Current and near future small molecules in epilepsy precisional therapy
And role of phenotype genotype correlations

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What is precisional therapy?

Traditional anticonvulsant treatment is based on the general effect of medications on function of ion channels and receptors related to the balance between excitation and inhibition processes in the brain.

Rational polytherapy: Based on symptomatology and the interaction between ASMs on the target and on pharmacokinetic interactions

Gradual shift in management from a population approach, based on epilepsy types and syndromes, to an individualized approach, where treatments could be targeted to genetically defined subgroups of individuals.

Precisional therapy : National Research Council: “the ability to classify individuals into subpopulations that differ in their disease susceptibility, biology and/or prognosis, or in their response to a specific treatment. …Interventions can then be concentrated on those who will benefit, sparing expense and side effects for those who will not.”

These treatments are either directly designed to the genetic diagnosis or found through clinical trials to have a significant effect in a particular genetic epilepsy (even if the mechanism of the therapy is not yet clear)

Different pathogenic variants of same gene can result in opposite functional effects, determining the effectiveness of certain medications.(GOF, LOF, Mixed and dominant negative)

Pharmacogenomics is another aspect of precisional medicine, involving consideration of genetic variants that do not necessarily directly contribute to the disease, but influence medication response and susceptibility to adverse reactions

Drug repurposing : using existing drugs for new therapeutic indications.

Epi4K Consortium and Epilepsy Phenome/Genome Project. (2013). "De novo mutations in epileptic encephalopathies." Nature, 501(7466), 217-221.

Six-tier approach in epilepsy treatment :proposed by Byrne et al. (2021) - Moving from reactive seizure control to proactive disease modification

Tier 1 (Phenotype-Based Therapy)

- Uses medications with historical efficacy for specific epilepsy syndromes, focuses on seizure reduction without addressing root causes. (Valproate in generalized epilepsies)

Tier 2 (Syndrome-Specific Therapy)

- Targets electroclinical syndromes : Symptom-focused but tailored to syndrome underlying characteristics (e.g., ACTH for infantile spasms)

Tier 3 (Pathophysiological Targeting)

- Addresses disrupted biological pathways (e.g., mTOR inhibitors in TSC's, KD for *SLC2A1)*

Tier 4 (Molecular Mechanism-Based Therapy) - drugs with known mechanisms tied to genetic dysfunction (e.g., Specific channels and receptor agonists and antagonist ,blockers and openers)

Tier 5 (Gene-Specific Therapy)

- Uses advanced genetic tools like antisense oligonucleotides (ASOs) to modulate gene expression (ASOS for Dravet, SCN2A)

Tier 6 (Phenotype Rescue Therapy)

- Fully correct genetic defects and restore normal function (Gene therapy including future CRISPR-based gene editing)

Six-tier approach to precision therapeutics in epilepsy, proposed by Byrne et al. (2021)

A reminder :

Diagnostic yield of genetic testing in patients with epilepsy: Exome sequencing led to a diagnosis in 24% of cases . It is highest in DEE's (27%) and in patients with epilepsy and neurodevelopmental disorders (27%).

Several studies investigated the effect of genetic studies results on the treatment of patients with epilepsy
Showing between 20-30% of diagnoses affecting treatment planning .

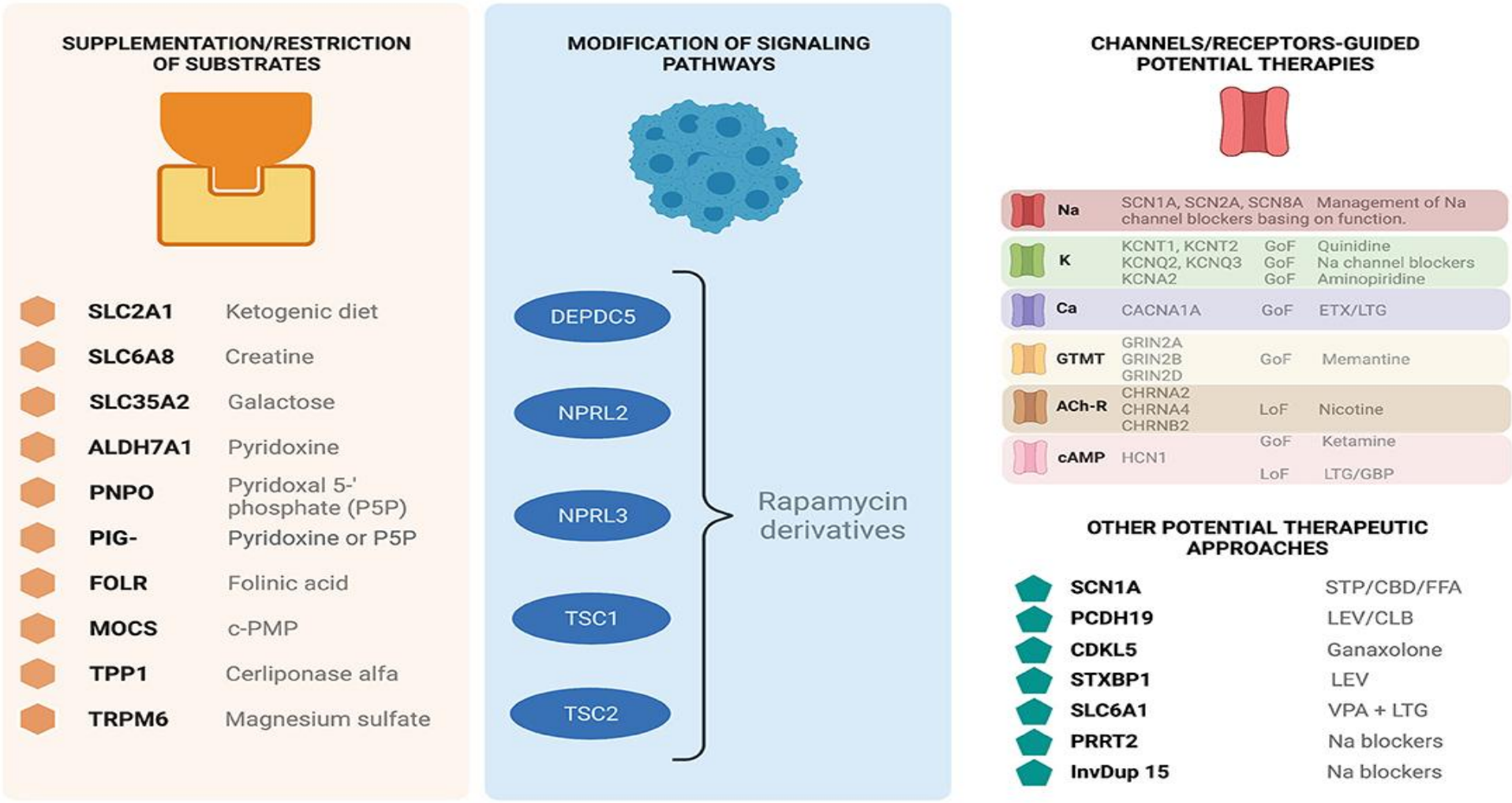
Age effect : Drug-repurposing and precisional treatment approach showed better results and even seizure freedom when targeted treatment started in early childhood rather than adolescence or adulthood

