De novo variants in *SNAP25* cause an early-onset developmental and epileptic encephalopathy

Chiara Klöckner,[1] Heinrich Sticht, PhD,[2] Pia Zacher, MD,[3] Bernt Popp, MD,[1] Holly E. Babcock, MS, CGC,[4] Dewi P. Bakker, MD, PhD,[5] Katy Barwick, PhD,[6] Michaela V. Bonfert, MD,[7] Carsten G. Bönnemann, MD,[8] Eva H. Brilstra, MD, PhD,[9] Care4Rare Canada Consortium,[10] Wendy K. Chung, MD, PhD,[11] Angus J. Clarke, DM, FRCP,[12] Patrick Devine, MD, PhD,[13] Sandra Donkervoort, MS, CGC,[8] Jamie L. Fraser MD, PhD, FACMG,[14]Jennifer Friedman, MD,[15] Alyssa Gates, MS, CGC,[16] Emma Hobson, MD,[17] Gabriella Horvath, MD, PhD,[18] Jennifer Keller-Ramey, PhD, FACMG,[19] Boris Keren, MD, PhD,[20] Manju A. Kurian, PhD,[6] Virgina Lee, MD,[21] Kathleen A. Leppig, MD,[16] Johan Lundgren, MD,[22] Marie T. McDonald, MD,[23] Amy McTague, PhD,[6] Heather C. Mefford, MD, PhD,[24] Cyril Mignot, MD, PhD,[25] Mohamad A. Mikati, MD,[26] Caroline Nava, MD, PhD,[27] F. Lucy Raymond, MD, PhD,[28,29] Julian R. Sampson, DM,[12] Alba Sanchis-Juan, PhD,[28,30] Vandana Shashi, MD,[23] Joseph T.C. Shieh, MD, PhD,[31] Marwan Shinawi, MD,[32] Anne Slavotinek, MB, BS, PhD,[31] Tommy Stödberg, MD,[33] Nicholas Stong, PhD,[34] Jennifer A. Sullivan, MS,[23] Ashley C. Taylor, MHS, PA-C,[35] Tomi L. Toler, MS,[32] Marie-José van den Boogaard, MD, PhD,[9] Saskia N. van der Crabben, MD, PhD,[36] Koen L.I. van Gassen, PhD,[9] Richard H. van Jaarsveld, PhD,[9] Jessica Van Ziffle, PhD, FACMG,[13] Alexandrea F. Wadley, MD,[37] Matias Wagner, MD,[38] Kristen Wigby, MD,[39] Saskia B. Wortmann, MD, PhD,[40,41] Yuri A. Zarate, MD, MBA,[42] Rikke S. Møller, PhD,[43,44] Johannes R. Lemke, MD,[1] Konrad Platzer, MD[1]

1 Institute of Human Genetics, University of Leipzig Medical Center, Leipzig, Germany.
2 Institute of Biochemistry, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany.
3 The Saxon Epilepsy Center Kleinwachau, Radeberg, Germany.
4 Rare Disease Institute, Children's National Hospital, Washington, DC, USA.
5 Department of Child Neurology, Amsterdam University Medical Centers, Amsterdam, The Netherlands.
6 Institute of Child Health, University Collge London, 30 Guilford St, Holborn, London WC1N 1EH, UK.
7 Department of Pediatric Neurology and Developmental Medicine and LMU Center for Children with Medical Complexity, Dr. von Hauner Children's Hospital, LMU - University Hospital, Ludwig-Maximilians-Universität, Munich, Germany.
8 Neuromuscular and Neurogenetic Disorders of Childhood Section, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland, USA.
9 Department of Genetics, University Medical Center Utrecht, Utrecht, The Netherlands.
10 Children's Hospital of Eastern Ontario Research Institute, University of Ottawa, Ottawa, Ontario, Canada.
11 Departments of Pediatrics and Medicine, Columbia University Medical Center, New York, NY, USA.
12 Division of Cancer & Genetics, School of Medicine, Cardiff University, Wales, UK.
13 Department of Pathology, University of California San Francisco, 1600 Divisadero Street Room B-620, San Francisco, CA 94115, USA.
14 Rare Disease Institute, Division of Genetics and Metabolism, Children's National Hospital, Washington, DC, USA.
15 Departments of Neurosciences and Pediatrics, University of California San Diego and Division of Neurology, Rady Children's Hospital, San Diego, CA, USA. Rady Children's Institute for Genomic Medicine, San Diego, CA, USA.
16 Department of Genetic Services, Kaiser Permanente Washington, Seattle, WA, 98122, USA.
17 Yorkshire Clinical Genetics Service, Chapel Allerton Hospital, Leeds, UK.
18 Department of Pediatrics, Division of Biochemical Diseases, University of British Columbia, Vancouver, Canada.
19 GeneDx, 207 Perry Parkway, Gaithersburg, MD 20877, USA.
20 APHP, Département de Génétique, Groupe Hospitalier Pitié Salpêtrière, Paris, France.
21 Department of Neurology, University of California San Francisco, San Francisco, California.
22 Institute of Clinical Sciences, Skane University Hospital, Lund, Sweden.
23 Division of Medical Genetics, Department of Pediatrics, Duke University Medical Center, Durham, North Carolina 27710, USA.
24 Division of Genetic Medicine, Department of Pediatrics, University of Washington, Seattle, WA, USA.
25 Département de Génétique, Centre de Référence Déficiences Intellectuelles de Causes Rares, Groupe Hospitalier Pitié Salpêtrière et Hôpital Trousseau, APHP, Sorbonne Université, Paris, France.
26 Division of Pediatric Neurology, Department of Pediatrics, Duke University Medical Center, T0913 Children's Health Center, DUMC Box 3936, Durham, NC, 27710, USA.
27 Sorbonne University, Paris Brain Institute, Inserm U1127, CNRS UMR 7225, AP-HP, Pitié Salpêtrière Hospital, Department of Genetics, F-75013, Paris, France.
28 NIHR BioResource, Cambridge University Hospitals NHS Foundation Trust, Cambridge Biomedical Campus, Cambridge, UK.
29 Department of Medical Genetics, Cambridge Institute for Medical Research, University of Cambridge, UK.
30 Department of Haematology, University of Cambridge, NHS Blood and Transplant Centre, Cambridge, UK.
31 Division of Medical Genetics, University of California, San Francisco, San Francisco, California. Institute for Human Genetics, University of California, San Francisco, San Francisco, California.
32 Department of Pediatrics, Division of Genetics and Genomic Medicine, Washington University School of Medicine, Saint Louis, Missouri, USA.
33 Department of Women's and Children's Health Karolinska Institutet Stockholm Sweden.
34 Institute for Genomic Medicine, Columbia University, New York, New York, USA.
35 Section of Genetics, Department of Pediatrics, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA.
36 Department of Clinical Genetics, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands.
37 University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma.
38 Institute of Neurogenomics, Helmholtz Zentrum Munich, Neuherberg, Germany.

