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Review

Calabria as a Genetic Isolate, a Model for the Study of Neurodegenerative Diseases

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Abstract: Although originally multi-ethnic in its structure, nowadays the Calabria region of southern Italy represents an area with a low genetic heterogeneity and a high level of consanguinity that allows rare mutations to be maintained due to the founder effect. A complex research methodology ranging from clinical activity to genealogical reconstruction of families/populations along the centuries, creation of databases, and molecular/genetic research, has been modelled on the characteristics of the Calabrian population for more than three decades. This methodology allows to the identification of several novel genetic mutations or variants associated with neurodegenerative diseases. In addition, in this population it has been reported a higher prevalence of several hereditary neurodegenerative diseases such as Alzheimer's disease, Frontotemporal dementia, Parkinson's disease, Niemann Pick type C disease, Spino-cerebellar ataxia, Creutzfeldt–Jakob disease and Gerstmann Straussler Scheincker disease. Thus, Calabria constitutes a model for the study of neurodegenerative diseases, a sort of "outdoor laboratory" useful for the advancement of knowledge in this field. Here, we summarize and discuss some results of research data supporting the view that Calabria is a genetic isolate and could represent a useful model for the study and characterization of neurodegenerative diseases.

Keywords: Calabria; Italy; neurodegenerative diseases; Alzheimer's disease; Frontotemporal dementia; Parkinson's disease; Niemann Pick type C disease; Spino-cerebellar ataxia; Creutzfeldt–Jakob disease; Gerstmann Straussler Scheincker disease

1. Introduction

Calabria is a region of southern Italy of 1 839 352 inhabitants [1], which constitutes the tip of the "boot" of Italy, the Strait of Messina separates it from Sicily, and it is suspended between two seas, the Ionian Sea and the Tyrrhenian Sea [2]. This strategic geographical position has favored various migratory flows both in pre-historical and historical times (e.g., Greek, Phoenician and Carthaginian colonization, Roman, Arab and Norman conquest) that have determined the presence of different genetic layers in the current population [3,4].

Although originally multi-ethnic in its structure, the Calabrian population remained relatively stable over the last three centuries, allowing its genetic "imprint" to remain constant over time. This phenomenon was determined by high flows of emigration and low immigration that, together with the geographical characteristics of the region (mountains and scarce and difficult communication routes), favored the maintenance of closed populations with high rate of inbreeding that are the characteristics of genetic isolates. Thus, nowadays Calabria represents an area with a low genetic heterogeneity and a high level of consanguinity that allows rare mutations - that originally represent the organism's response to a better environmental adaptation but become causative of very serious diseases

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in adults - to be maintained due to the founder effect [5,6]. A typical example is given by the presenilin 1 mutation which has protected over the centuries from very high perinatal mortality (the carriers of the mutation survived) but then developed hereditary Alzheimer's disease at about the age of 40 [5]. In addition, in Calabria it has been reported a higher prevalence of rare autosomal recessive disease with neurological features, such as fucosidosis [7], hereditary motor and sensory neuropathy [8], benign familial infantile seizures, familial paroxysmal kinesigenic dystonia and familial infantile convulsions with paroxysmal choreoathetosis [9] - as a genetic consequence of high consanguinity.

Beyond these autosomal recessive diseases and Alzheimer's disease, other rare and hereditary neurodegenerative diseases have been documented with high frequency such as Frontotemporal dementia, Parkinson's disease, Niemann Pick type C disease, Spinocerebellar ataxia) [10-13], Creutzfeldt–Jakob disease and Gerstmann Straussler Scheincker disease (*personal data*).

Here, we summarize and discuss some results of research data supporting the view that Calabria could be considered as a genetic isolate and could represent a model, a sort of outdoor laboratory – similar to other very few places in the world - useful for the advancement of knowledge of neurodegenerative diseases.

2. Neurodegenerative diseases, issues ad study methodologies

Neurodegenerative diseases are chronic, incurable and debilitating conditions originating from progressive degeneration and/or death of neurons. Depending on the brain areas involved and on its connections, neurodegenerative process can present with movement disorders and or cognitive - behavioral diseases such as dementias [14].

