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Two novel CAPN5 variants associated with mild and severe Autosomal Dominant Neovascular Inflammatory Vitreoretinopathy (ADNIV) phenotypes

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Abstract

Purpose—We report two new CAPN5 mutations associated with a phenotype of Autosomal Dominant Neovascular Inflammatory Vitreoretinopathy.

Methods—We performed next generation sequencing in two patients with ADNIV phenotype; the variants identified were explored further.

Results—Patient 1 was heterozygous for CAPN5 c.799G>A, p.(Gly267Ser). Patient 2 was heterozygous for CAPN5 c.1126G>A, p.(Gly376Ser). Both amino acids are highly conserved across species. Patient 1 had a severe phenotype and his mutation lies within the protein's catalytic domain. Patient 2 had a mild phenotype and her mutation is the first ADNIV-causing mutation to be described in the regulatory domain of Calpain-5.

Conclusions—Our findings potentially add two new ADNIV-causing CAPN5 mutations to the three previously described. We recommend CAPN5 genetic testing in all patients with a possible ADNIV phenotype, to develop our understanding of Calpain-5; a protein which could potentially provide therapeutically accessible targets for the treatment of many leading causes of blindness.

Keywords

ADNIV; Autosomal Dominant Neovascular Inflammatory Vitreoretinopathy; CAPN5; CALPAIN-5; UVEITIS

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

Introduction

Inherited retinal degenerations are the leading cause of untreatable blindness in working age adults¹ and the prevalence is predicted to increase with genetic drift over future generations. Gene therapy with adeno-associated viral (AAV) vectors has recently shown promise in a number of clinical trials targeting single gene replacement^{2–4}. One of the limiting factors in selecting patients for gene therapy is establishing which mutations are pathogenic as it can be difficult to distinguish a rare polymorphism from a true mutation.

Next-generation sequencing is identifying increasing numbers of potential candidate disease alleles and analysis of these new mutations can provide useful information. For example, even identifying a new premature stop codon in a known gene could confirm which exons are assembled to generate mRNA; a number of genes, such as RPGR are known to have splice variants in the retina that are not necessarily expressed elsewhere⁵.

Autosomal Dominant Neovascular Inflammatory Vitreoretinopathy (ADNIV) was first described as a cause of retinal degeneration in 1990⁶. Uniquely, it is an ocular autoimmune condition that may progress through several stages due to continued inflammation: uveitis, photoreceptor degeneration, retinal neovascularization, retinal fibrosis, retinal detachment, neovascular glaucoma, phthisis and ultimately blindness. Therefore, depending on time of presentation, it can be misdiagnosed as more common ophthalmic conditions such as autoimmune uveitis, retinitis pigmentosa (RP) and diabetic retinopathy.

The Genetic locus was mapped to chromosome 11q13 in 1992⁷ and *CAPN5* was identified as the causative gene in 2012⁸. The encoded 73 kDa protein Calpain-5 is a calcium-dependent intracellular cysteine protease involved in signal transduction for various cellular processes, targeting multiple intracellular proteins and pathways. It comprises 640 amino acids, with 2 catalytic domains and 2 regulatory domains. *CAPN5* is moderately expressed in photoreceptors⁹ and although it is widely expressed in other organs, no extra-ocular features are known to be associated with ADNIV.

Only three missense *CAPN5* mutations have been identified to date and these mutations are in close proximity to each other in the catalytic domain encoded by Exon 68,10. Here we report 2 new cases of ADNIV caused by novel mutations in *CAPN5*; one of which is in the regulatory domain and has a milder phenotype.

Methods

This study was conducted in accordance with the Declaration of Helsinki (2013). Formal consent for genetic testing was obtained as per standard NHS Trust policy, peripheral blood samples were taken for next-generation DNA sequencing.

Gene Enrichment and Next Generation Sequencing

Enrichment for the *CAPN5* gene was achieved as part of a customised HaloPlex enrichment system kit (Agilent technologies) designed to capture the coding exons and 10bp of the flanking introns of 111 retinal genes. HaloPlex reactions were prepared as per

manufacturer's instructions. Libraries were pooled into batches of 14 and sequenced on an Illumina MiSeq instrument (Illumina) using a MiSeq v3 kit as per manufacturer's instructions. Reads were aligned using BWA11 and variants called using Platypus12. All variants identified by next generation sequencing were confirmed by Sanger sequencing.