39 Department of Pediatrics, Division of Genetics, University of California, San Diego and Rady Children's Hospital-San Diego, San Diego, California, USA.

40 Amalia Children's Hospital , Radboud University Nijmegen, Nijmegen, The Netherlands.

41 University Childrens Hospital, Paracelsus Medical University, Salzburg, Austria.

42 Section of Genetics and Metabolism, Department of Pediatrics, University of Arkansas for Medical Sciences, Little Rock, Arkansas.

43 Institute for Regional Health Services, University of Southern Denmark, 5000 Odense C, Denmark.

44 Department of Epilepsy Genetics and Personalized Medicine, Danish Epilepsy Centre Filadelfia, 4293 Dianalund, Denmark.

Corresponding author: Konrad Platzer

Corresponding author's address: Philipp-Rosenthal-Straße 55

Institute of Human Genetics

University of Leipzig Medical Center

04103 Leipzig, Germany

Corresponding author's phone and fax: +493419723802

Corresponding author's e-mail address: konrad.platzer@medizin.uni-leipzig.de

Number of words in abstract (max. 200): 197

Number of words in main text (max. 4000): 3085

Number of Figures (max. 5): 2

Number of Tables: 1

CONFLICTS OF INTERESTS

JKR is an employee of GeneDx, Inc. NS is an employee and holds equity in Bristol Myers Squibb. The other authors declare no conflicts of interest.

ABSTRACT

Purpose

This study aims to provide the first comprehensive description of the phenotypic and genotypic spectrum of *SNAP25* developmental and epileptic encephalopathy (*SNAP25*-DEE) by reviewing newly identified and previously reported individuals.

Methods

Individuals harboring heterozygous missense or truncating variants in *SNAP25* were assembled through collaboration with international colleagues, matchmaking platforms and literature review. For each individual, detailed phenotyping, classification and structural modeling of the identified variant was performed.

Results

The cohort comprises 23 individuals with pathogenic or likely pathogenic de novo variants in *SNAP25*. Intellectual disability and early-onset epilepsy were identified as the core symptoms of *SNAP25*-DEE, with recurrent findings of movement disorders, cerebral visual impairment and brain atrophy. Structural modeling for all variants predicted possible functional defects concerning SNAP25 or impaired interaction with other components of the SNARE complex.

Conclusion

We provide a first comprehensive description of *SNAP25*-DEE with intellectual disability and early onset epilepsy mostly occurring before the age of two years. These core symptoms and additional recurrent phenotypes show an overlap to genes encoding other components or associated proteins of the SNARE complex such as *STX1B*, *STXBP1* or *VAMP2*. Thus, these findings advance the concept of a group of neurodevelopmental disorders that may be termed “SNAREopathies”.

Keywords: Neurodevelopmental disorder; epilepsy; seizures; movement disorder; SNARE

INTRODUCTION

The neuronal SNARE complex (soluble N-ethylmaleimide-sensitive-factor attachment receptor complex) plays a central role in the regulation of synaptic signaling by mediating membrane docking, priming and fusion of synaptic vesicles with presynaptic membranes. This ultimately leads to the release of neurotransmitters into the synaptic cleft.[1–3] During neuronal development, it promotes neurite outgrowth and the maturing process of synapses.[4]

The core SNARE complex comprises a four-helix bundle consisting of two SNAP25 helices (encoded by *SNAP25*), one syntaxin 1A helix (encoded by *STX1A*), and one synaptobrevin 2 helix (encoded by *VAMP2*). [1,5]

Pathogenic *de novo* variants disrupting SNARE proteins (*VAMP2*, MIM 618760) or SNARE complex associated proteins, such as *STXBP1* (MIM 612164), *STX1B* (MIM 616172) are a known cause for neurodevelopmental disorders consisting of an overlapping phenotype of developmental delay (DD), intellectual disability (ID) and epilepsy [6–10] that were recently grouped as “SNAREopathies”.[11]

SNAP25 showed a significant enrichment for *de novo* variants in a cohort study of individuals with neurodevelopmental disorders with epilepsy.[12] Six individuals with heterozygous variants in *SNAP25*, four of them of *de novo* origin, have been described in single case reports showing developmental delay, seizures and variable neurological symptoms.[13–17]

The aim of this study is to establish a first comprehensive description of the phenotypic spectrum of *SNAP25* developmental and epileptic encephalopathy (*SNAP25*-DEE). We report 19 individuals harboring *de novo* variants in *SNAP25* and review all four previously published individuals with *de novo* variants determined to be pathogenic or likely pathogenic. Through molecular modeling, we provide further insights into possible mechanisms through which the identified variants may disrupt SNAP25 and the SNARE complex.

