

Supplementary Materials.

Materials and Methods.

Figures and Figure legends.

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Mutagenesis and cloning.

All variants were engineered in the human cDNA of *KCNA2*. cDNA was transcribed into cRNA in vitro, which was injected into *Xenopus* oocytes. The human Kv1.2 in the pcDNA3.1 vector was kindly provided by Stephan Grissmer (Institute of Applied Physiology, Ulm University). Site-directed mutagenesis was performed using Quickchange™ (Agilent Technologies) and PCR (primers are available on request). The mutated clones were fully resequenced. cRNA for oocyte injection was prepared using the T7 mMessage kit from Ambion. For expression in CHO cells, human *KCNA2* wildtype or mutant cDNA was subcloned into an IRES-GFP-CBIG vector (provided by a generous gift of JD Macklis, Harvard Medical School (55)). Clones were sequenced to confirm the presence of wildtype (WT) or mutant cDNA coding sequence.

AAV production.

For the transfer plasmid human cDNA of *KCNA2* WT was ordered from IDT as gBlock and was cloned into pAAV-hSyn-EGFP, which was a gift from Bryan Roth (Addgene plasmid # 50465; <http://n2t.net/addgene:50465>; RRID:Addgene_50465). Mutagenesis for GoF *KCNA2* R297Q was performed using NEB Q5 Site-Directed Mutagenesis Kit (# E0554S) according to the manufacturer's instructions (Mut-R297Q-Primer-F: CGTGTCATCCAGTTGGTAAGAG, Mut-R297Q-Primer-R: GAGGATGGCCAGTGACAT). RepCap and pHelper sequences were combined on the plasmid pDP8.ape (Plasmidfactory). AAV8 Production in HEK293T Cells was performed as described here: <https://www.addgene.org/protocols/aav-production-hek293-cells/> and followed by AAV8 Purification by Iodixanol Gradient Ultracentrifugation described in detail here: <https://www.addgene.org/protocols/aav-purification-iodixanol-gradient-ultracentrifugation/>. The estimated titer was 8.3×10^{12} and 3.1×10^{12} viral genomes/ml for *KCNA2* WT and *KCNA2* R297Q, respectively. For experiments, primary hippocampal mouse cultures were transduced 10 days before first patch-clamp recordings.

Xenopus laevis oocytes.

K⁺ currents were recorded using two-microelectrode voltage clamping and analyzed as previously described (5, 11). Experiments were approved by the local Animal Care and Use Committee (Regierungspraesidium Tuebingen). *Xenopus laevis* oocytes were treated and stored as described. Fifty

nanolitres of cRNA encoding wild-type or mutated Kv1.2 subunits (1 mg/ml) was injected using Roboocyte2 (Multi Channel Systems). Oocytes were stored for 2 days (at 17°C) prior to the experiment. Amplitudes of currents of wild-type and mutant channels recorded on the same day were normalized to the mean value of Kv1.2 wild-type on that day to pool normalized data from different experiments. K⁺ currents in oocytes were recorded at room temperature (20–22°C) using two-electrode voltage-clamp with Roboocyte2. Electrode resistances were 0.4–1MΩ (1M KCl or 1.5M KAc). The holding potential was -80 mV. Oocytes were perfused with a ND96 bath solution containing (in mM): 93.5 NaCl, 2 KCl, 1.8 CaCl₂, 2 MgCl₂, 5 HEPES (pH 7.6). Currents were sampled at 5 kHz. Standard voltage-clamp protocols and analysis methods were used as described (11).

Voltage clamp protocols and data analysis: The membrane was depolarized to various test potentials from a holding potential of -80 mV to record K⁺ currents. The activation curve (conductance–voltage relationship) was derived from the current–voltage relationship that was obtained by measuring the peak current at various step depolarizations from the holding potential of -80 mV (10 mV increment, depolarized to +70 mV). The following Boltzmann function was fitted to the obtained data points:

$$g(V) = \frac{g_{\max}}{\left\{1 + \exp\left[\frac{(V - V_{1/2})}{k_v}\right]\right\}}$$

with $g(V) = I/(V - V_{\text{rev}})$ being the conductance, I the recorded current amplitude at test potential V , V_{rev} the K⁺ reversal potential, g_{\max} the maximal conductance, $V_{1/2}$ the voltage of half-maximal activation and k_v a slope factor.

For the pharmacological experiments, 4-AP (Sigma) stock (10 mM) was freshly prepared in ND96 solution and adjusted to pH 7.4. Before application of 4-AP, an activation protocol with increasing step depolarizations as described above was run and an additional 1 s depolarizing pulse from -80 mV to +20 mV applied three times every 30s. After washing in 4-AP in increasing concentrations (Fig. S2A), each for 5 min, the step protocol from -80 mV to +20 mV was run five times every 30 s to monitor the steady state block of Kv1.2 channels (Fig. S2A). The responses were normalized to control responses before 4-AP application to show the concentration- and use-dependent block of Kv1.2 channels by 4-AP (56). Subsequently, the activation protocol was repeated.

Mammalian CHO cells.

CHO cells were cultured and maintained in Gibco® F-12 Nutrient Mixture (F12) medium at 37 °C, with 5% CO₂ humidified atmosphere and sub-cultured into 35 mm petri dishes 1 day before the transfection. At least 50% confluence of the cells were ensured. Transfections using "TransIT®-LT1" reagent (Mirus Bio LLC., Madison, WI, USA) were performed for transient expression of WT or mutant subunits. An amount of 1 µg DNA was used for transfection.

Voltage clamp protocols and data analysis: Recordings were performed 24 hrs after transfection. Only the cells positive for green fluorescent protein fluorescence were used for electrophysiological measurements. Transfected cells were visualized and the expression of GFP was confirmed using a fluorescent inverted microscope (Leica Biosystems, Wetzlar, Germany). Standard whole-cell patch clamp recordings were performed using an Axopatch 200B amplifier, a Digidata 1320A digitizer and pCLAMP 10.2 data acquisition software (Axon Instruments, Union City, CA, USA). Leakage and capacitive currents were automatically subtracted using a pre-pulse protocol (-P/4). Currents were filtered at 2 kHz and sampled at 5 kHz. All recordings were performed at the room temperature of 21-23°C. Pipettes were pulled from borosilicate glass (Science Products GmbH) using a Sutter P97 Puller (Sutter Instruments), with resistances of 2-3 MΩ when filled with internal recording solution (see below). The residual series resistance was compensated up to 85%. The pipette solution contained (in mM): 90 KF, 10 KCl, 1 CaCl₂, 1 MgCl₂, 11 EGTA, 10 HEPES and 2 Na₂ATP (pH 7.2 with KOH). The bath solution contained (in mM): 135 NaCl, 5 KCl, 2 CaCl₂, 2 MgCl₂, 5 HEPES, 10 sucrose (pH 7.4 with NaOH). The liquid junction potential was calculated as +7.7 mV at 23°C, but was not corrected. The following voltage clamp protocols were used for recordings. The membrane was depolarized to various test potentials from a holding potential of -90 mV (500 ms steps from -110 mV to 60 mV with an increment of 10 mV). The above-mentioned Boltzmann function was fitted to the obtained data points.

For 4-AP application a perfusion insert (AutoMate Scientific) for a 35 mm petri dish was used. A Minipuls peristaltic pump (Gilson) provided a constant in and outflow (~3-4 ml/min) of bath solution containing different concentrations of 4-AP (10 µM, 100 µM, 1 mM, 5 mM, 10 mM). An incubation time of 5 min was always followed after application of the drug.

Primary neuronal cultures

Animal protocols for primary hippocampal cultures were approved by the local Animal Care and Use Committee (Regierungspraesidium Tuebingen, Tuebingen, Germany). Pregnant C57BL/6NCrl females

were cervically dislocated after asphyxiation by CO₂, and embryos were quickly taken out and immediately decapitated at embryonic Day 18 (E18) as described previously (57). Using microsurgical dissection methods, the hippocampus was isolated under a dissecting microscope (Olympus SZ 61, Shinjuku, Tokyo, Japan). Tissues were washed three times with 4°C magnesium- and calcium-free HBSS (PAA Laboratories GmbH) before treatment for 15 min with 2.5% trypsin. Subsequently, tissues were rinsed in DMEM with fetal bovine serum (Biochrom AG), L-glutamine (Invitrogen) and penicillin/streptomycin (Invitrogen) to block the trypsin reaction. Single neurons were obtained by mechanical dissociation using a pipette and a cell strainer (Becton Dickinson). Dissociated neurons were plated on 13-mm coverslips in 24-well culture plates filled with 500 µl DMEM supplemented with FCS and penicillin/streptomycin (Invitrogen). The coverslips were coated with poly-D-lysine prior to the embryo preparation. After six hours, during which neurons could settle on the cover slips in 5% CO₂ humidified atmosphere at 37°C, the culture medium was replaced by Neurobasal culture medium (Invitrogen) supplemented with B27 (Invitrogen), glutamine, and penicillin/streptomycin.

Voltage and current clamp recordings and data analysis: Whole cell patch clamp recordings were performed at 22°C using a Multiclamp 700B (Molecular devices) amplifier, a DigiData 1420 (Molecular devices) and pClamp 10.6 software (Molecular devices) as described before (57). Cover slips with cells were positioned in a submerged-type recording chamber (Scientifica, United Kingdom), continuously superfused with aCSF and visualized with a BX61WI Microscope (Olympus). The aCSF contained (in mM): 140 NaCl, 4.2 KCl, 1.1 CaCl₂, 1.0 MgSO₄, 0.5 Na₂HPO₄, 0.45 NaH₂PO₄, 5 HEPES, 10 glucose. The pH was set to 7.4 and osmolarity to 300 mosm/l. Pipettes were pulled from borosilicate glass (Science products) using a Sutter P97 Puller (Sutter Instruments), with resistances of 3-5 MΩ. Intracellular solution contained (in mM) 135 K-gluconate, 4 NaCl, 0.5 CaCl₂, 10 HEPES, 5 EGTA, 2 Mg-ATP, 0.4 Na-GTP and 0.2% Biocytin. The pH was set to 7.3 and osmolarity to 300 mosm/l. Calculated liquid junction potentials (LJP) were +15 mV ($V_m = V_p - V_l$; V_m : membrane potential, V_p : recorded potential at amplifier after offsetting, V_l : liquid potential). All data have been corrected for LJP offline. Recordings were sampled at 100 kHz and the data were filtered at 30 kHz. Series resistance (<20 MΩ) was monitored during the experiment. Cells showing unstable series resistance or resting membrane potential were discarded.

Superfusion of 100 µM 4-AP in aCSF was performed using a Minipuls peristaltic pump (Gilson). For cells recorded before and after application of 4-AP, an incubation time of 10 min was always followed after application of the drug.

Patients and mutation analysis.

One new (P6), and ten previously reported patients (P1 – P5 and P7 – P11; (5, 7, 11)) were included. All *KCNA2* variants were identified by routine genetic diagnostics performed in patients with DEE either by targeted gene panels or whole exome sequencing, and verified by Sanger sequencing as described previously (5, 11). *KCNA2* variants were assumed to be pathogenic if they were absent in control samples (Exome aggregation consortium, ExAC set of ~61 000 exomes; exac.broadinstitute.org/) and fulfilled one or more of the following criteria: (i) had arisen *de novo*; (ii) found in a patient with a suggestive phenotype with additional functional studies showing a clear functional effect; or (iii) has previously been identified as disease-causing.

Treatment of patients with 4-AP.

We performed n-of-1-trials in nine different centers worldwide judging treatment effects of 4-AP using longitudinal follow-up data from physicians and reports from parents for neurological symptoms, including seizures/seizure diaries, neurological examination, 24h- or routine electroencephalography (EEG) and video recordings. Seizure types were classified according to the latest ILAE classification system (58). 4-AP was prepared in capsules by local pharmacists for initial dose escalation or later given as commercially-available controlled-release formulation and was chosen due to better crossing of the blood-brain-barrier compared to 3-4 diaminopyridine (59), a K⁺ channel blocker which had been shown to be less effective in symptom management in MS compared to 4-AP (32). Patients and relatives were informed about known side effects of 4-AP, such as paraesthesia, dizziness, nausea/vomiting, falls/balance disorders, insomnia, urinary tract infections, asthenia, and seizures (45). All known side effects were systematically assessed at follow-up visits.

N-of-1 trials were performed according to the local regulations of each participating center, given individual treatment considerations, the use of 4-AP as a licensed agent in humans with a known safety profile, and lack of alternative available therapeutic options. Informed consent was obtained from all parents or legal guardians. The treating physicians had frequent contact during dose escalation, and neurological, EEG and ECG assessments were performed (see below). Each participating center collected clinical data for their patients. Ethical Boards in Germany do not consult n-of-1 trials ('named patient program'/'individueller Heilversuch'), since they are performed with full responsibility of the treating physician up to a number of four patients per center. Health insurances approved the off-label

treatment. In other countries, local authorities, such as the hospital's internal review board in Israel or medical regulatory bodies in the UK, approved the trials in single patients.

While Whereas the recommendation for dosing of 4-AP in adults is 10 mg b.i.d. (which corresponds to 0.29 mg/kg/d for a person of 70 kg), we here needed higher dosages per kg body weight to observe clinically beneficial effects. We increased the dosage slowly to minimize the risk for seizure exacerbation, a known side effect of 4-AP, in a seizure disorder. The initial dose for preschool-aged inpatient children were 2.5 (P11) or 5 mg/d depending on age, body weight and clinical setting and was chosen due to personal experiences from mainly episodic ataxia type 2 (EA-2), chronic ataxia (such as spinocerebellar ataxia type 6 (SCA6)) and some patients with multiple sclerosis. Dosages were increased every one to three days in inpatients, whereas rather cautious administration schemes with weekly to monthly increases were applied in an outpatient setting. For preschool-aged children (until 5 years of age), small increments of 2.5 mg were used, whereas 5 to 10 mg increments were used in school aged children and 10 mg steps in adults.

Assessment of treatment effects with 4-AP

We used the following scoring systems to quantify the functional abilities and progress of the patients' state.

