

# Angiostrongilus cantonensis an Atypical Presenilin: Epitope Mapping, Characterization, and Development of an ELISA Peptide Assay for Specific Diagnostic of Angiostrongyliasis

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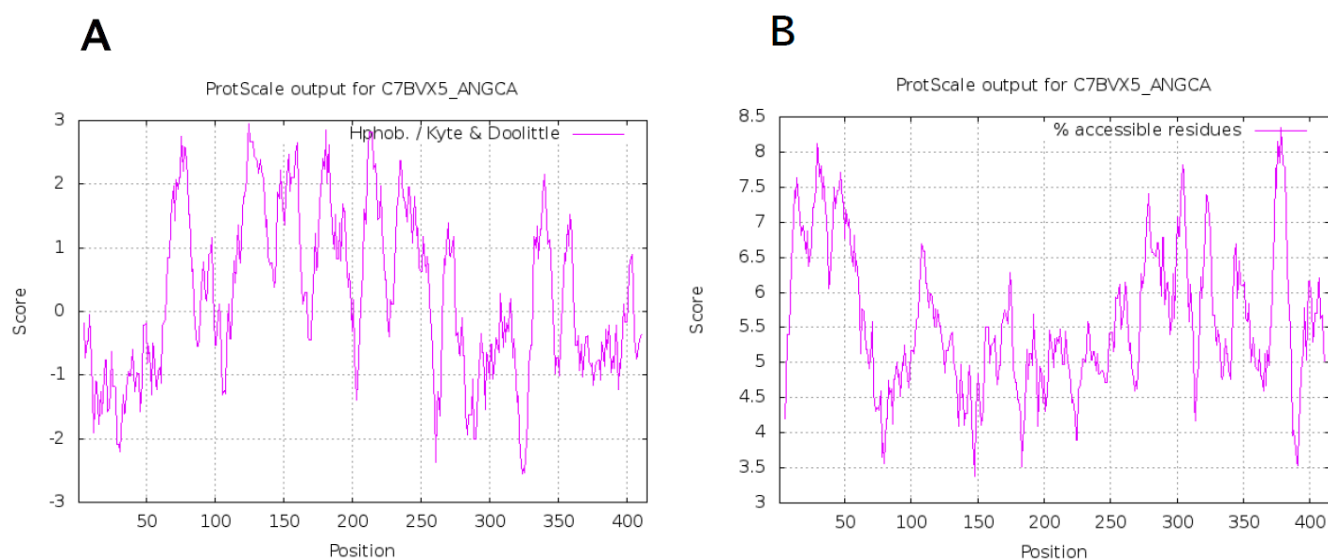
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Spot	Sequence	A29	KFYCFLTGFMFSTF	B29	EVKECTPVQDKVKS
A01	IHGRFVMDGSGSNT	A30	LTGFIMFSTFLLVSL	B30	TPVQDKVKSVPSSGR
A02	VMDGSGSNTDLKQE	B01	MFSTFLLVSLFSFVQ	C01	KVKSVPSGRFIESF
A03	SGSNTDLKQEVKTVN	B02	LLVSLFSFVQYQIL	C02	PSSGRFIESFRMPSL
A04	DLKQEVKTVNDETAS	B03	FSFVQYQILSDLV	C03	FIESFRMPSLKSND
A05	VKTVNDETASKSKER	B04	YQILSDLVNVPVSIV	C04	RMPSLKSNDERNIR
A06	DETASKSKERTISTT	B05	SDLVNVPVSIVFIGFL	C05	KSNDERNIRLGLGD
A07	KSKERTISTTARNRK	B06	PVSIVFIGFLHINLS	C06	ERNIRLGLGDFIFYS
A08	TISTTARNRKAASNT	B07	FIGFLHINLSAIELM	C07	LGLGDFIFYSLLVGT
A09	ARNRKAASNTGISEK	B08	HINLSAIELMSIFWK	C08	FIFYSLLVGTASTHG
A10	AASNTGISEKVKRLY	B09	AIELMSIFWKGPML	C09	LLVGTASTHGDWATT
A11	GISEKVKRLYGPTDM	B10	SIFWKGPMLQQASL	C10	ASTHGDWATTLALFR
A12	VKRLYGPTDMVKVVF	B11	GPMLQQASLILIAV	C11	DWATTLALFRVYLN
A13	GPTDMVKVFPVSIT	B12	QQASLILIAVTVLT	C12	LALFRVYLNRSWFHS
A14	VKVFPVSITMFIIV	B13	ILIAVTVLTIMQIL	C13	VYLNRSWFHSCSPSS
A15	PVSITMFIIVTCVRN	B14	TVTLTIMQILPQWTS	C14	SWFHSCSPSSSKGT
A16	MFIVVTCVRNFDIYH	B15	IMQILPQWTSWALLV	C15	CSPSSSKGTASTTN
A17	TCVRNFDIYHGVNLI	B16	PQWTSWALLVTLALW	C16	TSKGTASTTNIRHIW
A18	FDIYHGVNLIPTPYV	B17	WALLVTLALWDLFAV	C17	ASTTNIRHIWCDRYF
A19	GVNLIPTPYVIYNP	B18	TLALWDLFAVLTCPG	C18	IRHIWCDRYFSSRFA
A20	PTPYVIYNPAADPG	B19	DLFAVLTCPGPKLL	C19	CDRYFSSRFAMSKFA
A21	IYNPAADPGTKLLH	B20	LTPCGPKLLVETAE	C20	SSRFAMSKFAVELNR
A22	AADPGTKLLHAVANA	B21	PKLLVETAEERGED	C21	MSKFAVELNRKQIFI
A23	TKLLHAVANAATFLV	B22	VETAEERGEDLMPAI	C22	
A24	AVANAATFLVVVAIS	B23	ERGEDLMPAIITGS	C23	DREKLQERLAKLAG
A25	ATFLVVVAISTFALL	B24	LMPAIITGSASLPQ	C24	HPGSVNEFDF
A26	VVAISTFALLCLFYF	B25	IYTGASLPQSDSIE	C25	AVNFPNPPGKGGG
A27	TFALLCLFYKFYCF	B26	ASLPQSDSIESRRTS	C26	QEVRYF
A28	CLFYKFYCFLTGFI	B27	SDSIESRRTSEVKEC		
		B28	SRRTSEVKECTPVQD		

