Disease clustering: the example of ALS, PD, dementia and hereditary ataxias in Italy

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Summary

The “mini-epidemic” distribution of rare conditions (either sporadic, inherited or due to a transmissible agent) is frequently described as a cluster. Genetic abnormalities and environmental factors are usually investigated to explain the presence of a disease cluster. We have reported a cluster of amyotrophic lateral sclerosis (ALS) cases in a small area of central Italy, where an identical SOD1 gene mutation was found both in familial ALS (FALS) cases and in one apparently sporadic ALS individual. Along with this cluster of ALS patients, we review important clusters of neurological disorders in Italy and discuss the importance of an accurate estimation of their regional/local prevalence. This approach is likely to facilitate molecular investigations, the search for environmental agents and the analysis of gene-environment interaction in disease presentation and development. The organisation of national registers that record, in particular, the geographical distribution of neurological disorders, might represent a good research strategy.

KEY WORDS: Cluster, environmental factors, Italy, mutations, neurological disorders.

Introduction

Epidemiological studies constitute a powerful tool for the identification of clues to disease aetiology and for the evaluation of risk factors (e.g., environmental or occupational hazards) in public health management (1,2). Geographical epidemiology has become a distinct branch of epidemiology, with increasing attention being devoted to describing the health status of small, geographically-circumscribed populations (3).

In medical terms, a cluster is a “mini-epidemic” distribution of a pathological condition within a well-defined region, where it accounts for a higher-than-expected disease prevalence (i.e., above the estimated national prevalence). When, in some families, more “disease cases” occur than would be expected on the basis of the occurrence rate in the general population, we also talk of familial clustering. The identification of a cluster depends on the precise evaluation of several factors, including the expected prevalence of the disease in the given area and the geographical distribution of the patients under investigation. However, it can be argued that an “aggregation” of patients may also occur by chance or as a consequence of a statistical bias in the process of patient selection (2).

A genetic mutation with a recessive or dominant pattern of inheritance is often responsible for the appearance of a disease cluster, within a family or a geographical area. Exposure to environmental factors is another important cause of disease clustering. As postulated in Parkinson’s disease (PD), when the disease is not clearly related to a single gene mutation (4), the combined action of several genes together with exposure to environmental factors may be important pathogenically. In a situation where there is genetic predisposition to the disease and exposure to environmental precipitating factors (for members of some families but not for members of other families), a monogenic inheritance may be mimicked (5).

The importance of identifying disease clusters is increasingly debated. Systematic surveillance systems have been developed to identify foci of increased disease occurrence (together with possible risk factors), but despite this, their reliability remains a matter of discussion (6).

Social and cultural background can also play a part in the uneven distribution of several medical conditions (2). In addition to the aetiological factors, such as genetic abnormalities and/or environmental factors, socio-cultural habits and traditions appear to contribute to regional differences in general health status and in the development of several pathological disorders. A strong association between a population’s health status and social and economic characteristics has been shown by many methodological approaches, such as the “deprivation indices” developed in the United Kingdom (7). A significant association between antibodies against CMV and specific socio-demographic/geographical factors has also been found. The prevalence of these antibodies was significantly higher in females and in subjects residing in
the South of Italy (8). CMV infection may be a critical determinant for the appearance of neurological disorders, particularly in immune-compromised patients. To emphasize the importance of identifying regionally-confined clusters of patients suffering from a neurological condition, we describe our experience with a cluster of patients suffering from ALS. We have studied both the genetic and the environmental factors that may have played a role in the high occurrence of ALS in the area studied (9). The major clusters of neurological disorders in Italy are also reviewed, paying particular attention to analysis of genetic mutations, environmental hazards and gene-environment interaction. We discuss the importance of studying the geographical distribution of patients affected by a specific neurological disorder and the possible use of disease registers that would allow collection of and easy access to all the information regarding a patient population.

