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ORIGINAL COMMUNICATION

CADASIL in central Italy: a retrospective clinical and genetic study in 229 patients

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Abstract The objective of the study is to detail clinical and *NOTCH3* gene mutational spectrum in a large group of Italian CADASIL patients. Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is a familial cerebral small vessels disease caused by mutations in the *NOTCH3* gene on 19p13 usually presenting in young or middle adulthood. Characteristic features include migraine, recurrent lacunar stroke, subcortical dementia, mood disturbances and leukoencephalopathy. The disorder is often overlooked and misdiagnosed. CADASIL prevalence and disease burden is still undetermined. We retrospectively reviewed demographic, clinical, and mutational characteristic of all CADASIL patients diagnosed from January 2002 to December 2012 in three referral centers for neurogenetic and cerebrovascular diseases in central Italy. 229 *NOTCH3* positive subjects were identified. Mean age at diagnosis was 57.8 ± 14.7 years, and 48.6 ± 17.1 years at first symptom onset. Most frequent clinical symptoms were ischemic events (59 %) and psychiatric disturbances (48 %). The highest percentage of mutations were found on exons 4 and 19 (20.6 and 17.6 % respectively), the remaining being dispersed over the entire EGF-like region of the *NOTCH3* gene. 209 patients resided in a circumscribed geographic area which included three regions of the central Italy, yielding a minimum prevalence of 4.1 per 100.000 adult inhabitants. This is the most extensive study on CADASIL in Italy. Clinical phenotype showed several peculiarities in frequency and presentation of the main disease manifestations. Our study enlarges the number of pathogenic *NOTCH3* mutations and due to the heterogeneous mutational spectrum observed suggests that full sequencing of exons 2–24 is mandatory for CADASIL screening in the Italian population.

Keywords CADASIL · *NOTCH3* gene · Clinical spectrum · Prevalence

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Introduction

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is a familial cerebral small vessels disease caused by mutations in the *NOTCH3* gene [1] usually presenting in young or middle adulthood. Characteristic clinical features include migraine, frequently with aura, recurrent lacunar strokes, subcortical dementia and mood disturbances [2]. Brain magnetic resonance imaging (MRI) typically reveals signal abnormalities in the deep white matter, external capsules, and anterior pole of the temporal lobes and lacunar infarcts. For reasons that remain still unclear, there is a great variability in the clinical expression of CADASIL, both between and within families [3, 4]. This is rather surprising considering the highly stereotyped nature of *NOTCH3* disease causing mutations. Studies focusing on possible relationship between phenotype and *NOTCH3* mutation

site, with a few exceptions [5–7], failed to show significant correlations with either clinical presentation.

In general, CADASIL mutations occur in the exons 2–24 of the *NOTCH3* gene encoding for the epidermal growth factor-like repeats. Almost all mutations are missense mutations, but also several in-frame deletions and splice site mutations have been reported. Irrespective to their type, pathogenetic *NOTCH3* mutations lead to an odd number of cysteine residues within the EGF-like domain [8]. A worldwide common hotspot of *NOTCH3* mutations on exon 4 has been evidenced in single families and population studies [9]. A more variable mutational profile was found in the first Italian population studies [10, 11].

In spite of what previously thought, CADASIL does not seem to be an uncommon cause of cerebral small vessel disease, at least in some countries. However, population prevalence studies are still lacking being limited to circumscribed areas of the Scotland [12, 13] and the northern England [14]. The largest series of patients have been reported in European countries [3, 15–19]. More recently, an increasing number of patients have been reported in Asian countries and population-specific phenotypes have been suggested in this ethnic group for the finding of a higher rate of intracerebral hemorrhage and intracranial atherosclerosis than in Caucasians [20].

The aim of this study was to contribute to the genotype and phenotype characterization of CADASIL, by reporting new gene mutations and clinical details of a large group of Italian CADASIL patients and to give an approximate estimate of the prevalence of the disease in a specific area of central Italy.

