Clinical and Genetic Features in Patients With Reflex Bathing Epilepsy

Running head: Bathing epilepsy and SYN1 variants

Andrea Accogli1,2*, Gert Wiegand3,4*, Marcello Scala1,2, Caterina Cerminara5, Michele Iacomino1, Antonella Riva1, Barbara Carlini1, Letizia Camerota6, Vincenzo Belcastro7, Paolo Pronera8, Alberto Fernández-Jaén9, Nerses Bebek10, Paolo Scudieri1,2, Simona Baldassari1, Vincenzo Damiano Salpietro1,2, Giuseppe Novelli11,12,13, Chiara De Luca5, Celina von Stülpnagel14,15, Felicitas Kluger16, Gerhard Josef Kluger16, Gabriele Christine Wohlrab17, Georgia Ramantani17, David Lewis-Smith18,19, Rhys H. Thomas18,19, Ming Lai18,19, Alberto Verrotti20, Salvatore Striano21, Christel Depienne22,23, Carlo Minetti1,2, Fabio Benfenati24,25, Francesco Brancati6,26, Federico Zara1,2**, Pasquale Striano1,2**

This is an open access article distributed under the terms of the Creative Commons Attribution License 4.0 (CC BY), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Neurology® Published Ahead of Print articles have been peer reviewed and accepted for publication. This manuscript will be published in its final form after copyediting, page composition, and review of proofs. Errors that could affect the content may be corrected during these processes.
*These authors contributed equally to this work

**Corresponding authors:**

Pasquale Striano, MD, PhD

email: pasqualestriano@gaslini.org

and

Federico Zara, PhD

email: federicozara@gaslini.org

Figures: 2
Tables: 1

Supplemental data: https://doi.org/10.5061/dryad.w0vt4b8qr

Videos

Study funding

This work was supported by the Wellcome Trust [203914/Z/16/Z] to D.L.S. This work has been supported by the Italian Ministry of Health (grant n. RF-2016-02361949 to FZ).

Disclosure

P.S. has received speaker fees and participated at advisory boards for Biomarin, Zogenyx, GW Pharmaceuticals, and has received research funding by ENECTA BV, GW Pharmaceuticals, Kolfarma Srl., Eisai. D.L.-S. has no conflicts of interest to disclose. RHT has received honoraria from Arvelle, Eisai, GW Pharma, Sanofi, UCB Pharma, and Zogenix and meeting support from Bial, LivaNova, and Novartis. GW obtained honoraria for speaking engagements from Desitin (Hamburg, Germany) and Novartis (Nürnberg, Germany). He gave scientific advice for PTC Therapeutics (Frankfurt, Germany). The other authors do not report any conflict of interest.
Abstract

Objective: To describe the clinical and genetic findings in a cohort of subjects with bathing epilepsy, a rare form of reflex epilepsy.

Methods: We investigated by Sanger and targeted re-sequencing the SYN1 gene in 12 individuals from 10 different families presenting with seizures primarily triggered by bathing or showering. Additional twelve subjects with hot-water epilepsy were also screened.

Results: In all families with bathing epilepsy we identified 8 distinct pathogenic or likely pathogenic variants and 2 variants of unknown significance in SYN1, nine of which are novel. Conversely, none of the subjects with hot-water epilepsy displayed SYN1 variants. In mutated subjects, seizures were typically triggered by showering or bathing regardless of the water temperature. Additional triggers included fingernail-clipping, hair-cutting, or watching someone take a shower. Non-provoked seizures and a variable degree of developmental delay were also common.

Conclusion: bathing epilepsy is genetically distinct reflex epilepsy mainly caused by SYN1 mutations.

Keywords: SYN1, bathing epilepsy, showering, hot-water epilepsy, reflex seizures.
Introduction

Reflex epilepsies (REs) refer to conditions characterized by recurrent seizures primarily triggered by specific motor, sensory or cognitive stimulation\(^1\text{--}^2\). Acquired or genetic etiological factors are believed to underlie complex, and yet largely unknown, pathophysiological mechanisms which ultimately lead to hyperexcitability of cortical or subcortical neuronal areas in response to physiological stimuli\(^3\). The genetic background of REs is highly heterogeneous and only a few causative genes have been identified in humans\(^4\text{--}^7\).

Hot-water (HWE) and bathing (BE) epilepsies are among the most common REs in the pediatric population\(^8\text{,}^9\) and are considered as part of the same spectrum\(^10\). However, recent evidence suggests they are different entities with distinct genetics, triggers (hot vs. pouring water), clinical presentation, and comorbidities. Indeed, autosomal dominant pedigree with reduced penetrance allowed mapping two loci for HWE at chromosomes 10q21.3-q22.3\(^{11}\) and 4q24-q28\(^{12}\), and affected children have otherwise normal development. Conversely, BE has been associated with mutations in the X-linked gene *SYN1* that encodes one of the three Synapsins (SYN1-3), a family of phosphoproteins involved in synaptic development, function, and plasticity\(^{13\text{,}14}\). In addition to seizures triggered by water, *SYN1* mutations are responsible for a wide range of neurodevelopmental disorders, including cognitive impairment, autism spectrum disorders (ASD), and unprovoked seizures\(^{15\text{,}16}\). To date, *SYN1* mutations have been described in 9 patients with BE\(^{17\text{,}18\text{,}19}\) and one with HWE\(^{20}\).

