VARIABLE EXPRESSION IN SAMHD1-ASSOCIATED FAMILIAL AICARDI-GOUTIÈRES SYNDROME

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ABSTRACT

Aicardi-Goutières syndrome (AGS) is an encephalopathy of early childhood. This disorder is genetically heterogeneous, with mutations in seven genes having been identified to be disease-causing. Most patients with AGS present with poor developmental outcome and reduced survival in the neonatal period or early infancy. Significant variability can be found in the onset and phenotypic severity of the condition, sometimes even within the same family. Here we describe two sisters of mixed ancestry from the Western Cape province of South Africa presenting with skin manifestations of autoimmune disease resembling those of systemic lupus erythematosus (SLE) on histology but with negative serology. The two affected individuals carried a homozygous c.1681_1682delAG; p. Ser561Phefs*61 mutation in exon 15 of *SAMHD1* on chromosome 20. Both parents and the unaffected brother are heterozygous for this variant. The molecular investigation yielded a unifying diagnosis for an unusual combination of physical findings and differential phenotypic expression in the sisters. A confirmed diagnosis allowed for informed genetic counselling and targeted investigation and screening for complications such as glaucoma in the older sister.

Keywords: Aicardi-Goutières syndrome; intra-familial variability; *SAMHD1*; South Africa

INTRODUCTION

Aicardi-Goutières syndrome (AGS) is a Mendelian, autosomal recessive, neuro-inflammatory disorder that typically manifests in infancy, and is associated with severe developmental consequences.[1] Later onset and milder forms of the disease are also recognised. AGS encephalopathy is characterised by chronic cerebrospinal fluid (CSF) lymphocytosis, calcification of the basal ganglia, abnormalities of the white matter and cerebral atrophy.[2] There is an increased risk of early childhood death, and clinical manifestations include psychomotor retardation, dystonia, spasticity and microcephaly.[3] The combination of intra-cranial calcification, spasticity and microcephaly means that AGS may be mistaken clinically for congenital infection with cytomegalovirus or toxoplasmosis. AGS is genetically heterogeneous and mutations in seven genes, namely *TREX1*,[4] the three nonallelic components of the RNase H2 endonuclease complex (*RNASEH2B*, *RNASEH2C*, and *RNASEH2A*),[5] the deoxynucleoside triphosphate triphosphohydrolase *SAMHD1*,[6] the adenosine deaminase-1 *ADAR1*,[7] and the cytosolic dsRNA sensor *MDA5*[8] have been associated with the disease.

Mutations in *SAMHD1* are postulated to lead to the accumulation of nucleic acids in cells, mirroring a viral infection and subsequently leading to immune system activation.[6] This

activation of the immune system is considered to be the cause of the enhanced production of interferon-alpha (IFN-α) in the serum and CSF of AGS patients, which is considered a hallmark of the disease.[9] It is well documented that IFN-α levels are higher in the CSF than in the serum, thus indicating intrathecal production,[9,10] and astrocytes have been identified as a source of IFN-α and IFN-driven cytokines such as CXCL10.[10,11] AGS patients can also demonstrate elevated CSF levels of FMS-related tyrosine kinase 3 ligand, IL-12p40, IL-15, TNF-α and soluble IL-2 receptor.[12] It has been noted that most cytokines in the CSF decrease with age, but CXCL10 levels are continuously increased beyond early childhood.[12] Similar observations have been described for IFN-stimulated genes (ISGs) such as *IFI27*, *IFI44L*, *IFIT1*, *ISG15*, *RSAD2* and *SIGLEC1,*[13] with the presence of an 'interferon signature' representing a highly reliable disease biomarker. Here we describe two sisters of mixed racial ancestry from the Western Cape, South Africa, presenting to the Paediatric Rheumatology Clinic at Tygerberg Hospital. Subsequent molecular investigation established a diagnosis and allowed for appropriate screening for complications.

MATERIALS AND METHODS

The study was approved by the Health Research Ethics Committee of Stellenbosch University (study no N13/05/075). The parents granted their informed consent, which included the genetic evaluation of the siblings. The study adhered to the ethical guidelines as set out in the 'Declaration of Helsinki, 2013'.[14] Venous blood required for DNA extraction was drawn from both patients (1 mL), an unaffected brother and both parents (5 mL). DNA was purified from blood using the Nucleon BACC3 Kit (Amersham Biosciences, Buckinghamshire, UK).

