Diagnostic Opportunities for Rare Disease with NGS

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FORGE
CANADA CONSORTIUM

CAREforRARE

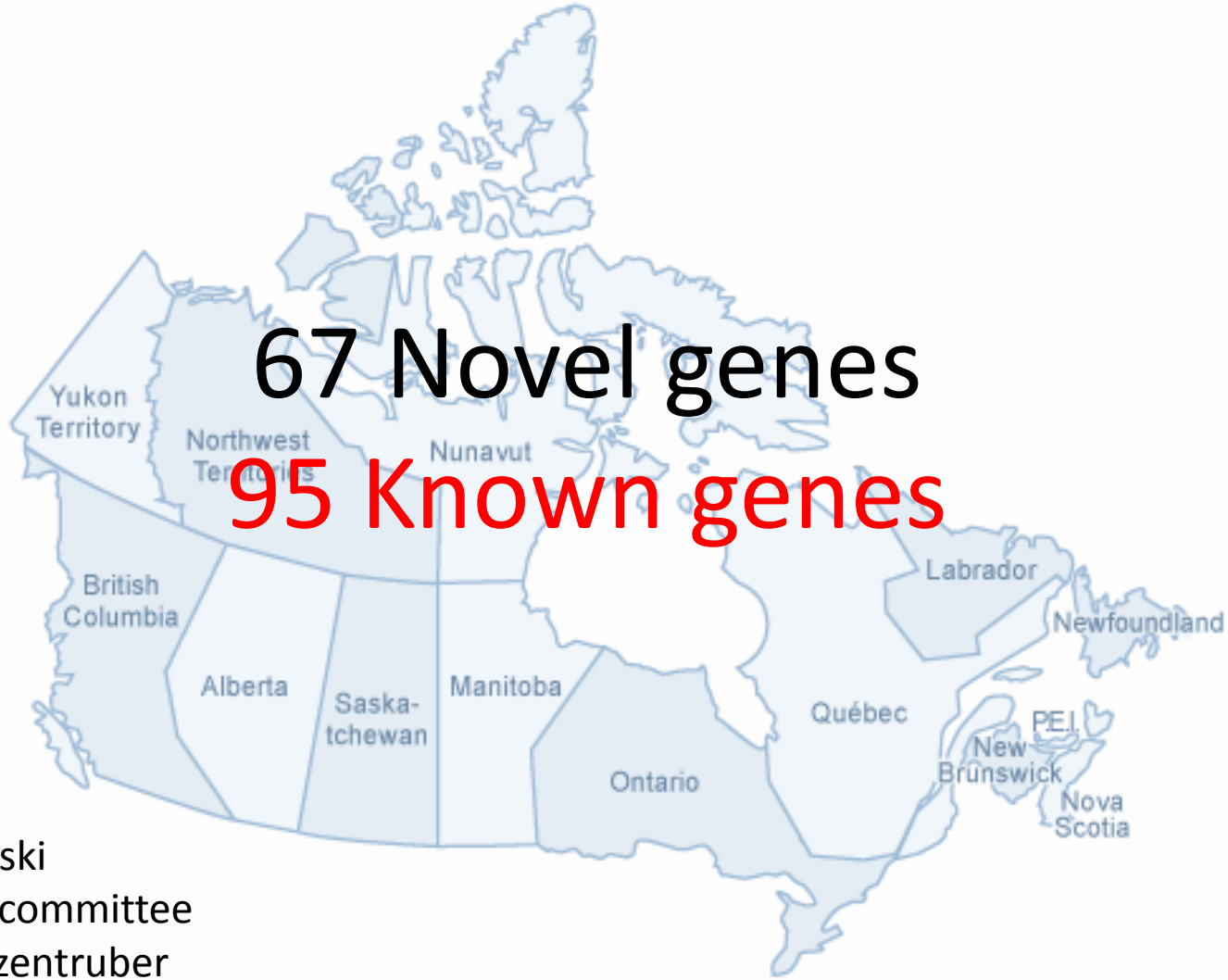
uOttawa
L'Université canadienne
Canada's university

NGS is changing the pace of diagnosis, reducing diagnostic odyssey for patients

Clinical opportunity to increase diagnostic rate for rare disease patients

Diagnosis for patient with rare disease is often laborious, time consuming, and frustrating for families

FORGE; Finding of rare disease genes



67 Novel genes

95 Known genes

Dr. Kym Boycott
Dr. Jacek Majewski
FORGE steering committee
Jeremy Schwartzentruber
Chandree Beaulieu

