GG: A domain involved in phage LTF apparatus and implicated in human MEB and non-syndromic hearing loss diseases

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Abstract Here, we report the identification of a novel domain – GG (domain in KIAA1199, FAM3, POMGnT1 and Tmem2 proteins, with two well-conserved glycine residues), present in eukaryotic FAM3 superfamily (FAM3A, FAM3B, FAM3C and FAM3D), POMGnT1 (protein O-linked mannose β -1,2-*N*-acetylglucosaminyltransferase), TEM2 proteins as well as phage gp35 proteins. GG domain has been revealed to be implicated in muscle–eye–brain disease and non-syndromic hearing loss. The presence of GG domain in Bacteriophage gp35 hinge connector of long tail fiber might reflect the horizontal gene transfer from organisms. And we proposed that GG domain might function as important structural element in phage LTF. © 2005 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.

Keywords: GG domain; LTF apparatus; Muscle–eye–brain disease; Non-syndromic hearing loss; Gp35; POMGnT1; KIAA1199; FAM3 superfamily

1. Introduction

"Viruses straddle the definition of life. They lie somewhere between supra molecular complexes and very simple biological entities" [1]. Bacteriophage T4 apparatus containing the longtail fibers (LTF) is responsible for host cell recognition and infection and initial attachment to susceptible bacteria [2,3]. Among the components of LTF, the distal half-fiber is composed of triple copies of gp34, gp36, gp37, respectively, as well as one copy of gp35 (30 kDa) [4]. In analyzing the protein sequence of gp35, we found a novel protein domain present in not only gp35 and but also a variety of eukaryotic proteins, including FAM3 superfamily, TMEM2 and POMGnT1.

2. Materials and methods

In analyzing the protein sequence of gp35, we found some homologues in similar phage strains. To find more homologues, we conducted PSI-BLAST searching at http://www.ncbi.nlm.nih.gov/blast/ [5] using the sequence of gp35 (gi]32753733, 52-157aa), with threshold

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value as 0.01. In the first two iterations, several phage proteins were retrieved, e.g., Enterobacteria phage JS98T4 gp35-like, tail fiber hinge (gi|52139849, E-value: 5e - 13), Enterobacteria phage RB69gp35 hinge connector of long tail fiber, proximal connector (gi]32753733, E-value: 3e - 50); Enterobacteria phage T4 gp35 hinge connector of long tail fiber, proximal connector (gi|5354251, E-value: 3e - 23), Enterobacteria phage T4 tail fiber protein gp35 - phage T4 (gi|2145006, E-value: 4e - 11); Enterobacteria phage gp35 tail fiber hinge (gi|66391730, Evalue: 3e - 07), Aeromonas phage 31 gp35 (gi|62114858, E-value: 3e - 05); Bacteriophage 44RR2.8t hinge connector of long tail fiber proximal connector; gp35 (gi|34733001, E-value: 3e - 05), Enterobacteria phage RB49hinge long tail fiber protein proximal connector (gi|33348149, E-value: 9e - 05). From the second to ninth iterations, many eukaryotic proteins were retrieved, such as FAM3A (Family with sequence similarity 3, member A), FAM3B, FAM3C, FAM3D, TEM2, KIAA1199, POMGnT1 and some other uncharacterized proteins, including Dictyostelium discoideum hypothetical protein DDB0204607; gi|66812802, D. discoideum hypothetical protein DDB0204608. Additionally, exhaustive searches against all available genome and protein database at GenBank demonstrated that no homologue with significant E-value in plants, fungi, insects, Archaea and bacteria.

After 9 iterations, the results converged and retrieved 75 non-redundant protein sequences totally, which were subjected to multiple sequence alignment with ClustalX software [6] and manual editing (Fig. 1A and B, and supplementary materials), colored with Chroma [7]. The phylogenetic tree of these sequences was constructed with ClustalX software. This conserved region was named GG domain (Domain in KIAA1199, FAM3, POMGnT1 and Tmem2 proteins with two well-conserved glycine residues). The secondary structure of GG domain was predicted by Jpred using the alignment profile [8].

3. Results and discussion

GG domain is composed of seven β -strands and two α helices, about 100 amino acid residues in size. It is present in a wide range of proteins, including FAM3 superfamily (FAM3A, FAM3B, FAM3C and FAM3D), POMGnT1, TEM2, phage gp35 proteins and some uncharacterized proteins. Functional roles of FAM3 superfamily almost remain unknown, although some pilot work suggested FAM3B could represent a novel class of effectors involved in the destruction of the β -cells and involved in the pathogenesis of type 1 diabetes [9]. Human *TMEM2* is expressed in cochlea and many other tissues, it is located in the ARNSHL (autosomal recessive non-syndromic hearing loss) linked region on (chromosome 9q13–q21) but no disease-causing mutations were found in the coding region [10].

