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TOWARDS PERSONALIZED THERAPIES FOR ALZHEIMER'S DISEASE: CHALLENGES FOR TRANSLATING MOLECULAR GENETICS AND SYSTEMS BIOLOGY INTO STRATEGIES TO PREVENT COMPLEX CHRONIC BRAIN DISORDERS

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ABSTRACT

The challenges for deciphering the etiologies and/or developing an intervention for the prevention of late-life complex chronic brain disorders, such as Alzheimer's disease (AD), stem from their (I) polygenic etiologies, (II) heterogeneity biological-behavioural signs and (III) prolonged degenerative processes beginning decadesbefore the onset of detectable symptoms. The crucial challenges for developing individualized therapies for AD dementia require the discovery, development, and validation of innovative technologies and complex algorithms, with a high degree of accuracy and/or prognostic certainty, for early detection of asymptomatic individuals in thegeneral population. The aim of this perspective is to discuss the merging evidence on a broad spectrum of putativerisk factors and outline the need for novel conceptual models or approaches regarding (I) research on thecomplexities of genetic-epigenetic-life style interactions in the expression of polygenic brain disorders, (II) theapplication of the systems biology framework to understand the emergence of AD and other related chronic braindisorders. Large population-scale analyses began to reveal an increasing number of genetic mutations andpolymorphisms, as well as a spectrum of putative epigenetic and lifestyle risk factors, which potentially play a central role in the expression of not only the rare inherited form of the early-onset familial type of AD but also the more prevalent late-onset sporadic form of the disease. A current working hypothesis speculates that the distinct endophenotypes of the sporadic form reflect the cascades of anomalous changes in molecular signalling

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pathways resulting from complex relations among several putative risks, e.g., gene-gene interactions, along with modulating influences of lifestyle, education, and the environment. The application of systems biology – using genomics and other omics sciences, such as metabolomics – along with advancing knowledge on genetic/epigenetic factors to test such hypothesis, is expected to yield new insights into the genesis and downstream pathophysiological mechanisms of AD and other complex brain disorders, therefore supporting the introduction of effective therapeutic strategies.

KEYWORDS: *Alzheimer's disease, brain disorder, deterioration, depression, Human Genome Project*

INTRODUCTION

Alzheimer's disease (AD) is an irreversible, progressive brain disorder that slowly destroys specific neural systems with the deterioration of some motor and brain functions. Symptoms include cognitive decline, behavioural problems including depression, delusions, agitation, and daily activities and self-care decline. The sporadic form of the disease, sporadic AD (sAD), is characterized by late onset, typically above 65 years of age, and accounts for about 95% of all cases.

The aetiology of this brain disorder is not entirely understood. However, growing evidence supports that the classic cluster of symptoms traditionally associated with the disease is not due to a single causative factor. Rather, it is the outcome of a complex combination of factors such as family history, genetics, comorbid conditions, and lifestyle. In addition, a growing body of evidence indicates that vascular risk factors for atherosclerosis, stroke and cardiac disease are significantly related to AD risk. These findings imply a central role in cerebrovascular dysfunction, which may promote neurodegenerative processes and precede the onset of sAD.

The relative importance of these putative risk factors in increasing the jeopardy or affecting the expression of the disease differs from individual to individual; progress in understanding the biology of such polygenic complex brain diseases leads to new debates about the clinical-biological phenotypes characterizing the disease. In general, it is widely known that:

(I) AD is highly heterogeneous regarding the onset of symptoms, patterns or a mix of clinical features, neuropathology, risk factors, genetics, biomarkers, and response to treatment.

(II) Gradual molecular alterations in the brain leading to the disease seem to begin decades before any symptoms can be detected.

(III) Genetic susceptibility and mutations are the most productive and promising (and also the most challenging) areas of AD exploration. In this regard, the Human Genome Project, following substantial technological developments, has led to a wealth of information on the genetics/epigenetics of AD (1,2).

There are promising prospects that advances in genetics/epigenetics and molecular biology will provide the critical breakthroughs needed to understand the disease pathophysiology and the knowledge required to develop effective therapies for AD and other chronic brain disorders.

The growing knowledge about the polygenic nature and the heterogeneity of AD requires a critical look at the role and influence of genetic and epigenetic factors. Such a careful reassessment of the genetics/epigenetics of complex brain disorders need to account for the distinct biological phenotypes and the heterogeneous behavioural-clinical phenotypes of AD. For instance, the challenge of discovering the causal relationships between various genetic mutations and the cascade of downstream biological (biochemical) events, including the expression of clinical phenotypes, needs to account for some unique features of the disease. From this standpoint, there are crucial aspects that need to be addressed, including:

(I) The means of translating accurate genetic and/or molecular information about a disease into effective treatments; for instance, the genetics of Huntington's disease has been well mapped; however, that knowledge has been difficult to translate into interventional approaches.

(II) Elucidating: (a) the specific mechanisms allowing various genetic mutations or susceptibility genes to explain the neuroanatomical specificity of the neurodegenerative processes and lesions as well as (b) the neurobiological mechanisms for the increased susceptibility of some neural structures for degeneration.

(III) Describing the specific combination of genetic/epigenetic factors that: (a) determine or affect the beginning and the rate of progression of the degenerative process and (b) account for the heterogeneity of clinical and neuropathologic features of AD.

(IV) The knowledge about the genetics of AD is based mainly on associations studies; in this regard, there is the need to develop the resources and technical capabilities for prospective validation of all putative genetic and/or epigenetic risk factors as prognostic tools for predicting the probability of disease onset.

(V) Evidence indicates that the disease's pathophysiological mechanisms begin decades before noticeable behavioural signs appear. Therefore, one of the crucial challenges for AD and other chronic brain disorders is to discover and, possibly, validate the optimal clusters of genetic/epigenetic risk factors for the accurate detection (and prediction) of individuals with an elevated risk of developing the disease in the pre-clinical asymptomatic stages of neurodegeneration.

(VI) The task for personalized interventions for at-risk individuals in the disease's asymptomatic phase is to discover how AD progresses from pre-clinical (asymptomatic) stages to the first expression of symptoms and beyond. The best opportunity to develop strategies for early detection is to scrutinize and map out, in longitudinal studies, the patterns of modifications in various biological indices in individuals carrying AD mutations. Several longitudinal studies, especially clinical trials, have recently been conducted to provide helpful information for further investigations. A relevant example is represented by the Dominantly Inherited Alzheimer's Network (DIAN), consisting of a long-term observational study of individuals dominantly carrying inherited mutations causing AD (3). Another important study is the Alzheimer's Prevention Initiative (API), a therapeutic prevention trial conducted in cognitively healthy asymptomatic individuals belonging to a large kindred from the Colombian state of Antioquia who carry the inheritable "Paisa" *PSEN1* E280A mutation (https://www.alzforum.org/mutations/psen1-e280a-paisa), the most frequent cause of early-onset familial AD (EOFAD) (4,5). Another interesting prototype for the longitudinal study of disease progression is represented by Down Syndrome (DS). Interestingly, patients with DS carry three copies of the amyloid precursor protein (*APP*) gene and are also at risk for AD; hence, they should be considered for presymptomatic discoveries (6-8).

Overview of the genetics of Alzheimer's disease

Studies on the genetics of AD led to uncovering of three broad categories of mutations and genetic variations associated with: (I) early-onset forms of AD, (II) late-onset sporadic forms of AD, and (III) susceptibility for AD (9,10). Here, we will consider some of the findings in each category that may have some bearing on the discovery and subsequent development of technologies for the early identification of individuals at risk and/or the introduction of disease-modifying therapies.

Early-onset AD (EOAD)

Early-onset AD (EOAD) cases have an early age of onset (<60 years) and account for approximately 5% of all AD cases. In particular, 35-65% of EOADs are familial, and the remaining are sporadic (11,12). A fraction of EOFAD cases, around 10-15%, are autosomal-dominant AD (ADAD) that follow an autosomal dominant inheritance pattern (13) due to highly penetrant mutations in three genes: (a) *APP* on chromosome 21q21.2, (b) presenilin-1 (*PSEN1*) on chromosome 14q24.3, and (c) presenilin-2 (*PSEN2*) on chromosome 1q42.13. Roughly 90% of the remaining EOFAD individuals are assumed to be due to an autosomal recessive inheritance pattern (14).

As of December 2022, the database available at https://www.alzforum.org/mutations, consisting of a repository of variants in genes implicated in AD, contains 479 genetic variants: (a) 63 genetic variants for *APP*, (b) 339 genetic variants for *PSEN1*, and (c) 77 genetic variants for *PSEN2*. *APP* and PSEN mutations both drive the amyloidogenic process in ADAD patients through alterations in brain metabolism of *APP* and amyloid beta (Aβ) peptides that support the development of toxic species and pathogenic aggregates (15), which ultimately may play a key role in neuronal cell death and dementia. Although the evidence concerning *APP*, *PSEN1*, and *PSEN2* mutations provides insights into the probable molecular mechanisms underpinning EOFAD, this data is inadequate to explain all cases of AD dementia fully.

Sporadic AD (sAD) or late-onset AD (LOAD)

The vast majority of AD cases (>95%) are sporadic AD (sAD) or late-onset AD (LOAD) with the usual onset after the age of 65 years (9,10). Evolving data indicates that multiple genes contribute to susceptibility or risk for LOAD. Susceptibility genes are identified by genetic association studies in which allele frequencies for polymorphisms at or near a gene are compared between cases and controls. Susceptibility genes are revealed when case and control frequencies differ significantly. Early attempts to identify these susceptibility genes were driven by selecting candidate genes based on the existing knowledge of disease pathogenesis (16). In 1993, Hypothesis-driven association studies on candidate genes led to the discovery of the association between the *APOE* gene that encodes for the apolipoprotein E (ApoE) and is located on chromosome 19q13.2 and AD (17, 18). *APOE* exhibits three isoforms (*ε2, ε3*, and *ε4*) and six genotypes (*ε2ε2, ε2ε3, ε2ε4, ε3ε3, ε3ε4*, and *ε4ε4*) resulting from a combination of two coding single-nucleotide polymorphisms (SNPs). The *APOE ε4* allele accounts for about one-third of the population-attributable risk for the disease (19). Despite efforts to elucidate the primary mechanisms, the same mode in which *APOE ε4* affects sAD onset and progression has yet to be verified. Current hypotheses on the pathogenic role of ApoE in AD revolve around the observation that Aβ accumulation is promoted in the brains of *APOE ε4* carriers and transgenic mice expressing the human *APOE ε4* allele

and mutant *APP* (20). ApoE does not modulate Aβ metabolism through direct binding to Aβ in solution but through its actions with other interacting receptors/transporters such as the low-density lipoprotein receptor-related protein 1 (LRP1)-dependent cellular uptake pathway in astrocytes (21,22). Moreover, ApoE participates in cholesterol transport and lipid metabolism and in addition to AD, the *APOE ε4* allele represents a confirmed risk factor in vascular disease, likely owing to its link to augmented plasma cholesterol concentrations (21).

In microglial cells, ApoE-induced intracellular Aβ degradation is mediated by the cholesterol efflux function of ApoE, which lowers cellular cholesterol levels and, subsequently, facilitates the intracellular trafficking of Aβ to lysosomes for degradation (21, 23).

Interestingly, a milestone study by Rhinn and colleagues (2013) analyzed whole-transcriptome cerebral cortex gene expression data in unaffected *APOE ε4* carriers and LOAD patients. *APOE ε4* carrier status was associated with a consistent transcriptomic shift resembling the LOAD profile. Furthermore, differential co-expression correlation network analysis of *APOE ε4* and LOAD transcriptomic changes enabled the detection of a set of candidate regulatory gene mediators. Several of these genes, including the amyloid beta A4 precursor protein-binding family A member 2 (*APBA2*), integral membrane protein 2B (*ITM2B*), fibroblast yes related novel (*FYN*), ring finger protein 219 (*RNF219*), and synaptic vesicle protein 2A (*SV2A*), encode known or novel modulators of *APP* endocytosis and metabolism. In addition, a genetic variant within *RNF219*, namely rs2248663, has been reported to affect Aβ deposition in the human brain and LOAD age-of-onset. These findings established the existence of an *APOE ε4*-associated molecular pathway that promotes LOAD (24).

Susceptibility for AD

Susceptibility genes/loci for LOAD have been inspected. Until about 2005, only a few genes could be investigated due to technical limitations. With a few exceptions, most studies of this type could not be replicated, likely because of inadequately evaluated population substructure, sample sizes that were too small to detect meaningful associations, and *P*-value thresholds that were insufficiently stringent. However, subsequent progress in genomic tools, in conjunction with gathering large sample cohorts, resulted in the initiation of genome-wide association studies (GWAS) aimed at recognizing novel genetic risk factors (25). Advances in genotyping platforms permitted the cross-examination of most of the genome for disease-associated variation in a single experiment. This method resulted in an exponential improvement in susceptibility gene discovery for a large number of diseases, disease-related traits, and associated phenotypes. Since the original report of *APOE* as a genetic risk factor for sAD, several hundreds of genes were tested for association with AD, leading to thousands of studies reporting positive associations, lack of association, replications, and refutations. Given the incessantly growing amount of published AD genetic studies, it became challenging to follow and interpret such a vast volume of published data (9,10,26). In order to address this problem, the AlzGene online database (27) was introduced to provide a comprehensive, unbiased, and routinely updated online catalogue and meta-analyses for the increasing list of AD candidate genes.

GWAS enabled the discovery of several SNPs associated with clinical AD or AD biomarkers. Compared with *APOE*, these SNPs induce only minor risk effects, which, when taken in combination, might substantially contribute to AD pathogenesis. Historically, the first large-scale GWAS executed (28-33) was successful in documenting at least ten novel genomic loci, in addition to *APOE*, as low-risk factors for sAD the following were also documented:

BIN1 (bridging integrator protein-1), *CLU* (clusterin, also known as apolipoprotein J), *ABCA7* (ATP-binding cassette, sub-family A, member 7), *CR1* (complement receptor type 1), *PICALM* (phosphatidylinositol-binding clathrin assembly protein), *MS4A4E* (membrane-spanning 4-domains, subfamily A, member 4E), *MS4A6A* (membrane-spanning 4-domains, subfamily A, member 6A), *CD33* (CD33 antigen, also known as Siglec [sialic acid-binding Ig-like lectin]-3), *CD2AP* (CD2-associated protein), and *EPHA1* (ephrin receptor EphA1).

Besides the association with the amyloidogenic cascade, these genes are linked with some basic physiological and pathophysiological interacting molecular pathways and exhibit patterns of putative functional relationships in sAD, namely: (a) cholesterol and lipid metabolism (*CLU* and *ABCA7*), (b) immune and complement systems/inflammatory response (*CR1*, *CLU*, *BIN1*, *MS4A4E/MS4A6A*, *CD33*, *ABCA7*, *CD2AP*, and *EPHA1*), (c) endocytosis pathways (*BIN1*, *PICALM*, and *CD2AP*), and (d) cell adhesion (*EPHA1*) (16). Furthermore, in line with the breakthrough results of these initial larger-scale GWAS, a high number of gene variants, not reaching genome-wide significance in sAD, were reported to be associated with the same category of pathways, particularly with the immune system and lipid metabolism, thus emphasizing a crucial role of these mechanisms in sAD pathophysiology (34).

The need for large-scale meta-analyses of GWAS and much larger sample sizes to enhance the identification of additional loci prompted an international initiative, the International Genomics of Alzheimer's Project (IGAP). This collaborative effort links the resources of four consortia focused on AD genetics/genomics: the Alzheimer's Disease Genetic Consortium (ADGC), the Cohorts for Heart and Ageing Research in Genomic Epidemiology (CHARGE) consortium, the European Alzheimer's Disease Initiative (EADI), and the Genetic and Environmental Risk in Alzheimer's Disease (GERAD) consortium (9,10). Following a meta-analysis conducted in a total sample of 74,046 participants (25,580 cases and 48,466 controls), this international partnership determined 11 novel genome-wide significant LOAD susceptibility loci: *INPP5D, MEF2C, HLA-DRB5/DRB1, NME8/GPR141, ZCWPW1/PILRA, PTK2B, CELF1/SPI1, SORL1, FERMT2, SLC24A4/RIN3*, and *CASS4* (35). Some of these loci stressed the prominent role of pathways already known to be linked to an amplified risk for AD, such as lipid metabolism, immune response and inflammatory pathways, cell migration, and mechanisms of endocytosis. More important, other genes emphasized the occurrence of different novel pathways underpinning AD, including hippocampal synaptic activity, cytoskeletal activity, axonal transport, and microglial and myeloid cell function (35). Moreover, the IGAP then performed a meta- analysis on a clinically evaluated enlarged sample of 94,437 participants (35,272 cases and 59,163 controls) and revealed 25 GWS loci, including five new ones (*IQCK, ACE, ADAM10, ADAMTS1*, and *WWOX*). Pathway enrichment analysis revealed lipid metabolism, immunity, *APP*, and tau binding proteins metabolism, thus highlighting that gene variants affecting *APP* and Aβ pathways are related to EOAD (ADAD) and LOAD (36). A very recent GWAS meta-analysis that combined a large, new case-control study with previous GWASs, allowed for examination of the most elevated number of AD patients (111,326 cases) together with controls (677,633) and reported 75 risk loci, many of which (specifically 42) were new. Pathway enrichment analysis confirmed the involvement of *APP* /Aβ peptide and tau-binding proteins metabolism, innate immunity, microglial activation, and tumor necrosis factor-alpha (TNF-α) signaling pathway in ADD (37).