We report the clinical and genetic findings of 12 subjects, from 10 unrelated families, affected by BE, all bearing variants in *SYN1*. The comprehensive analysis of our large cohort and additional cases reported in the literature indicate that bathing epilepsy is a genetically homogeneous distinct reflex epilepsy having SYN1 as its major causative gene.

Material and methods

Study design and participant recruitment
We enrolled 12 previously unreported probands from 10 unrelated families (Figure 1A) with reflex epilepsy induced by showering or bathing through the network therapy of rare epilepsies (NETRE). Inclusion criteria were patients with bathing/showering induced seizures documented either via video-EEG or home-video recordings by the parents. Clinical data including genetic findings, neurodevelopmental performance, epilepsy phenotype, and treatment response were collected using an anonymized, electronic questionnaire. Interictal/ictal (video)-EEG recordings, brain MRI, and neuropsychological tests were centrally reviewed. The neuropsychological and behavioral evaluation was assessed by the Wechsler Intelligence Scale for Children-IV (WISC-IV), Wechsler Preschool and Primary Scale of Intelligence-III (WPPSI-III), Autism Diagnostic Observation Schedule (ADOS), and Griffiths Mental Development Scale- Extended Revised (GMDS-ER).

**Standard Protocol Approvals, Registrations, and Patient Consents**

Ethical approval from the IRCCS ‘G. Gaslini’ Institute (Genova, Italy) was obtained for this study. We received written informed consent from all patients (or guardians of patients) participating in this study and authorization for disclosure (consent-to-disclose) of any recognizable persons in photographs and videos.

**Genetic investigations**

Genomic DNA was isolated from leukocytes of peripheral blood by using standard protocols. Target genetic analysis of SYN1 was performed by Sanger sequencing in subjects of families 1-4, 7, 8. Other subjects were investigated either by epilepsy gene panels (family 6, 10) or whole-exome sequencing (WES) (families 5, 9) and identified variants were confirmed by Sanger sequencing (additional details about sequencing process and data analysis are available in Supplemental Data). Variants were classified according to the ACMG guidelines\(^1\). In parallel, we screened for SYN1 mutations in a previously reported\(^1\) cohort of 21 HWE patients to gain further insights into genotype-phenotype correlations of water-related reflex epilepsies.
Data Availability

Supplemental data including clinical descriptions, methods of genetic testing, EEGs, tables of previously reported cases with BE and HBE are available on Dryad (https://doi.org/10.5061/dryad.w0vt4b8qr). Videos are available on the Neurology website. Additional anonymized data that support the findings of this study are available from the corresponding author (P.S.) on reasonable request. Not all of the data are publicly available because they contain information that could compromise children’s privacy and family consent.

Results

Clinical findings

The demographic and clinical features of our patients are summarized in Table 1. Extensive clinical details are available in Supplemental data. All but two subjects (II:3 from family 5, II:1 from family 9) were males. All affected individuals suffered from focal epilepsy with impaired awareness triggered by the experience of bathing or showering, regardless of water temperature. Seizures were typically triggered by pouring water over the head and consisted of behavioral arrest associated with pallor, cyanotic lips, buccal automatisms, and transient hypotonia (Supplemental data and videos). Evolution to bilateral tonic-clonic seizures was clearly described in four and loss of consciousness was reported in one. In two subjects, seizures were also triggered by rubbing with a towel after showering (Supplemental data, video 1). Seizures also occurred in one subject while washing hands and during the immersion of feet in the water, including seawater (II:1, family 1). Moreover, two patients also experienced a bilateral tonic-clonic seizure while watching their relatives take a shower or by the thought of bathing/showering (II:1, family 1; II:1, family 6). One adult (IV:2 of family 10) had an improvement in seizure control after predominantly showering rather than bathing in warm water. Additional triggers were fingernail clipping in two subjects (II:1, family 6; II:1, family 8), one of whom also experienced seizures provoked by hair cutting (II:1, family 6). The age at onset of provoked seizure ranged from 8 months to 15 years, with
weekly to monthly frequency.

Nine subjects also developed non-provoked seizures, including focal to bilateral tonic-clonic nocturnal seizures with autonomic features in five individuals. Three individuals reported febrile seizures, occurring before the onset of provoked seizures in two of them. One additional subject (II:3 of family 5) had no provoked focal seizures before the onset of BE.

All individuals received antiseizure medications. Half of them had a satisfactory response mainly to clobazam or valproic acid and three achieved complete control of seizures.

Ictal EEG recording showed high voltage polymorphic theta activity over the frontal-temporal areas in two subjects (Figure 2). Interictal findings in other subjects are available in the Supplemental data (Figure e-1, e-2). Brain MRI was performed in ten of the thirteen subjects with unremarkable findings.