MATERIALS AND METHODS

Ethics Statement

All examined individuals or their legal guardians provided informed written consent for testing and publication. In some cases, testing was done as part of routine clinical care and therefore institutional ethics approval was not required. If done in a research setting, testing was approved by the ethics committees of the respective centers.

Research Cohort and Identification of Variants

By using matchmaking platforms,[18] personal communication with colleagues and a literature review, 30 individuals harboring heterozygous missense or truncating variants in *SNAP25* were assessed. After thorough evaluation, we included 23 individuals harboring *de novo* variants determined to be pathogenic or likely pathogenic, including 19 previously unreported individuals. The remaining seven individuals harbored variants of unknown significance (VUS, Supplement Tables S2, S4.2 and S5.3) and were not included in the phenotypic description.

Phenotypic and genotypic information were obtained from the referring collaborators by using a standardized questionnaire to evaluate clinical, electroencephalography (EEG), and cranial magnetic resonance imaging (cMRI) findings as well as variant information. Variants were identified using trio exome sequencing (ES), trio genome sequencing (GS) or gene panel sequencing.

According to data from gnomAD, *SNAP25* (NM_130811.3) shows a reduced number of truncating and missense variants in controls: (1) a pLI score = 0.99 and a LOEUF = 0.23 for truncating variants with one nonsense variant deposited at amino acid residue 204, two codons before the canonical stop and (2) a z-score = 2.96 and LOEUF = 0.36 for missense variants. This indicates a selective constraint on these variant types in a control population not affected by early-onset NDD.[19] Therefore, causality of both truncating and missense

variants was assessed according to the guidelines of the American College of Medical Genetics (ACMG),[20] focusing on the following criteria: PS2 (*de novo* origin), PM2 (absent in population databases), PP3 (multiple lines of computational evidence support a deleterious effect on the gene/gene product). For *in silico* prediction of missense variants, the following tools were used: CADD, REVEL, MutationTaster, M-CAP, Polyphen-2, GERP++. [21–26]

Structural Modeling

The structural effects of SNAP25 variants were modeled with SwissModel (Version 4.1.0)[27] based on the experimental SNARE-αSNAP complex structures available (PDB codes: 3J96, 3J97, 3J98, 3J99, 6IP1, 6MDN).[28–30] RasMol (Version 2.7.5)[31] was used for structure analysis and visualization.

RESULTS

The key clinical findings in *SNAP25*-DEE comprise DD and/or ID, early-onset seizures and variable neurological symptoms such as muscular hypotonia, movement disorders (ataxia, dystonia or tremor), cerebral visual impairment (CVI) and brain volume loss. An overview of the main clinical symptoms is presented in Table 1 and Figure 1A.

Phenotypic spectrum

Global developmental delay / intellectual disability. All individuals presented with DD and a variable degree of ID, ranging between profound (4/20; 20%), severe (5/20; 25%), moderate (6/20; 30%), and mild (5/20; 25%).

Six individuals aged between 2 and 20 years remained non-verbal (6/17; 35%), with three being adolescent or adult. If language was acquired, individuals could either speak single words (2/17; 12%) or in sentences (9/17; 53%) with articulation noted to be poor or imprecise.

All individuals showed variable degrees of motor delay. Three individuals (3/15; 20%) aged older than three years were not able to walk, while four (4/15; 27%) needed assistance and eight individuals (8/15; 53%) were able to walk on their own.

Regression was reported in five individuals (5/17; 29%) with three of them showing signs of regression with the onset of seizures. This regression was primarily described as a loss of words previously learned.

Seizures. Seizures were reported in 17 individuals (17/23; 74%), while six individuals aged 2 months to 16 years had no history of seizures. The age of seizure onset ranged between the 7th day of life to 12 years, with a median age of 12 months. In all but three individuals, seizures started before age two years. A broad spectrum including epileptic spasms, generalized and focal seizures were reported with most individuals showing multiple seizure types over time. Primarily generalized or focal to bilateral tonic-clonic seizures were the most

common seizure type having occurred in seven individuals (7/17; 41%). Further frequently observed seizure types include absence-like seizures (6/17; 35%) and epileptic spasms (5/17; 29%). Myoclonic, tonic and atonic seizures were each reported for four individuals (4/17; 24%). Seizures reported in early childhood appeared to be more generalized in onset while older individuals aged 17 to 23 years predominantly exhibited (multi)focal epilepsies corresponding with respective EEG findings of multifocal epileptic discharges and generalized spike wave discharges. Seizure frequency ranged from numerous daily events (8/12; 67%) to isolated seizures (2/12; 11%). Response to antiepileptic drugs (AEDs) was inconsistent for individuals with recurrent seizures: seven of fourteen individuals (7/14; 50%), were treated with more than three AEDs and still had frequent seizures.

Brain MRI findings. cMRI was performed on 21 individuals and was unremarkable in 15 individuals (15/21; 71%). Focal or generalized brain volume loss was noted in four individuals (4/21; 19%) aged 7 months to 23 years and signs of a leukoencephalopathy were present in two individuals (2/21; 10%).