Transethnic GWAS, another GWAS-based approach for collecting results from multiethnic participants in existing analyses, was beneficial in revealing new loci for genetic traits and diseases (9). For instance, a recent transethnic GWAS meta-analysis, encompassing both Japanese (5,178 cases and 6,520 controls) and White participants from the 2019 IGAP stage 1 data, allowed the detection of the novel locus OR2B2 (38). Thus, transethnic GWAS can help identify several previously non-documented associations in investigations exclusively based on European participants (39,40). Therefore, upcoming transethnic GWAS for integrating much larger non-White cohorts are expected to support the identification of additional novel AD susceptibility loci (9).

In summary, AD is considered genetically heterogeneous, especially in late-onset forms showing polygenic risk inheritances. According to Kamboh (2022), the execution of large-scale GWAS and meta-analyses of GWASs allowed the disclosure of approximately 95 susceptibility loci for LOAD during the 2009-early 2021 period (9). In general, there is a clear indication for major cellular/molecular pathways dysregulation in AD, namely: (I) Aβ pathway progression, (II) inflammatory/immune response, (III) lipid homeostasis, (IV) modulation of endocytosis and vesicle-mediated transport, (V) regulation of cell cycle, (VI) oxidative stress response, and (VII) axon guidance (10,41).

Although GWAS represented a powerful method to discover risk loci in AD, a substantial proportion (up to 60%) of LOAD genetic variance remained unexplained (42). Rare or infrequent susceptibility genes are assumed to account for such a "missing heritability". The key platforms needed to identify rare genetic variants (population frequency <1%) with intermediate-to-high effect size in the disease are the next-generation sequencing (NGS) technologies, in the form of whole genome sequencing (WGS) and whole exome sequencing (WES) that can screen entire genomes and exomes, respectively. Large-scale NGS, such as WGS and WES, detected novel genes harbouring rare variants, usually missed by GWAS, associated with the disease (9,10,41). Notably, in order to progress the discovery of novel gene variants affecting AD risk, the National Institute on Aging (NIA) formed the Alzheimer's Disease Sequencing Project (ADSP) for sequencing and examining the genomes of a large number of well-characterized individuals to find an extensive range of AD risk (and protective) gene variants. The final aim is to accelerate the identification of new directions for AD pharmacological therapy research and prevention.

Benefits and pitfalls of pre-clinical models of Alzheimer's disease

Currently, no available animal model resembles all the cognitive, histopathological, biochemical, and behavioural alterations observed in AD patients. However, a partial reproduction of AD neuropathological changes and functional deficits have been obtained either with exogenous application of Aβ (43) or through genetically engineered mouse models expressing variants of APP, PSEN1, PSEN2, tau or ApoE proteins (44). These models provide excellent opportunities to examine the basis for the spatiotemporal evolution of the disease, as well as to identify novel targets for pharmacological intervention (45-47). Since memory loss is one of the major hallmarks of the disorder, the phenotypic characterization of these animals classically included electrophysiological studies to analyze synaptic transmission and long-term

potentiation (LTP) and behavioural tests to assess cognitive function. These are the most commonly used techniques to test animal disease models associated with cognitive decline and synaptic plasticity alterations (48-50). In addition, advances in histopathological and biochemical methods have been a powerful tool to address important questions about the pathogenic mechanisms of the disease. Most studies generally reported either inhibition of LTP or reduction in baseline fast excitatory transmission prior to plaque deposition. However, the relative importance of these changes and apparent discrepancies still needs to be resolved (51,52). Additionally, results from behavioural studies showed inconsistencies, possibly due to differences in the behavioral protocol, type of tests that were conducted, age of the animals, and the transgenic animals' genetic background (53).

So far, the amyloid cascade hypothesis has been the most important AD theory, postulating that the accumulation of Aβ species is the causative pathological event (54). Strong evidence that Aβ oligomers cause impairment of LTP was provided when naturally secreted soluble oligomers of human Aβ were injected intraventricularly into rats (55). These studies contributed to our knowledge of the cellular and molecular substrates involved in Aβ action. Understanding how Aβ accumulation and assembly compromise synaptic structure and function became the focus of therapeutically oriented research on the AD during the last two decades. Based on this hypothesis, interventions that reduce Aβ load in the brain would likely attenuate the neuropathological changes and functional deficits characterizing AD. Indeed, lowering the production of the peptide by inhibiting the enzymes responsible for Aβ generation, preventing the formation of Aβ aggregates, and increasing the rate of Aβ clearance from the brain have all proven successful in experimental models of AD (46).

In 2021, the U.S. Food and Drug Administration (FDA) approved aducanumab, a human IgG1 anti-Aβ monoclonal antibody, as the first disease-modifying treatment for AD. This regulatory decision raised controversies in the AD scientific community as it was based on a significant reduction of Aβ with no convincing data supporting clinical efficacy (56). For these reasons, the European Medicines Agency (EMA) and the Japanese Pharmaceuticals and Medical Devices Agency (PMDA) recently recommended the refusal of the marketing authorization for aducanumab. In addition, several other monoclonal antibodies targeting Aβ oligomers have been tested in clinical studies without producing encouraging results, thus raising concerns over the "amyloid cascade hypothesis" (57). Indeed, the development of transgenic AD mice carrying familial AD mutations has been useful in elucidating some of the mechanistic aspects of the disease, even though these models show poor predictive value (58). Several reasons might explain why these experimental studies failed to predict the outcome of clinical studies. First, transgenic mice carry familial AD mutations accounting for only 1-10% of all human AD cases. Also, while these models develop specific hallmarks of AD, they do not entirely recapitulate the complex human disease. For instance, it is questionable whether AD patients and transgenic mice share a similar temporal profile of disease progression. While in transgenic mice, cognitive impairment precedes plaque deposition, in AD patients, the latter is more likely to appear first. Also, determining the onset of memory deficits in AD transgenic mice is challenging since initial memory deficits are subtle, and memory tests may not be sensitive enough to detect early changes. Another weakness with transgenic mice is that only a few strains show evidence of significant cell death, different from the substantial neurodegeneration occurring in the human AD brain. Finally, differences between mouse/human species, including diversities in cerebrovascular anatomy, neuronal network physiology, disease susceptibility, and, most importantly, dynamics of drug-target interactions, all need to be considered in translation.

An Alzheimer's Association Business Consortium Think Tank was recently created to improve the translational predictability of such models (59). However, to better mimic the human condition, models or combinations of new models combining genetics with environmental interactions are needed. Also, the timing of disease development, heterogeneous mechanisms and pathways, comorbid conditions, and gender influence should be carefully considered.

Systems biology framework to Alzheimer's disease and complex polygenic brain disorders

In light of this, a systems biology framework for sporadic forms of AD consents to describing disease endophenotypes and examining commonalities among different neurodegenerative diseases (60,61). Indeed, systems biology is a holistic, integrative, and systems-level paradigm investigating how complex interactions among different molecular entities, including DNA-protein, transcript-protein, microRNA (miRNA)-protein, protein-protein, protein-metabolite interaction networks, occur across structurally/functionally organized networks and systems in both health and disease. Therefore, systems biology used at both the experimental and computational level (62,63) supports understanding the cellular/molecular pathways and the interactions involved in AD advancement (64-66).

Omics sciences, executed under the systems biology paradigm (67), are effective in illustrating and foreseeing the spatiotemporal trajectories of complex polygenic multi-factorial diseases, including cancer (68-70). A similar result is anticipated for AD (as well as other neurodegenerative diseases) and its pathomechanistic alterations (71,63). Hence, systems biology can support the incorporation of different diverging or converging molecular-cellular levels and time

phases of pathophysiological mechanisms, including inflammatory and immunological alterations, oxidative stress, protein misfolding, lipid dyshomeostasis, altered brain microvasculature, apoptosis, and autophagy (62,72).

Notably, among all the omics disciplines, comprehensive analyses focused on global metabolome profiling are gathering increasing consensus (71,73-75). Metabolomics designates the large-scale investigation of small molecules, usually known as metabolites, within biological fluids, cells, tissues, and organisms (71,73-75). Given that AD disrupts metabolic pathways, the long-term metabolic alterations produced can be reported in terms of metabolic signatures. Metabolome profiling can be effectively executed in peripheral tissues and biofluids, including CSF or, more recently, blood (plasma/serum), thus making this approach suitable for clinical applications. Various potential metabolic signatures have been documented for AD diagnosis (71,75) and are represented by numerous dysregulated metabolites whose levels fluctuate in the disease state or after drug exposure. The analysis of these metabolic patterns might offer significant information concerning disease pathophysiology (76,77).

In terms of technology, the advancement in analytical instrumentation associated with establishing standardized chemical fragmentation libraries and powerful data analysis algorithms made mass spectrometry-based metabolomics one of the most innovative platforms. Indeed, mass spectrometry is an excellent analytical platform, enabling the collection and simultaneous quantitation of femtomole amounts of cellular metabolites and their characterization, and exhibits an elevated sensibility and an extensive range of detectable metabolites. As a result, substantial improvement has been made in interpreting the major modifications occurring at both the mRNA, protein, and metabolite levels in tissues of AD organisms (73,74,78). Furthermore, as reviewed by Reveglia and colleagues (2021), biomarkers with different biological natures, including biogenic amines, oxylipins, lipid mediators, amino acids, oxidative stress markers, and metals, have been disclosed in AD research (73). Definitively, metabolomics will help effectively explore dynamic alterations in biological systems by providing appropriate information for clinical applications and translational medicine (73,74).

Genomics holds the promise of precision medicine in Alzheimer's disease

GWAS consortia enabled researchers to scrutinize several large datasets able to disclose genes that might have a subtler effect on AD risk. However, genetic clarification for a substantial portion of the heritability of AD pathology still needs to be revealed. Therefore, despite the relevant data attained from recent large-scale collaborative studies, novel strategies are required to identify novel risk loci. Optimistically, meta-analyses or pathway-based approaches will continue to discover new genes. To this aim, advances in high-throughput NGS technologies will continue to make WGS and WES approaches increasingly more affordable, thus allowing the opportunity to identify novel gene variants usually not documented in previously established GWAS marker panels. Furthermore, high-throughput NGS methods can potentially assess the effects of structural variations, such as copy number variations, and other large-scale structural rearrangements, which might help explicate the composite genetic architecture of AD (9,10,26,41).

Of note, besides SNPs results, researchers are developing and utilizing gene expression-based methods to increase the knowledge of AD pathogenesis (79). Ultimately, research in the area of AD epigenetics seems to be encouraging to explain the role of structural alterations in the neuronal DNA, due to DNA methylations or histone modifications, in supporting or delaying and/or preventing the expression of AD given an individual's genotype (80-82). Epigenetic processes are key regulators of interactions between the genome and the environment. Evidence suggests that sporadic forms of AD result from the combined effects of variation in several genes and environmental factors and from epigenetic aberrations (83,84). Notably, maps of epigenetic changes have been established to deliver more information on genetic variations and expression profiles for genes involved in AD. Therefore, targeting the epigenome has been thought helpful for establishing novel paths in AD therapy.

Furthermore, thanks to recent methodological advances, it is possible to profile genome-wide DNA methylation(DNA methylome) and the state of chromatin in the whole human genome in a high-throughput manner (85,86).Remarkably, chromatin immunoprecipitation (ChIP) approaches, combined with DNA microarray analysis (ChIP-chip) or followed by high-throughput DNA sequencing methods (ChIP-seq), enabled the introduction of epigenome-wide association studies (EWAS), hence highlighting a new era of epigenomics research in AD. Furthermore, such technologies can help discover epigenetic modifications to elucidate further disease mechanisms (85, 86). Hence, given that GWAS scrutinizing gene variants has been accomplished, a new generation of EWAS is foreseen to deliver unique, relevant information to enrich the comprehension of AD pathophysiology and, possibly, to support the elaboration of effective therapeutic approaches (85).

CONCLUSIONS

Definitively, there is clear evidence supporting that sporadic LOAD is caused by a chronic, non-linear, dynamic pathophysiological cascade of events that commences early in life and drives converging and diverging mechanisms that

result in neurodegeneration and late-stage clinical dementia. Moreover, unlike EOFAD, because there is a network of genes and gene variants interacting in these sporadic forms, the genetics of sporadic LOAD appear particularly intricate and multifaceted and seem not to be dependent on a major single-gene effect. In this connection, high-throughput NGS technologies constantly support the identification of the still elusive common and rare DNA sequence variants, believed to account for the "missing heritability" in AD (9,10,41).

Genomics holds the promise of the precision medicine paradigm, "tailoring" (ex. adapting) the management of patients to every individual's genetic profile. This framework could be helpful in rationally developing and targeting drugs to genes, transcripts, protein pathways, metabolic pathways, and molecular networks that participate in AD pathogenesis. As a result, upcoming developments in the study of AD complex genetic heterogeneity will probably allow clinicians to provide more efficacious targeted pharmacological treatments that will be "customized", that is to say, directed to the definite genetic profiles of their AD patients.

Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

1. Collins FS, Morgan N, Partinos A. The Human Genome Project: lessons from large-scale biology. *Science*. 2003;300(5617):286-90.
2. Manolio TA. Genome wide association studies and assessment of the risk of disease. *N Engl J Med*. 2010;363(2):166-76.
3. Bateman RJ, Aisen PS, De Strooper B, et al. Autosomal-dominant Alzheimer's disease: a review and proposal for the prevention of Alzheimer's disease. *Alzheimers Res Ther*. 2011;3(1):1.
4. Fuller JT, Cronin-Golomb A, Gatchel JR, et al. Biological and Cognitive Markers of Presenilin1 E280A Autosomal Dominant Alzheimer's Disease: A Comprehensive Review of the Colombian Kindred. *J Prev Alzheimers Dis*. 2019;6(2):112-20.
5. Tariot PN, Lopera F, Langbaum J, et al. The Alzheimer's Prevention Initiative Autosomal-Dominant Alzheimer's Disease Trial: A study of crenezumab versus placebo in pre-clinical PSEN1 E280A mutation carriers to evaluate efficacy and safety in the treatment of autosomal-dominant Alzheimer's disease, including a placebo-treated noncarrier cohort. *Alzheimers Dement (NY).* 2018;8(4):150-60.
6. Condello C, Maxwell AM, Castillo E, et al. Aβ and tau prions feature in the neuropathogenesis of Down syndrome. *Proc Natl Acad Sci USA.* 2022;119(46):e2212954119.
7. Iulita MF, Garzón Chavez D, Klitgaard Christensen M, et al. Association of Alzheimer Disease With Life Expectancy in People With Down Syndrome. *JAMA Netw Open*. 2022;5(5):e2212910.
8. Coppus AM, Schuur M, Vergeer J, Janssens AC, Oostra BA, Verbeek MM, van Duijn CM. Plasma β amyloid and the risk of Alzheimer's disease in Down syndrome. *Neurobiol Aging.* 2012;33(9):1988-94.
9. Kamboh MI. Genomics and Functional Genomics of Alzheimer's Disease. *Neurotherapeutics*. 2022;19(1):152-72.
10. Sims R, Hill M, Williams J. The multiplex model of the genetics of Alzheimer's disease. *Nat Neurosci*. 2020;23(3):311-22.
11. Cacace R, Sleegers K, Van Broeckhoven C. Molecular genetics of early-onset Alzheimer's disease revisited. *Alzheimers Dement*. 2016;12(6):733–48.
12. Zhu XC, Tan L, Wang HF, et al. rate of early onset Alzheimer's disease: a systematic review and meta-analysis. *Ann Transl Med*. 2015;3(3):38.
13. Jarmolowicz AI, Chen HY, Panegyres PK. The patterns of inheritance in early-onset dementia: Alzheimer's disease and frontotemporal dementia. *Am J Alzheimers Dis Other Demen*. 2015;30(3):299–306.
14. Wingo TS, Lah JJ, Levey AI, Cutler DJ. Autosomal recessive causes likely in early-onset Alzheimer disease. *Arch Neurol*. 2012;69(1):59–64.
15. Weggen S, Beher D. Molecular consequences of amyloid precursor protein and presenilin mutations causing autosomal-dominant Alzheimer's disease. *Alzheimers Res Ther.* 2012;4(2):9.
16. Schellenberg GD, Montine TJ. The genetics and neuropathology of Alzheimer's disease. *Acta Neuropathol*. 2012;124(3):305-23.