**Figure S1.** List of synthesized peptides covering the entire sequence of aspartyl protease (C7BVX5) from *Angiostrongilus cantonensis*. (F3, F4, F5, F11, F12, F13 positive controls and F9, F17 negative controls).

Entry	E-value	Score	Ident (%)	Organism	Protein name	Length
<a href="#">C7BVX5</a>	-	-	-	<i>Angiostrongylus cantonensis</i>	Presenilin	415
<a href="#">A0A0K0DB71</a>	0.0	1,584	77.5	<i>Angiostrongylus cantonensis</i>	Presenilin	414
<a href="#">A0A0D8XWV7</a>	1.3e-132	1,005	64.2	<i>Dictyocaulus viviparus</i>	Presenilin	315
<a href="#">A0A016U204</a>	5.4e-110	866	45.7	<i>Ancylostoma ceylanicum</i>	Presenilin	425
<a href="#">A0A016TZZ8</a>	9.6e-108	852	45.6	<i>Ancylostoma ceylanicum</i>	Presenilin	435
<a href="#">A0A0R3PEK8</a>	1.0e-128	972	79.8	<i>Angiostrongylus costaricensis</i>	Presenilin	409
<a href="#">G0N7M3</a>	6.5e-118	904	77.0	<i>Caenorhabditis brenneri</i>	Presenilin	444

**Figure S2.** Identity of the *A. cantonensis* aspartyl protease (C78VXS) used in our study with major other proteins deposited in data bank.



**Figure S3.** Hydropathicity (A) and amino acid accessibility (B) of amino acids of the *A. cantonensis* aspartyl protease.