CGI-I: The overall change of patient's health state was determined by the 7-point Clinical Global Impression – Improvement scale (CGI-I) (30) as an overall reference measure. This 7-point scale has been developed and recommended by the U.S. National Institute of Mental Health for use in clinical trials to provide a brief, stand-alone assessment of the clinician's view of the patient's global functioning prior to and after initiating a study medication. It records clinician-based ratings of a change in a health state. These changes are categorized into (i) very much improved, (ii) much improved, (iii) minimally improved, (iv) no change, (v) minimally worse, (vi) much worse or (vii) very much worse compared to the previous visit (last follow-up). It thus allows to capture patients' overall health state changes, independent from more specific functional symptom-specific scales (like for example the SARA score for ataxia) and neurophysiological measures (such as EEG recordings). While Whereas such functional scales and neurophysiological measures might seem to be more “objective” and better quantifiable, they might miss the patient's true overall change, as such measures only capture very partial functions (for instance SARA score only capturing ataxia, and not usable for young children) or surrogate parameters

of patients' overall health state (an EEG change might not reflect a change in the patient's overall health state) (60).

SCORE: For EEG assessment and report, we used *SCORE*, the Standardized Computer-based Organized Reporting of EEG, where for which standardized terms are used to report the features of clinical relevance, extracted while assessing the EEGs (61, 62). All available EEGs were quantified by counting all epileptic discharges in EEGs up to 60 minutes. For EEGs lasting four hours and more at least 60 minutes at representative episodes were manually counted (NREM sleep, REM sleep, awake). All counts were done by experienced and certified raters (CB, JS, SL, BZ).

WeeFIM: We used the standardized Functional Independence Measure for children (*WeeFIM*), the responsiveness of which to clinical changes in disabled children has been proven (31). The *WeeFIM* instrument provides an indication of functional outcomes in children and aims to measure changes in function over time. The *WeeFIM* has been developed by a multidisciplinary team of health, education, and rehabilitation professionals to measure patients' performance in self-care, continence, mobility, locomotion, communication, and social cognition (63) and covers 18 items that describe seven stages of independence (64). The scoring system is based on a seven-stage ordinal scale with high scores of 6–7 reflecting a child's ability to complete all components of a task without adult help or supervision in a safe and timely manner. Low scores (1 or 2) reflect that the child requires help for at least half of the task components (65). For more details on the different stages and tasks, see (66). Recent reports on the reliability and validity of the *WeeFIM* instrument indicate that the assessment has excellent consistency across raters and provides scores that are stable (67, 68). Good equivalence reliability has also been shown between *WeeFIM* ratings obtained from direct observation and from reports by parents and teachers (69).

In addition to standardized scores, treatment effects were differentiated into four categories (marked improvement, mild improvement, no effect, or worsening) on seizures, ataxia and cognition/speech (Table 1, Fig. S6, Tables S2-S5), based on medical examinations, histories taken by the parents, and videos before and after treatment:

1. For seizures, we classified the effect as marked improvement when the seizure frequency was reduced by more than 50% in the observation period (three to 50 months; 22.7 ± 5.4 months). Less than 50% seizure reduction within the observation period was ranged as mild improvement, no change as no effect and an increase in seizure frequency as worsening.

2. EEG classification into one of the four categories was performed due to the occurrence of epileptic activity and changes in the background activity. EEGs of patients in which epileptic activity disappeared almost completely after 4-AP treatment were classified as markedly improved. Reduction of epileptic discharges of more than one third or longer spike-free intervals or acceleration of slowed background activity were regarded as mild improvement. Increase of spike frequency of more than one third was regarded as worsening.

For classification of ataxia and cognitive impairment, the clinical assessments by physicians and parents were taken into account as well as standardized scores. Patients were classified as markedly improved, when large and undoubtable effects were observed after treatment with 4-AP (for example P1 speaking fluently, P2 and P3 using more words and forming longer sentences, P9 being able to walk independently). Treatment effects were denoted as mild, when there was a weaker, but noticeable improvement, and patients were able to perform tasks which were not possible before treatment with 4-AP (such as P10 being able to walk downhill).

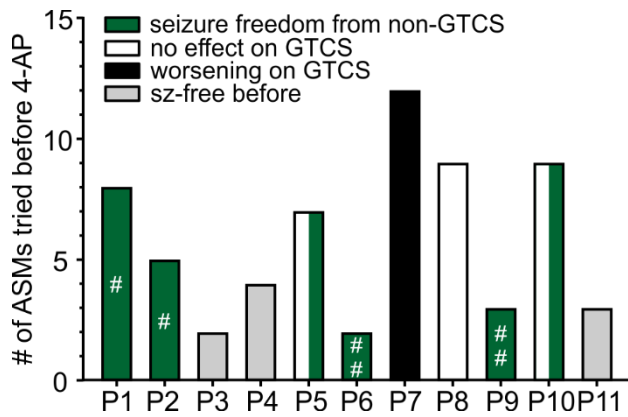


Fig. S1: Treatment history before starting 4-AP includes most important effects on seizure frequency upon treatment with 4-AP. Bars show the number of ASMs for each patient that have been tried before initiation of 4-AP treatment. Patients who showed a marked improvement in seizure frequency or gained seizure freedom from non-GTCS under 4-AP medication are shown in dark green, patients that have gained seizure freedom for all seizure entities before the initiation of 4-AP treatment are shown in grey. Worsening of seizure frequency (presented only with GTCS) during 4-AP medication is shown as a black bar. No effect on GTCS is shown as white bars. See also Table 2 of more information

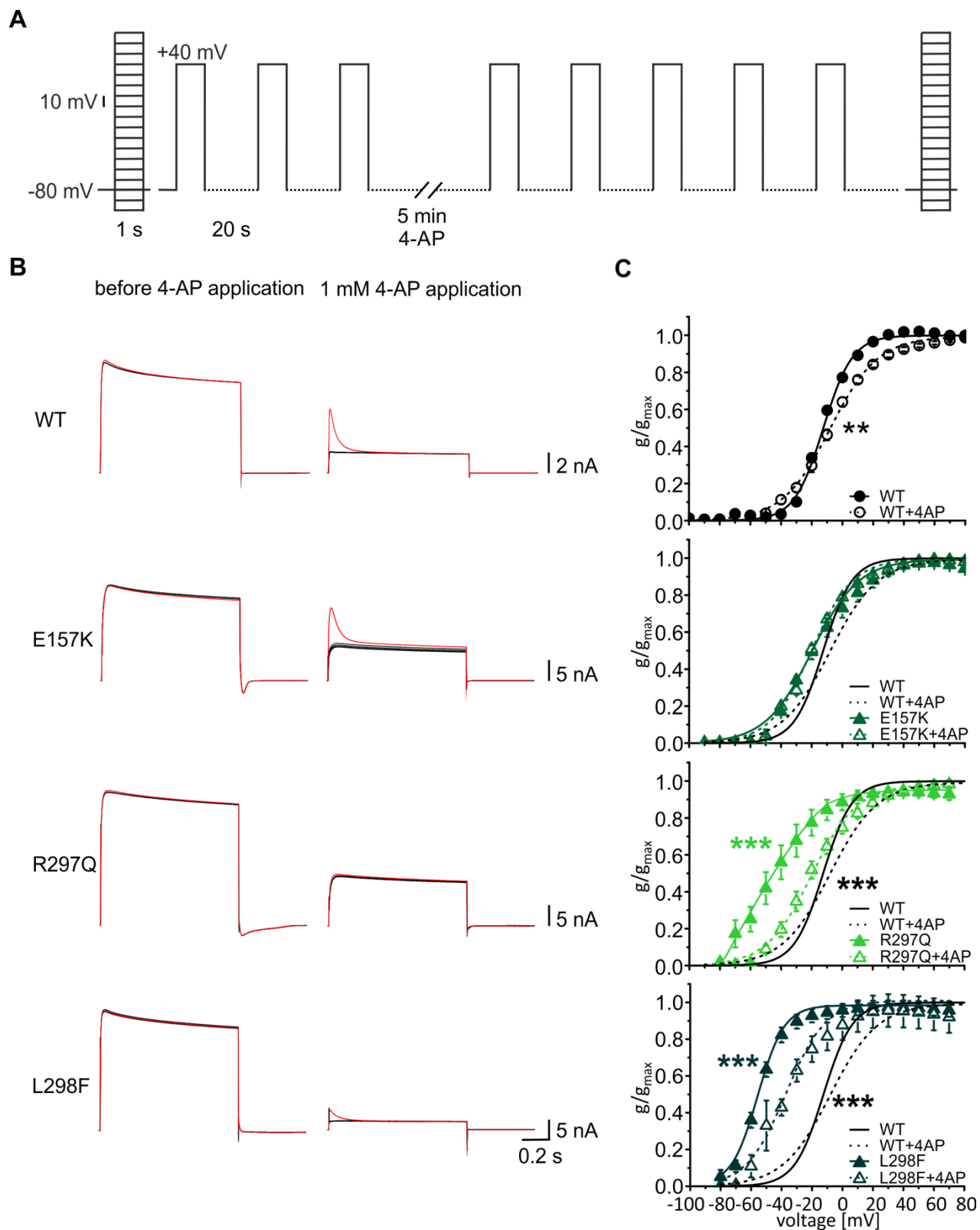


Fig. S2: 4-AP application affects current-voltage relationships of WT and GOF mutant Kv1.2 channels in *Xenopus* oocytes. (A) Voltage step protocols used for 4-AP application in *Xenopus*

Oocytes expressing WT or mutant channels. Activation voltage step protocols were followed by an additional 1 s depolarizing pulse from -80mV to +20mV applied three times every 30s. After washing in 4-AP, five depolarizing 1 s pulses from -80 mV to +20 mV were followed by the activation voltage step protocol. **(B)** Representative current traces of Kv1.2 wild-type (WT, top) and the GOF mutant channels p.(Glu157Lys) (E157K), p.(Arg297Gln) (R297Q), p.(Leu298Phe) (L298F) recorded in *Xenopus* oocytes during voltage steps with a duration of 1 s from -80 mV to +20 mV before (left traces) and after application of 4-AP for 5 min (right traces) as shown in Fig. S3A. Current traces elicited by the first voltage step after application of 4-AP are shown in red, the four following current traces are shown in black. **(C)** Voltage dependence of Kv1.2 channel activation for WT (top) and mutant channels (below) before and after application of 1 mM 4-AP. Shown are means \pm SEM. Lines represent Boltzmann functions fit to the data points. Activation curves for WT channels are shown as lines in each plot (before (solid) and after (dotted) 4-AP application). Coloured stars represent the significant difference for $V_{1/2}$ of activation curves of the respective mutant channel before and after 4-AP application. Black stars indicate a significant difference between $V_{1/2}$ of mutant channels after 4-AP application and $V_{1/2}$ of WT channels before 4-AP application (see Table S1). Significance with respect to controls is indicated as **P<0.01, ***P<0.001.

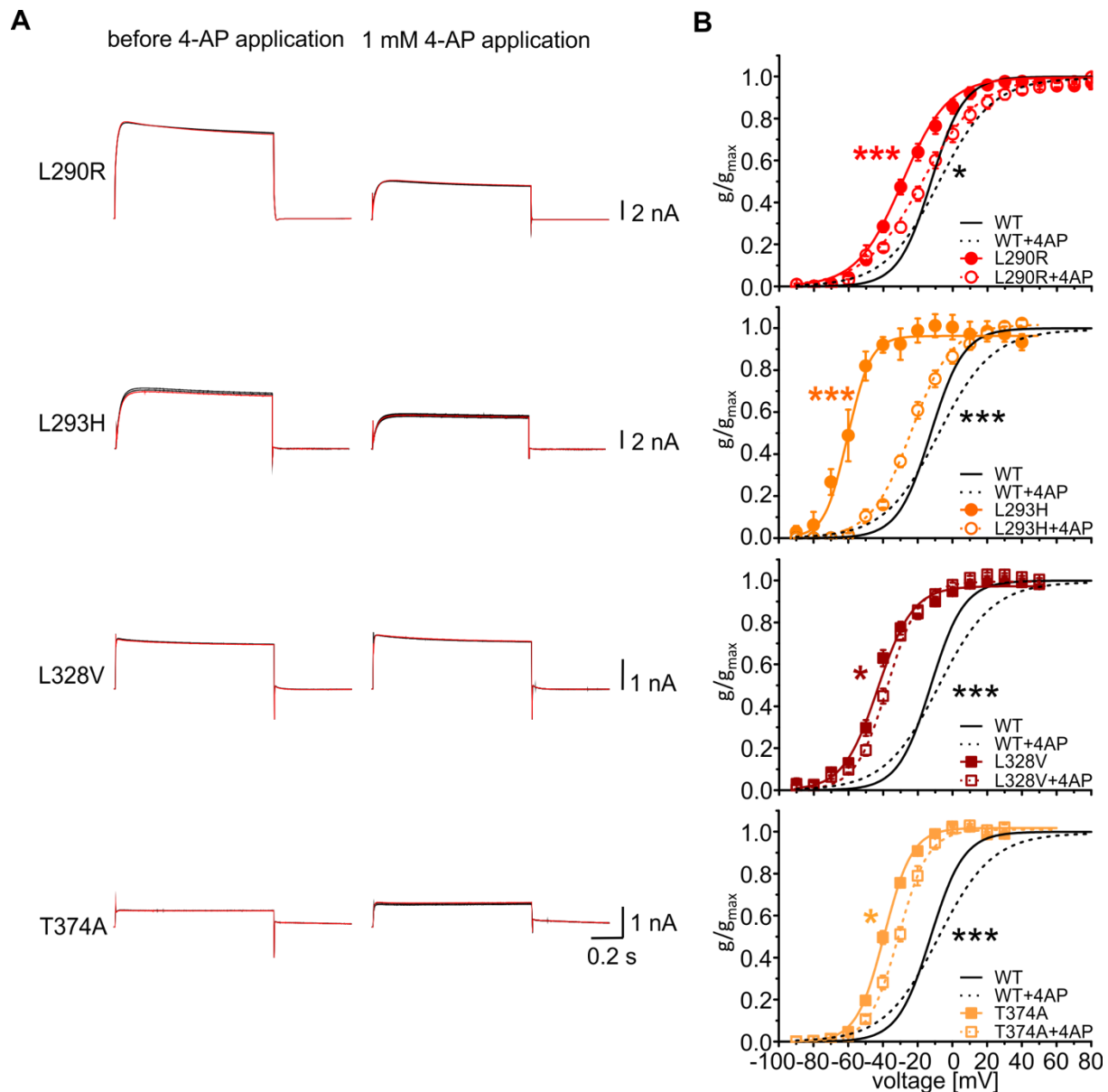


Fig. S3: 4-AP application affects current-voltage relationships of GOF+ LOF mutant Kv1.2 channels in *Xenopus* oocytes. (A) Representative current traces of the GOF+LOF mutant channels p.(Leu290Arg) (L290R), p.(Leu293His) (L293H), p.(Leu328Val) (L328V) and p.(Thr374Ala) (T374A) recorded in *Xenopus* oocytes during voltage steps with a duration of 1 s from -80 mV to +20 mV before (left traces) and after application of 4-AP for 5 min (right traces) as shown in Fig. S3A. Current traces elicited by the first voltage step after application of 4-AP are shown in red, the four following current traces are shown in black. For statistical tests of data see Table S1. (B) Voltage dependence of Kv1.2 channel activation for GOF+LOF mutant channels before and after application of 1mM 4-AP. Shown are means \pm SEM. Lines represent Boltzmann functions fit to the data points. Activation curves for WT

channels are shown as lines in each plot (before (solid) and after (dotted) 4-AP application). Coloured stars represent the significant difference for $V_{1/2}$ of activation curves of the respective mutant channel before and after 4-AP application. Black stars indicate a significant difference between $V_{1/2}$ of mutant channels after 4-AP application and $V_{1/2}$ of WT channels before 4-AP application (see Table S1). Significance with respect to controls is indicated as * $P < 0.05$, *** $P < 0.001$.