17. Strittmatter WJ, Saunders AM, Schmechel D, Pericak-Vance M, Enghild J, Salvesen GS, Roses AD. Apolipoprotein E: high-avidity binding to beta-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. *Proc Natl Acad Sci USA.* 1993;90(5):1977-81
18. Chartier-Harlin MC, Parfitt M, Legrain S, et al. Apolipoprotein E, epsilon 4 allele as a major risk factor for sporadic early and late-onset forms of Alzheimer's disease: analysis of the 19q13.2 chromosomal region. *Hum Mol Genet.* 1994;3(4):569-74.
19. Hardy J. A hundred years of Alzheimer's disease research. *Neuron*. 2006;52(1):3-13.
20. Vance JE, Hayashi H. Formation and function of apolipoprotein E-containing lipoproteins in the nervous system. *Biochim Biophys Acta*. 2010;1801(8):806-18.
21. Yamazaki Y, Zhao N, Caulfield TR, Liu CC, Bu G. Apolipoprotein E and Alzheimer disease: pathobiology and targeting strategies. *Nat Rev Neurol*. 2019;15(9):501-18.
22. Verghese PB, Castellano JM, Garai K, et al. ApoE influences amyloid-β (Aβ) clearance despite minimal apoE/Aβ association in physiological conditions. *Proc Natl Acad Sci USA.* 2013;110(19):1807-16.
23. Lee CY, Tse W, Smith JD, Landreth GE. Apolipoprotein E promotes β-amyloid trafficking and degradation by modulating microglial cholesterol levels. *J Biol Chem*. 2012;287(3):2032-44.
24. Rhinn H, Fujita R, Qiang L, Cheng R, Lee JH, Abeliovich A. Integrative genomics identifies APOE ε4 effectors in Alzheimer's disease. *Nature*. 2013;500(7460):45-50.
25. Gandhi S, Wood NW. Genome-wide association studies: the key to unlocking neurodegeneration? *Nat Neurosci*. 2010;13(7):789-94.
26. Pimenova AA, Raj T, Goate AM. Untangling Genetic Risk for Alzheimer's Disease. *Biol Psychiatry.* 2018;83(4):300-10.
27. Bertram L, McQueen MB, Mullin K, Blacker D, Tanzi RE. Systematic meta-analyses of Alzheimer disease genetic association studies: The Alz Gene database. *Nat Genet.* 2007;39(1):17-23.
28. Bertram L, Lange C, Mullin K, et al. Genome-wide association analysis reveals putative Alzheimer's disease susceptibility loci in addition to APOE. *Am J Hum Genet.* 2008;83(5):623-32
29. Harold D, Abraham R, Hollingworth P, et al. Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer's disease. *Nat Genet.* 2009;41(10):1088-93.
30. Lambert JC, Heath S, Even G, Campion D, Sleegers K, Hiltunen M, et al. Genome-wide association study identifies variants at CLU and CR1 associated with Alzheimer's disease. *Nat Genet.* 2009;41(10=:1094-99.
31. Seshadri S, Fitzpatrick AL, Ikram MA, et al. CHARGE Consortium; GERAD1 Consortium; EADI1 Consortium. Genome-wide analysis of genetic loci associated with Alzheimer disease. *JAMA.* 2010;303(18):1832-40.
32. Hollingworth P, Harold D, Sims R, et al. Common variants at ABCA7, MS4A6A/MS4A4E, EPHA1, CD33 and CD2AP are associated with Alzheimer's disease. *Nat Genet.* 2011;43(5):429-35.
33. Naj AC, Jun G, Beecham GW, et al. Common variants at MS4A4/MS4A6E, CD2AP, CD33 and EPHA1 are associated with late-onset Alzheimer's disease. *Nat Genet.* 2011;43(5):436-41.
34. Jones L, Holmans PA, Hamshere ML, et al. Genetic evidence implicates the immune system and cholesterol metabolism in the aetiology of Alzheimer's disease. *PLoS One.* 2010;5(11):e13950.
35. Lambert JC, Ibrahim-Verbaas CA, Harold D, et al. Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease. *Nat Genet.* 2013;45(12):1452-58.
36. Kunkle BW, Grenier-Boley B, Sims R, et al. Genetic meta-analysis of diagnosed Alzheimer's disease identifies new risk loci and implicates Aβ, tau, immunity and lipid processing. *Nat Genet.* 2019;51(9):414-30.
37. Bellenguez C, Küçükali F, Jansen IE, et al. New insights into the genetic etiology of Alzheimer's disease and related dementias. *Nat Genet*. 2022;54(4):412-36.
38. Shigemizu D, Mitsumori R, Akiyama S, et al. Ethnic and trans-ethnic genome-wide association studies identify new loci influencing Japanese Alzheimer's disease risk. *Transl Psychiatry.* 2021;11:5.
39. Bien SA, Wojcik GL, Hodonsky CJ, et al. The future of genomic studies must be globally representative: Perspectives from PAGE. *Annu Rev Genom Hum Genet.* 2019;20:181-200.

40. Wojcik GL, Graff M, Nishimura KK, et al. Genetic analyses of diverse populations improve discovery for complex traits. *Nature.* 2019;570:514-18.
41. Freudenberg-Hua Y, Li W, Davies P. The role of genetics in advancing precision medicine for Alzheimer's disease-a narrative review. *Front Med*. 2018;5:108.
42. Ridge PG, Mukherjee S, Crane PK, Kauwe, JSK. Alzheimer's disease: analyzing the missing heritability. *PLoS One*. 2013;8(11):e79771.
43. Chambon C, Wegener N, Gravius A, Danysz W. Behavioural and cellular effects of exogenous amyloid-β peptides in rodents. *Behav Brain Res*. 2011;225(2):623-41.
44. Myers A, McGonigle P. Overview of Transgenic Mouse Models for Alzheimer's Disease. *Current Protocols in Neurosc*. 2019;89(10):e81
45. La Rosa LR, Matrone C, Ferraina C, et al. Age-related changes of hippocampal synaptic plasticity in APP-null mice are restored by NGF through p75NTR. *J Alzheimers Dis*. 2013;33(1):265-72.
46. Nisticò R, Pignatelli M, Piccinin S, Mercuri NB, Collingridge G. Targeting Synaptic dysfunction in Alzheimer's disease therapy. *Mol Neurobiol*. 2012;46(3):572-87.
47. Balducci C, Mehdawy B, Mare L, et al. The gamma-secretase modulator CHF5074 restores memory and hippocampal synaptic plasticity in plaque-free Tg2576 mice. *J Alzheimers Dis.* 2011;24(4):799-816.
48. Mango D, Saidi A, Cisale GY, Feligioni M, Corbo M, Nisticò R. Targeting Synaptic Plasticity in Experimental Models of Alzheimer's Disease. *Front. Pharmacol*. 2019;10:778.
49. Nisticò R, Mango D, Mandolesi G, et al. Inflammation subverts hippocampal synaptic plasticity in experimental multiple sclerosis. *PLoS One*. 2013;8(1):e54666
50. Bonito-Oliva A, Pignatelli M, Spigolon G, et al. Cognitive Impairment and Dentate Gyrus Synaptic Dysfunction in ExperimentalParkinsonism. Biol Psychiatry 2013; 75(9):701-10.
51. Marchetti C, Marie H. Hippocampal synaptic plasticity in Alzheimer's disease: what have we learned so far from transgenic models? *Rev Neurosci*. 2011;22(4):373-402.
52. Middei S, Roberto A, Berretta N, et al. Learning discloses abnormal structural and functional plasticity at hippocampal synapses in the APP23 mouse model of Alzheimer's disease. *Learn Mem.* 2010;17(5):236-40.
53. Bryan KJ, Lee H, Perry G, Smith MA, Casadesus G. Transgenic Mouse Models of Alzheimer's Disease: Behavioral Testing and Considerations. In Methods of Behavior Analysis in Neuroscience. 2nd edition. Buccafusco JJ. Boca Raton, 2009.
54. Hardy JA, Higgins GA. Alzheimer's disease: the amyloid cascade hypothesis. *Science.* 1992;256(5054):184-85.
55. Walsh D, Klyubin I, Fadeeva J. et al. Naturally secreted oligomers of amyloid β protein potently inhibit hippocampal long-term potentiation *in vivo*. *Nature.* 2002;416(6880):535–39.
56. Nisticò R, Borg JJ. Aducanumab for Alzheimer's disease: A regulatory perspective. *Pharmacol Res.* 2021;171:105754.
57. Panza F, Lozupone M, Logroscino G, Imbimbo BP. A critical appraisal of amyloid-β-targeting therapies for Alzheimer disease. *Nat Rev Neurol*. 2019;15(2):73–88.
58. Mckean NE, Handley RR, Snell RG. A Review of the Current Mammalian Models of Alzheimer's Disease and Challenges That Needto Be Overcome. *Int J Mol Sci*. 2021;22(23):13168.
59. Vitek, MP, Araujo, JA, Fossel, M, et al. Translational animal models for Alzheimer's disease: An Alzheimer's Association Business Consortium Think Tank. *Alzheimer's Dement*. 2020;6(1):e12114.
60. Lista S, Khachaturian ZS, Rujescu D, Garaci F, Dubois B, Hampel H. Application of Systems Theory in Longitudinal Studies on the Origin and Progression of Alzheimer's Disease. *Methods Mol Biol*. 2016;1303:49-67.
61. Hampel H, Lista S, Khachaturian ZS. Development of biomarkers to chart all Alzheimer's disease stages: the royal road to cutting the therapeutic Gordian Knot. *Alzheimers Dement*. 2012;8(4):312-36.
62. Castrillo JI, Lista S, Hampel H, Ritchie CW. Systems Biology Methods for Alzheimer's Disease Research Toward Molecular Signatures, Subtypes, and Stages and Precision Medicine: Application in Cohort Studies and Trials. *Methods Mol Biol.* 2018;1750:31–66.

63. Castrillo JI, Oliver SG. Alzheimer's as a systems-level disease involving the interplay of multiple cellular networks. *Methods Mol Biol.* 2016;1303:3–48.
64. Seyfried NT, Dammer EB, Swarup V, et al. A Multi-network Approach Identifies Protein-Specific Co-expression in Asymptomatic and Symptomatic Alzheimer's Disease. *Cell Syst.* 2017;4(1):60–72.
65. Johnson CH, Ivanisevic J, Siuzdak G. Metabolomics: Beyond biomarkers and towards mechanisms. *Nat Rev Mol Cell Biol*. 2016;17(7):451–59.
66. Civelek M, Lusis AJ. Systems genetics approaches to understand complex traits. *Nat Rev Genet.* 2014;15(1):34–48.
67. Hampel H, Nisticò R, Seyfried NT. Alzheimer Precision Medicine Initiative (APMI). Omics sciences for systems biology in Alzheimer's disease: State-of-the-art of the evidence. *Ageing Res Rev*. 2021;69:101346.
68. Heitzer E, Haque IS, Roberts CES, Speicher MR. Current and future perspectives of liquid biopsies in genomics-driven oncology. *Nat Rev Genet*. 2019;20(2):71–88.
69. Johansson HJ, Socciarelli F, Vacanti NM, et al. Breast cancer quantitative proteome and proteogenomic landscape. *Nat Commun*. 2019;10(1):1600.
70. Mertins P, Mani DR, Ruggles KV, et al. Proteogenomics connects somatic mutations to signalling in breast cancer. *Nature*. 2016;534(7605):55–62.
71. Wilkins JM, Trushina E. Application of metabolomics in Alzheimer's disease. *Front Neurol*. 2017;8:719.
72. Noorbakhsh F, Overall CM, Power C. Deciphering complex mechanisms in neurodegenerative diseases: the advent of systems biology. *Trends Neurosci*. 2009;32:88-100.
73. Reveglia P, Paolillo C, Ferretti G, et al. Challenges in LC-MS-based metabolomics for Alzheimer's disease early detection: targeted approaches versus untargeted approaches. *Metabolomics.* 2021;17(9):78.
74. González-Domínguez R, Sayago A, Fernández-Recamales Á. High-Throughput Direct Mass Spectrometry-Based Metabolomics to Characterize Metabolite Fingerprints Associated with Alzheimer's Disease Pathogenesis. *Metabolites*. 2018;8(3):52.
75. Trushina E, Mielke MM. Recent advances in the application of metabolomics to Alzheimer's Disease. *Biochim Biophys Acta*. 2013;1842(8):1232-9.
76. Batra R, Arnold M, Wörheide MA, et al. Alzheimer's Disease Metabolomics Consortium (ADMC). The landscape of metabolic brain alterations in Alzheimer's disease. *Alzheimers Dement*. 2022;1-19.
77. Horgusluoglu E, Neff R, Song WM, et al. Alzheimer's Disease Neuroimaging Initiative (ADNI); Alzheimer Disease Metabolomics Consortium. Integrative metabolomics-genomics approach reveals key metabolic pathways and regulators of Alzheimer's disease. *Alzheimers Dement.* 2022;18(6):1260-78.
78. Mishur RJ, Rea SL. Applications of mass spectrometry to metabolomics and metabonomics: detection of biomarkers of aging and of age-related diseases. *Mass Spectrom Rev*. 2012;31:70-95.
79. Liang JW, Fang ZY, Huang Y, et al. Application of Weighted Gene Co-Expression Network Analysis to Explore the Key Genes in Alzheimer's Disease. *J Alzheimers Dis.* 2018;65(4):1353-64.
80. Nikolac Perkovic M, Videtic Paska A, Konjevod M, Kouter K, Svob Strac D, Nedic Erjavec G, Pivac N. Epigenetics of Alzheimer's Disease. *Biomolecules*. 2021;11(2):195.
81. Qazi TJ, Quan Z, Mir A, Qing H. Epigenetics in Alzheimer's Disease: Perspective of DNA Methylation. *Mol Neurobiol*. 2018;55(2):1026-44.
82. Balazs R, Vernon J, Hardy J. Epigenetic mechanisms in Alzheimer's disease: progress but much to do. *Neurobiol Aging*. 2011;32(7):1181-87.
83. Román GC, Mancera-Páez O, Bernal C. Epigenetic Factors in Late-Onset Alzheimer's Disease: MTHFR and CTH Gene Polymorphisms, Metabolic Transsulfuration and Methylation Pathways, and B Vitamins. *Int J Mol Sci*. 2019;20(2):319.
84. Bihaqi SW, Schumacher A, Maloney B, Lahiri DK, Zawia NH. Do epigenetic pathways initiate late onset Alzheimer disease (LOAD): towards a new paradigm. *Curr Alzheimer Res.* 2012;9(5):574-88.
85. Klein HU, Bennett DA, De Jager PL. The epigenome in Alzheimer's disease: current state and approaches for a new path to gene discovery and understanding disease mechanism. *Acta Neuropathol*. 2016;132:503–14.

86. Smith AR, Mill J, Smith RG, Lunnon K. Elucidating novel dysfunctional pathways in Alzheimer's disease by integrating loci identified in genetic and epigenetic studies. *Neuroepigenetics*. 2016;6:32–50.

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Case Report

EPITHELIOID GLIOBLASTOMA IN A CHILD WITH TUBEROUS SCLEROSIS

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ABSTRACT

Tuberous sclerosis (TS) is a rare autosomal dominant disease. This case report reports a 3-year-old boy with epitheloid glioblastoma in the TS region. The patient had right-sided hemiparesis and facial nerve palsy and underwent a frontotemporal craniotomy. The patient was treated with a bone graft, a very rare method. Therefore, a yearly MRI of the brain is recommended in TS.

KEYWORDS: *epithelioid glioblastoma, tuberous sclerosis, SEGA, TSC2, neurosurgery*

DISCUSSION

Tuberous sclerosis (TS) complex is a rare autosomal dominant disease. It occurs in 1 in 6,000 individuals (1). TS is associated with the manifestation of (sub)cortical tubers, calcified subependymal nodules and subependymal giant cell astrocytomas (SEGAs) (2). Brain tumors associated with TS are rather unusual, affecting only 6–14% of patients; SEGAs account for the majority (3).

Herewith, we report the case of a 3-year-old girl with epithelioid glioblastoma in the context of TS. Diagnosis was set with the Sanger sequencing method. A c.4846 C>T (p.Gln1616stop) mutation was identified during the neonatal age on the exon of the TSC2 gene. She also had suffered drug resistant epilepsy under triple antiseizure medication (oxcarbazepine, topiramate, and valproic acid).

Two weeks before her admission, she exhibited right sided hemiparesis and ipsilateral central facial nerve palsy. Magnetic Resonance Imaging (MRI) examination of the brain revealed a space occupying lesion frontotemporally on the left, with slight calvarial erosion (Fig.1).

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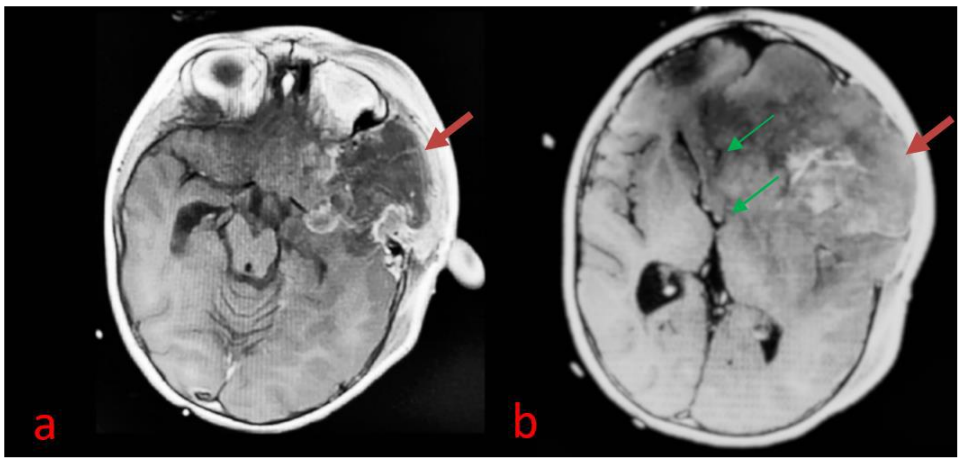

Fig. 1. *a): Space occupying lesion of left temporal pole and orbital gyri with bone erosion, adjacent to the upper orbital wall (red arrow); b): It is extraventricular, extends to inferior frontal gyrus (red arrow), and compresses the left frontal horn (thin green arrows). Histology exhibited epithelioid glioblastoma*.

Initially, she underwent a frontotemporal craniotomy under neuronavigation. The operation ceased due to circulatory instability and massive cerebral edema. Consequently, the bone flap was not placed back. In addition to that, a right-sided ventriculoperitoneal shunt system was implanted one week afterwards due to hydrocephalus. An adjunct second ventral catheter was inserted three weeks later due to persistent meningocele (Fig.2).

