ORIGINAL ARTICLE

Genetic Creutzfeldt-Jakob disease in Sardinia: a case series linked to the *PRNP* R208H mutation due to a single founder effect

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Abstract

In genetic prion diseases (gPrD), five genetic variants (E200K, V210I, V180I, P102L, and D178N) are responsible for about 85% of cases. The R208H is one of the several additional rare mutations and to date, only 16 cases carrying this mutation have been reported worldwide. To describe the phenotypic features of 5 affected patients belonging to apparently unrelated Sardinian (Italian) families with R208H gPrD, and provide evidence for a possible founder effect are the aims of this study. The R208H *PRNP* mutation has a much higher relative frequency in Sardinia than elsewhere in Italy (72% vs. 4.4% of gCJD cases). Our cohort shared similar phenotypic features to the previously described patients with R208H-129M haplotype with most patients showing the classical Creutzfeldt-Jakob disease (CJD) phenotype. The analysis of 10 controls and 5 patients by NGS sequencing identified 4 haplotypes, 3 associated with the wild type variant, and one (H1) shared by all patients carrying the 208His variant. This is the first report of a regional cluster for R208H mutation in gPrD and the first report of the presence of a common ancestor for this Sardinian R208H cluster, confirming the probable consequences of genetic isolation process even for rare diseases.

Keywords Prion · Creutzfeldt-Jakob disease · CJD · gCJD · Genetic · Sardinia · Founder effect

Introduction

Prion diseases (PrDs) are neurodegenerative disorders caused by a transmissible abnormal conformer of the prion protein (PrP^{Sc}), also known as prion, which stand for proteinaceous infectious particle [1]. Typically, human PrDs include sporadic forms (sPrD), with sporadic Creutzfeldt-Jakob disease (sCJD) and sporadic fatal insomnia; genetic forms (gPrD), with genetic CJD (gCJD), fatal familial insomnia, and Gerstmann-Sträussler-Scheinker; and acquired (aPrD) forms, including iatrogenic and variant CJD. SPrD account for 85–90%, gSPrD for 10 to 15%, and aPrD for < 1% of human PrD, respectively.

GPrDs are caused by mutations in the *PRNP* gene encoding for the prion protein and mapping to chromosome 20p12-pt [2]. *PRNP* variants include point mutations leading to amino acid substitution, premature stop codon, or insertion of octapeptide repeats in the N-terminal region of the gene. To date, more than 60 *PRNP* variants have been identified, but five of them (E200K, V210I, V180I, P102L, and D178N) account for about 85% of gPrD (data from nine major surveillance centers worldwide) [3]. Some variants rely on regional clusters due to a proven (E200K mutation clusters among Slovakians, Sephardic Jews from Libya and Calabria region in Italy, D178N in Spain, Germany, and Italy) or likely founder effect (the V180I mutation in Japan, and V210I in Italy) [4]. In contrast, other less common mutations have been only found in single or few isolated individuals usually in the absence of a positive family history for the disease [5, 6].

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Table 1   Incident cases of CJD in Italy and Sardinia, from 2006 to 2016

	Italy	Sardinia
No. of patients with CJD	1473	50
No. of patients with gCJD (%)	275 (18.6%)	7 (14%)
No. of patients with R208H (%)	12 (4.4%)	5 (71%)

The R208H substitution in the *PRNP* gene has been to date described in 16 cases from European countries, Japan, and China [3]. Here, we describe five new patients with R208H gCJD belonging to five apparently unrelated families from Sardinia, the second-largest island in Italy. Clinical and genetic characterization of this cohort revealed an unusually high prevalence of R208H mutation in Sardinia and a probable founder effect.

Methods

Patients

The present study patients were admitted to the neurology departments of AOU Policlinico Hospital and AO Brotzu Hospital in Cagliari and referred to the Italian Registry of CJD at the Istituto Superiore di Sanità, Rome, Italy (ISS) from 2006 to 2016. Demographic, clinical, and instrumental data were obtained by review of hospital records. CSF samples of the patients were analyzed for 14-3-3 protein by Western blot at the ISS Laboratory and stored at −80 °C. The detection of the pathological form of PrP^{Sc} by real-time quaking-induced conversion (RT-QuIC) [7] was performed retrospectively for the purpose of this study, since it has been only recently included in the diagnostic criteria for CJD [8]. The direct complete sequencing of the *PRNP* gene and the neuropathological assessment were performed at the moment of diagnosis according to published protocol [9, 10]. All subjects' or CJD patients' next of kin provided informed written consent. The diagnosis of probable or definite gCJD was made according to internationally recognized diagnostic criteria from the National UK CJD Research & Surveillance Unit Protocol [11].

Ten anonymous control DNA samples from the Sardinian population were included in the study.