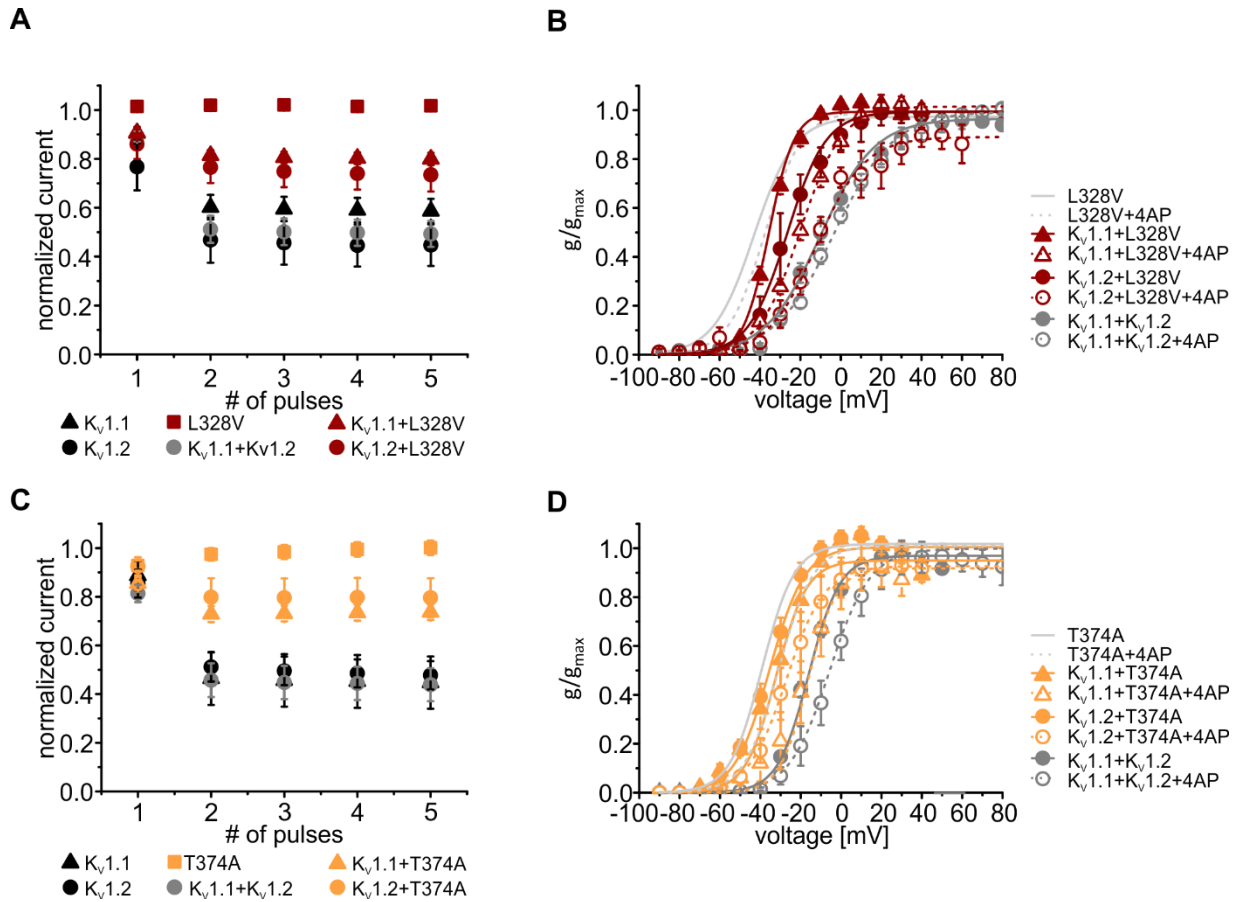


Fig. S4: Co-expression of GOF+ LOF together with $K_v1.1$ or $K_v1.2$ WT channels improved efficacy of 4-AP. (A, C) Normalized current response for WT $K_v1.2$ (black circle), WT $K_v1.1$ (black triangle), L328V (dark red box), T374A (yellow box) co-expression of WT $K_v1.2$ and WT $K_v1.1$ (grey circle), $K_v1.2$ together with mutant L328V (dark red circle) or T374A (yellow circle) and $K_v1.1$ together with mutant L328V (dark red triangle) or T374A (yellow triangle) recorded upon application of 1 mM 4-AP. Shown are current amplitudes for the five depolarizing pulses during 4-AP application (B, D) Voltage normalized to the mean values of the three pulses before 4-AP application for each cell.

dependence of channel activation for heterodimeric Kv1.1/Kv1.2 wildtype and GOF+LOF mutant channels before and after application of 1mM 4-AP. Shown are means \pm SEM. Lines represent Boltzmann functions fit to the data points. For statistical significance see Table S1.

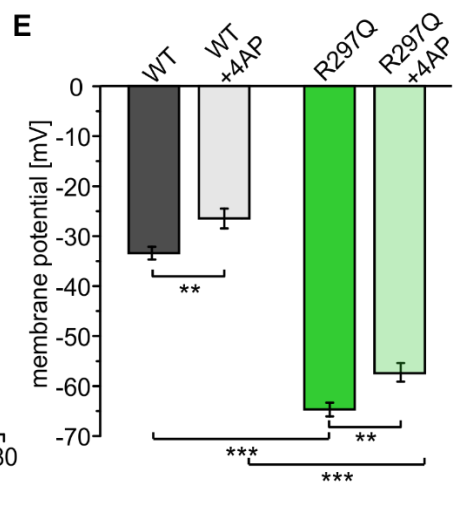
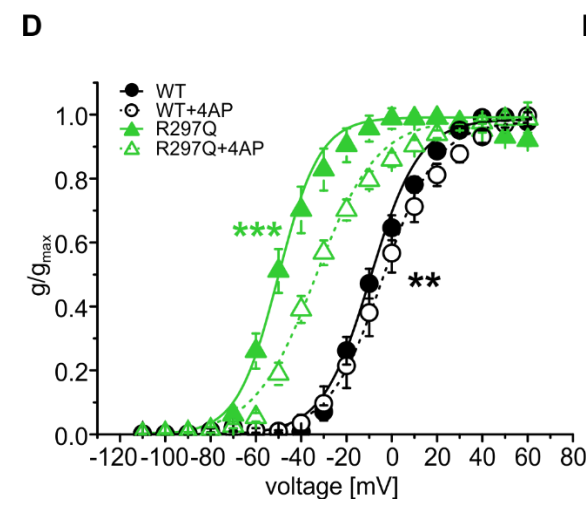
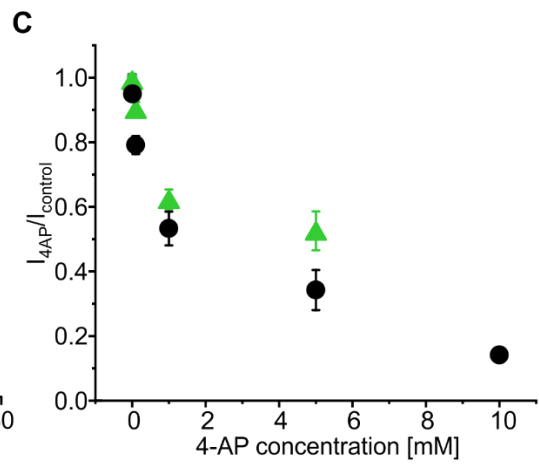
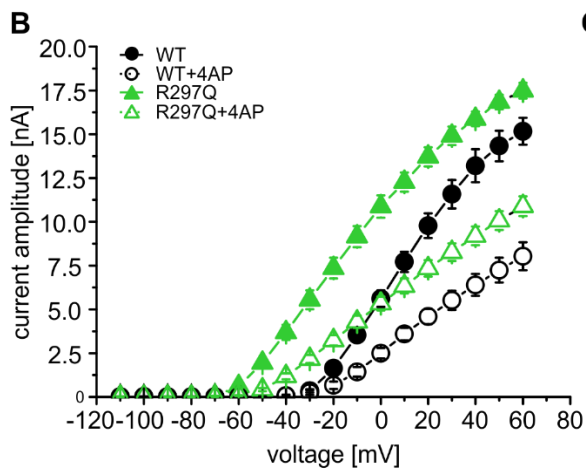
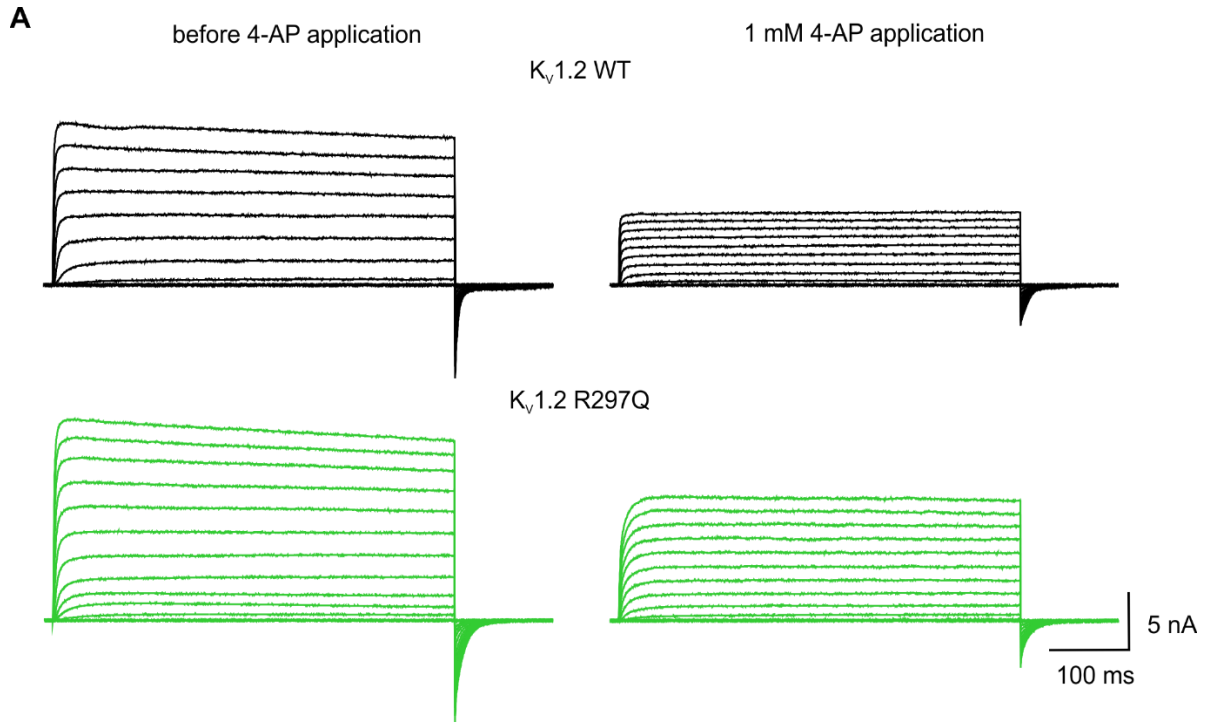


Fig. S5: 4-AP application also affects WT and GOF mutant p.(Arg297Gln) (R297Q) Kv1.2 channels in mammalian (CHO) cells. (A) Representative current traces of Kv1.2 wild-type (WT, top) and Kv1.2 R297Q (bottom) channels recorded in a CHO cells during voltage steps (500 ms steps from a holding potential of -90mV from -110 mV to 60 mV with an increment of 10 mV). (B) Current-Voltage relationship of WT and R297Q mutant channels before and after application of 4-AP. (C) Dose-response curve for WT and R297Q mutant channels recorded upon application of different 4-AP concentrations (in mM: 0.01, 0.1, 1, 5, 10) and normalized to the maximal response (0.01 mM) for each cell. Statistical significance between the IC50 values was verified by Mann-Whitney rank-sum and Dunnet's post hoc test. (D) Mean voltage dependence of Kv1.2 channel activation for WT and R297Q channels before and after application of 1mM 4-AP. Shown are means \pm SEM. Lines represent Boltzmann functions fit to data points. Green stars represent the significant difference for $V_{1/2}$ of activation curves of the mutant channel before and after 4-AP application. Black stars indicate a significant difference between $V_{1/2}$ of mutant channels after 4-AP application and $V_{1/2}$ of WT channels before 4-AP application (see Table S1) (E) Resting membrane potentials of CHO cells expressing WT and R297Q mutant channels before and after application of 4-AP. Significance with respect to controls is indicated as **P<0.01, ***P<0.001, respectively. For statistical significance see Table S1.