POMGnT1 is a glycosyltransferase, catalyzing the transfer of GlcNAc to O-mannose of glycoproteins. *POMGnT1* gene is implicated in muscle–eye–brain disease (MEB), caused by mutations in the *POMGnT1* gene, The frameshift mutations

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Jpred. Sec. 11	eu.	LLI	JEEEE	A LOLD LA		10.000						ELL.		
gi 3334194	95 GVKN	VG-R I	VALANGKT	G-EVLDTKY	DMWGG-DVA	PFIEF	LKAIQDGT	IVLMGTYDDO	GAT-KLNDE	ARRLIADL	TSITNLG	FRDNWVF	CGGKGIKTKSPFE	197
gi 37182476	92 PVKN	WG-R LN	ALVNGTT(G-AVLGQKA	DMYSG-DVM-	-HLVKF	LKEIPGGA	LVLVASYDDI	PGT-KMNDE	SRKLFSDL	SY <mark>A</mark> KQ <mark>L</mark> G	FRDSWVF	IGAKDLRGKSPFE	194
gi 57284179	96 SVKD	VGR LN	ALVNGVS	G-ELIEARA	DDMWAG-DVN	DLLKF	IRPLHEGT	LVFVASYDDE	PAT-KMNEE	TRKLFSEL	SRNAKELA-	FRDSWVF	VGAKGVQNKSPFE	198
gi 37182042	100 EQLG	VA-R IN	VIAIVNYVT(G-N <mark>VT</mark> ATRC	DMYEG-DNSG-	PMTKF	IQSAAPKS	LLFMVTYDDO	GST-RLNND	AKN <mark>AIEAL</mark>	SKEIRNMK	FRSSWVF	IAAKGLELPSEIQ	203
gi 17541586	236 KDLN	SAGR LN	LAMLEPKT	G-K <mark>VT</mark> AVAH	DTYED-ESH	GLEEW	LDAVPTGY	IVAVVSFDE/	ASN-QLSDM	ARRIFYEM	SMIDRLK-	FRASWYF	VGQKG <mark>I</mark> GAYTPFE	338
gi 12655225	122 DEAR	QG-R I	IVIVLNQAT(G-H <mark>VM</mark> AKRV	DTYSP-HEDE-	AMVLF	LNMVAPGR	VLICTVKDE0	GSF-HLKDT	AKALLRSL	QA <mark>G</mark> PALG	WRDTWAF	VGRKG <mark>G</mark> PVFGEKH	225
gi 55957834	258 TFEK	DFS-R LN	VRVIDQDT/	A-KILESER	DTHEYRNESR-	-RLQEF	LRFQDPGR	IVAI AVGDS/	AKSLLQGT	IQMIQERL	ELIQGLG-	YRQAWAL	VGV ID <mark>G</mark> GSTSCNE	363
gi 38564147	180 FERS	VGHR VI	(<mark>VHVI</mark> DPKS)	G-TVIHSDR	DTYRSKKESE-	RLVQY	LNAVPDGR	ILSV AVNDEO	GSR-NLDDM	ARKAMTKL	SKHFLHLG	FRHPWSF	LTVKGNPSSSVED	284
gi 38564147	1237 -KKY	PSSE-D IC	Q <mark>VVVI</mark> DGNQ(G-RVVSHTS	RNSILQGIPW-	QLFNY	VATIPDNS	IVLMASKGRY	VSRGP	WTRVLEKL	ADR <mark>G</mark> LKLK-	EQMAF	VGFKGSFRPIWV-	1336
gi 34733001	53S	SYSR I	ra <mark>v</mark> tispt-(GVAS-NQAT	DLYTRP-EHFI)T-LRSY	LMSAPSNN	IVVLVSYDAM	IKSTEAL	FDEFMYQY	RSWPGVP (1	11) YRSSYVA	IFNTSLKRFCYEN	163