Neurological findings. The most common neurological finding was muscular hypotonia (12/19; 63%), with severe hypotonia leading to feeding difficulties being observed in four individuals (4/19; 21%). Spasticity was noted in four individuals (4/21; 19%). Further recurrent findings include movement disorders such as ataxia (7/21; 33%), dystonia (4/21; 19%) and tremor (2/21; 10%). CVI was described in six individuals (6/21; 29%).

Behavior. Most individuals were reported to have no behavioral issues. However, three individuals showed signs of an autistic spectrum disorder (3/18; 17%) and four individuals presented with repetitive mannerisms such as hand-flapping (4/18; 22%).

Additional findings*.* Musculoskeletal findings include bilateral clubfeet (5/21; 24%), joint hypermobility (4/21; 19%) and hip dysplasia (2/21; 10%). Most individuals had no or only minor dysmorphic features, with epicanthus being reported for three individuals (3/21; 14%). Further findings included a high-arched palate (4/21; 19%) with abnormal dentition or dental

crowding (3/21; 14%; Supplemental Table S1 contains details on all phenotypic findings and Supplemental Table S3.1 lists all observed phenotypes ranked by frequency in standardized terminology according to the Human Phenotype Ontology).

Variant analysis

Out of the 23 enrolled individuals with *de novo* variants, 15 different missense variants were identified, in addition to four truncating variants (two nonsense variant and two splice donor variant).

All 19 variants were absent from the gnomAD database (last accessed July 2020). All pathogenic or likely pathogenic missense variants affected highly conserved amino acid residues (mean GERP++: 5.9) of the t-SNARE coiled-coil homology domain 1 (amino acid position 14-81) and t-SNARE coiled-coil homology domain 2 (amino acid position 135-202; Figure 1B and C).[32] All *de novo* missense variants were predicted to be deleterious by multiple *in silico* prediction programs (mean CADD: 29.6; for a complete overview of *in silico* analysis see Supplemental Tables S5.1 and S5.2).

Recurrent variants comprise the missense variant p.(Gly43Arg) identified in three individuals, the missense variant p.(Met71Thr) identified in two individuals, and the nonsense variant p.(Gln174*) identified in two individuals. Different missense variants affecting the same amino acid residue were also observed including p.(Asp166Gly) and p.(Asp166Tyr) as well as p.(Ala199Gly) and p.(Ala199Val).

***In silico* structural modeling**

SNAP25 is part of the neuronal SNARE complex. The structure of this complex is shown in Fig. 2_AB indicating the positions of the variants detected in the present study. The sidechains of most residues affected are oriented towards the core of the SNARE complex, whereas few are oriented to the outside and interact with αSNAP, a protein that is involved

in both SNARE assembly and disassembly after the completion of synaptic vesicle exocytosis.[33]

In order to better understand the effects of the identified variants, molecular modeling was performed. This analysis indicated that all pathogenic or likely pathogenic variants were predicted to destabilize the structure of SNAP25 by causing steric clashes: p.(Gly43Arg), p.(Leu57Arg), p.(Gln66Pro), p.(Gln174Pro), by weakening intramolecular interactions: p.(Leu50Ser), p.(Lys40Glu), p.(Ile192Tyr), or by enhancing backbone flexibility: p.(Asp166Gly), p.(Ala199Gly), p.(Gln197*). These structural effects are listed in more detail in the Supplemental Table S3.1. Other variants were predicted to predominantly destabilize the interactions with other components of the SNARE complex, namely STX1A: p.(Ile67Asn), p.(Met71Thr) or VAMP2: p.(Ala199Val). A third group of variants were predicted to result in disturbed interactions with αSNAP by causing steric clashes: p.(Val48Phe), p.(Asp166Tyr). It is important to note that some variants may both disturb SNAP25 structure and interactions, e.g. p.(Gly43Arg) or p.(Met71Thr). The structural effects of the p.(Gly43Arg) and p.(Ala199Val) variants are shown in detail in Fig. 2_CDEF. Taken together, despite differences in the proposed effects, all variants are expected to destabilize the SNARE complex itself or to affect its interactions with αSNAP (Supplemental Table S3.1).

DISCUSSION

We present the first sizeable cohort of individuals with pathogenic or likely pathogenic *de novo* variants in *SNAP25* and provide a comprehensive evaluation of an early onset developmental and epileptic encephalopathy.

Moderate to profound DD and/or ID and early-onset seizures were identified as the core symptoms of *SNAP25*-DEE. In addition, ataxia, dystonia, CVI, brain volume loss, muscular hypotonia and spasticity were identified as recurrent clinical symptoms. Individuals with the most severe course of *SNAP25*-DEE exhibited profound DD, onset of seizures in the first two months of life, spasticity, CVI, and brain volume loss.

Seizure semiology with a differentiation of focal or generalized onset can guide treatment options in individuals with epilepsy. An in-depth age-specific evaluation of seizure semiology was not possible in this comparatively small cohort, yet the adult individuals 5 and 12 exhibited mainly (multi)focal epilepsies. Video EEG data of individual 5 aged 20 years documented a focal epilepsy with parasagittal epileptic discharges corresponding with focal motor seizures (for the full video EEG report see Supplemental Table 1). This could indicate a possible evolution of seizures towards focal to bilateral tonic-clonic seizures in older individuals, but this will require further analysis of age-specific data on seizure semiology as well as their treatment to further elucidate *SNAP25*-DEE.