In general : GOF variants are better targets for pharmaceutical interventions than LOF variants.

Epi4K Consortium and Epilepsy Phenome/Genome Project. (2013). "De novo mutations in epileptic encephalopathies." Nature, 501(7466), 217-221.

Precisional small molecule drugs can be divided into 4 main groups based on mechanism of action

(1) Supplementation or restriction of substrates (vitamins and diets), (2) modifying signaling pathways (3) channel and receptor function modifiers 4) miscallenous



Klepper, J., et al. (2007). "The ketogenic diet in GLUT1 deficiency syndrome." Journal of Child Neurology, 22(9), 974-978.

Diet and Vitamins

Gene	Epilepsy syndrome	Suggested precision medicine	Therapeutic rationale	Status as precision medicine
ALDH7A1	Vitamin B6-deficient epilepsy	Pyridoxine, lysine-restricted diet	Impairment of lysine breakdown	Established (11)
CAD	DEE	Uridine	Disruption of pyrimidine metabolism	Established (15)
Folate cycle genes: FOLR-1, MTHFR, DHFR, PCFT	Cerebral folate transporter deficiency (ataxia and refractory myoclonic epilepsy)	Folinic acid, 5-methyltetrahydrofolate	Supplementation of active metabolite missing in folate cycle	Established (13, 14)
*PIGA**	X-linked recessive multiple congenital anomalies – hypotonia – seizures syndrome (MCAHS2), epileptic encephalopathy	Ketogenic diet	Unclear	Potential (10)
PNPO	Vitamin B6 – deficient epilepsy	Pyridoxal-5-phosphate	Supplementation of deficiency	Established (12)
PLPBP	Vitamin B6 – deficient epilepsy	Pyridoxine, pyridoxal-5-phosphate	Supplementation of deficiency	Established (92)
SLC2A1 (GLUT1)	*GLUT1* deficiency syndrome	Ketogenic diet	Alternate energy source	Established (8, 9)

*CAD AR disorder leading to defective multifunctional enzyme involved in *de novo* pyrimidine biosynthesis causing EE, DD and anemia with anisopoikilocytosis - oral uridine allowing recycling of pyrimidines led to significant developmental progress

**Antiquitin* is a dehydrogenase involved in lysine catabolism that when deficient leads to accumulation of metabolites inactivating pyridoxal 5'-phosphate (PLP)

**Occasional late* cases of *PNPO* deficiency and ones responding to pyridoxine (rather than PLP)

*Bi-allelic variants of *PLPBP* gene encoding proline synthetase co-transcribed homolog (PLP homeostasis protein) binds to PLP and is important for mitochondrial metabolism

*ketogenic diet in GLUt1 Def improves also motor and cognitive symptoms in *GLUT1-DS*

Klepper, J., et al. (2007). "The ketogenic diet in GLUT1 deficiency syndrome." Journal of Child Neurology, 22(9), 974-978

Channelopathies

Na channels and precisional therapy

Mutations in SCN1A, SCN1B, SCN2A, SCN3A, and SCN8A are responsible for a group of refractory genetic epilepsies developing from infancy to childhood

Dravet syndrome: >80% result of LOF mutations in SCN1A which impair mainly function of inhibitory, and less so excitatory neurons in several seizure-related brain regions

“old” precisional approach – **avoid Na channel blockers! (SCB)**

Role of Fenfluramine was retrospectively proven in DS mice model and connected to serotonin receptors role in SUDEP

(CBD and Stiripentol retrospectively showed positive effects in mouse models)

In the horizon :

LP352 (Longboard Pharmaceuticals) - a selective 5- HT2C superagonist with no effect on 5H2A/B receptors possibly avoiding negative cardiac effects - Currently studied in Dravet patients .

A peptide derived from spider venom (Hm1a) selectively activates the Nav1.1 channel with consequent improvement in seizure and mortality outcomes in a mouse model of DS

+ ASOs and Gene therapy ongoing trials

Dravet, C., et al. (2011). "Mutations in SCN1A, SCN1B, SCN2A, SCN3A, and SCN8A." Epilepsia, 52(1), 1-10.