Dementia is a clinical syndrome characterized by a progressive decline in cognitive functions associated with the loss of functional autonomy and behavioral-psychiatric symptoms, the etiology of which may depend on different diseases, both neurological and systemic [15]. Dementia affects about 50 million people worldwide and is related to dependence, poor quality of life, early institutionalization, and mortality [16]. Dementia has important social and economic implications both for direct medical and social care costs as well as for informal care. A global cost of dementia of US$ 1.3 trillion was estimated in 2019 and this amount is expected to increase even more as both people with dementia and care costs are expected to increase in the coming years [17].

The etiology of most neurodegenerative dementias has been considered multifactorial including both genetic and environmental factors. However, in many patients, the disease can be inherited as a Mendelian trait (i.e., monogenic form) [18]. Generally neurodegenerative diseases involve some common pathogenic mechanisms such as the accumulation, aggregation or altered folding of proteins and/or mitochondrial dysfunctions that cause damage to the nervous system [19]. These pathological features lead to the manifestation of several symptoms that often overlap between different neurodegenerative diseases. Sharing clinical characteristics makes diagnosis particularly difficult [18]. In addition, diagnosis is also made problematic by individual variability: patients with the same mutation in the same gene frequently manifest a range of different clinical symptoms [20] including variability of the age of onset [21]. Therefore, the use of a rigorous research methodology, focusing on hereditary neurodegenerative diseases caused by a unique specific mutation, can allow to overcome the clinical heterogeneity further permitting the building of large kindreds that are a useful model for neurodegeneration studies.

A complex research methodology, ranging from clinical activity to genealogical reconstruction of families/populations along the centuries, building of database, and molecular/genetic research, has been modelled on the characteristics of the Calabrian population. In particular, the study starts from the clinical and diagnostic assessment of living patients that permits the family's characterization. The genealogical reconstruction of the families/populations, in which the disease is transmitted, allows the identification of new affected surnames and consequently new patients with the same disease. Genealogical study requires the acquisition and analyses of birth, death and marriage certificates in the

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municipalities (available from 1809) and of baptism, burial, marriage and *Status Animarum* in the parishes (available from 1600). Data are further computerized in the e-database structure. The current database of the Regional Neurogenetic Centre contains over 190,000 records of subjects linked by transitive relations, filiation, and marriage and from different areas of Calabria [22,23].

3. Calabria as a genetic isolate for neurodegenerative diseases

Over the time of more than 25 years, through the methodology briefly summarized above, we identified many causative mutations of several neurodegenerative diseases that often manifest with atypical phenotypes. These mutations, moreover, have shown a different distribution along Calabria, as reconstructed by the geographical localization of the patients followed at Regional Neurogenetic Centre (**Figure 1**).

In the next sub-paragraphs, we will provide a description of the main research data collected on this topic.

3.1. Alzheimer's disease

Alzheimer's disease (AD) represents the most widespread type of dementia, clinically characterized both by cognitive and non-cognitive symptoms [24]. Main neuropathological features of AD are represented by extracellular deposits of amyloid beta (Aβ) peptide and other molecules (amyloid plaques) and intracellular deposits of the hyperphosphorylated form of the protein tau (neurofibrillary tangles)[25]. AD can be classified into sporadic (SAD) and familial (FAD) based on the presence or absence of a clear hereditary genetic component [25,26]. In the familial forms have been documented mutations in three main genes: Presenilin 1 (PSEN1), Presenilin 2 (PSEN2) and Amyloid Beta Precursor Protein (APP), with an autosomal dominant inheritance (autosomal dominant AD, ADAD) [27].

A clinical and genealogical study started in the eighty allowed the reconstruction of one of the few very large family in the world, known as N family [28], that in 1995 was instrumental, together with another italian family of Turin (TO family, [29]) in the identification and cloning of the PSEN1 gene, both families carrying the same Met146Leu mutation [30] thus demonstrating the same common origin.

Ancient clinical data for the N and TO families were found among medical records of the Psychiatric Hospital of Girifalco (Catanzaro) which operated from 1881 to 1978. It is worth mentioning the clinical record of Angela R., an ancestor of the N family – datable to 1904, before Alois Alzheimer's description of the first case of this disease - which showed a clinical picture consistent with a diagnosis of non-amnestic probable AD, matching the ''dysexecutive'' phenotype described in her descendants. The a posteriori diagnosis of AD was supported by the evidence of the causative genetic mutation PSEN1 Met146Leu as well as neuropathological AD features in her genealogically proven descendants [31].