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1: -----IHGRFVMEGSGSNTDLKQEVKTVDNETASKSKERTI-----STTARNskaASTNGISEKVKRLYGPTDMVKVF 69
2: -----0
3: -----MEDGSGSNTDLKQEVKTVDNETASKSKERTI-----STTARNskaASTNGISEKVKRLYGPTDMVKVF 63
4: MSSSTVCTEQELVLSGIDTSEE--SP-ITMANEARSRSNGENVNGSRPTTSAEVATTSAEIASSSRKEKNYGPQDMVKVF 77
5: MS-SSKSIGKTLVYKGDARHD--EE-DAITGEKKRRGENAAAAGSS-----RAKAKKSTSHRQHEKNYGPQDMVKVF 69
6: --P-----SCTS-----QPGTQEHPA-----TVRRTSTLGSGENEEEEAEELKYGASHVINLF 45
7: MPY-----SREP-----QDGGGNTSASSETHTT---YGTNLISNRSEPEEENQVEEAEELKYGASHVIHLF 57

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	PSAg1	PSAg2	PSAg3	
1:	VPVSITMFIVVTCVRNFDIYHGVNLIPTPYIYNPAADPGTKLLHAVANAATFLVVAISTFALLCLFYKFCFLTGF			149
2:	-----MIYTEPSDDAVTELLHAINASTFLLLVISTFLLLFLFYKFCFLHGF			50
3:	VPVSITMFIVVTCVRNFDIYHGVNLIPTPYIYNPAADPGTKLLHAVANAATFLVVAISTFLLCLFYKFCYCTG---			140
4:	VPVSICMAIVTVITRVEIYRQDVTIKTPYIYHDPPEAEPTKLWHSLVNAATFLCVIVVSTFGVLALFYKFCYRCLNFF			157
5:	VPVSICMTIIVIICTRNVEVYQKDIIILRTPYIYHDPPEAEPTKLWHSVMNAAVLLCVVVVATFGVLALFYCKCYRFLTIF			149
6:	VPVSIcMVMVFTMNTVTFYSYNDGRHLLYTPFVKETDSTSEKVLMSLGNALVMLCVVIIMTVILIVFYKYRCYKVITAW			125
7:	VPVSLCMALVFTMTITITFYSQNGRHLLYTPFVKETDSIVEKGLMSLGNALVMLSVVIVMTVLLIFFYKYKFKLIHGW			137

	PSAg4	PSAg5	
1:	IMFSTFLLVSLFSFVQYQIILSDLNVP-----VSIVFIGFLHINLSAIELMSIFWKGPMLRQQASLILIAVTV		217
2:	IMLSTFMLVTLFSFMQYQIILSDLNIP-----VSIVVMVFIHLNIAVQLMSIFWIGPMKVQQGSLILISVTV		118
3:	-----YFRQIILSDLNVP-----VSIVFIGFLHINLSAIELMSIFWKGPMLRQQASLILIAVTV		193
4:	IMFSTFMLITTASLMQYYEVLRAINFP-----ISSATVLLLHTNMAVIGLMSIFWKGPRLVQGGTLVLMAMV		225
5:	FMFATFMLITIMTALQYHEILRAINVP-----VSAVTFLLHLNVAVIGLMSIFWKGPMLVQATLILMAAMV		217
6:	LMVSSCLLLFLFSTIYFAVL-KSFTSCEFSFFQRSDEKAVETTASMFSLRFFLLDSTCLTHCH--PFFQFYLIAMSALM		202
7:	LIISFLLFLFTAIYVQEVLSKFDVS-----PSVILVLFGLGNYGVLGMMCIHWKGPLRLQQFYLIITMSALM		205

