterations. Some AD endophenotypes can be measured in vivo using neuroimaging techniques, which could be capable to highlight the presence of specific alterations usually observed in patients with clinically manifest AD. Among the pathological changes and the corresponding neuroimaging examinations, there are (Figure 2):
- morpho-structural brain changes, measurable with brain magnetic resonance imaging (MRI);
- brain synaptic disfunction, measurable using positron emission tomography (PET) with 18F-fluorodesoxyglucose (18F-FDG);
- deposition and accumulation of pathological proteins, such as amyloid and tau proteins, measurable using PET with amyloid tracers such as 11C-PiB.

Figure 2. Endophenotypes in the cascade from a genetic sequence variation and AD.



Maternal family history of AD

Several epidemiological studies in AD have shown that, besides AD heritability, maternal transmission may have a greater impact on the offspring's health than paternal transmission. This is not only due to the fact that AD affects more women than men [Green et al., 2002], but that maternal transmission is actually more frequent than paternal transmission of AD [Gomez-Tortosa et al., 2007]. A review of the AD literature [Duara et al., 1993; Edland et al., 1996; Heyman et al., 1983; Farrer et al., 1997] shows that approximately 20% of LOAD cases are maternally inherited and, in LOAD patients with one affected parent, the mother-to-father ratio is approximately 3:1 [Heyman et al., 1983, Duara et al., 1993, Edland et al., 1996, Farrer et al., 1997, Gomez-Tortosa et al., 2007]. Importantly, having an AD-affected mother was associated with poorer cognitive performance in late life [Schifitto et al., 2007] and with a more predictable age at onset of dementia in the offspring, as compared to having an AD-father [Duara et al., 1993, Gomez-Tortosa et al., 2007, Duara et al., 1996].

Among many possible explanations about this particular pattern of inheritance, the most likely genetic mechanisms to explain maternally inherited AD would appear to be (in alphabetical order):

- chromosome X-mediated transmission
- epigenetic imprinting
- mitochondrial DNA-mediated transmission
- sex-specific trinucleotide repeat expansions-mediated transmission.

Chromosome X-mediated transmission

Chromosome X-linked diseases are disorders that reflect the presence of defective genes on the X chromosome. The vast majority of X-linked diseases show recessive transmission with the following features:

1) males are more frequently affected than females because they are hemizygous and the gene defect is not balanced by a normal X chromosome;

2) females are rarely affected because they need to be homozygous for the defective gene to manifest the phenotype; 3) there is no male-to-male transmission;

4) transmission of the defective gene occurs from fathers to daughters, who become carriers;

5) males born to a carrier mother have a 50% probability of inheriting the X chromosome carrying the defective gene.

Classical examples of X-linked transmission are red–green colour blindness and haemophilia A.

The 'X-linked hypothesis' for a LOAD gene was supported by a recent genome-wide association study, which identified a strong association between LOAD and a mutation located on the PCDH11X gene on chromosome X.64 Female homozygotes were at significantly increased risk for AD compared with female non-carriers, female heterozygotes and male hemizygotes. Moreover, female heterozygotes were at greater risk than female non-carriers, and male hemizygotes were at higher risk than male non-carriers [Carrasquillo et al., 2009].

Although maternally inherited LOAD and X-linked diseases may share some characteristics, several properties of recessive X-linked disorders do not apply to AD. First, PCDH11X mutations were associated with higher risk in female homozygotes than male hemizygotes, which is contrary to what is usually found in X-linked recessive disorders. Secondly, there is no male-to-male transmission in X-linked inheritance, whereas father-to-son transmission has been reported in AD [Ehrenkrantz et al., 1999; Gomez–Tortosa et al., 2007].

Epigenetic imprinting

Genomic imprinting is a genetic process by which certain genes are expressed in a parent-of-origin specific manner, which does not follow classical Mendelian inheritance [Constancia et al., 2004]. One of the most common mechanisms of genomic imprinting is the silencing of one of the parental alleles, and gene expression is monoallelic. This silencing mechanism is known to involve epigenetic marking by allele-specific DNA methylation and/or histone modifications. Differential epigenetic marking of the parental chromosomes results in differential gene expression, predominantly from one parental allele.

For a gene that is normally imprinted with paternal silencing, a mutation in the maternal copy of the gene will result in disease, while a mutation in the paternal copy will have no effect, and vice versa. According to the parental conflict hypothesis, paternal imprinting appears to be growth promoting, while maternal imprinting would be growth limiting.

