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Review

New molecular approaches to Alzheimer's disease☆

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ABSTRACT

Alzheimer's disease is a neurodegenerative disorder and the most common and devastating form of dementia. It affects mainly older people, accounting for 50–80% of dementia cases. The age is the main associated risk factor and based on the onset age, early-onset (EOAD) or late-onset (LOAD) forms are distinguished. AD has a strong impact both on the life-style of patients and their families and on the society, due to the high costs related to social and medical care. So far, despite the great advances in understanding of the AD pathogenesis, there is no a cure for this form of dementia and current available treatments are limited to temporarily relieve symptoms.

In this review, firstly we give an overview of the current knowledge of the genetic basis of both forms of AD with a particular emphasis on the insights in the understanding of the pathogenic mechanisms of this disorder. Then we discuss the promising relevance of "omics sciences" and the open challenges of the application of Big Data in promoting precision medicine for AD.

1. General introduction

Alzheimer's disease (AD; OMIM #104300) is a neurodegenerative disorder and it is the most common and devastating form of dementia [1]. It affects mainly older people, accounting for 50–80% of dementia cases [2]. Today AD is recognized by the World Health Organization (WHO) as a primary issue for the public health all over the world. Indeed, it is estimated that so far AD affects nearly 50 million people worldwide and the number of affected patients is expected to triple in the next 30 years, due to the increase of number of older adults. In US 1 in 10 people over age 65 and 1 in 3 people over age 85 is affected with AD [3]. The percentage of deaths resulting from AD is higher (71%) than deaths from other pathologies, as stroke (23%) or prostate cancer (11%) [4].

From a clinical point of view, AD is characterized by a prodromal phase with a subsequent progressive decline of cognitive functions and loss of memory, leading to need of continuous medical care and ultimately complete dependency of patient [5]. Therefore AD has a strong impact both on the life-style of patients and their families and on the society, due to the high costs related to social and medical care [3]. So far, despite the great advances in understanding of the AD pathogenesis, there is no a cure for this form of dementia and current available treatments are limited to temporarily relieve symptoms [6]. The median survival time of patients from disease onset is < 10 years [7].

Neuroimaging examination can reveal the hippocampal and cortical atrophy in the AD brain. In particular the main neuropathologic hallmarks are intra-neuronal accumulation of neurofibrillary tangles (NFTs) of hyperphosphorylated tau protein and extracellular depositions of amyloid plaques of amyloid-β (Aβ) peptide, often surrounded by dystrophic neurites [8]. Moreover AD brain is also characterized by loss of synapse and neurons and reactive gliosis [9]. The pathological brain changes may begin 2–3 decade before the onset of the clinical symptoms [6].

The age is the main associated risk factor and the age of 65 years is used to distinguished AD patients with an early-onset (EOAD) or late-onset (LOAD) form [10]. The prevalence of EOAD is much lower than LOAD. Indeed, of all AD patients, 5% show cognitive impairment before age 65 [11,12]. Some studies described the presence of typical AD signs in a larger area of the brain and outside the medial temporal lobe in younger patients. However, the pathological features are similar in EOAD and LOAD, suggesting that at the end-stage of disease there are no other criteria to distinguish the two AD forms than the onset age [13].

From genetic point of view, AD is a complex disease with a multifactorial aetiology. Familial forms are present in < 1% of AD cases and the heritability is estimated about 60–80% [14].

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Currently associated risk factors are, for example, age, sex, cardiovascular and metabolic risk factors and family history [2,4].

In this review, firstly we give an overview of the current knowledge of the genetic basis of both forms of AD with a particular emphasis on the insights in the understanding of the pathogenic mechanism of this disorder. Then we discuss the promising relevance of "omics sciences" and the open challenges of the application of Big Data in promoting precision medicine for AD.

2. Genetic basis of EOAD: whole-genome linkage studies

Only 30–60% of EOAD cases have a positive family history for dementia and about 10–14% of familial cases present an autosomal dominant inheritance. Therefore classical mendelian AD forms are very rare, with an estimated prevalence < 1% [15,16]. Moreover, in addition to the autosomal dominant transmission, a considerable number of EOAD patients present a sporadic and genetically complex form of disease [3].