SCN2A

Coding alpha sub unit of voltage-gated sodium channels (Nav1.2) predominantly located in neocortical and hippocampal excitatory neurons and induce rapidly activating and inactivating currents. It changes its level and distribution of expression on neurons during development

Phenotypes : early SCN2A-related DEE and milder early infantile epilepsy – both caused by missense GOF mutations and respond well to SCB's particularly phenytoin and carbamazepine.

Late-onset epilepsy forms are associated with LoF truncation mutations aggravated by SCB's

60%-70% of published cases are DEE phenotype

Amino acid substitutions in BFNIS are milder because of exchange between physiochemically similar AA
while DEE missense mutations are of significantly different physiochemical properites

Transient/self limited phenotype of BFNIS explained by early expression of Nav1.2 in neurons of hippocampus and cortex which diminishes with time and gradually superseded by channel type Nav1.6 (demonstrated in mouse models)

High dosages of PHT needed to control seizures in GOF cases in severe DEE cases at onset and can be switched to another SCB later.
Phenytoin binds to the inactive pore site on the Na channel and decreases sodium influx and neuron excitability.
carbamazepine have similar working mechanismits but its affinity to inactivated sodium channels is ~ 3 times lower

West Syndrome presentation is usually not SCB responsive (LOF) as well as other severe late presentations

Class 1b antiarrhythmic agents (lidocaine, mexiletine) (different sodium channel blockers) occasionally useful in cases of classical SCB treatment failure.

SCN8A mutations related epilepsy

Coding for Nav1.6 channel subunits, mostly expressed in excitatory neurons, and less frequently in inhibitory neurons

De novo missense GoF mutations are responsible for the severe form of EIEE 13 and partially respond to SCB's

LOF variants lead to cognitive disorders/ ASM with milder or no epilepsy –seizures respond to other ASM's

A group of late onset Generalized epilepsies related to LOF mutations

Currently investigated drugs :

NBI-921352 (XEN901), selective Nav1.6 inhibitor designed to treat infantile epileptic encephalopathy related to GoF SCN8A mutations and focal seizures in adults – clinical trials are still analyzed and reconsidered

PRAX-562 is a selective persistent sodium current blocker currently tried in SCN2A GOF and SCN8A severe epilepsy patients in a friendly designed protocol

Precisional "pot pouree" : iPSC-derived excitatory cortical neurons from patients with SCN8A-related disorders showed variant-specific increases in persistent or resurgent sodium current responsive to riluzole selectively suppresses the late sodium current (INaL*INaL*) while sparing the transient current. Subsequent administration of riluzole to 2 individuals with the specific SCN8A variants led to substantial seizure reduction

PRRT2-Related Epilepsy – Na “channelopathy” related

PRRT2 (proline-rich transmembrane protein 2) encodes a pre-synaptic transmembrane protein that enables synaptic vesicle fusion by interacting with members of the SNARE complex

Haploinsufficient *PRRT2* disease-causing variants are a common genetic causes of epilepsy.

Presents in 3 major phenotypes (sometimes in same patient):

Self-limited familial infantile seizures

Paroxysmal kinesigenic dyskinesia (PKD)

Infantile convulsions with choreoathetosis

PKD was known for years to respond to low dose carbamazepine without explanation , an approach applied later successfully in *PRRT2*-related infantile seizures

Mechanism of action : PRRT2 related protein negatively modulates sodium channels (both Nav*v*1.2 and Nav*v*1.6) and by this pathway regulates calcium-mediated neurotransmitter release.

LOF mutations lead to increased neuronal excitability, spontaneous activity, and impaired synaptic plasticity

FGF (Nonsecreting fibroblast growth factors) **related DEE's**

FGF's : A group of 4 Na channel–binding proteins. Two recently associated with neurodevelopmental disorders with epilepsy

FGF12 and FGF13 gene products bind and interact with C-terminal tail of voltage gated Navs and modulate the channels' fast, and long-term inactivation

FGF12 disorder – Hot spot missense GOF (R52H) mutation leads to infantile DRE epilepsy with both focal, GTC's and tonic seizures ,abnormal EEG , DD and progressive cerebral and cerebellar atrophy

Part of the cases are germline somatic or related to parent mosaicism

FGF13 - an X linked disorder with both males and females affected with DRE and DD

Various mutations in a specific location on both proteins bind to NaV channels and strongly changes the voltage dependence of the inactivation gating

Phenytoin is the almost only effective drug is in both disorders

In our "local " experience 2 patients with FGF 12 are highly phenytoin dependant
While a girl with FGF13 mutation (diagnosed when presented with acute EE) showed dramatic change in EEG findings and general function in addition to seizure control which is ongoing (role of "acute" genetic diagnosis)

Creating a prediction tool of various missense mutation effects in order to choose the right treatment