PATIENT 1

The index patient was born at term to non-consanguineous parents. She presented at age three years with dysmorphic but not distinctive features of a syndrome, a history of idiopathic infantile hemiplegia, complex partial seizures in infancy and delayed milestones. Imaging of the brain was consistent with moyamoya disease (see Figure 1). At four years of age she was referred to the Dermatology and the Paediatric Rheumatology Services with interface dermatitis, vasculitic lesions on her ears and fingertips with threatened gangrene and recent onset of deteriorating gait. On examination, she had generalised signs of hypertonicity and global developmental delay and was unable to walk as a result of ankle contractures. She tested negative for human immune deficiency (HIV) disease, antinuclear antibody (ANA), anti-SM antibody and anti-double-stranded DNA (dsDNA) antibody, but tested positive for anti-beta2 glycoprotein 1 antibody. Treatment was started with Chloroquine, Azathioprine and Nifedipine to control extensive vasculitic and necrotic lesions of the extremities and sodium valproate was continued for seizure control. As a result of a poor response to treatment, and the finding of positive antiphospholipid antibody (anti-beta2 glycoprotein), she was anti-coagulated with Warfarin, which was associated with an improvement of her gait. However, she again deteriorated at age nine years and presented as unable to walk, with draining

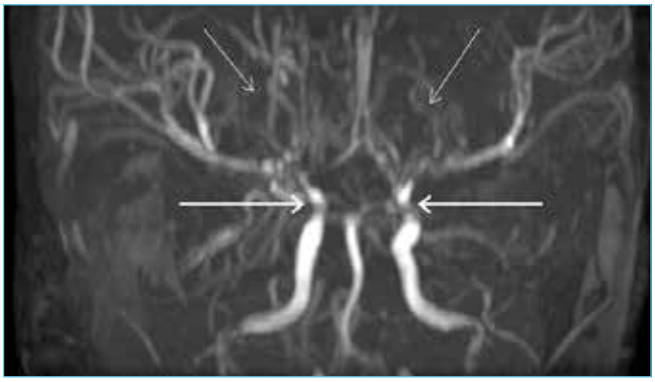

Figure 1: Three-dimensional time of flight (TOF) reconstruction demonstrating narrowed distal internal carotid arteries (ICA) bilaterally (thick white arrows) and lenticulo-striate collaterals (thin white arrows).

lesions of the spine as a presentation of extrapulmonary tuberculosis (TB). Multidrug-resistant tuberculosis (MDR TB) and *Staphylococcus aureus* were isolated from swabs of pus drained from the incision and drainage of lesions of the spine. Chest X-ray and sputa, including examination by GeneXpert, remained negative for TB. The patient responded well to MDR TB and antibacterial treatment, regaining the ability to walk. She is currently maintained on isoniazid prophylaxis and Warfarin.

PATIENT 2

The older sister of the index patient was brought to the attention of the Paediatric Rheumatology Clinic by her mother at age 14 years. She had a longstanding history of Raynaud's phenomenon, vasculitic skin lesions of the nose and auricles similar to her sister's and threatened gangrene of the fingertips on presentation. She was severely stunted, with a weight of 30.5 kg (less than the third centile for age) and height of 128.3 cm (less than the third centile for age), with delayed puberty, a feature not typically described in AGS. Her intellectual development appeared delayed but was not formally assessed. As in the case of her sister, a skin biopsy showed perivascular-interface dermatitis. On investigation she was HIV-negative and screening for autoimmune diseases, including SLE or antiphospholipid syndrome, was negative. She was diagnosed with glaucoma, a recognised feature of AGS. Further imaging for suspected orbital vascular malformations was refused by this patient She subsequently developed progressive contractures of her distal interphalangeal joints without bone resorption on X-ray. She responded well to Nifedipine, Chloroquine and low-dose Aspirin. TB prophylaxis with isoniazid was initiated in view of her sister's status. The presence of two similarly affected siblings suggested the possibility of a genetic disorder, and at this stage AGS was suspected.