Imprinted genes are susceptibility targets for numerous human pathologies because their functional haploid state enables a single genomic or epigenomic change to dysregulate their function. Recent evidence has highlighted the role of genetic imprinting on brain function for PraderWilli and Angelman syndromes, as well as more

common disorders like diabetes, obesity, breast cancer, autism, bipolar disorder, Tourette's syndrome, epilepsy and even AD [Constancia et al., 2004].
Maternal imprinting may be a genetic mechanism responsible for the increased maternal inheritance in AD cases. Some reports showed that the methylation process involved in imprinting is altered in AD patients, compared with controls [Payao et al., 1998]. A genome-wide screening of AD families reported a linkage between AD and a region close to the centromere of chromosome 10q only in families with an AD affected mother [Basset et al., 2002]. While these findings suggest imprinting, there is no clear evidence for the existence of imprinted genes in AD families on chromosome 10 or on other chromosomes.

Mitochondrial DNA

Mitochondrial disorders are entirely maternally inherited in humans and are characterised by variable phenotypic expression. Male and female progeny are equally likely to receive mutant maternal mitochondrial DNA (mtDNA).
The fact that mtDNA is exclusively maternally inherited in humans lends support to this hypothesis [Lin et al., 2006, Swerdlow et al., 2004].
Human mtDNA is a 16.569-kb circular, double-stranded molecule, containing 37 genes: 2 rRNA genes, 22 tRNA genes and 13 structural genes that encode electron transport chain (ETC) subunits. Of the approximately 80 proteins that make up the ETC, 13 are encoded by mtDNA and all others are encoded by nuclear DNA, such as complex II, coenzyme Q and cytochrome c. In contrast, the catalytic components of complex I, III, IV and V are primarily encoded by mtDNA, which contains 7 genes encoding for complex I (ND1-ND4, ND4L, ND5 and ND6), 1 for complex III (cytochrome b), 3 for complex IV (COX I-III) and 2 for complex V (ATPase6 and ATPase8). Although mtDNA encodes for only 2% of all proteins forming the subunits of the mitochondrial respiratory chain, all of them are essential for maintaining physiological oxidative phosphorylation (OXPHOS). For more than 15 years, evidence has been accumulating that AD is associated with mitochondrial dysfunction, as well as oxidative stress and increased reactive oxygen species (ROS) production. At post-mortem, extensive oxidative stress is found in AD brains, in which basically all cellular macromolecules (protein, DNA, lipids) are found in an oxidized form [Smith et al., 1995., Mecocci et al., 1994.]. Oxidative stress, particularly in the form of reduced COX activity, is most prominent in the brain regions showing degeneration in AD [Kish et al., 1992, Valla et al., 2001, Simonian et al., 1994.].

Reduced COX activity in AD was shown in several in vivo studies of blood platelets [Parker et al., 1994, Bosetti et al., 2002] and fibroblasts [Curti et al., 1997].
Studies that investigated the role of mtDNA mutations in AD have used three main approaches: i) cybrid analyses; ii) case-control mutation identification studies; iii) mitochondrial haplogroup association studies. Studies of cytoplasmic hybrid (cybrid) cells provide direct evidence for mtDNA involvement in the metabolic abnormalities characteristic of AD. Cybrids are obtained by mixing mtDNA from patients' platelets with cell lines depleted of their own endogenous mtDNA, resulting in cell lines containing mtDNA from the patient [Lucas et al., 1976]. Cybrid data in AD indicate that mtDNA at least partly accounts for impaired metabolism and increased oxidative stress in AD, as reflected in increased ROS production, mitochondrial respiratory enzymes defects, particularly affecting COX activity, increased changes in calcium homeostasis, decreased ATP production and enhanced Aß toxicity, and a vastly increased percentage of morphologically abnormal mitochondria [Swerdlow et al., 1997, Cardoso et al., 2004]. In addition to cybrid studies, several case-control mutation identification and mitochondrial haplogroup association studies suggested that maternally inherited mutations of mtDNA might play a pathogenic role in AD (Table 3). However, despite the observation of mtDNA mutations in AD vs controls and of associations observed between specific haplotypes and an increased risk for AD, the overall involvement of mtDNA in AD pathogenesis is equivocal because of conflicting results.

Table 3. Mitochondrial DNA mutations and haplotypes associated with LOAD.

Approach	Finding	Positive results	Negative results
Case-control mutation identification	T146G, T195C, T152C, A189G, T414G	Increased AD risk [Coskun et al., 2004]	No mutation found [Chinnery et al., 2001]
	4977 bp deletion	More frequent in AD patients [Corral-Debrinski et al., 1994.]	
	A4336G	More frequent in AD patients [Egensperger et al., 1997, Shoffner et al., 1993.]	No association with AD [Wragg et al., 1995.]
Haplogroup association	K, U	Decreased risk of AD, neutralizing the effect of ApoE genotype [Carrieri et al., 2001.]	No effect of K haplogroup in AD [van der Walt et al., 2004.]
	U	Increased risk in males, decreased risk in females [van der Walt et al., 2004.]	
	J	Increased in AD patients in a French-Canadian population [Chagnon et al., 1999.]	No AD haplogroup association in US population [Chinnery et al., 2001]
	T	Decreased AD risk in a French-Canadian population [Chagnon et al., 1999]	No AD haplogroup association in US population [Chinnery et al., 2001]