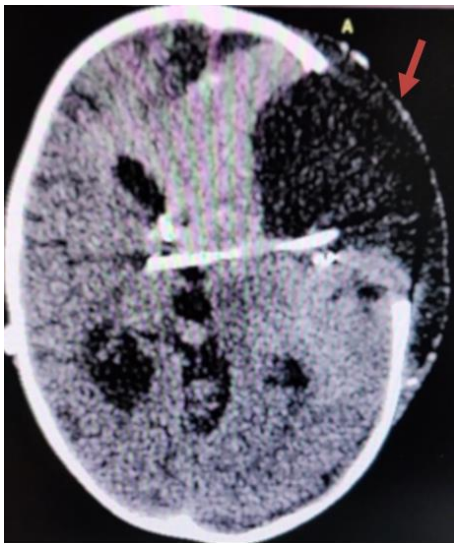

Fig. 2. *Ventriculoperitoneal system with two central catheters due to persistent meningocelcele (red arrow) and deficient communication between the two lateral ventricles.*

Histology revealed epithelioid glioblastoma. The child had undergone radiotherapy with 34.2 Grays total dose and three cycles of Temozolamide. Seven months afterwards, we implanted the custom-made bone graft and resected a new lesion of the left temporal pole, which had been considered local tumor recurrence; this lesion was identified as a SEGA (Fig.3).

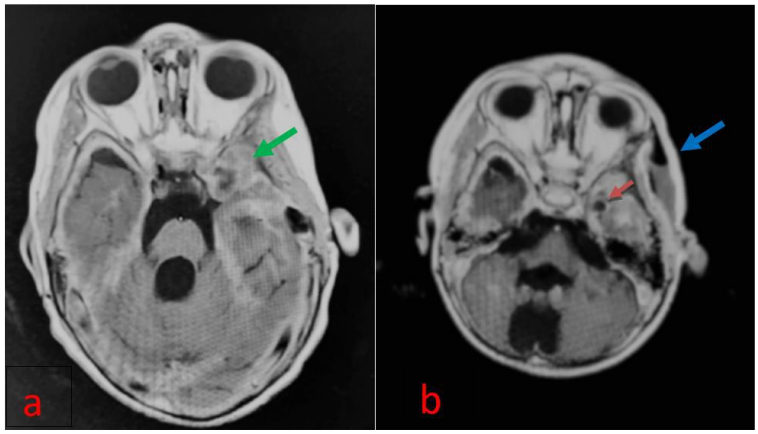

Fig. 3. ***a****): New lesion at the left temporal pole (green arrow) after the fourth surgical procedure;* ***b****): Bone graft implantation (thick blue arrow). The anatomical region of the excised left temporal pole lesion, which was identified as a SEGA (small red arrow).*

So far there are only seven cases of patients with occurrence of glioblastoma in the context of TS. In all of them, glioblastoma did not ensue as a transformation of the SEGA lesion (2). Malignant manifestations of TS have an incidence of 2% and afflict mostly younger patients (3). The association between high malignant tumors and primary lesions is not clear and they probably develop independently.

According to the updated surveillance criteria of TS, MRI of the brain should take place every one to three years (4). We advocate that the annual follow-up control should involve all TS patients and not only ones with large SEGAs.

Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

1. Northrup H, Krueger DA. International Tuberous Sclerosis Complex Consensus Group. Tuberous sclerosis complex diagnostic criteria update: recommendations of the 2012 International Tuberous Sclerosis Complex Consensus Conference. *Pediatr Neurol*. 2013;49(4):243-54. doi:10.1016/j.pediatrneurol.2013.08.001.
2. Vignoli A, Lesma E, Alfano RM, et al. Glioblastoma multiforme in a child with tuberous sclerosis complex. *Am J Med Genet A*. 2015;167A(10):2388-2393.
3. Sauter M, Belousova E, Benedikk MP, et al. Rare manifestations and malignancies in tuberous sclerosis complex: findings from the TuberOus Sclerosis registry to increase disease awareness (TOSCA). *Orphanet J Rare Dis*. 2021;16(1):301.
4. Northrup H, Aronow ME, Bebin EM, et al. International Tuberous Sclerosis Complex Diagnostic Criteria and Surveillance and Management Recommendations. *Pediatr Neurol*. 2021;123:50-66.

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HUMAN CYTOMEGALOVIRUS AND THE CNS: IMMUNITY AND INFLAMMATION

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ABSTRACT

Human cytomegalovirus (HCMV) is a highly widespread β-herpes virus that affects all age groups and is largely asymptomatic. The virus is transmitted through contact with bodily fluids and affects the central nervous system (CNS), causing neuropsychiatric disorders and contributing to immune activation and inflammation. HCMV infection can cause significant morbidity and be life-threatening for immunocompromised individuals and those taking immunosuppressant drugs. The virus can cause viral encephalitis in the immunocompromised, frequently resulting in brain damage. In addition, HCMV can cause serious infections affecting newborns with severe symptoms. In newborns, the virus can cross the blood-brain barrier (BBB), infecting brain cells and cerebrospinal fluid. Furthermore, HCMV can infect microglia, immune cells of the CNS with functions similar to macrophages, by activating toll-like receptors (TLRs) with the release of pro-inflammatory cytokines, including IL-1, TNF, and IL-6. This process leads to inflammation and neuropathic pain, which can be reduced by inhibiting these cytokines. Infection can also affect neurons and contribute to glioma and other brain pathologies. Here, the pathogenesis of HCMV infection involving the immune system and the effects on the CNS will be reported.

KEYWORDS: *cytomegalovirus, CNS, immunity, inflammation, microglia, infection*

INTRODUCTION

Human cytomegalovirus (HCMV) is a β-herpes virus that is widespread worldwide and primarily asymptomatic since the immune system contains it. HCMV is a virus that can affect any age group, with an incidence of about 50%-60% in developed countries (1,2), contributing to immune activation and inflammation (3). In addition, HCMV can affect the central nervous system (CNS), causing neuropsychiatric disorders (4) and the immune system, particularly in immunocompromised individuals and those taking immunosuppressant drugs (5,6).

The virus is transmitted from an infected individual to a healthy individual through contact with body fluids, including breast milk, saliva, urine, blood, sperm, tears, wounds, and pus. (7). HCMV infection can remain permanently latent, and its reactivation in subjects with immune pathologies can cause serious damage to vital organs (8). HCMV infection appears to affect immunodeficient patients but can also affect individuals with an efficient immune system. Therefore,

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HCMV infections can have different degrees of severity. It is one of the most frequent serious infections affecting newborns, estimated to be the leading cause of congenital infection in developed countries with an incidence between 0,3% and 2,3% of all births, presenting with cranial bone changes, mental retardation, microcephaly, jaundice, hearing loss, skin lesions, impaired neurodevelopment, and other symptoms (9,~~10~~). This infection most commonly affects children with Down syndrome (trisomy 21). HCMV is also transmitted in bodily fluids from infected but symptom-free individuals (10). Most newborns can render the infection asymptomatic, and the severity of the disease depends on the individual immune response. HCMV infections can be lethal, especially in immunosuppressed patients with impaired T lymphocyte response. In the most severe pathological cases, the CNS and the hematopoietic and respiratory systems can be altered, especially in the first months of the newborn's life (11).

HCMV Immunity and Inflammation

HCMV, present in body fluids, is primarily transported by myeloid cells and mainly affects certain types of cells, such as endothelial, epithelial, smooth muscle, and fibroblast cells (12). HCMV is transmitted from mother to child across the placenta, passes to the fetus, enters the bloodstream, and reaches the brain (13); the infection mainly affects newborns, while adults show a higher resistance. Since the newborn blood-brain barrier (BBB) is not yet formed, the virus can infect the brain cells and cerebrospinal fluid (14). The virus initially targets and replicates in astrocytes, cells important in synapse formation and supporting the BBB (11).

HCMV can cause viral encephalitis, a very common disease after brain infection, responsible for permanent CNS damage, which can even lead to patient death (15). Viral encephalitis can be caused by various infections mediated by the immune cells responsible for the inflammation. HCMV encephalitis affects most patients with compromised immune systems, and the disease can be diagnosed by polymerase chain reaction (PCR) analysis. Brain damage caused by HCMV is relatively frequent and, in certain circumstances, can cause IL-1beta-mediated fever, chills, fatigue syndrome, myalgia, motor deficits with paraplegia, hypoesthesia, paraesthesia, dysesthesia, anaesthesia, disorientation, brain fog, bilateral visual loss, urinary retention, constipation, and coma (Table I).

Table I. *Infections caused by HCMV.*

Most frequent manifestations:	• Colitis	• Encephalitis
	• Meningitis	• Myelitis
Pathological states of other organs:	• Hemolytic anemia	• Thrombocytopenia
	• Venous and/or arterial vascular thrombosis	• Uveitis
	• Pneumonia	• Splenic rupture
	• Coagulation	• Pancytopenia
	• Myelodysplastic changes	• Abdominal pain
	• Headache	• Jaundice

Microglia are long-lived immune cells in the brain that viruses, including HCMV, can activate. The innate immune defense of these cells protects against infection in the CNS, although in the case of HCMV, microglia are not the primary target. Microglia perform a function similar to peripheral blood monocytes in the CNS, protecting the brain from infection and inducing inflammation by releasing inflammatory mediators (16). Therefore, these cells appear to be not easily infected by HCMV. However, the virus can infect microglia by activating toll-like receptors (TLRs) involved in glial activation (17). It binds to TLR4, activating pro-inflammatory cytokines such as IL-1 beta, TNF-alpha and IL-6, causing inflammation and neuropathic pain (18,19) (Fig.1). These cytokines generated primarily by microglia, are the most studied inflammatory mediators in CNS insults, as several activators including viruses upregulate them. Their inhibition significantly reduces the inflammatory and neuropathic pain induced by pathogenic viruses. However, the exact mechanism(s) remain to be confirmed (20).

Neurons, which have little possibility of replication, can be weakly infected by viruses which exploit neuronal metabolism to replicate and induce apoptosis (21). In addition, neuronal stem cells are also resistant to HCMV infection. However, the ependymal glial cells of the CNS have cilia that move the cerebrospinal fluid of the CNS and are easily infected by the virus (11).

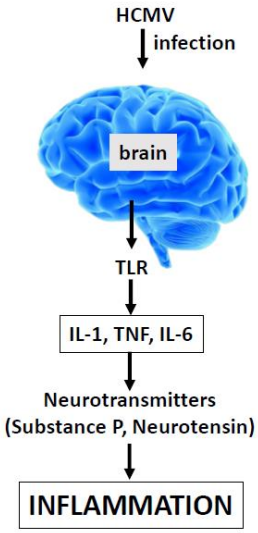

Fig. 1. *Here, we depict inflammation induced by HCMV in the brain, which provokes the generation of inflammatory cytokines and neurotransmitters such as substances P and neurotensin, leading to inflammation.*

An immunodeficiency involving CD8+ and/or CD4+ T cells is important for the reactivation of HCMV infection, an effect to be confirmed by future studies (22). Infection with HCMV can also promote the onset of tumors such as glioma and other brain pathologies (23). In addition, virus infection in the brain attracts T cells, exhibiting an immune and inflammatory effect. In some cases, specific antivirals can relieve symptoms, including systemic inflammation, and avoid death. (24,25).

However, the pathogenesis of HCMV infection is still unclear, and therefore, future studies, especially involving the immune system, are needed to unravel the many obscure points that arise in the CNS.

Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

1. Cytomegalovirus (CMV) and Congenital CMV Infection. [(accessed on 10 September 2022)]; Available online: https://www.cdc.gov/cmv/overview.html
2. Zuhair M, Smit GSA, Wallis G, et al. Estimation of the worldwide seroprevalence of cytomegalovirus: A systematic review and meta-analysis. *Reviews in Medical Virology*. 2019;29(3):e2034. doi:10.1002/rmv.2034
3. van de Berg Pablo J, Heutinck KM, Raabe R, et al. Human Cytomegalovirus Induces Systemic Immune Activation Characterized by a Type 1 Cytokine Signature. *The Journal of Infectious Diseases*. 2010;202(5):690-699. doi:10.1086/655472
4. Zhang XY, Fang F. Congenital human cytomegalovirus infection and neurologic diseases in newborns. *Chinese Medical Journal*. 2019;132(17):2109-2118. doi:10.1097/cm9.0000000000000404
5. Griffiths P, Reeves M. Pathogenesis of human cytomegalovirus in the immunocompromised host. *Nature Reviews Microbiology*. 2021;19(12):759-773. doi:10.1038/s41579-021-00582-z
6. Griffiths PD. Cytomegalovirus infection complicating immunosuppressive treatment. *Postgraduate Medical Journal*. 1988;64(747):1-3. doi:10.1136/pgmj.64.747.1
7. Cannon MJ, Schmid DS, Hyde TB. Review of cytomegalovirus seroprevalence and demographic characteristics associated with infection. *Reviews in Medical Virology*. 2010;20(4):202-213. doi:10.1002/rmv.655
8. Forte E, Zhang Z, Thorp EB, Hummel M. Cytomegalovirus Latency and Reactivation: An Intricate Interplay With the Host Immune Response. *Frontiers in Cellular and Infection Microbiology*. 2020;10. doi:10.3389/fcimb.2020.00130

9. Nassetta L, Kimberlin D, Whitley R. Treatment of congenital cytomegalovirus infection: implications for future therapeutic strategies. *Journal of Antimicrobial Chemotherapy*. 2009;63(5):862-867. doi:10.1093/jac/dkp083
10. Schottstedt V, Blümel J, Burger R, et al. Human Cytomegalovirus (HCMV) – Revised. *Transfusion Medicine and Hemotherapy*. 2010;37(6):365-375. doi:10.1159/000322141
11. Krstanović F, Britt WJ, Jonjić S, Brizić I. Cytomegalovirus Infection and Inflammation in Developing Brain. *Viruses*. 2021;13(6):1078. doi:10.3390/v13061078
12. Aguiar A, Galinato M, Bradley Silva M, Toth B, McVoy MA, Hertel L. Human Cytomegalovirus Replication and Infection-Induced Syncytia Formation in Labial, Foreskin, and Fetal Lung Fibroblasts. *Viruses*. 2021;13(12):2355. doi:10.3390/v13122355
13. Buxmann H, Hamprecht K, Meyer-Wittkopf M, Friese K. Primary Human Cytomegalovirus (HCMV) Infection in Pregnancy. *Deutsches Aerzteblatt Online*. 2017;114(4):45-52. doi:10.3238/arztebl.2017.0045
14. Alcendor DJ, Charest AM, Zhu WQ, Vigil HE, Knobel SM. Infection and upregulation of pro-inflammatory cytokines in human brain vascular pericytes by human cytomegalovirus. *Journal of Neuroinflammation*. 2012;9(1). doi:10.1186/1742-2094-9-95
15. Slavuljica I, Kveštak D, Csaba Huszthy P, Kosmac K, Britt WJ, Jonjić S. Immunobiology of congenital cytomegalovirus infection of the central nervous system—the murine cytomegalovirus model. *Cellular and Molecular Immunology*. 2015;12(2):180-191. doi:10.1038/cmi.2014.51
16. Bachiller S, Jiménez-Ferrer I, Paulus A, et al. Microglia in Neurological Diseases: A Road Map to Brain-Disease Dependent-Inflammatory Response. *Frontiers in Cellular Neuroscience*. 2018;12(488). doi:10.3389/fncel.2018.00488
17. Patrycy M, Chodkowski M, Krzyzowska M. Role of Microglia in Herpes virus-Related Neuroinflammation and Neurodegeneration. *Pathogens*. 2022;11(7):809. doi:10.3390/pathogens11070809
18. Compton T, Kurt-Jones EA, Boehme KW, et al. Human Cytomegalovirus Activates Inflammatory Cytokine Responses via CD14 and Toll-Like Receptor 2. *Journal of Virology*. 2003;77(8):4588-4596. doi:10.1128/jvi.77.8.4588-4596.2003
19. Jarvis MA, Borton JA, Keech AM, et al. Human Cytomegalovirus Attenuates Interleukin-1β and Tumor Necrosis Factor Alpha Proinflammatory Signaling by Inhibition of NF-κB Activation. *Journal of Virology*. 2006;80(11):5588-5598. doi:10.1128/jvi.00060-06
20. Tang XD, Ji TT, Dong JR, et al. Pathogenesis and Treatment of Cytokine Storm Induced by Infectious Diseases. *International Journal of Molecular Sciences*. 2021;22(23):13009. doi:10.3390/ijms222313009
21. Allsopp TE, Scallan MF, Williams A, Fazakerley JK. Virus infection induces neuronal apoptosis: A comparison with trophic factor withdrawal. *Cell Death & Differentiation*. 1998;5(1):50-59. doi:10.1038/sj.cdd.4400298
22. Brizić I, Šušak B, Arapović M, et al. Brain-resident memory CD8+T cells induced by congenital CMV infection prevent brain pathology and virus reactivation. *European Journal of Immunology*. 2018;48(6):950-964. doi:10.1002/eji.201847526
23. Hu M, Wang B, Qian D, et al. Human cytomegalovirus immediate-early protein promotes survival of glioma cells through interacting and acetylating ATF5. *Oncotarget*. 2017;8(19):32157-32170. doi:10.18632/oncotarget.17150
24. Devetag FC, Boscariolo L. Cytomegalovirus Meningoencephalitis with Paroxysmal Course in Immunocompetent Adults: A New Nosographical Entity. *European Neurology*. 2000;44(4):242-247. doi:10.1159/000008244
25. Eddleston M, Peacock S, Juniper M, Warrell DA. Severe Cytomegalovirus Infection in Immunocompetent Patients. *Clinical Infectious Diseases*. 1997;24(1):52-56. doi:10.1093/clinids/24.1.52

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ADVANCES IN HYPERTENSION, HEART DISEASE, AND THE CNS

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ABSTRACT

It is now known that hypertension is a cardiovascular risk factor, and a better understanding of the pathogenesis of this disease may lead to more specific therapies. Immune cells that cause inflammation may also be involved in hypertension by causing the activation of vasoactive molecules, including the cytokines IL-1, IL-6 and TNF. These inflammatory cytokines are also produced by microglia and can provoke cardiovascular disease, linking the nervous system with hypertension and heart disease. The inhibition of pro-inflammatory cytokines can be a therapeutic strategy in these pathologies.