FORGE: 264 Projects >500 families

67 Novel genes

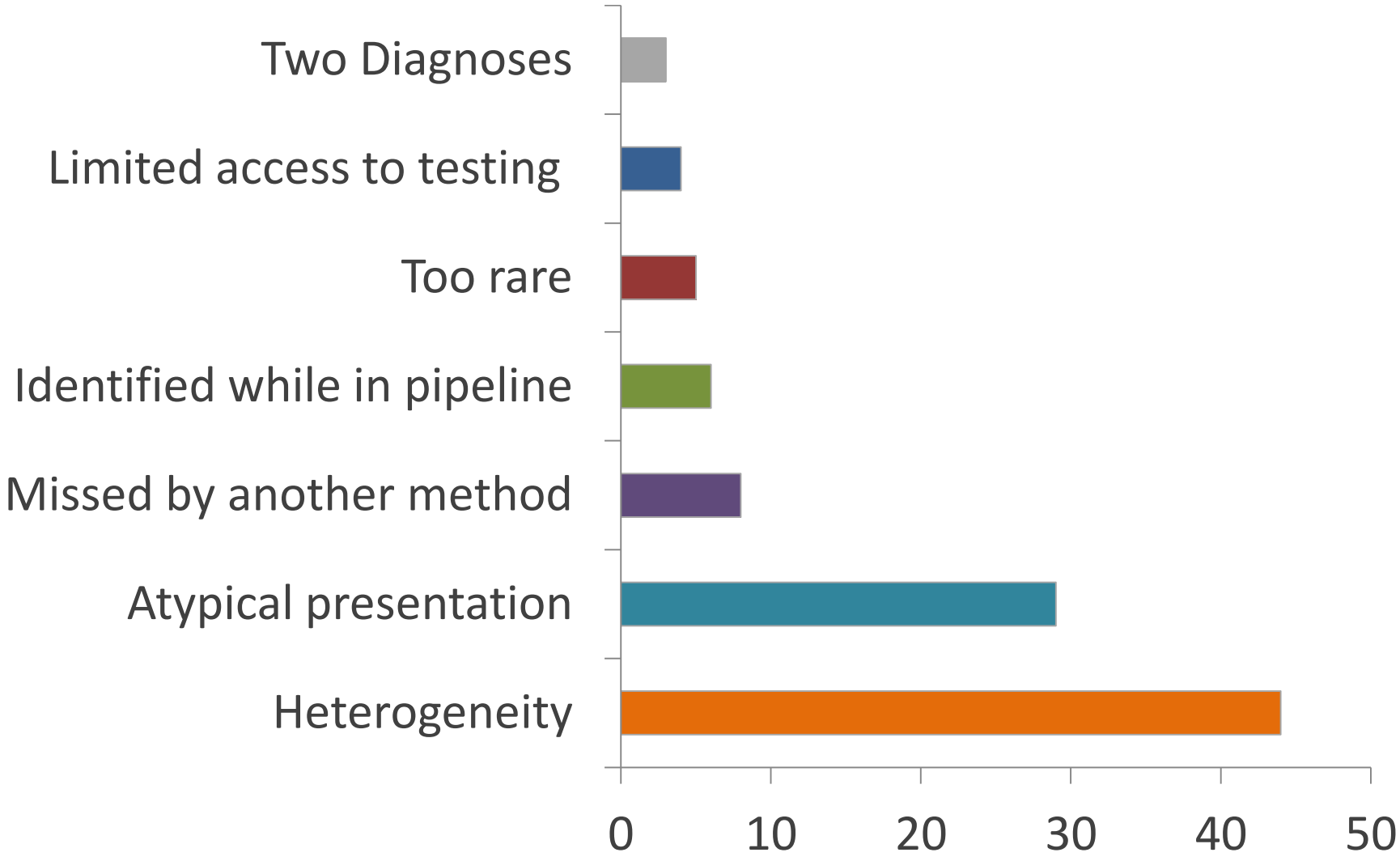
96 Known genes
100 diagnoses

PIK3R1

SRCAP

POMC, NNT, BRCA1, EFNB1, RAB3GAP1, SACS, ALG3, RTTN, MLL2, RPE65, SLC45A2, G6PC3, LRP5, IGHMBP2, CYP26C1, PLCB4, TERT, IDS, PRPS1, TRPV4, CORO1A, HSD17B4, OFD1, CEP290, CC2D2A, GRIN2A, EFTUD2, GNE, NDUFS2, SPTAN1, CHM, MUSK, C12orf65, MTO1, MUSK, SYNGAP1, TMPRSS6, ABCD1, PLA2G6, PYCR1, ZMYND10, SPAG1, LRRC6, MYOC, WDR36, NTF4, ASAH1, ATM, KAT6B, OTX2, COX10, SLC25A1, ALDH6A1, COL11A1, GRIN2A, AICDA, EP300, FRAS1, WNT5A, RARS2, PMM2, COQ9

Opportunities for patients with atypical presentations and heterogeneous disorders