Our studies demonstrated that the PSEN1 Met146Leu mutation was private and founder in Calabrian population shared among hundreds of affected subjects dispersed on several centuries and several continents due to Calabrian emigration flow. The N-TO families are still considered as a unique ADAD population reconstructed from present time back to the 17th century, over 11 generations and consisting of over 160 affected subjects who share a common ancestor calculated around the year 1000 [5]. The genealogical database reconstructed around the Calabrian kindreds contains subjects linked through the transitive filiation-marriage relationships and approximately 50.000 persons from 1600 to the present day [22]. Although these patients shared the same PSEN1 Met146Leu mutation, four different clinical pictures were identified: two classics for AD (memory deficits and spatial and temporal disorientation) and two characterized by symptoms pointing to frontal lobe involvement (apathetic and dysexecutive subgroups) [5].

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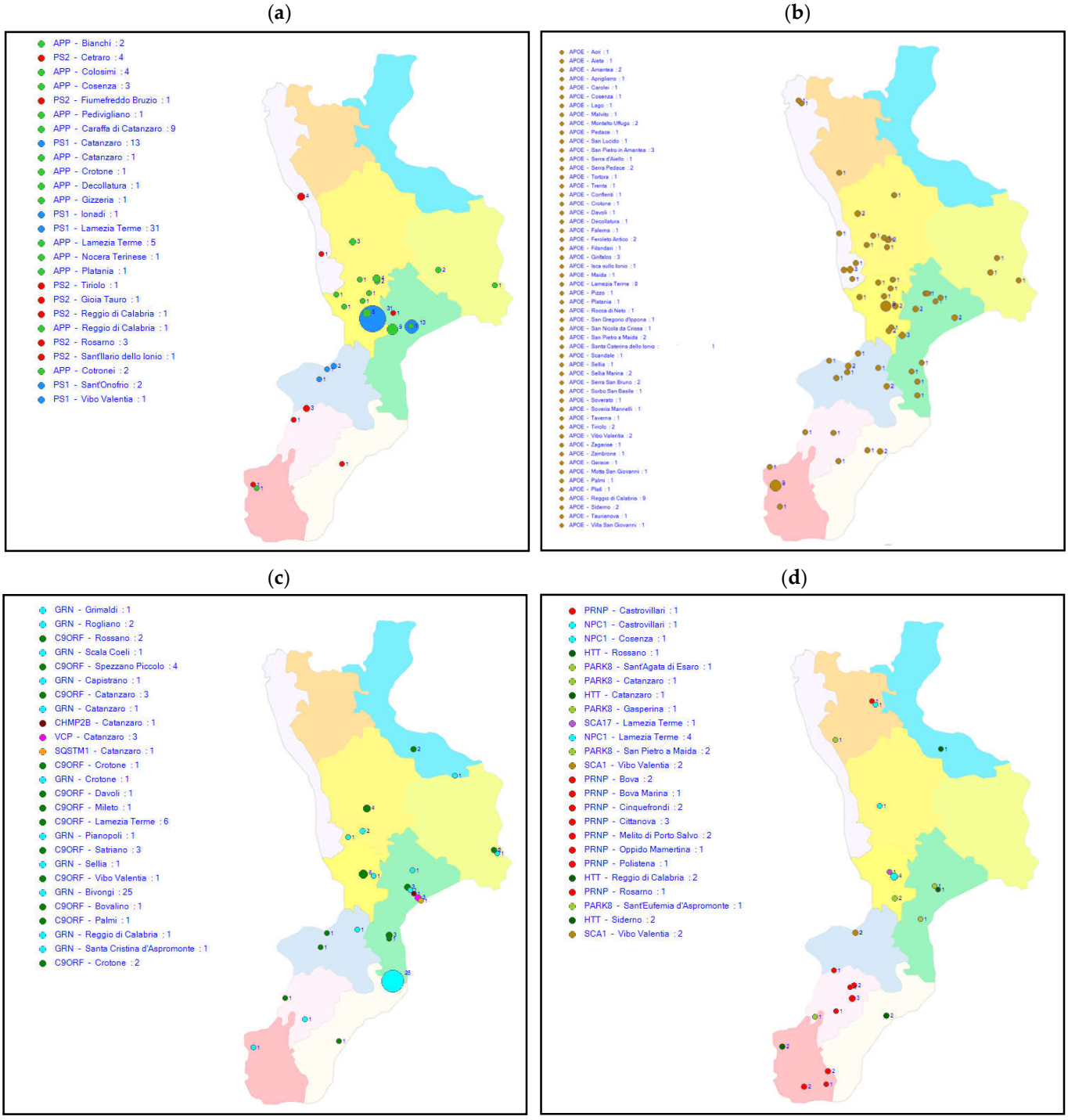

Figure 1. Geographical distribution of mutations in Calabria: (**a**) Mutations associated to Alzheimer's disease (**b**) APOEε4 carriers affected by Alzheimer's diseases; (c) Mutations associated to Frontotemporal dementia and Amyotrophic Lateral Sclerosis; (**d**) Mutation associated to other neurodegenerative diseases (*unpublished data*).