Prompted by similar case descriptions, oligonucleotide primers were designed to polymerase chain reaction (PCR)-amplify the exons of *SAMHD1*. Purified PCR amplification products were sequenced using BigDye terminator chemistry and an ABI 3130 DNA Sequencer.[6] Mutation description is based on the reference cDNA sequence NM_015474. A 303-base pair fragment containing the candidate variant was polymerase chain reaction (PCR)-amplified from genomic DNA from the patients,

Figure 2: Vasculitic skin lesions

an unaffected brother and both parents using the following primers: *SAMHD1*-F- 5'-AGTTAGGAGCCTAGGGACCAG-3' and *SAMHD1*-R- 5'-TGGGAACTTTTCAGCAGATAGAC-3'. Each amplicon was bi-directionally sequenced using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Perkin-Elmer, Applied Biosystems Inc, Foster City, California, USA), followed by electrophoresis on an ABI 3130XL Genetic Analyzer (Perkin-Elmer, Applied Biosystems Inc, Foster City, California, USA). All the automated DNA-sequencing reactions were performed at the Central Analytical Facility (CAF) at Stellenbosch University, Stellenbosch, South Africa. Following the positive identification of the variant in the affected family, a total of 322 ethnically matched controls were screened for the variant.

RESULTS

A homozygous single base pair deletion (c.1681_1682delAG; p. Ser561Phefs*61) was identified in exon 15 of *SAMHD1* in both affected siblings. The details of the variant are summarised in Table I. The variant leads to a frameshift, resulting in a premature stop codon 61 base pairs downstream, and *in silico* predictions of the variant (SIFT and Polyphen2) indicated that this variant is probably damaging (see Table I). The deletion was found in the heterozygous state in both parents and an unaffected brother (see Figure 3). The variant was absent from all 322 ethnically matched controls that were screened.

TABLE I: DETAILS OF THE CANDIDATE VARIANT IDENTIFIED IN EXON 15 OF *SAMHD1*

Chromosome	Chr 20
Position	35526290
Gene name	*SAMHD1*
RefSeq	NM_05474
Reference sequence	A
Mutation type	Frameshift
Mutation: DNA (HGVS nomenclature _c)	1681_1682del
Mutation: protein (HGVS nomenclature _p)	Ser 561Phe fs*61
Prediction <SIFT	Damaging
Prediction <PolyPhen-2	Probably damaging
Sanger verification	Yes

DISCUSSION

AGS is a rare genetic disorder in which mutations in seven genes have been associated with the autoinflammatory phenotype.[5,13,15] Here we identified a novel variant (c.1681_1682del p. Ser 561Phe fs*61) in *SAMHD1* in two sisters. Moreover, we documented marked intra-familial phenotypic variability associated with the same homozygous variant, where the index patient presented with early onset features of AGS, while her older sister presented incidentally with features of the disease that had not been recognised earlier. Certain genes that are mutated in AGS, namely *TREX1*, *SAMHD1* and *RNASEH2*, encode proteins that function as cellular nucleases,[13,15,16] mutations in which are hypothesised to result in a failure of cellular processing of endogenous nucleic acids and the subsequent induction of type I IFN-driven immune activation.[7] Mutations in *SAMHD1* have been associated with different forms of the AGS clinical phenotype, with later onset, increased survival and better intellectual preservation.[3,17,18] It has been suggested that *SAMHD1* might have a specific role in cerebral vascular homeostasis and blood-vessel integrity.[19,20,21] The marked phenotypic variability seen in this family may be as a result of the effect of environmental or genetic modifiers, which might be explained by cell-specific IFN-stimulated gene and cytokine responses.[10]

Mapping of the breadth of presentation and features of AGS raises the possibility that the disease may go undiagnosed in patients with a later onset or a milder course, or in those individuals who present with non-specific inflammatory features.[22] The genetic diagnosis of AGS related to *SAMHD1* mutations is important for affected families due to the 25% recurrence risk. Genetic counselling is essential for the family on prognosis, intra-familial variability, recurrence risks and risks for other family members. Careful clinical monitoring is indicated to evaluate for the presence or evolution of complications, including glaucoma or endocrine dysfunctions such as insulin-dependent diabetes mellitus (IDDM) or hypothyroidism. The observation of such intra-familial differences is highlighted in the family presented