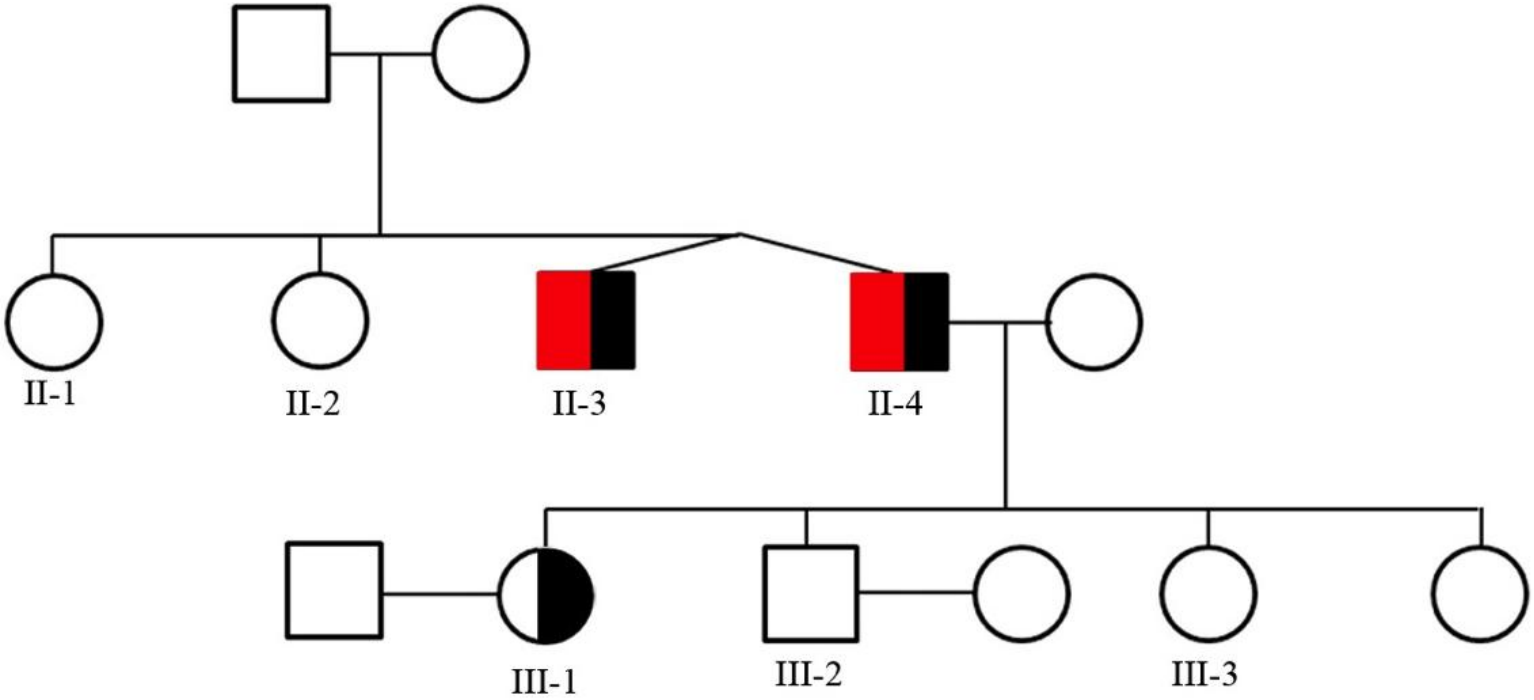


Two disorders in one patient: Fitzsimmons Syndrome

Hereditary spastic paraparesis and brachydactyly

Dr. Mohnish Suri, Dr. Christine Armour, Taila Hartley

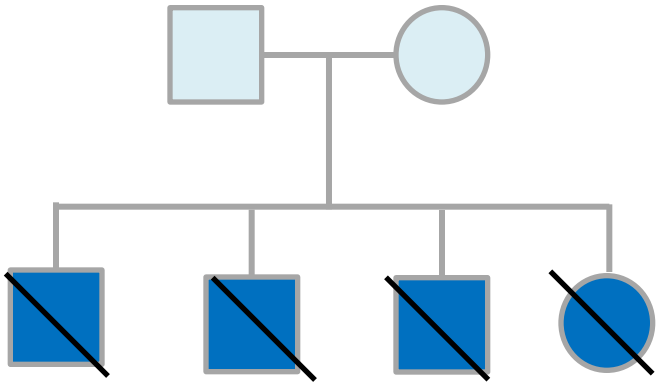
Trichorhinophalangeal syndrome, type III & Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS)



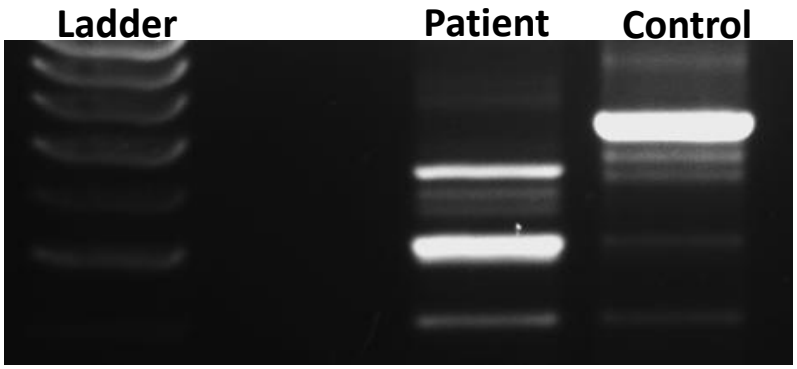
SACS: c.A2338T:p.K780X,
c.9346_9347:p.K3116delinsKLPK

TRPS1:c.276C>T:p.R921X

Ultra Rare: Neo-natal onset primary Coenzyme Q_{10} deficiency due to mutations in *COQ9*



COQ9: c.521+2T>C and c.711+3G>C



Lactic acidosis, hypotonia, cardiomyopathy, hypoplasia of corpus callosum, subventricular cysts around lateral ventricles and clenched hands.