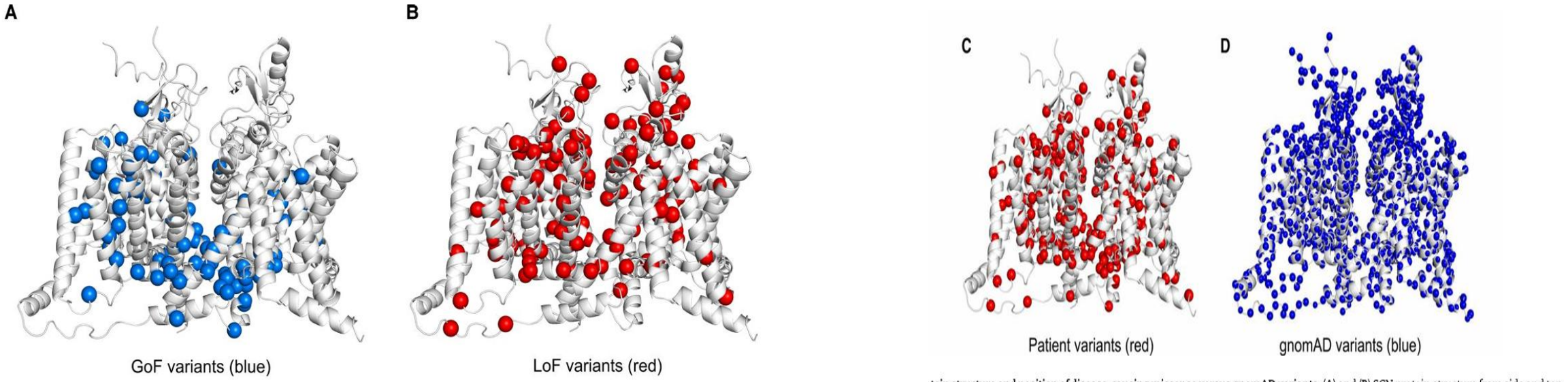
A brunklaus et al. suggested a new prediction tool currently available on the web

Patch clamp experiments on each new mutation is not practical

Hypothesis: Similarities in biophysical properties between different voltage-gated sodium channels can predict function and inform precision treatment across sodium channelopathies.

- Systematic literature search for already functionally assessed missense variants in all nine VGSC (until 2021) electrophysiologically characterized in mammalian cells by whole-cell patch-clamp recordings.
- Alignment of linear protein sequences of all Na channel genes and correlating variants by their overall functional effect on biophysical properties.
- 437 SCN variants properly studied (141 epilepsy-associated (SCN1/2/3/8A);the rest NM, cardiac or benign)
- Detected 38 missense variant pairs with an identical disease-associated variant in different sodium channel genes.
- 35/38 showed similar functional consequences - 92% biophysical agreement between corresponding sodium channel variants
- In-silico tool achieved 78.5% agreement in prediction of GoF properties and 75.0% agreement in LoF properties - Compared to gold standard whole-cell patch clamp experiments

*Brain 2022;*145; 12



gure 4 3D illustration comparing GoF with LoF locations across the SCN protein. (A) GoF variants are illustrated in blue. (B) LoF variants are illustrated red.

tein structure and position of disease-causing missense versus gnomAD variants. (A) and (B) SCN protein structure from side and top variants shown in red. (D) GnomAD variants shown in blue.

- Creating a GOF vs LOF topological map of SCN proteins indicating shared patterns of biophysical effects aiding variant analysis and guiding precision therapy. (A,B)
- Pathogenic missense variants are clustered in specific functional domains while population variants were more frequent across non-conserved domains (C,D)
- Free online webtool http://SCN-viewer.broadinstitute.org

This approach can probably work for other types of channelopathies and receptors but somebody (AI) has to do the work .

Brunklaus, A; Feng, T; Brünger, T; Perez-Palma, E; Heyne, H; Matthews, E; Semsarian, C; Symonds, JD; Zuberi, SM; Lal, D; Schorge, S; (2022) Gene variant effects across sodium channelopathies predict function and guide precision therapy.

Potassium channels K channels modulate neuronal excitability, controls modulation of action potentials evolution, axon repolarization, resting membrane potential maintenance, neurotransmitter release regulation and moderation of cell death/survival signaling pathways.

Activation leads to decreases neural excitability - inhibitory effects.

Most epilepsy related mutations have LoF characteristics.

KCNQ2

Protein product - Kv7.2 potassium channels

Forming homo- and heterotetrameric voltage-gated K channels when mixed with Kv7.3-subunits encoded by KCNQ3 (KV7.2/KV7.3) - Both co-expressed in same brain areas (with similar clinical phenotypes related to specific mutations)

Rresponsible for M-current - a non inactivating current that raises AP threshold.

Most common phenotype (80%) - LOF variants associated with benign familial neonatal epilepsy (BFNE) treated with SCB's: Carbamazepine and lamotrigine among the most effective

Explanation of response to SCB's unclear: co-localization of potassium and sodium channels? a common structural fragment in both Kv7 and sodium channels? – precisional retrospectively

Better precision : **K channel "openers":** Ezogabine (Retigabine) - selective Kv7 channel activator: Seizure reduction and developmental progress (out of market related to side effects)

Gabapentin !? has similar effect in vitro and was reported to lead to seizure freedom in a KCNq2 DEE patient!!!!

On the horizon: Phase 3 clinical trial of XEN496, a novel immediate-release formulation of retigabine .

Magura, I. S., Bogdanova, N. A., & Dolgaya, E. V. (2015). Potassium Channels and Signal Transduction Pathways in Neurons. *Neurophysiology*, 47, 71-761•

Part of KCNQ2 variants are GoF with increased channel activity, altered kinetics, and more severe clinical manifestations. SCB are not as effective and **K channel openers** aggravate clinical symptoms

KCNA2

Protein forms homomers and heteromers with Kv1.1 or Kv1.4- proteins creating channels conducts a voltage-dependent potassium "delay" (D-type) current activated even below threshold and delays the initiation of action potentials and prevents repetitive firing.

LoF variants result in focal seizures with prominent sleep activation.

