*Short Communication*

# Identification of Protein Complexes Associated with the Usher Syndrome 2C and Epilepsy-Associated Protein VLGR1 Applying Affinity Proteomics

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## SUMMARY

Authors aimed to identify novel VLGR1-associated protein networks to shed light on its integration into signaling pathways and the cellular compartments in which VLGR1 functions using high-resolution affinity proteomics based on tandem affinity purifications (TAPs).

## KEYWORDS

Adhesion GPCR; ADGRV1; Usher syndrome; USH2C; Epilepsy; Tandem affinity purification

| GO Terms/cellular modules | n |
|---|---|
| Retina development & homeostasis | 21 |
| Visual perception | 10 |
| Photoreceptor cells | 20 |
| Cilium organization | 16 |
| NS development / neurogenesis | 221 |
| Synapse structure or activity | 22 |
| Post-synaptic density (PSD) | 10 |
| Cell cycle, Cell division | 203 |
| Focal adhesion (FA) | 96 |
| mRNA splicing, Spliceosome | 30 |

**Table 1: Cellular modules based on GO terms of VLGR1 TAP prays.** n, number of proteins.

## BODY

Adhesion G protein-coupled receptors (aGPCRs) exert a dual function in cell-cell/cell-matrix adhesion and cellular signaling. VLGR1 (very large G protein-coupled receptor-1), also known as ADGRV1, GPR98 and MASS, is by far the largest aGPCR. Although VLGR1 expression is almost ubiquitous, it is concentrated in the nervous system [1]. Mutations in the human VLGR1 gene cause the human Usher syndrome (USH), the most common form of hereditary deaf-blindness [2]. In the inner ear and the retina, the extremely long adhesive extracellular domains of VLGR1 form fibrous inter-membrane links between neighbouring stereocilia of hair cells and inner segment and the connecting cilium of photoreceptor cells, respectively [3, 4]. In both types of sensory cells, VLGR1 is part of a larger membrane adhesion complex and protein network interacting with other USH proteins. In addition, there is growing evidence that defects in VLGR1 are also associated with epilepsy [5, 6] and osteoporosis [7]. So far little is known about its dual function in membrane-membrane adhesion and signaling [8] and therefore, the underlying pathomechanisms of diseases related to VLGR1 still remained largely elusive.

In our present project, we aimed to identify novel VLGR1-associated protein networks to shed light on its integration into signaling pathways and the cellular compartments in which VLGR1 functions. For this, we applied high-resolution affinity proteomics based on tandem affinity purifications (TAPs). We systematically tagged domains of VLGR1 and full-length VLGR1a with the Strep II Flag (SF)-tag (Figure 1A) and expressed these baits in HEK293T cells for TAPs of protein complexes related to VLGR1 [9, 10]. Subsequently, we determined the composition of eluted protein complexes by mass spectrometry.

By this strategy, we identified almost 1200 putative interaction partners of VLGR1 (Figure 1B, C). We compiled the data sets of the different VLGR1 baits, which revealed specific overlaps. For further analysis, we categorized the proteins into three groups: biological function, cellular component and molecular function by Gene Ontology(GO) term analyses using Cytoscape ClueGO (Table 1).

To exclude putative false positive hits we consulted the Contaminant Repository for Affinity Purifications, also known as CRAPome database, which contains a collection of common contaminants in affinity proteomic MS data [11]. Altogether, our bioinformatics analyses of the VLGR1 interactome revealed a landscape of protein clusters, which defines different, but overlapping functional cell modules.

We gathered evidence for involvement of VLGR1 in alternative non-canonical G protein in dependent pathways. A comparison with TAP data sets of other aGPCRs which we gathered in parallel showed certain overlaps in putative interaction partners indicating common non-canonical pathways for aGPCRs.