gi 62114858	53S	SYS-R I	ra <mark>vti</mark> spn-(GVAS-NQAT	PDLYTRP-EHFI)TLRSY	LMSAPSNN	IVVL VSYDAM	IKSTEAL	FDEFMNQY	RSWPGVP (1	1) YRSSYVA	IFNTSLKRFCYEN	163
gi 66391730	54 NRNT	RD-R I	LAIIDGT-	FLALL DYKT	FDMYGDPTTNGN	A-LRDY	LGSLPANR	IVCFYSYDA]	IGSNAN	FTAIMRKI	VAWP(1	10) RRSSYSA	IYSSTMKKICMEN	165
gi 5354251	53 — SQ	FSA	ILRVFDPST(G-ALVDSKS	YA <mark>F</mark> STSNDTTS/	A-FVSF	MNSLTNNR	IVAILTSGKV	VNFPPE	VVSWLRTA	TSAFP(6	5) RSDVS <mark>Y</mark> AA	FYTSSKRAIALEH	158
gi 2145006	1			M	LFRLQMLLHHQI	_LLLVF	MNSLTNNR	IVAILTSGKV	/NFPPE	VVSWLRTA	T		-TSSKRAIALEH	61
gi 32453733	52LQ	SFGIL	VRVINPEN	G-TIVDSKL	YN <mark>F</mark> APTNNATS#	A-FISF	VNTYADNF	IFAF ISNNKI	FNLPPE	I IEWFKAA	SVIP(6	5) LVDIS <mark>Y</mark> SA	FYVSGKNTIALEH	157
gi 52139849	52 — PQ	FQN L	LIEIDVSAL	EPK <mark>VV</mark> ANKT	YS <mark>F</mark> TKDF D VISE	EA-FITY	ISSIPANR	IVCL VSSGRI	LNASQN	LIDWFRAA	TA <mark>F</mark> P (6	5) RFEPSYSA	FYVSGRNTIVMEH	158
gi 33348149	59 - IV	GTGYEN L	FKVLTPTG	Q <mark>LH</mark> EEKV	YGRGAVLAMR-	DY	LSL <mark>L</mark> KGDY	TIAM ATHGEI	LFADPI	SDV <mark>V</mark> FSKM	SVS <mark>F</mark> PNHI (6	5) — RVS <mark>Y</mark> AA	IYSTKMGKIVCEG	162
gi 33414941	50 SLSP	SQT-K	CIHL TEDFI	KPTLMS	DFSVVTDTDR-	FTTW	VNSLATGI	-ILLMSHTEN	WTNDK	LNTYFDQ1	SVGWKYYW (3	5) TNRSSYVA	LIDCPLKKIMT	152
gi 66812800	50 — QQ	ÍLAKS <mark>R V</mark> I	A AFNPND	V-KT <mark>L</mark> KILA	EDTHHWVKEP-	(5) NLLSW	LKEIESFSPGW	VVAMVSNDD8	SSL-SMGGD	LKTYLSKF	LGFD	FRGSWLL	ALQTN <mark>G</mark> VNFKNLG	157
gi 66812802	48 — RT	ILLNG <mark>R I</mark> N	AFAFDPSNI	P-S <mark>VV</mark> KMLK	SDTYMEEPP	(7) GFTSF	VGEIKPRK-NW	VIAI VSLDDS	SYL-NMSED	VRQWFRGY	ISLS	YRGSYAL	VLQSN <mark>G</mark> LALNKIA	155
gi 17541586	26 — TD	IG-R I	AAILNGTT(G-DIIEKAT	DVTAS-DEK	LMTW	LLKVPHAS	LLVAASFGDV	VAE-HVSRQ	SRQLFAAF	AQKIDNWR-	VGNAYAI	I GQRG I RRGEAHE	125
gi 47213237	213 STLN	AG-V I	VIVVDGKT(G-K <mark>VT</mark> DTNH	DDMYSG-EVK-	-PLISF	LNAIEAGS	IVLIASFDEI	PAT-KLDNE	AKRLITEL	SSIGSLG-	FRDSWLF	VGAKG <mark>A</mark> AKKSLFE	316
gi 47213237	84 STLN	AGV	(TVVVDGKT)	S-KVTDTNH	DMYSG-EVK	PLISF	LNATEAGS	IVLIASFDER	PAT-KLDNE	AKRLITEL	SSIGSLG-	FROSWLF	VGPC1TKECPSDQ	186

