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Case Report

# Genetic study of the *NOTCH3* gene in CADASIL patients

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ABSTRACT

*Background:* Cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is a monogenic, hereditary, neurological syndrome characterized by small vessel disease (SVD), stroke, vascular cognitive impairment and dementia. It is caused by mutations in the *NOTCH3* gene, affecting the number of cysteines in the extracellular domain of the receptor, causing protein misfolding and receptor aggregation. The pathogenic role of cysteine-sparing *NOTCH3* missense mutations in patients with typical clinical CADASIL syndrome is unknown. No therapies are available for this condition.

*Objective:* Genetic study of the *NOTCH3* gene in CADASIL patients who were referred to the Fazeli-Sanati Genetics Laboratory.

*Subjects and methods:* Peripheral blood samples were collected from 10 CADASIL patients to extract genomic DNA. DNA sequences of exons 2–8, 11–12 and 18–19, where *NOTCH3* mutations are typically located; were amplified by using PCR and analyzed by direct sequencing.

*Results:* 11 *NOTCH3* exons were analyzed. Homozygous IVS7 + 15A>G mutation were found in five patients, Homozygous IVS7 + 16A>G mutation in one patient, Heterozygous for the Pro109Thr and Pro203His mutations in one patient, which were not reported previously. Heterozygous C395R and R153C mutations were found in two patients. One of the patients has no mutation in 11 analyzed *NOTCH3* exons.

*Conclusion:* We found four novel mutations (P109T, P203H, IVS7 + 15A>G and IVS7 + 16A>G) and 2 reported *NOTCH3* mutations. Exon 4 and Intron 7 are hotspots in the patients we examined with the *NOTCH3* mutations. These findings broaden the mutational spectrum of CADASIL.



## 1. Introduction

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is an adult-onset inherited small artery disease characterized by non-hypertensive, non-arteriosclerotic, small arterial granular degeneration [1,2].

The main clinical manifestations of CADASIL are progressive white matter lesions and recurrent strokes leading to disability, migraine with aura and dementia with early death [3–6].

In the case of CADASIL the presence of granular osmiophilic material (GOM), in close association with smooth muscle cells, pericytes and endothelial cells are very important for diagnosis [7,8]. The other critical point is analyzing all the exons of the *NOTCH3* gene to find the mutation that causes the pathology [6,9].

The mean age of onset of clinical symptoms is the mid-forties (for stroke onset is approximately 46 years; for dementia, the mean age of onset is 55) [10]. However, in recent studies, 52 years for males and 57 for females have been described [11]. CADASIL has been reported in various populations worldwide [5,12].

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**Table 1**
The *NOTCH3* gene mutations reported in the present study.

| Patient number | Gender | Age | NOTCH3 mutation | Exon | Intron | Homozygous/Heterozygous | Reported/Novel |
|---|---|---|---|---|---|---|---|
| 1 | Male | 71 | IVS7 + 15A>G | | 7 | Homozygous | Novel |
| 2 | Female | 32 | Cys 395 Arg T>C | 7 | | Heterozygous | Reported |
| 3 | Male | 48 | Arg 153 Cys C>T | 4 | | Heterozygous | Reported |
| 4 | Male | 42 | IVS7 + 16A>G | | 7 | Homozygous | Novel |
| 5 | Female | 51 | IVS7 + 15A>G | | 7 | Homozygous | Novel |
| 6 | Male | 70 | IVS7 + 15A>G | | 7 | Homozygous | Novel |
| 7 | Female | 22 | IVS7 + 15A>G | | 7 | Homozygous | Novel |
| 8 | Female | 44 | IVS7 + 15A>G | | 7 | Homozygous | Novel |
| 9 | Female | 26 | No mutation in 11 analyzed *NOTCH3* exons | – | – | – | – |
| 10 | Female | 57 | Pro109Thr | 3 | | Heterozygous | Novel |
| | | | Pro203His | 4 | | | |

This disease caused by mutation of the *NOTCH3* gene, leads to degeneration and loss of vascular smooth muscle cells [13–15]. *NOTCH3* gene is located on the 19p13 chromosome, which encodes a transmembrane receptor [6,16]. This single-pass transmembrane receptor consists of three domains: a large extracellular domain (ECD) with 34 tandem epidermal growth factor-like (EGF) repeats encoded by exons 2–24, where *NOTCH3* mutations are typically located; a transmembrane domain; and an intracellular domain (ICD) [7,17,18].

*NOTCH3* is composed of 33 exons and 2321 amino acids [13]. The presence of only one mutation in one of the two alleles leads to CADASIL, because the pathogenic mutations are dominant for this disease [19].

The studies done so far indicate that almost all of the pathogenic mutations are missense mutations that result in changes in the number of cysteines, with a strong clustering of mutations in exons 4 [14,19], although some studies indicate that there are mutations associated with CADASIL that do not affect the number of cysteines. However, the pathogenic role of these mutations is uncertain [7]. So far more than 230 mutations have been reported in the *NOTCH3* gene, which is associated with CADASIL [6].

