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Original Article

Dissecting Genetic Variant Contributions to Neurodegenerative Disorders through Targeted Gene Sequencing in a Sicilian Population

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Abstract

Background: Despite the technological advancements in modern genetic diagnosis, customized genetic panels are still frequently employed for diagnostic purposes due to their rapid, efficient, and cost-effective ability to detect genetic variants. **Methods**: In this study, we utilized a customized genetic panel designed to identify genetic variants associated with neurodegenerative disorders. The panel consisted of 61 genes and was applied to a cohort of 186 unrelated individuals diagnosed with different degenerative cognitive and movement disorders. The identified variants were filtered for a minor allele frequency of less than 1% and classified according to the American College of Medical Genetics (ACMG) guidelines. **Results**: Our results showed that 20.97% of individuals carried at least one likely pathogenic or pathogenic variant, with 35% of those individuals diagnosed with Alzheimer's disease (AD). The positive diagnostic yield of the panel was 16.67%, calculated based on variant zygosity, inheritance pattern, and concordance with the clinical phenotype. Furthermore, 34.41% of the individuals carried variants of uncertain significance (VUS), and 44.62% carried benign variants. Variants have been found only in 58 genes. Among these, 24.14% showed benign variants, 48.28% had VUS, and 27.59% carried pathogenic or likely pathogenic variants. Principal component analysis analysis based on the variables "age at onset" in addition to the phenotypic scores "MMSE" (global cognitive screening test), "IADL" (instrumental activities of daily living), and "ADL" (basic activities of daily living) distributed the individuals associated with the specific disease and variant in the plot. Notably, the individuals showed AD exhibited an average age at onset of 68 ±12.5 years and were differentiated in the plot. *GBA* gene exhibited the highest number of pathogenic variants (9) linked to AD, Parkinson's disease, early onset parkinsonism with epilepsy, fronto-temporal dementia, and mild cognitive impairment. **Conclusion**: The results highlighted a broad phenotypic heterogeneity associated with genes previously linked to only a limited number of neurodegenerative conditions, underscoring the value of the genetic testing performed. Translationally, although clinical exome sequencing has enabled novel gene discovery in neurodegenerative disorders, targeted genetic panels remain a cost-effective and clinically valuable approach for routine diagnostics. In this context, our study highlights the "real-world" utility and clinical impact of a focused panel-based strategy.

1. Introduction

Neurodegenerative disorders are a common and increasingly significant cause of morbidity and mortality worldwide, particularly among the elderly. According to the Alzheimer's Disease International database (https://www.alzint.org/), over 55 million people worldwide were living with dementia in 2020. This number is projected to nearly double every 20 years, reaching approximately 78 million by 2030 and 139 million by 2050 [1].

Although these conditions often share overlapping features, they are heterogeneous in their clinical presentations and underlying pathophysiology. Each disorder is characterized by distinct epidemiological patterns, clinical symptoms, laboratory and neuroimaging findings, neuropathological changes, and management strategies [2,3]. Neurodegenerative diseases can be classified according to primary clinical features (e.g., dementia, parkinsonism, or motor neuron disease), anatomic distribution of neurodegeneration (e.g., frontotemporal degenerations, extrapyramidal disorders, or spinocerebellar degenerations), or principal molecular abnormality [4,5]. Dementia risk is influenced by a broad range of factors, including frailty, lifestyle, and genetic predisposition [6]. Each of these elements plays a critical role in determining an

individual's likelihood of developing dementia, with genetic factors increasingly recognized as key contributors through the identification of specific variants linked to the condition.

Alzheimer's disease (AD) is the most prevalent form of dementia globally, accounting for 60%–80% of all dementia cases and affecting an estimated 24 million people [7]. The prevalence of AD increases significantly with age, typically occurring between 65 and 85 years [8]. Although it can develop in younger individuals, AD primarily affects the elderly. The estimated lifetime risk of developing AD dementia is 41.9% for women and 33.6% for men [9]. Parkinson' s disease (PD) is considered the second most common neurodegenerative disorder and has traditionally been described as a condition affecting the basal ganglia, leading primarily to motor disturbances, although non-motor symptoms are also clinically relevant and disabling, with some of them often preceding typical motor features.

Given the diverse spectrum of dementia aetiologies and the wide range of clinical presentations across different neurodegenerative conditions, the accurate and early diagnosis of dementia is of pivotal importance. Diagnostic precision not only allows for more reliable prognostication but also plays a critical role in guiding appropriate treatment and management strategies [10]. Therefore, distinguishing between the various forms of dementia is fundamental to providing effective and individualized clinical care. In this complex scenario, the employment of next-generation sequencing techniques enabled the identification of new genetic variants associated with this heterogenic condition [11]. The use a robust genetic panel, enables a fast screening of the wide array of genes associated with this condition. In fact, a robust and efficient genetic panel is essential for the rapid and comprehensive diagnosis of dementia-related disorders [11]. By targeting a wide array of genes known to be associated with various forms of dementia, these panels streamline the diagnostic process, reducing the time and complexity compared to traditional methods [12]. Importantly, the use of such panels not only increases diagnostic accuracy but also offers a cost-effective approach by consolidating multiple tests into a single, efficient analysis. This allows for the early identification of genetic variants, enabling timely interventions and tailored care strategies, ultimately improving patient outcomes.

According to the MalaCards database (https://www.malacards.org/) (accessed on 10 March 2025), the ten most strongly inferred genes associated with dementia are *PSEN1*, *PRNP*, *MAPT*, *GBA1*, *APP*, *APOE*, *ITM2B*, *VCP*, *TYROBP*, and *SNCA*. All these genes are annotated in the OMIM database with corresponding MIM phenotype codes, linking them to specific neurodegenerative phenotypes.

In this study, we analysed a cohort of 186 patients from the Sicilian population, all diagnosed with a broad spectrum of dementia phenotypes, using a targeted gene panel comprising 61 genes. Our aim was to evaluate the efficacy of an “ad hoc” genetic panel applied on a Sicilian population affected by different forms of degenerative cognitive and movement disorders by identifying any novel associated genetic variant.