Consensus/80%

..., p. t., RG1p1h11s., st. p1h..., FDha, s, p. p. ..., bhpa1p.h., sp. ..11hhss. Dcss... bp. php. hbppbGSp. h., 1..., aRpsasb1s. psh...s. bp



Fig. 1. Alignment (A) and phylogenic tree (B) of representative sequences with GG domain. In A, the sequences are: gi|3334194, *Homo sapiens* Protein FAM3C precursor; gi|37182476, *H. sapiens* FAM3D; gi|57284179, *H. sapiens* family with sequence similarity 3, member A; gi|37182042, *H. sapiens* FAM3B; gi|17541586, *Caenorhabditis elegans* putative protein family member, with a coiled coil-4 domain, of bilaterial origin (4D18); gi|2655225, *H. sapiens* O-linked mannose β -1,2-*N*-acetylglucosaminyltransferase; gi|55957834, *H. sapiens* transmembrane protein 2; gi|38564147, *H. sapiens* KIAA1199 protein; gi|34733001, *Bacteriophage* 44RR2.8t hinge connector of long tail fiber proximal connector; gi/2148066, *Enterobacteria phage* RB43 gp35 tail fiber hinge; gi|5354251, *Enterobacteria phage* T4 gp35 hinge connector of long tail fiber, proximal connector; gi|2145006, *Enterobacteria phage* T4 tail fiber protein gp35; gi|32453733, *Enterobacteria phage* RB69 gp35 hinge connector of long tail fiber, proximal connector; gi|2139849, *Enterobacteria phage* JS98 T4 gp35-like, tail fiber hinge; gi|33348149, *Enterobacteria phage* RB49 hinge long tail fiber protein proximal connector; gi|66812800, *Dictyostelium discoideum* hypothetical protein family member, with a coiled coil-4 domain, of bilaterial origin (4D18); gi|47213237, *T. nigroviridis* unnamed protein product. Fig. 1B is the Phylogenic tree of GG domain containing sequences retrieved by PSI-BLAST searches, KIAA1199 protein GG domain 1 denotes the N' terminal GG domain KIAA1199 and KIAA1199 protein GG domain 2 denotes the C' terminal GG domain in KIAA1199 and KIAA1199 protein GG domain 2 denotes the C' terminal GG domain.

(281C > T and 541 del T) in POMGnT1 result in truncated proteins missing both the GG domain and the GNT-I family region (Pfam entry: PF03071) (Fig. 2), and some other muta-

tions result in truncated proteins missing only the GNT-I family region [11]. GNT-I region occurs only in α -1,3-mannosylglycoprotein β -1,2-*N*-acetylglucosaminyltransferase family



Fig. 2. Domain architecture of representative proteins with GG domain. S indicates signal peptide; TM indicates transmembrane region; PFAM:GNT-I: GNT-I family (PF03071). GG domain occurs in related proteins as singlet or two copies.

(GNT-I, GLCNAC-T I) which transfers *N*-acetyl-D-glucosamine from UDP to high-mannose glycoprotein *N*-oligosaccharide. The catalytic domain in proteins of this family is located at the C-terminus [12,13].

KIAA1199 protein contains two GG domains, and the phylogenetic tree indicated that these two GG domains were originated from separate combination events, instead of intragenic duplication. In the two GG domains, the N' terminal one is more homologous to the phage gp35 proteins and *Dictyostelium* proteins (gi|66812800 and gi|66812802) (Fig. 1B). Murine *KIAA1199* is specifically expressed in Deiters' cells in the organ of Corti at postnatal day zero (Pn) P0 before the onset of hearing, but expression disappears by day P7 in those cells [14]. Abe et al. reported the R187C mutation of KIAA1199 protein in one family of non-syndromic hearing loss (2003), which is located at the N' terminal GG domain (Fig. 2). Arginine is negative charged and hydrophilic while cysteine is neutral and hydrophobic. Mutation of this residue might have impact on the structure and function of GG domain.

Noticeably, GG domain also occurs in T4 type phage gp35 proteins, component of the long-tail fibers (LTF) hinge. LTF is an apparatus for Bacteriophage T4 to recognize and infect host cells, and it is also responsible for its initial attachment to susceptible bacteria [4,15]. Among the components of LTF, the distal half-fiber is composed of triple copies of gp34, gp36, gp37, respectively, as well as one copy of gp35 (30 kDa). Gp35 forms the local non-equivalent hinge (the "knee-cap") between proximal and distal half-fibers [4]. Since gp35 proteins contain only GG domain (Fig. 2), we proposed that GG might be an important structural element for the role of gp35 proteins as asymmetrical "knee-cap" in LTF apparatus.

Virions including phage were originated from organisms, among which bacteriophages infect Eubacteria and Archaea [1]. In Enterobacteria phages, GG is present exclusively in gp35 proteins of T4-like phages. According to the available data, GG domain does not occur in bacteria. Therefore, it is reasonable to propose that the sequence coding GG domain in Enterobacteria phages was obtained from an unidentified Eubacteria or Archaea species, instead of vertical inheritance from common ancestor. The phylogenetic tree suggested that the nucleic acid sequences encoding GG domain in phages might be derived indirectly from the ancestor of genomic sequences encoding Dictyostelium proteins (e.g., gi|66812800 and gi[66812802), as well as the sequences encoding the C' terminal GG domain in KIAA1199 proteins (Fig. 1B and supplementary materials), prior to the transfer through the unknown Eubacteria or Archaea species.

Summarily, GG domain is a widely distributed protein motif, present in both eukaryotic proteins and T4 phage gp35 proteins. Mutations in KIAA1199 and POMGnT1 are associated with mammalian diseases including MEB and non-syndromic hearing loss, which suggest the physiological roles of GG domain. The GG domain in phage GG gp35 proteins might be crucially important structural element in LTF. And GG domain in the biological entities – T4 phages, might be acquired from organisms through horizontal gene transfer.

4. Supplementary data

Supplementary data for this paper are available online.

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