Sex–specific trinucleotide repeat expansions

Trinucleotide repeat disorders (TRD) are a class of molecular diseases characterised by the presence of unstable and abnormal expansions of DNA triplets (trinucleotides). Generally, the larger the expansions, the more likely they are to cause disease or increase the severity of disease. This property results in the phenomenon of genetic anticipation, as is evident in TRDs, in which there is a tendency for age of onset to decrease and severity of symptoms to increase through successive generations of an affected family, due to the expansion of these repeats [Brower et al., 2009].
Large increases or expansions in the number of trinucleotide tandem repeats are involved in a number of inherited neurodegenerative conditions, which appear to be specific to the gender of the transmitting parent. For example, in Huntington's disease there is an increased number of CAG repeats, and this condition is preferentially paternally inherited [Kehoe et al., 1999]. Some neurological TRDs show a preferential maternal transmission, such as fragile X syndrome [Mandel et al., 2004], Friedreich ataxia [La Pean et al., 2008], spinocerebellar ataxia type and myotonic dystrophy [Botta et al., 2009]. There is currently no evidence for trinucleotide repeat expansions in AD.

Hypotheses

Previous neuroimaging studies have shown that the specific brain alterations observed in AD patients occur also at the stage of mild cognitive impairment due to AD and even in normal individuals with genetic risk factors for AD, such as ApoE ε4 carriers.

In particular, several MRI studies in mild cognitive impariment and at-risk individuals demonstrated the presence of reduced grey matter volume in cortical regions usually affected in AD patients, such as precuneus/posterior cingulate and parieto-temporal regions. The grey matter volume reduction in these AD-vulnerable regions has been associated with increased risk for developing AD among normal individuals [Chen et al., 2007; Chetelat et al., 2005; Jack et al., 2005; Reiman et al., 1998].

Another consistent AD feature is the reduction of cerebral metabolism involving the parieto-temporal, precuneus/posterior cingulate cortices, and medial temporal lobes. Such metabolic reductions within these regions have been demonstrated to occur years before AD symptom onset, even in normal individuals at genetic risk of developing AD and predict clinical cognitive decline in mild cognitive impairment patients [Jagust et al., 2006; Mosconi et al., 2009].

Finally, a cardinal neuropathological feature in AD is fibrillar Aβ deposition in senile plaques. There are several PET tracers with high affinity for Aβ senile plaques; among them the most known is Pittsburgh Compound B (PiB). High PiB retention in amyloid-rich regions has been observed not only in AD patients, but also in many patients with mild cognitive impairment, and seems to be associated with conversion from mild cognitive impairment to AD [Kemppainen et al., 2007; Reiman et al., 2009].

Our hypothesis was that the presence of AD-specific alterations observed using neuroimaging techniques could be present also in cognitively normal individuals with a maternal family history of AD, thus providing a biological endophenotypes related to maternally transmitted risk of AD.

In particular, this project examinated wheter normal individuals with an AD-affected mother showed brain changes similar to those observed in AD patients, such as:

- reduced grey matter volume in AD-specific regions;
- cerebral metabolic reductions in AD-vulnerable regions;
- increased cortical Aβ load.

Materials and Methods

Subjects

Seventy-five clinically and cognitively normal subjects were recruited both at Azienda Ospedialiero-Universitaria Careggi, Florence, italy, and at New York University School of Medicine, New York, USA. These included individuals interested in risk consultation and research participation, spouses, family members, and caregivers of patients.
All subjects received a standard diagnostic evaluation that included medical, neuropsychological, and MRI examinations. Institutional Review Board (IRB)-approved informed consent was obtained from all subjects. Individuals with medical conditions or history of conditions that may affect brain structure or function, i.e., stroke, diabetes, head trauma, any neurodegenerative diseases, depression, hydrocephalus, intracranial mass, and infarcts on MRI, and use of psychoactive medications were excluded.
Subjects had age < 45 years, education>13 years, Clinical Dementia Rating (CDR)=0, Global Deterioration Scale (GDS)<2, Modified Hachinski Ischemia Scale scores<4, and Mini Mental State Examination (MMSE)>28. All subjects had normal cognitive test performance relative to normative values on neuropsycological examination.
Subjects with FHm (i.e., only the mother was affected with AD), FHp (i.e., only the father was affected with AD), and FH- (i.e., negative FH) were included in the study.
Demographic characteristics of the subjects under study are shown in Table 4. Age, gender, education were included as covariates in all analyses, as described below.
There were no differences across FH groups for age, gender, education, MMSE scores and neuropsychological test performance.

Table 4. Subjects demographic and clinical characteristics.