2. Results

NGS analysis using the specified gene panel was successfully performed on all 186 patients. The average of mapped reads was of 342,512 while the mean depth was 276.49. Across all 61 genes in the genetic panel, variants with a minor allele frequency (MAF) <1% were identified in 58 genes. Table S1 summarizes the relevant clinical and demographic data, as well as the main laboratory and instrumental findings, of the examined cohort of 186 patients. A total of 39 patients (20.97%) were classified as 'positive', having carried at least one likely pathogenic or pathogenic variant. Table S2 lists all 39 positive patients, highlighting clinical features, including Mini-Mental State

Examination (MMSE) scores, Activities of Daily Living (ADL), Instrumental Activities of Daily Living (IADL), and pharmacological treatments administered. Table S2 also lists the genetic findings of the positive patients, which are also reported in Figure S1. Additionally, 64 patients (34.41%) did not have pathogenic or likely pathogenic variants but carried at least one variant of uncertain significance (VUS). The remaining 83 patients (44.62%) had no pathogenic, likely pathogenic, or VUS variants. Concerning the classification of the 58 genes, 14 (24.14%) displayed only Likely Benign or Benign variants, 28 (48.28%) contained at least one variant of uncertain significance (VUS), and 16 (27.59%) harboured at least one Likely Pathogenic or Pathogenic variant. A total of 359 variants with MAF <1% were detected across the 186 patients. Among these, 217 (60.25%) were classified as benign or likely benign, 99 (27.58%) were VUS, and 43 (11.97%) were pathogenic or likely pathogenic (Figure 1).

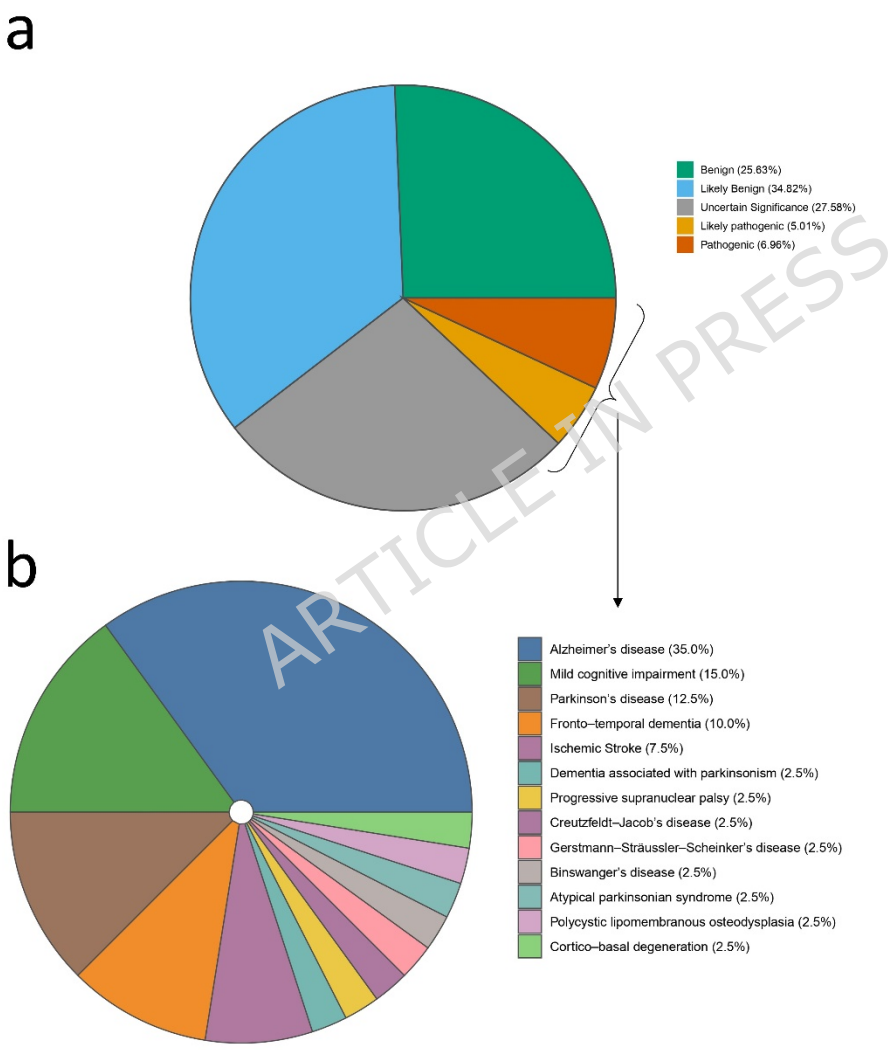

Figure 1. Graphical representation of the percentage distribution of classified variants identified across the 186 patients who underwent genetic testing in this study. (a) Percentage breakdown of the 359 genetic variants identified among the 186 examined individuals, categorized according to ACMG criteria. (b) Detailed view of the phenotypic distribution (in percentage) of the likely pathogenic and pathogenic variants observed in the 39 individuals who tested positive.

Figure 1b illustrates the classification of all identified variants across the genes included in the targeted genetic panel used in this study. Among individuals carrying pathogenic or likely pathogenic variants, 35% were diagnosed with Alzheimer's disease (AD), 15% had mild cognitive impairment (MCI), 12.5% were diagnosed with Parkinson's disease (PD), and 7.5% had ischemic stroke (Figure 1). Additionally, 10% were affected by fronto-temporal dementia (FTD). A smaller proportion (2.5%) were diagnosed with Lewy body dementia (LBD), progressive supranuclear palsy (PSP), Creutzfeldt-Jakob disease, Gerstmann-Sträussler-Scheinker disease, Binswanger's disease, early onset parkinsonism with epilepsy not further classified, polycystic lipomembranous osteodysplasia, or corticobasal degeneration. Table S1 shows the clinical characteristics and genetic findings of the 186 patients analysed in this study, including diagnosis, main neurological comorbidities, and variants identified by the targeted NGS gene panel.

Regarding the chromosomal distribution, 45 variants (12.53%) were found on chromosome 1. Specifically, this chromosome contained 5 Benign, 16 Likely Benign, 2 Likely Pathogenic, 10 Pathogenic variants, and 12 VUS. Notably, chromosome 1 accounted for the highest number of pathogenic variants. Figure S1 shows the chromosomal distribution of variants across the regions covered by the genetic panel used in this study, presented for descriptive purposes only.

The gene *ACE* had the highest number of variants, with a total of 27, comprising 9 benign, 6 likely benign, 3 pathogenic, and 9 VUS. POLG had the second highest number of variants (19), including 7 benign, 4 likely benign, 1 likely pathogenic, 5 pathogenic, and 2 VUS. Notably, *GBA*, with 15 variants, exhibited the highest number of pathogenic variants (9) within the cohort. Figure S2 illustrates the distribution of genetic variants across the 58 genes in which at least one variant was identified in this study.

With regard to the association between genes and diseases, the GBA gene displayed a total of five diseases associated with nine Pathogenic or Likely Pathogenic variants. Specifically, three variants were linked to AD, three to PD, one to early onset parkinsonism with epilepsy, one to FTD, and one to MCI. Similarly, *TREM2* was associated with a wide range of phenotypes. Among the five Pathogenic or Likely Pathogenic variants found in this gene, three were linked to AD, one to Binswanger's disease, one to MCI, and one to polycystic lipomembranous osteodysplasia. The Sankey plot depicted in Figure 2, illustrates the association between the genes in which likely pathogenic or pathogenic variants were identified and disease.