KEYWORDS: *hypertension, heart disease, CNS, neuroinflammation, microglia, cytokine, immune*

INTRODUCTION

Hypertension has an incidence of about 30% worldwide, is the most frequent cause of mortality, and can cause several other diseases (1). Hypertension occurs when blood pressure is higher than the normal population and it is an important risk factor in cardiovascular disease where it can lead to heart failure, vascular disease, and stroke (2). In addition, blood pressure rises with age and can lead to heart attack, aneurysm, and chronic kidney disease. Hypertension, which has increased in recent years, is the main cardiovascular risk factor, particularly in elderly Western subjects.

In the last twenty years, there has been considerable progress in the research on antihypertensive drugs and today the topic of hypertension is being tackled with greater therapeutic awareness. In hypertension, the smooth muscle cells of the arterial vessels are involved, responsible for regulating blood flow and therefore blood pressure (3). These muscle cells contract in high blood pressure to counteract the blood flow going to the tissues. Hypertension drugs are often rejected by patients because the disease is often asymptomatic, and the drugs can cause unwanted side effects.

In hypertension, the role of inflammation is an important issue linked to cardiovascular risk and other pathological phenomena. In this disease, the activation of immune cells, producing pro-inflammatory cytokines, can cause chronic low-grade inflammation, a topic that is still understudied by clinical research (4). IL-1 inhibition with new inhibitory cytokines such as IL-37, IL-38 or IL-1Ra, may improve clinical status by reducing C-reactive protein (CRP), and counteracting cardiovascular events, although they may not directly improve hypertension (5). The hypertensive phenomenon can activate vasoactive molecules, such as, for example, the NLRP3 inflammasome which could stimulate and lead to the generation of IL-1 by mediating chronic low-grade inflammation (6). IL-1 entering the circulation activates the macrophage cells of innate immunity, but also non-immune cells such as fibroblasts and endothelial cells (7). The

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activation of NLRP3 leads to the generation of IL-1, which causes the formation of fibrosis with renal vascular damage and hypertension (Fig.1).

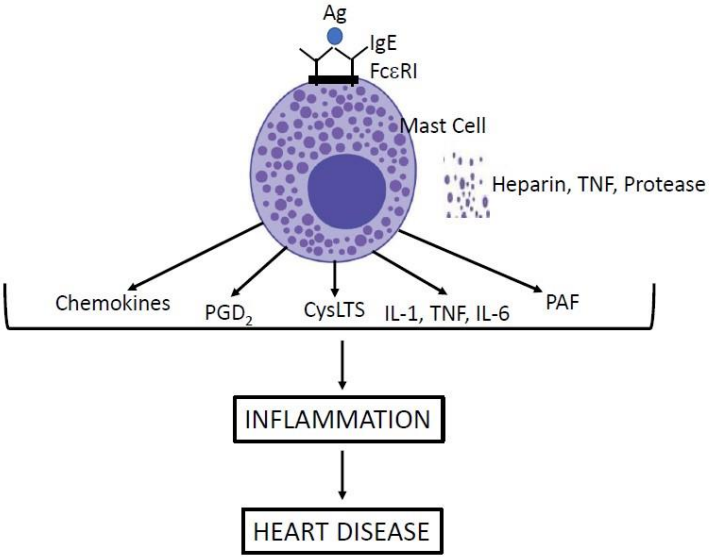

Fig. 1. *Activation of mast cells through its receptor (FcεRI) leads to the generation of chemical mediators such as heparin, TNF and protease, and other inflammatory compounds including chemokines, PGD_2, CysLTS, IL-1, TNF, IL-6 and PAF, that together, provoke inflammation and mediate heart disease.*

These studies demonstrate that innate immunity mediated essentially by macrophage cells producing IL-1 and other pro-inflammatory molecules, results in hypertension. In addition, immune cells such as innate lymphoid cells (ILCs), and gamma delta T cells that produce IL-17A, can damage organs and induce hypertension (8). Furthermore, the activation of lymphocytes and macrophages generates TNF, another very potent inflammatory cytokine. ILC cells produce inflammatory cytokines such as IFNγ, IL-17, and granulocyte-macrophage colony-stimulating factor (GMC-SF), also involved in hypertensive inflammation. CD4+ and CD8+ lymphocytes also participate in the pathological process in hypertension, producing inflammatory cytokines, while Treg lymphocytes (CD25), which have an immunosuppressive function and the ability to promote immunological tolerance, may play a protective role (9). In experimental mouse models where a Treg cell deficit was induced in the circulatory system and therefore in the organs, the animals showed an increase in blood pressure, demonstrating the importance of the activation of T lymphocytes in mediating hypertension (10).

Cardiovascular disorder is an inflammatory disease that can lead to myocardial infarction and stroke. Heart failure due to hypertension leads to ventricular hypertrophy and diastolic dysfunction.

Neuroinflammation in the central nervous system (CNS) is mainly fueled by microglia and contributes to the pathogenesis of many brain diseases. Therefore, microglia are protective immune cells similar to macrophages that reside in the CNS, but when activated by releasing substances such as cytokines that can inflame surrounding tissue, they can also enter the bloodstream (11).

Mast cells in cardiovascular disease

Cardiac tissue includes fibroblasts, pericytes, smooth muscle cells, endothelial cells, and cardiomyocytes, but immune cells are also present including macrophages, dendritic cells, T and B lymphocytes, and mast cells (MCs), which play an important role in maintaining proper regeneration and the healthy tissue physiological state (12). MCs are in the perivascular and participate in vascularized tissue reactions causing inflammation (13). Mast cells are bone marrow hematopoietic cells that are formed from CD34+ cells that are capable of migrating to all tissues including the heart where they reside near vessels. Histamine, released by MCs, acts on the endothelium, mediates vascular permeability, and promotes adhesion of platelets through the adhesion molecule. Activation of the FcεRI receptors of MCs can provoke the release of various pro-inflammatory compounds including IL-1 and other inflammatory cytokines and chemokines, which contributes to chronic inflammation. Antigen binding c-kit/CD117 receptor in cardiac mast cells also results in histamine secretion (14). Increased heart rate, mast cell count, and histamine levels mediate cardiac ischemia and cardiovascular spasm with myocardial damage (15).

MCs produce cytokines and proteases such as tryptase and chymase, promoting leukocyte infiltration into cardiac tissue and causing inflammation (Fig.2).

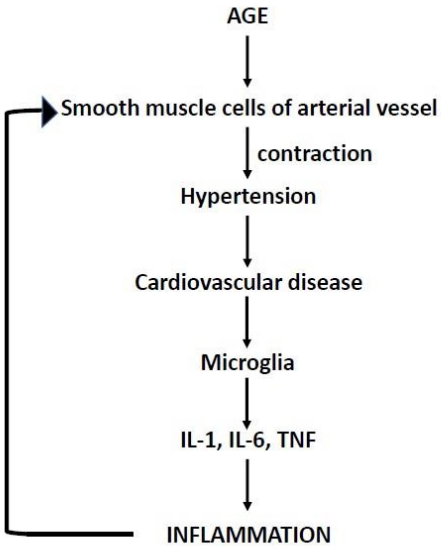

Fig. 2. *Smooth muscle cells of arterial vessel contraction in age can lead to hypertension and vascular disease, involving microglia which release inflammatory cytokines such IL-1, IL-6, and TNF causing inflammation which mediates hypertension.*

Activated MCs release tryptase from their granules which stimulates activate protease-activated receptors (PARs) present in sensory neurons near the MCs. This activation leads to the release of substance P from the afferent nerves, causing inflammation. MCs can proliferate in cardiac tissue and play an important role in heart failure and ischemia (15). However, it appears that no increase in the number of MCs results in myocardial infarction. They can proliferate in cardiac tissue and play an important role in heart failure and ischemia, although it has been reported that these cells may also play a protective role in cardiac tissue remodeling (16).

Hypertension, cardiovascular disease, and microglia

Hypertension, associated with cardiovascular morbidity, can lead to organ specific microvascular disease, mediated by sterile inflammatory processes. For instance, the activation of immune cells by an ischemic process leads to the recruitment of leukocytes with production of cytokines that mediate the sterile inflammatory process in cardiac tissue. Innate immunity, represented by monocytes CD14 and CD16 that maintain homeostasis, plays an important role in cardiovascular disease which is associated with various pathologies such as endocarditis, atherosclerosis, peripheral vascular disease, ischemic heart disease, hypertensive heart disease, hemorrhagic stroke, cardiomyopathy, and others (17).

Activated monocytes can generate several pro-inflammatory vasoactive mediators such as cytokines (IL-1, IL-6, and TNF), prostaglandins PGD2, cysteinyl leukotrienes (LTC4, D4, E4), platelet-activating factor (PAF), and chemokines. CD4+ immune T cells have a protective function in cardiovascular disease, as most of them resident in heart tissue are T regulatory (Treg) cells that produce IL-10, an anti-inflammatory cytokine that opposes heart disease (18). CD4+ cell intervention improves diseased heart tissue through monocyte/macrophage regulation.

In response to hypertensive insults, cardiovascular inflammation and neuroinflammatory processes can occur involving microglia which mediate the immune response in cooperation with neuronal cells and astrocytes. Glial cells are cells of mesodermal origin and non-neuronal cells of the nervous system. Glia is a word of Greek origin and means "glue" because these cells were thought to function only to hold the nervous system together. Glial cells are now known to play an important role in the nervous system such as astrocytes, which are glial cells responsible for regulating the levels of neurotransmitters, such as inflammatory substance P, around neurons (19). In addition, microglia, which are innate immune cells, are tissue-resident macrophages in the brain and perform their immunological function through phagocytosis, removing dead neurons and other harmful substances (Fig.3).

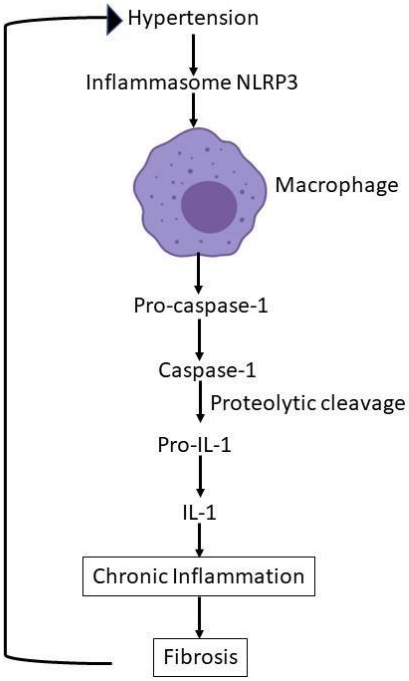

Fig. 3. *Inflammasone NLRP3 in hypertension activates macrophages to release caspase and therefore, IL-1, which mediate chronic inflammation and fibrosis involved in hypertension.*

In the nervous system, microglia also regulate synapses and the synthesis of myelin which surrounds neuronal axons. The cooperation between glial cells and neurons is important for a correct physiology of the nervous system.

The activation of microglia can occur in neurological and cardiovascular diseases and by acting on the microglia it is possible to obtain an improvement in these pathological states. In fact, microglia have been shown to mediate inflammatory diseases, including cardiovascular diseases, demonstrating a relationship between cardiac, neuroinflammatory, and hypertension diseases. The activation of microglia and the cardiovascular system leads to the production of pro-inflammatory cytokines such as IL-1, TNF, IL-6, and various chemokines (20). The M2 macrophage-like microglial phenotype produces anti-inflammatory cytokines such as IL-4 and IL-13 for tissue repair and healing. It is not yet known whether this population of M2 macrophage and microglial cells can generate the anti-inflammatory cytokines IL-37, IL-38 or IL-1Ra. Neuroinflammation affects blood pressure; In fact, infectious states activate microglia which produce pro-inflammatory cytokines, an effect that can be attenuated by inhibiting microglia activation. These reactions demonstrate a clear connection between hypertension and microglia. It has been reported that in acute hypertension, the number of synapses in contact with microglia increases, while in hypotension, the microglia decrease the number of synapses (21).

Microglia express the TLR4 receptor which can be activated by different antigens, resulting in an inflammatory response, a reaction that is downregulated by inhibiting TLR4. This demonstrates the importance of TLR4 in microglial cell response, hypertension, and cardiovascular disease, paving the way for therapeutic intervention. Myocardial infarction may also activate hypothalamic microglia with elevations of pro-inflammatory cytokines such as IL-1, IL-6, and TNF that activate the pituitary adrenal axis (22). Coronary injury with reperfusion also causes microglial activation, suggesting a connection between cardiac injury and microglial activation.

CONCLUSIONS

In this article, we have shown the interrelationship between hypertension, vascular disease, and neuroinflammation, demonstrating that the human body works as an orchestra where each instrument plays together to perform the correct music.

Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

1. Peixoto AJ. Acute Severe Hypertension. *N Engl J Med*. 2019;381(19):1843-1852. doi:10.1056/NEJMcp1901117
2. Latic N, Erben RG. Vitamin D and Cardiovascular Disease, with Emphasis on Hypertension, Atherosclerosis, and Heart Failure. *Int J Mol Sci*. 2020;21(18):6483. doi:10.3390/ijms21186483
3. Touyz RM, Alves-Lopes R, Rios FJ, et al. Vascular smooth muscle contraction in hypertension. *Cardiovasc Res*. 2018;114(4):529-539. doi:10.1093/cvr/cvy023
4. Agita A, Alsagaff MT. Inflammation, Immunity, and Hypertension. *Acta Med Indones*. 2017;49(2):158-165.
5. Madhur MS, Elijovich F, Alexander MR, et al. Hypertension: Do Inflammation and Immunity Hold the Key to Solving this Epidemic? *Circ Res*. 2021;128(7):908-933.
6. Griendling KK, Camargo LL, Rios FJ, Alves-Lopes R, Montezano AC, Touyz RM. Oxidative Stress and Hypertension. *Circ Res*. 2021;128(7):993-1020. doi:10.1161/CIRCRESAHA.121.318063
7. Conti P, Caraffa A, Mastrangelo F, et al. Critical role of inflammatory mast cell in fibrosis: Potential therapeutic effect of IL-37. *Cell Prolif*. 2018;51(5):e12475. doi:10.1111/cpr.12475
8. Zhang RM, McNerney KP, Riek AE, Bernal-Mizrachi C. Immunity and Hypertension. *Acta Physiol (Oxf)*. 2021;231(1):e13487. doi:10.1111/apha.13487
9. Huang N, Zhou R, Chen H, et al. Splenic CD4+ and CD8+ T-cells highly expressed PD-1 and Tim-3 in cirrhotic patients with HCV infection and portal hypertension. *Int J Immunopathol Pharmacol*. 2021;35:20587384211061051. doi:10.1177/20587384211061051
10. Radwan E, Mali V, Haddox S, et al. Treg cells depletion is a mechanism that drives microvascular dysfunction in mice with established hypertension. *Biochim Biophys Acta Mol Basis Dis*. 2019;1865(2):403-412. doi:10.1016/j.bbadis.2018.10.031
11. Richards EM, Li J, Stevens BR, Pepine CJ, Raizada MK. Gut Microbiome and Neuroinflammation in Hypertension. *Circ Res*. 2022;130(3):401-417. doi:10.1161/CIRCRESAHA.121.319816
12. Salei N, Rambichler S, Salvermoser J, et al. The Kidney Contains Ontogenetically Distinct Dendritic Cell and Macrophage Subtypes throughout Development That Differ in Their Inflammatory Properties. *J Am Soc Nephrol*. 2020;31(2):257-278. doi:10.1681/ASN.2019040419
13. Conti P, Caraffa A, Ronconi G, et al. Impact of mast cells in depression disorder: inhibitory effect of IL-37 (new frontiers). *Immunol Res*. 2018;66(3):323-331. doi:10.1007/s12026-018-9004-9
14. Galli SJ, Kalesnikoff J, Grimbaldeston MA, Piliponsky AM, Williams CM, Tsai M. Mast cells as "tunable" effector and immunoregulatory cells: recent advances. *Annu Rev Immunol*. 2005;23:749-786. doi:10.1146/annurev.immunol.21.120601.141025
15. Marone G, De Crescenzo G, Florio G, Granata F, Dente V, Genovese A. Immunological modulation of human cardiac mast cells. *Neurochem Res.* 1999;24: 1195–1202.
16. Clejan S, Japa S, Clemetson C, Hasabnis SS, David O, Talano JV. Blood histamine is associated with coronary artery disease, cardiac events and severity of inflammation and atherosclerosis. *J Cell Mol Med*. 2002;6: 583–592.
17. Vegting Y, Vogt L, Anders HJ, de Winther MPJ, Bemelman FJ, Hilhorst ML. Monocytes and macrophages in ANCA-associated vasculitis. *Autoimmun Rev*. 2021;20(10):102911. doi:10.1016/j.autrev.2021.102911
18. Granowitz EV, Clark BD, Vannier E, Callahan MV, Dinarello CA. Effect of interleukin-1 (IL-1) blockade on cytokine synthesis: I. IL-1 receptor antagonist inhibits IL-1-induced cytokine synthesis and blocks the binding of IL-1 to its type II receptor on human monocytes. *Blood*. 1992;79(9):2356-2363.
19. Zhang YY, Song N, Liu F, et al. activation of the RAS/B-RAF-MEK-ERK pathway in satellite glial cells contributes to substance p-mediated orofacial pain. *Eur J Neurosci*. 2020;51(11):2205-2218. doi:10.1111/ejn.14619
20. Wang M, Pan W, Xu Y, et al. Microglia-Mediated Neuroinflammation: A Potential Target for the Treatment of Cardiovascular Diseases. *J Inflamm Res*. 2022;15:3083-3094.
21. Kapoor K, Bhandare AM, Nedoboy PE, et al. Dynamic changes in the relationship of microglia to cardiovascular neurons in response to increases and decreases in blood pressure. *Neuroscience*. 2016;329:12–29.

