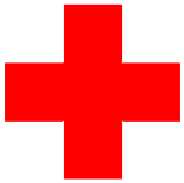
Precision Medicine in Epilepsy

King Chulalongkorn Memorial Hospital Experiences



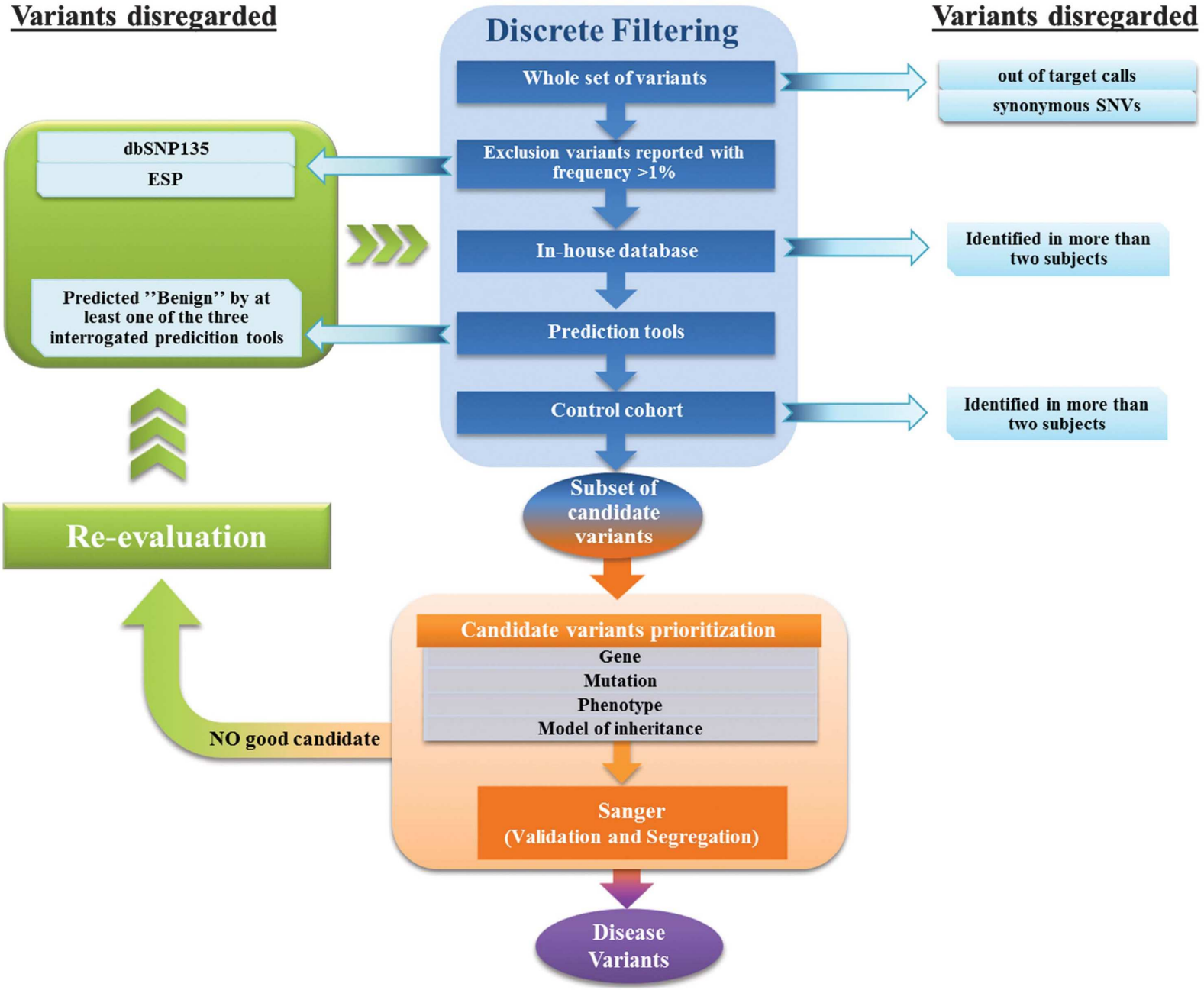
โรงพยาบาลจุฬาลงกรณ์
สภากาชาดไทย

Tayard Desudchit, Div. of Ped. Neurology
Faculty of Medicine, Chualongkorn U.

WHOLE EXOME SEQUENCING

Hypothesis free

- Rare variants
- Known chromosomal or single gene disease has been excluded
- Large genes
- Multigene testing is more expensive


Variants disregarded
Discrete Filtering
Variants disregarded
Whole set of variants
out of target calls
synonymous SNVs
dbSNP135
ESP
Exclusion variants reported with frequency >1%
In-house database
Identified in more than two subjects
Predicted ''Benign'' by at least one of the three interrogated predicition tools
Prediction tools
Control cohort
Identified in more than two subjects
Subset of candidate variants
Re-evaluation
Candidate variants prioritization
Gene
Mutation
Phenotype
Model of inheritance
NO good candidate
Sanger
(Validation and Segregation)
Disease Variants

The genetic landscape of the epileptic encephalopathies of infancy and childhood

Amy McTague, Katherine B Howell*, J Helen Cross, Manju A Kurian, Ingrid E Scheffer*

Lancet Neurol 2016; 1
Publish
Novembe
http://dx.doi.org
S1474-4422(1
*Authors contribut

Molecular Neu
(A McTagu
M A Kurian PhD) a
Neu
(Prof J H C
Developmental Neu
Programme, UCL In

Until 2001, the cause of epileptic encephalopathies was unknown, and they were thought to probably be due to a so-called symptomatic cause such as an acquired insult. A minority of cases undoubtedly have symptomatic causes in which a child has a structural aetiology such as a stroke or hypoxic-ischaemic encephalopathy underlying their epileptic encephalopathy. An exception is West syndrome, in which almost 30% of patients have an acquired aetiology.[19] The structural abnormality is associated with an epileptiform focus, leading to epilepsy and developmental regression. Similarly, malformations of cortical development can be associated with an epileptic encephalopathy, as exemplified by tuberous sclerosis complex. In these cases, the underlying cause of the malformation should still be sought and is often genetic,[20,21] although environmental causes are well recognised.[22]

of severe epilepsies
opmental slowing or
cific seizure types and
nderlying the epileptic
matic mosaicism and
conversely, one gene
have been implicated,
tions. Gene discovery
ways. These findings
of these devastating