Early death

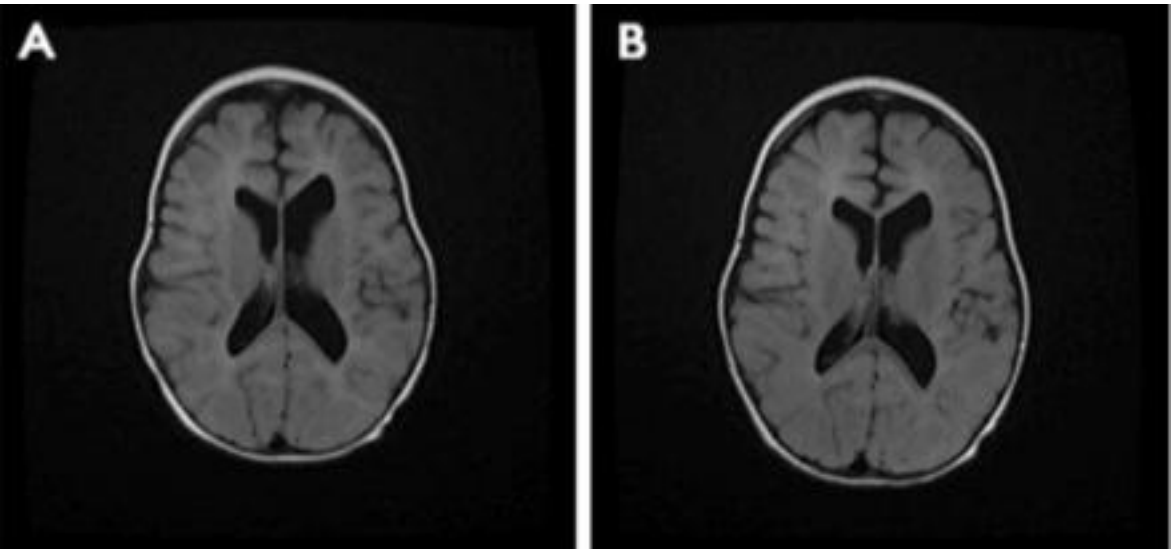
Mitochondrial disorder suspected

Neonatal presentation of coenzyme Q_{10} deficiency

Shamima Rahman, MRCP, Iain Hargreaves, PhD, Peter Clayton, MD, FRCP, and Simon Heales, PhD, MRCPath

Ultra Rare (n=5)

Mutations in *ALDH6A1* encoding methylmalonate semialdehyde dehydrogenase are associated with dysmyelination and transient methylmalonic aciduria



A. 13 months B. 21 months

Dr. Michael Geraghty,
Orphanet J Rare Disease 2013

3 reported cases with molecular confirmation

Variably elevated lactates

Delayed myelination

Developmental delay

Atypical Presentation of a known disorder

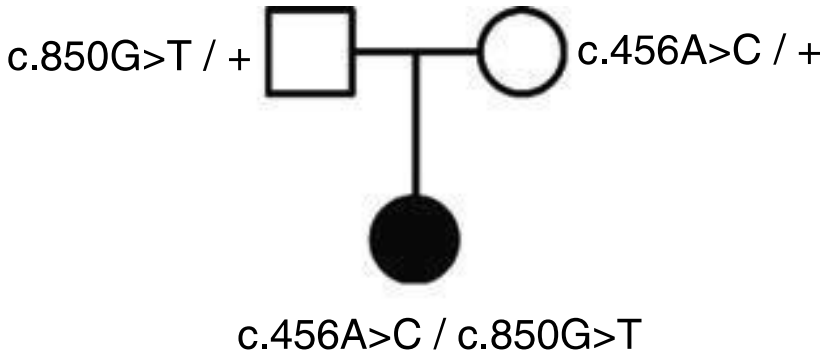
Presented with absence and atonic seizures with sudden falls at age **10**

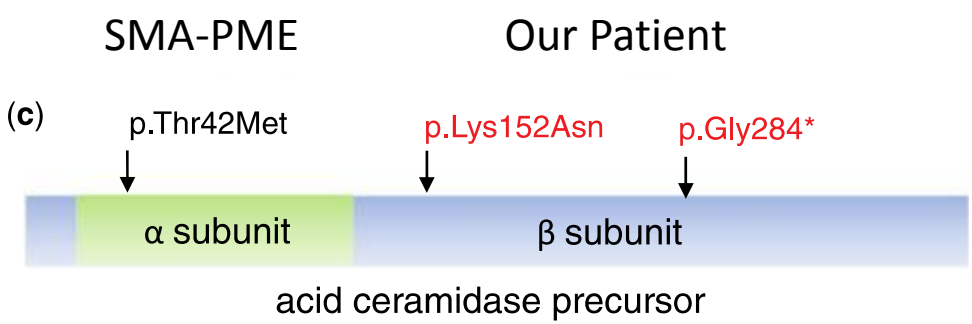
Frequent myoclonic jerks of upper and lower extremities