Of special interest for future clinical predictions is comparing the clinical course of individuals with recurrent variants or variants affecting the same amino acid residue: (1) The *de novo* missense variant p.(Gly43Arg) was identified in three individuals. It is remarkable that all individuals showed a rather mild phenotype comprising mild to moderate ID, sporadic generalized seizures that did not require treatment (individual 4) or showed good response to AEDs (individual 2) as well as ataxia and tremor. These finding suggests that this specific disruption of amino acid residue 43 could present with a milder variant-specific clinical course within *SNAP25*-DEE, while other individuals with pathogenic or likely pathogenic

variants nearby in residues 40 and 48 presented with a more severe clinical course. (2) Two *de novo* variants affecting the amino acid residue 166, p.(Asp166Gly) and p.(Asp166Tyr) were identified in individual 12 and 13 aged 17 and 23 years, respectively. They presented with mild to moderate ID, focal and generalized seizures and did not show additional neurological symptoms. (3) A different picture is seen for the recurrent *de novo* variant p.(Met71Thr) that was identified in individual 10 and 11. Individual 10 was able to speak in sentences at the age of eight years and had no history of seizures while individual 11 was only able to speak single words at age seven years and had daily seizures since the age of two years and six months.

All pathogenic or likely pathogenic missense variants are located in the coiled-coil homology domains 1 and 2 of *SNAP25,* representing potential hotspots, also considering that little variation is observed in population databases in these domains (see Figure 1). The variants of four individuals aged two to 23 years with notable brain volume loss or signs of a leukoencephalopathy were located in the t-SNARE coiled-coil homology domain 2 (amino acid position 140-202), indicating a possible location-specific clinical observation (4/7; 57% of individuals with variants in this domain).

Future studies with additional individuals with *SNAP25*-DEE will shed more light onto the underlying clinical course. Recent modeling suggests an incidence of *de novo* variants in *SNAP25* in live births of approximately 1:100.000 for missense variants and 0.1:100.000 for nonsense variants.[34]

The underlying disease mechanisms for “SNAREopathies” have recently been summarized as very diverse, including many examples of haploinsufficiency due to loss-of-function (LoF) and missense variants, as well as instances of a dominant negative mechanism.[13] Data on functional analyses of variants in SNAP25 is scarce as of now and is only available for p.(Ile67Asn) .[14] Cotransfection of chromaffin cells with mutant cDNA or with wild-type plus mutant cDNA both suppressed vesicle fusion to the same extent, indicating a dominant

negative mechanism rather than haploinsufficiency for this variant. In the absence of comprehensive functional analyses, structural modeling of missense variants using published crystal structures can help in predicting the underlying mechanisms by which variants disrupt either SNAP25 or its interaction with other proteins of the SNARE complex. In some instances, individuals harboring variants for which similar structural changes were predicted showed a similar course of disease. (1) The variants identified in individuals 10, 11 (p.(Met71Thr)) and 15 (p.(Ile192Thr)) are both predicted to cause a reduced packing with each other. All three individuals presented with moderate to severe ID while motor development seemed to be only mildly affected with all of them being able to walk on their own. In addition, individuals 11 and 15 presented with a rather late onset of seizures at 2,5 years and 12 years respectively, while individual 10 did not have a history of seizures. (2) Structural modeling of the likely pathogenic variants p.(Gln174Pro) and p.(Arg198Pro) indicated a disruption of the helix of SNAP25 for both variants. Individuals 14 (p.(Gln174Pro)) and 16 (p.(Arg198Pro)) presented with a severe course of disease with a seizure onset at age 2 months, severe to profound DD, CVI and spasticity. (3) Of further interest are two variants affecting the amino acid residue 199, p.(Ala199Gly) (individual 17) and p.(Ala199Val) (individual 18) with regards to a possible interaction with the SNARE complex protein VAMP2. The variant p.(Ala199Val) is predicted to cause a steric clash with the amino acid residue 77 of VAMP2. In a recent study, three individuals with variants affecting the amino acid positions 75, 77 and 78 of VAMP2 were described.[7] All individuals showed overlapping clinical symptoms to the two individuals of the current *SNAP25* cohort comprising moderate to severe ID, onset of seizures in the first year of life, muscular hypotonia, ASD, stereotypic hand movements, CVI, absent speech and unremarkable brain imaging.[7] These findings indicate that the disruption of these structural domains in either SNAP25 or VAMP2 may cause a similar downstream functional effect and result in a similar clinical course. Putting these clinical observations in *SNAP25*-DEE in context to other known disease genes of the neuronal SNARE complex, it becomes clear that a shared clinical

course has been repeatedly described, suggesting a shared phenotypic spectrum of the neuronal “SNAREopathies” (see Supplemental Table S6 and Figure S1)[7,10,35]

The diverging clinical presentations of individuals with LoF variants, allow some hypotheses to be drawn concerning the underlying molecular disease mechanism. Individuals with nonsense variants located in the last and next to last exon of *SNAP25* show a more severe clinical course that contrast rather mildly affected individuals with splice variants located in the second and third exon. The nonsense variant p.(Gln174*) is located 32 base pairs from the end of exon seven of eight, so nonsense mediated mRNA decay (NMD) cannot be readily assumed, and the variant more likely leads to the translation of a truncated protein.[36] The same can be assumed for p.(Gln197*). Supporting causality for both nonsense variants is the fact that they are predicted to truncate SNAP25 by 33 or ten highly conserved amino acids, respectively. In addition, multiple pathogenic or likely pathogenic missense occur downstream of both positions. By which mechanism other than haploinsufficiency these variants could infer an altered protein function remains to be investigated, but a dominant negative mechanism is possible. The two essential splice site variants c.72+1G>A and c.114+2T>G, p.(?) are located at donor site of in-frame exons two and three. Both variants could lead to an in-frame exon skipping possibly resulting in a non-functional gene product on RNA or protein level that is quickly degraded resulting in haploinsufficiency (for a report on *in silico* splice prediction see Supplemental Table S5.2). Another mildly affected individual with mild ID and no seizures inherited the frameshift variant c.464delG p.(Gly155Alafs*84) from his unaffected mother was classified as a VUS (see individual V6, Supplemental Table 2). Although this variant also likely escapes NMD, the substantial change in the amino acid sequence more likely results in a non-functional gene product that is quickly degraded and thereby acting via haploinsufficiency. Mouse models support the potential role of haploinsufficiency in the origin of *SNAP25*-DEE as SNAP25(+/-) mice display a susceptibility to induced seizures, EEG abnormalities and cognitive deficits.[37] Take together, haploinsufficiency of *SNAP25* may therefore lead to a rather mild phenotype but the