This variability could be due to the localization of the early AD lesions in different cortical areas or to different unknown genetic modifiers [5] and underline the broad phenotypic spectrum of Calabrian founder mutations.

In addition, the research on the N Family led to the identification of Nicastrin, a transmembrane glycoprotein that is part of the gamma secretase protein complex, which is one of the proteases involved in processing amyloid precursor protein (APP) to form the Aβ peptide [32]. The protein was so called to acknowledge the importance of the N

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Family, originating from the Calabrian village named Nicastro (today part of Lamezia Terme) [33].

Moreover, the characterization of other Calabrian families and subjects also allowed to identify other novel mutations or variants associated with AD, such as: PSEN1 I143V [34], M84V [35] and E318G [36] and PSEN2 Val139Met [37] and Ser130Leu [38].

Another important result, which reinforce the view of Calabria as a genetic isolate for neurodegenerative diseases, is related to the APP_{A713T} mutation. APP_{A713T} was initially described by Armstrong et al. [39] as a rare polymorphism with dominant inheritance associated with AD. However, from a first study that we carried out in 2004 emerged an association of this mutation with early-onset dementia with multiple strokes in several members of a family coming from a village of central Calabria. The neuropathological study of the proband revealed the presence of AD with severe cerebral amyloid angiopathy (CAA) and multiple infarcts [12]. Considering that other studies also revealed that some APP mutations (i.e., p.Ala692Gly, p.Glu693Lys, and p.Asp694Asn) can be associated with CAA [40], we decided to investigate the presence of APP_{A713T} mutation in 59 Calabrian patients affected by early-onset AD with cerebrovascular lesions (CVLs), a family history of dementia and a neuroradiological evidence of white matter lesions (WMLs) or hypodensities. The results of this study showed the presence of the APP_{A713T} mutation in heterozygosis in three late-onset unrelated patients living in different areas of the Calabria region (i.e., prevalence of 5%) [41]. The same mutation in homozygous affected subjects was also identified in another unrelated Calabrian family coming from a village of central Calabria affected by autosomal dominant AD with CVLs due to CAA [41]. Interestingly, both heterozygous and homozygous cases show a similar clinical picture characterized by memory loss, absence of insight, and behavioral and personality changes [40,41]. More recently, we have been reported the same mutation in a Belgian AD patient of presumed Italian descent and in another AD patient identified in Northern Italy with Calabrian origin. Thus, we used a population genomics approach to estimate the inheritance from a common ancestor of the APP_{A713T} mutation in the Belgian and northern Italy patients and in six patients that were representative of all apparently unrelated APP_{A713T} Calabrian families. The results showed that all carriers fell into the genetic variability of Southern Italy. In addition, five out of seven patients shared a 1.7 Mbp-long DNA segment centered on the APP_{713T} mutation, making it possible to estimate the time of the most recent common ancestor for its common origin in the Calabrian region dating back over 1000 years [26].

Despite these evidence, in Alzforum database the APP_{A713T} mutation is considered of uncertain significance [42]. However, the bioinformatical analysis on varsome [43] showed that it can be classified as "likely pathogenetic" according to the American College of Medical Genetics (ACMG) criteria [44]: PM1) is located in a mutational hot spot and/or critical and well-established functional domain; PP3) Multiple lines of computational evidence support a deleterious effect on the gene or gene product; PP5) Reputable source recently reports this variant as pathogenic but the evidence is not available to the laboratory to perform an independent evaluation. Further studies are thus needed to fill the knowledge gap in APP_{A713T} mutation and pathogenicity at least in Calabrian population.

