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Peculiar CADASIL phenotype in monozygotic twins carrying a novel *NOTCH3* pathogenetic variant

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Abstract. – BACKGROUND: Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is an autosomal dominantly inherited cerebral small vessel disease caused by Neurogenic locus notch homolog protein 3 (*NOTCH3*) gene mutations. The main clinical features include migraine with aura, recurrent ischemic strokes and dementia. Brain MRI typically shows multiple small lacunar infarcts and severe, diffuse, symmetrical white matter hyperintensities (WMHs), with characteristic involvement of the anterior temporal pole, external capsule, and superior frontal gyrus. Reports of twins with CADASIL are scarce. Herein we describe a pair of monozygotic twins with peculiar CADASIL phenotype, carrying a new *NOTCH3* variant.

CASE PRESENTATION: Twin A was a 45-year-old male suffering from migraine, obesity, arterial hypertension, and polycythemia (with negative genetic analysis), who complained of a transient, short-lasting (~ 5 minutes) episode of speech difficulties. Brain MRI showed diffuse, symmetrical, confluent periventricular WMHs involving frontal, parietal, and temporal lobes and external capsules, with sparing of anterior temporal poles. Genetic analysis of *NOTCH3* gene demonstrated the presence of missense c.3329G>A, p.(Cys1110Tyr) variant, confirming CADASIL diagnosis. Twin B, affected by migraine and polycythemia, as well as his monozygotic twin, presented with a 2-month history of trigeminal neuralgia. Brain MRI demonstrated diffuse WMHs with a pattern of distribution like his twin. Genetic analysis revealed the same *NOTCH3* pathogenic variant.

CONCLUSIONS: Our monozygotic twins have a strikingly similar neuroimaging picture with sparing of anterior temporal poles. They also have a peculiar phenotype, both presenting polycythemia without genetically confirmed cause. Twin B had trigeminal neuralgia, that is unusual in CADASIL. The possible association of the peculiar findings with the newly reported *NOTCH3* variant needs to be confirmed with further observations.

Key Words:
CADASIL, Temporal pole sparing, Polycythemia, Trigeminal neuralgia, White matter hyperintensities.

Background

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL; OMIM #125310) is a rare genetic small vessel disease caused by mutations in the Neurogenic locus notch homolog protein 3 (*NOTCH3)* gene located on chromosome 19 (19p13.2-p13.1)[1,2]. It represents the most common hereditary cause of adult-onset ischemic stroke and vascular dementia. The main clinical features include migraine with aura in early adulthood, recurrent subcortical ischemic attacks (either transient ischemic attack or stroke), mood disturbances, and progressive cognitive decline in middle age[2,3]. Brain MRI shows multiple small lacunar infarcts and severe, diffuse, symmetrical white matter hyperintensities (WMHs), with typical involvement of the anterior temporal pole, the external capsule, and the superior frontal gyrus[2,4,5]. However, atypical phenotypes and a wide variability of both clinical and neuroimaging manifestations, even within the same families, have been reported[2,6]. In literature, reports of twins with CADASIL are scarce. To the best of our knowledge, CADASIL has been previ-

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ously reported only in two pairs of monozygotic twins[7,8]. Herein, we report the clinical and neuroimaging findings of a pair of monozygotic twins with CADASIL carrying a novel heterozygous c.3329G>A (p.Cys1110Tyr) *NOTCH3* pathogenic variant.

Case Reports

Twin A

A 45-year-old man was referred to the Emergency Department of our hospital because of a sudden, transient, short-lasting (~ 5 minutes) episode of speech difficulties. He suffered from obesity, arterial hypertension, migraine since his twenties, and polycythemia (genetic analysis for essential polycythemia, i.e., JAK2, BCR/ABL, and *MPL* gene, was negative). There were no additional vascular risk factors. At admission, neurological examination was normal. Head CT scan revealed extensive leukoencephalopathy. Brain MRI showed diffuse, symmetrical, confluent periventricular white matter vascular lesions in the frontal, parietal, and temporal lobes as well in external capsules, with sparing of anterior temporal poles (Figure 1 A-D). Clinical and neuroimaging data suggested the diagnosis of CADASIL. Neuropsychological evaluation was unremarkable. Genetic analysis of *NOTCH3* gene by next-generation sequencing (NGS) revealed the presence of missense c.3329G>A (p.Cys1110Tyr) variant in the exon 21. *In silico* analysis was performed, and this variant was predicted to be deleterious by PolyPhen-2 (score: 1.000), Mutation Taster, Align-GVGD (class C55), and SIFT (score: 0.00) prediction tools. The patient was prescribed acetylsalicylic acid 100 mg/day and atorvastatin 20 mg/day.