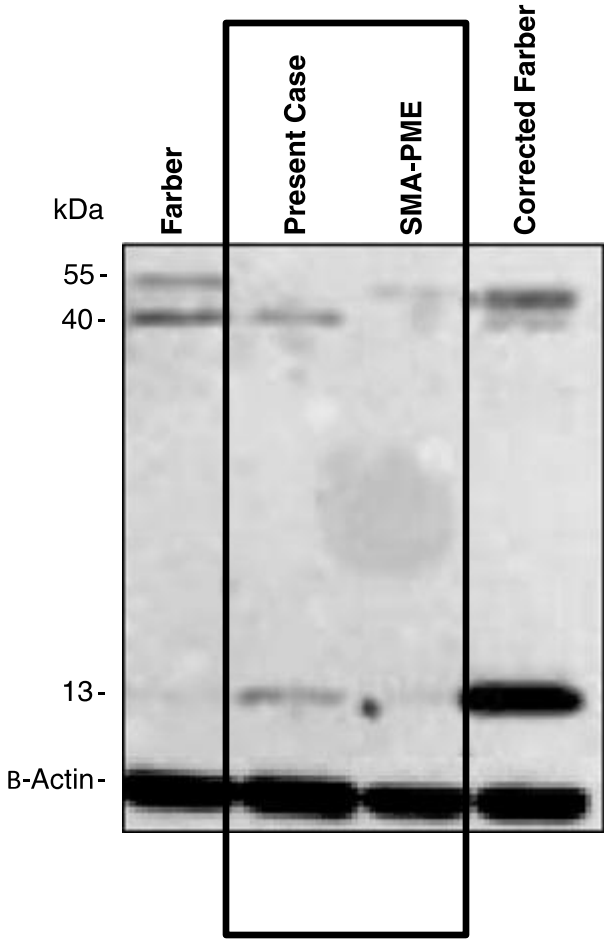
Seizures reached >100 per day, classified as myoclonic-absence epilepsy

Bilateral sensorineural hearing loss

Spinal muscular atrophy with progressive myoclonic epilepsy (SMA-PME): *ASAH1*







Atypical Presentation of a known disorder

Microcephaly
TEF
Cleft palate
Choanal atresia

Deafness
Heart defect

Normal *CDH7*

CHARGE-like

Haploinsufficiency of a Spliceosomal GTPase Encoded by *EFTUD2* Causes Mandibulofacial Dysostosis with Microcephaly

Matthew A. Lines,[1] Lijia Huang,[1] Jeremy Schwartzentruber,[2] Stuart L. Douglas,[1] Danielle C. Lynch,[1] Chandree Beaulieu,[1] Maria Leine Guion-Almeida,[3] Roseli Maria Zechi-Ceide,[3] Blanca Gener,[4] Gabriele Gillessen-Kaesbach,[5] Caroline Nava,[6] Geneviève Baujat,[6] Denise Horn,[7] Usha Kini,[8] Almuth Caliebe,[9] Yasemin Alanay,[10,11] Gulen Eda Utine,[10] Dorit Lev,[12] Arthur W. Grix,[14] Dietmar R. Lohmann,[15] Ute Hehr,[16] Detlef Böhm,[13] Jacek Majewski,[18] Dennis E. Bulman,[19] Dagmar Wieczorek,[15,20] and Kym M. Boycott[1,20,*]

FORGE Canada Consortium

EFTUD2 deletion

Mandibulofacial dysostosis and microcephaly-*EFUTD2*

Phenotypic spectrum not well understood

Three patients separately identified with mutations in this gene!

Need for non-biased methods for diagnosis

Missed by another method: Emery-Dreifuss-like (*COL6A1*)

FINAL DIAGNOSIS:

Biopsy, Skeletal muscle (immuno only) - Immunopositive for alpha-dystroglycan and collagen VI

Staff Pathologist

*****Electronically Signed Out by *****

CLINICAL HISTORY
No clinical history provided with specimen.

TISSUE SUBMITTED:
7 Unstained frozen slides

GROSS DESCRIPTION:
Received at the request o e Children's Hospital of Eastern Ontario are 7 frozen slides for Immunostaining.

MICROSCOPIC DESCRIPTION:
IMMUNOFLUORESCENCE
The muscle cells how sarcolemmal immunopositivity for alpha-dystroglycan and Collagen VI, the latter is normally co-localised with laminin.