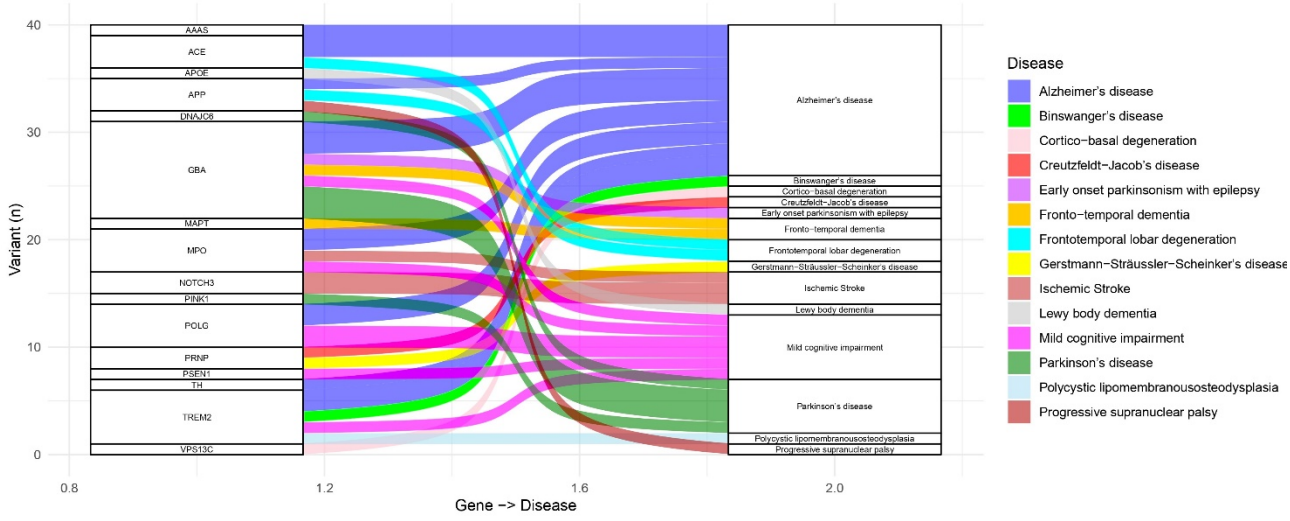

Figure 2. Sankey plot depicting the association between the genes carrying at least one likely pathogenic or pathogenic variant and diseases.

In the cohort examined in this study, several recurrent variants were identified. The most frequent alterations were the pathogenic *GBA* variant c.721G>A and the likely pathogenic *TREM2* variant c.482+2T>C. The *GBA* c.721G>A variant was detected in the heterozygous state in patients 17, 18, 19, and 20, and was associated with heterogeneous clinical presentations, including PD, AD, and FTD (Figure 3).

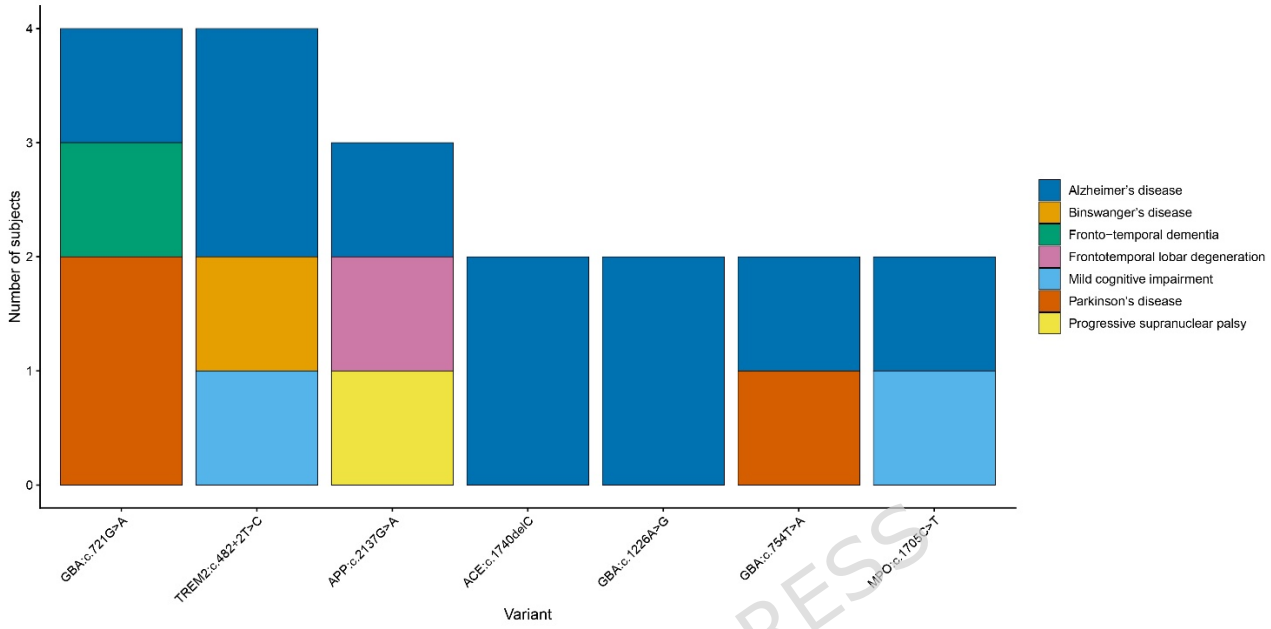

Figure 3. Association between recurrent genetic variants and corresponding clinical phenotypes. This figure displays the recurrent variants identified in multiple patients and their association with specific dementia-related phenotypes observed in this study. Each variant is linked to one or more clinical presentations, highlighting potential genotype–phenotype correlations. The visualization emphasizes how certain mutations may be repeatedly associated with particular neurodegenerative manifestations, supporting their clinical relevance.

Similarly, the *TREM2* c.482+2T>C variant was identified in the heterozygous state in patients 4, 8, 9, and 30, and was linked to diverse phenotypes, including Binswanger's disease, AD, and MCI. Furthermore, the *APP* variant c.2137G>A was detected in the heterozygous condition in patients 7, 25, and 37, and was associated with progressive supranuclear palsy, frontotemporal lobar degeneration, and AD, respectively.
Pearson's correlation analysis among the variables "Pathogenicity," the phenotypic scores IADL, ADL, and MMSE, as well as Age at onset and Pathogenicity predicted using VarSome in accordance with the ACMG criteria, revealed that only the phenotypic variables were significantly correlated with each other (**Figure 4**).