preliminary findings reported in this cohort must be complemented by future analyses. Similar observations have been made concerning *STX1B*, where LoF variants leading to haploinsufficiency cause mild ID with epilepsy whereas missense variants causing altered protein function cause a more severe form of DEE.[35] This phenomenon of LoF variants resulting in a rather mild course of disease is also known for multiple other NDD genes encoding ion channels, e.g. *GRIN2A* or *KCNQ2*.[38,39]

In summary, *de novo* variants in *SNAP25* cause an early onset developmental and epileptic encephalopathy mainly characterized by ID and epilepsy. *SNAP25*-DEE demonstrates a strong phenotypic overlap with disorders caused by the disruption of other components or associated proteins of the SNARE complex, including movement disorders, cerebral visual impairment and brain atrophy. These findings add to the delineation of a group of disorders that may be called “SNAREopathies”.

.**Acknowledgements**

We thank the patients and their families for their participation and support of this study. We especially thank Elizabeth Dellureficio for her continuous efforts to bring affected families together.

Work on individual 5 was supported in part by grants from SFARI and the JPB Foundation.

We thank the clinicians involved with the care of these patients including Dr Stephen Nirmal, Dr Alasdair Parker and Dr Manali Chitre, UK. AM, KB and MAK are funded by the NIHR GOSH BRC. The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health.

Individual 7 was enrolled in the NIHR BioResource research study, supported by the Cambridge Biomedical Research Centre and the National Institute for Health Research (NIHR) for the NIHR BioResource (grant number RG65966).

Individual 17 was ascertained in the Duke Genome Sequencing Clinic. Funding for the Duke Genome Sequencing Clinic which is supported by the Duke University Health System.

Individual V6 was enrolled in Care4Rare Canada Consortium, funded by Genome Canada, Ontario Genomics Institute (OGI-147), Canadian Institutes of Health Research, Ontario Research Fund, Genome Alberta, Genome British Columbia, BC Children's Hospital Foundation, BC Children's Hospital Research Institute, BC Provincial Health Services Authority, Genome Quebec, and Children's Hospital of Eastern Ontario Foundation.

This study makes use of data generated by the DECIPHER Consortium. A full list of centres who contributed to the generation of the data is available from https://decipher.sanger.ac.uk/ and via email from decipher@sanger.ac.uk. Funding for the project was provided by the Wellcome Trust.

R.S.M. was supported by a grant from the Lundbeck Foundation (R277-2018-802).

Research reported in this publication was supported by the National Human Genome Research Institute of the National Institutes of Health under Award Number U01HG009599.

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. Amendola et. al. American Journal of Human Genetics. 2018 Sep 6;103(3):319-327. doi: 10.1016/j.ajhg.2018.08.007

We thank Katherine R. Chao for her help with the exome data analysis. The work in C.G. Bönnemann's laboratory is supported by intramural funds from the NIH National Institute of Neurological Disorders and Stroke. Sequencing and analysis were provided by the Broad Institute of MIT and Harvard Center for Mendelian Genomics (Broad CMG) and was funded by the National Human Genome Research Institute, the National Eye Institute, and the National Heart, Lung and Blood Institute grant UM1 HG008900 to Daniel MacArthur and Heidi Rehm.

References

1. Rizo J, Xu J. The Synaptic Vesicle Release Machinery. *Annu Rev Biophys*. 2015;44:339-367. doi:10.1146/annurev-biophys-060414-034057

2. Jahn R, Fasshauer D. Molecular machines governing exocytosis of synaptic vesicles. *Nature*. 2012;490(7419):201-207. doi:10.1038/nature11320

3. Ramakrishnan NA, Drescher MJ, Drescher DG. The SNARE complex in neuronal and sensory cells. *Mol Cell Neurosci*. 2012;50(1):58-69. doi:10.1016/j.mcn.2012.03.009

4. Hepp R, Langley K. SNAREs during development. *Cell Tissue Res*. 2001;305(2):247-253. doi:10.1007/s004410100359

5. Han J, Pluhackova K, Böckmann RA. The Multifaceted Role of SNARE Proteins in Membrane Fusion. *Front Physiol*. 2017;8:5. doi:10.3389/fphys.2017.00005

6. Saitsu H, Kato M, Mizuguchi T, et al. De novo mutations in the gene encoding STXBP1 (MUNC18-1) cause early infantile epileptic encephalopathy. *Nat Genet*. 2008;40(6):782-788. doi:10.1038/ng.150

7. Salpietro V, Malintan NT, Llano-Rivas I, et al. Mutations in the Neuronal Vesicular SNARE VAMP2 Affect Synaptic Membrane Fusion and Impair Human Neurodevelopment. *Am J Hum Genet*. 2019;104(4):721-730. doi:10.1016/j.ajhg.2019.02.016

8. Schubert J, Siekierska A, Langlois M, et al. Mutations in STX1B, encoding a presynaptic protein, cause fever-associated epilepsy syndromes. *Nat Genet*. 2014;46(12):1327-1332. doi:10.1038/ng.3130