Materials and methods

The present study is a retrospective review of all patients with genetically confirmed diagnosis of CADASIL performed during the years 2002–2012 in the only reference centres (Siena, Firenze, and Ascoli Piceno) for diagnosis of CADASIL in a wide area of central Italy which includes the regions of Tuscany, Marche, and Umbria. No specific screening selective criteria were applied and *NOTCH3* gene mutation analysis was performed in patients (aged >18 years) with clinical suspicion of an inherited cerebral ischemic small vessel disease. All patients gave informed consent for genetic testing.

Demographic and clinical data were collected for each patient by means of a standardized form outlining the detailed clinical information required for genetic analysis. In particular, the following data were recorded: gender, age, regional residence, presence and age at onset of the following phenotypic features including cerebrovascular events, migraine, cognitive impairment/dementia, seizures, and psychiatric disturbances. The occurrence of typical disease features in the family (history considered positive when at least one typical symptom was present in at least one proband's first degree relative) and presence of vascular risk factors were also recorded.

Stroke and TIA were defined according to currently used clinical criteria [21, 22].

Stroke was defined by acute onset of symptoms and/or clinical signs of focal (or, in specific cases, global) neurologic dysfunction, lasting more than 24 h, with no other cause than vascular origin [21]. TIA is defined as a neurological deficit lasting <24 h due to focal ischemia in the brain or retina [22].

Psychiatric disorders were recorded as present on the basis of previous diagnosis by a certified specialist and/or previous or current use of antipsychotic and/or antidepressant drugs (or psychotherapy), or mood or behavior disorders referred by the patient or his/her family. Cognitive disorders were recorded in the presence of a previous diagnosis of cognitive impairment (mild cognitive impairment or dementia) made by a specialist (neurologist, geriatrician or psychiatrist). Migraine with and without aura were classified according to the International Headache Society Criteria [23].

Seizures were defined according to the International League Against Epilepsy Commission Report [24]. The following cardiovascular risk was assessed: hypertension, hypercholesterolemia, smoking status, diabetes mellitus, and hyperhomocysteinaemia. Hypertension was defined as systolic blood pressure >140 mmHg, diastolic blood pressure >90 mmHg, or the prescribed use of antihypertensive medication [25]. Hypercholesterolemia was defined as an age adjusted non-fasting cholesterol >5.2 mmol/L or drug treatment for hypercholesterolemia [26].

Smoking was recorded as present in case of current or previous history. Diabetes was defined according to the American Diabetes Association criteria [27]. Hyperhomocysteinaemia was defined as non-fasting serum homocysteine >15 mmol/L.

Prevalence was calculated only for definite CADASIL cases (index cases and relatives) residing in the year 2012 in the area of Central Italy referring to our neurological centres for CADASIL diagnosis, based upon adult population (age 18–85 years) from the 2011 national census (http://www.istat.it/en/censuses).

Genetic study

Following informed consent, total genomic DNA was extracted from peripheral blood leukocytes using standard procedures, polymerase chain reaction (PCR) was performed with primers (comprising intron–exon boundaries) specific for exons 2–24 of the *NOTCH3* gene (primers and

PCR condition available on request). Following purification of PCR products, sequencing was performed using the automated sequencer ABI 3730 (Applied Biosystems, Foster City, CA, USA).

Results

Demographic features

We identified 229 *NOTCH3* positive subjects, 117 men and 112 women, with a mean age at the diagnosis of 57.8 ± 14.7 years, from 150 different and unrelated families. A positive family history was present in 131 out of 136 families for which sufficient history data were available.

The number of CADASIL patients resident in the defined area of central Italy including the regions of Tuscany, Marche, and Umbria was 209 with a prevalence of 4.1/100.000 (total population 18–85 years 5.084.186 residents, Istat 2011).

Clinical characteristics

The clinical characteristics of the 229 CADASIL patients are reported in Table 1. Among the main features, acute cerebrovascular events (TIA/stroke) were found in 59 % of patients, psychiatric disturbances in 48 %, migraine in 42 %, in approximately half of cases with aura, cognitive impairment (MMSE <23) in 38 %, and seizures in 8 %.

Mean age at onset of first symptom (Table 1) was 48.6 ± 17.1 (range 15–84) years. The most frequent presenting symptom recorded in 196/229 patients was TIA/stroke in 51 %, migraine in 23 %, in 59 % of which with aura, psychiatric disorders in 9.7 %, epilepsy in 5.7 %, and cognitive decline in 3.6 %. A few patients (7 %) presented with other manifestations including vertigo, cranial nerves paresis and gait disturbances.