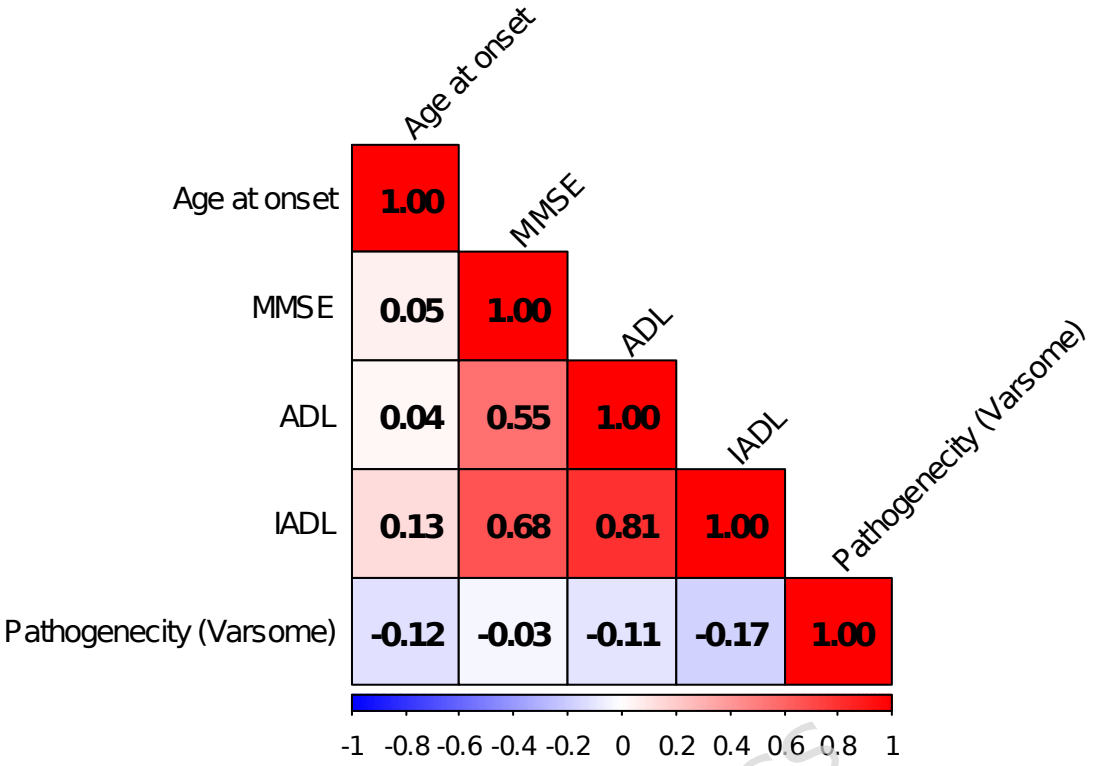

Figure 4. Pearson's correlation among the examined traits. The traits analysed were the Age at onset, MMSE, ADL, IADL and Pathogenicity predicted using VarSome in accordance with the ACMG criteria (scored by 1 = likely pathogenic and 2 = pathogenic).

Principal component analysis (PCA) conducted on the analysed variables (IADL, ADL, MMSE, and Age at onset) showed that the cumulative variance explained by the first three principal components (PC1, PC2, and PC3) was approximately 95.55%. PC1, accounting for about 56.29% of the total phenotypic variance, was positively correlated with the IADL, ADL and MMSE scores (Figure 5).

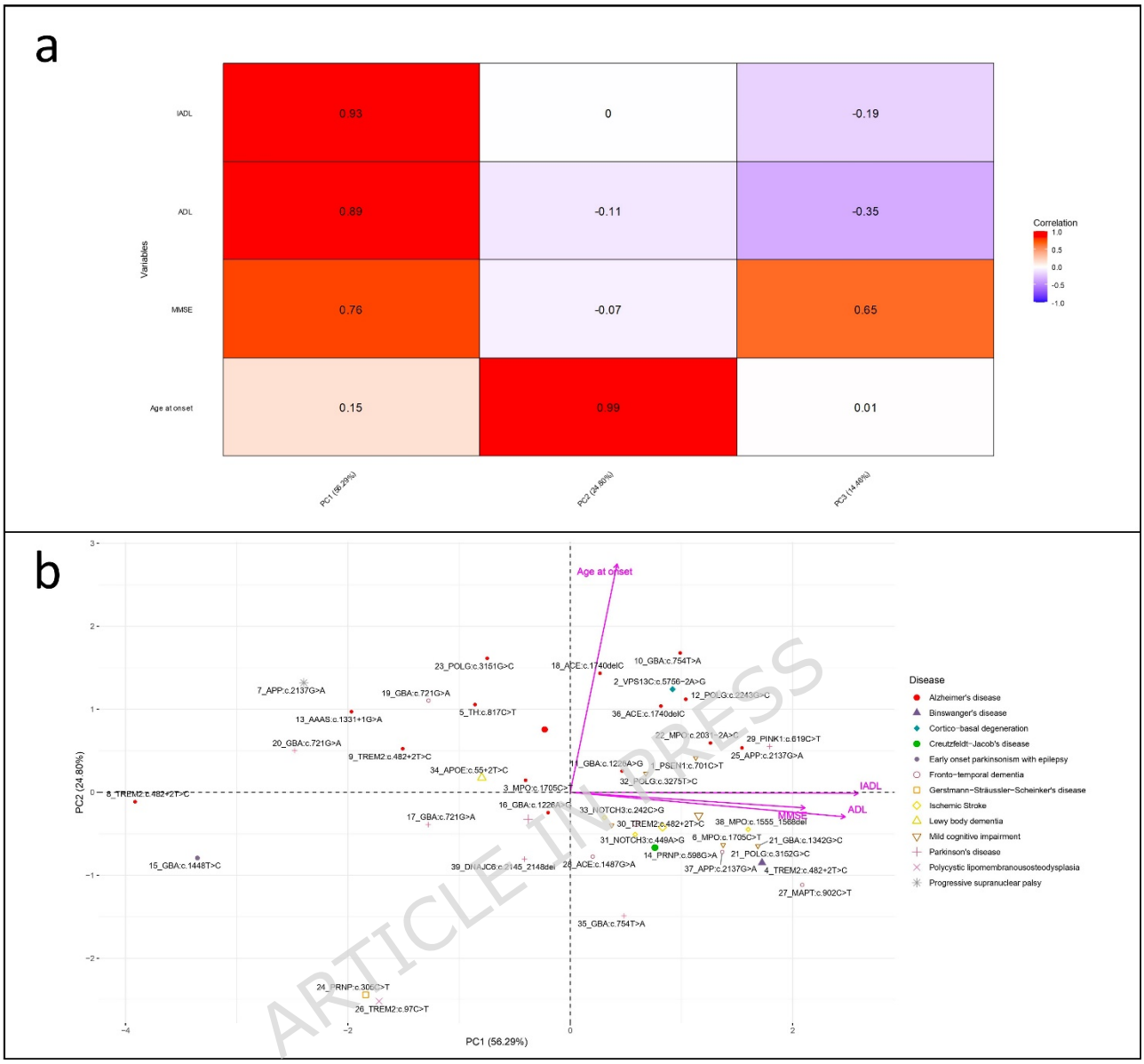

Figure 5. Principal Component Analysis (PCA) of the 39 individuals who tested positive for pathogenic or likely pathogenic variants and presented with various dementia-related phenotypes. (a) Heatmap showing the correlation matrix between the examined variables and each extracted principal component. (b) PCA plot illustrating the distribution of the 39 individuals according to the first two principal components, PC1 and PC2, which account for 56.29% and 24.90% of the total phenotypic variance, respectively. Each data point is labelled with the individual's unique identifier (as listed in Table S1) followed by the corresponding genetic variant. Different symbols represent distinct clinical phenotypes associated with each variant.