In the 1990s genetic analysis of informative and large autosomal dominant AD pedigrees allowed the identification of high-penetrant mutations in three genes: *APP* [17,18], *PSEN1* [19–22] and *PSEN2* [22–24]. Mutations in these genes are responsible of 5–10% of EOAD cases [25,26].

In particular, whole-genome linkage studies identified the first causative genes in AD patients and families, encoding for the amyloid β precursor protein (*APP*) and localized on chromosome 21q21.2-21q21.3 [27]. In the amyloidogenic pathway, amyloid β precursor protein is proteolytically processed by β- and γ-secretase in Aβ peptide and it is reported that pathogenic mutations on *APP* alter the length and the production of Aβ peptide, leading to an abnormal self-aggregation [28].

So far, 52 causative mutations in APP have been identified in 119 autosomal dominant AD cases [29]. Most of these pathogenic heterozygous variants are missense and 25 APP genomic duplications have been identified in autosomal dominant families. However, also recessive mutations, respectively a one amino acid deletion (p.E693D) and a missense mutation (p.A673V), have been associated to EOAD with dominant negative effect on amyloidogenesis [30].

Interestingly, the same variant p.A673V in heterozygous state and a different amino acid change at the same site (p.A673T), described in the Icelandic population, showed a reduction of APP cleavage *in vitro*, reducing AD risk and suggesting a protective effect [31,32].

APP mutations are associated with a disease onset between 45 and 60 years. However, while the missense mutations present a near-complete penetrance, the identified APP genomic duplications are associated with an incomplete penetrance and higher variability in the disease age [33].

Segregation studies in EOAD families negative for APP mutation identified new loci associated to EOAD on chromosome 14q24.3 and 1q31-q42, specifically *PSEN1* and *PSEN2* genes [23,34]. They are homologous genes, encoding for proteins of the γ-secretase complex. Mutations on these genes impair the cleavage of APP, leading to an increase production of Aβ peptide and supporting the hypothesis of the involvement of amyloidogenic pathways in EOAD pathogenesis [35,36]. Mutations on PSEN genes are different types of heterozygous variants, including missense, small indels and a genomic deletion specifically on *PSEN1*. *PSEN1* is the most frequently mutated genes of all three associated EOAD genes, with 215 mutations identified in 475 autosomal dominant cases [29]. *De novo* mutations in *PSEN1* have been described in probands with an early disease onset (28 years) [37,38]. *PSEN1* mutations are associated with a more severe phenotype, a complete penetrance and an onset age between 30 and 50 years while in probands carrying *PSEN2* mutations the onset age is more variable (39–83 years) and the penetrance is incomplete [1].

To summarize, the mutation frequency for *APP* gene is < 1%, 6% and 1% respectively for *PSEN1* and *PSEN2,* meaning that a large amount of EOAD cases remain genetically unsolved [39]. For sure, phenotypic heterogeneity complicates the genetic studies but it is plausible to continue efforts in order to identify other causative genes or modifier genetic factors involved into the EOAD pathogenesis.

3. Genetic basis of LOAD: twin-studies and GWAS

From a genetic point of view, LOAD is a complex disorder with a multifactorial aetiology. Therefore, it is not possible to define a single model of inheritance and genetic mutations or modulators may interact with environmental factors.

Twin studies estimated the heritability of LOAD about 58–79% [40]. In 1990's *APOE* gene has been associated with a major genetic risk factor for this form of AD. It encodes a polymorphic lipid carrier (ApoE) that is expressed in nervous system, liver, monocytes and macrophages [41]. It is responsible for cholesterol transport and it is involved in the neuronal growth, nerve tissue injury repair, immunoregulation and nerve regeneration [1]. *APOE* gene presents three different haplotypes (ε2, ε3, ε4) that differ in site 112 and 158 of the amino acid sequence and encode for three different isoforms (ApoE2, ApoE3, ApoE4) [42]. In particular, the APOE ε4 allele increases risk for AD in familial and sporadic cases with a threefold effect for heterozygous patients and 15-fold for homozygous carriers in a dose dependent manner [42]. In Caucasian population, AD patients carrying homozygous ε4 allele show an higher risk than the homozygous ε3 carriers [43]. On the other hand, ε2 allele is thought to be protective against AD, also delaying onset age [44]. Moreover, the presence of at least one ε4 allele also increases risk for EOAD patients with a positive family history [45].