	FH-	FHp	FHm
N	25	25	25
Age, years	65.5±8.9	62.9±12.1	63.0±7.8
Gender (male/female)	14/11	13/12	15/10
Education, years	16.4±2.2	16.9±2.0	17.2±1.6
MMSE	29.4±0.8	29.6±0.8	29.6±0.8
Values are mean ± SD.			

MRI acquisition and processing

All subjects received an MRI study on a 1.5 T MRI scanner. The scan was a 124 slice T1-weighted fast-gradientecho acquired in a sagittal orientation as 1.2-mm thick sections. These scans were used to rule out MRI evidence of hydrocephalus, intracranial mass, strokes, subcortical gray matter lacunes, nonspecific white matter disease, and focal white matter hyperintensities. MRI images were analyzed with voxel-based morphometry (VBM) using MATLAB 7.8 (the MathWorks, Natick, MA, USA) and Statistical Parametric Mapping (SPM8) (Wellcome Trust Centre for Neuroimaging, University College London). For all subjects, MRI images were spatially normalized to the SPM template in the stadardized anatomical space, which conforms to the Montreal Neurological Institute (MNI) space, by estimating the optimum (least squares) 12-parameter affine transformation, followed by 7x8x7 discrete cosine functions. A masking procedure was used to weight the normalization to brain rather than non-brain tissue. The spatially normalized MRI images were segmented into gray matter (GM), white matter, and cerebrospinal fluid images using SPM8's unified tissue segmentation procedure which rely on an iterative process to determine proportions of GM and white matter in each voxel, after removal of all non-brain voxels and application of image intensity nonuniformity correction. The GM images were retained for analyses and normalized to an a priori GM template by using the same normalization parameters described above. To preserve GMV within each voxel, the images were modulated by the Jacobian determinants derived from the spatial normalization, and then smoothed using an 8-mm full width at half maximum (FWHM) isotropic Gaussian kernel. GM, white matter, and cerebrospinal fluid segmentations for each image were used to calculate the total intracranial volume (TIV) for each

subject. The TIV was computed in cubic centimeters and examined examined as a covariate in subsequent analyses.

18F-FDG PET acquisition and processing

Each subject received a brain PET scan using 18F-FDG as the tracer. Subjects received 5–8 mCi of 18F-FDG intravenously while lying in a dimly lit room. Each subject's head was positioned by using two orthogonal laser beams and imaged with the scanner tilted 25° negative to the canthomeatal plane. PET images were obtained 30 min after injection and acquired over 20-minutes acquisition. Data were reconstructed by using filtered back-projection and corrected for attenuation, scatter, and radioactive decay, yielding a 128x128 matrix.
Image Analysis. Statistical parametric mapping (SPM5; Wellcome Department of Neurology, London, U.K.) was used for image analysis. 18F-FDG-PET images were spatially normalized to a standard FDG-PET brain template in the Montreal Neurological Institute space, which approximates the Talairach and Tournoux space. The spatial normalization process involves estimating the optimum least-squares 12-parameter affine transformation, followed by an iterative estimate of local alignment based on a family of 7x8x7 discrete cosine functions. The spatially normalized PET images were then resampled with a voxel size of 1.5x1.5x1.5 mm and smoothed with a 12-mm FWHM Gaussian filter. Only voxels with values>80% of the whole mean metabolic values were included in the analysis, and only clusters exceeding an extent threshold of 30 voxels (i.e., more than two times the FWHM) were considered significant. Global calculation was obtained with respect to the mean voxel value while accounting for global metabolism. Anatomical location of brain regions showing significant effects was described by using the Talairach and Tournoux coordinates using Talairach Daemon 12.0 after coordinates conversion from the Montreal Neurological Institute space to the Talairach space using linear transformation.

11C-PiB PET acquisition and processing

N-methyl[11C]2-(4′-methylaminophenyl)-6-hydroxy-benzothiazole (PiB) as the tracer. PiB was synthesized on site from the reaction of 6-OH-BTA-0 and [11C]methyl triflate. Radiochemical purity of the radioligand was >98%. Before PET imaging, an antecubital venous line was positioned for isotope injection. Subjects were rested with eyes open and ears unplugged in the quiet and dimly lit scan room. After injection of