Results

Patient 1 (P1)

The first patient was a 42 year-old Caucasian male with a severe phenotype and advanced disease. He had progressive visual problems from infancy with myopia, congenital nystagmus, optic nerve myelination, and progressive vitreoretinopathy with recurrent vitreous haemorrhages since 19 years old. He had multiple cryotherapy treatments, left eye ruthenium plaque brachytherapy and underwent bilateral pars plana vitrectomies. The retinal vasoproliferation continued to progress with exudation, sub-retinal fluid and fibrosis leading to bilateral tractional retinal detachments. He then developed left rubeotic glaucoma with recent insertion of a glaucoma drainage tube. His only past medical history of note was psoriasis.

At presentation to our service his visual acuity was 6/60 in the right eye and 4/60 in the left eye. On OCT imaging he had gross disturbance of normal retinal morphology due to unusual retinal nerve fibre layer (RNFL) myelination and fibrosis. Autofluorescence was decreased centrally from masking by the RNFL pathology and retinal degeneration. Wide-field laser imaging demonstrated the predominantly inferotemporal vasoproliferative abnormality. There was no immediate family history of eye problems and his mother and son were examined who both had normal ocular appearances.

Next-generation sequencing identified that the patient was heterozygous for *CAPN5* c.799G>A, p.(Gly267Ser). This variant has been identified at an allele frequency of 0.023% and 0.047% in European and Finnish populations respectively within the Exome Aggregate Consortium (ExAC) database12. p.Gly267 is highly conserved amongst vertebrates and glycine to serine is a non-polar to polar amino acid change. This mutation is in the catalytic domain of the protein encoded by exon 6, similar to previously described mutations. His mother, as the only surviving parent subsequently underwent genetic testing and was negative for the CAPN5 mutation.

Patient 2 (P2)

The second patient was a 19 year-old female of South Asian extraction with a milder phenotype and earlier disease. She was asymptomatic and an incidental finding of irregular retinal hyperpigmentation and peripheral vascular changes at a routine optometrist appointment. She was fit and well with no significant past medical history. Her visual acuity was 6/6 in the right eye and 6/5 in the left eye. Fundus fluorescein angiography (FFA) showed patchy asymmetrical pigmentary retinopathy and also a small area of periphlebitis with leakage temporally in the left eye. She was an only child with no family history of eye problems. She was initially diagnosed with retinitis pigmentosa, however her asymmetrical

clinical findings, FFA and ERG were not consistent with this diagnosis. Therefore, following referral to our service, genetic testing was undertaken.

The *CAPN5* variant c.1126G>A, p.(Gly376Ser) was identified in this patient. This variant is present at an allele frequency of 0.017% in the South Asian population within the Exome Aggregate Consortium (ExAC) database¹³. p.Gly376 is highly conserved amongst vertebrates and glycine to serine is a non-polar to polar amino acid change. Both parents were symptom free but declined undergoing ocular examination and genetic testing.

Discussion

Two new missense heterozygous mutations in *CAPN5* were identified in patients with ADNIV phenotype. Both of these mutations led to glycine to serine amino acid changes at points which are highly conserved across species. The two patients have differing phenotype severity: P2 at 19 years old is asymptomatic whereas P1 at the same age already had significant visual impairment. The mutation in P1 at p.Gly267 lies within the catalytic domain similar to those previously described. The mutation in P2 at p.Gly376 however is the first to be described within a regulatory domain. ADNIV does demonstrate phenotypical heterogeneity, however it is reasonable to hypothesise that a mutation in the regulatory domain may have a less severe effect on protein function than that in the catalytic domain. The prevalence of 0.017% on the ExAc database would equate to a carrier rate of about 1 in 6,000 in the South Asian population, but it is likely that many of these patients would have subclinical disease, or other broad clinical diagnoses, such as multifocal choroiditis in which peripheral vascular lesions have been described¹⁴. Further screening of patients for variants in *CAPN5*, with correlation to phenotype and protein structure modelling, will provide more insight into the effect on protein function resulting in disease.