One hypothesis of the pathogenic role of ApoE4 in AD is related to the reduction of cholesterol transport and of the efficiency of the Aβ clearance, leading to Aβ aggregation, increasing neuronal atrophy and synaptic activity [46]. Furthermore, recent studies have demonstrated also an involvement of ApoE4 in tau pathogenesis and tau-mediated neurodegeneration, independently of amyloid-β pathology [47]. For sure, so far ApoE4 has a well-established effect in AD risk and it is the strongest genetic risk factor, even if it accounts only for 27.3% of the disease heritability of LOAD [48].

So, in order to highlight other susceptibility loci underlying the part of heritability still unexplained, large-scale collaborative GWAS studies has been performed. GWAS is a powerful approach of study that allows to identify common variants in genes likely involved in a complex disease [49]. More than 30 additional AD-associated risk loci have been identified and specifically they encode proteins involved in immune response and inflammation(*CLU, CR1, ABCA7, MS4A, CD33, EPHA1, MEF2C, HLA-DRB1/DRB5, TRIP4,TREM2*), lipid metabolism (*CLU, ABCA7,SORL1*), synapse activity (*PICALM, BIN1, CD33, CD2AP, EPHA1, INPP5D, PTK2B, SORL1,SLC2A4*), Aβ metabolism (APP, CLU, CR1, ABCA7, INPP5D, SORL1), and tau pathology (BIN1) [50]. A list of published GWAS studies on LOAD has been created in the NHGRI-EBI catalog [51].

Therefore, a number of potential risk genomic loci have been identified but it is evident that for some variants there is a strong association data, replicated by several GWAS or meta-analysis, while other genetic loci are described in only one single study and further evidences are necessary to demonstrate the likely aetiological role. However, the real advantage of these studies is that the obtained data have shed light upon the principal pathways involved in the AD aetiology, allowing to hypothesize pathogenic mechanisms underlying the disease. In particular, it becomes evident that, besides the involvement of amyloid cascade or tau pathology, lipid metabolism, innate immunity and inflammatory response, endocytosis and vesicle tracking are the other main involved pathways [49]. Other more recent identified categories are related to the regulation of cell cycle (*RANBP2*), oxidative response (*MEF2C*) and axon guidance (*UNC5C*) [3].

However so far there is no a clear genotype-phenotype correlation for these risk candidate genes, considering for example the onset age,

the severity of the disease or the clinical features [52].

Being AD a complex disorder, also the interaction of different genes cannot be excluded as underlying pathogenic mechanisms but further studies are necessary to validate this hypothesis [3].

However, despite efforts on GWAS studies, the identified candidate risk loci account only for a small fraction of LOAD heritability and the majority of AD sporadic cases remains largely unexplained [6]. One possible explanation could be that GWAS approach is for sure a good method to identify risk loci, but it is not suitable for the detection of rare genetic variants.

In this field an important contribution could come from high-throughput sequencing [53], that will be discuss in the next paragraph.

4. Study of AD genetic basis exploiting next generation sequencing

So far, the main genes associated with EOAD are *APP*, *PSEN1* and *PSEN2*, that account only 5–10% of cases, leaving a large fraction of familial cases genetically unsolved [26]. Also the current knowledge of the LOAD genetic risk factors presents several limitations [3], suggesting that other causative genes are still to be identified.

In this context, the powerful technique of next generation sequencing can give a great contribution in gaining new insights in the study of genetic basis of AD, exploiting different approaches of study, like the sequencing of the entire genome (whole-genome sequencing, WGS) or exome (whole-exome sequencing, WES). In particular, it allows to investigate small families genetically unsolved or groups of unrelated AD patients with an extremely severe phenotype.

One of the first WES study on a genetically undiagnosed EOAD family identified a mutation in *NOTCH3* gene, previously associated with a form of dementia, with different clinical features overlapping the EOAD [54].