15 mCi (~550 MBq) of PiB, subjects were positioned in the scanner using laser light beams for head alignment. Total scanning time was 90 min. All images were corrected for photon attenuation, scatter, and radioactive decay and were reconstructed into a 128x128 matrix spaced every 4.25 mm. Image processing and data analyses were performed at NYU. Summed PET images corresponding to the 60–90 min of PiB data were created for both data sets and were coregistered to T1-MRI using a surface-fitting algorithm implemented in MIDAS 1.9. Standardized uptake values (SUV) were determined on a voxel-wise basis. A cerebellar GM region was delineated on MRI and used as the reference to correct (normalize) for nonspecific tracer binding by dividing each voxel's SUV into the cerebellar SUV, yielding parametric PiB SUV ratio (SUVR) images. Parametric PiB SUVR images were processed using SPM5 and automated ROI. Briefly, subjects' MRIs were normalized spatially to a standardized MRI brain template image, which approximates the Talairach and Tournoux space, by estimating the optimum least-squares, 12-parameter affine transformation, followed by 7x8x7 cosine functions. These parameters were applied to MRI-coregistered PiB scans to generate spatially normalized PiB SUVR images. Automated ROIs were used to sample GM within AD-related brain regions, including the anterior putamen, IPL, LTL, medial frontal gyrus, PCC/precuneus, PFC, OCC, and thalamus. A cortical PiB retention mask (AD-mask) also was created by combining the cortical ROIs. Spatially normalized PiB images then were smoothed with a 12-mm Gaussian filter and were examined for voxel-wise effects acrossgroups. As a result of the cerebellar normalization of PiB data, no proportional scaling or grand mean scaling was performed. A GM mask was generated from the SPM5 a priori GM template by retaining voxels with values ≥0.8 (i.e., the probability of the voxel's contents beingGMwas≥80%) and was included as an explicit mask to perform group comparisons exclusively within GM voxels. Only clusters of 30 or more voxels were considered significant.

Statistical analysis

SPSS and SPM were used for data analyses. Differences in clinical and neuropsychological measures between FH groups were examined with chi-square tests and the general linear model (GLM) with post hoc least significant difference (LSD) tests, as appropriate. Results were considered significant at $p<0.05$.
For SPM analysis, the GLM/univariate analysis with post hoc t tests was used to test for GMV differences across FH groups. Additionally, analyses were repeated

correcting for other potential risk factors for AD, such as age, gender, education, by including these variables as nuisance variables (i.e., covariates) in the GLM.

Results

MRI

FHm subjects showed reduced GMV compared with both FH- and FHp (Table 5). As compared with FH-, FHm subjects showed significant GMV reductions in the inferior parietal cortex and precuneus of the left hemisphere, and in superior temporal cortex and inferior frontal gyrus of the right hemisphere ($p<0.05$, FWE-corrected) (Figure 3, Table 5).

As compared with FHp, FHm subjects showed reduced GMV in the left precuneus ($p<0.05$, FWE-corrected) (Figure 3, Table 5).

FHm had on average 15% reduced GMV compared with FH- (ranging from 13% in superior temporal to 16% in inferior frontal gyrus), and 15% reduced GMV in precuneus compared with FHp.

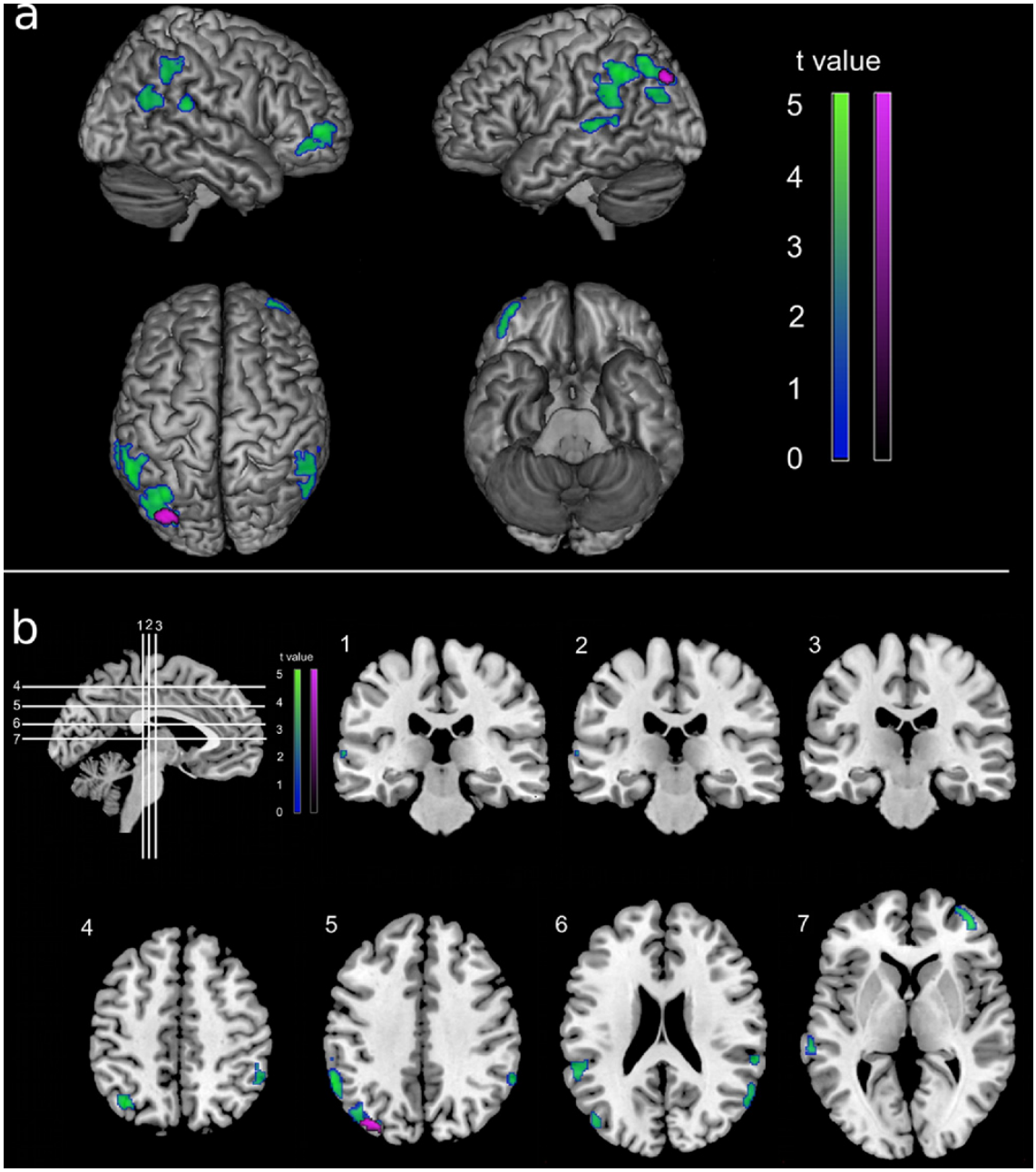
FHp subjects showed no regions of reduced GMV as compared with FH- and FHm.