Epilepsy of infancy v
KCNT1
SCN2A, SCN1A
PLCB1, QARS, SCN8A,

epsy–aphasia spectrum
2A

Other predominantl
Onset 0–1 years: *EEF1*
Onset >1 year: *CHD2,*

Other predominantl
Onset 0–6 months: *A*
Onset 6–12 months:
Onset >1 year: *ARHGE*

0 months | 3 months | 6 months | 1 year | 2 years | 4 years

G-protein-coupled transduction | cAMP | Na^+ | HCN1

	Genes (and approximate proportion of syndromic cases where known)	Sex affected and incidence	Age at onset	Seizures at onset	EEG	Treatment	Epilepsy evolution and outcome	Syndrome differential diagnosis	Development
(Continued from previous page)									
Epilepsy with myoclonic–atonic seizures	*SLC2A1* in 5%; *SLC6A1* in 4%; *CHD2*, *GABRA1*, *GABRG2*, *SCN1A*, *SCN1B*, *KCNA2*	2:1 (boys:girls) when onset at age >1 year; equal when onset at age <1 year; 1:10 000	7 months–6 years (peak 3–4 years)	Several seizure types: myoclonic–atonic with or without myoclonic, absence, or tonic–clonic seizures, and episodes of non-convulsive status epilepticus	Interictal: hypersynchronous theta or delta slowing; generalised spike-wave or generalised polyspike-wave activity, increasing in sleep; photosensitivity in some	Most patients resistant to several antiepileptic drugs; beneficial: ketogenic diet (>50% improve), corticosteroids	Remission in most within 3·5 years of onset; persistent seizures in more severe cases, usually as nocturnal tonic or tonic vibratory seizures	Benign myoclonic epilepsy of infancy, Dravet syndrome, Lennox-Gastaut syndrome, atypical benign rolandic epilepsy, late-onset epileptic spasms, other myoclonic epilepsies	Early development normal in most; regression often occurs with epilepsy onset; outcomes vary from normal intellect (26–67%) to severe intellectual disability
Lennox-Gastaut syndome	*ALG13*, *CACNA1A*, *CDKL5*, *CHD2*, *DNM1*, *FLNA*, *GABRB3*, *GRIN2B*, *HNPRNU*, *HNRNPH1*, *IQSEC1*, *iQSEC2*, *KCNQ3*, *MTOR*, *SCN1A*, *SCN2A*, *SCN8A*, *STXBP1*	Equal; 1:200 000	1–8 years (peak 3–5 years); rare adult-onset cases	Several seizure types: tonic seizures with or without atypical absence, atonic, myoclonic, or generalised tonic–clonic seizures, spasms, focal seizures, episodes of tonic or non-convulsive status epilepticus	Interictal: slow background, slow (<2·5 Hz) spike-wave, generalised paroxysmal fast activity in sleep; ictal: electrodecrement or low-voltage fast activity (tonic seizures), slow spike-wave (atypical absences), generalised spike-wave or polyspike-wave activity (myoclonic seizures)	Resistant to several antiepileptic drugs; if focal lesion, surgical resection might be curative	Seizures persist into adulthood in ~80%	Epilepsy with myoclonic–atonic seizures, Dravet syndrome, epilepsy-aphasia spectrum	Developmental delay precedes epilepsy onset in 20–60%; cognitive impairment in 90% by 5 years after seizure onset; learning difficulties in remainder
Epilepsy-aphasia spectrum (including Landau-Kleffner syndrome, epileptic encephalopathy with continuous spike-wave discharges in slow wave sleep, and atypical benign rolandic epilepsy)	*GRIN2A* in 10–20%	Unknown for whole epilepsy-aphasia spectrum; 3:2 (boys:girls) for benign epilepsy with centrotemporal spikes	3–7 years	Landau-Kleffner syndrome: rolandic seizures in 70%; epileptic encephalopathy with continuous spike-wave discharges in slow wave sleep: rolandic seizures; atypical benign rolandic epilepsy: rolandic seizures, negative myoclonus, atonic seizures	Atypical benign rolandic epilepsy : centrotemporal spikes, often bilateral, becoming synchronous and increasing in sleep; Landau-Kleffner syndrome and CSWS: electrical status in sleep (>85% non-REM sleep)	Resistant to several anti-epileptic drugs; beneficial: steroids, benzodiazepines sodium valproate, sulthiame, ethosuximide, levetiracetam; exacerbating: carbamazepine	Epilepsy is age limited, resolving by mid-teens in almost all patients	Lennox-Gastaut syndrome	Pre-seizure development normal in most; regression occurs with seizure onset in many (language, global, or motor); outcome varies from normal to severe delay

REM=rapid eye movement. All genes are described in further detail in the appendix. *Most cases have a syndrome that can be readily distinguished from Dravet syndrome. † Lamotrigine and carbamazepine are exacerbating in the context of *SCN1A*-mutation-positive Dravet syndrome.