All but two subjects (II:1 family 4; II:1 family 7) had a variable degree of developmental delay and cognitive impairment. Specifically, eight individuals had a global developmental delay (GDD) and five of them were found to have intellectual disability (ID) that ranged from mild to severe. Of note, six subjects were diagnosed with attention-deficit/hyperactivity disorder (ADHD) and one had hyperactivity. Three subjects had autism spectrum disorder (ASD). Behavioral issues such as aggressive behavior were noticed in two subjects. One adult (IV:2 of family 10) now lives and works with minimal support. The clinical features of 21 patients with hot-water-induced epilepsy are summarized in table e-1.

Twenty subjects had focal seizures, eight of whom also developed focal to bilateral seizures. Non-provoked seizures occurred in 62% of cases. Seizure activity was mainly recorded over unilateral temporal regions. Seizure control was achieved by reducing the temperature and duration of the bath or shower and by antiseizure medications such as carbamazepine\textsuperscript{10}.

**Genetic findings**

We identified 8 distinct pathogenic variants in \textit{SYN1} (NM_006950.3)c.1264 C>T p.(Arg422*) in case II:1 of family 1; c.1439dupC p.(Leu481fs202*) in case III:1 of family 2; c.774 +2T>C in case II:1 of family 3; c.436 -1G>C in case II:1 of family 4 and c.1406dupA
p.(Pro470Alafs*214) in cases II:1,II:2,II:3 of family 5; c.1472_1473insT p.(Gln491Hisfs*193) in case II:IV of family 6; c.1647_1650dupCGCC p.(Ser551Argfs*134) in case II:1 of family 8; c.1266delA p.(Gln423Serfs*244) in case II:2 of family 10 and two VUS (c.929C>A p.(Ala310Asp) in case II:3 of family 7; c.1760_1771dup p.(Arg587_Pro590dup) in case II:2 of family 9)(Table 1, Figure 1B). All variants were absent in the gnomAD database. The c.1264 C>T p.(Arg422*) variant, previously reported, occurred de novo, while all other variants were novel and maternally inherited, or presumed to be maternally inherited in family 10. The c.436 -1G>C and c.774 +2T>C variants are predicted to severely affect the protein structure through aberrant mRNA splicing. The c.1264 C>T p.(Arg422*) is predicted to undergo nonsense-mediated mRNA decay or result in a truncated protein. All the frameshift variants (c.1439dupC p.(Leu481fs202*), c.1406dupA p.(Pro470Alafs*214), c.1472_1473insT p.(Gln491Hisfs*193), c.1647_1650dupCGCC p.(Ser551Argfs*134) and c.1266delA p.(Gln423Serfs*244)) lead to new reading frames predicted to give rise to a truncated or degraded protein. Although the missense c.929C>A p.(Ala310Asp) and the in-frame insertion c.1760_1771dup p.(Arg587_Pro590dup) variants are classified as VUS, they are predicted to have a deleterious effect by multiple in-silico analysis and evolution conservation tools. The clinical association with BE further supports their likely pathogenic role. WES failed to identify additional pathogenic or likely pathogenic variants in any other OMIM genes in all tested subjects. No pathogenic or likely pathogenic variants in SYN1 were identified in the HWE cohort.

Discussion

Genetic spectrum of SYN1-related reflex epilepsy

In the first original description of SYN1 family, Garcia et al. did already mention the possible occurrence of water-induced seizures in a patient carrying the p.(W356*) SYN1 variant. However, since the first report of 7 subjects belonging to the same large French-
Canadian family, carrying the truncating variant p.(Gln555*)\textsuperscript{17}, only two additional subjects harboring distinct nonsense p.(Arg422*)\textsuperscript{18} and missense p.(Ile319Thr) variants\textsuperscript{19} in SYN1 were described (Table e-2). All patients had reflex seizures triggered by bathing or showering and variable neurodevelopmental disorders ranging from dyslexia or specific language impairments to pervasive developmental disorders. Here, we described the largest cohort of patients with BE carrying \textit{SYN1} mutations.

Apart from the previously reported nonsense c.1264 C>T p.(Arg422*) variant\textsuperscript{18}, all other identified alleles are novel and include two splicing site, five distinct frameshift, one missense, and one \textit{in-frame} insertion variants (Fig. 1b). The nonsense, frameshift, and splicing variants are predicted to act through a loss-of-function mechanism like most \textit{SYN1} mutations linked to BE. Although we did not functionally assess the impact of the missense and \textit{in-frame} variants, they affect the highly conserved residue of the protein and are located in important functional domains. Overall, \textit{SYN1} variants related to BE are clustered in the Pro-rich regulatory domain, while the few ones not associated with BE are also found in other protein domains. However, given the limited number of subjects reported so far, further studies are needed to corroborate this observation and provide further insights into the genotype-phenotype correlations (Figure 1B). Moreover, we observed intrafamilial variability as pointed out by the segregation analysis in family 2 in which the maternal uncle displayed non-provoked seizure and ASD but not BE. This is in line with the previous evidences\textsuperscript{17}, suggesting that all subjects harbouring \textit{SYN1} mutations have variable neurodevelopmental impairments yet not all develop BE.