Twin B

His monozygotic twin came to our attention six months later for episodes of multiple, brief (1-2 seconds), intense, sharp shooting left facial pain lasting from two weeks. His past medical story revealed migraine and polycythemia (genetic analysis was negative for essential polycythemia; see twin A). No other additional vascular

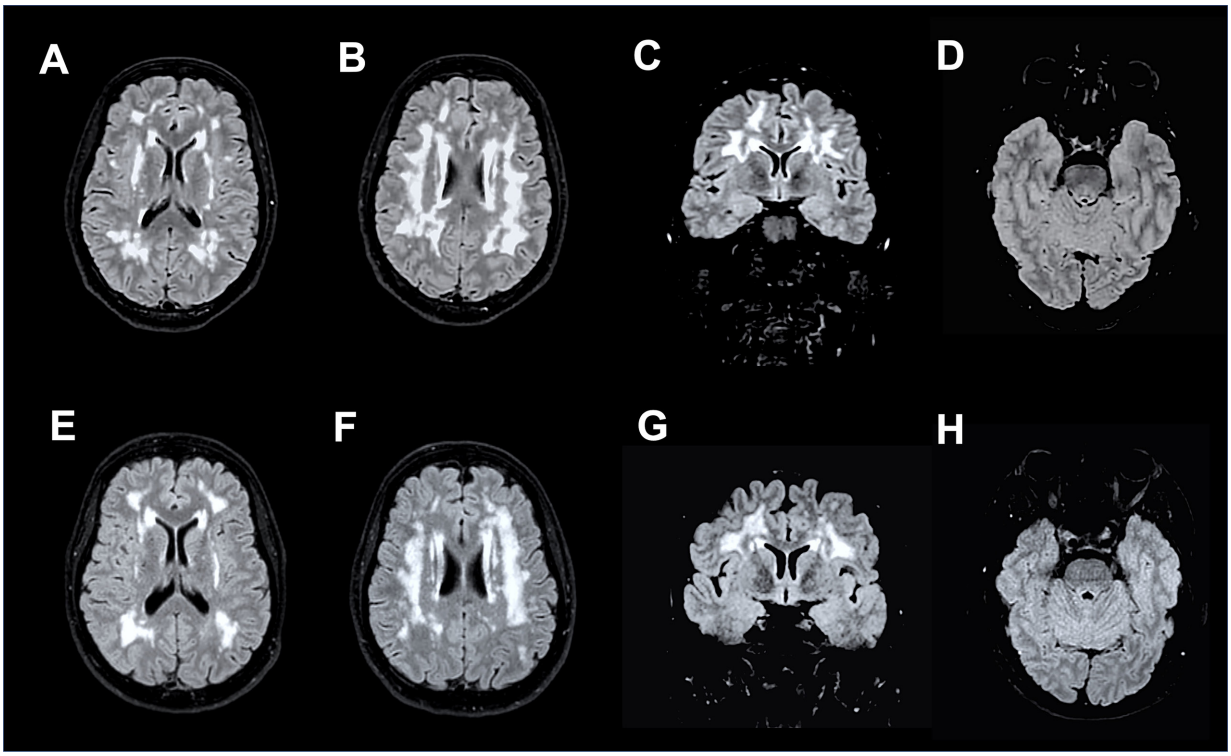

Figure 1. Main neuroradiological findings in our twins with CADASIL. MRI FLAIR sequences of twin A (**A, B**, axial sequences; **C**, coronal sequence) and twin B (**E**, **F**, axial sequences; **C**, coronal sequence) showing extensive, bilateral, confluent, subcortical and periventricular white matter hyperintensity involving bilateral external capsule and frontal, parietal and temporal lobes. Note anterior temporal poles sparing in FLAIR axial sequences in both twins (**D**, twin A; **H**, twin B).