Furthermore, seven rare mutations in *SORL1* gene have been identified in 29 probands in a WES study [55]. In AD patients a reduction of the expression of SORL1 transcript has been demonstrated using a microarray approach, suggesting its involvement in the AD pathogenesis due to its function as neuronal apolipoprotein E receptor and the impairment of Aβ metabolism [56,57]. Both common and rare variants on *SORL1* have been associated with LOAD risk, suggesting that the screening of this gene in case-control studies can contribute to clarify also the role of rare variants in LOAD [58].

More recent NGS analysis of LOAD cohorts allowed the identification of other low frequency variants in novel genes, such as *TREM2*, encoding for triggering receptor expressed on myeloid cells 2. In particular, the rare variant R47H on this gene is associated with an increased AD risk in the European population. This result hasn't been replicated in Asian population, showing that specific rare risk variants may be population-specific [1]. Other identified genes with rare LOAD associated variants are *PLD3*, *UNC5C*, *AKAP9* and *ADAM10* [59–61].

For sure, numerous large-scale NGS studies on AD are ongoing and further gene discoveries will help to fill the current gap of our knowledge of genetics of this disorder.

5. Other “omics” approaches in AD

Genomic studies are crucial for the identification of novel genes associated with AD. However also other “omic” approaches can be exploited to shed light on new insights in the disease aetiology.

For example, epigenomic studies on AD have been recently published. “Epigenomics” is the analysis of DNA methylation or histone modification, that can influence the gene expression and that can be altered in pathological conditions. Since the moderate concordance of disease among twins, there is the idea that also non-hereditable genetic changes, or epigenetic modifications, that accumulate over time, may influence the occurrence of AD.

Studies on DNA methylation highlighted that several AD risk genes are methylated in promoter regions and new loci with differential methylation have been identified, such as ANK1, RPL13, RHBDF2, suggesting a possible association with AD susceptibility [62,63]. On the other hand, studies on histone acetylation gave discordant results, that have to be further validated. It can be also due to the cell-type specific modifications associated with AD [64].

Therefore, further studies on the single-cell DNA methylation profiling are necessary to investigate how epigenetic mechanisms can influence genome accessibility and affect the gene transcription, in order to better clarify the AD pathogenic mechanisms.

Moreover, the most recent NGS technologies have been used for the study of the entire set of RNA transcripts, also called “Transcriptomics”, allowing the identification of alternative splicing, non-coding RNA or novel transcripts. Specifically, in AD several studies have been performed on the whole genome miRNA profile, that is the analysis of small non-coding RNAs with a regulatory function on the post-transcriptional gene expression and responsible for an abnormal expression of specific genes in pathological conditions. Several miRNA associated with inflammation and AD pathogenesis have been identified *post-mortem* in brain tissue but these findings have to be validated also in other biological fluids in order to evaluate its utility as possible biomarker [65]. Indeed, it is possible to investigate miRNAs with high accuracy in blood cells, plasma or cerebrospinal fluid (CSF), suggesting promising application in non-invasive diagnosis. Since so far different sets of miRNAs have been associated with AD with conflicting results, further analysis and replication are necessary on a large number of patients and controls but the potential translational impact is evident.

In order to identify new useful biomarkers in AD, also proteomic approach has been exploited. “Proteomics” allows the analysis of the entire set of proteins produced in an organism and the development of mass spectrometry-based proteomic technologies led great advantages in this field. In plasma, CSF or brain tissue of AD patients, protein levels have been investigated using this technique, highlighting new possible biomarkers related to Aβ-peptide metabolism pathway or neuronal adhesion and suggesting their direct involvement in the AD pathogenesis [66–68]. However, also in proteomic studies obtained findings are discordant, likely due to technical limitations in experimental process, the great inter-individual variability in protein levels or to the big variability in the clinical phenotype. So a validation in larger and independent cohorts of AD patients is needed [69]. Moreover, due to the complexity of AD pathogenic mechanisms, we cannot exclude the involvement of possible protein-protein interactions underlying the phenotypic heterogeneity. Therefore, it is necessary to evaluated changes in a panel of proteins rather than in one single protein, assessing also a possible phenotype correlation and leading to a classification of subgroups of patients for targeted therapies [69–71].