\textit{Phenotypic spectrum of \textit{SYN1}-related reflex epilepsy}

The main clinical features of our cohort are consistent with the core phenotype of BE (Table 1). All but one subject presented with seizures provoked by showering or bathing regardless of water temperature. Interestingly, one subject experienced recurrent seizures provoked by immersion of feet in the water, and not by pouring of water over the head. Additional triggers
were rubbing with a wet towel, fingernail cutting, and hair-cutting in some subjects. In two individuals, seizures were also provoked by watching someone taking bathing or just thinking about having a bath.

Of note, we report the first two females with BE. They also displayed a variable degree of developmental delay, from mild GDD to severe cognitive impairment associated with non-provoked seizures. We hypothesize that skewed X-inactivation occurring in the brain tissues could explain the expression of the disease in these female carriers.

Nine of the thirteen subjects also developed non-provoked seizures. In two of them, febrile seizures preceded the onset of bathing seizures. Other non-provoked seizures occurred at night in five subjects and were mostly focal or focal to bilateral with autonomic features. Only one subject had a prolonged seizure resulting in status epilepticus.

Antiseizure treatment was required in all subjects and partial or complete control of seizures was achieved in six cases. Clobazam and valproic acid were the most effective drugs. Ictal EEG recorded in two subjects showed rhythmic theta activity over the frontocentral/temporal regions in keeping with the previous reports. Variable cognitive impairment was noticed in ten out of thirteen subjects. ASD, ADHD, and behavioural issues were also predominant features in several subjects.

Overall, the clinical and electrophysiological findings in our patients overlap those described in previous BE cases, suggesting that this condition is a specific and preventable RE related to contact with water.

**Role of SYN1 in bathing epilepsy**

SYN1 encodes a neuron-specific phosphoprotein implicated in the regulation of neurotransmitter release and synaptogenesis. Its role in epilepsy has been elucidated by studies in the Syn1 knockout mouse model showing impaired synaptic vesicle trafficking and impairment of GABA release through a loss-of-function mechanism that results in higher network excitability and firing activity. Although the exact pathophysiology of SYN1-
related BE is currently unknown, the ictal SPECT findings in some subjects have suggested insular cortex involvement\textsuperscript{17}. Indeed, the insula is a key integrative multisensory area, well connected with the temporal lobe\textsuperscript{24} and potentially able to generate motor and autonomic symptoms, like those observed in BE cases, if functionally perturbed by genetic mutations\textsuperscript{17}. Accordingly, SYN1 mutations may lead to imbalances between excitatory and inhibitory influences at the synaptic level, thus entraining temporal and insular areas into a seizure activity after water pouring\textsuperscript{17}.

**Hot water vs. bathing epilepsy and correlation with SYN1 mutations**

REs represent a spectrum of conditions characterized by broad clinical and genetic heterogeneity and several overlapping features\textsuperscript{1,6}. Apart from SYN1, a few additional REs-related genes have been reported, including SYNGAP1 in subjects with chewing reflex\textsuperscript{25,26}, CDKL5-related disorders exhibiting diaper change reflex\textsuperscript{27}, SCN1A in somatosensory reflex seizures\textsuperscript{28}, and CHD2 in photosensitive seizures\textsuperscript{5}. It is likely that in all these epilepsy types, the genetic defect eventually results in abnormal hyperexcitability of cortical areas that are physiologically activated during specific sensory stimulation, acting as triggers.

The recent report of HWE in a subject carrying a splice variant (c.527+1G>T) in SYN1 may argue whether BE and HWE belong to the same phenotypic spectrum\textsuperscript{20}. Moreover, to specifically address this issue we screened a cohort of cases showing HWE and found no evidence of pathogenic variants in SYN1.

HWE, largely reported in Southern India, is induced by bathing with hot water usually over 37°C\textsuperscript{29}. Seizures often occur when subjects are seated and hot water is poured from a washtub or basin over their heads\textsuperscript{10,29}. Seizures may also start with self-induction in some patients as they enjoy this situation. Similar to BE, there is a male-to-female predominance and about 20-40\% of subjects with HWE may develop spontaneous seizures\textsuperscript{8}, but, unlike BE, the majority of subjects have normal development. Several studies including EEG and functional MRI have suggested a predominant temporal lobe involvement, with the possible
contribution of parietal and occipital areas\textsuperscript{30,31}. SPECT studies have demonstrated ictal hypermetabolic uptake in the medial temporal structures and hypothalamus\textsuperscript{32}. Although the physiopathology of HWE remains unknown, it has been suggested it could be related to a hyperthermic kindling involving the thermoregulation centre of the hypothalamus that triggers seizures\textsuperscript{33,34}.

Despite a similar ictal semiology and EEG, our data suggest that BE and HWE are likely distinct epileptic disorders with different genetics, seizure triggers (pouring vs hot water), and hyperexcitability of cortical circuits. The improvement observed after decreasing water temperature in HWE\textsuperscript{33} and the report of a few subjects with BE who also experienced seizure precipitated by rubbing the face with a wet cloth or nail clipping\textsuperscript{17} further support our thoughts. Similarly, two of our patients also experienced seizures triggered by rubbing with a towel after showering. The other two individuals had seizures triggered by fingernail clipping and one of them also by hair cutting. Taking together, these findings suggest that BE is intrinsically related to a somatosensory stimulus rather than the simple water temperature, as instead observed in HWE. Hence, it may be possible that the reflex seizure reported in two \textit{SYN1} cases, apparently after hot water exposure, was instead precipitated by the somatosensory stimulus of water and the temperature just played a confounding role.