Amyotrophic lateral sclerosis

A 41-year-old man presenting with rapidly progressive weakness of the lower limbs was admitted to our clinic. The patient had a family history of ALS, and detailed investigation of the pedigree led to the identification of a large kindred with an autosomal dominant inheritance for ALS (FALS). The proband's mother died of ALS at 36 after 4 years of illness and his grandfather died at 55 after a 12-year disease course. In this particular family, we identified a total of 13 cases spanning back 6 generations to the beginning of the 19th century. Data were collected from interviews with family members, medical records, and church/civic records of births and deaths. With the cooperation of local general practitioners (GPs), we also analysed the distribution of other ALS cases in the area where the proband was identified: an area of approximately 400 km² located in the north of the central Italian province of Ascoli Piceno, including the valleys around Fermo (the area). A total of 12 different ALS cases (2 familial and 10 sporadic) were identified in a population numbering 166,451 people (1997). The prevalence of the disease according to our findings was 7.8/100,000, higher than the national frequency (which ranges between 1.56 (10) and 5.4 (11) per 100,000).

The SOD1 gene molecular analysis of the FALS cases (including our proband) yielded a missense point mutation causing the substitution of Leucine with Phenylalanine in exon 4 (L84F). We extended the molecular investigation to the sporadic ALS cases and to those members of the pedigrees at risk of inheriting the disease. The L84F SOD1 gene mutation was also detected in a 60-year-old sporadic ALS patient with unknown father and with clinical features that overlapped those of the FALS cases. Furthermore, the L84F mutation was also found in a 65-year-old unaffected woman belonging to the proband's family. One of the two new FALS patients was linked to the family lineage of our proband. In this pedigree a case of conjugal ALS was present in a previous generation, but no subject homozygous for the L84F mutation was detected. Due to the low population prevalence of ALS, this event may suggest that the disease had been inherited by two apparently unrelated individuals in a small community or that they had been exposed to the same environmental agent. A total of nine cases of conjugal ALS have been previously described (12), two of these from Italy (southern Italy and Sardinia). All these couples, including our family, originated from small, inbred rural communities.

The presence of a significant number of individuals sharing the same rare molecular abnormality in a small isolated population may suggest a common founder for the SOD1 gene mutation and a high level of inbreeding within the population in the area. The presence of ALS cases bearing a SOD1 gene mutation increased the total number of affected individuals in the area, and ultimately, the local disease prevalence to 7.8/100,000. The prevalence of sporadic ALS in the area was 4.8/100,000, which is within national estimated range. In this small population the SOD1 gene mutation spreads vertically through apparently distant lineages and distinct family pedigrees. Environmental factors were also investigated in search of other elements explaining the “apparently” high occurrence of ALS cases in this area. A putative external agent was unlikely to explain the appearance of the disease in subsequent generations (transmitted in an autosomal dominant fashion). Therefore, if an exogenous agent had been responsible for the occurrence of the disease throughout the generations, a “subtle” element, perhaps linked to a well-established local practice, could be postulated. We discovered that shoe manufacturing spread rapidly within a well-defined period in the area and that many individuals, including our FALS cases, were employed in this activity (whereas in the past, agriculture had been the most important form of occupation for the area's population). Nevertheless, no evidence of increased exposure to toxicants in these individuals or in the general population of the area emerged. Furthermore, analysis of local aqueduct water did not reveal high concentrations of manganese or magnesium, as reported in the previous investigations on environmental hazards in ALS (13,14). Finally, we were unable to find environmental factors that could explain the trend of generational anticipation that was present in these families.

Few other studies of the regional distribution of the disease are available in the literature. As for other neurological disorders, Sardinia has been a focus of epidemiological studies because it is isolated from foreign influence and has communities, throughout the island, that have maintained close kinship ties. Giagheddu et al. (15) analysed the occurrence of ALS cases in various areas of the island, and noted a significantly heterogeneous distribution of the disease. Maurelli et al. (16) reported 3 families in the province of Pavia, an area in northern Italy. Reconstruction of the pedigree was difficult and the SOD1 gene analysis was not carried out. Prior to our study, only one family harbouring a SOD1 gene mutation had been reported in Italy (17), while more recently, six other mutations have been identified in seven ALS families (18). The authors do not comment on the pattern of territorial distribution of these families.