Lastly, the newest developed approach is the “metabolomics”, that is the identification of metabolites involved in several biological processes and their quantification in a biological specimen. Levels of metabolites, such as sphingomyelins and glycerophospholipids, have been found significantly increased in CSF of AD patients [72]. Interestingly other studies assessed a correlation between metabolic changes and the presence of clinical features, identifying a panel of metabolites, that belong to important metabolic pathways regulating oxidative stress, inflammation and nitric oxide bioavailability, and that are associated with the age-related memory impairment [73].

6. How to translate genetic findings in clinical management of AD patients

Today new insights into the knowledge of AD pathogenesis have been achieved and now the main challenge is their translation into the clinical practice (Fig. 1).

For sure the first applications are the genetic diagnostic and predictive testing for the known mutations in the three main genes (*APP*, *PSEN1* and *PSEN2*) associated with the familial EOAD cases. Even if

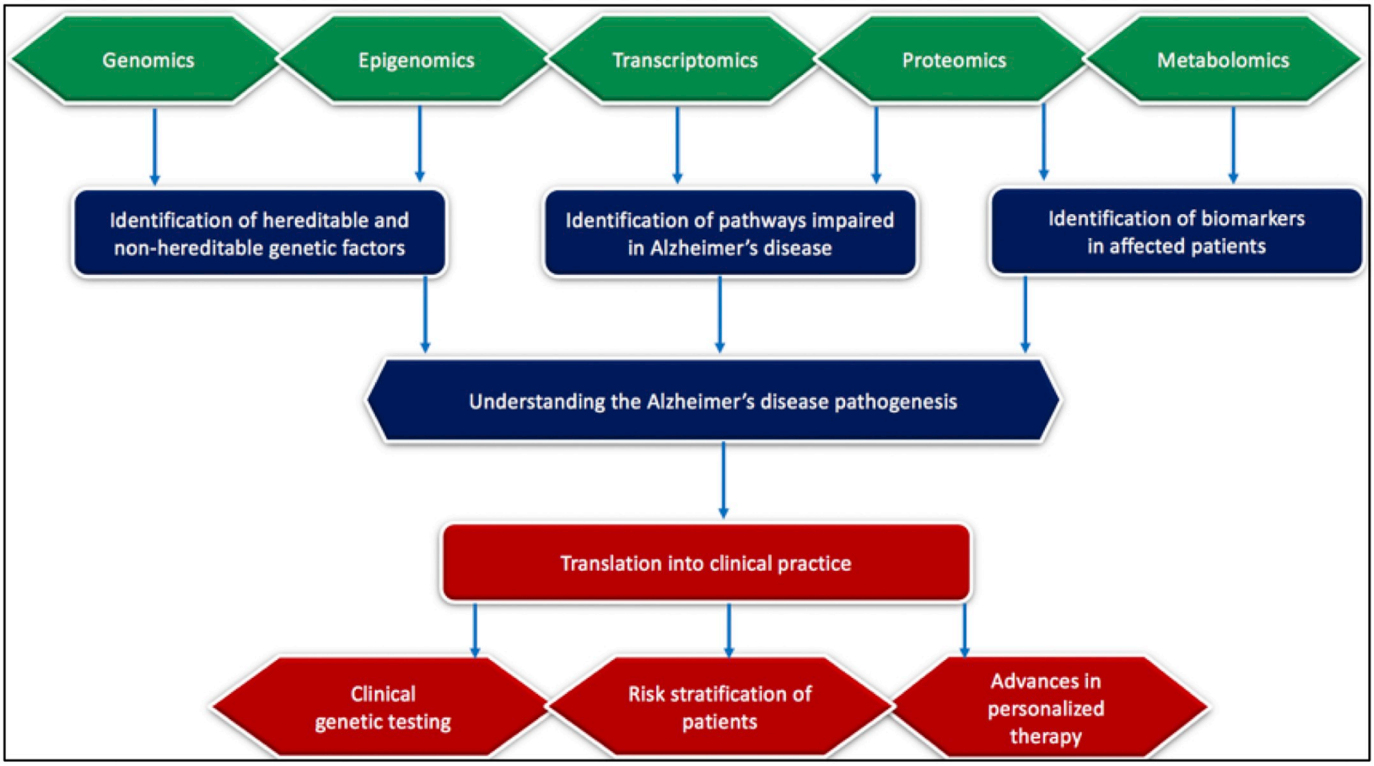

Fig. 1. The investigation of molecular basis of AD in the omics era. The figure summarizes the main goals of each omics approach applied to AD studies (genomics, epigenomics, transcriptomics, proteomics and metabolomics) and the principal applications of the achieved results into the clinical practice, as explained in the text.