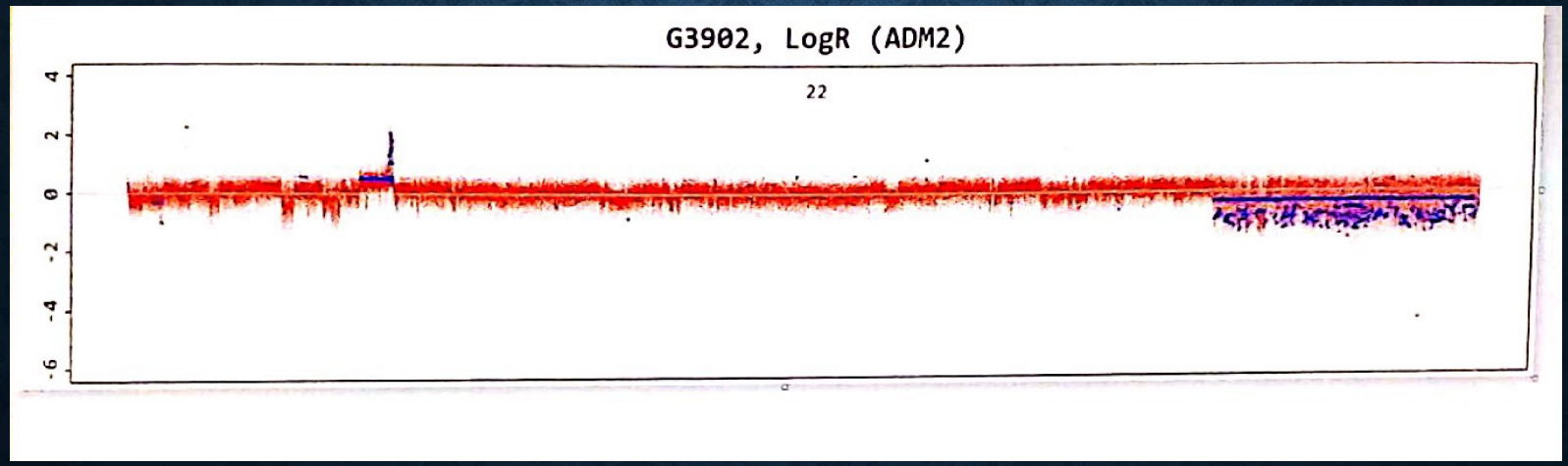
Table: **Epileptic encephalopathies—electroclinical syndromes and known genetic determinants**

	GABRA1, *GABRG2*, *HCN1*,* *STXBP1*				common	clobazam, levetiracetam, ketogenic diet; exacerbating: carbamazepine, lamotrigine†	epilepticus; from second decade: brief nocturnal convulsive seizures with or without focal dyscognitive seizures,	Gastaut syndrome	with episodes of status epilepticus; outcome mild-to-severe delay (rare cases of normal development reported)

MUTATION ANALYSIS

WES: No candidate variant found

Array CGH



Whole Exome Sequencing in Intractable Pediatrics Epileptic Patients: King Chulalongkorn Memorial Hospital Experiences

Tayard DESUDCHIT[1)], Chupong ITTIWUT[2)], Ponghatai DAMRONGPHOL1[1,2)], Kanya SUPHAPEETIPORN[2)], Vorasuk SHOTELERSUK[2)]

- Whole exome sequencing (WES) were performed in 36 intractable pediatrics epilepsy patients (19 Males, 17 female) at King Chulalongkorn Memorial Hospital from January 2015- August 2016.
- Twenty-three exome results (63.9%, 10 males, 13 females) revealed mutations in epilepsy related genes.

Whole Exome Sequencing in Intractable Pediatrics Epileptic Patients: King Chulalongkorn Memorial Hospital Experiences

Tayard DESUDCHIT[1)], Chupong ITTIWUT[2)], Ponghatai DAMRONGPHOL1[1,2)], Kanya SUPHAPEETIPORN[2)], Vorasuk SHOTELERSUK[2)]

- Negative in 13 patients (36.1%, 9 males, 4 females)
- Positive
- sodium channels muations (9, 25 %, including SCN1A (5, 13.9%), SCN2A (2, 5.6%), SCN8A (2, 5.6%).

- 1 cases each of pyridoxal-5-phosphate responsive epilepsy, (PNPO, 1 , 2.8 %),
- 3 ALDH7A1(17 year old, 1 from Rajburi, 1 from Ayuthaya)
- 2 congenital hypotonia-seizure due to PIGA mutation (2 , 5.6%)
  - NK: A novel hemizygous missense c.268T>C (p.Tyr90His)
  - NA: A heterozygous C1030_1032delCTT(p.Leu344del)
- 2 siblings with progressive-photosensitive-myoclonic epilepsy due to FARS2 mutation (2 patients, 5.6%).

- 5+1 more patients (13 %) were identified with different epilepsy related mutations
  - PCDH19,
  - KCNA2,
    - Onset 5m, MRI Nl, 1st EEG Nl-> Later Gen swc VPA/LEV/FYCOMPA
  - KCNMA1,
    - Onset 7m,cataplexy, ini EEG Nl->gen SZ, MRI Nl,LEV, upgaze palsy
  - KCNT1,
    - Status epilepticus, 10Hz eye blink (Facial myokymia)
  - SLC1A2,
    - onset 3m, MRI Nl, LEV/TMP Sz stopped Dr. Thitiporn, heterozygous missense c.244G>A (p.Gly82Arg)
  - GABRA1
    - Onset 6m+fever, MRI Nl, Novel chr5:161309645 G/A heterozygous, depth:403/193,GABRA1 gene

“Daignosed clinically”

- 2 x pyridoxine dependent epilepsy,
- 1 x pyridoxal-5-phosphate responsive epilepsies
- 2/5 : SCN1A were diagnosed clinically (5, 13.9 %) and exome results provide confident to adjust, reduce or discontinue anticonvulsants.

“Benefit on Pt Care”

- 18 x patients (50 %), exome ->
  - new diagnosis -> better seizure control
  - SCN2A
  - SCN1A
  - myoclonic epilepsies (KCNA2 and FARS2).

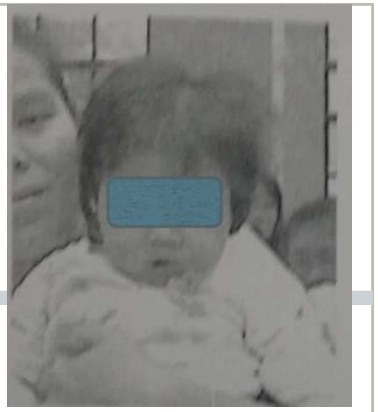
“Negative Result”

- 13 x patients with no identifiable mutation,
- three patients have early infantile epileptic encephalopathies,
- three patients with myoclonic epilepsies,
- two patients had autism with epilepsies,
- four patients had status epilepticus at various ages and
- one with clinical nocturnal frontal lobe epilepsy.