3.2. Frontotemporal dementia

Frontotemporal dementia (FTD) represents the most prevalent neurodegenerative disorder with a presenile onset [45]. Based on clinical presentation, it can be classified into: (i) behavioral variant FTD (bvFTD); (ii) non-fluent primary progressive aphasia (PPA); (iii) and semantic variant primary progressive aphasia (svPPA) [46]. Recent estimates indicate that most cases of FTD are sporadic (55-75%) whereas the remaining 25-55% of cases are familial in which mutations in three main genes have been documented: chromosome 9 open reading frame 72 (*C9ORF72*), microtubule associated protein tau (*MAPT*) and progranulin (*GRN*) [47]. These mutations give rise to a high heterogeneity of clinical and

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neuropathological manifestations with a varying degrees of frontal and temporal lobe neuronal loss, atrophy, gliosis and proteins accumulation [48]. In particular, *MAPT* mutations mainly determine the deposition of the microtubule-associated protein tau whereas *C9ORF72* mutations of the transactive response (TAR) DNA binding protein of 43 kDa (TDP43). Instead, *GRN* mutations give rise to a predominance of diffuse granular neuronal cytoplasmic inclusions (NCIs) [48]. Many other mutated genes have also been associated to frontal dementia (e.g., *VCP, CHMP2B, TARDBP, FUS, SQSTM1, CHCHD10, TBK1, OPTN, CCNF, TIA1*) enlarging the genetic and phenotypic spectrum of this disease [49].

In 2002, applying the same methodology previously adopted for Alzheimer's disease, we reconstructed a large pedigree - known as B family - for 15 generations back to 16th century in the village of Bivongi (Reggio Calabria). The corresponding database encompasses about 8,000 persons from both the affected lineages and the unaffected (spousal) lineages. Thirty-four persons (19 females) over four consecutive generations have been identified as affected by FTD. All FTD patients have been linked to the same ancestors who lived in the early 18th century. Interestingly, although an autosomal dominant transmission was evident, none of the affected individuals had mutations of the *MAPT* gene - the only gene so far identified at that time as being associated with FTD [50]. n a subsequent door to door FTD study targeting all subjects in the B village who were ≥ 50 years of age, 78 patients (23 belonging to B family) were included [10], and we investigated the *GRN* gene, which was discovered to be associated with FTD in other countries [51]. Surprisingly, we found a very high and unusual prevalence rate of FTD (3.5%) and three different and novel mutations: one truncating *GRN* mutation (c.1145insA) and the two A266P and C126W. All mutations resulted associated with a very variable age of onset (between 35 and 87 years) [10] and also to three distinct phenotypes (behavioral, affective and delirious type) underlining the high prevalence and clinical variability that characterize the neurodegenerative diseases in the Calabrian population.

The characterization of other Calabrian families and subjects also allowed to identify other novel mutations or variants associated with FTD), *MAPT* Val75Ala [52], V363I [53], IVS10+4A > C and IVS9−15T > C [54], *GRN* Cys139Arg [55] and c.1145insA [56] and Cys139Arg [57], also in genes not generally associated to FTD such as *PRNP* P39L [58], *PSEN2* Arg62His [52], *PSEN1* Val412Ile [55].

More interestingly, we have recently identified a novel mutation (D395A) in *VCP* gene associated with early-onset FTD in a Calabrian family of central area [59]. Previous data reported that mutations in this gene cause a rare multisystem proteinopathy known as inclusion body myopathy (IBM) associated with Paget's disease of bone (PDB) and early-onset FTD (IBMPFD) [60]. *VCP* mutations have also been reported in patients with amyotrophic lateral sclerosis (ALS) [61], Charcot-Marie-Tooth type 2 (CMT2) disease [62], and hereditary spastic paraplegia (HSP) [63]. In addition, Saracino et al. [64] documented a *VCP*-mutated patient with FTD that did not develop clinical symptoms of PDB or IBM. In our case, the FTD was developed by three siblings without PDB and IBM signs underlining the autosomal dominant transmission of this new mutation and, once again, the lack of a "classic" genotype-phenotype correlation of a mutation in members of the Calabrian population. To our knowledge, our study represented the first description of *VCP*-related FTD phenotype in patients belonging to the same family, suggesting that a *VCP* analysis should be considered for the genetic screening of familial FTD with an early-onset also in absence of clinical sings of IBM or PDB, at least in Calabrian population [59].