End of Report

Missed by another method: Emery-Dreifuss *COL6A1*

Project# And Disorder	Gene name	Patient ID	Mutation Type	Mutation	If match with Exome Sequencing
C4R_468	COL6A1	Affected (CH0048)	Het Splicing	COL6A1(NM_001848:exon14:c.1003-3C>G)	Yes
Emery-Dreifuss like		Mother (CH0047)	Normal	Normal	NA
		Father (CH0049)	Normal	Normal	NA

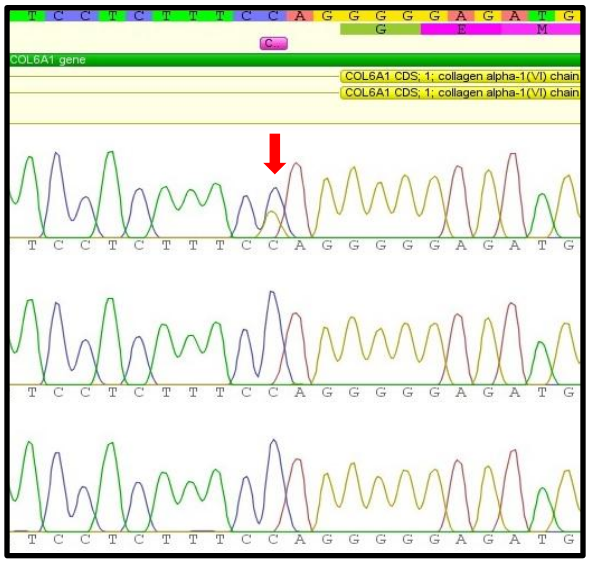
COL6A1(NM_001848:exon14:c.1003-3C>G)

De Novo

Affected

Mother

Father



Genetic Heterogeneity: Ataxia

Feature of >100 neurological disorders with childhood onset

Standard-of-care genetic testing for patients with ataxia: SCA panel (repeats), Friedreich's ataxia (FXN), ARSACS (*SACS*)

Retrospectively selected all FORGE Canada projects that included cerebellar ataxia as a feature; 28 families

Diagnosed 11 out of 28= 39%

DISORDER	# AFF	GENE	DISORDER
Congential cerebellar atrophy	2	PMM2	Congenital disorders of glycosylation
Holmes syndrome	2	RNF216	No change
AR spinocerebellar ataxia childhood onset	1	SACS	ARSACS
Perrault syndrome	2	HSD17B4	D-bifunctional protein deficiency
Ataxia with cognitive impairment	2	SETX	AR spinocerebellar ataxia
Cerebellar atrophy	3	HSD17B4	D-bifunctional protein deficiency
Marinesco Sjogren	1	RAB3GAP1	Warburg Micro syndrome
Ataxia, DD, seziures	1	SYNGAP1	Intellectual disability
Ataxia	1	SACS	ARSACS
Epilepsy with ataxia	3	KCTD7	Progressive myoclonic epilepsy
DD and hypotonia	1	PLA2G6	Neurodegeneration with brain iron accumulation

BRIEF REPORT

Human Mutation

OFFICIAL JOURNAL
HGVS
HUMAN GENOME VARIATION SOCIETY
www.hgvs.org

Exome Sequencing as a Diagnostic Tool for Pediatric-Onset Ataxia

Sarah L. Sawyer,[1] Jeremy Schwartzentruber,[2] Chandree L. Beaulieu,[1] David Dyment,[1] Amanda Smith,[1] Jodi Warman Chardon,[1] Grace Yoon,[3] Guy A. Rouleau,[4] Oksana Suchowersky,[5] Victoria Siu,[6] Lisa Murphy,[6] Robert A. Hegele,[7] Christian R. Marshall,[8] FORGE Canada Consortium, Dennis E. Bulman,[1] Jacek Majewski,[9] Mark Tarnopolsky,[10†] and Kym M. Boycott[1*†]