Parkinson's disease (PD)

Unlike ALS, Parkinson's disease (PD) shows a much higher prevalence, varying in Italy from 74 to 257 cases per 100,000 members of the population (19). The crude
prevalence of idiopathic PD in Italy varies considerably, depending on the method of study utilised (20-22). An important “aggregation” of PD cases was detected in Sicily, where a door-to-door method of investigation was adopted (23).

Despite the presence in the southern Italian Campania and Molise regions (including the Salerno province) of an overall prevalence that is comparable to that of other western populations, a large kindred of autosomal dominant PD was found in a small village in the Salerno province (24). The procedures for identification and characterisation of the families living in this area were similar to those used in our study on the ALS familial cluster. Initially, two large kindreds were found in the village of Contursi (Salerno province) where the rate of occurrence of the illness was significantly high. The disease in the two kindreds showed a phenotypic homogeneity. Further investigation revealed a common origin of the two families and an autosomal dominant pattern of inheritance of the disease. Molecular studies confirmed the presence of a mutation in exon 4 in the α-synuclein gene with Ala to Thr substitution at position 53 (25,26). As in our experience with the ALS cluster, the genetic investigation confirmed a genetic mutation to be responsible for the significant increase in the number of PD cases in the above PD familial cluster.

Prior to the publication of these results, De Michele et al. analysed PD-related environmental and genetic risk factors in these regions of southern Italy. Family history was found to be the major risk factor for the disease, with 33% of the patients having had at least one affected relative (27). No environmental risk factor has been reported to act on the genetic background described in the Contursi families. The search for an external toxic agent in PD has focused in the past on the study of MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), which shows a selective toxicity for the neurones of the substantia nigra (28). However, no PD clusters caused by MPTP-related environmental toxins have, to date, been described (29). Several studies have also analysed susceptibility markers within important metabolic pathways, to identify genetic polymorphisms which might aggregate with the disease. Of these, the “pharmacogenetic” marker P450D6 debrisoquine 4-hydroxylase (a cytochrome P450-dependent enzyme which metabolises over 25 drugs) can be present in the “extensive” and “poor” metaboliser apolipoprotein E (ApoE) epsilon 4 allele (30). Several foci of high incidence of Creutzfeldt-Jacob disease (CJD) have been reported in France, Chile, Slovakia, Israel and among Libyan Jews (3,35,36). All of these resulted from high incidence of familial CJD in a restricted area. In Italy, a large familial cluster of CJD was found in the Calabria region, where, as mentioned above, the prevalence of dementia-related disorders (including the prevalence of early-onset FAD) linked to different genetic mutations, has been found to be significantly high. The CJD cluster in this region was later found to bear the E200K Prp gene mutation (37,38).

Autosomal dominant spinocerebellar ataxias (ADCAs)

Autosomal dominant spinocerebellar ataxia type 1 (ADCA1), the most common form of dominant ataxia, is another inherited neurological condition described mainly in the southern part of Italy, particularly in Sicily (39). Genetic heterogeneity is a specific hallmark of this condition where different loci have been found to bear a pathological expansion of a trinucleotide repeat. Molecular analysis in 51 families from these regions harbouring this disorder identified an SCA2 expansion in 30 families (39). In particular, molecular studies performed in 12 of 15 families with ADCA type I originating from mid-eastern Sicily identified the SCA2 mutation in 11 of them (91.6%) (40). Despite the lack of a clear analysis of the territorial distribution of these families in this part of the island, the presence of a substantial number of ADCA1 pedigrees with the same molecular abnormality in a small territory would strongly support the idea of a clustering distribution, where the disease and the mutation have spread through apparently unrelated families. Another pedigree with mutations in the SCA2 gene has been reported to have a southern Italian ancestry (41).
Concluding remarks

We have emphasised the importance of an accurate analysis of the territorial distribution of patients affected by neurological disorders. For a number of reasons, including socio-cultural factors, historical traditions and the particular regional/geographical conformation of the country, the Italian peninsula emerges as an extraordinary pool of inherited disorders. In certain regions of southern Italy, the rural populations are geographically isolated, which explains the significantly increased presence of several genetic disorders, most of them neurological. These diseases can appear in familial clusters and in some cases, a genetic abnormality has been found to cause the illness.