Exome in Ped. Neuro @Chula

- Paroxysmal kinesogenic Dyskinesia
- Non Paroxysmal kinesogenic Dyskinesia(KC)
- 2 x PANTOTHENATE KINASE-ASSOCIATED NEURODEGENERATION; PKAN (MV): NBIA1
- Many more “Unknown” : Infantile spasm x 2, Ohtahara,?? Unknown significant

Case 1: PJ

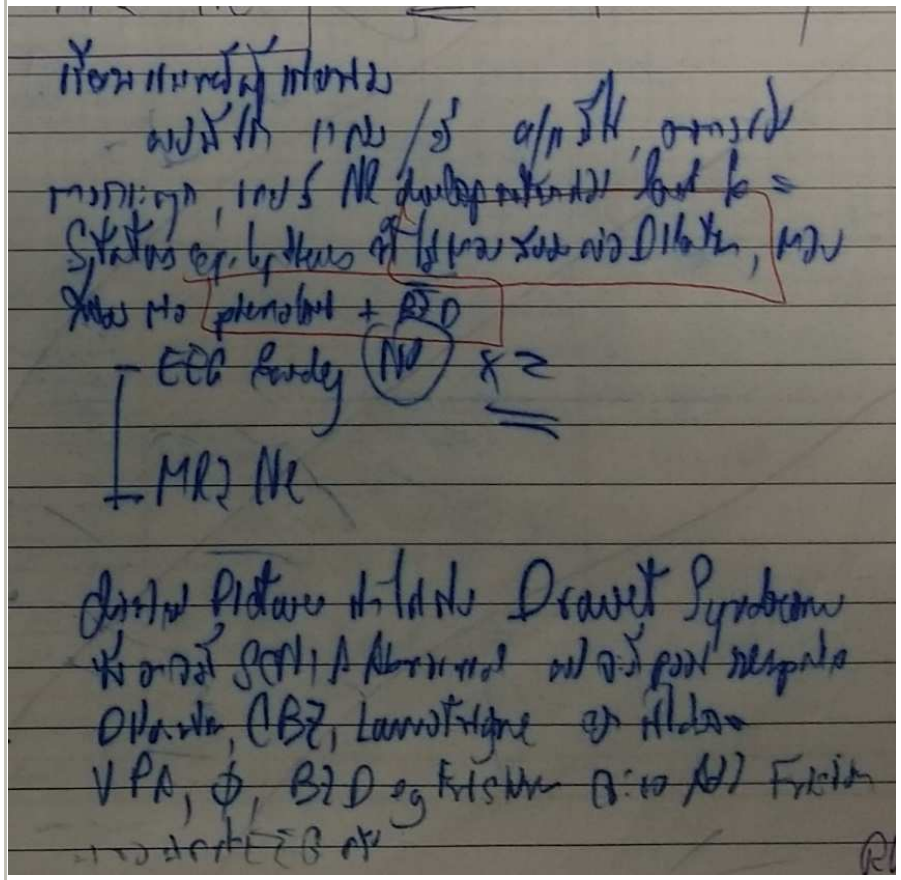


- A four month old Thai girl with prolong seizure without fever > 30 min, precipitated by fever. Rx:IV Diazepam & Dilantin 20 mg / kg & 6 hours intubation. CSF / CT brain (-)
- Second episode of seizure with fever > 10 min five days later, with fever 39 C. Rx DZP/ PHT, D/C home on PHT
- Seen @ Chula at five month old. , VPA started & increased. Had 2-3 Sz/ month. Can sit @ 5m/o.

Case 1: PJ

- DZP added -> Seizure freq. decreased, still had 2 sz with fever /3 months.
- EEG Normal x 2 @ 3 months and 5 months.
- MRI Normal @ 1 year old.
- Had another status epilepticus @ 1 year old w/o fever, Rx PHY 20 mg/ kg x 2 at BRH-> not improved -> Rx Phenobarbital & Midazolam drip.
- What is your Dx & Management ?

Case 1 : PJ



- Presumptive Dx : Dravet Syndrome
- Please avoid “Strong Sodium Channel AED” PHT/CBZ/LTG !
- Rx : Phenobarbital / BZD / TPM
- Developmental stimulaton
- Hearing / Vision scrrening

Case 1 : PJ

- Seizure free 1-2 years with break through seizure with fever
- Develpmental delayed
- Exome test 2015 : SCN1A

Lab ID	G0053202		
Clinical Dx	*epiletic encephalopathy*	**Source of Specimen**	*DNA*
Date of Birth	-	**Date of Specimen Received**	*28 April 2015*
Family ID	-	**Date of Analysis**	*25 September 2015*
Phone	-	**Date of Print**	*25 September 2015*

METHOD:

Genomic DNA was isolated from peripheral blood by ArchivePure DNA Blood kit. The mutation was identified by whole exome sequencing and confirmed by PCR-direct sequencing.

RESULTS:

A heterozygous mutation, c.3637C>T (p.R1213X), of the *SCN1A* gene was identified. The c.3637C>T (p.R1213X) mutation was not found in her parents (attached picture).

INTERPRETATION:

The heterozygous c.3637C>T (p.R1213X) mutation in the *SCN1A* gene was identified. This mutation in the *SCN1A* gene has been identified in patients with severe myoclonic epilepsy in infancy (Fujiwara T, Brain. 2003 (126):531-46).

COMMENT:

The c.3637C>T (p.R1213X) is a known mutation.

Rungnapa Ittiwut

Rungnapa Ittiwut, Ph.D.
Research scientist
29 | Sep | 2015

Chupong Ittiwut

Chupong Ittiwut, Ph.D.
Research Scientist
| |

Kanya Suphapeetiporn

Kanya Suphapeetiporn, M.D., Ph.D., FABMG
Laboratory co-director
29 | Sept | 2015

Vorasuk Shotelersuk

Vorasuk Shotelersuk, M.D., FABMG
Laboratory co-director
29 | Sep | 2015

Dravet Syndrome
Evolution of symptoms

- Initial presentation: Febrile seizures in 1st year of life
  - Often prolonged febrile seizures/febrile status
  - Evolve to Hemiclonic (alternating) or generalized tonic-clonic seizures
  - Seizures provoked by modest hyperthermia (e.g. hot bath)
  - Rarely have fever without seizure
  - Development usually normal at time of onset
- By age 2y, unprovoked seizures may begin including Myoclonic, GTC, Complex partial, absence, atonic
  - Patients experience frequent admissions with status epilepticus initially, both convulsive and nonconvulsive
  - EEG may be normal initially, but progressively worsens to generalized slowing, generalized spike/polyspike wave, multifocal independent spike wave
  - Development plateaus then progressively declines around 1 year of age or with the appearance of other seizure types