No GMV decreases were found in FH- subjects compared with FHm or FHp subjects.

Table 5. Brain regions showing significant differences in gray matter volume between FH groups

Cluster extent	Coordinates*	Z value**	Functional area	Brodmann Area
Reduced gray matter volume in FHm compared to FH-				
4049	-57 -47 38	4.88	left Inferior Parietal Lobule	40
	-36 -60 47	4.76	left Inferior Parietal Lobule	7
	-40 -65 40	4.45	left Inferior Parietal Lobule	39
x	x	x	Left Precuneus	19
1175	40 50 -1	4.72	right Inferior Frontal Gyrus	10
	48 36 22	4.40	right Middle Frontal Gyrus	46
	36 56 -3	4.34	right Middle Frontal Gyrus	10
Reduced gray matter volume in FHm compared to FHp				
317	-34 -74 39	4.54	left Precuneus	19
* Coordinates (*x*, *y*, *z*) from the atlas of Talairach and Tournoux [17];				
**Z value at the peak of statistical significance, $P \leq 0.05$, FWE-corrected.				

Figure 3. Statistical parametric maps showing gray matter volume (GMV) reductions in cognitively normal individuals (NL) with maternal family history (FHm) compared with NL with negative family history (FH_) (in blue-green) and paternal family history (FHp) (in violet).



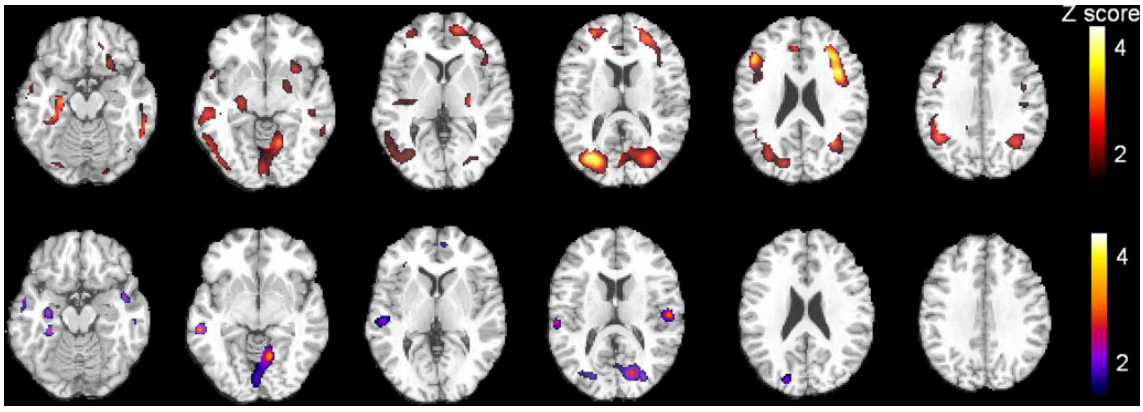
From Berti et al. Neurobiol Aging 2011

18F-FDG PET

As compared with FH-, FHm subjects showed brain metabolic reductions bilaterally in the inferior parietal lobe and middle temporal gyrus, and in the PCC/precuneus, superior frontal gyrus, and medial temporal lobe of the left hemisphere (P<0.05, SVC) (Table 6 and Figure 4). Metabolic reductions ranged from 8% in the left superior frontal gyrus to 22% in the left PCC.