risk factors were reported. Neurological examination was otherwise normal, except for left facial hyperalgesia. Head CT scan showed diffuse leukoencephalopathy. Brain MRI demonstrated a pattern of lesion distribution similar to his twin (Figure 1 E-H), with an adjunctive T2-FLAIR hyperintense lesion area in the left middle cerebellar peduncle along the intracisternal course of the fifth cranial nerve. Neuropsychological evaluation was normal. Genetic analysis showed the same *NOTCH3* gene variant as his twin. He was given carbamazepine up to 600 mg/day with mild almost complete pain relief. Moreover, he started treatment with acetylsalicylic acid 100 mg/day and atorvastatin 20 mg/day.

Discussion

Here we report a not previously identified missense c.3329G>A (p.Cys1110Tyr) pathogenic variant in the exon 21 of the *NOTCH3* gene in a pair of twins with clinical and neuroimaging CADASIL features.

CADASIL is a genetically determined cerebral small vessel disease caused by a degenerative change of vascular smooth muscle cells related to mutations in *NOTCH3* gene[1,2]. *NOTCH3* gene encodes for a single-pass transmembrane protein of 2321 amino acids, predominantly expressed in vascular smooth muscle cells[2,9]. To date, more than 300 different *NOTCH3* mutations have been reported[10]. Gene variants, mainly consisting of missense substitutions leading to loss or gain of a cysteine residue within one of the EGF-like repeats of *NOTCH3* extracellular domain, cause receptor misfolding, aggregations of its extracellular domain and deposition in smooth muscle cells in the walls of small arteries[11]. This phenomenon leads to arterial structure abnormalities and perivascular space dilation, with subsequent small subcortical infarction and diffuse white matter hyperintensity[3,9,12].

In our family, the occurrence of transient ischemic attack in early age in twin A and the concomitant history of migraine along with neuroimaging findings suggested the diagnosis of CADASIL. No signs of cognitive impairment were demonstrable in either of the twins, probably due to their young age. Of note, twin B came to our attention because of trigeminal neuralgia, which doesn't represent a typical CADASIL feature. To the best of our knowledge, no other case of trigeminal neuralgia in the context of CADASIL is reported in the literature.

Both twins were affected by polycythemia of undetermined etiology. Data about the association between CADASIL and polycythemia are rare; only one patient has been described in the literature[7], although authors hypothesized that polycythemia may be uncommonly associated with CADASIL[13]. In our monozygotic twins, the etiology of polycythemia is more likely to be genetic. It remains to be elucidated if polycythemia is a casual genetic comorbidity or represents a manifestation of this new *NOTCH3* variant, expanding the spectrum of CADASIL phenotype. Moreover, it may be assumed that increased blood viscosity secondary to polycythemia may play a role in further impairing blood flow in the affected blood vessels and increasing brain damage.

The presence of WMHs on T2-weighted and fluid-attenuated inversion recovery MRI images, mostly in the deep white matter, external capsules, and anterior pole of the temporal lobes, with symmetrical and bilateral distribution is a hallmark of CADASIL[4,5,14]. Other standard MRI features are lacunar infarcts, cerebral microbleeds, enlarged perivascular space, and brain atrophy[15]. Anterior temporal lobe WMHs, although not mandatory, are highly suggestive of CADASIL and have been reported in up to 95% of Caucasian patients[5,16]. Sparing of anterior temporal poles represents an uncommon finding, mainly occurring in cysteine-sparing *NOTCH3* mutations and the Asian population[17,18]. Brain MRI showed a strikingly similar pattern of lesions load and distribution between our twins. Neuroimaging showed diffuse, symmetrical, confluent hyperintensities in the frontal, parietal, and temporal lobes' white matter and in external capsules. Noteworthy, sparing of anterior temporal poles occurred in both of them. Further studies are needed to establish if the sparing of temporal poles may be due to this new *NOTCH3* variant reported in our family.

Similar clinical and neuroimaging phenotypes in twins have also been reported by Ragno et al[8]. On the contrary, Mykkänen et al[9] found apparent different clinical and neuroimaging findings in their two twins with CADASIL and suggested an effect of environmental factors on the disease due to different lifestyles. Indeed, the role of the vascular risk factors in determining CADASIL phenotype is well known, and recent European guidelines have highlighted the need for strict control of cardiovascular risk factors in CADASIL patients[19]. However, the matter is still debated in the literature[20,21] and the role of environmental factors needs to be considered case by case.