Dravet Gait

Some children with Dravet Syndrome will develop a characteristic apraxic gait demonstrated below

From: **Progressive Gait Deterioration in Adolescents With Dravet Syndrome**

Arch Neurol. 2012;69(7):873-878. doi:10.1001/archneurol.2011.3275

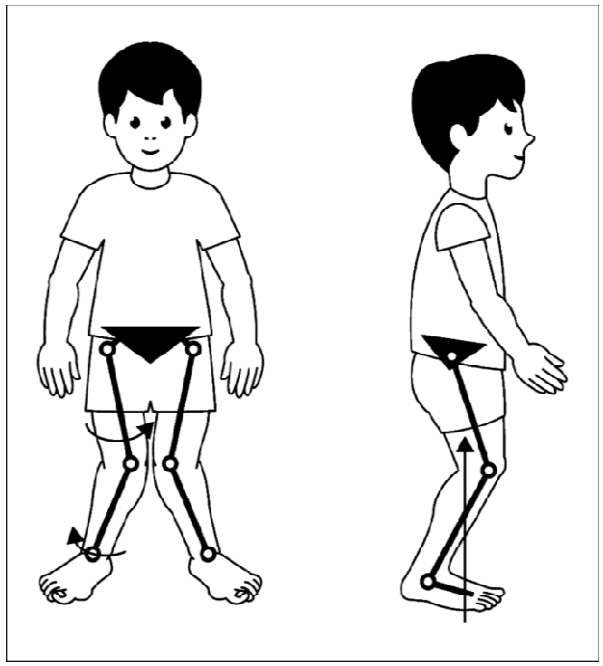


Figure Legend:

Figure 2. Crouch gait is characterized by increased hip and knee flexion and ankle dorsiflexion in the sagittal plane throughout the stance phase and is accompanied by bony malalignment in the transverse plane of medial femoral torsion, lateral tibial torsion, and planoabductovalgus of the feet.

Evaluation: Dravet Syndrome

- EEG: may be normal at initial presentation, but typically shows generalized slowing, generalized and multifocal spike wave discharges by the time unprovoked seizures begin
- MRI: often normal, but may show some cerebral atrophy or hippocampal sclerosis
- Genetic testing: SCN1A- at least 70% of patients have mutation in SCN1A, the vast majority are de novo
- Other mutations are also rarely reported to be associated with Dravet phenotype including:
- SCN2A, SCN1B, SCN8A, SCN9A, GABRG2, STXBP1

SCN1A

- Location: 2q21-34
- Function: voltage gated Na channel (NaV1.1) – mutations may lead to increased Na influx, thus excitation
- Most mutations are frameshift, nonsense, or splice-site mutations which produce nonfunctional protein
- Missense mutations are also found
- Testing strategies: DNA sequencing or Deletion Testing
- 73-92% of mutations are detectable by DNA sequencing
- 8-27% have large scale or whole gene deletions
- Microdeletions within SCN1A present in 2-3%
- Rare reports of duplication or amplification
- Location of mutation within the gene is important
- Approximately 15% of Dravet syndrome have no mutation identified

Treatment Strategies-SCN disorders

- Abnormal SCN1A channels disproportionately affect GABA neurons (Yu, et al 2006)
- Benzodiazepines
- Clobazam –dosed 0.2-1mg/kg/d divided bid/tid
- Valproic acid
- Stiripentol – not FDA approved in US
- Topiramate
- Phenobarbital – not as well tolerated secondary to cognition
- DRUGS TO AVOID: carbamazepine, lamotrigine, oxcarbazepine may worsen seizures – specifically, myoclonus

Associated SCN1A Conditions: Genetic Epilepsy with Febrile Seizures Plus

- Age of onset: 6m-6y
- Seizure types: classic febrile seizures which persist beyond 6 years, often with afebrile GTC, absence, myoclonic and focal seizures of variable frequency.
- EEG: variable findings. May be normal or often have generalized spike wave
- Key features: febrile seizures beyond 6 years and strong family history of similar febrile and afebrile seizures
- Prognosis: varies, though often the phenotypes of family members is similar
- Treatment: varies. Decision to start treatment rests on the need based on frequency of seizures.

Exome in Epilepsy

EXOME TESTING TO FIND THE CAUSE HAS MANY BENEFITS

Personal Usefulness to Patients & Families[8]

- Enables risk identification in family members
- Allows for reproductive planning
- Ends the quest for a diagnosis
- Ameliorates parental guilt or shame
- Allows for connection to resources and community

Medical Usefulness to Doctors[8]

- In some cases, enables changes in medical management
- Allows for prediction of epilepsy progression
- Enables genetic counseling (many mutations shown to be *de novo**)
- Enables enrollment in clinical trials and research
- Can decrease the time/cost of diagnostic and treatment odyssey

**De novo* – A spontaneous gene alteration that arises in the developing child; not inherited.

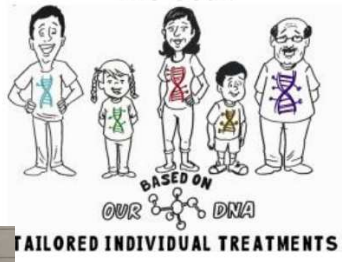
EXOME TESTING CAN HELP ADVANCE RESEARCH

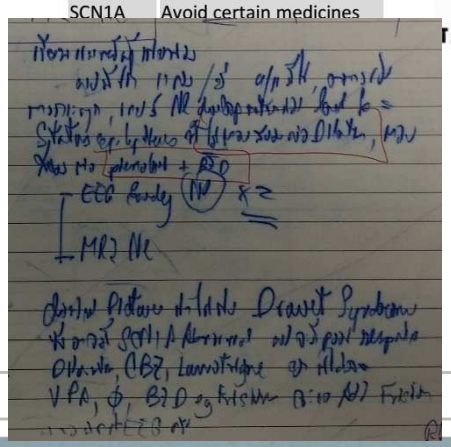
Advancing our understanding of the genetic causes of epilepsy will allow us to improve the ways we anticipate, prevent, diagnose, and treat epilepsy.