PC2, which explained around 24.80% of the total variance, was positively correlated with Age at onset, whereas PC3, contributing 14.46% of the total variance, was positively correlated with MMSE. This analysis allowed for the distribution of individuals carrying pathogenic or likely pathogenic variants along the two axes represented by PC1 and PC2. In the higher part of the PCA plot, individuals with a higher age at onset were distributed. Conversely, in the lower part of the PCA plot, individuals with a lower age at onset were distributed. On the right side of the graph, individuals with higher phenotypic scores for IADL, ADL and MMSE were observed, while those with lower phenotypic scores were positioned on the left.

In the GBA protein, all pathogenic variants in the GBA gene result in missense mutations affecting key functional domains. Specifically, the Gly241Arg, Phe252Ile, Asn409Ser, and Asp448His variants are located within the Glycosyl hydrolase family 30 TIM-barrel domain (amino acids 117–466), as annotated in the InterPro database (IPR033453) Figure S4 illustrates the specific position of each variant within the GBA protein. The Leu483Pro variant is found within the Glycosyl hydrolase family 30 beta-sandwich domain (amino acids 469–531) (IPR033452). Regarding the *POLG* gene, the pathogenic Gly1051Ala, Gly1051Arg, Phe1092Ser, and Ser1176Leu variants are found within the Pol gammaA domain, a family A polymerase responsible for DNA replication and repair in mitochondria (amino acids 785–1203) (IPR047580) (Figure S5). In contrast, the Trp748Ser variant is located outside functional domains. With regards to the pathogenic variants in ACE protein, the variants Arg496Gln and Trp581fs are both located within the functional Peptidase M2 1 domain (amino acids 40-624) (PRU01355), which is crucial for regulating various physiological processes (Figure S7). Notably, the Arg496Gln variant was associated with fronto-temporal dementia in one patient analyzed in this study, while the Trp581fs variant was linked to AD in two individuals.

3. Discussion

3.1 Main findings

In this study, we report on a cohort of 186 patients affected by different neurodegenerative conditions, analyzed using a customized targeted genetic panel of 61 genes. The panel was designed based on the MRC Dementia Gene Panel [13]. Additional genes from the MRC Dementia Gene Panel were selected based on their association, as reported in the MalaCards database, with AD, PD, and dementia, or were selected because of their documented association with cognitive decline. Our findings revealed that 20.97% of individuals carried at least one likely pathogenic or pathogenic variant, aligning with previous reports of diagnostic yields ranging from 10% to 40% for targeted panels in neurodegenerative disorders [12,14]. Nevertheless, the estimation of positive diagnostic yield must take into account variant zygosity, inheritance patterns, and concordance with the clinical phenotype. After applying these criteria, only 31 patients, rather than 39, were considered to have a diagnostically relevant finding, as we included only pathogenic or likely pathogenic variants compatible with a causal interpretation, primarily in genes with autosomal dominant inheritance. Patient 26 was retained in the diagnostic group because this individual carries a homozygous variant in *TREM2*, a gene associated with autosomal recessive disease. This approach reduced the positive diagnostic yield of the panel from 20.97% to 16.67%. The relatively modest yield is consistent with the multifactorial nature of these conditions, which often involve complex interactions between genetic and environmental factors. Based on these considerations, we consider the use of a targeted gene panel as an effective and clinically relevant approach in the routine genetic evaluation of dementia when molecular testing is indicated. The inclusion of VUS in 34.41% of patients suggests the potential for additional pathogenic mechanisms that remain to be elucidated [11,15]. VUS, despite their unresolved clinical impact, should not be underestimated, as they may also act as potential contributors to disease susceptibility. Moreover, periodic re-evaluation of VUS in light of emerging evidence may increase the diagnostic yield of gene panel.

Among the 39 “positive” patients, 13 individuals (33.3%) carried a single variant classified as pathogenic or likely pathogenic according to ACMG criteria, without additional rare variants in other genes included in the panel. In these cases, the

identified variants involved genes with well-established associations to the corresponding clinical phenotypes, supporting a potential causal role. Specifically, as indicated in Table S1, Table S2 and Figure S3, two patients with AD carried pathogenic variants in *GBA* (c.1226A>G, p.Asn409Ser; patient 11) and *POLG* (c.3151G>C, p.Gly1051Arg; patient 23), while another Alzheimer's disease patient harbored a likely pathogenic variant in *APP* (c.2137G>A, p.Ala713Thr; patient 25). A further Alzheimer's disease case carried a pathogenic frameshift variant in *ACE* (c.1740delC, p.Trp581GlyfsTer5; patient 36). One patient with Parkinson's disease carried a pathogenic *GBA* variant (c.721G>A, p.Gly241Arg; patient 17). The patient diagnosed with fronto-temporal dementia carried a pathogenic *MAPT* variant (c.902C>T, p.Pro301Leu; patient 27), whereas the individual affected by Gerstmann–Sträussler–Scheinker disease harbored the pathogenic *PRNP* variant c.305C>T (p.Pro102Leu; patient 24). Two patients with ischemic stroke carried likely pathogenic variants in *NOTCH3* (c.449A>G, p.Tyr150Cys; patient 31; and c.242C>G, p.Pro81Arg; patient 33), consistent with a CADASIL-like phenotype. The patient affected by polycystic lipomembranous osteodysplasia carried a homozygous pathogenic nonsense variant in *TREM2* (c.97C>T, p.Gln33Ter; patient 26). In addition, one patient with mild cognitive impairment carried a likely pathogenic *POLG* variant (c.3275T>C, p.Phe1092Ser; patient 32), and one patient with Lewy body dementia harbored a likely pathogenic splice-site variant in *APOE* (c.55+2T>C; patient 34). In these 13 individuals, the concordance between the affected gene, the nature of the variant, and the observed clinical phenotype suggests that these alterations represent the primary genetic determinant of disease. However, interpretation of causality should be approached within the methodological framework of the study. Parental DNA was not available, precluding segregation analyses and determination of whether the identified variants were inherited or arose *de novo*. Additionally, as is inherent to panel-based approaches, deep intronic and regulatory regions were not systematically analyzed, preventing the identification of variants potentially affecting gene expression or splicing. Therefore, although these 13 variants represent the only genetic findings plausibly able to explain the phenotype in the respective patients, an additional contribution from other undetected genetic factors cannot be excluded. In contrast, the remaining 26 patients (66.7%) harbored additional genetic variants beyond the pathogenic or likely pathogenic finding, which may plausibly act as contributory modifiers of the observed phenotype.