Calpain-5 targets multiple intracellular pathways¹⁵ and activates necrotic, inflammatory cell death cascades¹⁶. The pathogenic mechanism causing ADNIV is unknown as the specific protein substrates for Calpain-5 have not been identified. However, it is likely that mutations in *CAPN5* could alter its activity for multiple discrete signalling cascades which could explain the array of pathological features which are unusual in occurring simultaneously in ADNIV patients. This is supported by studies which found that intraocular steroids suppress inflammatory cells and neovascularisation but not retinal fibrosis or degeneration in ADNIV patients^{17,18}. This may indicate different pathogenic pathways for the various phenotypic characteristics of the disease.

Current opinion is that *CAPN5* mutations lead to gain of function – leading to unregulated activation of potentially multiple signalling pathways and ocular inflammation – rather than haploinsufficiency^{8,10,19,20}. This is supported by mice studies where h*CAPN5*-R243L-mutant mice elicit ADNIV-like disease. In a lentiviral vector transduction study h*CAPN5*-R243L-mutant mice demonstrated CD3+ T-cell migration into the retina, altered expression of cytokines and transcription factors involved in inflammation, reduction of ERG b-wave and photoreceptor degeneration¹⁹. A subsequent study using transgenic mutant mice additionally demonstrated upregulation of several biomarkers for uveitis including members of the Toll-like receptor pathway, chemokines and cytokines involved in innate and adaptive

immune responses. Histologically, the transgenic *hCAPN5-R243L* mice eyes exhibited vitritis, vitreoretinal fibrosis, photoreceptor degeneration and neovascularisation²⁰. These findings are in contrast to *CAPN5* knockout mice which have no observed phenotype²¹.

CAPN5 mutations cause a spectrum of disease related to many significant causes of blindness; notably *CAPN5* is the only identified monogenic cause for nonsyndromic uveitis. Therefore, further insight into the pathogenic mechanisms at play could improve our understanding not just of ADNIV but of autoimmune uveitis, retinitis pigmentosa, proliferative vitreoretinopathy and diabetic retinopathy.

It will be interesting to know if *CAPN5* polymorphisms are risk factors for these diseases and moreover whether modifying calpain expression can alter their progression. There is already evidence that inhibition of calpain activity can rescue other types of retinal degeneration^{22–25}. It has been shown that *CAPN5* mutation expression in the eye is sufficient to cause the disease¹⁹, therefore it could be a potential candidate for gene therapy.

Our findings add to the 3 previously identified *CAPN5* mutations associated with ADNIV. We would recommend *CAPN5* genetic testing in all patients with potential ADNIV phenotype, particularly those with unusual inflammation or atypical retinitis pigmentosa. This may also include patients who have a phenotype of familial exudative vitreoretinopathy (FEVR) in which genetic testing for the typical FEVR genes is negative. Whilst genetic testing is becoming mainstream in RP clinics, uveitis specialists may also need to consider ADNIV in unusual cases because a clinically significant dominant history is not always evident. Identifying more ADNIV patients and their *CAPN5* variants will further contribute to our understanding of Calpain-5; a protein which could potentially provide therapeutically accessible targets for the treatment of many leading causes of blindness.

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A. CAPN5 Domain Structure

B. Patient 1 Mutation

C. Patient 2 Mutation

Figure 1. Two Novel CAPN5 mutations identified associated with ADNIV

A. Calpain-5 is a 640 amino acid, 73 kDa protein composed of four domains. The mutations previously described are located in catalytic domain IIb.

B. P1 mutation CAPN5 c.799G>A missense change equivalent to p.(Gly267Ser) or replacement of hydrophobic glycine for serine which is polar at the corresponding amino acid position.

C. P2 mutation CAPN5 c.1126G>A missense change equivalent to p.(Gly376Ser). This is the first ADNIV- causing mutation identified in the regulatory domain of the protein.

A. Patient 1

B. Patient 2

Figure 2. ADNIV Phenotype

A. Patient 1 has a severe phenotype: optomap shows bilateral vasoproliferative vitreoretinal pathology.

B. Patient 2 has a mild phenotype: colour photographs show patchy areas of retinal pigmentary change.

Figure 3. Patient 2 Fundus Fluorescein angiogram

Fluorescein angiography reveals patchy asymmetrical pigmentary retinopathy and a small area of periphlebitis in the left eye (white arrow).