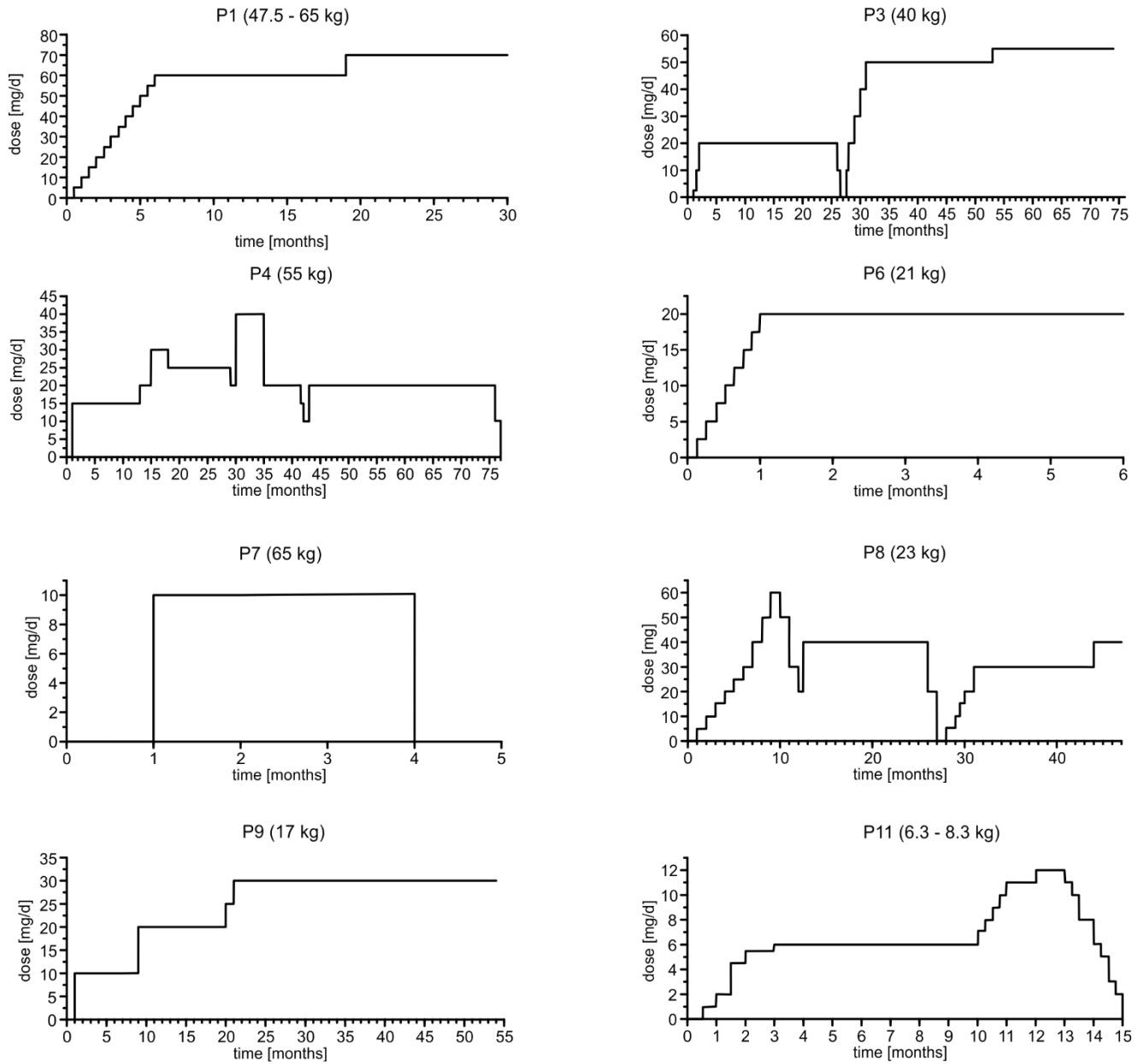


Fig. S6: Dosage of 4-AP was adjusted during treatment. 4-AP dosage in mg/d are shown for patients P1, P3, P4, P6, P7-9 and P11, the weight of the patients is given in brackets.

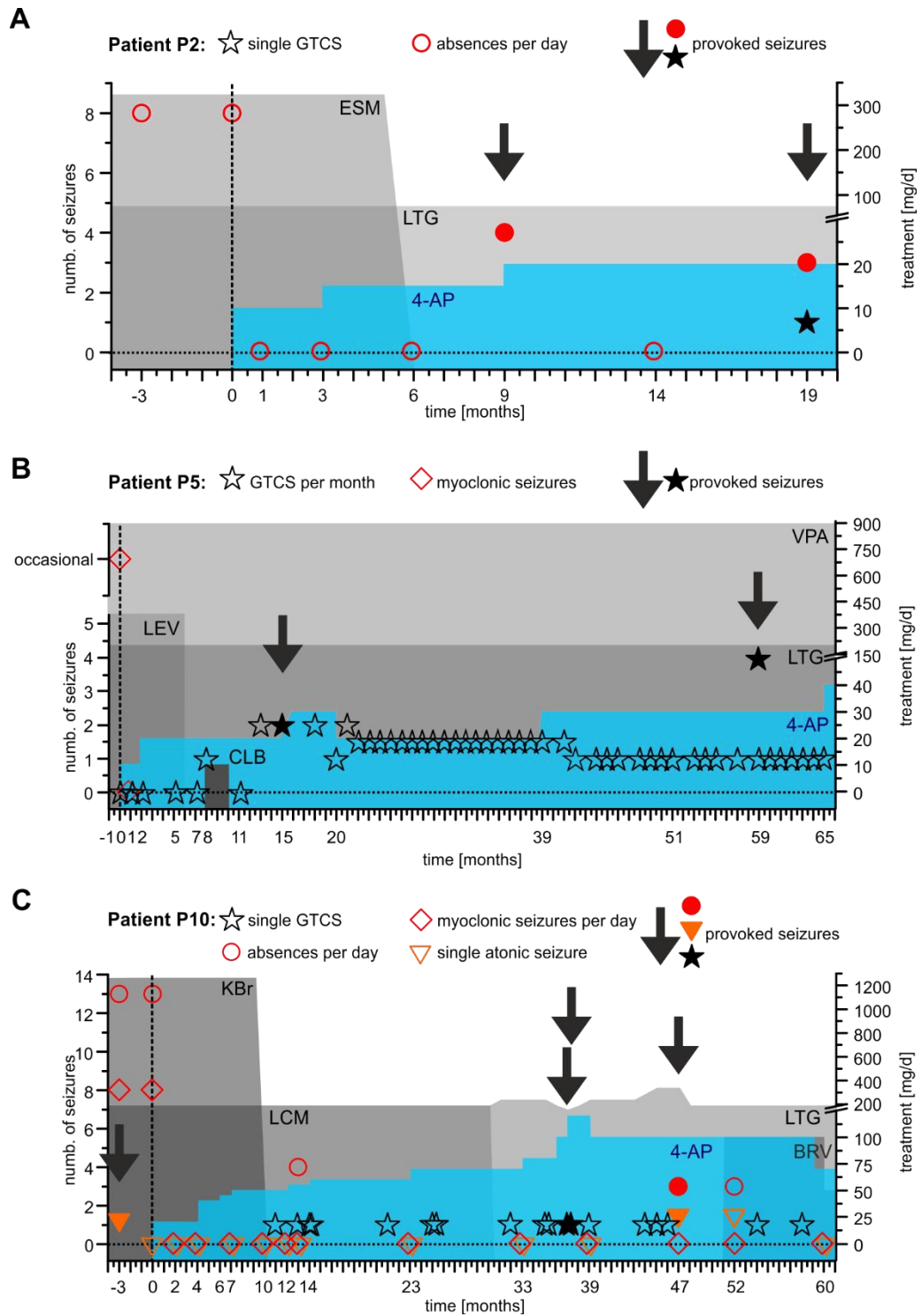


Fig. S7: Course of seizures and administered ASMs changed before and during treatment with 4-AP. Frequency of different seizure types are shown for patients P2 (A), P5 (B) and P10 (C). All administered ASMs during last follow-up before start of 4-AP treatment and during the 4-AP treatment period (blue) are shown. For P5 (B) the frequency of myoclonic seizures could not be specified since

they occurred occasionally, as described on the y axis, probably a few per month. Vertical dashed lines indicate start of 4-AP treatment. Seizure types are indicated with the symbols shown in each panel. Arrows and open symbols show provoked seizures after a vaccination without fever, reduction of concomitant medication, short-term illness such as gastritis, fever or urinary tract infection or sleep deprivation.

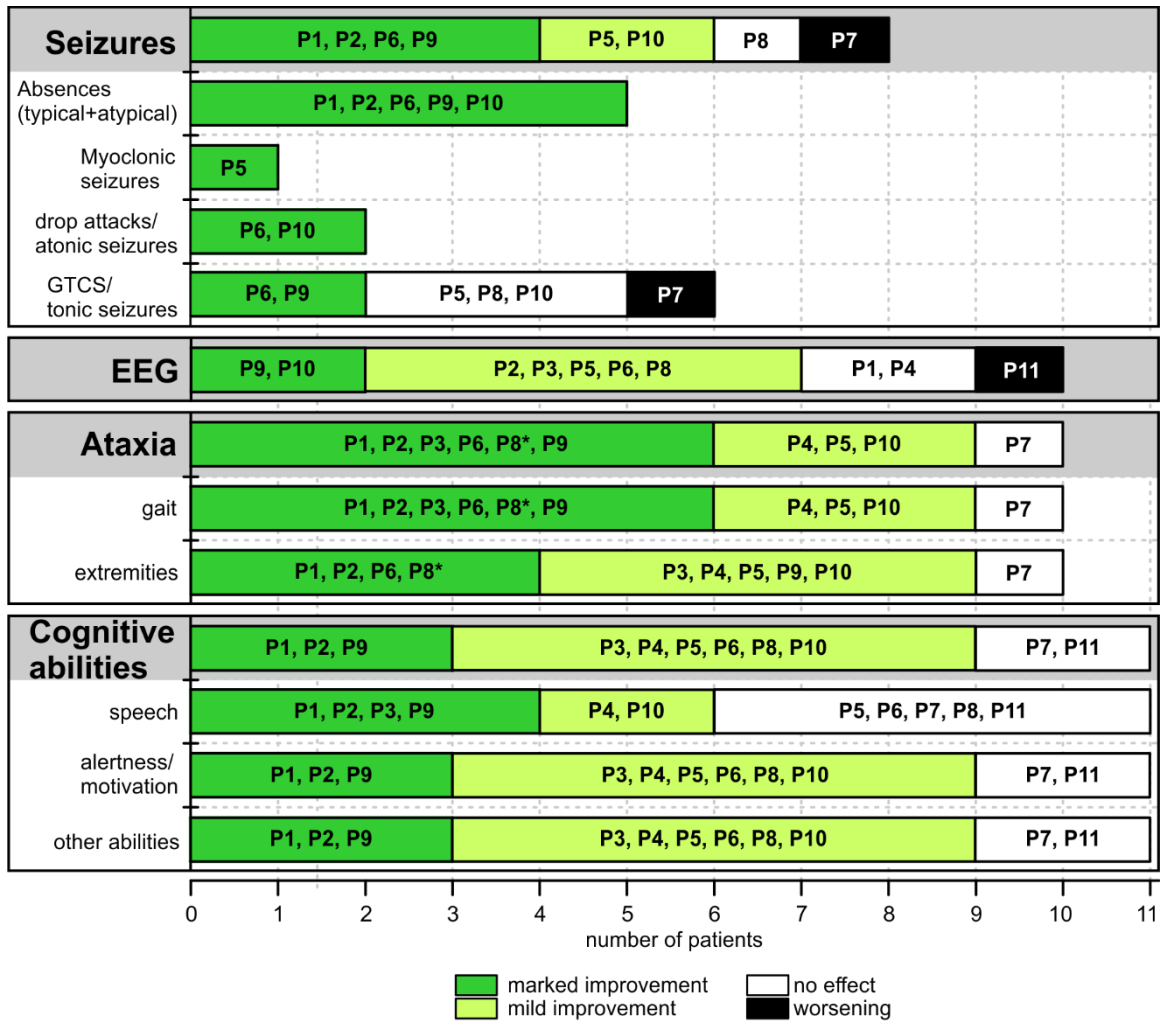


Fig. S8: Treatment with 4-AP improved seizures, ataxia and cognitive abilities. All treated patients (also non-responders P7 and P11) and their clinical outcome according to the judgement of the treating physician with start of 4-AP treatment. Columns on gray background show the general clinical outcome for each category, whereas the outcome on more specific symptoms is indicated on a white background. If less than eleven patients are shown, the respective symptoms did not apply to all, for example patients which were seizure-free before 4-AP treatment did not have absence seizures; P11 was too young and

not far enough developed to diagnose ataxia. *After an initial marked improvement, the effects of 4-AP on ataxia were attenuated gradually in P8 after six months (further details in Results text).