Nevertheless, the primary aim of our study was to evaluate the positive diagnostic rate among individuals analysed using this specific targeted genetic panel, highlighting its clinical utility while also acknowledging its limitations. An important aspect of variant interpretation in this study concerns both zygosity and clinical relevance. All identified variants were detected through a targeted gene panel, which represented the sole molecular diagnostic tool used for these patients. While pathogenic and likely pathogenic variants can be considered causative or strongly contributory to disease, their presence should be interpreted within a broader multifactorial framework. In this context, these variants are best viewed as risk factors that may contribute to the patient phenotype rather than as fully deterministic findings. Although variants in genes such as *GBA*, *MAPT*, *MPO*, and *TREM2* are often discussed as susceptibility factors, some alterations may exert a direct pathogenic role in specific clinical contexts. In particular, the *MAPT* c.902C>T (p.Pro301Leu) variant has been previously reported in association with FTD [16,17], with strong supporting evidence from functional studies and curated resources such as Alzforum. Accordingly, this variant should be interpreted as disease-causing rather than a susceptibility factor. In our analysis, we found pathogenic and likely pathogenic variants in genes which have

disease model of autosomal recessive transmission. For instance, *TREM2* is annotated in OMIM as a gene associated with autosomal recessive disorders, including Nasu–Hakola disease. The likely pathogenic splice-site variant identified in our cohort (c.482+2T>C) is predicted to trigger nonsense-mediated mRNA decay (NMD) according to the NMDEsc predictor, supporting a loss-of-function mechanism. Importantly, *TREM2* represents a paradigmatic example of how the same gene can be implicated in disease through distinct mechanisms depending on the type of variant and its zygosity. While biallelic loss-of-function variants cause a rare early-onset neurodegenerative disorder, heterozygous missense variants (e.g., p.Arg47His and p.Arg62His) have been associated with increased risk of Alzheimer's disease and frontotemporal dementia [18,19]. In our cohort, patients carrying the TREM2 c.482+2T>C variant in the heterozygous state (patients 4, 8, 9, and 30) also harbored additional genetic variants that may plausibly contribute to their phenotypes, suggesting that this variant alone is unlikely to be fully explanatory. In contrast, patient 26, who carries the TREM2 nonsense variant c.97C>T (p.Gln33Ter) in the homozygous state, represents a case in which this single variant is likely sufficient to explain the observed phenotype. Although computational predictions suggest a potential dominant-negative effect for TREM2 (pDN = 0.706, DECIPHER), the available evidence supports a model in which variant-specific effects—particularly loss-of-function versus missense variation and zygosity—are critical determinants of clinical outcome.

Across the likely pathogenic and pathogenic variants, the highest percentage (35%) was associated with AD. According to large Italian data retrieved from the ISTAT database (ISTAT 2021-2023), the ratio females/males related to the individuals diagnosed with AD in Sicily region is 2.2. In our study, this ratio was 1.8 considering the positive patients. This observation aligns with literature, emphasizing the gender difference in this neurodegenerative condition [20,21]. This finding underscores the strong genetic component of AD and the relevance of the selected gene panel in identifying individuals at risk, as also widely supported by previous similar studies [22]. Despite an overall diagnostic yield of 16.67%, the targeted gene panel demonstrated clear clinical relevance, particularly in late-onset patients. In our study, the selection of a cohort from various provinces across the Sicilian region was also crucial, as previously reported in a study on dementia in a rural Sicilian community [23]. Educational and occupational levels were shown to influence the clinical severity and presentation of the disease, potentially reflecting different subtypes of neurodegenerative disorders.

Additionally, 15% of cases were linked to MCI, suggesting a potential genetic predisposition in the early stages of neurodegeneration, especially towards AD or mixed pathology. Other conditions, such as PD (12.5%), ischemic stroke (7.5%), and various forms of FTD and early onset parkinsonism with epilepsy, were also observed. As documented, many conditions with overlapping phenotypes stem from distinct genetic causes, highlighting the need for genetic analysis in differential diagnosis [24]. Moreover, phenotypic similarities can sometimes lead to clinical misinterpretation, especially when family members are not adequately investigated, further emphasizing the importance of a genetic approach in accurately classifying neurodegenerative diseases [25].

Chromosome 17 exhibited the highest number of total variants, harboring 57 in total. However, only six were classified as pathogenic and two as likely pathogenic, affecting the *MAPT*, *MPO*, and *ACE* genes, all located on the long arm (17q). We emphasize that the chromosomal localization of the variants was provided solely for descriptive

purposes. We acknowledge that this representation is not fully informative and is inherently biased by the arbitrary selection of genes included in the targeted panel.

The *GBA* gene, located on chromosome 1, exhibited the highest number of pathogenic variants (n = 9). This gene has been described with specific MIM phenotype numbers associated with susceptibility to “Lewy body dementia” (#127750) and “Parkinson disease, late onset” (#168600), both with an autosomal dominance inheritance pattern. The pathogenic variants in the *GBA* gene described in this study were identified in individuals presenting a wide spectrum of neurodegenerative phenotypes, including AD, MCI, early onset parkinsonism with epilepsy, FTD, and PD (Figure 2 and Figure S1).