Precision therapies are already available for some patients[8]

Gene	Treatment
SLC2A1	Keto diet
POLG	Avoid certain medicines
ALDH7A1	Vitamin B6
SCN1A	Avoid certain medicines

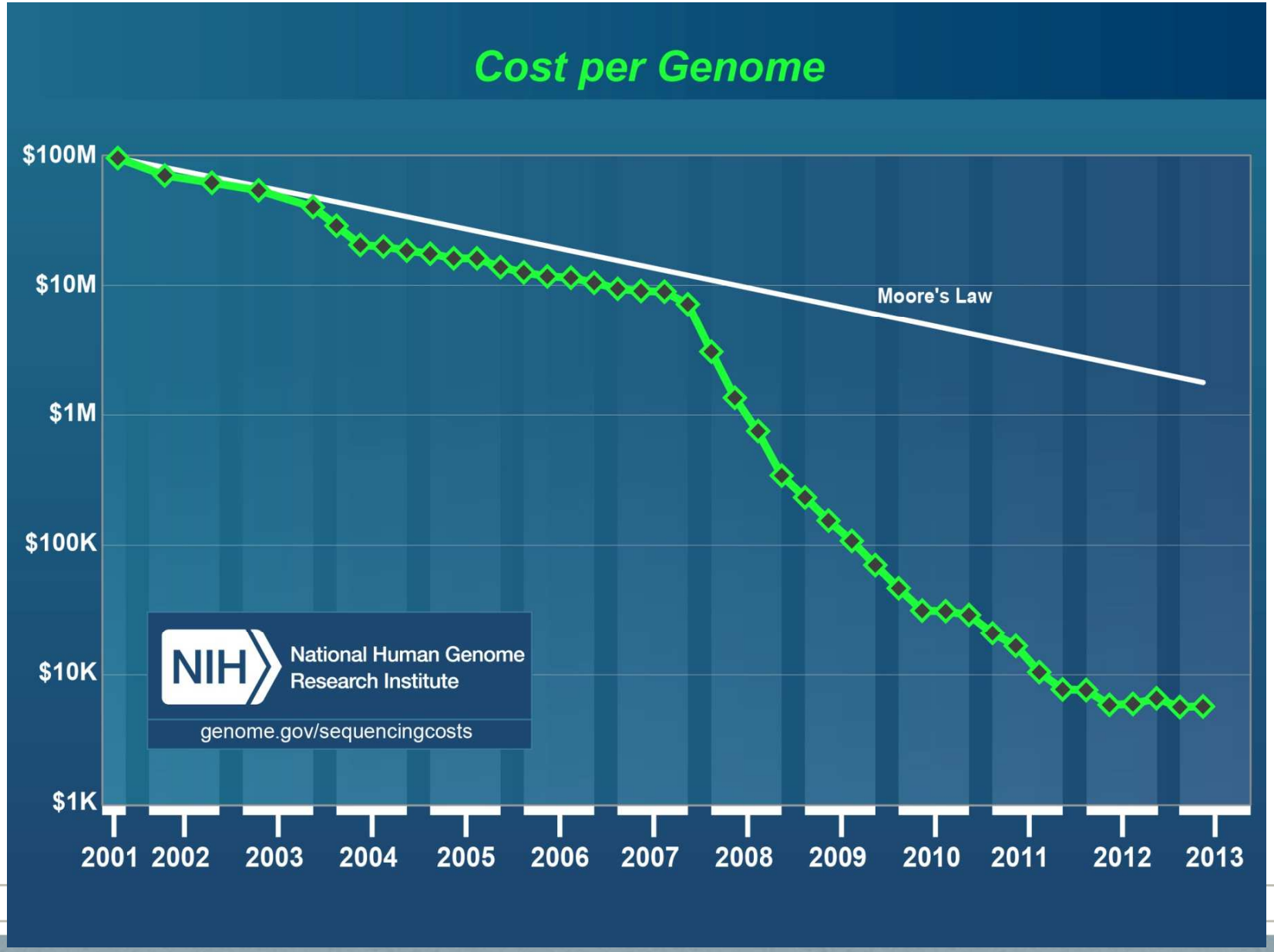
The Goal:



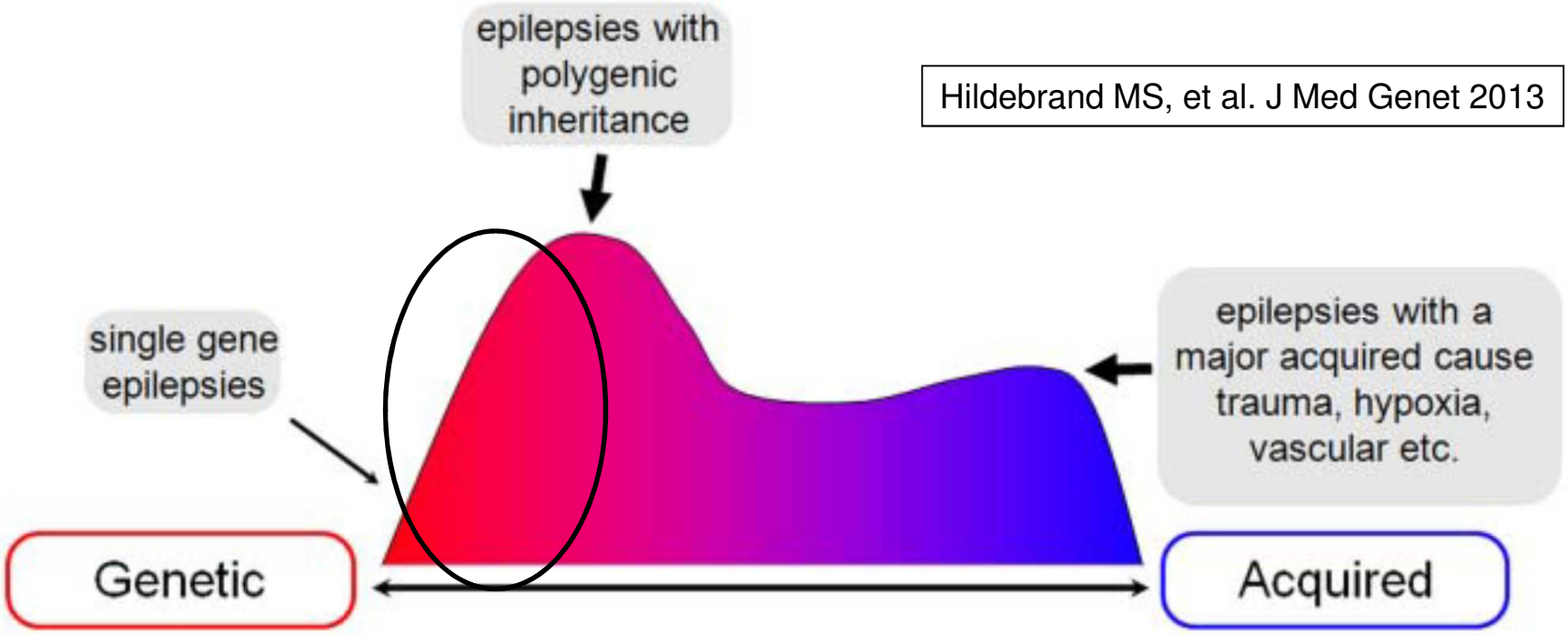


www.CUREepilepsy.org/EGI

Whole Genome Sequencing



No diagnosis obvious……(most cases)



Hildebrand MS, et al. J Med Genet 2013

Same model applicable to intellectual disability and autism

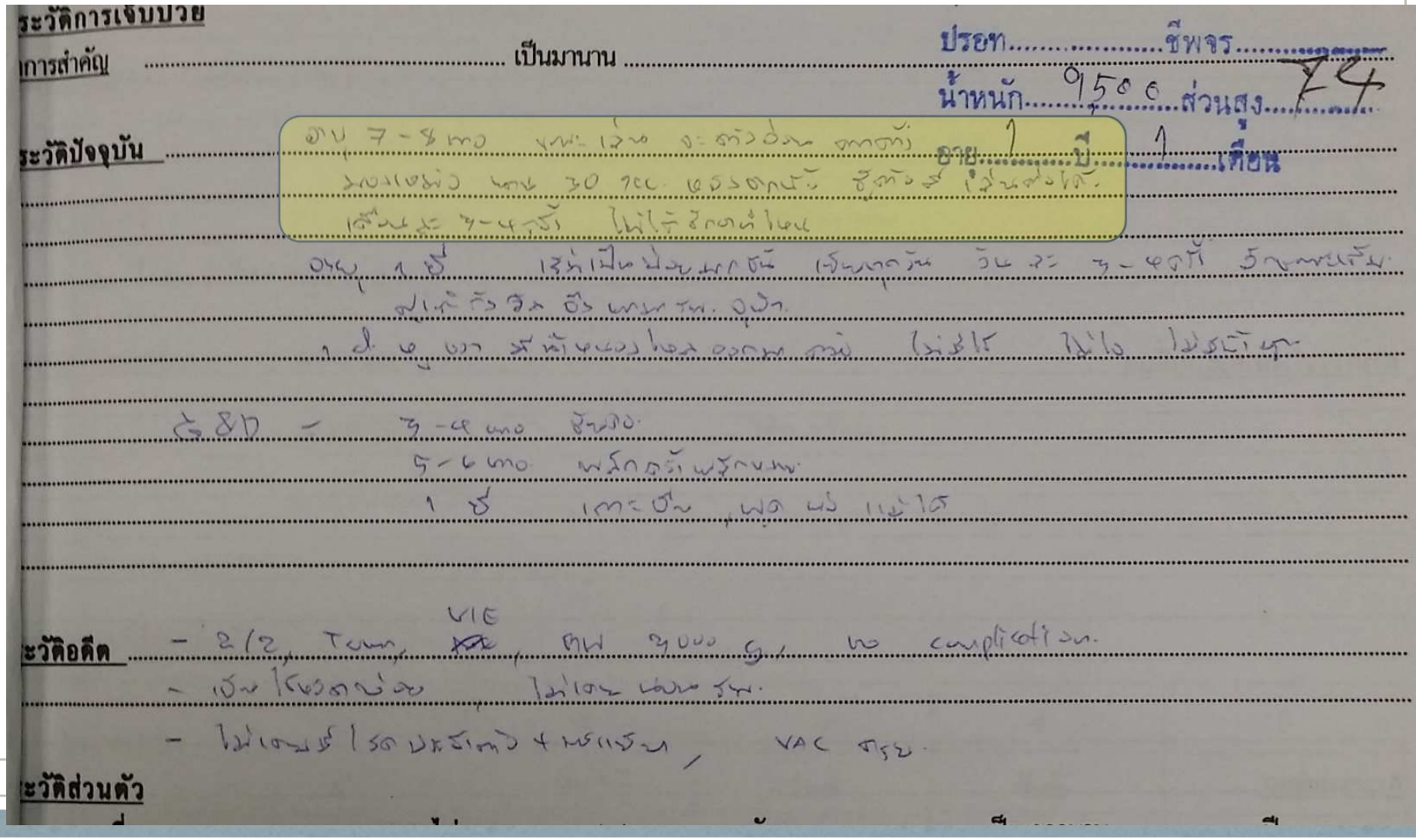
Look for the specific

- SCN1A : Look for the "Dravet Gait"
  - Avoid SC : PHT,CBZ,
  - Rx VPA,TPM,Frisium, fypompa
- "Myoclonus"
  - PME, Lafora
  - KCNA2 : Lev,VPA,LTG,Fycompa2
  - FARS2