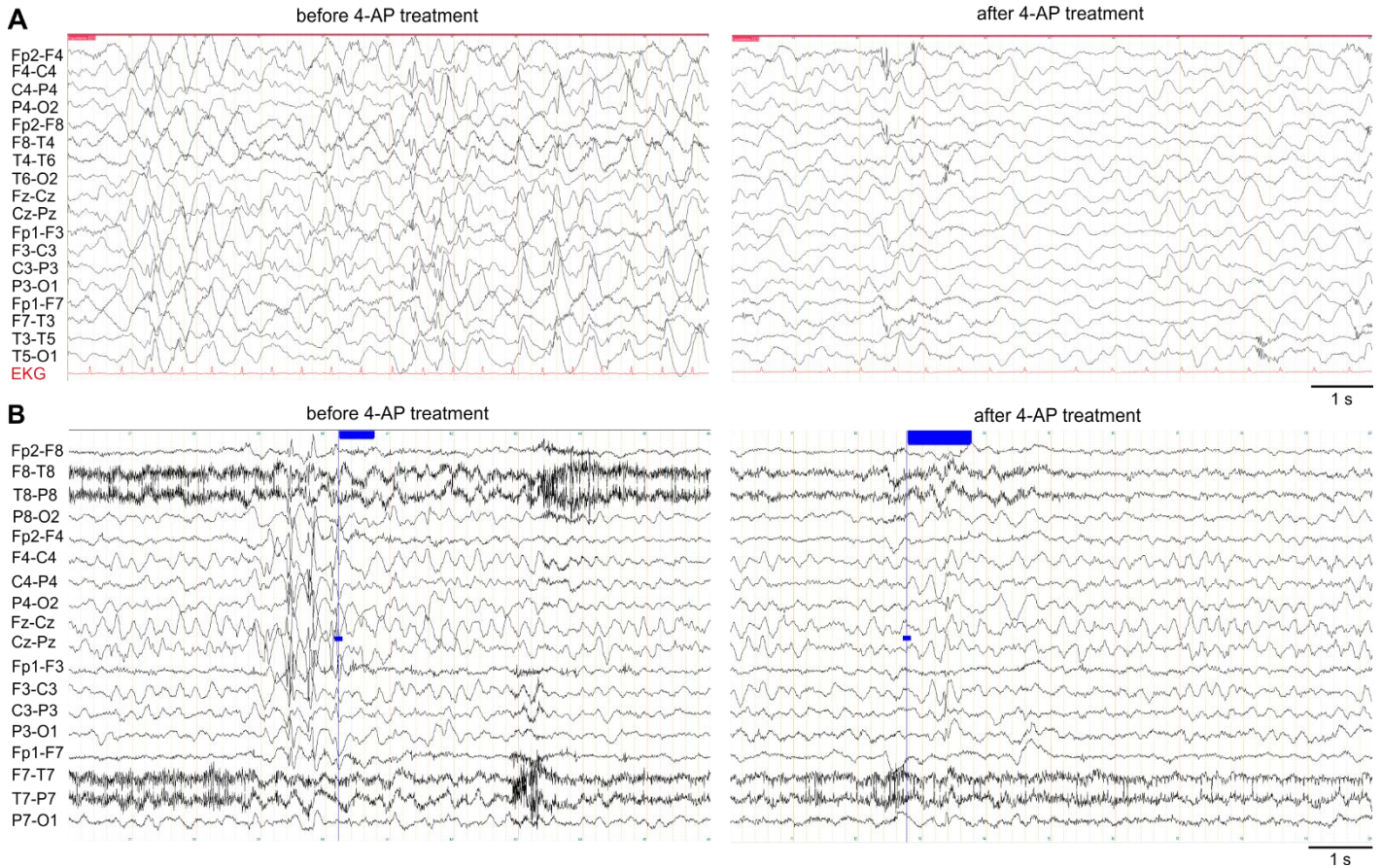



Fig. S9: 4-AP improved clinical outcome of a young and an adolescent patient. (A) Representative EEG Recordings of P6 (4 years of age, p.(Leu298Phe) variant) before and after 4-AP treatment. **Left:** Before treatment, awake, in the morning, no signs of tiredness: Approx. 10 absences per day with and without atonic falls, as well as clusters of GTCS every four weeks. EEG: paroxysmal 2.5 Hz slow waves; posterior bilateral spike and sharp waves. **Right:** Only one week after treatment with 1 mg/kg/day (20 mg/d), awake, in the morning, no signs of tiredness: No absences, no drops anymore, only one cluster of GTCS after three months. EEG: marked improvement with some slow waves and rare posterior small spikes. No change in concomitant anti-seizure medication (valproate 570 mg/d). **(B)** Representative EEG Recordings of P10 carrying the p.(Leu328Val) variant before and after 4-AP treatment, respectively. **Left:** Before treatment (0 mg 4-AP), awake, in the morning, no signs of tiredness: Approx. 10-15 absence seizures per day, approx. five myoclonic seizures per day, atonic falls only during febrile episodes, as well as rare GTCS (1-3/year). EEG: generalized Spike waves every 10

to 20 seconds, multifocal Delta slow waves. **Right:** Ten weeks after treatment with 0.75 mg/kg/day (30 mg/d), awake, in the morning, no signs of tiredness: No absences, no atonic falls, only occasional myoclonic jerks, no GTCS (but observation period for GTCS too short). EEG: marked improvement with some Delta slow waves and rare Spikes (two in 20 minutes). No change in concomitant anti-seizure medication (Lamotrigine 225 mg/d, Lacosamide 200 mg/d, Bromide 1275 mg/d).



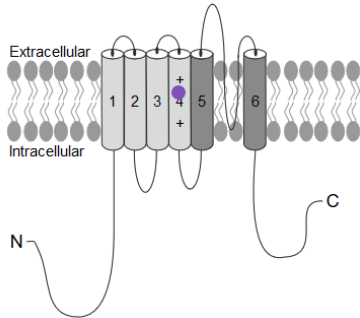
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Treatment: possible!

Kv1.2 position:	R297Q
Studied in channel:	Kv1.2
Mutation:	R297Q
Localization:	S4
Overall functional consequences:	GOF

Activation

Effect:	hyperpol. Shift (~ -40 mV)
Mutation $V_{1/2}$ [mV]:	-51.1 ± 0.5
WT $V_{1/2}$ [mV]:	-12.8 ± 1.1
Slope:	increased
Slope Mutation:	8.5 ± 0.2
Slope WT:	6.8 ± 0.3
Functional consequences:	GOF



Alignment:

Kv1.2	AMSLAAILRVI	R	LVRVFRIFKL
Kv1.1	ATSLAAILRVI	R	LVRVFRIFKL
Shaker	AMSLAAILRVI	R	LVRVFRIFKL

Reference:
[Nat Genet. 2015 Apr;47\(4\):393-399. doi: 10.1038/ng.3239. Epub 2015 Mar 9.](#)

Fig. S10: Online tool supports treatment decisions for patients with *KCNA2* variants. www.kcna2-treatment.com; user name: KCNA2; password: 4APtreatment

Tables

Table S1: Normalized current amplitudes and gating parameters of oocytes injected with WT RNA or mutant RNA and CHO cells transfected with WT or mutant DNA before and after application of 1 mM 4-AP.

	Norm current				Activation							
Oocytes												
					V _{1/2} [mV]			k-slope				
	Control	1 mM 4-AP	Sign.	N	Control	1 mM 4-AP	Sign.	Control	1 mM 4-AP	Sign.	N	
WT	0.999 ± 0.001	0.385 ± 0.019	***	58	-12.9 ± 1.4	-7.4 ± 1.9***	n.s.	8.2 ± 0.2	15.2 ± 1.5***	-	23	
GOF												
E157K	1.012 ± 0.004	0.546 ± 0.038	***	6	-18.5 ± 1.5	-16.9 ± 2.4	n.s.	15.4 ± 1.2	14.3 ± 0.9	n.s.	6	
R297Q	0.990 ± 0.007	0.628 ± 0.023	***	17	-52.8 ± 9.2	-21.3 ± 3.1***	***	15.5 ± 1.7	13.3 ± 0.8	n.s.	12	
L298F	0.974 ± 0.008	0.416 ± 0.037	***	11	-57.5 ± 1.9	-37.1 ± 1.1***	***	9.2 ± 0.7	12.7 ± 0.5			
GOF + LOF												
L290R	1.036 ± 0.007	0.512 ± 0.066	***	13	-26.4 ± 1.8	-15.2 ± 2.5***	*	13.7 ± 0.8	16.6 ± 1.0	n.s.	13	
L293H	1.008 ± 0.010	0.373 ± 0.057	***	5	-60.4 ± 3.1	-23.48 ± 1.76***	***	6.6 ± 1.3	11.8 ± 0.9*	*	5	
L328V	1.003 ± 0.002	1.017 ± 0.013	0.056	21	-43.5 ± 1.3	-38.1 ± 1.0*	***	10.0 ± 0.6	9.0 ± 0.4	***	21	
T374A	0.993 ± 0.002	1.005 ± 0.019	0.278	11	-39.2 ± 0.9	-30.3 ± 1.6*	***	7.7 ± 0.2	8.5 ± 0.4	n.s.	10	
Coexpression L328V												
K _v 1.1+K _v 1.2	1.003 ± 0.003	0.493 ± 0.053	***	10	-15.0 ± 3.5	-4.2 ± 1.4***		9.9 ± 1.3	14.9 ± 0.7*		10	
K _v 1.1+ L328V	1.010 ± 0.003	0.824 ± 0.021	***	14	-34.3 ± 1.1	-17.5 ± 1.6***	***	6.2 ± 0.4	10.6 ± 0.8***	**	14	
K _v 1.2+L328V	0.992 ± 0.007	0.735 ± 0.068	**	5	-28.8 ± 3.7	-15.9 ± 3.0**	***	6.2 ± 1.2	6.2 ± 2.1	***	5	
Coexpression T374A												
K _v 1.1+K _v 1.2	1.000 ± 0.002	0.439 ± 0.068	***	13	-16.0 ± 1.1	-5.7 ± 3.3***		7.7 ± 0.3	10.0 ± 0.8		10	
K _v 1.1+T374A	0.994 ± 0.002	0.736 ± 0.032	***	9	-32.3 ± 3.7	-16.4 ± 2.7***	*	7.6 ± 0.8	6.9 ± 1.1	n.s.	8	
K _v 1.2+T374A	1.003 ± 0.002	0.796 ± 0.080	*	7	-35.5 ± 2.2	-23.2 ± 4.3*	***	8.6 ± 0.7	8.3 ± 1.6	n.s.	6	
CHO cells												
WT	1.00 ± 0.00	0.53 ± 0.05	***	7	-9.4 ± 2.1	-3.9 ± 3.4		10.5 ± 0.4	12.0 ± 0.7	n.s.	7	
R297Q	1.00 ± 0.00	0.62 ± 0.03	***	9	-50.1 ± 3.3	-32.9 ± 2.4***	**	9.1 ± 1.3	13.7 ± 1.2*	n.s.	9	

Data are presented as mean ± SEM, k slope factor; *marked values are significantly different from the respective WT values. Values for normalized currents were tested using paired t-tests, values for activation were tested with two-way ANOVA with Tukey post hoc test. * p<0.05; ***p<0.001

Table S2: Patients' demographic and seizure outcome of all described patients with variants in the *KCNA2* gene. All so far treated patients were listed above according to their functional consequences (gain-of-function [GOF] or gain-of-function and loss-of-function [GOF+LOF]) and their age at start of treatment.

Patient # (Patient # in (5)) Variant	Gender, age, origin, Clinical Center	Age at epilepsy onset/sz type	Other sz types	Start of 4-AP treatment (age) and duration	Current ASM / Last medication (all ASM before)	Sz freq. before 4-AP	Sz freq. after 4-AP treatment
P1 (Patient 9) <i>p.(Glu157Lys)</i>	M, 17 y, American- Swedish; Mayo Clinic, MN, USA	9 m, febr. GTCS	GTCS, Atypical Abs	17 y 8 m	VPA 875 mg/d LTG 175 mg/d ACZ 1500 mg/d 4-AP 40 mg/d (CBZ, TPM, CLZ, CLB, GBP)	3 to 21 clusters of atypical Abs weekly (15 to 17 y old). 2 GTCS (at age 9 m and 13 y)	Sz-free for GTCS (since 4 y) and for Abs (since 8 m) with one interruption of 3-4 Abs (when changed to compounded immediate-release 4-AP for a few days): Markedly improved
P2 (Patient 14) <i>p.(Arg297Gln)</i>	F, 5 y, French- Spanish; UAM, Madrid, Spain	1 m, inf. Spasms	Abs (with MC & atonic sz), GTCS (often febr.)	3 y 15 m	LTG 87.5 mg/d 4-AP 20 mg/d (VPA, ESM, LEV, ZNS)	Abs (with MC & At) 7-8/day; 2 GTCS at 12 m and 1 GTCS at 22 m; Infantile spasms daily only in first months after birth	Sz-free for GTCS and Abs; only one cluster of Abs after 2 y due to relative dose reduction, with adapted dose again seizure-free: Markedly improved
P3 (Patient 13) <i>p.(Arg297Gln)</i>	F, 8 y, ovodonation; Elizalde Children's Hospital, Buenos Aires, Argentina	10 m, Abs	GTCS	4 y 39 m	VPA 500 mg/d 4-AP 55 mg/d (LCM)	Sz-free from 2.5 y of age.	Remained sz-free
P4 (Patient 10) <i>p.(Arg297Gln)</i>	M, 30 y, German; University Clinic Tuebingen, Germany	5 m, febr. sz	Abs, GTCS (often febr.), MC	25 y 50 m	LTG 350 mg/d ZNS 300 mg/d 4-AP 20 mg/d (PRM, VPA)	Sz-free from 24 y of age.	Remained sz-free

P5 (Patient 12) <i>p.(Arg297Gln)</i>	F, 40 y, Danish; Danish Epilepsy Center, Filadelfia, Denmark	10 m, febr. sz	GTCS, MC	37 y 44 m	VPA 900 mg/d LTG 200 mg/d 4-AP 30 mg/d (LEV, LCM, OXC, CLZ, PHT)	Since introduction of LEV in 2014, no GTCS, only mild MC during sleep. With LEV behavioral disturbances	Still rare GTCS/y: No clear effect; but no MC anymore: Markedly improved
P6 <i>p.(Leu298Phe)</i>	M, 4 y, German; University Clinic Tuebingen, German	10 m, tonic sz	GTCS clusters, drop Sz, Abs	4 y 3 m	VPA 570 mg/d 4-AP 20 mg/d (ESM)	Approx. 2-3 GTCS/m, approx. 100 drop sz/m, approx. 300 Abs/m (at age 4 y)	3 m sz-free for all entities; 1 Cluster of GTCS shortly before next increase of dose, no Abs, no drop sz anymore: Markedly improved
P7 (Patient 17) <i>p.(Leu298Phe)</i>	M, 38 y, British; UCL Queen Square, London & Chalfont Centre for Epilepsy, Bucks, UK	6 m, febr. GTCS	MC, atypical Abs, GTCS, eye deviation, eyelid flickering	38 y 3 m	LEV 1500 mg/d VPA 800 mg/d CBZ 800 mg/d ESM 750 mg/d (PHB, PHT, LTG, CLB, TPM, OXC, LCM, MSX)	12-17 GTCS/year over preceding 10 years at least	3 GTCS/m: Worsening
P8 (Patient 19) <i>p.(Leu290Arg)</i>	F, 11 y, Irish; National University of Ireland Galway, Ireland	2 m, suspicious events; 9 m Abs	Atypical Abs (no Abs in years prior to 4-AP), GTCS	10 y 15 m	LTG 350 mg/d CLB 22.5 mg/d ACZ 250 mg/d ESM 500 mg/d 4-AP 40 mg/d (STM, VPA, ZNS, CBZ, LEV)	1 GTCS/m.	Until over 1 year after 4-AP still 1 GTCS/m (then became sz-free for 6 m on 40mg 4- AP/day, but LTG increased also): gradual recurrence of seizures/ataxia profile. (active puberty) No clear effect
P9 (Patient 20) <i>p.(Leu293His)</i>	F, 4 y, Ashkenazi Jewish; Sheba Medical Center, Israel	3 m, gen. conv. SE	Abs, GTCS, MC	2 y 23 m	PHT 125 mg/d CLB 2.5 mg/d 4-AP 30 mg/d (PHB)	3-4 GTCS/w, daily 5-10 Abs., MC only during first year of life (stopped after administration of PHT)	On 10 mg/d 1-2 GTCS/w, no Abs; On 25 mg/d 1 GTCS/w, no Abs; On 30 mg/d sz free for 6 m; since then 1GTCS/m (!), no Abs anymore: Markedly improved

<p>P10 (Patient 21) <i>p.(Leu328Val)</i></p>	<p>M, 15 y, German; Epilepsy Center Kork, Kehl, Germany; University Clinic Tuebingen, Germany</p>	<p>6 m, febr. SE</p>	<p>Abs, MC, atonic Sz, GTCS</p>	<p>12 y 43 m</p>	<p>LTG 250 mg/d 4-AP 120 mg/d (VPA, ESM, STM, LEV, TPM, CLB, LCM, BRM)</p>	<p>Several Abs/d, 1-3 GTCS/y, atonic sz (only with fever) at age 13.5 y</p>	<p>Sz-free for Abs, MC, atonic sz: Markedly improved; 2-5 GTCS/y: No clear change, GTCS under dose reduction of co-medication (BRM, LCM, LTG)</p>
<p>P11 (Patient 24) <i>p.(Thr374Ala)</i></p>	<p>M, 1 y, American; University Clinic Tuebingen, Germany</p>	<p>1 m, MC</p>	<p>Tonic Sz</p>	<p>7 m 12 m</p>	<p>TPM 25 mg/d 4-AP 12 mg/d (LEV, PHB)</p>	<p>Sz-free since the age of 4 m (with TPM), had only tonic sz between 6 w and 4 m (start of 4-AP with 5 m). Myoclonic jerks only at age 1 m</p>	<p>Remained sz-free</p>

Abbreviations: Abs: absences, d: days, F: female, febr.: febrile, (s)GTCS: (secondary) generalized tonic-clonic seizure, M: male, m: months, MC: myoclonies, mg: milligram, s: seconds, sz: seizure, y: years.

