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Molecular testing of CADASIL in SWTRGL

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Introduction

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) [MIM 125310] is an adult-onset disorder characterised by migraine, recurrent subcortical strokes, cognitive impairment, psychiatric disturbances and dementia. Cerebral magnetic resonance imaging (MRI) abnormalities in CADASIL include diffuse white matter hyperintensities, multiple lacunar infarcts and microbleeds. Skin biopsies show deposition of granular osmiophillic material (GOM) close to the basement membrane of the degenerating smooth muscle cells. This is unique to CADASIL and occurs in all small arteries of the body in CADASIL patients[1]. The average age of onset is 45 and fully penetrant by age 60. The disease is caused by pathogenic variants in the *NOTCH3* gene on chromosome 19 that has 33 exons and contains 34 EGF-like (epidermal growth factor-like repeat) repeats of 40-50 amino acids present in the extracellular domain[2]. Each repeat has six conserved cysteine residues and the vast majority of causative mutations found in CADASIL patients result in the creation or loss of a cysteine (Cys) residue in this gene[1]. Here we present how the molecular testing of CADASIL is conducted in SWTRGL and the rate of accurate diagnosis achieved during the history of this disease service. The clinical manifestations of patients referred to this laboratory are described, including an audit of type of cases received: diagnostic, confirmation, predictive and prenatal. Data about variant clustering and most common Cys changes found in SWTRGL will also be presented including variants of uncertain significance (VUS) that have been reported in house. Interesting cases will be discussed and compared to relevant existing literature.

Methods

CADASIL referrals and their results were collated from 2013 to 2019 in SWTRGL. A total of 863 referrals have been received and table 1 presents the number of referrals by category. The current testing strategy in this laboratory consists in the analysis of exons 2-26 and intron-exon boundaries of *NOTCH3* by Sanger sequencing. The majority of referrals received are diagnostic and require full screen of the *NOTCH3* gene. Pre-screen testing is offered when requested by sequencing exons 3 and 4 as approximately 45% of pathogenic variants are present in these two exons. Testing for a specific variant is also available for predictive, confirmation, prenatal referrals; a positive familial control is always used when available.

Pre - screen	Full screen	Predictive	Confirmation	Prenatal
96	637	115	11	4

Table 1: Total number of referrals by category received from 2013 to 2019 in SWTRGL.

Early studies noticed a strong clustering in the first 5 EGF-like domains encoded by exons 3 and 4 with 60-70% of mutations found in these exons. Until recently no studies have been carried out to define the mutational spectrum in a specific population, except the Finns where one mutation accounts for all cases. Population data in SWTRGL is in line with the information available, 41% of pathogenic variants have been found in exon 4; however 16% of them are equally shared between exon 3 and 11 (Fig 1).

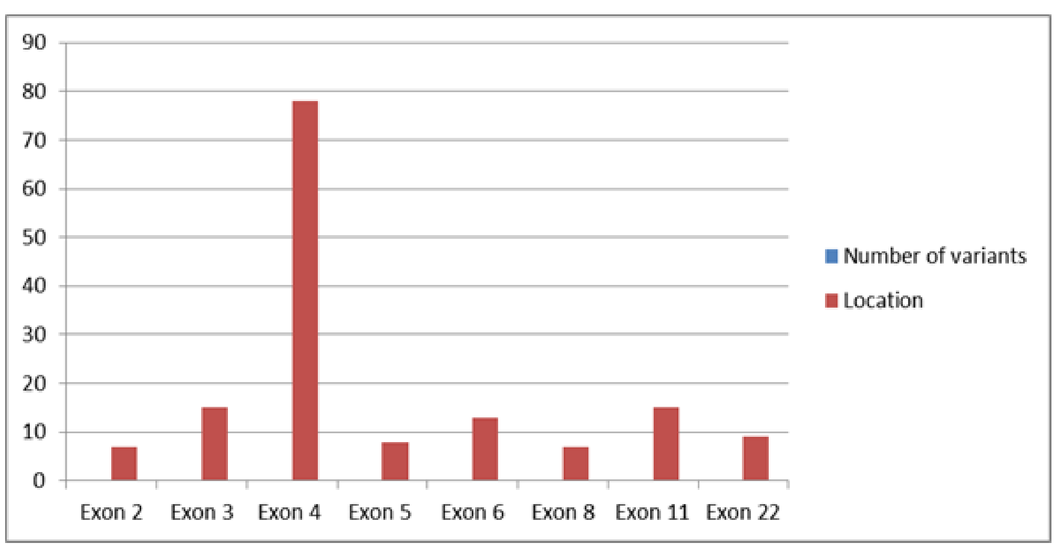

Figure 1: Number of pathogenic variants by exon.