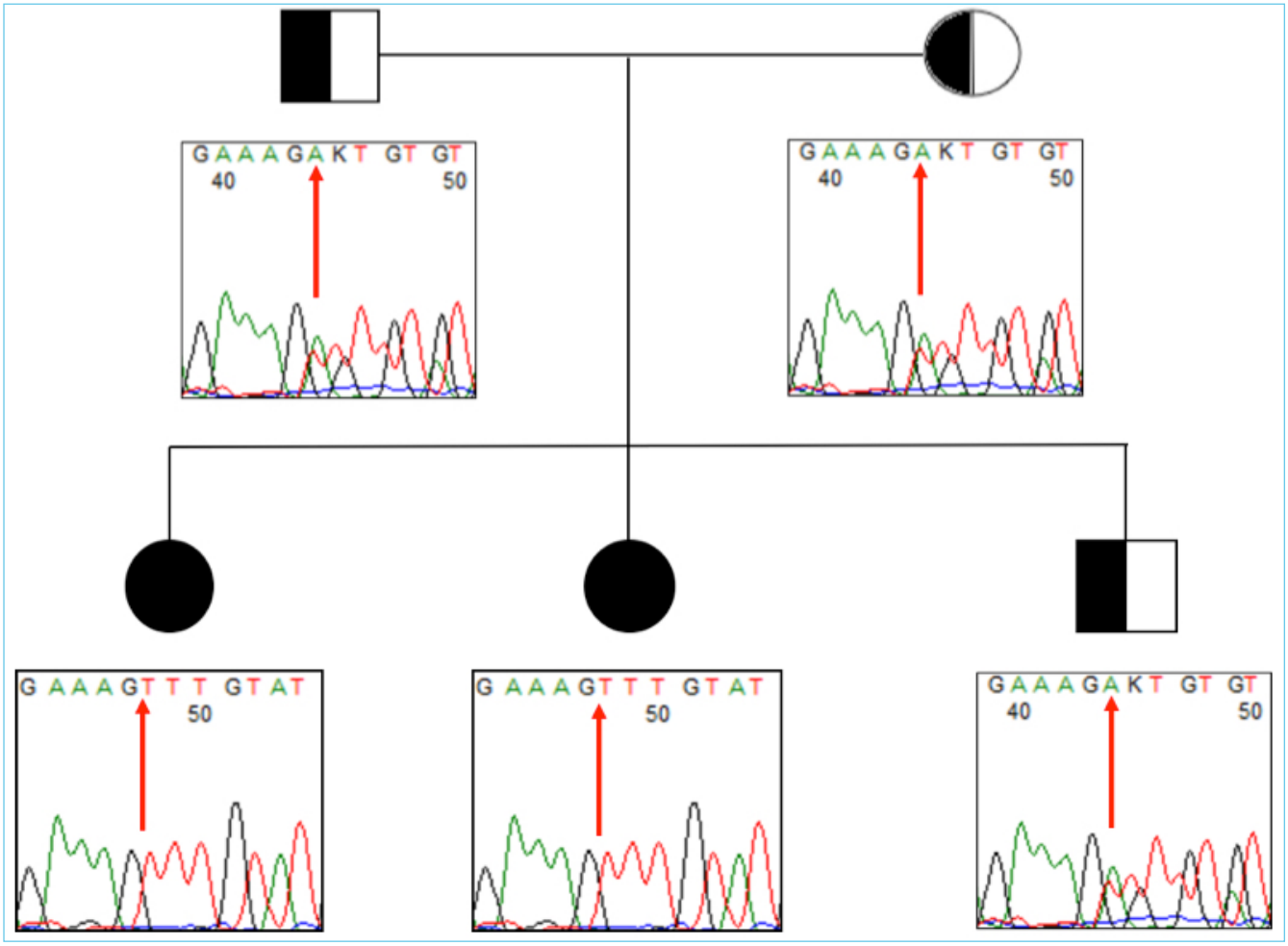

Figure 3: Sanger sequencing of Exon 15 of SAMHD1. Pedigree and sequence chromatograms of affected family. (The red arrow indicates the position of AG deletion.)

here, with an expanding range of clinical phenotypes being reported in this innate immune defect and other primary immune deficits. Whether patients with the *SAMHD1* mutation are also more susceptible to TB, as observed in our index patient, or whether carriers may be at increased risk for autoimmune disease, remains to be investigated on follow-up.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

This article has been peer reviewed.

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