Epidemiologic data on CJD in Italy and Sardinia, from 2006 to 2016, were obtained from the Italian Registry of CJD, as summarized in Table 1. In the selected time period, 50 cases of CJD (including our own patients) were diagnosed in Sardinia and 1473 in Italy.

NGS sequencing and gametic phase reconstruction

Molecular and genetic studies were performed at the Genetica Medica Laboratory, R. Binaghi Hospital, Cagliari.

Specific libraries were prepared for each individual included in the study. These were produced using a long-read PCR protocol (LR-PCR) and NGS library prepared using the Nextera library preparation kit (Illumina). For this purpose, 10 ng of high molecular weight DNA were amplified with specific primers described in Table 2. These produced a fragment of 7055 bp which includes the exon 2 of the *PRNP* gene and the regions flanking it. Table 2 shows the primer sequences and the genomic positions referring to the hg19 release. The LR-PCRs were purified using the AMPure paramagnetic beads (Beckman Coulter s.r.l. Milan, Italy), and 1 ng of amplification product was fragmented for library preparation. Each library was labeled using two index sequences (Nextera Index kit A). The libraries were normalized to the concentration of 4 nM and verified for the correct composition of fragments in a fragment analyzer system (Agilent techn. Santa Clara, USA). The libraries were then pooled and loaded onto a MiSeq Reagent Kits v3 (Illumina Inc. Milan, Italy) flow cell/cardrige system of an Illumiana MiSeq sequencer. The FastQ files produced by the MiSeq sequencer were analyzed using the MiSeq Reporter package (Illumina Inc. Milan, Italy) in order to obtain the VCF and BAM output files containing the identified variants. These were inspected with the Variant Studio 3 (Illumina) and IGV (Integrative Genomics Viewer-Broad Institute) software. The gametic phase and therefore the structure of the haplotypes was reconstructed by inspecting the BAM files containing the reads. The variants were considered in phase when present in the same read.

Table 2   Features of the primers used for the long read PCRs

Name	Sequence (5′-3′)	Position (5′)	Position (3′)	tm
LR-*PRNP*-F	AGACGCTTTACAGTTTGGTTGT	4677014	4677035	58.72
LR-*PRNP*-R	CACCGTAGGAGGTTGGCATT	4684068	4684049	60.04

PRNP, is the human gene encoding for the major prion protein PrP (protease-resistant-protein, Pr for prion, and P for protein)

Table 3 Clinical and laboratory features of the study cohort (Pt1-Pt5) and of the published patients [10–20] with R208H mutation. For neuropathology in the study cohort (Pt1-Pt5), positive patients showed mild to moderate spongiform change, neuronal loss, and gliosis in cerebral cortex, striatum, thalamus, and cerebellum. *Pos* positive, *Neg* negative, *NA* data not available, *MV* methionine-valine, *MM* methionine-methionine, *VV* valine-valine

	Pt1	Pt2	Pt3	Pt4	Pt5	Mastrianni (1996)	Capellari (2005)	Roeber (2005)	Nozaki (2010)
Country	ITA	ITA	ITA	ITA	ITA	USA	ITA	Germany	Japan
Sex/age at onset	M/67	M/54	F/58	M/61	F/73	M/60	F/58	F/69	M/74
Family history	None	None	None	None	None	None	None	None	None
Disease duration (months)	3	11	2	28	2	7	7	12	3
Prodromes (years)	0	0	0	4	1	2	0	0	NA
Symptoms at onset	Cognitive aphasia	Ataxia, dysarthria	Ataxia dysarthria	Cognitive ataxia aphasia	Cognitive ataxia aphasia	Cognitive	Anorexia aphasia	Left hand cognitive	NA
Clinical signs									
Cognitive	Yes	No	No	Yes	Yes	Yes	No	Yes	NA
Cerebellar	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	NA
Visual	No	Yes	No	No	No	Yes	Yes	No	NA
Pyramidal/extrapyramidal	No	No	Yes	No	Yes	No	Yes	Yes	NA
Myoclonus	Yes	Yes	Yes	Yes	No	Yes	Yes	No	NA
Akinetic mutism	No	No	No	Yes	Yes	No	Yes	No	NA
Diagnostic features									
EEG	Neg	Neg	Pos	Pos	Pos	Pos	Pos	Pos	Pos
Brain MRI (high DWI signal)	Pos (cortex, basal ganglia)	Neg	Pos (basal ganglia)	Neg (0.5 T)	Neg (0.5 T)	Pos	Neg	Neg	Pos
14-3.3.	Pos	Pos	Pos	Pos	Pos	NA	Pos	Pos	Pos
RT-QuIC	Pos	Pos	Pos	Pos	Pos	NA	NA	NA	NA
Neuropathology	Pos	NA	NA	Pos	Pos	Pos	Pos	Pos	NA
PrP^{CJD}Type	Type 1	NA	NA	Type 1	Type 1	NA	Type1	Type 1	Type 1
Codon 129	MV	MM	MM	MM	MM	MM	MM	MM	MM