Vascular risk factors and/or laboratory data were not available in all patients. Elevated blood pressure was recorded in 67/188 (35.6 %) patients, increased cholesterol values in 65/188 (34.6 %), diabetes in 24/188 (12.7 %), hyperhomocysteinemia in 25/158 (15.8 %), and cigarette smoking in 25/160 (15.6 %).

NOTCH3 gene mutation analysis

Forty-five different missense mutation sites, one small deletion and one splice site mutation were found in the 150 index cases. Among these, nine new mutations were identified (p.Cys262Arg, p.Cys329Tyr, p.Cys379Tyr, p.Cys455Phe, p.Cys493Ser, p.Arg767Cys, p.Arg951Cys, p.Cys1099Phe and p.Cys1298Phe) (Table 2). Most missense mutations were detected in exon 4 (20.6 %), followed by exon 19 (17.6 %), exon 11 (11.5 %) and exon 10 (10.8 %). Five mutations were found with a relatively high frequency: the p.Arg1006Cys mutation is the most frequently observed (16.1 %), followed by p.Arg1231Cys (12.2 %), p.Arg607Cys and p.Gly528Cys (7.4 % each).

Discussion

We reviewed the clinical and *NOTCH3* mutational spectrum of 229 adult CADASIL subjects diagnosed in our reference centres in the period January 2002–December 2012. We also estimated a minimum prevalence in a circumscribed geographic area of 4.1/100.000.

CADASIL is commonly considered the most frequent monogenic cause of cerebral ischemic small-vessels disease. CADASIL families have been increasingly described worldwide and especially in many European countries, where the most large series of patients have been reported. However, with the exception of a few prevalence studies performed in the west Scotland [12, 13] and northern England [14], epidemiological data on prevalence of

Table 1 Clinical features of 229 CADASIL patients

Clinical manifestations	Frequency of different symptoms in 229 CADASIL subjects Number of patients (%)	Frequency of first (presenting) symptoms (available in 196/229 patients) Number of patients (%)
TIA or stroke	**136 (59)**	**100 (51)**
Migraine	**95 (42)**	**46 (23)**
Migraine with aura	42/95 (44)	27/46 (59)
Epilepsy	**19 (8)**	**11 (6)**
Cognitive decline/dementia	**88 (38)**	**7 (4)**
Psychiatric disturbances	**111 (48)**	**19 (10)**
Depression	77/111 (69)	
Anxiety disorder	27/111 (24)	
Psychotic disorder	8/111 (7)	

Table 2 *NOTCH3* mutations in this series of Italian CADASIL families

Mutation	Exon	EGF-like repeat	Number of families	References
p.Cys49Tyr	2	1	2	Joutel [8]
p.Arg54Cys	2	2	1	Escary [28]
p.Cys65Tyr	2	2	2	Bianchi [29]
p.Arg90Cys	3	2	3	Joutel [8]
p.Cys108Ser	3	2	2	Rufa [30]
p.Arg110Cys	3	2	2	Joutel [8]
c.341-26_24delAAC; p.Gly114_Pro115ins25	–	–	1	Bianchi [31]
p.Pro127 fs	4	3	1	Dotti [32]
p.Arg133Cys	4	3	2	Joutel [8]
p.Cys134Tyr	4	3	1	Adib-Samii [18]
p.Arg141Cys	4	3	7	Joutel [8]
p.Cys144Phe	4	3	1	Grigg [33]
p.Arg153Cys	4	3	2	Joutel [8]
p.Arg169Cys	4	4	3	Joutel [34]
p.Cys174Tyr	4	4	2	Dichgans [35]
p.Cys183Ser	4	4	1	Dichgans [36]
p.Cys185Gly	4	4	2	Joutel [8]
p.Arg207Cys	4	5	9	Lesnik Oberstein [37]
p.Cys233Arg	5	5	1	Adib-Samii [18]
p.Tyr258Cys	5	6	1	Joutel [8]
p.Cys262Arg	5	6	1	New
p.Cys329Tyr	6	8	1	Mizuno [38]
p.Arg332Cys	6	8	6	Oliveri [39]
p.Cys338Arg	6	8	1	Dotti [10]
p.Cys379Tyr	7	9	1	New
p.Cys428Arg	8	10	1	Dotti [10]
p.Cys435Arg	8	11	1	Lesnik Oberstein [40]
p.Cys440Arg	8	11	1	Opherk [6]
p.Cys455Phe	8	11	1	New
p.Cys493Ser	9	12	1	New
p.Cys511Tyr	10	13	4	Bianchi [41]
p.Gly528Cys	10	13	11	Dotti [10]
p.Arg532Cys	10	13	1	Bianchi [29]
p.Arg558Cys	11	14	3	Joutel [8]
p.Arg578Cys	11	14	3	Joutel [8]
p.Arg607Cys	11	15	11	Escary [28]
p.Arg728Cys	14	18	2	Joutel [8]
p.Arg767Cys	15	19	1	New
p.Arg951Cys	18	24	1	New
p.Arg1006Cys	19	26	24	Joutel [8]
p.Tyr1021Cys	19	26	2	Kalimo [17]
p.Arg1076Cys	20	27	4	Lesnik Oberstein [42]
p.Cys1099Phe	20	28	1	New
p.Cys1131Trp	21	29	1	Pescini [43]
p.Arg1231Cys	22	31	18	Joutel [8]
p.Cys1298Phe	24	33	1	New
p.Cys1315Tyr	24	33	1	Valenti [44]