In the GBA protein, all pathogenic variants resulted in missense mutations affecting key functional domains. Specifically, the Gly241Arg, Phe252Ile, Asn409Ser, and Asp448His variants are located within the Glycosyl hydrolase family 30 TIM-barrel domain (Figure S4). Interestingly, the same variant can sometimes be associated with different phenotypes. For example, the *GBA* variant c.754T>A (p.Phe252Ile) was linked to AD in patient 10 and PD in patient 35. Defects in protein having this specific domain are associated with a wide range of neurodegenerative disorders, such as Huntington’s, Gaucher’s and Krabbe [26,27]. Based on these considerations, *GBA* should be regarded primarily as a genetic risk factor. Accordingly, we propose that specific mutations within GBA may confer differential susceptibility to neurodegenerative phenotypes rather than directly determining disease onset. In our cohort, among the 10 patients carrying pathogenic or likely pathogenic GBA variants (patients 10, 11, 15, 16, 17, 18, 19, 20, 21, and 35), only two individuals (patients 11 and 17) harboured GBA as the sole rare genetic finding identified by the targeted gene panel. The remaining patients carried additional rare variants in other genes that may plausibly contribute to their clinical phenotype. While the literature and the OMIM database associate GBA mutations primarily with PD and LBD, our cohort analysis revealed that GBA variants linked to PD were correlated with an earlier age at onset. Specifically, patients with pathogenic and likely pathogenic GBA variants associated with AD had an average age at onset of 68 ±12.5 years, whereas PD patients exhibited an earlier onset, with an average age of 54.3 ±10 years. On the other hand, the subject who experienced early onset parkinsonism with epilepsy had an age at onset of 47 years. As documented, LBD symptoms often overlap with those of tauopathies such as AD [28]. We cannot exclude that, despite their similarities, some cases diagnosed as AD may, in fact, be LBD. However, consistently with our findings, LBD often exhibits features of PD or atypical parkinsonism. In this context, atypical parkinsonism shares clinical features with PD, but among its differential diagnostic criteria, the significant and long-lasting response to levodopa strongly suggest PD rather than atypical parkinsonian syndromes.

Another key gene in which we identified genetic variants associated with both AD and MCI is *POLG*. This gene is associated with mitochondrial DNA depletion syndrome 4A Alpers type (MIM #203700) and MNGIE type (MIM #613662), both of which exhibit autosomal recessive inheritance. Additionally, *POLG* is linked to progressive external ophthalmoplegia, in both autosomal dominant (MIM #157640) and autosomal recessive (MIM #258450) forms. Beyond these Mendelian conditions, existing literature has also implicated *POLG* in early-onset parkinsonism, Alzheimer’s disease, and neuronal loss [29–31]. We believe that the present study further supports the association of *POLG* with neurodegeneration and may serve as a basis to inspire further research into this important topic.

As outlined by Pearson’s correlation analysis (Figure 4), the phenotypic indices ADL and IADL exhibited a strong linear correlation. Both indices assess an individual's

ability to perform daily tasks and are widely used to evaluate functional decline in neurodegenerative disorders. Notably, both ADL and IADL scores are negatively associated with quality of life in older adults. As highlighted in this study and extensively reported in the literature, the IADL/ADL scale is hierarchical, unidimensional, and unbiased by age [32,33]. Similarly, the MMSE scale scores we obtained did not seem to be significantly affected by age [34]. For instance, in the cohort examined in this study, individuals with an MMSE score higher than 25/30 had an average age of 57.5 ± 8.74 years. It is worth noting that all phenotypic scores were normalized for age and educational level. Notably, Pearson's correlation analysis among traits revealed no significant association with age at onset.

PCA analysis summarized the total phenotypic variability of the individuals carrying pathogenic and likely pathogenic variants (Figure 5). The plot graphically allowed the distribution of individuals diagnosed with a specific neurodegenerative disease harboring a specific genetic variant. It is worth mentioning that the individuals having a higher age at onset were located in the upper side of the PCA plot. In fact, as expected, the individual diagnosed with AD were graphically located in the positive quadrants (Figure 6). The age at onset of AD can vary due to a combination of genetic and non-genetic factors. Specific genetic variations may influence disease susceptibility and progression, while environmental exposures, lifestyle, and epigenetic mechanisms can further modulate the timing of symptom onset [35,36]. This variability highlights the complex interplay between genetic predisposition and external influences in neurodegenerative disorders. In our dataset, the patient with the earliest age at onset of AD was patient 8, who carried the TREM2:c.482+2T>C variant and developed symptoms at the age of 54. It is worth noting that individuals with MCI were positioned in the lower quadrants of the PCA plot. MCI is recognized as an early manifestation of dementia [37] and, in most cases it can precede the onset of AD or other degenerative cognitive disorders [38].

This genetic panel enabled the identification of the ultrarare prion diseases Gerstmann-Sträussler-Scheinker (GSS) and Creutzfeldt-Jakob disease (CJD), both classified as transmissible spongiform encephalopathies (TSEs). Diagnosing these disorders is particularly challenging due to their rarity and the considerable clinicopathological overlap among conditions such as AD, LBD, FTD [39].

In our cohort, the individual diagnosed with GSS was positioned in the lower section of the PCA plot, reflecting an early onset at 30 years, aligning with literature that reports an onset range of 30 to 60 years (average: 42 years). The identified genetic variant, PRNP c.305C>T (p.Pro102Leu), is well-documented in ClinVar [40]. However, GSS exhibits a broad phenotypic variability [41].

Conversely, the CJD case was associated with the PRNP c.598G>A (p.Glu200Lys) variant, with an onset at 54 years. This individual displayed high functional scores (ADL: 6/6, IADL: 6/8) and an above-average MMSE (18/30). Notably, both GSS and CJD share clinical manifestations such as cognitive decline, ataxia, motor dysfunction, and the presence of amyloid plaques in the brain [42].

As documented in the OMIM database, the ACE gene has been associated with renal tubular dysgenesis (MIM #267430), following an autosomal recessive inheritance, and with hemorrhagic stroke (MIM #614519), though its inheritance pattern remains unknown. However, according to the MalaCards database and several studies [43–45], ACE has been identified as a susceptibility gene and risk factor for neurodegenerative conditions.

Both variants identified in this study are located within the Peptidase M2 functional domain (amino acids 40-624) (PRU01355), crucial for regulating various physiological processes (Figure S5). We hypothesize that these variants may not be directly

causative of the observed phenotypic conditions, which could instead be influenced by unknown genetic mechanisms, environmental factors, or linkage with other causative variants.

Notably, the Arg496Gln variant has been reported in the ClinVar database (VCV000424035.7), but no associated phenotype was provided. In contrast, the Trp581fs variant is absent from ClinVar but exhibits a very low allele frequency (0.000006206) in the GnomAD database, having been identified exclusively in the European (non-Finnish) population.

The homozygous stop-gain variant c.97C>T (p.Gln33Ter) in the *TREM2* gene was identified in a single individual in our study, who presented with polycystic lipomembranous osteodysplasia. This patient exhibited a severe and early-onset form of the disease, with symptoms beginning at age 29. This variant has been previously reported in patients with similar clinical features [46], many of whom also had an early onset. This variant was also annotated in ClinVar database with the accession code VCV000005219.11. The variant p.Gln33Ter was localized within the Immunoglobulin V-set domain (Figure S7). As annotated, this functional domain is involved in cell-cell recognition, cell-surface receptors, muscle structure and the immune system. As documented in the OMIM database (MIM phenotype #618193), *TREM2* is associated with polycystic lipomembranous osteodysplasia, following an autosomal recessive inheritance pattern. However, previous studies and data from the GeneCards database suggest that *TREM2* may exhibit incomplete penetrance, and several causative autosomal dominant variants have been reported [47–49]. Consistently, in our study, likely pathogenic variants in *TREM2* were identified in heterozygous individuals diagnosed with AD and Binswanger's disease.