GoF mutations: severe infantile generalized seizures with ataxia and cerebellar atrophy

Early-onset phenotypes, with generalized or focal seizures and developmental impairment caused by both GoF and LoF mutations

4-aminopyridine - a potassium channel blocker antagonize GOF defects in the *KCNA2* gene.

N-of-1 trial in 9 centers, 9/11 patients showed improvement in seizure burden, gait, ataxia, alertness, and cognition which is long lasting – Age was the main predictive factor for positive effect (younger-better) (UBS Hedrich et al Sci Trans medicine 2021)

*GOF variants in KCNA1 (KV1.1 subunit) associated with episodic ataxia type 1 or epilepsy – reports on similar positive response to 4-aminopyridine

Existing online support tool (www.kcna2-treatment.com, user: KCNA2, password: 4APtreatment) based on electrophysiological results obtained from variants in highly conserved KV1.1 and KV1.2 subunits and in evolutionary conserved shaker channel of drozophila. Inserting respective position and AA change in KCNA2/KV1.2, will indicate all functional changes that have been found in the three different channels and whether 4-AP treatment should be considered

Xie, C., Kessi, M., Yin, F. *et al.* Roles of *KCNA2* in Neurological Diseases: from Physiology to Pathology. *Mol Neurobiol* **61**, 8491–8517 (2024).

KCNT1 gene

ligand-gated potassium channel (KNa1.1) – **Slack** -“sequence like a calcium activated K+ channel” activated by intracellular sodium concentration.

GOF Mutations in KCNT1 are related to :

1. Most common and severe phenotype of autosomal-dominant nocturnal frontal lobe epilepsy (ADNFLE)
2. Most severe form of encephalopathy with malignant migrating focal seizures of infancy (EIMFS)

Quinidine- a pore blocker of Slack channels investigated as a treatment option.

- Response unpredictable, slightly better when started early in ADNFLE (under 4 y).
- A study in *KCNT1*-related epilepsy noted a > 50% seizure reduction in 20% of patients, with a few achieving transient seizure freedom.
- Mutation location did not correlate with response
- Significant SAE: QT prolongation
- Failure of trials : Poor BBB penetration of quinidine , frequent QT prolongation, variability in electroclinical syndromes, ages, and treatment regimens.

Conclusion : road to a successful therapy can sometimes be more complex than initially expected.

Lim CX, Ricos MG, Dibbens LM*, et al*
KCNT1 mutations in seizure disorders: the phenotypic spectrum and functional effects
Journal of Medical Genetics 2016;**53:**217-225.

GABA receptors (GABAAR)

- Comprising five protein subunits surrounding a chloride channel
- Responsible for inhibitory neurotransmission
- Channels of GABAAR complex opens in response to positive stimulation of binding sites leading to chloride ions inflow to neurons causing membrane hyperpolarization ,elevating seizure threshold.
- Most mutations LOF causing hyperexcitability, and seizures
- Some **dominant negative -**loss of more than 50% of function.
- Mutations in 4/5 subunits GABRA1, GABRB3, and GABRG2, GABRG5) lead to various monogenic epilepsies
- Precision : use of Gabaergic drugs

A very rare AR disorder- GABA TRANSAMINASE deficiency (ABAT) : Excessive GABA levels partially responds to Flumazenil- a GABA receptor blocker

Ghit, A., Assal, D., Al-Shami, A.S. *et al.* $GABA_A$ receptors: structure, function, pharmacology, and related disorders. *J Genet Eng Biotechnol* **19**, 123 (2021).

Ionotropic Glutamate Receptors

Glutamate binds to both ionotropic and metabotropic glutamate receptors (iGluRs and mGluRs, respectively).

4 groups of ionotropic receptors : NMDA (N-methyl-d-aspartate), AMPA (Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid), kainate,, and GluD (delta)- differing in physiological roles and properties

NMDA receptors (NMDARs)

- Involved in excitatory synaptic transmission and relate to long-term potentiation and memory.
- Heteroterameric ion channels : Two obligatory **GluN1** subunits with glycine as co-agonist and other 2 either both GluN2 or GluN2/ GluN3 subunits with glutamate binding site
- Activation requires two molecules of glycine and two molecules of glutamate.
- At resting membrane potentials channels are blocked by extracellular magnesium ions.
- Depolarization removes magnesium block and enables the inward movement of sodium and calcium ions
- GOF mutations in GRIN1, GRIN2A, GRIN2B, and GRIN2D leads to long abnormal excitation and seizure generation
- GOF GRIN1 variants: several seizure phenotypes (epileptic spasms and tonic, focal, myoclonic, focal migrating seizures) and profound EEG abnormalities
- GRIN2A/B LOF and GOF mutations:benign temporal lobe epilepsy, atypical benign partial epilepsy, EE with CSWS and LKS occasionally accompanied by motor and speech disorders
- GOF GRIN2B mutations related to West syndrome and other early DEEs
- Small series and case reports of **GOF mutations** in these genes responding to **NMDA antagonists**:Memantine (non competitive antagonist), dextromethorphan, dextrorphan, amantadine, ketamine and Felbatol

NMDA LOF mutations

Trials of L-Serine - **NMDAR co-agonist** (1.N Juliá-Palacios et al, Brain 2024; 2.I. Kreyet al , Neurotherapeutics (2022)

- phase 2A trial: L-Serine treatment in 24 pts with GRIN LOF variants, 2–18 y -52 weeks
- Primary outcome measures: Developmental and behavioural
- At baseline : 3 -drug-resistant epilepsy, 16 Abnormal epileptiform EEG
- Epilepsy related outcome : normalized EEG pattern - 5/16 , reduced seizure frequency 1/3
- Response level was severity dependant : all GRIN2A carriers, GRIN2B with missense/truncating/frameshift variants causing a reduction of NMDAR surface density = milder phenotype.
- Poor response of GRIN1 missense variants carriers
- German retrospective L-Serine study (9 pts with GRIN2A and GRIN2B) :Behaviour improvements in 8 (89%), development in 4 (44%), and/or in EEG or seizure frequency in 4 (44%).