NOTCH3 mutation carriers are still limited. The minimum prevalence estimated in our area is significantly higher than that originally reported in two geographic areas of the UK and accounting for 1.3 and 1.9/100.000 (England) respectively [14]. The prevalence we actually found corresponds to that "predicted" by Razvi et al. [12] and subsequently confirmed as "true" by Moreton et al. [13] in the West Scotland. The ~4/100.000 prevalence was also hypothesizes by Kalimo et al. [45] in Finland.

Table 3 lists the phenotypic details of single country CADASIL studies with more than 40 patients obtained from a systematic MEDLINE search from 1990 to 2012 using the key word "CADASIL", and including the present series. The extensive Opherk et al. [6] review study on disease progression and causes of death in 400 German CADASIL cases, was not included for lack of phenotypic details. Clinical data of the sixty-one Italian CADASIL patients reviewed by Pescini et al. [19] were not listed in Table 3 because they were included in the present series. Similarly, the Singal et al. [46] review of 127 British patients was excluded and substituted by the larger and more recent series of 200 patients reported in the same country [18].

When compared to the main series available in the literature (Tables 3, 4), demographic, clinical, and genetic data of our patients suggest some considerations. First, it is noteworthy that the relevant number of *NOTCH3* mutation carriers, 229 subjects from 150 families, reviewed in our survey which represents a considerable group of patients in itself, and even more if we consider the limited area of reference. This may be due to either the 2–24 exons of the *NOTCH3* gene screening we currently performed or the presence of a real "cluster" of the disease in this specific Italian area.

Second, in our population the mean age at onset is higher than usually reported in the other studies (range 34–45 years). This may be partly due to the less frequent occurrence of migraine, namely with aura, as the first symptom. In fact, migraine, if present, is usually the first symptom with an average age at onset of 30 years [2]. The mean age at diagnosis in our population (57.8 years, with respect to a range of 46–51) is higher but the latency from disease onset is comparable in all studies, including the present one (from 3 to 14 years).

Third, the population-related phenotypic profile is quite variable with respect to the frequency of the cardinal manifestations. The frequency of acute cerebrovascular events we registered is in line with the mean of the other European populations, ranging from 51 % of the British study [18] to 71 % [16]. The high percentage (84 %) of the

Table 3 Demographic features and frequency of clinical symptoms in the main series of CADASIL patients