In real-world clinical practice, particularly in genetically heterogeneous and late-onset neurodegenerative disorders, our findings support the clinical–translational relevance of the tested gene panel, highlighting its feasibility, diagnostic impact, and practical applicability in routine settings.

3.2 Limitations

Nowadays, WES is widely employed in the diagnosis of neurodegenerative disorders, including those analysed in this study. In fact, WES exhibits a higher mutation rate compared to targeted genetic panels, allowing for the identification of novel genes that may be associated with dementia [50]. Nevertheless, the currently low incremental diagnostic yield of research exome sequencing supports the use of panel-based testing, which is more cost-effective and reduces issues related to the incidental detection of clinically relevant variants [12]. In this context, our study is distinguished by its clinical relevance, the expected clinical impact, its particular utility in late-onset or diagnostically uncertain patients, and the added value of a targeted gene panel. However, in the present study we applied a specifically customized targeted genetic panel, which was developed as part of an on-going research project at the Oasi Research Institute-IRCCS of Troina (Italy) started in 2018. Although the panel used in the present study analyses fewer genes compared to WES, it offers a faster diagnostic approach while maintaining a moderate diagnostic yield. We emphasize that the future of genetic diagnostic protocols will likely rely on whole-genome sequencing (WGS) or long reads sequencing approaches, as these technologies enable the detection of not only chromosomal abnormalities but also causative deep intronic variants that may be missed by more targeted methods [51].

Another potential limitation of this study is the absence of parental genetic data, which would have provided valuable insights into the inheritance patterns of the identified

variants, including the distinction between inherited and de novo mutations. De novo events, in particular, may play a significant role in the development of dementia phenotypes. Unfortunately, this limitation is common in neurodegenerative disorder research, as the advanced age of many affected individuals often precludes access to parental samples.

4. Materials and methods

4.1. Participants and assessment

In this study, a customized genetic panel was tested on a total of 186 consecutive patients (85 females and 101 males) presenting with an adult-onset progressive cognitive and/or movement disorder. Subjects were recruited between 2018 and 2022 from the following centres: Oasi Research Institute–IRCCS in Troina (Italy). All participants were Caucasian and of Sicilian ancestry, in order to minimize the potential effect of any environmental factor. The presence of a neurodegenerative cognitive (i.e., MCI, AD, LBD, FTD) and/or movement disorder (i.e., PD, early onset parkinsonism) was considered an inclusion criterion, whereas patients with secondary forms of dementia or movement disorders, as well as those with other neurological, neuropsychiatric, or systemic diseases with secondary CNS involvement, were excluded; additional exclusion criteria were: age < 18 years and refusal to provide informed consent or genetic test. Family history was collected through a detailed interview with a first-degree relative or the proband's partner. Clinical and past medical history of each patient was collected, and all the available documents related to the patient and any other affected family member (e.g., medical records, certificates, and drug prescriptions) were acquired. All clinical diagnoses were provided by trained neurologists, in accordance with current international diagnostic criteria.
All subjects, or their legal guardians, gave written informed consent for inclusion before their inclusion in the experimental protocol. The study was conducted in accordance with the Declaration of Helsinki of 1964 and its later amendments and was approved by the Ethics Committee of the Oasi Research Institute–IRCCS of Troina, Italy (approval code: 2018/07/18/CE-IRCCS-OASI/14).

4.2. Genomic DNA extraction and genetic panel design

Genomic DNA was extracted from peripheral blood leukocytes of the individuals under investigation, as previously described [52]. The genetic panel used in this study was based on the MRC Dementia Gene Panel [13], which originally comprised 16 genes and C9orf72. In our customized panel, we included all genes from the original panel and added 44 additional genes that were manually selected based on prior associations with dementia, Alzheimer's disease, Parkinson's disease as reported in the MalaCards database. The *AAAS*, *CYP17A1*, and *RAI1* genes were also added to the panel because of their reported associations with neurodevelopmental disorders and cognitive impairment. Table S3 includes all the genes used in the gene customized gene panel, their associated diseases and the respective inheritance annotated in OMIM database. Next-generation sequencing (NGS) was conducted using the Ion Torrent PGM platform. Template preparation, clonal amplification, recovery, enrichment of template-positive Ion Sphere™ Particles, and loading onto Ion 314 semiconductor chips were performed using the Ion Chef™ System. Sequencing was carried out with the Ion S5 Sequencing Kit (Thermo Fisher Scientific).
Post-run data processing was conducted using Ion Torrent Suite 5.10, Variant Caller 5.10, Coverage Analysis 5.10, and Ion Reporter. The reference genome used was hg19. Variants were filtered according to the following criteria: (i) recessive, de novo, or X-

linked inheritance patterns; (ii) minor allele frequency (MAF) < 1%, based on the 1000 Genomes, ESP6500, ExAC, and gnomAD databases. Variant filtering and prioritization were performed using VarAft software, and candidate variants were visualized with the Integrated Genomics Viewer (IGV). All candidate variants were validated by Sanger sequencing. The selected variants were classified according to the guidelines established by the American College of Medical Genetics and Genomics (ACMG) [53], using the Varsome platform [54]. Individuals were considered positive only if they carried variants classified as likely pathogenic or pathogenic.

4.3. Data analysis

Pathogenic and likely pathogenic variant data were statistically analyzed using Pearson's correlation and principal component analysis (PCA). For these analyses, the R packages ggplot2, corrplot, FactoMineR, and factoextra (RStudio version 3.4.3) were employed. The alluvial Sankey diagram was generated using the ggalluvial package (RStudio version 3.4.3) to illustrate the relationship between genes and associated phenotypes. The UniProt database (https://www.uniprot.org/, accessed on 10 March 2025) was used to retrieve information on the proteins GBA (P04062), POLG (P54098), ACE (P12821), and TREM2 (Q9NZC2), including their respective protein identifiers. These identifiers were subsequently used in UCSF ChimeraX software (version 1.8) to obtain structural models for each protein. UCSF ChimeraX is developed by the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco, with support from NIH grants R01-GM129325 and the Office of Cyber Infrastructure and Computational Biology, NIAID (https://www.cgl.ucsf.edu/chimerax/, accessed on 10 March 2025). To investigate the functional domains of the aforementioned proteins, the InterPro database (https://www.ebi.ac.uk/interpro/, accessed on 10 March 2025) was employed. The NMDEscPredictor (https://nmdprediction.shinyapps.io/nmdescpredictor/, accessed on 20 January 2026) was used for the in silico evaluation of nonsense-mediated mRNA decay (NMD) affecting the *TREM2* transcript as a result of the c.482+2T>C variant.

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