	Chen (2011)	Shi(2015)	Mitrova (2015)	Basset(2006)	Matej (2012)	Vita(2013)	Tiple (2019)
Country	China	China	Slovakia	France	Czech R	ITA	ITA
Sex/age at onset	M/45	NA	F/73	M/61	F/62	F/62	F/63
Family history	None	NA	None	None	None	None	Yes
Disease duration (months)	4	NA	7	7	16	9	30
Prodromes (years)	0	NA	9	3	1	0	0
Symptoms at onset	Cognitive tremor	NA	Cognitive insomnia	Psychiatric	Gait	Akinesia, psychiatric	Psychiatric
Clinical signs							
Cognitive	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Cerebellar	Yes	No	NA	Yes	No	No	Yes
Visual	No	Yes	NA	No	No	No	No
Pyramidal/extrapyramidal	Yes	No	Yes	Yes	Yes	Yes	Yes
Myoclonus	No	Yes	Yes	No	Yes	No	Yes

Table 3 (continued)

Akinetic mutism	No	Yes	NA	Yes	No	Yes	Yes
Diagnostic features							
EEG	NA	NA	Neg	Neg	Pos	Neg	Pos
Brain MRI (high DWI signal)	Neg	Pos	NA	Neg	Pos	Pos	Pos
14-3-3.	Neg	Pos	NA	Neg	Pos	Neg	Pos
RT-QuIC	NA	NA	NA	NA	NA	Neg	Pos
Neuropathology	NA	NA	Pos	Pos	Pos	NA	Pos
PrP^{CJDType}	NA	NA	Type 1	Type2A	Type2A	NA	Type2A
Codon 129	MM	MM	MV	VV	VV	VV	VV

Table 4 Allelic structure of the haplotypes identified in five patients and ten controls

4677092 rs6107516 Intronvariants	4677207 rs6116474	4677369 rs6116475	4680251 rs1799990 Met129Val	4680489 rs74315412 Arg208His	4682507 rs12625444 Downstream genevariants	4683196 rs2181021	4683295 rs2065706	
G	T	A	A	A	C	T	T	H1
G	T	A	A	G	C	T	T	H1WT
A	C	G	G	G	C	A	C	H2WT
G	T	A	A	G	C	T	T	H3WT

Met Methionine, *Val* Valine, *Arg* Arginine, *His* Histidine

Results

Patients

All clinical, genetic, neuroimaging, and laboratory features of the 5 study patients are described in Table 3. There were 3 men and 2 women, with a mean age at symptom onset of 63 years old (range 54–73 years old). All cases were apparently unrelated, and their family history was unremarkable for CJD or other neurodegenerative diseases. Clinical prodromes (including depression and slight memory impairment) in the year preceding clinical onset were reported by 2/5 patients. The most common symptoms at onset were ataxia (4/5 cases), cognitive impairment (3/5 cases), and language disturbances (aphasia in 3/5 cases and dysarthria in 2/5 cases). Further signs/symptoms that developed over the disease course were visual symptoms (1 patient), pyramidal or extrapyramidal motor signs (3 patients), myoclonus (4 patients), and akinetic mutism (2 patients). (Table 3).

On laboratory and instrumental investigations, 3 patients showed typical periodic and triphasic sharp waves at EEG, and 2 patients had a positive brain MRI (it is worth noting that 2/3 patients who showed a normal brain MRI underwent a low-field 0.5 T MRI). All patients had positive 14-3.3 protein in CSF and a positive prion RT-QuIC CSF assay. Autopsy was performed in 3/5 patients: all of them showed mild to moderate spongiform change, neuronal loss, and gliosis mainly involving the cerebral cortex, striatum, thalamus, and cerebellum as in the typical, most common sCJD subtype linked to homozygosity for methionine (MM) or methionine/valine heterozygosity (MV) at codon 129 and PrP^{Sc} type 1 [9]. (Table 3).

The R208H mutation was found associated with MM in 4 patients, and with MV in one case at codon 129. R208H-129M haplotype was found in all patients.

According to internationally recognized diagnostic criteria [11], 3 of our patients were diagnosed as definite gCJD, while 2 patients satisfied the criteria for probable gCJD.

Genetic and molecular testing

NGS sequencing of the region was conducted in the five patients described in this study and in the 10 control samples.

The presence of the NM_000311.3 variant: c.623G > A (NP_000302.1: p. Arg208His) in heterozygosity was confirmed in all patients (Table 4).