they account only a small fraction of patients affected by an autosomal dominant form, the detection of a causal mutation segregating in an affected family allows to provide important advantages in the early identification of at-risk individuals. However, due to the complicated genetic basis of AD, only few clinicians are prepared to manage and discuss the genetic data with their patients. So, the American College of Medical Genetics and the National Society of Genetic Counselors provided practice guidelines in order to evaluate which patients may benefit from genetic testing [74]. Indeed, it is important to take into account the limited predictive value of the positive genetic test in a carrying asymptomatic individual, given the incomplete penetrance and phenotypic variability in AD [75].

In the "Omics era" the advent of high-throughput sequencing has brought the great advantage of analyzing multiple panel of genes in the same testing. It is particularly relevant in a genetically heterogenous disorder [76], such as AD. Indeed it allows to investigate at the same time not only the principal causative genes but also low-frequency mutated genes, previously reported in pedigrees with clinical symptoms of AD [77,78]. NGS testing is a fast and cost-effectiveness approach in clinical setting but on the other hand it presents disadvantages related to the complexity of genetic data interpretation and new ethical issues. These aspects depend on the possible detection of variants of unknown significance, whose clinical interpretation is complex, and of incidental findings, whose clinical management is still debated [78–80]. Therefore, new complete counselling guidelines are needed to improve the application of genetic testing in autosomal dominant AD cases. On the other hand, the clinical testing for common variants identified in GWAS is not recommended and it has a limited relevance, because these variations are not disease causal mutations, but they confer only a small relative risk of developing AD. So, there is no a relevant clinical utility for patient management [78].

As discussed above, Omics studies allow to clarify the molecular mechanisms underlying AD pathogenesis, exploiting epigenomic or transcriptomic approach, or to identify novel biomarkers, in proteomic or metabolomic projects, useful for the early diagnosis or for predicting disease progression 80.

Moreover, Omics results may be useful to open new frontiers in the personalized therapy. Indeed so far, the only available treatment is aimed at treating symptoms but not the cause of disease or its progression [81]. Moreover, despite undiscussed progress in the knowledge of AD pathogenesis, in recent years > 400 drug trials have been performed but unfortunately failed [81]. It may be due to the heterogeneity of the recruited groups of patients in phase III clinical trials or to the too advanced stage of disease at the time of treatment [82].

Advances in computational and bioinformatic tools opened the new era of the "Big Data", that are large data sets containing large amount of clinical information, diagnostic test results, medication history or lifestyle factors of healthy individuals or affected patients.

In the context of autosomal dominant forms of AD, a huge international multicenter registry has been created by the Dominantly Inherited Alzheimer Network (DIAN), comprising cognitive, biomarkers and brain imaging information, clinical and genetic data of individuals, carriers or non-carriers of a mutation in the principal causal genes (*APP*, *PSEN1* and *PSEN2*), with a positive family history for AD. Under the assumption that the treatment efficacy is higher in a pre-symptomatic stage, DIAN launched the first prevention trial for at-risk families with the autosomal dominant form of AD. The main goal is the identification of drugs that could potentially change the course of the disease. Even if results from DIAN will bring benefit only to the small portion of patients carrying the mendelian mutations, it represents a big effort in the use of large-scale data sets in order to identify at risk subject and to optimized the pharmacological treatment [83].

7. Conclusion

So far, great forward steps have been made in the knowledge of the molecular basis of different forms of AD. Moreover, the recent advent of "omics" has led to a significant progress in the study of complex

disorders, such as AD. In particular the great opportunity of integrating massive data obtained by omics approaches, such as genomic sequencing, epigenomic and transcriptomic studies, as well as metabolomics, offers the possibility of shedding light in the knowledge of pathways implicated in AD pathogenesis. For sure, there are still many gaps to fill, but however there is the optimistic idea that this approach may be useful for the advance of precision medicine, allowing a melioration in prevention, diagnosis and treatments of AD.

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