22. Francis J, Chu Y, Johnson AK, et al. Acute myocardial infarction induces hypothalamic cytokine synthesis. *Am J Physiol Heart Circ Physiol*. 2004;286(6):H2264–71.

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NEUROLOGICAL COMPLICATIONS ASSOCIATED WITH SCUBA DIVING (WITH AN EMPHASIS ON TAU PROTEIN)

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ABSTRACT

Scuba diving is practised as a recreational sport and for commercial and military purposes. Divers breathe gas mixtures at partial pressure and are subjected to stress from the hyperbaric environment. Decompression illness (DCI) is a serious risk for divers and can affect the nervous system. Cerebral white matter (WM) lesions and cognitive impairment have been identified in divers, and although some cases are linked with DCI, others showed no apparent connection. It has been proposed that the act of diving may have negative neurological consequences regardless of history of DCI, but studies have been inconclusive and hard to interpret. Further research is needed, and blood tau protein levels could be a promising tool to assess neuronal stress associated with diving, in order to determine the long-term neurologic consequences related to scuba diving.

KEYWORDS: *scuba, diving, decompression, neurological, brain, lesion, cognitive, DCI, DCS, AGE*

INTRODUCTION

Scuba diving is practiced by commercial divers, military members, professionals, and recreational sports divers worldwide. Every year in the United States alone, approximately 3 million people practice diving recreationally, and the sport is gaining in popularity (1). Divers breathe a mix of gases at partial pressure and are subjected to changes in hydrostatic and atmospheric pressure that is exerted on the body as they descend and ascend.

There are certainly benefits for those who practice diving, such as the physical activity, social interaction, and stress reduction it can provide, as well as the opportunity to immerse in nature and open blue spaces of water (2). However, the human body is very sensitive to changes in ambient pressure, with hyperbaric conditions producing pulmonary, circulatory, and cardiac changes during the compression and decompression stages of immersion in water. There are serious risks as well, and apart from drowning, cold temperatures, and possible equipment failure, physiological changes can lead to complications such as oxygen toxicity, nitrogen narcosis, barotrauma to the lungs and sinuses, and decompression illness (DCI).

DCI can affect the nervous system, and some studies have indicated that diving itself, even in the case of undocumented DCI, may cause alterations to cerebral white matter (WM) and affect cognitive functions. However, results have been conflicting and hard to interpret, with some showing CNS lesions without a clear cause. Currently, no definite consensus has been reached and further research is necessary to validate the investigations presented.

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This review will look at the correlation between neurological complications and cerebral damage that has been presented as of now, and the importance this may have for the long-term health outcome of those who partake in scuba diving.

Decompression illness

DCI is caused by the formation of intravascular or extravascular gas bubbles as a result of decompression. This term encompasses both decompression sickness (DCS) and arterial gas embolism (AGE), the two main decompression pathologies that afflict scuba divers (3). Both DCS and AGE are caused by bubbles; In DCS gas bubbles are formed in venous blood and tissue, and in AGE the bubbles enter the arterial circulation. Each causes damage to the particular afflicted tissue by ways of different mechanisms (4). DCS tends to have a delayed onset, and progressively worsen after the dive, whereas that of AGE is sudden (5).

Scuba divers breathe a mixture of gases that include oxygen, nitrogen, and sometimes helium. During descent, the increase in ambient pressure causes nitrogen to become dissolved in bodily fluids and tissues, and the amount of nitrogen bubbles that is accumulated depends on the bottom time (time at reached depth) and the level of depth, in addition to numerous individual factors of variability. During ascent, the change from a high to a lower-pressure environment draws the dissolved inert nitrogen out of these fluids and tissues. A slow, controlled ascent can allow nitrogen to be eliminated safely, but a critical amount of buildup with an uncontrolled or rapid ascent can cause it to revert to a gas and form bubbles in the blood or tissues, causing DCS (6). DCS is a multisystem disorder and symptoms can vary greatly, ranging from harmless joint pains to serious complications such as cerebral gas embolism and death (7).

The extent of bubble formation, the gas load, predicts the severity of DCS, which can be classified as two types. Type I DCS, the milder form, involves the musculoskeletal system and skin, causing joint and limb pain, and rashes or itching (8). Type II DCS, the serious form, involves the central nervous system (CNS) and the thoracic level spinal cord is commonly affected. Although the spinal cord can be affected by Type II DCS, it is rare to have cerebral injury in addition to spinal cord injury (9). Although rare, cerebral DCI is very serious and can be life-threatening (10). Nitrogen is far more soluble in fat when compared to blood. It is five times more soluble in lipids and adipose tissue, and fat will act as a nitrogen reservoir, suggesting that obesity could be a risk factor for DCS (11). The WM of the spinal cord is particularly sensitive as nitrogen is highly soluble in myelin (9).

AGE occurs when bubbles enter directly into arterial circulation, possibly due to an overexpansion injury such as pulmonary baurotrauma, where they can result in emboli in distal arterioles and cause tissue or organ damage (12). Usually, the lungs can filter small bubbles in the venous system, and they are unnoticeable. However, pulmonary baurotrauma can be caused by breath-holding during ascent, uncontrolled ascent, or by lung diseases such as asthma or bronchitis, and leads to lung hyperexpansion, which allows gas bubbles to enter directly into the blood stream (13).

The brain is predominantly affected by arterial bubbles, as it receives a large proportion of blood flow (5,14). If gas bubbles are arterialized into the brain, it can cause stroke-like symptoms and a transient embolism, and the diver will typically lose consciousness within 10 minutes of surfacing. Embolism will occur in multiple areas, with different lesions causing crossed neurological deficits. Neurological symptoms can appear minor, such as tingling or numbness, motor weakness, or difficulty in thinking, or include paralysis, sensory loss, visual disturbances, and convulsions (14).

"Right-to-left" cardiac or pulmonary shunts could allow otherwise asymptomatic vascular gas bubbles to enter the arterial circulation and cause AGE. An increased risk has been associated with the presence and size of patent foramen ovale (PFO) of the heart, which acts as a "right-to-left" shunt where bubbles can cross into arterial circulation (15,16).

An AGE in the brain stem causes blood pressure to increase and dilatation of cerebral arterioles, leading to cerebrovascular autoregulation, cardiac arrhythmias, and possibly cardiac arrest and respiratory depression (13). Immediate death can result if the gas bubbles in the brain stem are large enough to block blood flow (13).

Both DCS and AGE, enveloped within the term DCI, follow the same course of treatment. Mild symptoms after diving may tend to be ignored or be attributed to other causes initially, but treatment must be initiated as soon as possible to improve the likelihood of a good outcome (14). The first course is breathing 100% oxygen by mask, followed by recompression with hyperbaric therapy (5). Treatment has high rates of success, although serious cases require more recompression sessions and there can be residual deficits and brain damage (3).

Proper dive safety procedures have been implemented to control the slow release of N during the ascent phase to minimize the risk of DCS (17), but apart from accidents and failure to adhere to protocol, there are numerous individual factors that are risks for the development of DCS. Some of the potential risk factors include alcohol consumption, dehydration, overexertion, obesity, injury and fatigue, thermal stress, and performing multiple ascents, and consecutive dives and days of diving (13).

CNS involvement in decompression illness

DCS includes many different symptoms, ranging from mild to severe, but neurological symptoms are well-documented and considered the hallmark of serious cases (9,18). Neurological symptoms usually appear one hour after resurfacing and may include confusion, difficulty in concentration and coordination, paresthesia and dysesthesia, lethargy, vertigo, motor weakness, bowel and bladder disfunction, and paralysis (8,19). Entrapped vascular bubbles may cause cellular injury, increased permeability of the blood-brain barrier (BBB), cerebral edema (20), and stroke-like symptoms (3). There is also a greater risk of neurological DCS for divers with PFO, especially if it is large (15).

Recreational divers breathe compressed air, which is commonly 79% nitrogen. In the CNS, nitrogen could be accumulated by myelin, the lipid-rich substance produced by glial cells, under hyperbaric conditions, although myelin alterations have not been studied in divers as of yet (21). Nitrogen is highly soluble in fat and due to the high amount of blood flow to the brain, and in the presence of hyperbaric conditions, the gas is carried and dissolves in the myelin sheaths of neurons, where the bubbles may cause mechanical disruption and affect the functioning of WM (21,22).

A recent study by Coco et al. of 54 professional divers utilized Diffusion Tensor Imaging and neuropsychological testing to study the effects of diving on brain WM and cognitive abilities. Anterior WM alterations were present, as well as impaired attention and memory functions of the prefrontal cortex, suggesting that repeated dives may build-up micro-lesions in the CNS, presumably affecting the myelin sheet of neurons (21).

Different theories have been proposed to explain how bubbles damage the CNS. Arterial occlusion, venous infarction, and in situ nitrogen toxicity have been proposed as the cause. The presence of cerebral lesions from AGE similar to those of stroke, the fact that cerebral blood flow can be obstructed by bubbles, the higher risk of DCI in those with a PFO, and hypoperfused areas identified by single-photon emission computed tomography (SPECT) give support for damage by arterial occlusion (10,23,24). Microvascular damage may progress over time by "silent embolism", when inert gas bubbles cause slight damage that can accumulate with repeated dives. The "silent" bubbles could cause subclinical cerebral vasculopathy, without the presence of DCI, and lead to some unexplained findings in some studies (25). Venous infarction may also be a cause and has been supported by different radiologic and histopathologic findings (26-28). And finally, the theory regarding in situ nitrogen toxicity claims that bubbles can alter nerve conduction and be toxic to neurons, leading to cytotoxic edema and cell death (29). But these mechanisms appear to be interlaced in patients and the complexity of neurological DCI has yet to be elucidated.

Cognitive impairment and white matter damage

Numerous studies have been conducted to identify the neurological effects of diving. Neuropsychological and neurobehavioral tests, electroencephalograms (EEGs), and SPECT scans have been used in studies to determine the neurological function and extent of effects, and magnetic resonance imaging (MRI) has been used to assess areas where WM damage has occurred.

It has been seen that DCI can lead to nervous system damage, which could have possible long-term neurologic effects. One study by Bast-Pettersen et al. found no long-term neuropsychological effects in recreational divers after a 12-year follow-up, but impaired memory and neuropsychiatric symptoms were shown to affect divers who had a history of DCI (30). EEG has also shown abnormalities in the temporal regions of commercial saturation divers, which was exacerbated by a history of DCI (31). However, another study by Murrison et al. found no abnormalities in EEG in divers who had experienced DCI, and no evidence to support involvement of the brain (32).

The presence of a PFO, especially a large one, is a risk factor in diving, as it has been linked to higher rates of DCI and brain lesions (33), likely caused by an AGE that enters the arterial circulation through the PFO. A study by Reul et al. found a high percentage of brain lesions, and multiple lesions, came from a subgroup of 27% of studied divers, which could suggest the involvement of the PFO, which is present in 10-30% of the general population (34). Knauth et al. were able to link multiple brain lesions with the presence of a large PFO, despite the absence of DCI (33). Still, another study by Balestra et al. showed that divers with PFO showed no greater prevalence of WM lesions when compared to non-PFO divers (16).

However, most dives are asymptomatic, meaning that DCI does not occur, and it is still unclear how the act of asymptomatic diving itself could affect the nervous system in the long-term, as studies have shown mixed results. MRI and EEG have provided conflicting results, and it could be that these methods are not sufficiently sensitive to detect cerebral changes associated with diving (35).

Different studies have shown negative effects in asymptomatic diving (21,25,36-43).

A study of 113 military divers by Erdem et al. investigated the prevalence of lesions in divers with similar parameters (blood pressure, smoking, alcohol consumption, history of head trauma or migraine) against a non-diving control group. They found a higher incidence of cerebral WM lesions, which was not affected by age or dive history (39). An MRI study

by Gempp et al. in military divers showed a higher prevalence of brain hyperintense spots and WM changes in divers when compared to a control group, and especially in divers with right-to-left shunting (40).

Very deep dives, usually performed by commercial saturation divers, could exacerbate the damage and repeated deep diving can have more severe effects (41,42). Long-term effects may be influenced by extreme conditions, number of dives, and deep depth (38,44-46). While engaging in dives to depths of 50 meters or more, during the compression phase bottom time, and immediately after resurfacing, neurological and neurophysiologic effects have been shown in divers (43).

Some studies have shown a correlation between impaired cognitive function and higher number of dives (21,25). Coco et al. found that WM alterations and mild associated cognitive impairment increased with a high number of dives, independent of the age of divers, when compared to a non-diving control group (21).

In contrast to these studies, others have shown little neurologic effects associated with asymptomatic diving (47-50).

An experimental rodent study investigated the effects of severe decompression, such as that experienced by commercial saturation divers, on the brain. It was seen that there were circulatory changes in the brain during the acute phase of decompression, but structural or cellular injury to brain tissue was not present, even after 2 weeks follow-up (47). A 2000 study with MRI showed no differences in WM damage between a group of experienced elderly divers and a control group of non-divers (48). Another study of the same year by Cordes et al. found no abnormal neurologic findings with neuropyschometric test results, and no increased prevalence of cerebral lesions in military divers (49). Finally, interesting research by Hemelryck et al. compared the cognitive functioning of scuba divers with a healthy control group as well as to professional boxers, who are at high risk of brain damage. The divers showed memory deficits when compared to the control group, but performed much better than the boxers, who had the lowest results and showed the most cognitive function deficiency (50).

In summary, as to the relationship of diving having negative long-term effects on the brain, affecting cognitive function, and causing lesions, studies until now have provided conflicting results and have so far been inconclusive.

Tau protein

Some recent research to determine the harmful effects of the hyperbaric environment on the CNS has begun to focus on tau protein levels, as tau may be an indicator of neuronal stress in diving.

Breathing partial pressures of oxygen and nitrogen at depth could increase reactive oxygen species (ROS) production and oxidative stress, which could cause neuronal damage (51), and could be observed by biochemical markers such as tau.

Tau protein is a microtubule-associated protein (MAP) found in the neurons of the CNS, and their dysfunction, and successive formation of neurofibrillary tangles, is linked to different neurodegenerative diseases such as Alzheimer's disease and chronic traumatic encephalopathy (52). It can be released after axonal damage and increased neuronal activity in response to stress. Increased tau levels in the blood have also been seen in association with traumatic brain injuries (53) and in contact sports where concussions are common, such as boxing (54,55). High intensity interval training and breath-hold diving have been seen to raise tau levels after activity (56,57), and tau seems to be unrelated to DCI (58), which could prove useful for focusing on CNS damage inflicted by the act of diving itself.

Studies by Rosén et al. have found increased blood tau protein levels after diving, with no identified correlation between absolute tau concentrations and venous gas loads in divers. A small, 2019 pilot study of 10 divers, who performed repeated deep dives between 52 and 90 meters over four days, found serum tau concentration was increased after diving by 2.5 times (59). In another recent study, Rosén et al. measured the blood tau levels of 32 divers in a water-filled hyperbaric chamber, for a time of 10 minutes, to simulate a dive pressurized to 42 meters. Blood was sampled from the divers before diving and two intervals afterwards at 35-40 and 120 minutes. Tau levels were seen to increase after diving at the 35-40 min, and were further increased at 120 min, and the study was repeated with uniform results (60).

Future research using blood biomarkers such as tau could be useful for investigating neuronal damage from scuba diving.

Significance

Scuba diving is a relatively new activity and has been growing in popularity as a recreational sport. Because of this, the long-term effects of continuous diving are now being investigated and, considering the growing number of people who are participating in diving globally and the aging population of divers, it is becoming increasingly important to determine the neurological health effects that may be associated with it.

As discussed, studies have provided conflicting results, with some showing the potential neurologic damage that could be incurred by diving and others being unconclusive. Some have shown that cognitive impairment and WM lesions have

been linked to diving in people with a history of DCI and even in those without. It may be possible that the higher prevalence of cerebral lesions seen in some studies of divers without a history of DCI could be due to cumulative, subclinical injury to the neurological system caused by inert nitrogen gas bubbles during diving (39).

WM lesions are a common characteristic seen by MRI in adults (61), especially in the aging brain, with 90% prevalence in people 65 years of age and older (62). Lesions have been associated with dementia, depression, Alzheimer's Disease, and cognitive decline (63-65). They may be non-specific, but sources of lesions are many, and can include vascular diseases, untreated chronic hypertension, migraine, inflammatory disorders, infectious diseases, alcohol abuse, metabolic disorders, and traumatic brain injuries, amongst others. Just because lesions are revealed in divers by MRI does not mean they are the direct consequence of diving.