A. Patient 1 Gly267

Human	E	A	R	L	H	G	A	W	T	L	H	E	D	P	R	Q	N	R	G	G	G	C	I	N	H	K	D	T	F	F
Chimp	E	A	R	L	H	G	A	W	T	L	H	E	D	P	R	Q	N	R	G	G	G	C	I	N	H	K	D	T	F	F
Macaque	E	A	R	L	H	G	A	W	T	L	H	E	D	P	R	Q	N	R	S	G	G	C	I	N	H	K	D	T	F	F
Rat	E	A	R	L	R	G	A	W	T	R	H	E	D	P	Q	Q	N	R	S	G	G	C	I	N	H	K	D	T	F	F
Mouse	E	A	R	L	H	G	A	W	T	R	H	E	D	P	Q	Q	N	R	S	G	G	C	I	N	H	K	D	T	F	F
Dog	E	A	R	L	R	G	A	W	T	R	H	E	D	P	Q	K	N	R	S	G	G	C	I	N	H	K	D	T	F	F
Cat	E	A	R	L	R	G	A	W	T	R	H	E	D	P	R	K	N	R	S	G	G	C	I	N	H	K	D	T	F	F
Cow	E	A	R	L	R	G	A	W	T	R	H	E	D	P	L	Q	N	R	S	G	G	C	I	N	H	K	D	S	F	F
Opossum	E	A	K	I	R	G	E	W	K	F	H	E	S	P	L	K	N	R	T	G	G	C	T	N	H	K	E	T	F	F
Platypus	E	A	K	L	R	G	A	W	T	R	S	D	D	P	L	K	N	R	S	G	G	C	I	N	H	K	S	T	F	L
Chicken	E	A	V	L	H	G	A	W	T	R	H	D	D	P	L	K	N	R	S	G	G	C	I	N	H	K	D	T	F	L
Tetraodon	E	A	T	I	R	G	S	W	R	R	H	D	D	P	L	L	N	R	A	G	G	C	I	N	N	R	S	S	F	L
Baker's Yeast	K	N	R	A	E	N	G	H	H	S	Y	G	S	P	Q	S	Y													

B. Patient 2 Gly376

Human	C	G	L	V	K	G	H	A	Y	A	V	T	D	V	R	K	V	R	L	G	H	G	L	L	A	F	F	K	S	E
Chimp	C	G	L	X	K	G	H	A	Y	A	V	T	D	V	R	K	V	R	L	G	H	G	L	L	A	F	F	K	S	
Macaque	C	G	L	V	K	G	H	A	Y	A	V	T	D	V	R	K	V	R	L	G	H	G	L	L	A	F	F	K	S	E
Rat	C	G	L	V	K	G	H	A	Y	A	V	T	D	V	R	K	V	R	L	G	H	G	L	L	A	F	F	K	S	E
Mouse	C	G	L	V	K	G	H	A	Y	A	V	T	D	V	R	K	V	R	L	G	H	G	L	L	A	F	F	K	S	E
Dog	C	G	L	V	K	G	H	A	Y	A	I	T	D	V	R	K	V	R	L	G	H	G	L	L	A	F	F	K	S	E
Cat	C	G	L	V	K	G	H	A	Y	A	I	T	D	V	R	K	V	R	L	G	H	G	L	L	A	F	F	K	S	E
Cow	C	G	L	V	K	G	H	A	Y	A	I	T	D	V	R	K	V	R	L	G	H	G	L	L	A	F	F	K	S	E
Opossum	C	G	L	V	R	G	H	A	Y	A	I	T	D	V	R	K	I	R	L	G	H	G	L	L	A	F	F	K	S	E
Platypus	C	G	L	V	K	G	H	A	Y	A	I	T	D	V	R	K	V	R	L	G	H	G	L	L	S	F	F	K	S	E
Chicken	C	G	L	V	K	G	H	A	Y	A	V	T	D	V	R	K	V	R	L	G	H	G	L	L	S	F	F	K	S	E
Tetraodon																														
Baker's Yeast						K	K	L	K	Y	A	A	G	D																

Figure 4. The novel CAPN5 mutated residues are highly conserved amongst species
 Alignment of calpain-5 orthologs show high evolutionary conservation of both patients' CAPN5 mutations.

A. Patient 1 nucleotide change position Gly267 is indicated in green

B. Patient 2 nucleotide change position Gly 376 is indicated in red.