As compared with the FHp group, the FHm group showed reduced cortical metabolism bilaterally in the middle and superior temporal gyrus, and in the left PCC/precuneus and medial temporal lobe (P<0.05, SVC) (Table 6 and Figure 4). Metabolic reductions ranged from 5% in the right middle temporal gyrus to 27% in the PCC/precuneus.

Figure 4. Statistical parametric maps showing CMRglc reductions in normal FHm subjects as compared with FH- (Upper) and FHp (Lower) subjects.



From Mosconi et al. PNAS

Table 6. Brain regions showing significant hypometabolism in subjects with maternal family history of AD as compared with the other groups

Cluster extent	Coordinates*	Z values	Functional area	BA
CMRglc reductions in FHm subjects as compared with FH- subjects				
1,056	_4, _70, 24	2.86	Precuneus	7
	_3, _67, 20	2.75	PCC	31
710	_43, _35, 39	2.69	Inferior parietal lobe	40
	_39, _56, 30	2.63	Inferior parietal lobe	40
	_61, _48, _4	2.60	Superior temporal gyrus	21
569	_28, _23, _8	2.70	Hippocampus	
	_20, _21, _12	2.65	Parahippocampal gyrus	35
	_21, _24, _8	2.35	Parahippocampal gyrus	28
357	52, _52, 32	3.23	Inferior parietal lobe	40
300	_23, 54, 4	2.75	Superior frontal gyrus	10
237	62, _43, _8	2.55	Superior temporal gyrus	21/37
	63, _46, _4	2.52	Middle temporal gyrus	21
171	_55, 2, _8	2.97	Middle temporal gyrus	21
CMRglc reductions in FHm subjects as compared with FHp subjects				
231	_25, _29, _8	3.08	Hippocampus	
	_19, _28, _8	2.39	Parahippocampal gyrus	28/35

676	_65, _16, 8	4.09	Middle temporal gyrus	22
	_64, _32, _4	3.38	Superior temporal gyrus	21
873	7, _61, 8	3.63	Cingulate gyrus	23/30
	4, _58, 12	3.49	Cingulate gyrus	23
510	64, _26, _8	3.40	Middle temporal gyrus	21
410	_4, _71, 24	2.69	Precuneus	7
	_3, _65, 21	2.55	PCC	31

11C-PiB PET

As compared to FH-, FHm subjects showed higher PiB retention in middle frontal gyrus, prefrontal cortex, inferior frontal gyrus, PCC/precuneus, anterior cingulate cortex (ACC), temporal, parietal, and occipital cortex, and fusiform gyri bilaterally, and in basal ganglia and thalamus of the right hemisphere.

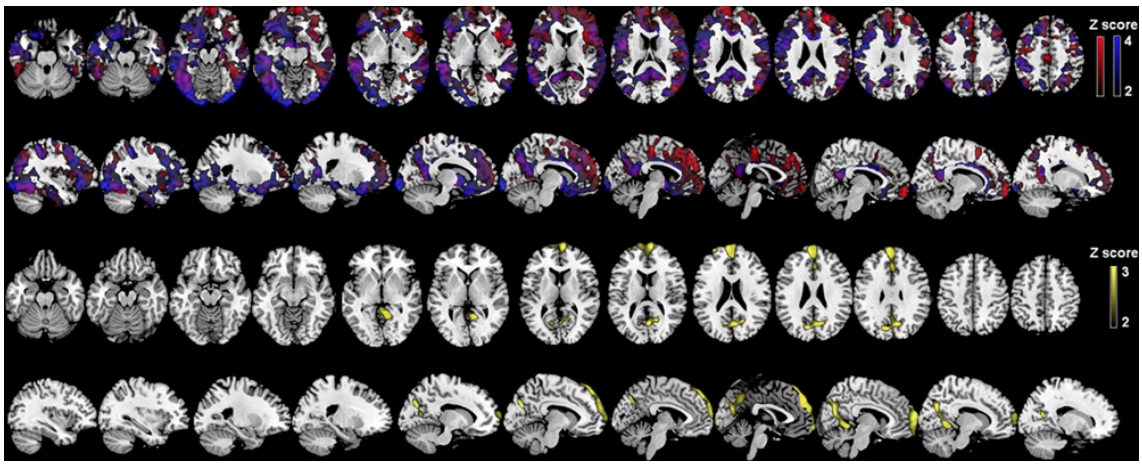
As compared to FHp, FHm subjects showed higher PiB retention in the bilateral PCC/precuneus, inferior parietal lobe, prefrontal cortex, ACC, and in the left temporal, occipital, middle frontal gyrus, and inferior frontal gyrus (Figure 5).