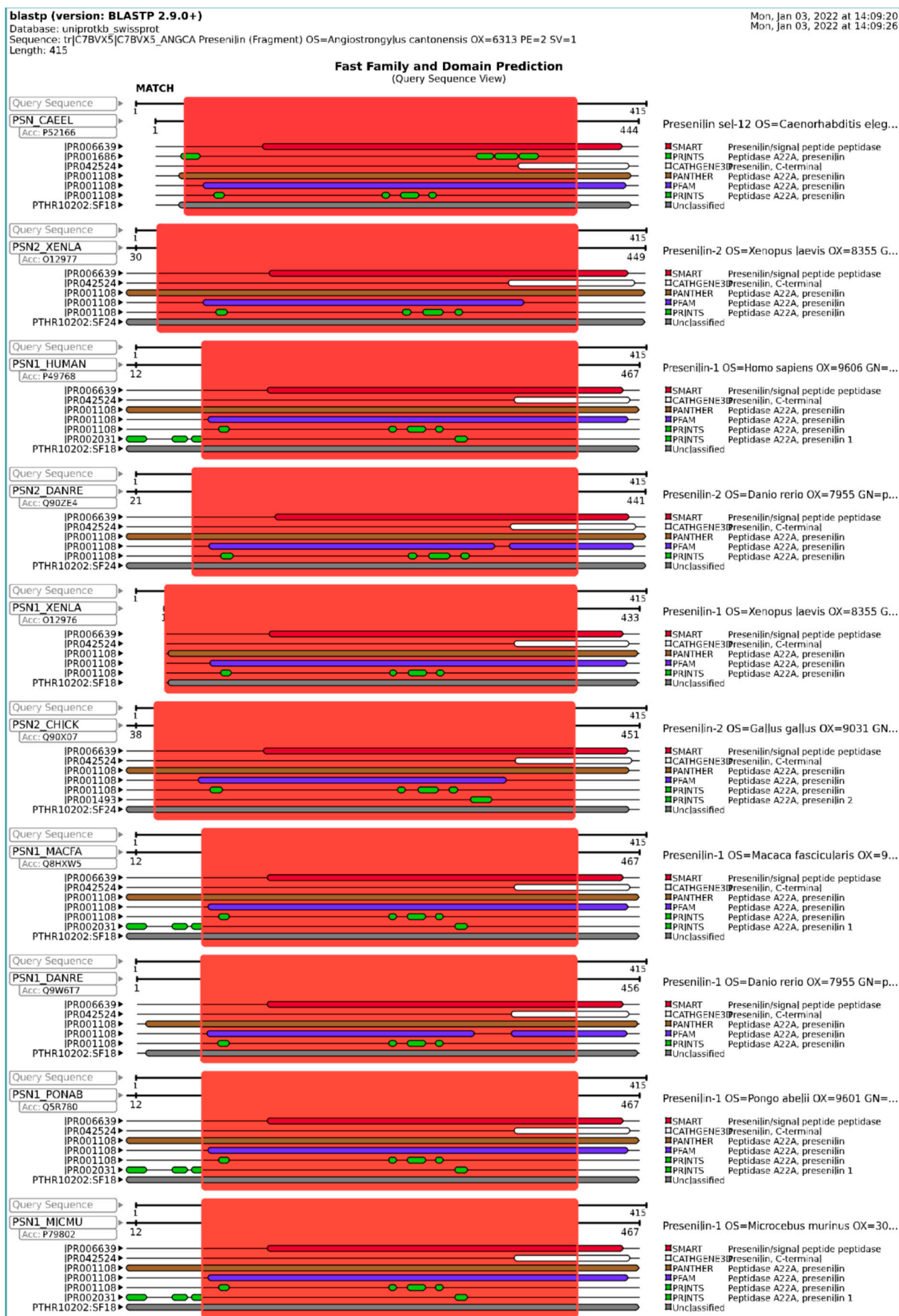
	PSAg6	PSAg7	
1:	TLTIMQILPQWTSWALLVTLALWDLFAVLTPCGPLKLLVETAERGEDLMPAIIYTGASLPQSDSI-----		284
2:	TLTIMQILPKWTSWLLVTLALWDLFAVLSPCGPLKLLVETAERGEDLMPAMIYTGIAFLQSTSSK-----		186
3:	TLTIMQILPQWTSWALLVTLALWDLFAVLTPCGPLKLLVETAERGEDLMPAIIYSMLVLLHFHNQTLNRVARQKLK-N		272
4:	TLTIMRVLPRTNWMVILLAFWDLCAVLSPCGPLKILVETAERGEDLMPAIIYTGAAISIEKTESTQ-----		293
5:	TLTIMRVFPKWTSAIVILLAFWDIFAVLSPCGPLKILVEIAERDEELMAAIIYSTQPFEEKKLKLYDI---CIS-A		293
6:	ALVFIKYLPEWTVWVFLVVISVWDLVAVLTPKGPLRYLVETAQERNEPIFPALIYSS-GIIPYVVLVAVKNSEEGTEMP		281
7:	ALVFIKYLPEWTVWVFLVVISVWDLVAVLTPKGPLRYLVETAQERNEPIFPALIYSS-GIIPYVVLVAVKNSEEGTEMP		384