Moreover, the report of two subjects with seizures provoked by watching someone bathing or showering suggests that the pathophysiology of \textit{SYN1}-related RE could be even more complex, likely involving mirror-like activities and yet unknown and tightly regulated neuronal circuits.

Recent simulation theories in cognitive neuroscience emphasize that sensorimotor capacities and cognitive processes are inseparable as the simulation process involves the same sensorimotor neural correlates that are active during the action execution or interaction with the actual object or entity itself\textsuperscript{35}. Accordingly, watching someone else bathing or showering, or imagining bathing or showering may involve the same neuronal circuits that trigger the seizure when acting.
Conclusion

Bathing epilepsy is a clinically and genetically homogeneous distinct RE and should be considered a handle for the molecular diagnosis of SYN1-related epilepsy. The early identification of the molecular defect may help start early intervention strategies to optimize function and quality of life and prevent comorbidities in affected patients. Future studies using advances in electrophysiology and imaging data acquisition will help to define the genotypic-phenotypic spectrum and understand the underlying pathomechanisms of this rare reflex epilepsy to eventually develop effective and targeted therapeutic strategies.

Acknowledgments

This work was developed within the framework of the DINOGMI Department of Excellence of MIUR 2018-2022 (legge 232 del 2016).
**APPENDIX 1: Authors**