Epidemiological searches in confined regions, where patients with a family history of a neurological disorder can be identified, appear to be of primary importance. The validity of this approach is confirmed by our experience with an ALS cluster in central Italy. In our study we identified a concentration of familial and sporadic ALS patients confined within a restricted area, i.e., a cluster of the disease. Subsequent studies discovered an underlying genetic defect (a SOD1 gene mutation) causing the spread of the pathological condition within “apparently” non-related pedigrees (9). In a previous molecular investigation of several individuals suffering from sporadic and familial ALS and originating from different Italian regions, we failed to identify SOD1 gene mutations (42). These findings support the idea that the evaluation of patients within well-characterised clusters may represent a priority in the quest to find either known molecular defects or new aetiological clues.

Clusters of patients suffering from a specific neurological disorder may differ as regards the clinical features of the illness and the severity of its evolution. In the reported ALS pedigrees and in the clusters of familial CJD, individuals carrying the same genetic mutation show extreme variability in age at onset and disease duration (3,9,26,35,43,44). A comparative study of these clusters may provide further insight into genetic and/or environmental factors influencing the disease phenotype. The geographical location of a disease cluster can also be highly informative with regard to the role of toxic agents in the disease aetiology. To date, no significant relationship between a specific neurological disorder and a well-defined neurotoxic element has been identified, with the exception of some toxic polyneuropathies (45). An increased concentration of different elements, including metals in certain areas of the CNS of patients affected by different neurological disorders, has been suspected (46,47). Studies of the basal ganglia, which included measurement of the levels of iron, potassium, silica, sodium, sulphur, zinc and aluminium, as well as assessment of exposure to other environmental agents (particularly pesticides, industrial solvents and MPTP), did not reveal any pathological accumulation (48). Single gene abnormalities or environmental factors may cover the entire range of possible aetiologies of a neurological disorder. However, a complex interplay between various genes and environmental xenobiotics is likely to explain, at least in some cases, both the heterogeneous distribution of a neurological disorder and the occurrence of areas (i.e., clusters) with a higher-than-expected prevalence of the disorder. The islands of Sardinia and Sicily and the southern part of the Italian peninsula show an undoubtedly high prevalence of various neurological disorders and a cluster-like distribution of some of these conditions. In Sardinia, for example, diseases like myasthenia gravis, multiple sclerosis and insulin-dependent diabetes mellitus show frequencies up to 3-5 times higher than those found in the rest of Italy (49). The social context in these areas, characterised by large family groups and people traditionally not inclined to emigrate, may have favoured the spread of gene alypotypes involved in the disease presentation and development. As we have pointed out, subsequent migration from these areas with high rates of inherited disorders has, as reported above for AD and PD, resulted in the appearance of the same hereditary diseases (and possibly of associated genetic mutations) in other recipient regions and countries.

The relationship between genetic determinants and environmental factors is currently the focus of study through the project “Colombo 2000”, an analysis of the phenomenon of massive migration from Italy to Argentina. The project represents a “natural experiment” into gene-environment interactions in disease pathogenesis. Data on incidence, prevalence and mortality rates in the original and in the migrant population (exposed to different environmental factors) may be compared to elucidate risk factors, as well as protective influences, for different neurological disorders. Preliminary data from this study have shown differences in mortality rates for brain tumours, Parkinson’s disease, Alzheimer’s disease, motor neuron diseases, stroke and alcohol-related diseases, between Italy and Argentina (50,51).

In conclusion, we suggest that the observations outlined above underline the importance of careful analysis of the “territorial distribution” of patients afflicted by various neurological disorders. The implementation of regional and national disease registers, which report the origins of cases, would greatly favour such investigations and represent a crucial step towards the elucidation of the role of genetic mutations, environmental factors and/or gene-environment interaction in the pathogenesis of specific neurological disorders.

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