Identification of new genes responsible for Nocturnal Frontal Lobe Epilepsy (NFLE): WES analysis in a large cohort.

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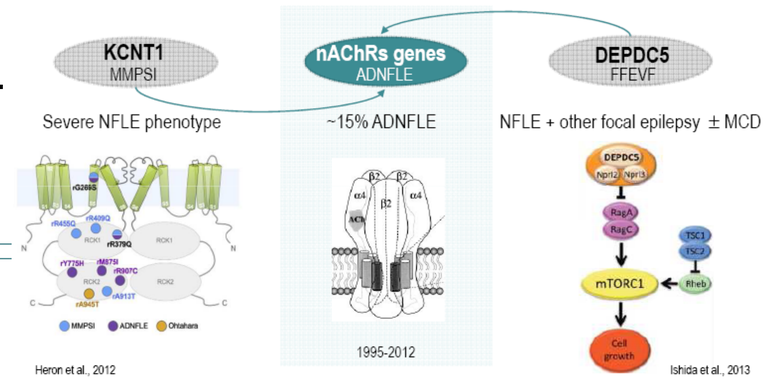
PURPOSE: Autosomal Dominant NFLE (ADNFLE) shows **genetic heterogeneity.**

NFLE is also one of the most common epilepsy phenotype of FFEVF (Familial Focal Epilepsy with Variable Foci). We conducted a clinical and genetic study of NFLE patients, sporadic and familial, negative for mutations in nAChRs genes at the previous screening by denaturing High Performance Liquid Chromatography (dHPLC) to: (i) Identify novel genes for NFLE/FFEVF; (ii) Evaluate the mutation rate of KCNT1 and DEPDC5 in NFLE.



nAChRs: neuronal nicotinic Acetylcholine receptor subunits; MMPSI: malignant migrating partial seizures of infancy; ADNFLE: autosomal dominant NFLE; FFEVF: familial focal epilepsy with variable Foci; MCD: malformation of cortical development.

MATERIALS AND METHODS:

Population Inclusion criteria

(i) all consenting patients diagnosed with NFLE on clinical and Video-EEG criteria were enrolled:
- from a pool of NFLE patients diagnosed and followed up in our Institute, negative for nAChRs genes mutations
- newly-diagnosed cases, referred to our Institute since 2012
- patients referred from other Italian Epilepsy Centers (genetic commission of LICE)

(ii) sporadic and familial cases (≥1 relative within II grade of relatedness with NFLE/other focal epilepsy →ADNFLE and FFEVF pedigree

Clinical study: detailed electro-clinical data were collected in an ad hoc database

Genetic study: WES analysis performed in: 1) familial cases: the proband and at least one of the affected members, when available;
2) sporadic cases: TRIO approach.

WES analysis – details:
HiSeq 2000, 91PE, 12G clean data 100X/sample.
Variants retained:
(i) Exonic, nont synonymous
(ii) absent in public database
(ii) GERP score ≥2
Familial cases: CADD scaled >10
TRIO: de novo probability>0.5 by DeNovoGear.

RESULTS

Population:

Recruitment flow-chart

Pool of NFLE patients diagnosed and followed up in our Institute n= 139

PRELIMINARY GENETIC STUDY
Analysis of nAChRs genes: 71 patients, all negative

NFLE probands enrolled in WES study n= 41

Additional cases n=21
- newly-diagnosed n=9
- referred by other Centers n=12

Relatives included n= 78
- affected n=23
- unaffected (from TRIOs) n=55

Total of NFLE probands included in WES analysis n= 62

15 families: - 6 ADNFLE
- 9 NFLE + other focal
47 sporadics: - TRIOS: 28
- incomplete TRIOS : 1
- only proband: 18

Total of individuals included in WES analysis n= 140

Genetic findings: summary

MUTATIONS

- new genes	1 NPRL2	(pedigree 1)
- known genes	4 DEPDC5	(pedigrees 2-5)
	1 KCNT1	(pedigree 6)
	3 CHRNA4	(pedigrees 7-9)