Conclusions

We described two monozygotic twins with CADASIL due to a novel *NOTCH3* missense variant, with a strikingly similar brain MRI and a probable genetic form of polycythemia. Additional investigations are necessary to confirm the relationship between polycythemia and CADASIL.

Conflict of Interest
The authors declare that they have no relevant financial or non-financial interests to disclose that are relevant to the content of this article.

Ethical Approval
Not applicable.

Informed Consent and Consent to Participate
Written informed consent has been obtained from patients. The participants have consented to submitting the case report to the journal.

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Authors' Contribution
Angelo Pascarella: data collection, interpretation of data, manuscript writing; Lucia Manzo: data collection, interpretation of data; Oreste Marsico: data collection, interpretation of data; Sara Gasparini: data collection, interpretation of data; Elena Falcone: data collection, interpretation of data; Simona Cammaroto: data collection, interpretation of data; Umberto Sabatini: data collection, interpretation of data; Umberto Aguglia: revision of manuscript for intellectual content; Edoardo Ferlazzo: data collection, interpretation of data, manuscript writing, revision of manuscript for intellectual content.

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Data Availability Statement
Data sharing does not apply to this article as no datasets were generated or analyzed during the current study.

References

1) Joutel A, Corpechot C, Ducros A, Vahedi K, Chabriat H, Mouton P, Alamowitch S, Domenga V, Cécillion M, Marechal E, Maciazek J, Vayssiere C, Cruaud C, Cabanis EA, Ruchoux MM, Weissenbach J, Bach JF, Bousser MG, Tournier-Lasserve E. Notch3 mutations in CADASIL, a hereditary adult-onset condition causing stroke and dementia. Nature 1996; 383: 707-710.
2) Chabriat H, Joutel A, Dichgans M, Tournier-Lasserve E, Bousser MG. Cadasil. Lancet Neurol 2009; 8: 643-653.
3) Federico A, Bianchi S, Dotti MT. The spectrum of mutations for CADASIL diagnosis. Neurol Sci 2005; 26: 117-124.
4) Chabriat H, Levy C, Taillia H, Iba-Zizen MT, Vahedi K, Joutel A, Tournier-Lasserve E, Bousser MG. Patterns of MRI lesions in CADASIL. Neurology 1998; 51: 452-457.
5) O'Sullivan M, Jarosz JM, Martin RJ, Deasy N, Powell JF, Markus HS. MRI hyperintensities of the temporal lobe and external capsule in patients with CADASIL. Neurology 2001; 56: 628-634.
6) Dichgans M, Mayer M, Uttner I, Brüning R, Müller-Höcker J, Rungger G, Ebke M, Klockgether T, Gasser T. The phenotypic spectrum of CADASIL: clinical findings in 102 cases. Ann Neurol 1998; 44: 731-739.
7) Mykkänen K, Junna M, Amberla K, Bronge L, Kääriäinen H, Pöyhönen M, Kalimo H, Viitanen M. Different clinical phenotypes in monozygotic CADASIL twins with a novel NOTCH3 mutation. Stroke 2009; 40: 2215-2218.
8) Ragno M, Sanguigni S, Manca A, Pianese L, Paci C, Berbellini A, Cozzolino V, Gobbato R, Peluso S, De Michele G. Parkinsonism in a pair of monozygotic CADASIL twins sharing the R1006C mutation: a transcranial sonography study. Neurol Sci 2016; 37: 875-881.
9) Domenga V, Fardoux P, Lacombe P, Monet M, Maciazek J, Krebs LT, Klonjkowski B, Berrou E, Mericskay M, Li Z, Tournier-Lasserve E, Gridley T, Joutel A. Notch3 is required for arterial identity and maturation of vascular smooth muscle cells. Genes Dev 2004; 18: 2730-2735.
10) Hu Y, Sun Q, Zhou Y, Yi F, Tang H, Yao L, Tian Y, Xie N, Luo M, Wang Z, Liao X, Xu H, Zhou L. NOTCH3 Variants and Genotype-Phenotype Features in Chinese CADASIL Patients. Front Genet 2021; 12: 705284.
11) Rutten JW, Haan J, Terwindt GM, van Duinen SG, Boon EM, Lesnik Oberstein SA. Interpretation of NOTCH3 mutations in the diagnosis of CADASIL. Expert Rev Mol Diagn 2014; 14: 593-603.