<table>
<thead>
<tr>
<th>Name</th>
<th>Location</th>
<th>Contribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Andrea Accogli, MD</td>
<td>Istituto Giannina Gaslini, Italy</td>
<td>Data acquisition, drafting the initial manuscript, analysis and interpretation of data and finalizing the manuscript with all authors’ input.</td>
</tr>
<tr>
<td>Gert Wiegand, MD</td>
<td>University Medical Centre Schleswig-Holstein, Kiel, Germany</td>
<td>Data acquisition, analysis and interpretation of data, drafting and finalizing the manuscript</td>
</tr>
<tr>
<td>Marcello Scala, MD</td>
<td>Istituto Giannina Gaslini</td>
<td>Drafting and final revision of the manuscript</td>
</tr>
<tr>
<td>Caterina Cerminara, MD</td>
<td>Tor Vergata University, Roma, Italy</td>
<td>Patients recruitment, data analysis and final revision of the manuscript</td>
</tr>
<tr>
<td>Michele Iacomino, PhD</td>
<td>Istituto Giannina Gaslini</td>
<td>Genetic analysis, data analysis</td>
</tr>
<tr>
<td>Antonella Riva, MD</td>
<td>Istituto Giannina Gaslini</td>
<td>Patients recruitment, clinical assessment, final revision of the manuscript</td>
</tr>
<tr>
<td>Barbara Carlini, MSc</td>
<td>Istituto Giannina Gaslini</td>
<td>Genetic analysis</td>
</tr>
<tr>
<td>Letizia Camerota, MD</td>
<td>University of L’Aquila, L’Aquila, Italy</td>
<td>Patients recruitment, clinical assessment and final revision of the manuscript</td>
</tr>
<tr>
<td>Vincenzo Belcastro, MD</td>
<td>Child Neuropsychiatry Unit, Department of Mental Health, ASST-LARIANA, Como, Italy</td>
<td>Patients recruitment, clinical assessment and final revision of the manuscript</td>
</tr>
<tr>
<td>Paolo Prontera, MD</td>
<td>Medical Genetics Unit, “S. Maria della Misericordia” Hospital, Perugia, Italy</td>
<td>Patients recruitment, clinical assessment and final revision of the manuscript</td>
</tr>
<tr>
<td>Alberto Fernández-Jaén, MD, PhD</td>
<td>Hospital Universitario Quirónsalud and Universidad Europea de Madrid, Madrid, Spain</td>
<td>Patients recruitment, clinical assessment and final revision of the manuscript</td>
</tr>
<tr>
<td>Nerses Bebek, MD</td>
<td>Istanbul University Istanbul Faculty of Medicine Department of Neurology, Istanbul, Turkey.</td>
<td>Recruitment of patients with HWE</td>
</tr>
<tr>
<td>Paolo Scudieri, PhD</td>
<td>Istituto Giannina Gaslini, Italy</td>
<td>Genetic testing</td>
</tr>
<tr>
<td>Simona Baldassari, PhD</td>
<td>Istituto Giannina Gaslini, Italy</td>
<td>Genetic testing</td>
</tr>
<tr>
<td>Vincenzo Salpietro, MD</td>
<td>Istituto Giannina Gaslini, Italy</td>
<td>Patient evaluation, clinical assessment and final revision</td>
</tr>
<tr>
<td>Giuseppe Novelli, PhD</td>
<td>Tor Vergata University of Rome, 00133 Rome, Italy</td>
<td>Genetic testing</td>
</tr>
<tr>
<td>Chiara De Luca, MD</td>
<td>Human Genetics, Department of Life, Health and Environmental Sciences, University of L’Aquila, Italy</td>
<td>Patients recruitment, clinical assessment and final revision of the manuscript</td>
</tr>
<tr>
<td>Celina von Stülpnagel, MD</td>
<td>Department of Pediatrics, University Hospital Munich, Munich, Germany</td>
<td>Patients recruitment, clinical assessment and final revision of the manuscript</td>
</tr>
<tr>
<td>Felicitas Kluger, MD</td>
<td>Epilepsy Center for Children and Adolescents, Vogtareuth, Germany</td>
<td>Drafting and revision of the manuscript</td>
</tr>
<tr>
<td>Name</td>
<td>Institution/Department</td>
<td>Task</td>
</tr>
<tr>
<td>-------------------------------------------</td>
<td>----------------------------------------------------------------------------------------</td>
<td>----------------------------------------------------------------------</td>
</tr>
<tr>
<td>Gerhard Josef Kluger, MD</td>
<td>Epilepsy Center for Children and Adolescents, Vogtareuth, Germany</td>
<td>Patients recruitment, clinical assessment and final revision of the manuscript</td>
</tr>
<tr>
<td>Gabriele Christine Wohlrab, MD</td>
<td>Department of Neuropediatrics, University Children's Hospital Zurich, Zurich, Switzerland</td>
<td>Patients recruitment, evaluation and final revision of the manuscript</td>
</tr>
<tr>
<td>Georgia Ramantani, MD</td>
<td>Department of Neuropediatrics, University Children's Hospital Zurich, Zurich, Switzerland</td>
<td>Patients recruitment, evaluation and final revision of the manuscript</td>
</tr>
<tr>
<td>David Lewis-Smith, MD</td>
<td>Translational and Clinical Research Institute, Newcastle University, Newcastle Upon Tyne, UK</td>
<td>Patients recruitment, evaluation and final revision of the manuscript</td>
</tr>
<tr>
<td>Rhys H. Thomas, MD</td>
<td>Translational and Clinical Research Institute, Newcastle University, Newcastle Upon Tyne, UK</td>
<td>Patients recruitment, evaluation and final revision of the manuscript</td>
</tr>
<tr>
<td>Ming Lai, MD</td>
<td>Translational and Clinical Research Institute, Newcastle University, Newcastle Upon Tyne, UK</td>
<td>Patients recruitment, evaluation and final revision of the manuscript</td>
</tr>
<tr>
<td>Alberto Verrotti, MD, PhD</td>
<td>Department of Pediatrics, University of L'Aquila, Via Vetoio 1, 67100, Coppito, L'Aquila, Italy</td>
<td>Patients recruitment, evaluation and final revision of the manuscript</td>
</tr>
<tr>
<td>Salvatore Striano, MD, PhD</td>
<td>Epilepsy Center, Federico II University, Napoli, Italy</td>
<td>Patients recruitment, evaluation and final revision of the manuscript</td>
</tr>
<tr>
<td>Christel Depienne, MD, PhD</td>
<td>YInstitut du Cerveau et de la Moelle épiniere (ICM), Sorbonne Université, UMR S 1127, Inserm U1127, France</td>
<td>Patients recruitment, evaluation and final revision of the manuscript</td>
</tr>
<tr>
<td>Carlo Minetti, MD, PhD</td>
<td>Istituto Giannina Gaslini, Italy</td>
<td>Patients recruitment, evaluation and final revision of the manuscript</td>
</tr>
<tr>
<td>Fabio Benfenati, MD, PhD</td>
<td>Center for Synaptic Neuroscience and Technology, Istituto Italiano di Tecnologia, Genoa, Italy</td>
<td>Critical revision and final revision of the manuscript</td>
</tr>
<tr>
<td>Francesco Brancati, MD, PhD</td>
<td>Human Genetics, Department of Life, Health and Environmental Sciences, University of L’Aquila, Italy</td>
<td>Patients recruitment and evaluation genetic testing, final revision of the manuscript</td>
</tr>
<tr>
<td>Federico Zara, PhD</td>
<td>Istituto Giannina Gaslini, Italy</td>
<td>Principal investigator, coordinator of the genetic testing, revision of final the manuscript</td>
</tr>
<tr>
<td>Pasquale Striano, MD, PhD</td>
<td>Istituto Giannina Gaslini, Italy</td>
<td>Principal investigator, lead of the design and conceptualization of the study. Critical revision of the manuscript</td>
</tr>
</tbody>
</table>
References


I, Aguilera-Albesa S. Ictal Video-Electroencephalography Findings in Bathing Seizures: Two New Cases and

electrophysiologic findings based on 21 cases. Epilepsia. 2001; 42(9):1180-1184.

dominant reflex epilepsy precipitated by hot water maps at chromosome 10q21.3-
q22.3. Hum Genet. 2009;125(5-6):541-549.

reflex epilepsy triggered by hot water maps to 4q24-q28. Hum Genet. 2009;
126(5):677-683.


origin of the excitation/inhibition imbalance in the hippocampus of synapsin I/II/III

a mutation in synapsin I, a synaptic vesicle protein, in a family with epilepsy. J

mutation in non-syndromic X-linked intellectual disability affects synaptic vesicle


