Broadening the Genetic Horizons of CADASIL: New Variants of the NOTCH3 Gene Revealed and their Association with CADASIL

Commentary

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is an angiopathy caused by pathogenic mutation in the NOTCH3 gene on chromosome 19.[1] CADASIL affects the small blood vessels in the brain, leading to recurrent strokes, cognitive impairment, and other neurological symptoms.[2,3] Migraine with aura, acute reversible encephalopathy, ischemic episodes, cognitive impairment, and psychiatric disturbances are the common presenting symptoms of CADASIL. The onset of symptomatic disease typically occurs in adulthood,[1] but cases have also been reported in children and adolescents.[4,5] The NOTCH3 gene plays a vital role in cell fate decisions during embryonic development, as well as in vascular smooth muscle cell differentiation and vascular development in adults. Over 400 pathogenic variants have been identified in patients with CADASIL, Majority of which are located in the extracellular region of the NOTCH3 transmembrane receptor.[6] The spectrum includes missense variants, splice site variants, and small in-frame deletions. Approximately 95 percent of patients have pathogenic missense variants.(Locatelli, 2020). These variants involve cysteine residues and result in an odd number of cysteine residues within wild-type epidermal growth factor (EGF)-like repeat domains. The extracellular domain of the Notch3 receptor accumulates within blood vessels, primarily affecting the brain's leptomeningeal and long penetrating arteries. Accumulation takes place at the cytoplasmic membrane of VSMCs and pericytes in close vicinity to the granular osmiophilic material (GOM) that characterize the disease.[7] This leads to reduced internal diameter and increased arteriolar wall thickness, and the specific anatomy of the blood-brain barrier may also contribute to the brain's vulnerability to CADASIL.[8,9]

Brain MRI is the most useful imaging method to demonstrate the radiologic features of CADASIL, including recent lacunar infarctions, chronic lacunes, and white matter hyperintensities. The following MRI signs may help to identify patients with CADASIL: temporal lobe and external capsule hyperintensities, subcortical lacunar lesions, cerebral microbleeds, and brain atrophy.[10]

There is no specific disease-modifying treatment for CADASIL, and only limited information is available regarding the management of the major manifestations of the disorder like primary and secondary prevention of stroke, symptomatic management of migraine, pseudobulbar palsy, cognitive impairment, and neuropsychiatric manifestations.[11]

Several therapeutic approaches for CADASIL are currently in preclinical development, including immunotherapy, [NOTCH3 receptor ($NOTCH3^{ECD}$) immunotherapy,[12] immunotherapy using agonist antibody A13-specific for the NOTCH3 receptor],[13] antisense mediated NOTCH3 exon skipping,[14] and treatment with stem cell factor and granulocyte colony-stimulating factors.[15]

In this issue of the journal, Gorukmez *et al.*, 2023, conducted a comprehensive genetic analysis of NOTCH3 in 368 Turkish patients with symptoms and neuroimaging findings suggestive of CADASIL using the next-generation sequencing (NGS).[16] They identified heterozygous NOTCH3 variants, mostly missense mutations, in about 12% of the patients (44 out of 368 patients). Interestingly, 30 different variants were identified, 17 of which were novel, suggesting that there may be more genetic diversity in CADASIL than previously recognized. The majority of variants were missense mutations, 16 cysteine-altering variants, four non-cysteine-altering variants, and four variants outside the epidermal growth factor-like repeat (EGFr) region (two pathogenic and two likely benign—P2249P and L1518M). The synonymous variant P2249P was detected in two patients with bilateral parietal WMH and in the mother of one of these patients who complained of migraine. The L1518M variant was associated with severe white matter lesions and was detected in a 36-year-old female patient with severe neurological symptoms and in a 47-year-old male patient. Neuroimaging of these unrelated patients showed extensive white matter involvement. The L1518M variant, outside of EGFr, has been previously reported by Park *et al.*,[17] in a patient with CADASIL who had associated positive GOM.

Recent studies have examined the correlation between NOTCH3 variant position and clinical features and molecular markers of CADASIL. In a cohort of 38 probands, Hu *et al.*[18] found 23 different NOTCH3 variants, with patients carrying cysteine-sparing pathogenic variants experiencing later symptom onset and milder temporal lobe involvement. Cho *et al.*[19] discovered that variants in EGFRs 1-6 were associated with earlier onset of stroke and encephalopathy, while variants in EGFRs 7-34 were linked to lower stroke risk. Gravesteijn *et al.*[20] reported lower levels of NOTCH3 aggregation in patients with EGFr 7-34 variants. Almeida *et al.*[21] identified 15 different heterozygous variants in 24 Portuguese families with suspected CADASIL, suggesting

that genetic testing should focus on exons 4, 8, and 11 in this population.

Despite these recent advances, there is still a knowledge gap in understanding the exact relationship between specific NOTCH3 pathogenic variants and disease manifestations. For example, it is not fully clear why the vascular complications are largely limited to the brain in CADASIL, despite it being a generalized angiopathy. Additionally, the exact mechanisms by which accumulation of the extracellular domain of the NOTCH3 receptor leads to vascular dysfunction and brain damage are not fully understood. There is also a need for better genotype–phenotype correlations, as the association between specific pathogenic variants and disease manifestations is still disputed.[22,23]

The present study has several strengths, including its large sample size, detailed clinical and radiological data, and systematic genetic analysis involving NGS of all exons, splicing sites, and UTR regions of NOTCH3. The bioinformatic analysis was performed on a reliable platform, and the pathogenicity scoring for novel changes was performed according to the American College of Medical Genetics and Genomics (ACMG) criteria. The results of this study provide valuable insights into the genetic landscape of CADASIL, as 17 novel variants were detected. The study also highlights the association of B (benign) and LB (likely benign) changes, according to the ACMG criteria, with the disease.

However, the study has some limitations. Firstly, it was conducted in a single center and had a retrospective study design, which may limit the generalizability of the findings, and there may be a recall bias. Secondly, the study only included patients with suspected CADASIL, which may have led to selection bias. Thirdly, the study did not investigate the functional consequences of the identified variants.

In conclusion, this study provides important information on NOTCH3 variants in patients with suspected CADASIL using NGS. The identification of novel variants expands the current knowledge of the genetic landscape of CADASIL, and the clinical and radiological heterogeneity of CADASIL among individuals, regardless of the NOTCH3 variant, underscoring the importance of a comprehensive evaluation of patients with suspected CADASIL. Furthermore, this study could inform the development of new diagnostic and therapeutic approaches for CADASIL, such as targeted gene therapy or precision medicine.

However, it is also important to acknowledge the ethical challenges that arise from genetic testing, particularly in the context of hereditary diseases like CADASIL. Patients and families may face difficult decisions regarding disclosure of test results, reproductive options, and life planning. Therefore, it is crucial that genetic testing is accompanied by appropriate counseling and support services to ensure that patients are fully informed and empowered to make informed decisions. Ultimately, a multidisciplinary approach that combines genetics, neurology, and psychosocial support is essential for optimizing the care of patients with CADASIL and other hereditary disorders.

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Submitted: 08-Apr-2023 **Revised:** 18-Apr-2023 **Accepted:** 21-Apr-2023
Published: 12-Jun-2023

DOI: 10.4103/aian.aian_301_23