Q&A:

Question: L-serine as activator of glutamatergic transmission why it does not aggravate seizures?

Answer : “paradoxical” effect maybe due to glutamate potentiation of inhibitory interneurons releasing GABA.

Do not attempt L serine in GOF mutations!!! -

A function defining (GOF or LOF) approachable tool is required!!!!

Julia-Palacios N, Olivella M, Sigatullina Bondarenko M, et al. L-serine treatment in patients with GRIN-related encephalopathy: a phase 2a, non-randomized study. *Brain*. 2024;147(5):1653-1666.

On the horizon : (for GOF mutations)

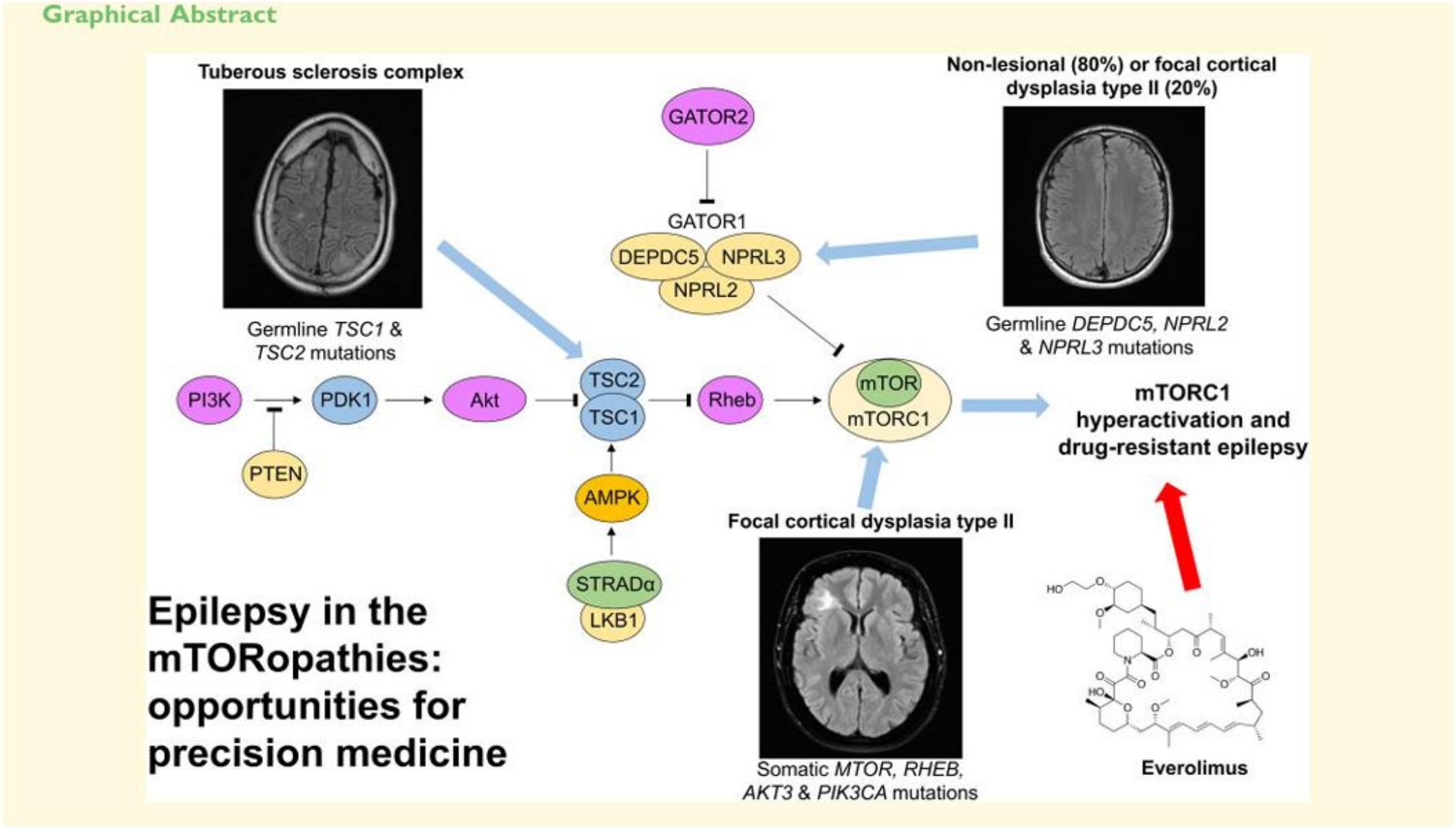
Radiprodil - negative allosteric modulator of the N-methyl-d- aspartate (NMDA) receptor NR2B (GluN2B) subunit - always part of the NMDA receptor

- Demonstrated favorable safety, tolerability, and pharmacokinetic profiles in animal models and adults
- **phase1 Honeycomb Study**: Radiprodil reduced seizures by a median of 86% among patients with GRIN-related disorders: One became seizure-free, nearly 50% - seixure reductions> 90%.
- Improvements in non-seizure symptoms
- Currently studied in Europe in pediatric GOF GRIN patients