3.3. Parkinson's disease

Parkinson's disease (PD) is the second most common neurodegenerative disease, with a prevalence of 1–2% in the population over 60 years of age [65], characterized by the presence of Lewy bodies and the loss of dopaminergic neurons in the *pars compacta* of the substantia nigra [66]. The main clinical features are represented both by motor (e.g.,

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bradykinesia, rigidity, postural instability, and resting tremor) and non-motor symptoms (e.g., hallucinations, anxiety, depression, cognitive dysfunction, sleep impairment, fatigue and urinary disturbance) [67,68]. Although the etiology of PD is multifactorial, many causative genes have been identified such *α-synuclein*, *PARK2*, *PARK7*, *PINK1*, and *LRRK2* [69]. Between 2006 and 2011 we performed a screening to verify the presence of *LRRK2* mutations in 88 Calabrian patients affected by PD (63 sporadic and 25 with a family history of PD or other neurodegenerative diseases) [12]. In our cohort we reported a prevalence of 10.2% of *LRRK2* mutations that appears higher compared to previous epidemiological studies on other population [70]. In the same way, the frequency of the most common worldwide mutation, p.Gly2019Ser, was 3.2% higher in our sporadic cohort with respect to previously reported data [71] and it could be explained by the different ethnicity and by the relative genetic isolation of the Calabrian population. In addition, this study allows to identify three novel missense variations of *LRRK2* gene (p.Phe1227Leu, p.Gly1520Ala, and p.Ile2020Ser) associated with PD in Calabrian patients underlying, once again, the peculiar genetic characteristics of this population, useful for provide additional genetic insight into PD 12].

In recent decades, another research group has tried to characterize the genetics of PD in the Calabrian population, showing its peculiarities. For example, in Calabrian patients with PD, it has been reported a lower frequency of mutations in several genes, such as *CHCHD2* [72], *TARDBP* [73], *LRP10* [74] and *DNAJC13* [75], which instead are common in other cohort of PD patients [76-79]. Conversely, in Calabrian PD patients' data shown a higher prevalence of mutation on *GBA* gene [80]. These evidence further strengthening the view of Calabria as a genetic isolate for neurodegenerative diseases and suggest continuing the search for novel mutations for PD in this population.

3.4. Niemann-Pick type C disease

Niemann-Pick type C disease (NPC) is a rare autosomal recessive inherited lipid storage disease caused by a defect in the intracellular trafficking of cholesterol [81]. The genes responsible for NPC are the *NPC1* gene, located on chromosome 18 and mutated in 95% of patients, and the *NPC2* gene located on chromosome 14 [82]. These gene encode for the NPC1 and NPC2 proteins that cooperatively mediate the egress of cholesterol from endosomes/lysosomes [83]. Due to the high heterogeneity of the clinical presentation, the disease is classified into 5 different forms based on the age of onset: perinatal (<2 months), early infantile (<2 years), infantile (<5 years), juvenile (<15 years) and adults (15-70 years) [84]. In adults, NPC often presents with a slowly worsening evolution typical of chronic neurodegenerative diseases.

Until few years ago, the heterozygous status for *NPC1* or *NPC2* mutations was considered non-pathogenic and heterozygous carriers were not supposed to develop any neurological symptoms during their life. However, in 2013 a prevalence study [85] revealed a frequency of 3.6% for heterozygous mutations in *NPC* genes in a population of adults affected by dementia, parkinsonism, or psychosis. These data prompted us to speculate that, depending on the type of mutation, the heterozygous condition may induce an alteration of lipid metabolism and therefore a "benign" phenotype of NPC, with onset in adulthood-old age and slowly progressive course, when the structure of the protein is altered. Instead, when the mutations do not alter the protein structure, heterozygous status could represent a risk factor for neurodegenerative diseases. To verify this hypothesis, we conducted a screening study for *NPC1* and *NPC2* mutations in 50 Calabrian patients affected by dementia with atypical clinical presentations or dementia plus, in which progressive and invalidating cognitive impairment was the main clinical feature associated with other neuropsychiatric and systemic symptoms [12]. Sequencing analysis revealed four different and known heterozygous mutations in *NPC1* (p. F284LfsX26 and c.1947+8G>C) and *NPC2* (p.V30M and c.441+1G>A) genes. The p.F284LfsX26 mutations was associated with a picture of progressive supranuclear palsy-like syndrome whereas the other three with a corticobasal syndrome. The results of our study demonstrated that

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heterozygous mutations of *NPC1* and *NPC2* genes could contribute to dementia plus, at least in a subset of Calabrian patients.