Clinical manifestations comparison with other reports	Families	Syntomatic patients/ Total patients	Mean age (SD) at assessment, years	Mean age (SD) at onset, years	TIA or stroke (%)	Migraine (%)	Migraine with aura[b] (%)	Psychiatric disturbances (%)	Cognitive decline/ dementia (%)
Chabriat [15]	7	45/45	51.3 ± 11.1	45 ± 10.6	84	–	22	20	–
Dichgans [18]	29	83/102	49.7 ± 0.13	37 ± 13.5	71	38	87	30	48
Desmond [3]	33	105/105[a]	47.6 ± 12.6	36.7 ± 12.9	68	43	67	21	60
Singhal [46]	65	119/127	48 (range 21–82)	–	60	92	–	31	19
Kim [47]	9	14/29	48.2 ± 11.7	–	93	7	1/1	36	50
Adib-Samii [18]	124	200/200	47.7 ± 11.4	33.6 ± 14.1	51	75	90	35	17
Wang [20]	33	57/57	45.6 ± 9.9	42.7 ± 9.12	82	–	5	7	60
This study	**150**	**229/229**	**57.8 ± 14.7**	**48.5 ± 17.1**	**59**	**42**	**44**	**48**	**38**

[a] Heterogeneous study group: 34 France, 16 Italy, 39 other european countries, 13 United States, 2 El salvador, 1 Japan

[b] Subset of migraine

Table 4 Frequency of presenting symptoms in the main series of CADASIL patients

Presenting symptoms comparison with other reports	TIA or stroke (%)	Migraine (%)	Migraine with aura (%)	Psychiatric disturbances (%)	Cognitive decline/ dementia (%)	Other manifestations (%)
Desmond [3]	43	40	71	8.5	6	3 Epilepsy
Adib-Samii [18]	19.5	67	90	8.5	3	1 Epilepsy
Wang [20]	70	–	6	6	12	3 Vertigo
Italian cases included in this study	**51**	**23**	**59**	**9.7**	**3.6**	**5.6 Epilepsy**

first Chabriat's paper [15], is presumably related to the selection of patients to address to CADASIL genetic analysis. Worth noting a similar high percentage (80 %) of TIA/stroke recently reported by some of us [19] in a subgroup of cases (61 patients) also included in the present larger series of patients (229 cases). We think that excessive stroke/TIA rate may be due to the selection of the patients reported in the previous study exclusively performed in a tertiary cerebrovascular disease centre comprising a stroke unit.

As previously mentioned, we found a history of migraine in a percentage that is sensibly lower than that reported in the British survey [18], but slightly higher than that found in the German population of Dichgans et al. [16]. With respect to both these series, however, the percentage of Italian patients with migraine with aura was significantly lower (about a half). On the contrary, we found the highest percentage of patients with psychiatric disturbances. As previously reported, this may be due to the considerable number in our population of patients with mutations in the exon 10, in whom a possible genotype-phenotype correlation was hypothesized [7].

Finally, the present retrospective study confirms the peculiarity of the mutational spectrum of the *NOTCH3* gene in the Italian patients [10, 11] with mutations in exon 4 representing only 20.6 % of the total, compared with a frequency ranging between 60 and 70 % in other populations of European and Asian origin [9, 18, 20]. The sum of exon 3 and 4 mutations (25 %) is again unremarkable with respect of about 80 % of the recent UK study of Adib-Samii et al. [18]. Typically, in our population, CADASIL mutations are dispersed over the entire EGF-like region of *NOTCH3* gene. The heterogeneous mutational spectrum observed in this study suggests that full sequencing of exon 2–24 is a better diagnostic approach for Italian CADASIL patients. Interestingly, the most common mutation reported in our study (p.Arg1006Cys) is only found in pedigrees from the Marche region of Italy. The peculiar geographical distribution of this mutation is probably due to a founder effect or a geographical isolation [48].

In conclusion, this is one of the largest population studies on CADASIL performed until now. Its results are noteworthy for several reasons. First, the high minimum prevalence of the disease we found in the defined geographical area of the central Italy is remarkable. Nevertheless, it is presumable that this number still underestimates the real disease burden and that it can further improve if an educational campaign mainly addressed to general practitioners and specialists of the stroke units will be performed. Second, clinical phenotype of our Italian group of patients showed some peculiarities in frequency and age of presentation of the main disease manifestations with respect to the other population studies. Finally, the heterogeneous mutational spectrum we observed suggests that full sequencing of exons 2–24 is mandatory for CADASIL screening at least in the Italian population.

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Conflicts of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

Ethical standard On behalf of all the authors, the corresponding author states that we acted in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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