The majority of variants described in the literature are missense variants that lead to the loss or gain of a cysteine residue in one of the EGFr domain of *NOTCH3.* This results in an uneven number of cysteine residues in the given domain, most likely most likely modifying the tertiary structure of the protein. A few splice-site variant, insertions and deletions have been described, also resulting in an uneven number of cysteine residues within EGFr. [3,4]

A total of 191 variants have been identified since 2013 in SWTRGL. To date the most common pathogenic variants seen in this laboratory are presented in table 2. Variants of uncertain significance (VUS) that were detected have been interpreted using the most current ACGS guidelines[9]; none of these include Cys changes.

Pathogenic Variant	Location	Episodes
c.544C>T p.(Arg182Cys)	Exon 4	16
c.421C>T p.(Arg141Cys)	Exon 4	15
c.505C>T p.(Arg169Cys)	Exon 4	11
c.268C>T p.(Arg90Cys)	Exon 3	10

Table 2: Most common pathogenic variants by exon and times seen in SWTRGL.

Interesting cases

Case A: Female patient referred for CADASIL diagnostic testing, clinical details include leukodystrophy and ischaemia/infarcts on MRI. *NOTCH3* gene full screening detected two variants that create a cysteine residue: c.1594C>T p.(Arg532Cys) and c.3298C>T p.(Arg1100Cys). Also a male patient presenting extensive white matter changes, bilateral reduced hearing for several years and aphasia/encephalopathy was referred for *NOTCH3* gene full screening. Sanger sequencing detected two heterozygous pathogenic variants c.2149C>T, p.(Arg717Cys) and c.3296G>T, p.(Cys1099Phe).

Parental testing has not been performed in either case so phase has not yet been established. If the variants are *in trans* all their offspring will be at risk of CADASIL. Patients that are compound heterozygous for two different *NOTCH3* pathogenic variants have not been reported in the literature; therefore it is not possible to make robust conclusions from a single case. However the presence of Cys changes confirms a diagnosis of CADASIL.

Case B: Female patient referred for CADASIL diagnostic testing, clinical details include migraine, stroke like episodes and MRI typical of CADASIL. *NOTCH3* gene full screening detected an in-frame deletion: c.1549_1566del p.(Arg517_Cys522del). Although this variant has not been reported in the literature, it is predicted to affect cysteine residues within EGFr, which is consistent with a diagnosis of CADASIL.

Case C: Female patient experiencing leukoaraiosis and cognitive decline was referred for *NOTCH3* gene full screening. Sequencing analysis revealed this patient is homozygous for the c.3691C>T p.(Arg1231Cys) pathogenic variant. Few cases of patients with homozygous pathogenic variants have been published and these are not thought to be more severely affected than patients with heterozygous pathogenic variants[5]. This finding confirms a diagnosis of CADASIL.

Conclusions

- The prevalence of CADASIL is unknown, but it seems likely that it is underdiagnosed, often being confused with Multiple Sclerosis and Alzheimer's. Clinical diagnosis usually relies on a characteristic brain MRI scan and skin biopsy. This is reflected in the number of patients that have accurately being diagnosed in SWTRGL. Approximately only 20% of patients have been found to have a pathogenic variant that is consistent with the clinical diagnosis. CADASIL is a good example of a genetic disease where both laboratory and clinical input is essential.
- CADASIL has a classical autosomal dominant pattern of inheritance, for which one single heterozygous pathogenic variant is sufficient to cause the disease. In accordance with this, patients that are compound heterozygous or harbour homozygous variants seem to be no more severely affected than cases with single heterozygous variants [6, 7, 8]. However, further family studies in these patients are recommended before definite conclusions can be drawn.
- In Caucasian patients with CADASIL, the majority of *NOTCH3* pathogenic variants are found in exon 4, followed by exons 3, 5, 6 and 11. However, the 16% of pathogenic variants found in the SWTRGL local population are equally shared between exon 3 and 11. Novel and previously reported VUS are difficult to assess and need further functional investigations in order to make robust conclusions. These 2 findings confirm the heterogeneity of CADASIL and the need of tailoring pre-screening for each population as mutational clustering seems to be closely linked to ethnicity.

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