And the presence of brain lesions does not correlate directly with reduced cognitive functioning, although it has been shown there may be a relationship in some studies. More research combining MRI and neuropsychological testing is needed to define the relationship of these brain lesions to neurologic performance.

Causation and correlation must be established in further studies by limiting for the various independent factors and methodological consistency. Numerous independent factors can interfere with results, including age, the presence of a PFO, prior head injuries and brain damage, and cardiovascular diseases including high cholesterol and hypertension.

Many studies conducted as to date have methodological flaws and biases, which could explain the inconclusive results between similar studies. Selection biases, varying degrees of age, of diving experience, and number of dives, and insufficient detection sensitivity of MRI are all factors that could be responsible for the conflicting results from studies (16). Furthermore, these studies are methodologically diverse, each reaching an independent conclusion instead of a collective result. Finally, psychometric function testing should be correlated to imaging-detected cerebral points of interest for verification (16).

Tau protein levels may be a useful tool for indicating neuronal stress caused by diving, and research should be continued to provide further insight. More studies are needed to elaborate the correlation of WM damage with diving, establish the causation, and determine the significance of lesions for the long-term neurological health outcome of divers.

CONCLUSIONS

The commercialization of diving has led to high safety standards and diving today is considered relatively safe when safety protocol is followed (66). However, DCI is a great risk for divers, even when depth and time regulations are followed, due to accidents, rapid ascent, and a high variability of independent factors that are associated with its occurrence. DCS and AGE can have serious health consequences for divers, but the risk can be minimized by attentively following safety protocol.

Studies on the possible neurological effects related to diving have been conflicting, with some showing WM lesions and cognitive impairment in divers, and others showing no evidence of this. Overall, the causation of such research cannot be clearly related to diving, as those studies regarding the neurological consequences of diving have not yet been able to prove a direct correlation between cerebral damage and asymptomatic diving. Numerous variables must be accounted for to determine the underlying cause of WM damage and related cognitive deficits. Further research is needed to clarify the long-term, cumulative neurological effects that could be caused by the act of diving. Biochemical markers of neuronal damage, such as tau protein, could be useful for assessing increased neuronal activity in response to stress.

Deep diving and diving in severe conditions causes a high level of decompression stress on the body and carries a higher risk of DCI, and it could also be true that this carries a higher risk for long-term health outcome, as some studies have shown possible neurological effects. Therefore, DCI should remain the biggest concern and divers should aim to practice their sport in a conscientious and prudent manner to avoid the occurrence of DCS and AGE.

Conflict of interest

The author declares that they have no conflict of interest.

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REFERENCES

1. (SFIA) Sports and Fitness Industry Association. Participation in Recreational Diving Report. Silver Spring, MD.: Sports and Fitness Industry Association. 2015.
2. Carreño A, Gascon M, Vert C, Lloret J. The Beneficial Effects of Short-Term Exposure to Scuba Diving on Human Mental Health. *International Journal of Environmental Research and Public Health*. 2020;17(19):7238. doi:10.3390/ijerph17197238
3. Vann RD, Butler FK, Mitchell SJ, Moon RE. Decompression illness. *The Lancet.* 2011;377(9760):153-164. doi:10.1016/s0140-6736(10)61085-9
4. Bosco G, Rizzato A, Moon RE, Camporesi EM. Environmental Physiology and Diving Medicine. *Frontiers in Psychology*. 2018;9. doi:10.3389/fpsyg.2018.00072
5. Gorman DF. Arterial gas embolism as a consequence of pulmonary barotrauma. Diving and Hyperbaric Medicine, European Underwater Biological Society. 1984:348-368.
6. Moon RE, Vann RD, Bennett PB. The Physiology of Decompression Illness. *Scientific American.* 1995;273(2):70-77. doi:10.1038/scientificamerican0895-70
7. Stephensen JC. Pathophysiology, Treatment and Aeromedical Retrieval of SCUBA - Related DCI. *Journal of Military and Veterans' Health*. 2009;17(3):10-19.
8. Hawes J, Massey EW. Neurologic Injuries from Scuba Diving. *Physical Medicine and Rehabilitation Clinics of North America.* 2009;20(1):263-272. doi:10.1016/j.pmr.2008.10.018
9. Jallul S, Osman A, El-Masry W. Cerebro-spinal decompression sickness: report of two cases. *Spinal Cord.* 2007;45(1):116-120. doi:10.1038/sj.sc.3101923
10. Kamtchum Tatuene J, Pignel R, Pollak P, Lovblad KO, Kleinschmidt A, Vargas MI. Neuroimaging of Diving-Related Decompression Illness: Current Knowledge and Perspectives. *American Journal of Neuroradiology*. 2014;35(11):2039-2044. doi:10.3174/ajnr.a4005
11. Gabler-Smith MK, Westgate AJ, Koopman HN. Microvessel density, lipid chemistry and N2 solubility in human and pig adipose tissue. *Undersea and Hyperbaric Medicine.* 2020;47(1):1-12. doi:10.22462/01.03.2020.1
12. Kemper T, Rienks R, van Ooij P, van Hulst R. Cutis marmorata in decompression illness may be cerebrally mediated: a novel hypothesis on the aetiology of cutis marmorata. *Diving Hyperb Med.* 2015;45(2):84-88.
13. Gorman DF. Decompression Sickness and Arterial Gas Embolism in Sports Scuba Divers. *Sports Medicine.* 1989;8(1):32-42. doi:10.2165/00007256-198908010-00004
14. Thalmann E. Decompression Illness. Divers Alert Network. https://dan.org/health-medicine/health-resources/diseases-conditions/decompression-illness-what-is-it-and-what-is-the-treatment
15. Denoble P, Holm J. Guidelines for Patent Foramen Ovale and Fitness. Proceedings Summary | DAN/UHMS PFO and Fitness to Dive Workshop. www.DAN.org.
16. Balestra C, Germonpré P. Correlation between Patent Foramen Ovale, Cerebral "Lesions" and Neuropsychometric Testing in Experienced Sports Divers: Does Diving Damage the Brain? *Frontiers in Psychology*. 2016;7:696. doi:10.3389/fpsyg.2016.00696
17. Howle LE, Weber PW, Hada EA, Vann RD, Denoble PJ. The probability and severity of decompression sickness. West J, ed. *PLOSONE*. 2017;12(3):e0172665. doi:10.1371/journal.pone.0172665
18. Moon R, Gorman D, Treatment of the decompression disorders. In: Brubakk AO, Neuman TS (eds*). Bennett and Elliott's Physiology and Medicine of Diving.* 5th ed. (Bennett PB, Elliott DH, Brubakk AO, eds.). Saunders; 2007:600-651.
19. Newton H, Padilla W, Burkart J, Pearl D. Neurological manifestations of decompression illness in recreational divers - the Cozumel experience. *Undersea Hyperb Med.* 2007;34(5):349-357.
20. Hjelde A, Nossum V, Steinsvik M, Bagstevold JI, Brubakk AO. Evaluation of cerebral gas retention and oedema formation in decompressed rats by using a simple gravimetric method. *Scandinavian Journal of Clinical and Laboratory Investigation.* 2002;62(4):263-270. doi:10.1080/003655102760145816
21. Coco M, Buscemi A, Perciavalle V, et al. Cognitive Deficits and White Matter Alterations in Highly Trained Scuba Divers. *Frontiers in Psychology*. 2019;10. doi:10.3389/fpsyg.2019.02376

22. Francis T, Pezeshkpour G, Dutka A, Hallenbeck J, Flynn E. Space occupying lesions in experimental spinal cord decompression sickness may be evidence of autochthonous bubble injury. *Undersea Biomedical Research* 14 (Suppl). 1987;6.
23. Leitch D, Green R. Pulmonary barotrauma in divers and the treatment of cerebral arterial gas embolism. *Aviat Space Environ Med.* 1986;57(10 pt 1):931-938.
24. Koch A, Kirsch H, Reuter M, Warninghoff V, Rieckert H, Deuschl G. Prevalence of patent foramen ovale (PFO) and MRI-lesions in mild neurological decompression sickness (type B-DCS/AGE). *Undersea Hyperb Med.* 2008;35:197-205.
25. Kowalski J, Varn A, Röttger S, et al. Neuropsychological deficits in scuba divers: an exploratory investigation. *Undersea Hyperb Med.* 2011;38(3):197-204.
26. Vollmann R, Lamperti M, Magyar M, Simbrunner J. Magnetic Resonance Imaging of the Spine in a Patient with Decompression Sickness. *Clinical Neuroradiology.* 2011;21(4):231-233. doi:10.1007/s00062-011-0053-x
27. Kim RC, Smith HR, Henbest ML, Choi BH. Nonhemorrhagic venous infarction of the spinal cord. *Annals of Neurology.* 1984;15(4):379-385. doi:10.1002/ana.410150413
28. Sparacia G, Banco A, Sparacia B, et al. Magnetic resonance findings in scuba diving-related spinal cord decompression sickness. *Magma: Magnetic Resonance Materials in Physics, Biology, and Medicine.* 1997;5(2):111-115. doi:10.1007/bf02592241
29. Kei PL, Choong CT, Young T, Lee SH, Lim CCT. Decompression Sickness: MRI of the Spinal Cord. *Journal of Neuroimaging.* 2007;17(4):378-380. doi:10.1111/j.1552-6569.2007.00122.x
30. Bast-Pettersen R, Skare Ø, Nordby KC, Skogstad M. A twelve-year longitudinal study of neuropsychological function in non-saturation professional divers. *International Archives of Occupational and Environmental Health.* 2014;88(6):669-682. doi:10.1007/s00420-014-0991-0
31. Todnem K, Skeidsvoll H, Svihus R, et al. Electroencephalography, evoked potentials and MRI brain scans in saturation divers. An epidemiological study. *Electroencephalography and Clinical Neurophysiology.* 1991;79(4):322-329. doi:10.1016/0013-4694(91)90127-p
32. Murrison AW, Glasspool E, Pethybridge RJ, Francis TJ, Sedgwick EM. Electroencephalographic study of divers with histories of neurological decompression illness. *Occupational and Environmental Medicine.* 1995;52(7):451-453. doi:10.1136/oem.52.7.451
33. Knauth M, Ries S, Pohimann S, et al. Cohort study of multiple brain lesions in sport divers: role of a patent foramen ovale. *BMJ.* 1997;314(7082):701-701. doi:10.1136/bmj.314.7082.701
34. Reul J, Jung A, Thron A, Weis J, Willmes K. Central nervous system lesions and cervical disc herniations in amateur divers. *The Lancet.* 1995;345(8962):1403-1405. doi:10.1016/s0140-6736(95)92598-8
35. Grønning M, Risberg J, Skeidsvoll H, et al. Electroencephalography and magnetic resonance imaging in neurological decompressionsickness. *Undersea Hyperb Med.* 2005;32(6):397-402.
36. Peters BH, Levin HS, Kelly PJ. Neurologic and psychologic manifestations of decompression illness in divers. *Neurology.* 1977;27(2):125-125. doi:10.1212/wnl.27.2.125
37. Curley M, Schwartz H, Zwingelberg K. Neuropsychologic assessment of cerebral decompression sickness and gas embolism. *Undersea Biomed Res*. 1988;15(3):223-236.
38. Tetzlaff K, Friege L, Hutzelmann A, Reuter M, Höll D, Leplow B. Magnetic Resonance Signal Abnormalities and Neuropsychological Deficits in Elderly Compressed-Air Divers. *European Neurology.* 1999;42(4):194-199. doi:10.1159/000008106
39. Erdem I, Yildiz S, Uzun G, et al. Cerebral White-Matter Lesions in Asymptomatic Military Divers. *Aviation, Space, and EnvironmentalMedicine*. 2009;80(1):2-4. doi:10.3357/asem.2234.2009
40. Gempp E, Sbardella F, Stephant E, et al. Brain MRI Signal Abnormalities and Right-to-Left Shunting in Asymptomatic Military Divers. *Aviation, Space, and Environmental Medicine.* 2010;81(11):1008-1012. doi:10.3357/asem.2786.2010
41. Vaernes R, Aarli J, Kløve H, Tønjum S. Differential neuropsychological effects of diving to 350 meters. *Aviat Space Environ Med.* 1987;58(2):155-165.
42. Vaernes R, Kløve H, Ellertsen B. Neuropsychologic effects of saturation diving. *Undersea Biomed Res.* 1989;16(3):233-251.
43. Grønning M, Aarli JA. Neurological effects of deep diving. *Journal of the Neurological Sciences.* 2011;304(1-2):17-21. doi:10.1016/j.jns.2011.01.021

44. Slosman D, De Ribaupierre S, Chicherio C, et al. Negative neurofunctional effects of frequency, depth and environment in recreational scuba diving: the Geneva "memory dive" study. *British Journal of Sports Medicine.* 2004;38(2):108-114. doi:10.1136/bjsm.2002.003434
45. Taylor CL, Macdiarmid JI, Ross JA, et al. Objective neuropsychological test performance of professional divers reporting a subjective complaint of "forgetfulness or loss of concentration." *Scandinavian Journal of Work, Environment & Health.* 2006;32(4):310-317. doi:10.5271/sjweh.1015
46. Leplow B, Tetzlaff K, Höll D, Zeng L, Reuter M. Spatial orientation in construction divers - are there associations with diving experience? *International Archives of Occupational and Environmental Health.* 2001;74(3):189-198. doi:10.1007/s004200000155
47. Havnes MB, Widerøe M, Thuen M, Torp SH, Brubakk AO, Møllerløkken A. Simulated dive in rats lead to acute changes in cerebral blood flow on MRI, but no cerebral injuries to grey or white matter. *European Journal of Applied Physiology.* 2012;113(6):1405-1414. doi:10.1007/s00421-012-2565-8
48. Hutzelmann A, Tetzlaff K, Reuter M, Müller-Hülsbeck S, Heller M. DOES DIVING DAMAGE THE BRAIN?: MR control study of divers' central nervous system. *Acta Radiologica.* 2000;41(1):18-21. doi:10.1080/028418500127344894
49. Cordes P, Keil R, Bartsch T, et al. Neurologic outcome of controlled compressed-air diving. *Neurology.* 2000;55(11):1743-1746. doi:10.1212/wnl.55.11.1743
50. Hemelryck W, Germonpré P, Papadopoulou V, Rozloznik M, Balestra C. Long term effects of recreational SCUBA diving on higher cognitive function. *Scandinavian Journal of Medicine & Science in Sports.* 2013;24(6):928-934. doi:10.1111/sms.12100
51. Mrakic-Sposta S, Vezzoli A, D'Alessandro F, Paganini M, Dellanoce C, Cialoni D, Bosco G. Change in Oxidative Stress Biomarkers During 30 Days in Saturation Dive: A Pilot Study. *Int J Environ Res Public Health.* 2020;17(19):7118. doi: 10.3390/ijerph17197118
52. Sinsky J, Pichlerova K, Hanes J. Tau Protein Interaction Partners and Their Roles in Alzheimer's Disease and Other Tauopathies. *International Journal of Molecular Sciences*. 2021;22(17):9207. doi:10.3390/ijms22179207
53. Walker KR, Tesco G. Molecular mechanisms of cognitive dysfunction following traumatic brain injury. *Frontiers in Aging Neuroscience.* 2013;5. doi:10.3389/fnagi.2013.00029
54. Zetterberg H, Hietala MA, Jonsson M, et al. Neurochemical Aftermath of Amateur Boxing. *Archives of Neurology.* 2006;63(9):1277. doi:10.1001/archneur.63.9.1277
55. Shahim P, Tegner Y, Marklund N, Blennow K, Zetterberg H. Neurofilament light and tau as blood biomarkers for sports-related concussion. *Neurology.* 2018;90(20):e1780-e1788. doi:10.1212/wnl.0000000000005518
56. Gren M, Shahim P, Lautner R, et al. Blood biomarkers indicate mild neuroaxonal injury and increased amyloidβ production after transient hypoxia during breath-hold diving. *Brain Injury.* 2016;30(10):1226-1230. doi:10.1080/02699052.2016.1179792
57. Di Battista AP, Moes KA, Shiu MY, et al. High-Intensity Interval Training Is Associated With Alterations in Blood Biomarkers Related to Brain Injury. *Frontiers in Physiology.* 2018;9. doi:10.3389/fphys.2018.01367
58. Shahim P, Arnell P, Kvarnström A, et al. Cerebrospinal fluid markers of central nervous system injury in decompression illness – a case-controlled pilot study. *Diving Hyperb Med.* 2015;45(4):240-243.
59. Rosén A, Oscarsson N, Kvarnström A, et al. Serum tau concentration after diving – an observational pilot study. *Diving and Hyperbaric Medicine Journal*. 2019;49(2):88-95. doi:10.28920/dhm49.2.88-95
60. Rosén A, Gennser M, Oscarsson N, et al. Protein tau concentration in blood increases after SCUBA diving: an observational study. *European Journal of Applied Physiology.* 2022;122(4):993-1005. doi:10.1007/s00421-022-04892-9
61. Weidauer S, Wagner M, Hattingen E. White Matter Lesions in Adults – a Differential Diagnostic Approach. *RöFo - Fortschritte auf dem Gebiet der Röntgenstrahlen und der bildgebenden Verfahren.* 2020;192(12):1154-1173. doi:10.1055/a-1207-1006
62. de Leeuw F-E. Prevalence of cerebral white matter lesions in elderly people: a population based magnetic resonance imaging study. The Rotterdam Scan Study. *Journal of Neurology, Neurosurgery & Psychiatry*. 2001;70(1):9-14. doi:10.1136/jnnp.70.1.9
63. Vermeer SE, Prins ND, den Heijer T, Hofman A, Koudstaal PJ, Breteler MMB. Silent brain infarcts and the risk of dementia and cognitive decline. *The New England Journal of Medicine.* 2003;348(13):1215-1222. doi:10.1056/NEJMoa022066

64. Barber R, Scheltens P, Gholkar A, et al. White matter lesions on magnetic resonance imaging in dementia with Lewy bodies, Alzheimer's disease, vascular dementia, and normal aging. *Journal of Neurology, Neurosurgery & Psychiatry*. 1999;67(1):66-72. doi:10.1136/jnnp.67.1.66

65. Debette S, Markus HS. The clinical importance of white matter hyperintensities on brain magnetic resonance imaging: systematic review and meta-analysis. *BMJ*. 2010;341(jul26 1):c3666-c3666. doi:10.1136/bmj.c3666

66. Dimmock K, Cummins T. "History of scuba diving tourism," in Scuba Diving Tourism eds Musa G, Dimmock K. *Oxon*;14-28.