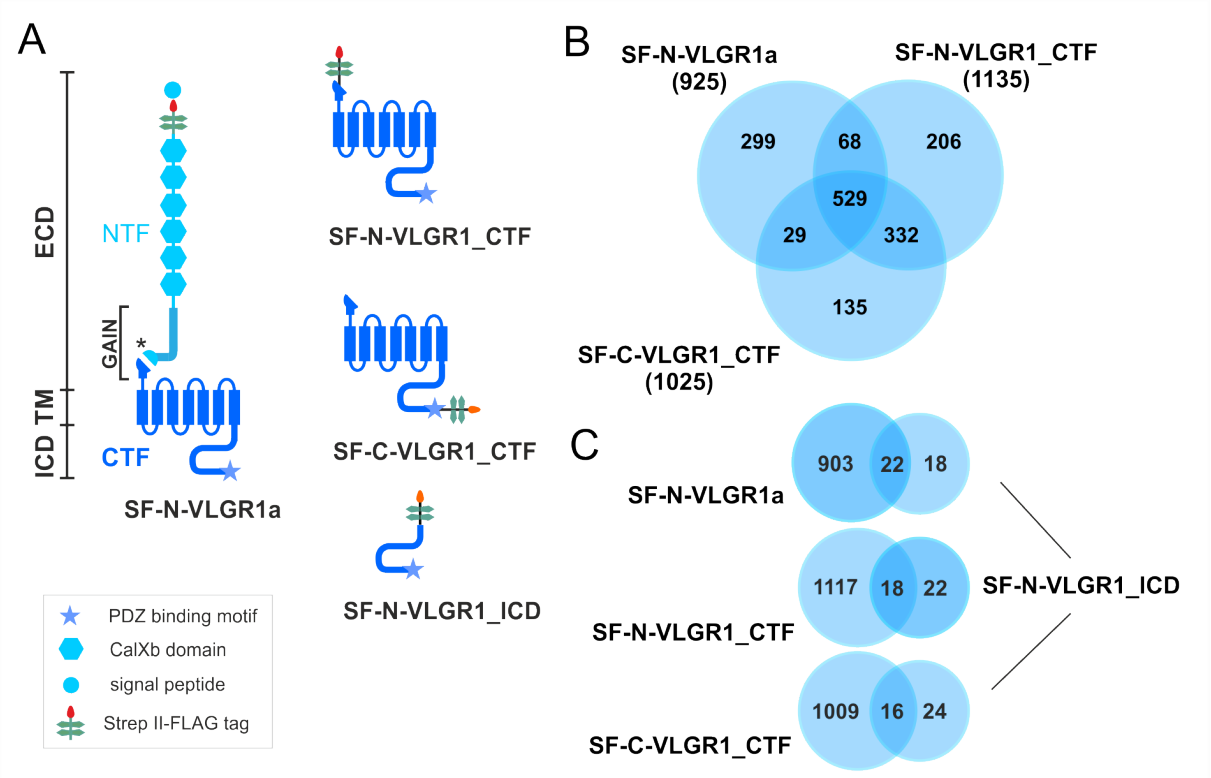


**Figure 1: VLGR1 interactome.** (A) SF-tagged baits used in TAPs. Overlap between identified putative complex partners (preys) of VLGR1 in TAPs for VLGR1a and VLGR1_CTF (B) and with VLGR1_ICD (C).

Our TAP strategy also affirmed previously identified interactions of VLGR1 with other USH proteins. Furthermore, we found a high degree of overlap between TAP data sets for VLGR1 and the USH protein CIB2 (USH1J) indicating a common interactome for both proteins. Interestingly, the VLGR1-CIB2 interactome includes proteins related to actin cytoskeleton remodeling and molecular transport, as well as all eight subunits of the TRiC/CCT chaperon in complex. The latter complex is essential for the modification of ciliary proteins [12].

Current work in the lab focuses on the validation of the molecular interaction indicated bythe TAP data by independent complementary protein-protein interaction assays. Furthermore we analyse functional relevances of the identified interactions using a repertoire of molecular and cell biology tools. All in all our affinity proteomics strategy provided novel insights in the function of VLGR1 and thereby underlying pathomechanisms of diseases related to VLGR1.

## ACKNOWLEDGEMENTS

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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