THE UNIVERSITY OF CHICAGO GENETIC SERVICES

Ataxia exome panel: 332 genes

AAAS	ARL13B	BCKDHA	CLN5	CTSA	EIF2B4	FOXG1	HSPD1	L2HGDH	NDUFA10	NDUFV1	PC	PMP22	RARS2	SH3TC2	STUB1	TMEM70	WDR62
ABCB7	ARX	BCKDHB	CLN6	CTSD	EIF2B5	FOXRED1	IFT140	LARGE	NDUFA11	NDUFV2	PCDH19	PNKD	REEP1	SIL1	SUCLG1	TPK1	WDR81
ABCD1	ASL	BCS1L	CLN8	CUL4B	ELOVL5	FTL	INPP5E	LIG4	NDUFA12	NEU1	PDHA1	PNPLA6	RELN	SLC16A2	SUOX	TPP1	WWOX
ABHD12	ASS1	BEAN1	COA5	CWF19L1	EOMES	FXN	ISPD	LMNB1	NDUFA2	NHLRC1	PDHB	POLG	RNF170	SLC17A5	SURF1	TSEN2	XPA
ACO2	ATCAY	BSCL2	COG4	CYP27A1	EPM2A	GAN	ITM2B	LRPPRC	NDUFA9	NIPA1	PDSS1	POLR3A	RNF216	SLC19A3	SYNE1	TSEN34	ZFYVE26
ADCK3	ATL1	BTD	COL18A1	CYP7B1	ERCC3	GBA2	ITPR1	LRSAM1	NDUFAF1	NKX2-1	PDSS2	POLR3B	ROGDI	SLC1A3	SYT14	TSEN54	ZFYVE27
AFG3L2	ATM	C10orf2	COQ2	DARS2	ERCC4	GBE1	KCNA1	MAN2B1	NDUFAF2	NOP56	PDX1	POMGNT1	RPGRIP1L	SLC33A1	TBC1D24	TTBK2	ZIC1
AHI1	ATN1	C12orf65	COQ4	DBT	ERCC5	GCH1	KCNC3	MAPK10	NDUFAF3	NPC1	PDYN	POMGNT2	RRM2B	SLC46A1	TBP	TTC19	ZIC4
ALDH5A1	ATP1A3	C5orf42	COQ6	DKC1	ERCC6	GCLC	KCND3	MARS2	NDUFAF4	NPC2	PEX1	POMT1	RTN2	SLC52A2	TCF4	TTC21B	ZNF592
ALG6	ATP7B	CA8	COQ9	DLAT	ERCC8	GFAP	KCNJ10	MECP2	NDUFAF5	NPHP1	PEX10	POMT2	SACS	SLC6A19	TCTN1	TTPA	
AMACR	ATP8A2	CACNA1A	COX14	DLD	ERLIN2	GJC2	KCNQ2	MFSD8	NDUFAF6	NUBPL	PEX2	PPP2R2B	SCARB2	SLC9A6	TCTN2	TTR	
ANO10	ATPAF2	CACNB4	COX15	DNAJC19	EXOSC3	GLB1	KCTD7	MLC1	NDUFB3	NUP62	PEX26	PPT1	SCN1A	SPAST	TCTN3	TYMP	
AP4B1	ATXN1	CAMTA1	COX6B1	DNAJC5	FA2H	GOSR2	KIAA0196	MPV17	NDUFS1	OFD1	PEX7	PRICKLE1	SCN2A	SPG11	TDP1	UBE3A	
AP4E1	ATXN10	CASK	CP	DNM2	FASTKD2	GPR56	KIAA0226	MPZ	NDUFS2	OPA1	PHYH	PRICKLE2	SCN8A	SPG20	TGM6	UQCRB	
AP4M1	ATXN2	CC2D2A	CPS1	EEF2	FGF14	GRM1	KIF1A	MRE11A	NDUFS3	OPA3	PIK3R5	PRKCG	SCN9A	SPG21	TINF2	UQCRQ	
AP4S1	ATXN3	CDKL5	CRAT	EGR2	FKRP	GRN	KIF1B	MTPAP	NDUFS4	OPHN1	PLA2G6	PRNP	SDHA	SPG7	TMEM138	UROC1	
AP5Z1	ATXN7	CEP290	CSTB	EIF2B1	FKTN	HEXA	KIF5A	MTTP	NDUFS6	OTC	PLEKHG4	PRPS1	SDHAF1	SPR	TMEM216	VAMP1	
APOB	AUH	CEP41	CTC1	EIF2B2	FLVCR1	HEXB	KIF7	MVK	NDUFS7	OTUD4	PLP1	PRX	SEPSECS	SPTAN1	TMEM237	VLDLR	
APTX	B4GALNT1	CHMP1A	CTDP1	EIF2B3	FMR1	HLCS	L1CAM	NDUFA1	NDUFS8	PAX6	PMM2	PSAP	SETX	SPTBN2	TMEM67	VRK1	

Lessons learned-what works!

1. WES diagnosed patients with disorders with significant genetic heterogeneity

2. Relatively non-biased approaches to testing identified patients with atypical presentations and ultra- rare disorders

Lessons learned-what works!

1. WES diagnosed patients with disorders with

Therapies were adjusted or initiated for 6 patients given a clinical diagnosis

A molecular diagnosis provides an opportunity to treat patients with rare disease