Look for the specific

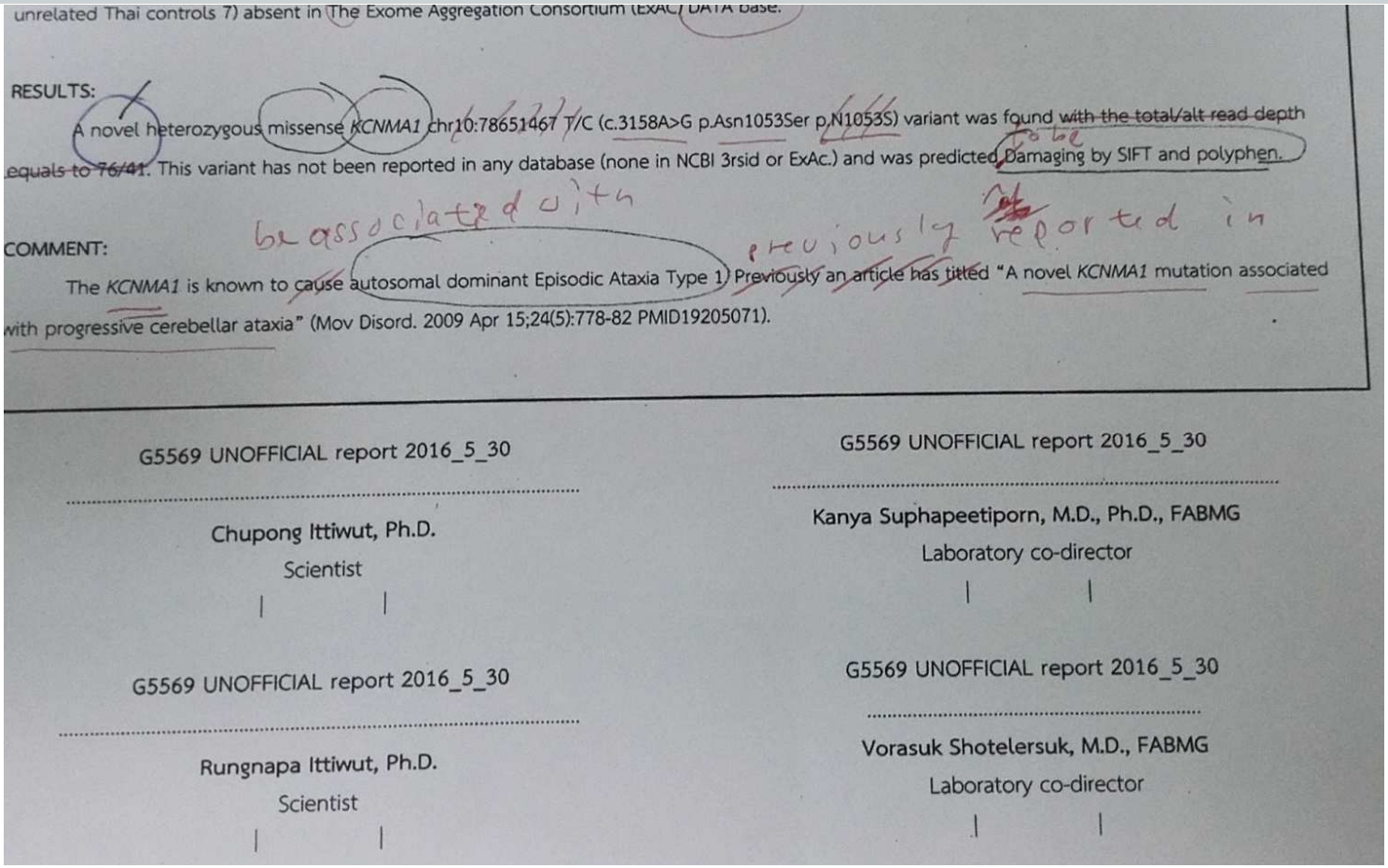
- SCN1A : Look for the "Dravet Gait"
  - Avoid SC : PHT,CBZ,
  - Rx VPA,TPM,Frisium, fypompa
- "Myoclonus"
  - PME, Lafora
  - KCNA2
  - FARS2 : VPA, Clona,Dec Pheno, add fycompa

Case KPC



- EEG : initially Normal-> Gen SWC in 2558(9 yo)
- Video EEG : Recorded events has no EEG correlate
- Clinical Dx : Cataplexy DDx
  - MRI : normal
  - Neimann-PICK screen send to Taiwan : Normal
  - No response to all cataplexic drugs / AEDs

Exome : KCNMA1



unrelated Thai controls 7) absent in The Exome Aggregation Consortium (EXAC) DATA base.

RESULTS:

A novel heterozygous missense *KCNMA1* chr10:78651467 T/C (c.3158A>G p.Asn1053Ser p.N1053S) variant was found with the total/alt read depth equals to 76/41. This variant has not been reported in any database (none in NCBI 3rsid or ExAc.) and was predicted damaging by SIFT and polyphen.

COMMENT:

The *KCNMA1* is known to cause autosomal dominant Episodic Ataxia Type 1. Previously an article has titled "A novel *KCNMA1* mutation associated with progressive cerebellar ataxia" (Mov Disord. 2009 Apr 15;24(5):778-82 PMID19205071).

G5569 UNOFFICIAL report 2016_5_30

Chupong Ittiwut, Ph.D.
Scientist

G5569 UNOFFICIAL report 2016_5_30

Kanya Suphapeetiporn, M.D., Ph.D., FABMG
Laboratory co-director

G5569 UNOFFICIAL report 2016_5_30

Rungnapa Ittiwut, Ph.D.
Scientist

G5569 UNOFFICIAL report 2016_5_30

Vorasuk Shotelersuk, M.D., FABMG
Laboratory co-director

Novel mutation KCNMA1:
Generalized epilepsy with Cataplexy

.0001 GENERALIZED EPILEPSY AND PAROXYSMAL DYSKINESIA

KCNMA1, ASP434GLY [dbSNP:rs137853333] [ClinVar]

In all 13 affected members of a family with generalized epileptic seizures and paroxysmal nonkinesigenic dyskinesia or both (GEPD; 609446), Du et al. (2005) found a 1301A-G transition in exon 10 of the KCNMA1 gene, resulting in an asp434-to-gly (D434G) amino acid change in the regulator of conductance for K+ (RCK) domain. In affected members of this family, alcohol triggered dyskinesia. Du et al. (2005) suggested that the gain-of-function mutation D434G may have a synergistic effect with ethanol in the triggering of symptoms.

Thank you