9. Redler S, Strom TM, Wieland T, et al. Variants in CPLX1 in two families with autosomal-recessive severe infantile myoclonic epilepsy and ID. *Eur J Hum Genet EJHG*. 2017;25(7):889-893. doi:10.1038/ejhg.2017.52

10. Baker K, Gordon SL, Melland H, et al. SYT1-associated neurodevelopmental disorder: a case series. *Brain J Neurol*. 2018;141(9):2576-2591. doi:10.1093/brain/awy209

11. Verhage M, Sørensen JB. SNAREopathies: Diversity in Mechanisms and Symptoms. *Neuron*. 2020;107(1):22-37. doi:10.1016/j.neuron.2020.05.036

12. Heyne HO, Singh T, Stamberger H, et al. De novo variants in neurodevelopmental disorders with epilepsy. *Nat Genet*. 2018;50(7):1048-1053. doi:10.1038/s41588-018-0143-7

13. Rohena L, Neidich J, Truitt Cho M, et al. Mutation in SNAP25 as a novel genetic cause of epilepsy and intellectual disability. *Rare Dis Austin Tex*. 2013;1:e26314. doi:10.4161/rdis.26314

14. Shen X-M, Selcen D, Brengman J, Engel AG. Mutant SNAP25B causes myasthenia, cortical hyperexcitability, ataxia, and intellectual disability. *Neurology*. 2014;83(24):2247-2255. doi:10.1212/WNL.0000000000001079

15. Liang J-S, Wang J-S, Lin L-J, Yang M-T, Hung K-L, Lu J-F. Genetic Diagnosis in Children with Epilepsy and Developmental Delay/Mental Retardation Using Targeted

Gene Panel Analysis. *Neuropsychiatry*. 2018;08(05). doi:10.4172/Neuropsychiatry.1000494

16. Hamdan FF, Myers CT, Cossette P, et al. High Rate of Recurrent De Novo Mutations in Developmental and Epileptic Encephalopathies. *Am J Hum Genet*. 2017;101(5):664-685. doi:10.1016/j.ajhg.2017.09.008

17. Fukuda H, Imagawa E, Hamanaka K, et al. A novel missense SNAP25b mutation in two affected siblings from an Israeli family showing seizures and cerebellar ataxia. *J Hum Genet*. 2018;63(5):673-676. doi:10.1038/s10038-018-0421-3

18. Sobreira N, Schiettecatte F, Valle D, Hamosh A. GeneMatcher: a matching tool for connecting investigators with an interest in the same gene. *Hum Mutat*. 2015;36(10):928-930. doi:10.1002/humu.22844

19. Lek M, Karczewski KJ, Minikel EV, et al. Analysis of protein-coding genetic variation in 60,706 humans. *Nature*. 2016;536(7616):285-291. doi:10.1038/nature19057

20. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med Off J Am Coll Med Genet*. 2015;17(5):405-424. doi:10.1038/gim.2015.30

21. Rentzsch P, Witten D, Cooper GM, Shendure J, Kircher M. CADD: predicting the deleteriousness of variants throughout the human genome. *Nucleic Acids Res*. 2019;47(D1):D886-D894. doi:10.1093/nar/gky1016

22. Ioannidis NM, Rothstein JH, Pejaver V, et al. REVEL: An Ensemble Method for Predicting the Pathogenicity of Rare Missense Variants. *Am J Hum Genet*. 2016;99(4):877-885. doi:10.1016/j.ajhg.2016.08.016

23. Schwarz JM, Rödelsperger C, Schuelke M, Seelow D. MutationTaster evaluates disease-causing potential of sequence alterations. *Nat Methods*. 2010;7(8):575-576. doi:10.1038/nmeth0810-575

24. Jagadeesh KA, Wenger AM, Berger MJ, et al. M-CAP eliminates a majority of variants of uncertain significance in clinical exomes at high sensitivity. *Nat Genet*. 2016;48(12):1581-1586. doi:10.1038/ng.3703

25. Adzhubei I, Jordan DM, Sunyaev SR. Predicting functional effect of human missense mutations using PolyPhen-2. *Curr Protoc Hum Genet*. 2013;Chapter 7:Unit7.20. doi:10.1002/0471142905.hg0720s76

26. Cooper GM, Stone EA, Asimenos G, et al. Distribution and intensity of constraint in mammalian genomic sequence. *Genome Res*. 2005;15(7):901-913. doi:10.1101/gr.3577405

27. Guex N, Peitsch MC. SWISS-MODEL and the Swiss-PdbViewer: an environment for comparative protein modeling. *Electrophoresis*. 1997;18(15):2714-2723. doi:10.1002/elps.1150181505

28. Zhao M, Wu S, Zhou Q, et al. Mechanistic insights into the recycling machine of the SNARE complex. *Nature*. 2015;518(7537):61-67. doi:10.1038/nature14148

29. Huang X, Sun S, Wang X, et al. Mechanistic insights into the SNARE complex disassembly. *Sci Adv*. 2019;5(4):eaau8164. doi:10.1126/sciadv.aau8164

30. White KI, Zhao M, Choi UB, Pfuetzner RA, Brunger AT. Structural principles of SNARE complex recognition by the AAA+ protein NSF. *eLife*. 2018;7. doi:10.7554/eLife.38888

31. Sayle RA, Milner-White EJ. RASMOL: biomolecular graphics for all. *Trends Biochem Sci*. 1995;20(9):374. doi:10.1016/s0968-0004(00)89080-5

32. The UniProt Consortium. UniProt: a worldwide hub of protein knowledge. *Nucleic Acids Res*. 2019;47(D1):D506-D515. doi:10.1093/nar/gky1049