Thus, heterozygosity can be a risk factor for dementia, and this is also confirmed by the high risk of neurodegenerative diseases, especially AD, in the parents of NCP patients [86] and by the links between lipid metabolism and Aβ [87]. Therefore, even the study of very rare forms can help to identify pathogenetic pathways of neurodegeneration that have not yet been fully elucidated.

3.5. Spinocerebellar Ataxia Type 17

Autosomal dominant cerebellar ataxia encompasses a group of neurodegenerative diseases clinically characterized by ataxia, ophthalmoplegia, pyramidal and extrapyramidal signs, and peripheral neuropathy. Dementia occurs only in some forms of spinocerebellar ataxia (SCA), such as SCA1, SCA2, SCA3 and SCA12, developing in the latest stages of the disease, while in SCA17 dementia is a constant feature of the phenotype [88]. A CAG repeat expansion in the TATA boxbinding protein (*TBP*) gene on chromosome 6 has been identified as the cause of SCA17 in some familial and sporadic cases, resulting in cerebellar ataxia and followed by dementia, parkinsonism, and dystonia, with onset in childhood and adulthood [89,90].

Starting from the end of the 1990s our clinical and research attention was attracted by a large autosomal dominant Calabrian family with a complex neurologic syndrome that comprises early-onset dementia, psychotic features, extrapyramidal and cerebellar signs and epilepsy [91]. The genealogic reconstruction of this family initially included 57 individuals (14 affected, 7 personally observed) in 5 generations. Since the clinical picture seemed to mimic different forms of neurodegenerative diseases in an atypical way, we decided to carry out on this family a large linkage analysis on 26 genes until then known to cause hereditary dementias (i.e., *APP, PSEN1, PSEN2, FTDP-17, BRI, PI12, FND, HD-like, SCA1, SCA2, SCA3, SCA4, SCA5, SCA6, SCA7, SCA8, SCA10, SCA11, SCA12, SCA13, PARK1, PARK2, PARK3, HD, DRPLA, PRNP*). Surprisingly, then molecular analyses excluded the presence of mutations in these genes [91]. In subsequent years, we continued to reconstruct the genealogy of this family, including a total of 230 members across 5 generations, among whom 16 individuals were affected (4 men, 12 women; 11 personally observed). The observation of further cases allowed to better characterize the clinical picture, which was characterized by early and prominent behavioral disorder that, together with the strong reduction of verbal fluency, was followed by a definite picture of frontal lobe dementia. However, also cerebellar signs were noticed later but were eventually masked by extrapyramidal signs such as dystonia and rigidity. Myoclonus and epilepsy were characteristic of the late stages of the disease. The main neuropathological characteristics of the autopsied case were a low brain weight, atrophy of the frontotemporal cortex, nerve cell loss in the precentral gyrus, the primary visual cortex, the striatum, and the thalamic dorsomedial nucleus, pseudo-hypertrophic degeneration of the inferior olive and severe loss of Purkinje cells. These evidence led us to hypothesize that it could be an atypical clinical picture of SCA17. The molecular analysis performed on *SCA17* gene confirmed our hypothesis. In particular, we found a stable 52 TBP CAG repeat expansion in this gene, despite the reported differences in the age of onset among generations 3, 4, and 5 (from 17 to 53) [13].Thus, the characteristics of this Calabrian family broaden the clinical picture of SCA17: initial presenile dementia with behavioral symptoms should be added to ataxia, rigidity, and dystonic movements, which are more commonly encountered.

4. Conclusion

Neurodegenerative diseases are highly variable and heterogeneous concerning causes and phenotypes [92]. Being moreover age-related diseases, metabolic and vascular risk factors are frequently co-occurring thus increasing confusion and complicating clinical and pathological aspects. The clinical and genetic research conducted on neurodegenerative diseases can simplify and reduce the variability by using a “simple model” that is

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constituted by the large families/ kindreds and populations in which neurodegenerative mendelian diseases segregate. The genotype-phenotype correlation studies have largely improved the clinical knowledge of these diseases and the relatively recent epigenetic and epigenomic research have addressed the undeniable precision medicine [93].

Despite the enormous advancement of knowledge concerning etiology and mechanisms of pathogenesis, many ways are still obscure and to date there is no cure stopping or modifying neurodegenerative disorders fatal course. However, the enormous socio-economic impact of these diseases due to the global aging of the populations, makes more and more urgent to better clarify mechanisms, identify all links of chains and possibly find one (or many) cure [94].