In the region of more than 7 Kb of the *PRNP* gene analyzed, there were 684 SNPs (Genome Aggregation Database-gnomAD). Of these, almost all consisted of rare variants, whereas 7 SNPs in the European population showed a percentage frequency greater than 20% (Fig.1). These SNPs resulted in 14 different haplotypes in the European population of which one showed a frequency > 50% and 5 others a frequency between 3 and 18%.

In all the analyzed individuals, we determined the gametic phase with certainty and therefore the structure of the haplotypes. Overall, in the 5 patients and 10 controls, we identified the presence of 4 haplotypes (Tab. 3). Of these, the haplotype indicated as H1 was present in all patients carrying the (A) 208His variant, while the other haplotypes (H1WT, H2WT, and H3WT) were all associated with the wild type (G) variant. Interestingly, variant (A) 208His was always associated with the variant A 129M. These data show a common founder for all Sardinian patients carrying the 208His variant.

Discussion

The 5 patients herein reported from Sardinia, Italy, were apparently unrelated to each other up to 2 generations. Nevertheless, genetic analysis revealed that all patients were positive for the R208H mutation and exhibited a large risk haplotype across the R208H locus. The latter observation indicated that they were part of a larger kindred and probably shared a common ancestor.

To date, we are aware of a total of 16 patients carrying the R208H mutation reported worldwide, [3] even though detailed information was available only for 11 cases [12–22]. These 11 patients showed different codon 129 genotypes: 6 patients were MM [12–16, 20], 4 patients were VV [17, 18, 21, 22], and 1 patient was MV [19]. It is worth noting that the R208H VV cases showed different clinical/laboratory features in comparison to the R208H MM and R208H MV cases, i.e., longer disease duration (12.5 months), higher frequency of psychiatric symptoms at onset, and a reduced sensitivity of 14-3-3 (6 positive out of 9 tested)
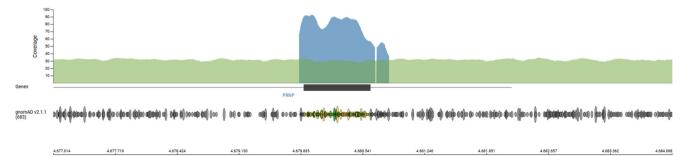

Fig. 1 Genomic map of the region analyzed in this study. SNPs are represented by oval figures

and RT-QuIC (1 positive out of 2 tested) assays. (Table 3). Considering codon 129 haplotype, in our cohort no patient was VV, 4 patients were MM, and one patient carried MV heterozygosity. This is the second report of a patient with MV c129 gCJD with *PRNP* R208H mutation. Overall, the clinical features showed by our patients were similar to those of patients with the same *PRNP* R208H-129M haplotype reported in the literature (Table 3) and to sCJD patients [23].

While E200K represents by far the most common *PRNP* mutation worldwide, in Italy, the gCJD cases carrying the V210 mutation outnumbered those carrying E200K, representing 42% of all gCJD cases recorded in the period 2006–2016. In contrast, the R208H mutation accounted for only 12/275 (4.4%) of the gCJD identified in Italy in the same period (including the 5 cases herein described, three patients from the literature, and 4 unpublished cases recorded by the ISS registry). When the analysis is limited to Sardinia, however, of the 7 gCJD cases identified in 2006–2016 period, 5 (71%) carried the R208H mutation, one (14.5%) the V210I mutation, and one (14.5%) the E200K mutation. Sardinia, with a population of 1.7 million, is the second-largest Italian island, located 120 miles west of the main Italian coastline. Despite numerous invasions over the centuries, many studies indicate that Sardinians are phylogenetically distinct from other European populations, including mainland Italians [24, 25]. Thus, the greater frequency of the R208H mutation in Sardinia probably reflects genetic isolation. The genetic analysis revealed that our 5 patients all shared a large risk haplotype across the R208H locus, an observation indicating that they had a common ancestor and were part of a larger kindred. This founder effect is comparable with that described for the E200K mutation clusters worldwide [26] and it is the first report for this effect for R208H. Interestingly, despite an uneven geographical distribution of gCJD due to the V210I mutation has been observed in Italy, with an excess of cases in Campania and Apulia, a common ancestor for these patients has not been formally demonstrated yet. [4, 5]

In conclusion, we reported the first regional familial cluster of patients carrying the R208H-129M haplotype worldwide. We also expanded the knowledge about the origin of mutations in the *PRNP* gene, identifying a common ancestor and showing that our patients, both the previously reported R208H patients and the sCJD patients, shared similar phenotypic features. The future perspectives based on the present results include the study of other R208H patients in Italy to determine whether they also share the same common ancestor of the Sardinian cluster and to evaluate the frequency of R208H mutation in the entire Sardinian population, in order to identify the potential risk and protective factors for the development of the disease.

Compliance with ethical standards

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