AMPA receptors

- Ionotropic receptors predominantly expressed in postsynaptic neuronal membrane, mediating fast excitatory synaptic neurotransmission
- De novo mostly LOF mutations in GRIA2 gene (AMPA RECEPTOR) - significant DEE and EE.
- GOF GRIA2 variants are also related to seizures (tonic convulsions) – successfully treated with **perampanel (Fycompa), a negative allosteric modulator of AMPARs**, possible combination with decanoic acid (product in ketogenic diet) acting negatively on a different site leading to synergistic effect on the overacting receptor
- FCD2 surgical specimens show abundance of **vesicular glutamate transporter 1 (VGLUT1)– positive synapses** leading to increased cortico- cortical excitatory input in dysplastic regions. **Perampanel** , capable of maintaining closure of AMPA receptors in the presence of elevated glutamate shown to be effective in reducing seizure- like activity in this neocortical slices suggesting potential benefit for its use in treating pharmacoresistant epilepsy in this patient group.
- Observational multicenter study show efficacy and safety for perampanel as an adjunctive therapy in a drug- resistant focal epilepsy patient cohort that had a significant number of participants with focal cortical dysplasias

Overactive Cellular Signaling mechanism



Moloney, P. B., Cavalleri, G. L., & Delanty, N. (2021). Epilepsy in the mTORopathies: opportunities for precision medicine. *Brain* .1 *Communications*, 3(4), fcab2221

- mTOR complex is a key regulator of cell growth, proliferation, and survival
- Pathway upregulation leads to malformations of cortical development, medically refractory epilepsies, and neurodevelopmental disorders = **mTORopathies**
- mTORopathies: TSC, focal cortical dysplasia type II (FCD II), hemimegalencephaly (HME), and Pretzel syndrome(PMSE): polyhydramnios, megalencephaly, and symptomatic epilepsy
- mTORC activation disrupt formation of proper neural circuits, resulting in excitatory/inhibitory imbalance and net synaptic hyperexcitability - **Activation alone without significant cortical malformations sufficient to generate seizures**
- mTORopathies stem from GOF variants in mTOR pathway activators (PI3KCA, AKT3, RHEB, MTOR) or LOF variants in inhibitors of mTOR (TSC1, TSC2, DEPDC5, NPRL3, NPRL2, PTEN, STRADA)
- In more detail : mTORC1 is negatively regulated by an upstream protein complex GATOR1, composed of three subunits DEPDC5, NPRL2, and NPRL3 which repress the complex in response to amino acid depletion

- ***Most common and studied : TSC1/2* mutations leading to hyperactivity of mTOR complex 1 (mTORc1)**

- Rapamycin: Allosteric inhibitor of the mTORC given to *Tsc1*-inactivated mice prevented development of epilepsy and premature death compared to untreated mice; late administration suppressed seizures in seizing mice and prolonged survival

- **Exist -3:** randomized, double-blind, placebo-controlled phase 3 trial: Everolimus (a rapamycin analog with better pharmacokinetic properties) was effective in short and long term seizure control . Approved in TSC1/2 patients >2yr 2 ASM failure as adjunctive therapy . Investigating its effect <2y seems promising
- Prospective cohorts of children and teen agers with TSC (4–21y) did not show improvment in neurocognitive functioning, autism or neuro psychological deficits

- Sirolimus led to a statistically significant decrease in seizure frequency compared to the standard-of-care with high number of seizure free patients (alternative for patients unable to tolerate or get everolimus)

- Case reports and small series of epilepsy patients with other disorders of cellular proliferation:Sturge-Weber syndrome (*GNAQ), NPRL3*-related CMDs, DEPDC5, and *PIK3CA*-related overgrowth syndromes responding to “rapalogs” like sirolimus and everolimus.

Gene	Epilepsy syndrome	Suggested precision medicine	Therapeutic rationale	Status as precision medicine
GATOR1 complex (DEPDC5, NPRL2, NPRL3)	Familial focal epilepsy with variable foci	mTOR inhibitors (everolimus)	Inactivation of mTOR pathway	Potential (1, 93)
GNAQ	Sturge-Weber-related epilepsy	mTOR inhibitors (sirolimus)	Inactivation of mTOR pathway	Potential (27)
PIK3CA	Intractable epilepsy	PI3K inhibitors	Suppression of PI3K signaling	Potential (29)
TSC1, TSC2	Tuberous sclerosis, focal cortical dysplasia	mTOR inhibitors (sirolimus, everolimus, 1,3,5-triazine derivatives)	Inactivation of mTOR pathway	Established (19)

- 50% of individuals with GATOR1-related epilepsies have DRE even without MCD - most mutations are LOF
- Some missense mutations function is not clear - mostly related to non lesional epilepsies and at least for **DEPDC5 missense mutations poorer response to rapamycin reported**

In FCD patients mTOR inhibitors may be used as bridging therapy before epilepsy surgery , alternative therapeutic strategy when FCS is inaccessible or for persistent seizures after epilepsy surgery

- PIK3CA related overgrowth syndromes: mosaic multisystem disorders characterized by congenital lipomatous overgrowth, vascular malformations and skeletal abnormalities. Somatic PIK3CA mutations have been detected in FCD type II, HME and polymicrogyria
- **Alpelisib** - selective PIK3CA inhibitor improved clinical outcomes in individuals with PIK3CA-related overgrowth syndromes even in ones that ad previously failed treatment with rapamycin

- Diagnosing the underlying mosaic mutation in FCD patients may have a role in treatment planning - surgery specimens and CSF DNA studies being a method for diagnosis pre and post surgery

Additional precisional options in mTORopathies

- FCD type II and cortical tubers retain immature GABA signalling mechanisms which requires additional GABA for their inhibitory effect
- Vigabatrin shown to have additional inhibitory effects on mTOR pathway in a TSC mouse model explaining its unique effect on seizures compared to other Gabaergic drugs

EPISTOP study : Vigabatrin treatment at onset of epileptiform abnormalities on EEG delayed onset of seizures, reduced the severity of epilepsy and reduced the frequency and severity of neurodevelopmental delay compared with those who received vigabatrin after their first seizure.