In the present study, we examined the *NOTCH3* gene mutations in ten diagnosed CADASIL patients and report four novel splicing mutations of the *NOTCH3* gene.

## 2. Subjects and methods

A total of 10 CADASIL patients, who were referred to the Fazeli-Sanati Genetics Laboratory for the detection of the *NOTCH3* gene mutations in genomic DNA were chosen. The subjects participated in this study with their written informed consent.

Whole blood was collected by venipuncture in tubes containing EDTA. Genomic DNA was extracted from peripheral blood using routine procedures. The concentration and purity of DNA samples were determined by spectrophotometric analysis.

Sequencing of the exons 2–8 and 11, 12, 18, 19 (both coding regions and intron/exon boundaries), which are mutational hotspots of the *NOTCH3* gene, was undertaken using PCR amplification and direct Sanger sequencing. The amplified PCR products of the *NOTCH3* gene visualized on a 2% agarose gel. https://blast.ncbi.nlm.nih.gov/ was used to align sequences data with *NOTCH3* reference DNA sequence to identify pathogenic mutations.

## 3. Results

Ten patients diagnosed with CADASIL were selected for this study. The mean age at the time of collection of CADASIL patients was 46.3 years. Six out of the ten patients were females (60%) and four were males (40%). All of the patients were of Iranian origin.

In the total of ten patients, DNA molecular analysis was performed for 11 exons of the *NOTCH3* gene. Direct sequencing of exons 2–8, 11, 12 and 18, 19 and intron/exon boundaries in clinically suspected patients identified 6 mutations.

The mutations were: IVS7 + 15A>G, IVS7 + 16A>G, C395R, R153C, P109T and P203H (Table 1). Heterozygous missense mutations were identified in exons 7 and 4 (p.Cys395Arg, p.Arg153Cys); Patient number nine did not involve the mutation in examined exons of the *NOTCH3* gene.

The highest numbers of mutations were found in intron 7 (n = 6), followed by exon 4 (n = 2), Exons 3 and 7, each had one mutation in two patients, and the examined mutations of the *NOTCH3* gene are shown in Table 1. The most common mutation was IVS7 + 15A>G in intron 7.

In total six variants including two known mutations and four novel mutations in ten patients were identified.

## 4. Discussion

CADASIL is an inherited arteriopathy and a rare neurological disorder in the general population, but the most frequent non-atherosclerotic monogenic hereditary degenerative vascular cerebral disease [13,20,21]. In 1997 the *NOTCH3* gene was found to be the cause of CADASIL that is composed of 33 exons and its product is a 2321 amino acid [13,22,23].

Ten patients with CADASIL from Iranian population were recruited in the present study and performed genetic sequencing of the 11 exons of the *NOTCH3* gene.

In our study we identified four novel mutations, two homozygous mutations IVS7 + 15A>G in intron 7 of the *NOTCH3* gene and two heterozygous mutations predicting amino acid change from proline to threonine at position 109 (Pro109Thr) and proline to histidine at nucleotide 203 (Pro203His). Furthermore we identified 2 heterozygous missense mutations (p.Cys395Arg, p.Arg153Cys) respectively in exons 7 and 4, which do not affect the number of cysteines, unlike almost all of the pathogenic mutations associated with CADASIL syndrome. However one of the patients had no mutation in the considered exons of the *NOTCH3* gene.

Our findings broaden the mutation spectrum of *NOTCH3* and suggest that in patients with CADASIL should consider the sequencing of the 11 exons in *NOTCH3* gene.

In German, Greek, Caucasian and Japanese populations the *NOTCH3* mutations were previously showed to cluster in exons 2–6, especially in exon 4 [24–30]. In Chinese populations higher frequency of mutations were clustered in exons 3, 4, 11 [20,31]. The *NOTCH3* gene analysis revealed mutations on exons 22 in Kashmir and Italian patients [2,32,33].

In Italian studies the highest percentage of mutations were found in exons 3 and 4, but in exons 4 and 11 was noted in the center and characterized by a recurrent mutation p.Arg607Cys [20,34]. In another study a heterozygous deletion mutation was identified in intron 3 (c.341-26_24delAAC) [35].

In a recent report of 29 Korean mutation carriers the exon 11 contributed the most and p.Arg544Cys were relatively common, while the ratio of mutations in exon 4 was extremely low [20,36].

In our study which included patients from Iran, the intron 7 and exon 4 were considered to be a "hot region". Nevertheless the mutation IVS7 + 15A>G was ranked in the first place of the mutational spectrum in our subjects.

Analyses of four novel mutations were identified in this study in other family members of the patients to confirm these findings and complete investigation of the *NOTCH3* gene, using NGS method, would be needed in future studies.

In addition, for one of the patients who had no mutations in her examined exons, despite the presence of symptoms of CADASIL, analysis of other exons of the *NOTCH3* gene is suggested.

## 5. Conclusion

We found four novel mutations and 2 reported *NOTCH3* mutations in CADASIL patients that expand the genetic spectrum of this disease. Our work also indicates that *NOTCH3* mutations are clustered in exon 4 and intron 7 in the patients we examined.

## Conflict of interest

None.

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