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HIDDEN IN PLAIN SIGHT: IMPROVING EARLY RECOGNITION AND INTERVENTIONS IN HIGH-FUNCTIONING AUTISM

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ABSTRACT

Autism Spectrum Disorder (ASD) is a heterogeneous neurodevelopmental condition characterized by deficits in social interaction and communication, restricted and repetitive behaviours, and sensory and learning defects. The category includes autistic, pervasive development, and Asperger's disorders, with a spectrum of impairment of symptoms ranging from mild to severe. High-functioning autism (HF ASD) refers to the milder form of the disorder, in which individuals can use language superficially and have a normal or above-average IQ. This milder presentation of ASD often goes unrecognized in females, children with high intellectual capacity, and those with less severe symptoms. Individuals with HF ASD experience difficulties with emotional regulation, and there is a higher prevalence of depression, anxiety, self-harm, and suicide attempts. In addition, HF ASD comes with higher rates of misdiagnosis and psychiatric hospitalizations, and individuals face daily challenges to function in their environment. In order to improve recognition of HF ASD, clinicians should pay close attention to complicated or unclear presentations of ASD. This paper aims to confront the factors contributing to poor recognition of HF ASD and outlines early recognition and intervention improvements.

KEYWORDS: *autism spectrum disorder, high-functioning, neurodevelopmental, disorder, recognition, intervention*

INTRODUCTION

Autism Spectrum Disorder (ASD) is a heterogeneous, highly heritable, neurodevelopmental condition that includes autistic, pervasive development, and Asperger's disorders. It is characterized by dysfunctional social interaction and communication, repetitive behaviours, and sensory and learning defects (1). ASD is a common neurodevelopmental disorder, and diagnosis rates have increased dramatically over the past two decades, with prevalence rates steadily increasing from 1 to 150 children in 2000 to 1 to 44 children in 2018 (2-3).

The category of ASD contains a broad range of impairments, presenting with severe to mild symptoms. The milder form was referred to in the past as high-functioning autism (HF ASD), thus, it will be referred to as such in this article.

The diagnosis's severity depends on the degree of social impairment and repetitive behaviours, criteria outlined in the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) (1).

ASD is a lifelong condition and is normally diagnosed early, with the onset of symptoms usually presenting within the first three years of life. Behavioural and development presentations are used for diagnosis, including clinical specifiers related to language, movement, adaptation, cognitive skills and comorbidity.

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Autism presents differently in milder and higher-functioning individuals who require minimal daily support. Individuals with HF ASD have average, sometimes above-average IQs and can use language, albeit superficially.

However, the term "high-functioning" is misleading, as it refers solely to the context of autistic disorders. Compared to the general public, these individuals are no longer "high-functioning" and face many challenges regarding their actual ability to function in their environment. People with this type of autism have significant difficulties with emotional regulation (4), higher rates of depression (5), anxiety (6-7), self-harm (8), and suicide attempts (9). In addition, there are higher rates of psychiatric hospitalizations in previously undiagnosed autism (10). For these reasons, it is important to improve early recognition and interventions in HF ASD.

Factors contributing to poor recognition of high-functioning ASD

People with HF ASD have an average or above-average IQ and can use language for communication, although it may be in a superficial manner; this may contribute to a missed or late diagnosis, as a mild presentation of autism is often missed. Females and children with high intellectual capacity are at higher risk for poor recognition. ASD has a prevalence rate of 3-4 males to every female (11), which could affect diagnosis by gender bias. Females may also be less diagnosed due to concealing behaviours, such as masking.

Many factors can contribute to a missed or late ASD diagnosis, including camouflaging behaviours such as masking and mimicking, internalizing behaviours, mistaken social competence, and advanced verbal language skills. Masking behaviours, hiding certain personality traits, may be utilized to adjust to the surrounding social environment. For example, mimicking, the imitation of gestures, facial expressions, movement, and speech, may be used for peer acceptance in social situations. Individuals may have trained themselves to use eye contact, smile, or utilize certain phrases and gestures, for example, for social engagement. These behaviours help individuals "appear to be normal", camouflage their actual social-communication difficulties, and lead to a mistaken social competence (12-13). Mimicking and masking are commonly seen in females with HF ASD (14-15).

Internalization can result in poor self-esteem and lead to emotional disorders, such as depression or anxiety. In addition, co-occurring psychiatric disorders may conceal the actual neurodevelopmental disorder of ASD, resulting in late diagnosis and improper support.

The complexity of social demands and expectations advances as children grow, so as time goes on, problems may become apparent and result in a late diagnosis.

Presentation of high-functioning ASD

Areas of difficulty in individuals with HF ASD often fall in the following triad: cognitive rigidity, social-communication deficits, and sensory deficits.

Cognitive rigidity is the "inability to mentally adapt to new demands or information" (16). There may be a "black and white" viewpoint, with little space for alternatives, whether that be viewpoints or ideas. In daily life, the inflexible individual may be described as "stubborn", "defiant", "difficult", "does not go with the flow", "insists on having things done only in a specific way", and "refuses to cooperate". They think in literal terms and show difficulties with adapting to unexpected change.

Social-communication deficits present difficulties in integrating social norms and context, as well as social perspective-taking. Social reputation is not recognized and does not serve as a motivator (17). There is difficulty in social contexts in relating to the feelings and beliefs of others, with failure in perspective taking. Someone with HF ASD may be referred to as "unfiltered", speaking without regard to the situation or individual in their presence. It may be noted that they do not understand sarcasm, due to poor understanding of situational context, communication tone and body language. Instead, metaphors or irony may be interpreted literally and are misinterpreted. Although there may be a desire to engage socially, there may be a failure to initiate interactions due to a lack of social skills (18-19).

Over-responsivity to sensory stimuli encountered in daily life is another difficulty commonly presented. Sensory deficits include sensitivity to food and clothing textures, loud sounds, deep pressure affinity, light, and differences in pain perception.

The individual with HF ASD may be a picky eater, displaying strong food preferences and selectivity. Tactile sensitivity presents with food and clothing preferences, as there may be an inability to tolerate certain materials and textures. In addition, hypersensitivity to human touch may also be present and someone with HF ASD may pull away from light touches or be calmed by deep embraces like hugs. Hyperacusis, heightened sensitivity to sound, can be observed with over-reaction to loud noises that most people can normally tolerate. The individual may cover their ears, become upset, or try to leave the area. Bright lights or certain types of lighting, such as fluorescent lights or LED, may

cause distress. And pain responsivity can be different in these individuals as well, with some displaying hyposensitivity, reduced pain sensitivity, and others showing hypersensitivity, increased pain sensitivity.

The importance of appropriate identification and treatment

It is important to identify HF ASD and initiate treatment as early as possible. Misdiagnosis rates are high for people on the milder scale of ASD. These individuals are often diagnosed with oppositional defiant disorder (ODD), disruptive mood dysregulation disorder (DMDD), bipolar disorder, or borderline personality disorder (BPD), diagnoses which may mask their actual autistic condition and prevent them from receiving proper support (20).

There is also a high level of psychiatric comorbidity with HF ASD. One study of 122 adults with normal-intelligence ASDs revealed that significant percentages of patients experienced depression, anxiety, attention deficit hyperactivity disorder (ADHD), obsessive–compulsive disorder (OCD), tic disorders, and psychotic disorder (5). This leads to polypharmacy and higher risks of negative outcomes.

Because an individual with undiagnosed HF ASD can manage and execute daily tasks, they may not be identified as different and are expected to function normally in their environment. There may be minimal to no accommodation by schools or family and support systems, and these individuals are expected to perform with tools they may not possess. The minimal adaptation of the environment and treatments explicitly targeting social pragmatics makes it harder to cope and succeed socially, especially as a child enters adolescence, when demands increase. A misdiagnosis that masks an ASD can create confusion and frustration for an individual regarding their skills and social competence, damaging self-esteem and leading to psychiatric problems such as anxiety and depression.

Improving recognition and support

What can be done to improve the recognition of HF ASD? Because individuals with HF ASD show milder symptoms, recognition and diagnosis can be difficult. They may show a normal IQ and deficits in social interaction and communication may not be immediately visible. Furthermore, coping mechanisms may be used to hide these deficits and appear as "normal". Therefore, clinicians should maintain a high index of suspicion in cases with complicated and unclear presentations. Subtle signs of social deficits may not be apparent at first, and further observation may be necessary.

The gold standard for diagnosis remains a clinical one using DSM-5 criteria, which outline deficits in social communication and interaction, restricted or repetitive behavior patterns and interests, and gives clear guidelines for diagnosis (1). It is imperative to obtain a thorough developmental history that includes social pragmatics and adaptive functional skills, and it is important to use screening tools, such as the Social Responsiveness Scale (SRS), to aid and support the clinical suspicion. The SRS measures the severity of symptoms regarding mannerisms and social contexts including deficits, cognition, communication, and motivation, and can be particularly useful for identifying autistic traits in milder forms of ASD.

Once a diagnosis is confirmed, support can be improved with therapy intervention, accommodation, the explicit teaching of skills, and modelling of inclusivity and acceptance.

Psychoeducation should follow diagnosis, starting with the child, as well as parents, the school and supports. This aims to improve self-understanding and awareness of autistic symptoms. Awareness can allow for the reframing of behaviors. The difficult behaviors of the individual before diagnosis must be relabeled. Refusal, stubbornness, defiance, and opposition can be redescribed; they now become "unable to" and "doesn't have the required skills currently to meet the demands being asked".

The environment should be accommodated to set the children up for success, rather than placing further challenges and difficulties upon their shoulders. Sensory breaks, moments of time dedicated to reducing stimuli overload, can be introduced into the daily schedule. Unexpected changes or transitions can be very difficult for autistic children, so in order to support them any upcoming changes should be previewed. Schedules with routines will help these individuals cope better. Peer models can be very useful as well, as peer-based interventions using typically developing peers as social models have been shown to improve social skills (21).

It is important to teach explicitly what neurotypical children learn implicitly; this includes social pragmatic skills, self-regulation skills, and complex adaptive skills ("street smarts").

Stigma affects the autistic community but can be overcome by understanding of the disorder. The environment should be a model of inclusivity and acceptance, providing welcoming conditions and opportunities for the autistic individual.

CONCLUSIONS

In the spectrum of autistic disorders, HF ASD presents with milder symptoms and for this it may not be initially apparent. Individuals with this type of autism face many challenges in daily life, where they are expected to perform in

their environment but lack the tools to function. Individuals with HF ASD often have difficulty due to cognitive rigidity, social-communication deficits, and sensory deficits. Communication and social challenges increase as these individuals advance in age, further increasing these challenges and leading to poor self-esteem. They may suffer from depression, anxiety, or other mood disorders, have problems with emotional regulation, and psychiatric hospitalization rates are higher for those who go undiagnosed. For these reasons, it is vital to improve recognition of HF ASD in order to extend support to these individuals.

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Conflict of interest

The author declares that they have no conflict of interest.

REFERENCES

1. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders: DSM-5. 5th ed. American Psychiatric Publishing; Arlington, VA, USA: 2013
2. Maenner MJ, Shaw KA, Bakian AV, et al. Prevalence and Characteristics of Autism Spectrum Disorder Among Children Aged 8 Years— Autism and Developmental Disabilities Monitoring Network, 11 Sites, United States, 2018. *MMWR Surveillance Summaries*. 2021;70(11):1-16. doi:10.15585/mmwr.ss7011a1
3. Autism and Developmental Disabilities Monitoring Network Surveillance Year 2000 Principal Investigators; CDC. Prevalence of autism spectrum disorders—Autism and Developmental Disabilities Monitoring Network, six sites, United States, 2000. *MMWR Surveill Summaries.* 2007;56(No. SS-1).
4. Laurent AC, Rubin E. Challenges in Emotional Regulation in Asperger Syndrome and High-Functioning Autism. *Topics in Language Disorders*. 2004;24(4):286-297. doi:10.1097/00011363-200410000-00006
5. Hofvander B, Delorme R, Chaste P, et al. Psychiatric and psychosocial problems in adults with normal-intelligence autism spectrum disorders. *BMC Psychiatry*. 2009;9(1). doi:10.1186/1471-244x-9-35
6. Ghaziuddin, M, Zafar S. Psychiatric comorbidity of adults with autism spectrum disorders. *Clinical Neuropsychiatry*. 2008;5(1):9-12.
7. Mazzone L, Ruta L, Reale L. Psychiatric comorbidities in asperger syndrome and high functioning autism: diagnostic challenges. *Annals of General Psychiatry*. 2012;11(1):16. doi:10.1186/1744-859x-11-16
8. Maddox BB, Trubanova A, White SW. Untended wounds: Non-suicidal self-injury in adults with autism spectrum disorder. *Autism*. 2016;21(4):412-422. doi:10.1177/1362361316644731
9. Hedley D, Uljarević M. Systematic Review of Suicide in Autism Spectrum Disorder: Current Trends and Implications. *Current Developmental Disorders Reports*. 2018;5(1):65-76. doi:10.1007/s40474-018-0133-6
10. Tromans S, Chester V, Kiani R, Alexander R, Brugha T. The Prevalence of Autism Spectrum Disorders in Adult Psychiatric Inpatients: A Systematic Review. *Clinical Practice & Epidemiology in Mental Health*. 2018;14(1):177-187. doi:10.2174/1745017901814010177
11. Baio J, Wiggins L, Christensen DL, et al. Prevalence of Autism Spectrum Disorder Among Children Aged 8 Years — Autism and Developmental Disabilities Monitoring Network, 11 Sites, United States, 2014. *MMWR Surveillance Summaries*. 2018;67(6):1-23. doi:10.15585/mmwr.ss6706a1
12. Lai MC, Lombardo MV, Ruigrok AN, et al. Quantifying and exploring camouflaging in men and women with Autism. *Autism*. 2016;21(6):690-702. doi:10.1177/1362361316671012
13. Tubío-Fungueiriño M, Cruz S, Sampaio A, Carracedo A, Fernández-Prieto M. Social Camouflaging in Females with Autism Spectrum Disorder: A Systematic Review. *Journal of Autism and Developmental Disorders*. 2020;51. doi:10.1007/s10803-020-04695-x

14. Bargiela S, Steward R, Mandy W. The Experiences of Late-diagnosed Women with Autism Spectrum Conditions: An Investigation of the Female Autism Phenotype. *Journal of Autism and Developmental Disorders*. 2016;46(10):3281-3294. doi:10.1007/s10803-016-2872-8
15. Baldwin S, Costley D. The experiences and needs of female adults with high-functioning autism spectrum disorder. *Autism*. 2015;20(4):483-495. doi:10.1177/1362361315590805
16. Cohen SJ. Cognitive Rigidity, Overgeneralization and Fanaticism. *Encyclopedia of Personality and Individual Differences*. Published online 2017:1-7. doi:10.1007/978-3-319-28099-8_834-1
17. Izuma K, Matsumoto K, Camerer CF, Adolphs R. Insensitivity to social reputation in Autism. *Proceedings of the National Academy of Sciences*. 2011;108(42):17302-17307. doi:10.1073/pnas.1107038108
18. Myles BS, Simpson RL. Asperger Syndrome. *Focus on Autism and Other Developmental Disabilities*. 2002;17(3):132-137. doi:10.1177/10883576020170030201
19. Stichter JP, Herzog MJ, Visovsky K, et al. Social Competence Intervention for Youth with Asperger Syndrome and High-functioning Autism: An Initial Investigation. *Journal of Autism and Developmental Disorders*. 2010;40(9):1067-1079. doi:10.1007/s10803-010-0959-1
20. Tebartz van Elst L, Pick M, Biscaldi M, Fangmeier T, Riedel A. High-functioning autism spectrum disorder as a basic disorder in adult psychiatry and psychotherapy: psychopathological presentation, clinical relevance and therapeutic concepts. *European Archives of Psychiatry and Clinical Neuroscience*. 2013;263(S2):189-196. doi:10.1007/s00406-013-0459-3
21. Reichow B, Volkmar FR. Social Skills Interventions for Individuals with Autism: Evaluation for Evidence-Based Practices within a Best Evidence Synthesis Framework. *Journal of Autism and Developmental Disorders*. 2009;40(2):149-166. doi:10.1007/s10803-009-0842-0