The FHp group showed higher PiB retention than the FH- group in the middle frontal gyrus and PCC/precuneus, mostly of the left hemisphere.

No brain regions showed significantly higher PiB retention in the FH- group as compared with the FHm or FHp groups or in the FHp group as compared with the FHm group.

Overall, PiB retention was higher in all brain regions in the FHm group, than in the FHp and FH- groups.

Figure 5. SPMs showing higher PiB retention in FHm subjects than in FH– and FHp subjects (Top Two Rows) and in FHp subjects than in FH– subjects (Bottom Two Rows).



From Mosconi et al. PNAS 2010

Discussion

The present neuroimaging study demonstrated the presence of cerebral changes, which are tipically observed in AD patients, in adult children of parents affected with AD. Specifically, cognitively normal individuals with AD maternal family history consistently showed brain alterations, such as brain atrophy, cortical hypometabolism in AD-vulnerable regions and increased Aβ accumulation as compared with subjects of similar demographic characteristics with paternal family history and negative family history for AD.

Previous studies have already shown that precuneus/posterior cingulate and parieto-temporal regions are affected in AD patients compared with controls. AD patients demonstrated variable degree of cortical atrophy involving precuneus/posterior cingulate cortex, medial temporal lobes and parieto-temporal regions [Convit et al., 2000; Hirata et al., 2005]. Another main characteristic of AD patients is the reduction of cerebral metabolism in the same specific brain areas, such as precuneus and posterior cingulate [Minoshima et al., 1997], parieto-temporal cortex [Friedland et al., 1983] and medial temporal cortices [Mosconi et al., 2006], and in advanced stages of the disease in the frontal lobe [Foster et al., 1984]. Finally, high 11C-PiB retention in amyloid-rich regions is observed consistently in AD patients, involving extensively cortical and subcortical areas [Klunk et al., 2004].

Our findings in FHm subjects are consistent with the presence of AD-typical brain changes, as shown by MRI, 18F-FDG and 11C-PiB PET, observed in presymptomatic individuals carrying genetic mutations responsible for early onset familial AD, as well as normal subjects carriers of the ApoE-ε4 allele, a major genetic risk factor for AD [Reiman et al., 1998; Reiman et al., 1996; Reiman et al., 2009].

Atrophy and hypometabolism in AD-vulnerable regions and cerebral accumulation of fibrillar amyloid also accurately predict decline from mild cognitive impairment to AD, as recently confirmed in several neuroimaging studies [Chetelat et al., 2005; Mosconi et al., 2009; Okello et al., 2009].

Togheter with such previous findings, our results of AD-typical brain changes in cognitively normal FHm individuals compared with FH- and FHp suggest that having a mother affected by AD may be associated with increased risk for preclinical brain volume loss, preclinical brain metabolic impairment and preclinical accumulation of

fibrillar Aβ; they may be associated also to an increased risk for developing AD, compared with having a father affected with AD.
Moreover, according to our data, FHm subjects appear to be at a more advanced stage of both brain amyloidosis, metabolic impairment and cortical atrophy as compared to FHp and FH- individuals.
Collectively, our MRI, 18F-FDG and 11C-PiB results demonstrated that FHm individuals have significant cortical atrophy, metabolic reductions and increased Aβ load in AD regions as compared to FH- subjects, whereas FHp individuals of the same age range have some Aβ deposits in the absence of hypometabolism and atrophy. These data could suggest that amyloidosis may precede neuronal dysfunction in FHp subjects. On the other hand, the presence of both Aβ pathology, hypometabolism and atrophy in middle-aged to old normal FHm subjects indicates that these brain abnormalities and thus the process leading to neuronal dysfunction and subsequent loss may have developed at younger ages in these subjects; it is also relatively independent from other possible risk factors for AD such as age, gender, education, and ApoE status..
Longitudinal studies are needed to determine wheter the observed brain abnormalities in normal FHm subjects are predictive of future cognitive impairment in these individuals.

Conclusion

In conclusion, during this project we observed using neuroimaging techniques the presence of AD-specific brain abnormalities in cognitively normal individuals with a maternal family history of AD, thus providing a biological endophenotypes related to maternally transmitted risk of AD.

In particular, our data demonstrated that normal individuals with an AD-affected mother showed brain changes similar to those observed in AD patients, such as:

- reduced grey matter volume in AD-vulnerable regions;
- cerebral metabolic reductions in AD-vulnerable regions;
- increased cortical Aβ load.

These findings may indicate that cognitively normal FHm subjects may be a group of individuals at particularly high risk for AD by virtue of developing brain hypometabolism, atrophy, and amyloid-beta pathology prior to their age-matched peers with different family histories.

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