	PSAg8	
1:	-----ESRRTSEVKECTPVQDK---VKSVPs---SGRFIESFRMPS-----LKS-NDDERNIRLGLGD	335
2:	-----SPTSAEVEGSSPIKDK---IKT-KS---SGNTVESLRMPS-----LNS-EAEERNIRLGLGD	235
3:	GIEIFDDGKPAISYKFIVPRDCTPVQDK---VKSVPs---SGRFIESFRMPS-----LKS-NDDERNIRLGLGD	334
4:	G---SQTPP-----TAVDRSPTNEK---RSASRS---PLRRSGSQPIPS-----NSVSAEEESNIRLGLGD	345
5:	GSATLSRTEPKQSDSLASGEESPTK-E---RRSSRS---SVRTVESLRIPS-----FSETDEEDRNIRLGLGD	355
6:	IS-----SNLPLE-----FVFS-CFEACFTAGMVPsFLVLHN-----VVQICNISEGVKLGLGD	329
7:	TTSSASSSQPSNVPKKTTKVKRIPQKVQIETNVSSNPQGETPVARVERERQPIVNVEPVQYVRHEDYQEEKGVKLGLGD	364

	PSAg9	PSAg10	PSAg11	PSAg12	PSAg13	
1:	FIFYSLLVGTASTHGDWATTLALFRVYLNRSWFHSCSPSSTSGKTASTTNIRHIWCDRYFSSRFAMSKFAVELNRKQIFI					415
2:	FIFYSLLVGSASVDGDWVTTLACFVSILTGLGFTLVLLVILQKALPALPISITFGVLAYFSSRYAVSKFVLELNRKLMFL					315
3:	FIFYSLLVGTASTHGDWATTLACFVSILTGLGFTLVLLVILQKALPALPISVTFGVIAFYSSRFAMSKFAVELNRKQIFI					414
4:	FIFYSLLVGNATVLADWTTIVACAVSILVGLAFTLVLLVIFQKALPALPISIAFGAIAFFSSKFVTSKYLDRLNSEQIFL					425
5:	FIFYSILVGNAAVLADLTTLACFVSILVGLGFTLILLVIVQKALPALPISIAFGAITFFVSKLVTSKLIQDLNKEAIFL					435
6:	FIFYSVLVGKASSYFDWNTTVACYVAILVGLCFTLLLLAVFRRALPALPISIFAGLLFYFCTRWIVTPYVSEITRRQWVY					409
7:	FIFYSVLLGKASSYFDWNTTLACYVAILIGLCFTLVLLAVFKRALPALPISIFSGLLIFYFCTRWIITPFVTKFTQNCLLY					444

**Figure S4.** Cross-reactivity of the six-presenilin nematode proteins deposited in the database with a higher degree of identity (45–80%) with *A. cantonensis*. BLAST was carried out in the UniProt (Universal Protein) database of the EBI (European Bioinformatics Institute), using the C7BVX5 protein from the organism “*Angiostrongylus cantonensis*” as input. (1) C7BVX5 (*Angiostrongylus cantonensis*); (2) A0A0D8XWV7 (*Dictyocaulus viviparus*); (3) A0A0K0DB71 (*Angiostrongylus cantonensis*); (4) A0A016U204 (*Ancylostoma ceylanicum*); (5) A0A016TZZ8 (*A. ceylanicum*); (6) A0A0R3PEK8 (*A. costaricensis*); (7) G0N7M3 (*Caenorhabditis brenneri*).





**Figure S5.** Multiple sequence alignment for functional prediction of AgPS.