33. Ma L, Kang Y, Jiao J, et al. α-SNAP Enhances SNARE Zippering by Stabilizing the SNARE Four-Helix Bundle. *Cell Rep*. 2016;15(3):531-539. doi:10.1016/j.celrep.2016.03.050

34. López-Rivera JA, Pérez-Palma E, Symonds J, et al. A catalogue of new incidence estimates of monogenic neurodevelopmental disorders caused by de novo variants. *Brain J Neurol*. 2020;143(4):1099-1105. doi:10.1093/brain/awaa051

35. Wolking S, May P, Mei D, et al. Clinical spectrum of STX1B-related epileptic disorders. *Neurology*. 2019;92(11):e1238-e1249. doi:10.1212/WNL.0000000000007089

36. Popp MW-L, Maquat LE. Organizing principles of mammalian nonsense-mediated mRNA decay. *Annu Rev Genet*. 2013;47:139-165. doi:10.1146/annurev-genet-111212-133424

37. Corradini I, Donzelli A, Antonucci F, et al. Epileptiform activity and cognitive deficits in SNAP-25(+/-) mice are normalized by antiepileptic drugs. *Cereb Cortex N Y N 1991*. 2014;24(2):364-376. doi:10.1093/cercor/bhs316

38. Strehlow V, Heyne HO, Vlaskamp DRM, et al. GRIN2A-related disorders: genotype and functional consequence predict phenotype. *Brain J Neurol*. 2019;142(1):80-92. doi:10.1093/brain/awy304

39. Weckhuysen S, Mandelstam S, Suls A, et al. KCNQ2 encephalopathy: emerging phenotype of a neonatal epileptic encephalopathy. *Ann Neurol*. 2012;71(1):15-25. doi:10.1002/ana.22644

40. Lex A, Gehlenborg N, Strobelt H, Vuillemot R, Pfister H. UpSet: Visualization of Intersecting Sets. *IEEE Trans Vis Comput Graph*. 2014;20(12):1983-1992. doi:10.1109/TVCG.2014.2346248

Web Resources

DECIPHER, https://decipher.sanger.ac.uk/

gnomAD, http://gnomad.broadinstitute.org/

GeneMatcher, https://genematcher.org/

GenBank, https://www.ncbi.nlm.nih.gov/genbank/

OMIM, http://www.omim.org/

R, http://www.r-project.org/

UniProt database, https://www.uniprot.org/

Figures, Titles and Legends

Fig. 1: Overview of *SNAP25* variants and phenotypes (A) Upset-Plot[40] of recurrent phenotypic combinations. To assess the broader phenotypic spectrum of *SNAP25*-DEE, symptoms of all included individuals were grouped under certain phenotypic aspects corresponding with the Human Phenotype Ontology (HPO) terms HP:0100704 Cerebral visual impairment, HP:0100022 Abnormality of Movement, HP:0001250 Seizures and HP: 0001249/HP:0001263 Intellectual Disability/Global Developmental Delay. The colored bars show the absolute number of observations of a certain phenotype in this cohort. The black bars indicate how many individuals presented with a certain phenotypic combination.

(B) Location of missense and truncating variants in *SNAP25* with respect to domain structure (GenBank: NM_130811.3). Variants above protein scheme are *de novo* variants reported in this cohort with red circles indicating missense variants and orange squares indicating truncating variants. Below the protein scheme are missense variants in gnomAD with allele count and the degree of coloring being proportionate to the allele count with the lightest grey indicating singletons. Abbreviations are as follows: t-SNARE c-c h 1= t-SNARE coiled-coil homology domain 1; t-SNARE c-c h 2 = t-SNARE coiled-coil homology domain 2.

(C) Density plot of all missense variants (*de novo* pathogenic or likely pathogenic variants in red and variants present in gnomAD in blue).

Fig. 2: Location and structural effects of the SNAP25 variants detected in this study.

(A) Top view of the SNARE-αSNAP complex. The individual proteins are shown in ribbon presentation and in different colors: SNAP25 (blue), Syntaxin (green), VAMP2 (cyan), αSNAP (yellow, orange, red, purple). Residues, for which variants were detected, are shown in space-filled presentation and colored according to their atom type (cpk coloring). (B) Side view of the complex shown in (A).

(C) Interactions of Gly43 in the wildtype. Gly43 (gray) is located at a sterically demanding position of the four-helix bundle close to Leu160 of SNAP25 and Phe216 of syntaxin. (D)

The longer sidechain of the p.(Gly43Arg) variant forms steric clashes (red dotted circles) with the Leu160 and Phe216 sidechain thereby destabilizing the helix bundle.

(E) Interactions of Ala199 in the wildtype. Ala199 (gray) is located in spatial proximity to Phe77 of VAMP2. (F) The longer sidechain of the p.(Ala199Val) variant forms steric clashes (red dotted circle) with the Phe77 sidechain thereby destabilizing the SNAP25-VAMP2 interaction.

Tables

Table 1. Summary of clinical findings in individuals with pathogenic or likely pathogenic *de novo* variants in *SNAP25.* Variant nomenclature corresponds to GenBank: NM_130811.3. Abbreviations: +, phenotype present (not specified); -, phenotype not present; ASD, autism spectrum disorder; AT, ataxia; CVI, cerebral visual impairment; DD, developmental delay; ES , epileptic spasms; F, female; FS , focal seizures; GS , generalized seizures; ID, intellectual disability; M, male; m, months; MH, muscular hypotonia; n/a, not available; w, weeks; y, years.

Supplemental Data

Supplemental Data includes nine Supplemental Tables and one Supplemental Figure.