On the horizon

- **Basimglurant** – a first-in-class negative allosteric modulator of the metabotropic glutamate receptor 5 (mGluR5) found to correct aberrant protein synthesis via activation of the MEK/ERK pathway and thought to potentially reduce mTOR dependent protein synthesis in TSC
- Noema Pharma is conducting the GALENE Phase 2B : Efficacy and safety of NOE-101 (basimglurant), for seizure control in TSC pediatric and adult patients.

Fang, Y., (2025).. *Acta Epileptologica*, 7, Article number: 121 Jóźwiak, S., et al. (2021). Early diagnosis and epilepsy prevention: Results of the EPISTOP study. *Tuberous Sclerosis Association*3

Molecular Chaperones

Protein conformational changes caused by mutations in specific genes may lead to epilepsy and neurodevelopmental disorders usually through misfolding and abnormal aggregation of the mutated protein

Chemical chaperones—small molecules that can stabilize misfolding and aggregating abnormalities of mutated proteins and in this way prevent deleterious interactions with other proteins, or modify activity of endogenous chaperones

STXBP1 (Syntaxin-Binding Protein 1) related to vesicular neurotransmitter release: AD de novo mutations lead to DEE with 75 – 89% seizures at some point (typically first year of life), 90% - intellectual disability ,ASD and movement disorder

SLC6A1- encodes GAT-1- a synaptic GABA reuptake transporter: heterozygote mutations cause childhood onset epilepsy usually generalized (91%) developmental delays (84%), usually mild to moderate range (64%) , ASD (24%) and movement disorders

4 Phenyl butyrate (used in urea cycle disorders) -acts as chemical chaperone that stabilizes protein folding

- Pre clinical studies in cells with STXBP1 mutations: Rescues protein misfolding and aggregation
- In cellular models of SLC6A1 mutations reduced endoplasmic reticulum retention of mutant protein, improved trafficking of the wild-type protein, and directly activated SLC6A1 and in mutant heterozygous mice show reduced spike-wave discharges

Zummo, L.;et al. Molecular Chaperones and miRNAs in Epilepsy: Pathogenic Implications and Therapeutic Prospects. *Int. J. Mol. Sci.* **2021**, *22*, 8601

A multicenter open-label study involving 20 children (10 with STXBP1 ,9 with SLC6A1 mutations)

Safety: (primary outcome) was proven ; side effects were : somnolence (35%), appetite changes (25%), and honey-like body odor (20%). Metabolic acidosis in one resolved after discontinuation

Efficacy: STXBP1 participants – 60% reduced seizure frequency within 10 weeks with two becoming seizure-free at target doses

- Long-term follow-up (2-3 years) : 30% SF, 60% with reduced seizure burden - EEG improvements correlated with clinical response . None of the IS patients responded
- SLC6A1 : 3/9- SF 4/9 > 90% reduction at 10 weeks .
  Clinical benefit continued through last visit (1-3 years after enrollment) , 6/9 no longer taking additional anti-seizure medications

Remark: 2 patients (one of each gene) fully haploinsufficient (microdeletions)- suggesting 4PB improved wild-type function or has a different effect (is it still a chaperon effect ??) .

European Commission granted orphan drug designation to glycerol phenylbutyrate for STXBP1-DEE in 2023, potentially accelerating further development

Grinspan, Z. M., Burré, J., Cross, J., Ross, M. E., Stone, A., Basma, N., Gao, K., Kang, J.-Q., Lim, J., Dubow, E., Abila, M., Miele, A., & Demarest, S. (2024). 4-Phenylbutyrate for STXBP1 and SLC6A1: Safety, tolerability, seizure, and EEG outcomes. A case series at 2 centers. medRxiv.1

Towards realizing the vision of precision medicine: Ail based prediction of clinical drug response j.de.jong et al brain 2021!!!

Reading suggestion :the role of AI (machine learning) use in precisional therapy by using WGS of populations to predict treatment response to a specific drug well studied mechanism of action

Brivaracetam and epilepsy patients!!

Identifying clinical and genetic characteristics that predispose patients to poor drug response

Combining high-dimensional genetics data with clinical data can improve drug response prediction even in relatively small samples which has potential implications for clinical trial design

*Precisional therapy “private” initiatives- Studying in a high throughput fashion the effects of small molecules on computational mutated protein structure models, transcriptome changes and other methods +/- in vivo proof models – currently mainly sponsored by families and parents’ associations.

De Jong, J., et al. (2021). "AI-based prediction of clinical drug response." Brain, 144(1), 1-10.

Summery and conclusions :

1. Early genetic diagnosis can lead to better treatment and prognosis
2. Both the affected gene and the specific mutation role are important for a “smart” precisional decision
3. The role of N-1 and N-some studies in rare and ultra-rare disorders
4. Real need for available prediction tools for different mutations especially novel
5. Studying similarities between mechanisms of monogenic disorders and multifactorial epilepsies may lead to precisional treatment decisions in patients with multifactorial epilepsies too
6. Epileptologists should stay tuned to new practical developments in the field
7. Appropriate collection of data on patients’ epilepsy , mutation location and response to treatment may be significantly useful for future machine learning studies with practical conclusions for treatment