Anti-seizure medications (ASMs): ACZ: Acetazolamide, BRM: Bromide, CBZ: Carbamazepine, CLB: Clobazam, CLZ: Clonazepam, ESM: Ethosuximide, GBP: Gabapentin, LCM: Lacosamide, LEV: Levetiracetam, LTG: Lamotrigine, MSX: Methosuximide, OXC: Oxcarbazepine, PHB: Phenobarbital, PHT: Phenytoin, PRM: Primidone, STM: Sulthiame, TPM: Topiramate, VPA: Valproic acid, ZNS: Zonisamide.

Table S3: Electrographic features before and during treatment with 4-AP. Reporting supported by SCORE (Standardized computer-based organized reporting of EEG) (61). Quantification of interictal epileptic activity is provided as spikes per hour (Sp/h), where available.

Patient # Variant	EEG <u>before</u> 4-AP treatment	EEG <u>after</u> 4-AP treatment	Rating of change after 4- AP treatment
P1 <i>p.(Glu157Lys)</i>	<p>Routine-EEGs (30 min, awake, hyperventilation, photic stimulation):</p> <ul style="list-style-type: none"> • 4 y: Normal PDR, frequent generalized spike-and-slow-waves, recorded Absences • 13 y: Abnormal PDR (4-6 Hz). Frequent generalized spike-and-slow-waves (on CLB). • 15 y: Abnormal PDR (4-6 Hz). Abundant generalized spike-and-slow-waves, runs of 20 s w/o clear correlate (off CLB) • 16 y: Abnormal PDR (3-7 Hz). Generalized spike-and-slow-waves in runs < 2s • 16.5 y: Abnormal PDR (3-7 Hz). Rare generalized spike-and-slow-waves • 17 y: Abnormal PDR (5-6 Hz). Frontal and occipital intermittent rhythmic delta activity. Rare to frequent right occipital sharp waves and generalized spike-and-slow-waves 	<p>Routine-EEGs (30 min, awake, hyperventilation, photic stimulation):</p> <ul style="list-style-type: none"> • 17.5 y, 6 m after 4-AP (40 mg/d; 0.8 mg/kg/d): Abnormal PDR (3-7 Hz). Frequent rhythmic trains of bilateral occipital spike-and-slow-waves 	No clear change
P2 <i>p.(Arg297Gln)</i>	<p>Routine-EEGs (30 min, awake, hyperventilation, photic stimulation):</p> <ul style="list-style-type: none"> • First months: Normal. No epileptic activity. <p>10 h video-EEG monitoring:</p> <ul style="list-style-type: none"> • 1.8 y: Abnormal PDR (2-3 Hz). Bilateral posterior sharp waves with high persistence in wakefulness and sleep (Quantification was not possible because this study was performed in another center) • 2.7 y: Abnormal PDR (3-5 Hz). Intermittent bilateral posterior RDA in wakefulness and sleep. Frequent (90x/1h) bilateral posterior sharp waves at 2-3 Hz with low persistence in wakefulness and sleep present in 900 sec of record, which is approx. 2.5% of time 	<p>24 h video-EEG monitoring:</p> <ul style="list-style-type: none"> • 3.5 y, 4 d after 4-AP (5 mg/d, 0.25 mg/kg/d): Unchanged. <p>4 h video-EEG monitoring:</p> <ul style="list-style-type: none"> • 4 y, 6 m after 4-AP (10 mg/d, 0.5 mg/kg/d): Unchanged • 4.7 y, 15 m after 4-AP (20 mg/d; 1.0 mg/kg/d): Abnormal PDR (3-6 Hz). Occasional posterior bilateral sharp waves with low persistence, only seen frequently (45x/h) during sleep present in 900 sec of record, which is approx. 1.25% of time 	Mildly improved
P3 <i>p.(Arg297Gln)</i>	<p>Routine-EEGs (30 min, awake, hyperventilation, photic stimulation):</p> <ul style="list-style-type: none"> • 1.5 y: GSW 2-3 Hz, during sleep GPSW, Occipital sharp waves, generalized Spike-and-slow-waves with 2-3 Hz and during sleep generalized polyspike-and-slow-waves 	<p>Routine-EEGs (30 min, awake, hyperventilation, photic stimulation):</p> <ul style="list-style-type: none"> • 5.7 y, 10 m after 4-AP (50 mg/d; 1.25 mg/kg/d): Reduction of generalized polyspike-and-slow-waves during sleep (<50%) 	Mildly improved
	<p>Routine-EEGs (30 min, awake, hyperventilation, photic stimulation):</p>	<p>Routine-EEGs (30 min, awake, hyperventilation, photic stimulation):</p> <ul style="list-style-type: none"> • 26 y, 2nd day after 4-AP initiation (10 mg/d; 0.18 mg/kg/d): 	No clear change

P4 <i>p.(Arg297Gln)</i>	<ul style="list-style-type: none"> • 6 y: generalized spike-and-slow-waves and generalized polyspike-and-slow-waves (no more information available) • 26 y: Abnormal PDR (3-4 Hz). Frequent rhythmic trains of right parieto-occipital sharp waves and, partly continuously and rhythmic; but better after activation, no status epilepticus pattern 	<p>No change to previous EEG (before 4-AP). Abnormal PDR (3-4 Hz). Frequent rhythmic trains of right parieto-occipital sharp waves and, partly continuously and rhythmic; but better after activation, no status epilepticus pattern</p> <ul style="list-style-type: none"> • 27.5 y, 1.5 y after 4-AP (25 mg/d; 0.5 mg/kg/d): Abnormal PDR (3-5 Hz). Frequent right occipital sharp waves 	
P5 <i>p.(Arg297Gln)</i>	<p>Routine-EEGs (60 min, awake and light sleep, photic stimulation):</p> <ul style="list-style-type: none"> • 37 y: Abnormal PDR (7 Hz), poorly organized. Multifocal frontal and midline epileptic activity: Occasional spikes (18x/1h), uncommon spike-and-slow-waves (4x/1h), sharp waves (3x/1h) and rhythmic trains of polyspikes (4x/1h) 	<p>Routine-EEGs (30 min, awake, hyperventilation, photic stimulation):</p> <ul style="list-style-type: none"> • 38 y, 1 y after 4-AP (30 mg/d; 0.6 mg/kg/d): Abnormal PDR (7 Hz). Multifocal frontal and midline epileptic activity: Uncommon spikes (2x/30 min) and sharp waves (1x/30 min) 	Mildly improved
P6 <i>p.(Leu298Phe)</i>	<p>Video-EEG (60 min, awake, hyperventilation, photic stimulation):</p> <ul style="list-style-type: none"> • 4 y: Abnormal PDR (4 Hz). Frequent bilateral posterior sharp waves (230x/1h). Focal to bilateral tonic-clonic seizure with diffuse seizure onset over the right posterior hemisphere 	<p>Routine-EEGs (60 min, awake, hyperventilation, photic stimulation):</p> <ul style="list-style-type: none"> • 4 y, 3 m after 4-AP (20 mg/d; 1.0 mg/kg/d): Abnormal PDR (4-5 Hz). Frequent bilateral fronto-central sharp waves and sharp-and-slow-waves (210/1h; with longer spike-free intervals of up to 15 s) <p>Routine-EEGs (20 min, awake):</p> <ul style="list-style-type: none"> • 4 y, 6 m after 4-AP (20 mg/d; 1.0 mg/kg/d): Abnormal PDR (4-5 Hz). Frequent bilateral fronto-central sharp waves and sharp-and-slow-waves (63x/20 min) 	Mildly improved
P7 <i>p.(Leu298Phe)</i>	<p>Routine-EEGs (30 min, awake, hyperventilation, photic stimulation):</p> <ul style="list-style-type: none"> • 22 y: Abnormal PDR. Frequent generalized spike-and-slow-waves 	N/A	
P8 <i>p.(Leu290Arg)</i>	<p>Routine-EEGs (25 min, awake, photic stimulation):</p> <ul style="list-style-type: none"> • 0.5 y: Focal sharp waves and generalized spike-and-slow-waves (3 Hz) • 9 y: Abnormal PDR (3-5 Hz), disorganized. Occasional left central and temporal sharp waves (6x/25 min) 	<p>Routine-EEGs (30 min, awake, photic stimulation):</p> <ul style="list-style-type: none"> • 10 y, 1 y after 4-AP (20 mg; 1.74 mg/kg/d): Abnormal PDR (3-5 Hz), poorly organized. Uncommon left central and temporal sharp waves (4x/30 min) • 12 y, 2 y after 4-AP (30 mg): Abnormal PDR (5-6 Hz), poorly organized. Uncommon left central and temporal sharp waves (3x/30 min) 	Mildly improved
P9 <i>p.(Leu293His)</i>	<p>Routine-EEGs (30 min, awake, hyperventilation, photic stimulation):</p> <ul style="list-style-type: none"> • 2 y: Abnormal PDR, disorganized. Infrequent multifocal sharp waves 	<p>Routine-EEGs (30 min, awake, hyperventilation, photic stimulation):</p> <ul style="list-style-type: none"> • 4.5 y, 1 y 8 m after 4-AP (30 mg/d; 1.76 mg/kg/d): Abnormal PDR poorly organized. >50% reduced interictal epileptic activity anymore! 	Markedly improved

<p>P10 <i>p.(Leu328Val)</i></p>	<p>Routine-EEGs (20 min, awake, hyperventilation, photic stimulation):</p> <ul style="list-style-type: none"> • 0.5 y: Right occipital spikes, propagating to centro-parietal right; FIRDA • 12 y: Abnormal PDR (4 Hz); FIRDA, multifocal spikes (2x/20 min) <p>24h-EEG (incl. sleep-EEG):</p> <ul style="list-style-type: none"> • 12.5 y: Abnormal PDR (4 Hz), FIRDA. Frequent (186x/1h) rhythmic trains of generalized and multifocal spikes and sharp waves, duration up to 4 s. In NREM sleep increased (<50%) to abundant rhythmic trains (900x/1h) 	<p>24h-EEG (incl. sleep-EEG):</p> <ul style="list-style-type: none"> • 12.75 y, 3 m after 4-AP (40 mg/d): • Abnormal PDR (4-7 Hz), FIRDA. Markedly reduced interictal epileptic activity (>50%), with only occasional multifocal parieto-central spikes, sharp waves, and generalized spike-and-slow-waves (21x/1h) • 15.5 y, 3 y after 4-AP (100 mg/d): Stable to 24h-EEG at the age of 12.75 y; abnormal PDR (4-7 Hz), FIRDA. Slightly reduced interictal epileptic activity, with occasional multifocal parieto-central spikes, sharp waves, and generalized spike-and-slow-waves (16x/1h) <p>Routine-EEGs (20 min, awake, hyperventilation, photic stimulation):</p> <ul style="list-style-type: none"> • 13 y, 6 m after 4-AP (50 mg/d): Abnormal PDR (4 Hz), FIRDA. No epileptic activity. • 14.5 y, 2 y after 4-AP (60 mg/d): Abnormal PDR (4-7 Hz), FIRDA. Frequent bilateral sharp waves fronto-central & generalized spike-and-slow-waves • 14.5 y, 2 y after 4-AP (70 mg/d): Abnormal PDR (4-7 Hz), FIRDA. Frequent bilateral sharp waves fronto-central & generalized spike-and-slow-waves • 15.5 y, 3 y after 4-AP, few days after dose increase (100mg/d to 120 mg/d) and 24h-EEG (s. above): No epileptic activity in short recording (approx. 10 min), due to incomppliance of the patient, FIRDA 	<p>Markedly improved in 24h-EEG</p>
<p>P11 <i>p.(Thr374Ala)</i></p>	<p>Routine-EEGs (60 min, awake, hyperventilation, photic stimulation):</p> <ul style="list-style-type: none"> • 6 m: PDR normal for age (3-5 Hz). Bilateral fronto-central slowing (6 Hz). Frequent to abundant bilateral fronto-central sharp waves (360x /1 h) 	<p>Routine-EEGs (60 min, awake, hyperventilation, photic stimulation):</p> <ul style="list-style-type: none"> • 6 m, 3 d after 4-AP (4.5 mg; 0.6 mg/kg/d): No change to previous EEG. • 6 m, 10 d after 4-AP (6.5 mg; 1.0 mg/kg/d): No change to previous EEGs; abnormal PDR (3-4 Hz). Abundant diffuse bilateral and left centro-parietal sharp waves and spike-and-slow-waves with left temporal diffusion (1660 /1 h), but after seizure occurred directly before • 1.5 y, 12 m after 4-AP (9 mg/d; TPM withdrawal): Rather worsening of EEG; abnormal PDR (3-5 Hz). Abundant diffuse bilateral and left centro-parietal sharp waves and spike-and-slow-waves with left temporal diffusion (1800x /1 h) 	<p>Worsening</p>

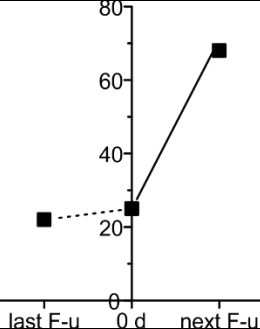
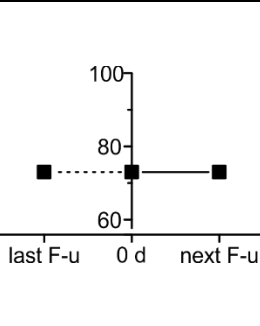
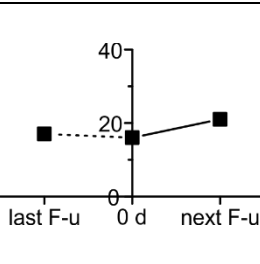
Incidence (for single discharges): Only once; Rare (less than 1/h); Uncommon (1/5 min to 1/h); Occasional (1/min to 1/5min); Frequent (1/10 s to 1/min); Abundant (>1/10 s).