Figure 1. Pedigrees and SYN1 mutations of affected patients

(A) Pedigree of the 10 families showing affected members with bathing epilepsy (shaded in grey) and healthy female carriers (indicated with a central dot); the uncle of family 2 (II:3) with non-provoked seizure is coloured in light grey. (B) Nonsense, frameshift, and missense variants in SYN1 (NM_006950.3) are depicted along with the Synapsin-1 structure (Bottom; protein domains: A; B, linker; C, actin-binding and synaptic-vesicle binding; D, Pro-rich linker; E) and splicing variants are displayed along the genomic locus (top). SYN1 variants related to Bathing epilepsy are in red and those linked to other clinical presentations different than Bathing Epilepsy are in black. Variants identified in our cohort with bathing epilepsy are in bold.
Figure 2. Ictal EEG in two SYN1 patients

Ictal-video EEG (after bathing) of subject II:4 of family 6 showing the onset of seizure with initial theta high voltage polymorphic activity over the frontal-temporal region (A). Ictal-video EEG (after bathing) of subject II:3 of family 7 showing high voltage polymorphic theta activity over the right frontal-temporal area (B).
Table 1. Genetic and phenotypic features of subjects with SYN1 variants and bathing epilepsy

<table>
<thead>
<tr>
<th>Family</th>
<th>Subject</th>
<th>Family 1</th>
<th>Family 2</th>
<th>Family 3</th>
<th>Family 4</th>
<th>Family 5</th>
<th>Family 6</th>
<th>Family 7</th>
<th>Family 8</th>
<th>Family 9</th>
<th>Family 10</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>H:1</td>
<td>H:1</td>
<td>H:1</td>
<td>H:2</td>
<td>H:1</td>
<td>H:1</td>
<td>H:1</td>
<td>H:1</td>
<td>H:1</td>
<td>IV:1:2</td>
</tr>
<tr>
<td>Age, sex</td>
<td></td>
<td>18y M</td>
<td>3 y M</td>
<td>9 y M</td>
<td>15 y M</td>
<td>2.5 y M</td>
<td>2.5 y M</td>
<td>7 y F</td>
<td>5.5y M</td>
<td>2y M</td>
<td>13y M</td>
</tr>
<tr>
<td>Detection</td>
<td>Family analysis</td>
<td>Sanger sequencing</td>
<td>Sanger sequencing</td>
<td>Sanger sequencing</td>
<td>Maternal</td>
<td>WES Maternal</td>
<td>Gene panel Maternal</td>
<td>Sanger sequencing</td>
<td>Maternal</td>
<td>WES Maternal</td>
<td>Gene panel Presumed Maternal</td>
</tr>
<tr>
<td>FH of BA</td>
<td>No</td>
<td>Yes, maternal uncle</td>
<td>No</td>
<td>Yes, sister</td>
<td>Yes, brother</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes, maternal aunt</td>
<td>Yes, 7 maternal male relatives</td>
<td></td>
</tr>
<tr>
<td>Development</td>
<td>Speech delay, agressive behavior, ADHD</td>
<td>Speech delay, hyperactivity</td>
<td>GDD, moderate ID, ASD, motor stereotypy aggressive behavior, echocardiopathy</td>
<td>Normal</td>
<td>Mild GD, speech delay, ADHD</td>
<td>Mild GD, speech delay, ADHD</td>
<td>Mild GD, speech delay, ADHD</td>
<td>GDD, moderate ID, ASD, ADHD</td>
<td>Normal</td>
<td>GDD, Mild ID, ASD, motor stereotype ADHD</td>
<td>Mild GD, speech delay mild ID, autistic traits</td>
</tr>
<tr>
<td>RE - Onset</td>
<td>5 y</td>
<td>2 y</td>
<td>7 y</td>
<td>6 y</td>
<td>22 m</td>
<td>14 m</td>
<td>2 y</td>
<td>4y7m</td>
<td>1y3m</td>
<td>8 m</td>
<td>8 m</td>
</tr>
<tr>
<td>Features</td>
<td>Impaired awareness, pallor, cyanosis, oral automatisms, hypotonia</td>
<td>Impaired awareness, lip cyanosis, buccal automatisms, hypertonia</td>
<td>Impaired awareness, lip cyanosis, buccal automatisms, hypertonia</td>
<td>Impaired awareness, lip cyanosis, buccal automatisms, hypertonia</td>
<td>Impaired awareness, lip cyanosis, buccal automatisms, hypertonia</td>
<td>Impaired awareness, lip cyanosis, buccal automatisms, hypertonia</td>
<td>Impaired awareness, lip cyanosis, buccal automatisms, hypertonia</td>
<td>Autonomic seizures with apnea, cyanosis, loss of consciousness, automatism</td>
<td>Autonomic features, atonic seizures, pallor, staring, cyanosis</td>
<td>Autonomic seizures with apnea, cyanosis, loss of consciousness, automatism</td>
<td>Autonomic features, atonic seizures, pallor, cyanosis, oro-buccal automatisms</td>
</tr>
</tbody>
</table>