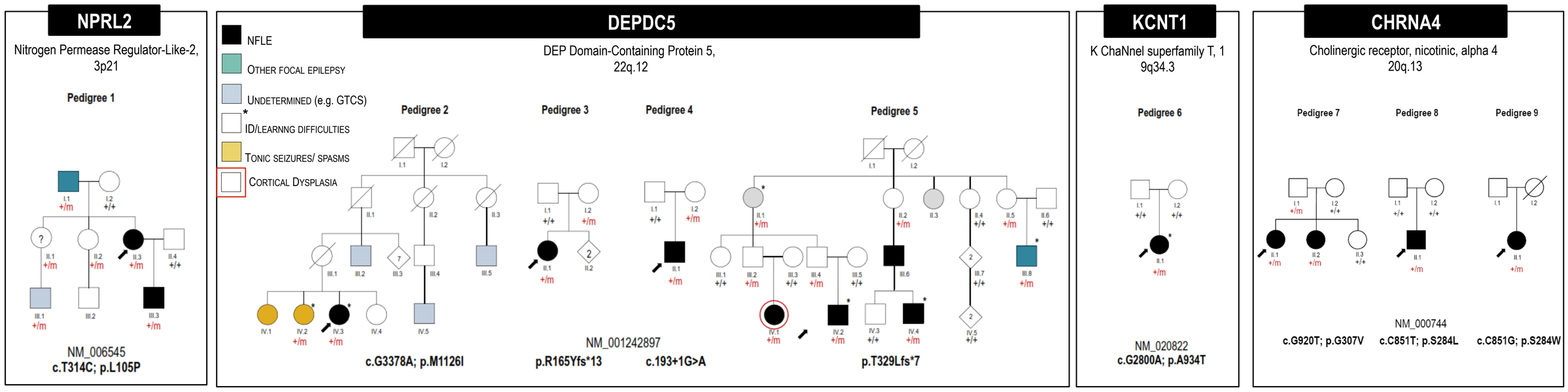
VUS (Variants of uncertain significance)

- known genes	2 DEPDC5	c.T3688C, p.F1230L, inherited
		c.G814A, p.V272I, inherited
		c.1525C>T, p.R509C, inherited
	2 CHRNB2	c.1191G>C, p.Q397H, inherited
		c.C1126T, p.L376F, inherited

NOVEL CANDIDATE GENES

11 variants in 11 different genes

Genetic findings: details



	NPRL2	DEPDC5				KCNT1	CHRNA4		
CLINICAL FEATURES	**Family 1**	**Family 2**	**Case 3**	**Case 4**	**Family 5**	**Case 6, 20 y**	**Family 7**	**Case 8**	**Case 9**
▪ Age at onset	11.5 y (5-19)	8.3 y (3-19)*	10 y	9 y	5.9 y (3m-12 y)	9 y	7.5 y (7-8)	3 y	12 y
▪ Seizure type	HS (2); focal undet (1); GTCS (1)	AT(1), S (2), undet (3)	HS	AT	AT (1), HS (3); T (1), undet (2)	AT	AT (1), PA (1)	HS	AT
▪ Daytime seizures	Yes (1) rare	na	Yes	Yes	Yes (3)	-	Yes (1) rare	-	Yes
▪ Drug resistance	Yes (1); 3 patients SF on AED	-	Yes	-	Yes (4)	Yes (rr)	Yes (1) rr	Yes	Yes
▪ ID/psychiatric disorders	-	Yes (2)	-	-	Yes (4)	Yes (IQ:76)	-	-	Yes (IQ:79)
▪ Epileptiform EEG	Yes (3)	Yes (1) L F-T	Yes, F-C-P >L	R F-T	Yes (5) biT(2), R F-T (2) L T(1)	Yes , bi F-C	Yes (1) bi F	Yes, F-T >L	Yes L F
▪ Abnormal MRI	-	Yes (1) atrophy L H	-	-	Yes (2) 1 with FCD	-	-	-	-
▪ Penetrance	67%	na	na	na	~55%	De novo	67%	De novo	na

Abbreviations: AT: Asymmetric Tonic; HS: Hypermotor Seizures; PA: Paroxysmal Arousals; S: spasms; T: temporal; H: hemisphere; SF: seizure free; AED: antiepileptic drug; F-C-P: fronto-centro-parietal; F-T: fronto-temporal; bi: bilateral; L: left; R: right; ID: intellectual disability; rr: relapsing remitting; na: not applicable/ not available. * data available on 3/6 affected

CONCLUSIONS:

We identified: - a novel gene, NPRL2, acting in mTOR pathway.
- mutations in known genes in 13% of the cases analyzed: DEPDC5 showed the highest mutation rate, strengthening its role in familial focal epilepsies, possibly associated with focal cortical dysplasia; KCNT1 is confirmed to be involved in NFLE associated with ID.

The unexpected detection of mutations in CHRNA4, confirms the low sensitivity of dHPLC.

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