Prevalence (for trains/bursts): Rare (<1%); Occasional (1-9%); Frequent (10-49%); Abundant (50-89%); Continuous (>90%).

Abbreviations: bilat.: bilateral, centr.: central, dom.: dominant, FIRDA: frontal intermittent rhythmic delta activity, foc.: focal, front.: frontal, gen.: generalized, GSW: generalized spike-wave complexes, GPSW: generalized polyspike-wave complexes, Hz: Hertz, occ.: occipital, NREM – Non-rapid eye movement sleep, OIRDA: occipital intermittent rhythmic delta activity, par.: parietal, PDR: posterior dominant rhythm, post.: posterior, REM: rapid eye movement, Sp: Spikes, SW: sharp waves, temp.: temporal, w/o: without.

Table S4: Ataxia and motor function of all described patients with variants in the KCNA2 gene.

Patient # Variant	Ataxia & Motor function <u>before</u> 4-AP treatment	Ataxia & Motor function <u>after</u> 4-AP treatment (dose)	Change of Sara Score (patients > 7ys)	Rating of change after 4-AP treatment	Motor function – WeeFIM subscore @ last follow-up (F-u) before treatment, start of treatment (0 d) and next F-u at stable dose								
P1 <i>p.(Glu157Lys)</i>	<p>Onset: 3 y</p> <p>Gait: Progressive coordination deficits with broad-based ataxic gait</p> <p>Extremities: impaired Coordination and fine motor skills, dysmetria of all extremities, dysdiadochokinesia</p> <p>Other: Large jerks and tremor of all extremities (myoclonus), head titubation</p>	<p>Start of treatment: 17 y</p> <p>6 m after 4-AP (40 mg/d, 0.8 mg/kg/d):</p> <p>Gait: Improved coordination, smoother gait with improved posture and control:</p> <p>Extremities: Dysmetria and poor alternating hand movement right greater than left</p> <p>Other: Large jerks and tremors of extremities</p>	-2 [9.5→7.5 of 40]	<p>Gait: Markedly improved</p> <p>Extremities: Markedly improved</p>	<table border="1"> <caption>WeeFIM subscore for Patient P1</caption> <thead> <tr> <th>Time Point</th> <th>WeeFIM Subscore</th> </tr> </thead> <tbody> <tr> <td>last F-u</td> <td>~45</td> </tr> <tr> <td>0 d</td> <td>45</td> </tr> <tr> <td>next F-u</td> <td>~85</td> </tr> </tbody> </table>	Time Point	WeeFIM Subscore	last F-u	~45	0 d	45	next F-u	~85
Time Point	WeeFIM Subscore												
last F-u	~45												
0 d	45												
next F-u	~85												
P2 <i>p.(Arg297Gln)</i>	<p>Onset: since birth, was noticed when first sat and walked</p> <p>Gait:</p> <p>19 m: Started walking, no previous crawling, not able to jump or run Since first steps ataxia with broad-based ataxic gait, walks alone with difficulty and frequent falls</p> <p>Extremities: Parents always noticed impaired coordination and fine motor skills, limb dysmetria (since first months of life)</p> <p>Other: Episodes of worsening of motor skills without any clear trigger with a variable duration of hours up to several days</p>	<p>Start of treatment: 3.5 y.m</p> <p>6 m after 4-AP (15 mg/d):</p> <p>Gait: No falls, greater speed.</p> <p>15 m after 4-AP (20 mg/d; 1.0 mg/kg/d):</p> <p>Gait: No falls, can jump and run.</p> <p>Extremities: Can draw simple figures, no dysmetria:</p>	Not valid, since patient < 7ys	<p>Gait: Markedly improved</p> <p>Extremities: Markedly improved</p>	<table border="1"> <caption>WeeFIM subscore for Patient P2</caption> <thead> <tr> <th>Time Point</th> <th>WeeFIM Subscore</th> </tr> </thead> <tbody> <tr> <td>last F-u</td> <td>~30</td> </tr> <tr> <td>0 d</td> <td>35</td> </tr> <tr> <td>next F-u</td> <td>~60</td> </tr> </tbody> </table>	Time Point	WeeFIM Subscore	last F-u	~30	0 d	35	next F-u	~60
Time Point	WeeFIM Subscore												
last F-u	~30												
0 d	35												
next F-u	~60												

<p>p3 <i>p.(Arg297Gln)</i></p>	<p>Onset: 15 m 3y 2m: Gait: Progressive coordination deficits with broad-based ataxic gait 3y 6m: Walks alone Extremities: Impaired fine motor skills, ataxia of all extremities, dysdiadocho-kinesia</p>	<p>Start of treatment: 4.10 y.m 12 m after 4-AP (50 mg/d; 1.25 mg/kg/d): Gait: Gait and walking is much more stable, less broad-based, gained speed. increased the speed of movement and safety when walking, with a decrease in falls due to tripping. Extremities: Moderate improvement in dysmetria and dysdiadochokinesia in the upper extremities.</p>	<p>Not done</p>	<p>Gait: Markedly improved Extremities: Mildly improved</p>	 <table border="1"> <caption>Data for Patient p3 Graph</caption> <thead> <tr> <th>Time Point</th> <th>Value</th> </tr> </thead> <tbody> <tr> <td>last F-u</td> <td>~20</td> </tr> <tr> <td>0 d</td> <td>~25</td> </tr> <tr> <td>next F-u</td> <td>~70</td> </tr> </tbody> </table>	Time Point	Value	last F-u	~20	0 d	~25	next F-u	~70
Time Point	Value												
last F-u	~20												
0 d	~25												
next F-u	~70												
<p>p4 <i>p.(Arg297Gln)</i></p>	<p>Onset: 1 y 2 m Gait: 2y: Markedly ataxic gait. 3y: Progressive coordination deficits with broad-based ataxic gait. 26y: Persistent strong gait ataxia. Extremities: 3y: Impaired fine motor skills, dysdiadochokinesia. 26y: Strong ataxia of all extremities.</p>	<p>Start of treatment: 26 y 1.5y after 4-AP (25 mg/d; 0.5 mg/kg/d): Gait: More stable and less broad-based ataxic gait; deterioration after reduction of 4-AP (to 10mg/d), and again improvement after re-starting 4-AP. However, no change after a second trial of stopping the medication. Extremities: Less upper limb dysmetria, improved lifting of a glass to the mouth, improved tying of his shoelaces.</p>	<p>-1 [16/40 before treatment → 15/40 with 30 mg 4-AP/d]</p>	<p>Gait: Mildly improved Extremities: Mildly improved</p>	 <table border="1"> <caption>Data for Patient p4 Graph</caption> <thead> <tr> <th>Time Point</th> <th>Value</th> </tr> </thead> <tbody> <tr> <td>last F-u</td> <td>~70</td> </tr> <tr> <td>0 d</td> <td>~75</td> </tr> <tr> <td>next F-u</td> <td>~75</td> </tr> </tbody> </table>	Time Point	Value	last F-u	~70	0 d	~75	next F-u	~75
Time Point	Value												
last F-u	~70												
0 d	~75												
next F-u	~75												
<p>p5 <i>p.(Arg297Gln)</i></p>	<p>Onset: 13 m Gait: Broad-based gait, walks only with help (rollator or person), no autonomous walking</p>	<p>Start of treatment: 37 y 3 y after 4-AP (20 mg/day; 0.4 mg/kg/d): Gait: Mild improvement of balance & coordination, little less wide-based, but not autonomous (still needs rollator). (Upper) Extremities: mild improvement of the coordination especially when eating, and of the tremor.</p>	<p>Not done</p>	<p>Gait: Mildly improved Extremities: Mildly improved</p>	 <table border="1"> <caption>Data for Patient p5 Graph</caption> <thead> <tr> <th>Time Point</th> <th>Value</th> </tr> </thead> <tbody> <tr> <td>last F-u</td> <td>~20</td> </tr> <tr> <td>0 d</td> <td>~20</td> </tr> <tr> <td>next F-u</td> <td>~25</td> </tr> </tbody> </table>	Time Point	Value	last F-u	~20	0 d	~20	next F-u	~25
Time Point	Value												
last F-u	~20												
0 d	~20												
next F-u	~25												
<p>p6 <i>p.(Leu298Phe)</i></p>	<p>Onset: 1 y 8 m Gait: Walks freely without assistance, but uncertain gait with a pronounced tendency to fall (undirected), can run (also tendency to fall), can climb up a slide with help and slides down independently. Extremities: Reduced spontaneous activity, puzzle not possible due to severe dysmetria. Tries to fill sand in a bucket.</p>	<p>Start of treatment: 4.3 y.m 3 m after 4-AP (20mg, 1.0 mg/kg/d): Gait: Gait with less falls. Extremities: Climbs better, unscrews devices, pins photos on the wall (all things he couldn't do before 4-AP), can do puzzles, more alert and therefore more activities. All features have improved from age 4 y on (start 4-AP). Dysmetria is still present, but only mild.</p>	<p>Not valid, since patient < 7ys</p>	<p>Gait: Markedly improved Extremities: Markedly improved</p>									

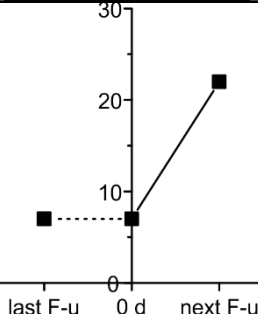
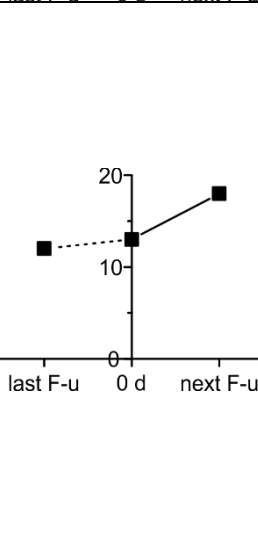
<p>P7 <i>p.(Leu298Phe)</i></p>	<p>Onset: 20 y Gait: Marked ataxia, totally dependent, chairbound.</p>	<p>Start of treatment: 38 y 3 m after 4-AP (10 mg/d ; 0.15 mg/kg/d): No change during short period of time.</p>	<p>Not done</p>	<p>No effect</p>									
<p>P8 <i>p.(Leu290Arg)</i></p>	<p>Onset: 18 m (when walked first) Gait: 11 y: Walks 8 m in 13 s with considerable staggering, but without support. Extremities: Dysmetric finger chase, remarkable dysdiadochkinesia, unable to perform fast alternating hand movements or a single heel-shin slide</p>	<p>Start of treatment: 10 y 1 y after 4-AP (40 mg; 1.74 mg/kg/d): Gait: More stable in limb movements and gait (for example balance standing better, not “rolling” on feet, fluidity of movements is better, less bumping due to clumsiness, less falling and more confidence: Extremities: Improved dysmetric finger chase and dysdiadochkinesia, able to perform fast alternating hand movements and single heel-shin slide.</p>	<p>-10.5 [24/40 →13.5/40]</p>	<p>Gait: Markedly improved* Extremities: Markedly improved*</p>	<table border="1"> <caption>Data for P8 Graph</caption> <thead> <tr> <th>Time Point</th> <th>Value</th> </tr> </thead> <tbody> <tr> <td>last F-u</td> <td>35</td> </tr> <tr> <td>0 d</td> <td>35</td> </tr> <tr> <td>next F-u</td> <td>40</td> </tr> </tbody> </table>	Time Point	Value	last F-u	35	0 d	35	next F-u	40
Time Point	Value												
last F-u	35												
0 d	35												
next F-u	40												
<p>P9 <i>p.(Leu293His)</i></p>	<p>Onset: 20 m when trying to stand Gait: 20 m: Could not walk independently, ataxic stance Extremities: 20 m: Choreoathetosis.</p>	<p>Start of treatment: 2.10 y.m 1 y 8 m after 4-AP (30 mg/d; 1.76 mg/kg/d): Gait: Walks independently. Extremities: Less choreoathetotic movements.</p>	<p>Not valid, since patient < 7ys</p>	<p>Gait: Markedly improved Extremities: Mildly improved</p>	<table border="1"> <caption>Data for P9 Graph</caption> <thead> <tr> <th>Time Point</th> <th>Value</th> </tr> </thead> <tbody> <tr> <td>last F-u</td> <td>25</td> </tr> <tr> <td>0 d</td> <td>25</td> </tr> <tr> <td>next F-u</td> <td>50</td> </tr> </tbody> </table>	Time Point	Value	last F-u	25	0 d	25	next F-u	50
Time Point	Value												
last F-u	25												
0 d	25												
next F-u	50												
<p>P10 <i>p.(Leu328Val)</i></p>	<p>Onset: 3 y (when walked first) Gait: Could walk short distances without help and assistance, often along walls, standing still not possible, could not walk downhill without stumbling Extremities: Severe ataxia with dysmetria, problems with finding a keyhole.</p>	<p>Start of treatment: 12 y 6 m after 4-AP (60 mg/d; 1.5 mg/kg/d): Gait: Can walk downhill, faster and without stumbling, can walk and stand on a sofa (not possible before 4-AP). Extremities: Can pick up objects from the ground, can eat more independently, finds key whole. Other: uses a bicycle as a wheel. 2 y 8 m after 4-AP (120 mg/d; 3.0 mg/kg/d): Gait: Can walk and stand on a sofa (not possible under 60 mg/d), smoother gait, less fluctuating. Extremities: Less upper limb dysmetria, better coordination of hands, especially when eating: 3 y 5 m after 4-AP (reduction to 80 mg/d; 1.7 mg/kg/d):</p>	<p>-2 [18/40→16/40]</p>	<p>Gait: Mildly improved Extremities: Mildly improved</p>	<table border="1"> <caption>Data for P10 Graph</caption> <thead> <tr> <th>Time Point</th> <th>Value</th> </tr> </thead> <tbody> <tr> <td>last F-u</td> <td>60</td> </tr> <tr> <td>0 d</td> <td>60</td> </tr> <tr> <td>next F-u</td> <td>70</td> </tr> </tbody> </table>	Time Point	Value	last F-u	60	0 d	60	next F-u	70
Time Point	Value												
last F-u	60												
0 d	60												
next F-u	70												