---

Copyright © 2021 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of the American Academy of Neurology.
<table>
<thead>
<tr>
<th>Seizure frequency at the onset</th>
<th>Week ly</th>
<th>Monthl y</th>
<th>1-2/week</th>
<th>Wee kly</th>
<th>Wee kly</th>
<th>2-8/month</th>
<th>1-2/wee k</th>
<th>2-3/week</th>
<th>2-3/week</th>
<th>1-2/week</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Feverle seizure</strong></td>
<td>Yes (4y9 m)</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes (3y)</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td><strong>Other sz</strong></td>
<td>Nocturnal TCS at 6 y</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Focal impaired awareness sz, 2.5y</td>
<td>Focal autonomic sez, at 5y</td>
<td>Nocturnal autonomic sz, SE, 7y</td>
<td>Infantile spasm, 5m; TCS with automatism, 2y; atonic atypical absence sz</td>
<td>Focal to bilateral TCS, nocturnal TCS in cluster</td>
</tr>
<tr>
<td><strong>EEG</strong></td>
<td>R temporal, L anterior temporal</td>
<td>R temporal, L frontotemporal</td>
<td>R central, temporal</td>
<td>Normal</td>
<td>Bilateral temporal</td>
<td>Bilateral temporo</td>
<td>Bilateral centrotemporal</td>
<td>Normal</td>
<td>R and L temporal</td>
<td>Bursts of slow spike-wave</td>
</tr>
<tr>
<td><strong>Ictal</strong></td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>Rhythmic theta seizure pattern right temporal</td>
<td>Rhythmic theta left temporal</td>
</tr>
<tr>
<td><strong>ASMs</strong></td>
<td>CLB, VPA</td>
<td>CLB, VGB, CBZ, CLB</td>
<td>N/A</td>
<td>VPA</td>
<td>VPA</td>
<td>VPA, STM, LTG</td>
<td>CBZ</td>
<td>OCX, STM, VPA, LTG</td>
<td>LG, VPA, VGB, LAC, LEV, BRV, ZNS, steroids, KD, CBL, RUF</td>
<td>CBZ, LTG, VPA</td>
</tr>
<tr>
<td><strong>Response to medications</strong></td>
<td>Decreased seizures frequency</td>
<td>Partial response</td>
<td>Decreased seizure frequency</td>
<td>N/A</td>
<td>Poor response</td>
<td>Poor response</td>
<td>Seizure-free</td>
<td>No</td>
<td>Seizure-free, avoidance of warm water</td>
<td>Seizure-free</td>
</tr>
</tbody>
</table>

ADHD, attention deficit hyperactivity disorder; ASMs, antiseizure medications; ASD, autism spectrum disorder; BE, bathing epilepsy; BRV, brivaracetam; CBZ, carbamazepine; CLB, clobazam; GDD, global developmental delay; EEG, electroencephalogram; FH, family history; GTCS, generalized tonic-clonic seizures; ID, intellectual disability; KD, ketogenic diet, LAC, lacosamide, LEV, levetiracetam, LTG, lamotrigine, M, male; m, months, MRI, magnetic resonance imaging; N/A, not available; OCX, oxcarbazepine, RE, reflex epilepsy, RUF, rufinamide, STM, sz, seizure; sulthiamine, TCS, tonic-clonic seizures; VGB, vigabatrin; VPA, valproic acid; ZNS, zonisamide, y, years. † A vagus nerve stimulator was also placed, resulting in a further decrease in the frequency of unprovoked seizures, but not affecting bathing seizures. SE, status epilepticus, WES, whole-exome sequencing.
Videos of patients with SYN1 mutations

Video 1: Video of case II:1 of family 1
Case II:1 presenting a reflex seizure at the age of 6 years triggered by rubbing with a towel after showering. The episode was characterized by impairment awareness, pallor, buccal automatism and losing of axial tone lasting less than a minute.

Video 2: Video of case II:1 of family 2
Case II:1 presenting a reflex seizure at age of 1 year during bathing, characterized by impaired awareness, eye blinking and transient hypotonia.

Video 3: Video of case II:1 of family 3
Case II:1 presenting a reflex seizure at age of 8, characterized by impaired awareness, losing of axial tone, uprolling eye movements and lips cyanosis.

Video 4: Video of case II:1 of family 6
Ictal video EEG recording during bath in subject II:1. A focal dyscognitive seizure starts (00:08) with a staring and left head deviation followed by oral automatisms, pallor and loss of consciousness. The seizure lasts over than 3 minutes and ictal EEG shows an initial tetha high voltage polymorphic activity over the frontal temporal region. During seizure course the EEG recording shows a predominant high voltage delta activity over the posterior (R>L) brain areas and it ends (03:40) with a slowed activity over the posterior right hemisphere. EKG also noted a prolongation of QT interval during the seizure.

Video 5: Video of case II:1 of family 7
Ictal video EEG recording during and after bath in subject II:1. A focal dyscognitive seizure starts after bathing (01:12) with a staring, head deviation to the right, oral automatism and pallor. Ictal-video EEG shows high voltage polymorphic theta activity over the right frontal-temporal regions.