Genetic isolates are characterized by geographical and cultural isolation and low genetic variability due to lack of immigration and, consequently, high inbreeding. These populations offer advantage to finely characterize the genetic architecture of complex disorders, due to the high frequency of the common(s) pathological trait(s) and, therefore, the possibility to reconstruct genealogical families' trees, building large kindreds, giving the possibility to trace back mutation in the centuries and, rather easily, conduct wide-ranging molecular genetics analysis to isolate causative mutations in pathological genes.

In the rest of the world, only few places have been described as genetic isolate and have made possible to achieve important results on several diseases [95]. An Italian example, is represented by Sardinia in which founder mutations have been described in several medical areas, including neurological disorders, such as Wilson's disease [96], amyotrophic lateral sclerosis [97], and Parkinson's disease [98].

The clinical and research work done in about thirty years in Calabria, and developed with several research groups in the world, demonstrated the validity of the model for all the neurodegenerative diseases studied that undoubtedly increased the advancement of knowledge in this field. The study on Autosomal dominant Alzheimer's Disease (ADAD) mainly contributed to the isolation and cloning of *PSEN1* gene in 1995 [30], to the identification of the novel gamma secretase component protein "Nicastrin" [32], to the amyloid cascade hypothesis and to the description of one of the largest families carrying ADAD in the world [5]. The genotype-phenotype correlation studies on ADAD patients gave the possibility to better characterize early phenotypes showing frontal involvement or other atypical presentation and these clinical specific aspects were only successively recognized by the NIAA–AA criteria, for the AD diagnosis, in 2011 [99]. Clinical follow-up of Dominantly Inherited Alzheimer Network (DIAN) cohorts showed that the biological disease starts in brains of subjects carrying mutations approximatively 20 to 25 years before the clinical onset of the disease [100].

Frontotemporal dementia in the B. area of Calabria shows a prevalence largely higher compared to Alzheimer disease and no report exists to date in other countries of the world. Some causes have been identified in three different *GRN* mutations [10], but numerous cases remain to be determined in the etiology and epigenetic aspects need to be studied. Peculiar environment, rich of metal mines could be a trace to follow in the future.

Almost all the neurodegenerative diseases studied in Calabria show peculiarities. They are frequently associated with many novel causative or associated mutations or variants that often manifest with atypical phenotypes compared to the "classical" clinical pictures and at younger age. These data contributed to the results of a recent metanalysis that reported a worldwide higher prevalence of early-onset dementia (119 per 100.000 inhabitants) [101].

Often, in early-onset Calabrian patients the cognitive impairment is accompanied by a higher frequency of neuropsychiatric symptoms, such as apathy, agitation, depression, hallucinations, anxiety, disinhibition, and eating disorders [102]. The cause for increased neuropsychiatric symptoms in these patients is likely multifactorial and includes both social and biological factors.

Although the different genetic background of genetic isolate areas generally has been considered of relatively scarce impact due to the rarity of the "rare diseases" [103], this

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cannot be applied to Calabrian population. Knowing the peculiarities of Calabria as genetic isolate and its neurodegenerative diseases can be important for the international medical and scientific community as the emigration to different places in Italy and the world (e.g., Australia, North and South America, Canada, Europe, England, etc.) occurred during the last centuries [104] and especially in the recent years [105]. Thus, emigration constantly brings out of this region thousands of people and therefore, individuals carrying rare mutations with their potential diseases, risk to be difficulty diagnosed out of their geographic informed context.

In addition, we must remember that we are facing with dominant diseases whose onset is in adulthood when the subjects, in most cases, have already children. Receiving a diagnosis of a neurodegenerative disease is surely more emotionally difficult for these patients due to their younger age, more responsibilities within their families such as taking care of children and holding down a job [106]. These specific aspects suggest that health services should be built for young onset forms of dementia (e.g., family counseling services, social services). General practitioners also need to be trained on all these diseases for an early diagnosis and intervention. Not to be outdone, the community must be informed and made aware of the existence of the early-onset forms that require not only medical and social care but also a reduction of social stigma to improve the quality of life of both patients and their families. Last but not least, these patients (and also at-risk subjects) could accept experimental clinical trials such as the novel disease modifying drugs as already done in prevention trials kicked off by DIAN-TU [107].

In conclusion Calabrian population could represent a useful model not only for the characterization of etiology and pathophysiology of several debilitating, and currently incurable, neurodegenerative diseases but also for the development of new treatments suitable all over the world.

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