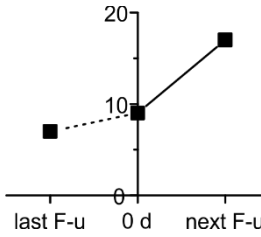
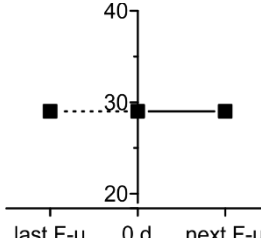
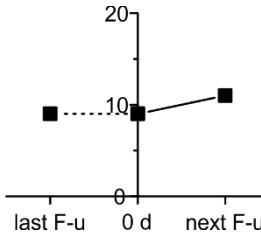

		<p>Gait: gait stability worse than with 120, and much worse than with 100 mg/d.</p> <p>3 y 6 m after 4-AP (increase to 100 mg/d; 2.2 mg/kg/d):</p> <p>Gait: gait stability improves again, less falls.</p>			
<p>P11</p> <p><i>p.(Thr374Ala)</i></p>	<p>No ataxia obvious. Most severely affected from birth</p> <p>5 m:</p> <p>Persistent choreatic movements of arms and legs, proximal and distal, including finger movements, no asymmetry, opisthonic movements, no head control, no turning of the body</p>	<p>Start of treatment: 0.7 y.m</p> <p>6 m after 4-AP (12 mg/d; 1.5 mg/kg/d): No ataxia</p> <p>Other symptoms (chorea, hypotonia): No change after 6 m.</p>	Not valid, since patient < 7ys	No effect	

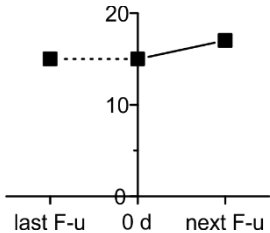
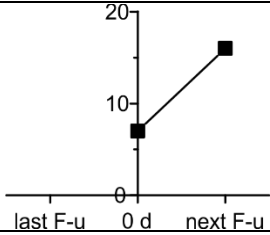
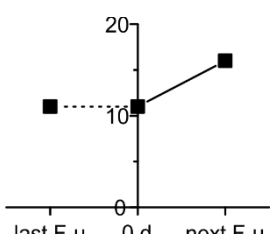
*ataxia initially markedly improved, but effect attenuated after 6 months and became gradually mild and abated (deterioration during puberty – see text).

The WeeFIM instrument and its motor subscore provides an indication of functional outcomes and aims to measure changes in patients' performance in self-care, continence, mobility and locomotion over time. Specific information, which was asked for with the WeeFIM questions, were integrated in the overall clinical assessment of patients and their individual improvements and can differ from single subscores in the WeeFIM. Therefore, substantial improvements in the WeeFIM can be associated with only mild improvements in the overall rating of change after 4-AP treatment (P3 and P9) and no clear changes in the WeeFIM score can be associated with mild clinical overall improvements of ataxia (P4).

Table S5: Cognitive abilities of all described patients with variants in the *KCNA2* gene.

Patient # Variant	Cognitive abilities & speech before 4-AP treatment	Cognitive abilities & speech after 4-AP treatment	Rating of change after 4-AP treatment	Cognition – WeeFIM subscore @ last follow-up (F-u) before treatment, start of treatment (0 d) and next F-u at stable dose								
P1 <i>p.(Glu157Lys)</i>	<p>Onset of developmental delay at <3 y</p> <p>Speech: Slow speech, labored and broken, scanning speech with flat intonation, uneven speech cadence, short responses, speech was unintelligible to most outside family members and friends</p> <p>Alertness/motivation: Not motivated to engage in conversations, IQ score 47 (14 y) and 57 (15 y)</p>	<p>6 m after 4-AP (40 mg/d; 0.8 mg/kg/d):</p> <p>Speech: Faster and smoother, improved cadence, 70% intelligible, improved functions and more deliberate in his answers.</p> <p>Alertness/motivation: More spontaneously and alert, speech much more fluid.</p>	<p>Speech: Markedly improved</p> <p>Alertness/motivation: Markedly improved</p>	 <table border="1"> <caption>WeeFIM Subscore Data for Patient P1</caption> <thead> <tr> <th>Time Point</th> <th>WeeFIM Subscore</th> </tr> </thead> <tbody> <tr> <td>last F-u</td> <td>~5</td> </tr> <tr> <td>0 d</td> <td>~8</td> </tr> <tr> <td>next F-u</td> <td>~22</td> </tr> </tbody> </table>	Time Point	WeeFIM Subscore	last F-u	~5	0 d	~8	next F-u	~22
Time Point	WeeFIM Subscore											
last F-u	~5											
0 d	~8											
next F-u	~22											
P2 <i>p.(Arg297Gln)</i>	<p>Onset of deviation in development at < 2 y (first assessment)</p> <p>Speech: 2 y: First simple words 2 y 8 m: Single words 3 y 5 m: Single words, does not construct sentences, does not understand simple orders, episodes of worsening of language without any clear trigger with a variable duration of hours up to several days</p> <p>Alertness/motivation: Good eye contact, but inconstant behavior, sometimes restless, irritable and aggressive, very shy, spontaneous smiling</p> <p>Other abilities: Not able to count objects, frequent awakenings during sleep</p>	<p>15 m after 4-AP (20 mg/d; 1.0 mg/kg/d):</p> <p>Speech: 2 to 3-word-sentences, incomplete structure of sentences with frequent omission of verbs.</p> <p>Alertness/motivation: More interactive although continues with sociability problems, perhaps autistic behaviour/ASD.</p> <p>Other abilities: Improvement in sleep with less sleep interruptions/awakenings.</p>	<p>Speech: Markedly improved</p> <p>Alertness/motivation: Markedly improved</p> <p>Other abilities: Markedly improved</p>	 <table border="1"> <caption>WeeFIM Subscore Data for Patient P2</caption> <thead> <tr> <th>Time Point</th> <th>WeeFIM Subscore</th> </tr> </thead> <tbody> <tr> <td>last F-u</td> <td>~10</td> </tr> <tr> <td>0 d</td> <td>~12</td> </tr> <tr> <td>next F-u</td> <td>~18</td> </tr> </tbody> </table>	Time Point	WeeFIM Subscore	last F-u	~10	0 d	~12	next F-u	~18
Time Point	WeeFIM Subscore											
last F-u	~10											
0 d	~12											
next F-u	~18											

<p>P3 <i>p.(Arg297Gln)</i></p>	<p>Onset of deviation in development at 6 m, all milestones later Speech: 3 y: First speech 4 y 10 m: Phrases of 1-3 words. Others: IQ score 41</p>	<p>12 m after 4-AP (50 mg/d; 1.25 mg/kg/d): Speech: Sustained improvement over 2.5 y, much better vocabulary, longer sentences (5-6 words), more fluent, faster responses. Alertness / motivation: Manifests behavioral disorders with poor tolerance for frustration.</p>	<p>Speech: Markedly improved Alertness/motivation: Mildly improved</p>	 <table border="1"> <caption>Graph Data for P3</caption> <thead> <tr> <th>Time Point</th> <th>Score</th> </tr> </thead> <tbody> <tr> <td>last F-u</td> <td>5</td> </tr> <tr> <td>0 d</td> <td>10</td> </tr> <tr> <td>next F-u</td> <td>18</td> </tr> </tbody> </table>	Time Point	Score	last F-u	5	0 d	10	next F-u	18
Time Point	Score											
last F-u	5											
0 d	10											
next F-u	18											
<p>P4 <i>p.(Arg297Gln)</i></p>	<p>Onset of deviation in development at 2 y Speech: Impairment of communicative skills. 16 y: Slowed speech 26 y: Scanning speech, no aphasia, unaffected speech comprehension, repetition of simple sets w/o problems, regular vocal loudness Alertness/motivation: Reduced direct eye contact, not immediately fixed the eyes of the opposite, reduced verbal and cognitive engagement with interaction partners and surrounding, spontaneously smiling and laughing, but always points to the ground Others: IQ score 42</p>	<p>1.5y after 4-AP (25 mg/d; 0.5 mg/kg/d): Speech: Slightly more fluent, single 2- to 3-word-sentences, partly half sentences, incomplete sentence structure with omission of verbs. Alertness/motivation: More direct eye contact, more verbal and cognitive engagement with interaction partners and surrounding, no abstract or conceptual thinking possible.</p>	<p>Speech: Mildly improved Alertness/motivation: Mildly improved Other abilities: Mildly improved</p>	 <table border="1"> <caption>Graph Data for P4</caption> <thead> <tr> <th>Time Point</th> <th>Score</th> </tr> </thead> <tbody> <tr> <td>last F-u</td> <td>28</td> </tr> <tr> <td>0 d</td> <td>30</td> </tr> <tr> <td>next F-u</td> <td>30</td> </tr> </tbody> </table>	Time Point	Score	last F-u	28	0 d	30	next F-u	30
Time Point	Score											
last F-u	28											
0 d	30											
next F-u	30											
<p>P5 <i>p.(Arg297Gln)</i></p>	<p>Onset of deviation in development at 13 m 37 y: Speech: Very limited language capabilities, not able to count or spell words Other abilities: Stubborn and sometimes irritable/aggressive behaviour</p>	<p>3 y after 4-AP (20 mg/day; 0.4 mg/kg/d): Speech: No change. Alertness/ motivation: According to the mother and caregivers, she is more alert. Other abilities: Little more autonomous, cognition and behaviour unchanged.</p>	<p>Speech: No effect Alertness/motivation: Mildly improved Other abilities: Mildly improved</p>	 <table border="1"> <caption>Graph Data for P5</caption> <thead> <tr> <th>Time Point</th> <th>Score</th> </tr> </thead> <tbody> <tr> <td>last F-u</td> <td>10</td> </tr> <tr> <td>0 d</td> <td>10</td> </tr> <tr> <td>next F-u</td> <td>12</td> </tr> </tbody> </table>	Time Point	Score	last F-u	10	0 d	10	next F-u	12
Time Point	Score											
last F-u	10											
0 d	10											
next F-u	12											
<p>P6 <i>p.(Leu298Phe)</i></p>	<p>Onset of deviation in development at < 1 y 4 y 2 m: Speech: Speaks single words (mum, dad, vocabulary around 10 words), unspecific phonations Alertness/motivation:</p>	<p>3 m after 4-AP (20mg, 1.0 mg/kg/d): Speech: No effect Alertness/ motivation: More initiative and constructive playing, more concentrated and focused, for example when doing</p>	<p>Speech: No effect Alertness/motivation: Mildly improved</p>	 <table border="1"> <caption>Graph Data for P6</caption> <thead> <tr> <th>Time Point</th> <th>Score</th> </tr> </thead> <tbody> <tr> <td>last F-u</td> <td>10</td> </tr> <tr> <td>0 d</td> <td>10</td> </tr> <tr> <td>next F-u</td> <td>10</td> </tr> </tbody> </table>	Time Point	Score	last F-u	10	0 d	10	next F-u	10
Time Point	Score											
last F-u	10											
0 d	10											
next F-u	10											

	Good eye contact, interested in toys, short time of focusing and concentration Other abilities: Little constructive playing, little initiative, restless activity, changing moods	puzzles, follows instructions more promptly, more awake and active, better eye contact: Other abilities: moods more positive	Other abilities: Mildly improved	
P7 <i>p.(Leu298Phe)</i>	Onset of deviation in development at < 1 y (first assessment) Speech: No speech (lost language skills soon after acquisition)	During short period of time on low dosage of 4-AP (10 mg/d; 0.15 mg/kg/d): No change regarding speech, alertness or other abilities.	No effect	
P8 <i>p.(Leu290Arg)</i>	Onset of deviation in development (initial years) Speech: Can have a dialogue, but slower at processing, dysarthria, excellent receptive language Other abilities: 11 years: Counting (1-10) 11 seconds, calculating 1(+1,2+2,3+3 etc.) 50 seconds, 4 wrong answers, naming & spelling "car" "cat" "book" 35 seconds, mental arhythmic slow	1 y after 4-AP (40 mg; 1.74 mg/kg/d): Speech: Unchanged dysarthria. Other abilities: 12 y: Counting (1-10) 8 seconds (better fluency, can also do backwards quickly), calculating 1+1,2+2,3+3, etc (48 seconds; again some wrong answers), naming & spelling "Car" "cat" "book" 21 seconds, concentration 'better', numerical calculation/ arithmetic not better).	Speech: No effect Other abilities: Mildly improved*	
P9 <i>p.(Leu293His)</i>	Onset of deviation in development at < 3 m Speech: 2 y 10 m: Few words Other abilities: Moderate ID	1 y 8 m after 4-AP (30 mg/d; 1.76 mg/kg/d): Speech: vocabulary of many words, short sentences. Alertness/motivation: More alert, faster responses, very cooperative. Other abilities: Very active, not at all autistic.	Speech: Markedly improved Alertness/motivation: Markedly improved Other abilities: Markedly improved	
P10 <i>p.(Leu328Val)</i>	Onset of deviation in development at 6 m Speech: 12 y: Phrases of 1-3 words Other abilities: Cannot count or do simple additions	2 y 8 m after 4-AP (120 mg/d; 2.6 mg/kg/d): Speech: Faster responses, more words and better pronunciation. Alertness/motivation: Can occupy himself for > 30 min. Other abilities: More active and independent. 3 y 5 m after 4-AP (reduction to 80 mg/d; 1.7 mg/kg/d): Psychomotor speed reduced. Aggressive and rejecting behavior. 3 y 6 m after 4-AP (increase to 100 mg/d; 2.2 mg/kg/d): Psychomotor speed increased again, best gait stability.	Speech: Mildly improved Alertness/motivation: Mildly improved Other abilities: Mildly improved	

<p>P11 <i>p.(Thr374Ala)</i></p>	<p>Onset of deviation in development from birth on Speech: No speech, only rare phonation Other abilities: Severe developmental delay, no eye contact (optic nerve atrophy). 6 m: Restless and agitated, no eye contact, sleeps well</p>	<p>6 m after 4-AP (12 mg/d; 1.5 mg/kg/d): Speech + alertness/motivation + other abilities: Unchanged.</p>	<p>No effect</p>	
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* became less obvious over time

Data File1. Primary data for main text and supplementary figures.

Movie S1. Improvement of gait in an infantile patient treated with 4-AP.

Movie S2. Improvement of gait in an adolescent patient treated with 4-AP.

Movie S3. Improvement of dysdiadochokinesia due to 4-AP treatment.