12) Yamamoto Y, Liao YC, Lee YC, Ihara M, Choi JC. Update on the Epidemiology, Pathogenesis, and Biomarkers of Cerebral Autosomal Dominant Arteriopathy With Subcortical Infarcts and Leukoencephalopathy. J Clin Neurol 2023; 19: 12-27.

13) Tikka S, Baumann M, Siitonen M, Pasanen P, Pöyhönen M, Myllykangas L, Viitanen M, Fukutake T, Cognat E, Joutel A, Kalimo H. CADASIL and CARASIL. Brain Pathol 2014; 24: 525-544.

14) Bersano A, Bedini G, Markus HS, Vitali P, Colli-Tibaldi E, Taroni F, Gellera C, Baratta S, Mosca L, Carrera P, Ferrari M, Cereda C, Grieco G, Lanfranconi S, Mazucchelli F, Zarcone D, De Lodovici ML, Bono G, Boncoraglio GB, Parati EA, Calloni MV, Perrone P, Bordo BM, Motto C, Agostoni E, Pezzini A, Padovani A, Micieli G, Cavallini A, Molini G, Sasanelli F, Sessa M, Comi G, Checcarelli N, Carmerlingo M, Corato M, Marcheselli S, Fusi L, Grampa G, Uccellini D, Beretta S, Ferrarese C, Incorvaia B, Tadeo CS, Adobbati L, Silani V, Faragò G, Trobia N, Grond-Ginsbach C, Candelise L; Lombardia GENS-group. The role of clinical and neuroimaging features in the diagnosis of CADASIL. J Neurol 2018; 265: 2934-2943.

15) Di Donato I, Bianchi S, De Stefano N, Dichgans M, Dotti MT, Duering M, Jouvent E, Korczyn AD, Lesnik-Oberstein SA, Malandrini A, Markus HS, Pantoni L, Penco S, Rufa A, Sinanović O, Stojanov D, Federico A. Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL) as a model of small vessel disease: update on clinical, diagnostic, and management aspects. BMC Med 2017; 15: 41.

16) Markus HS, Martin RJ, Simpson MA, Dong YB, Ali N, Crosby AH, Powell JF. Diagnostic strategies in CADASIL. Neurology 2002; 59: 1134-1138.

17) Muiño E, Gallego-Fabrega C, Cullell N, Carrera C, Torres N, Krupinski J, Roquer J, Montaner J, Fernández-Cadenas I. Systematic Review of Cysteine-Sparing NOTCH3 Missense Mutations in Patients with Clinical Suspicion of CADASIL. Int J Mol Sci 2017; 18: 1964.

18) Ueda A, Ueda M, Nagatoshi A, Hirano T, Ito T, Arai N, Uyama E, Mori K, Nakamura M, Shinriki S, Ikeda K, Ando Y. Genotypic and phenotypic spectrum of CADASIL in Japan: The experience at a referral center in Kumamoto University from 1997 to 2014. J Neurol 2015; 262: 1828-1836.

19) Mancuso M, Arnold M, Bersano A, Burlina A, Chabriat H, Debette S, Enzinger C, Federico A, Filla A, Finsterer J, Hunt D, Lesnik Oberstein S, Tournier-Lasserve E, Markus HS. Monogenic cerebral small-vessel diseases: diagnosis and therapy. Consensus recommendations of the European Academy of Neurology. Eur J Neurol 2020; 27: 909-927.

20) Singhal S, Bevan S, Barrick T, Rich P, Markus HS. The influence of genetic and cardiovascular risk factors on the CADASIL phenotype. Brain 2004; 127: 2031-2038.

21) Mizuno T, Mizuta I, Watanabe-Hosomi A, Mukai M, Koizumi T. Clinical and Genetic Aspects of CADASIL. Front Aging Neurosci 2020; 12: 91.