Therapies were adjusted or initiated for 6 patients given a clinical diagnosis

~6%

Intractable Epilepsy

de novo GRIN2A mutation

Subunit of N-methyl D-aspartate
Mediates excitatory transmission in the CNS

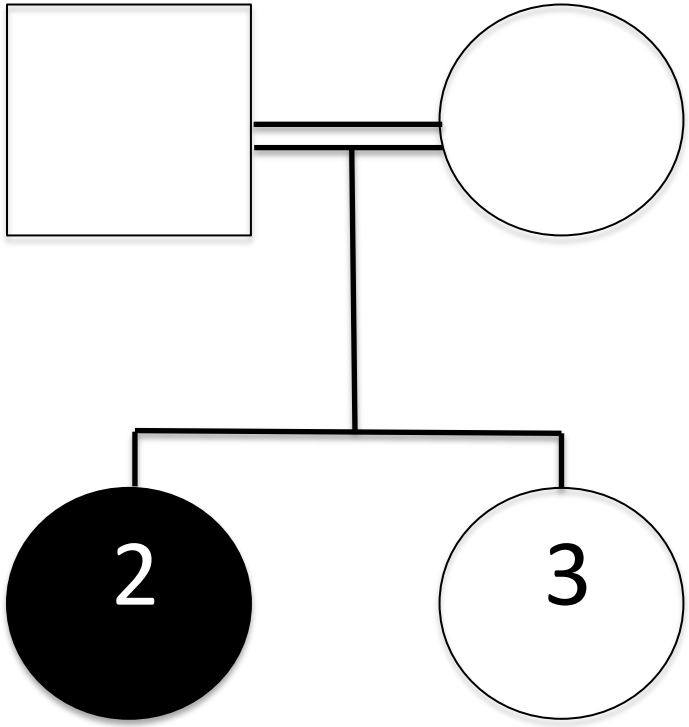
Adjusted therapy: Topiramate enhances GABA evoked current

10 months of significant seizure reduction

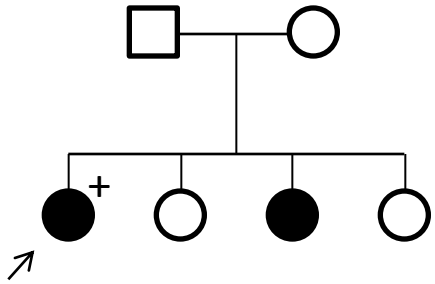
Dr. David Dyment, Epilepsia in press

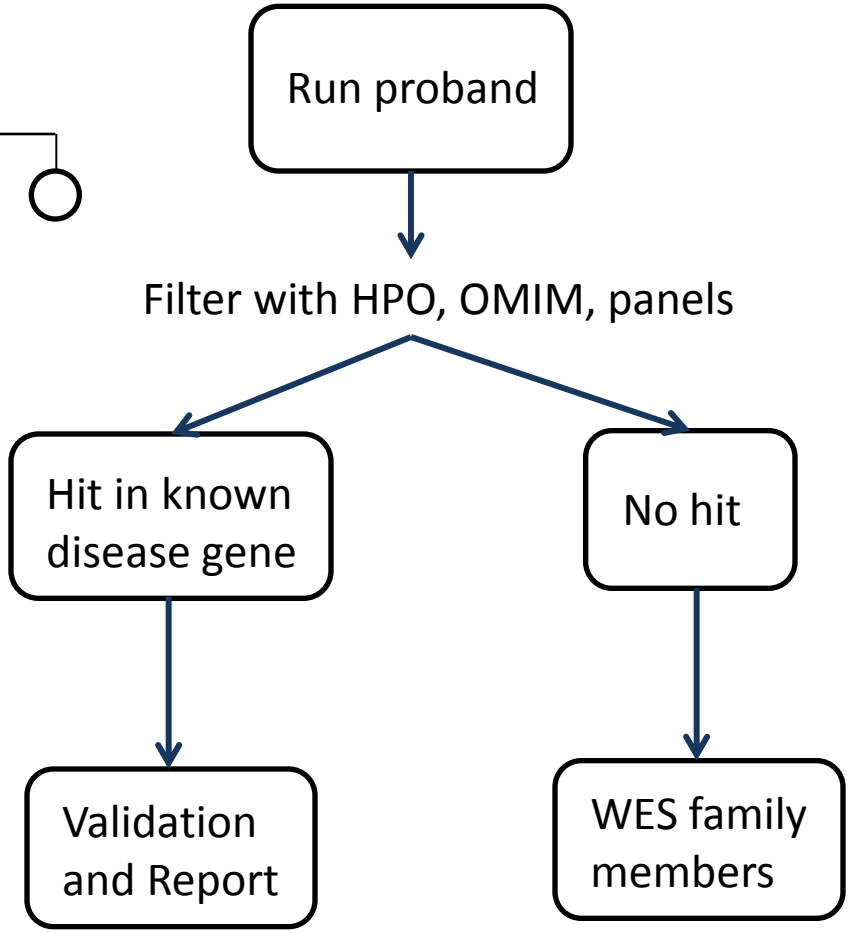
Cerebral folate transporter deficiency

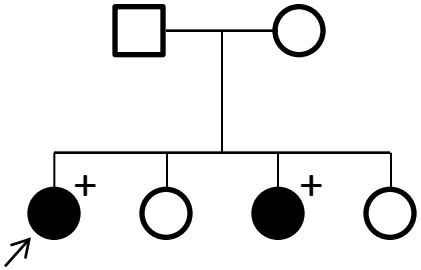
Seizures and intellectual disability, onset at 2y
Extensive white matter damage
Homozyous mutations in FOLR1
Rx: Improved seizure control on folinic acid



WES staged approach







Clinical implementation for Canada

Defined Diagnostic Utility

CCMG Position Statement

Approach to Incidental Findings

CCMG Best Practice Guidelines

Economic Impact

Education

Impact of diagnosis on the health care system

1. Shorten the diagnostic odyssey
2. Some disorders are treatable!

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Instituts de recherche en santé du Canada

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The Future is in Our Genes.

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