NEXT GENERATION SEQUENCING (NGS) ANALYSIS OF PATIENTS WITH IDIOPATHIC EPILEPSY: DESIGN OF DIFFERENT GENETIC PANELS

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Objectives

In order to analyze a large series of patients with different types of epilepsy, we designed a NGS genetic panel for the analysis of 46 disease genes. This genes were selected due to their involvement in different forms of epilepsy, such as treatable forms, epileptic encephalopathies, benign epilepsy, myoclonic epilepsy, progressive epilepsy, epilepsy with febrile seizures, neuronal migration disorders, subcortical dysplasia, bilateral perisylvian polymicrogyria, heterotopic periventricular nodular microcephaly.

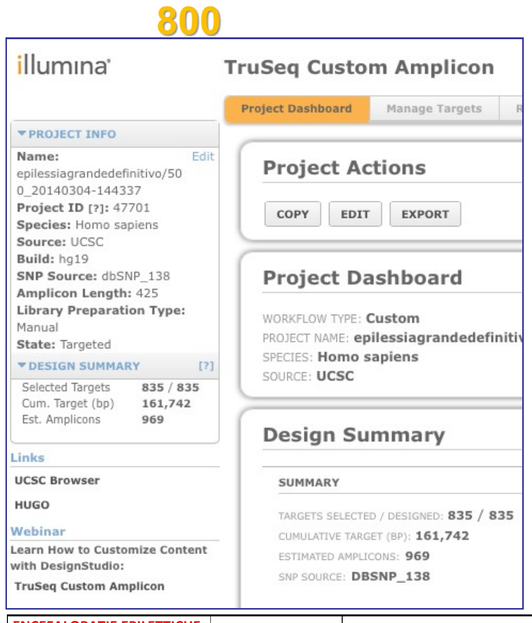
Materials and methods

We conducted a preliminary study in 50 patients from 49 unrelated families. The analysis was carried out by a NGS method using the TruSeq Custom Amplicon Illumina technology and the MySeq Illumina apparatus.

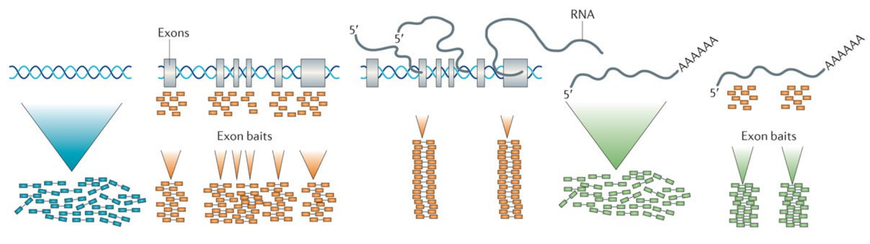
Results The primary filtering process showed the presence of a total of 100 variants presenting a MAF<1% (on average 2 variants for patient). The secondary filtering process allowed us to extract about 63 exonic or splicing variants of possible pathogenic significance. In 41 patients (82%) we report at least one variant with unknown significance and in 12 patients (24%) we reported one mutation of possible pathological role. These variants were present in the following genes: GRIN2A, GRIN2B, POLG, SLC6A8, ATP1A3, PRRT2, TUBB8, PPT1, CDKL5, RELN, FOXG1, SLC2A1, KCNQ3 and COL4A1. Two mutations, one in the FOXG1 gene and one in the CDKL5 gene, are nonsense mutations, two other mutations, one in the ATP1A3 gene and one in the TUBB8, are intron splicing variants, while all the other variants are missense mutations. All mutations were novel and were confirmed by Sanger sequence analysis.

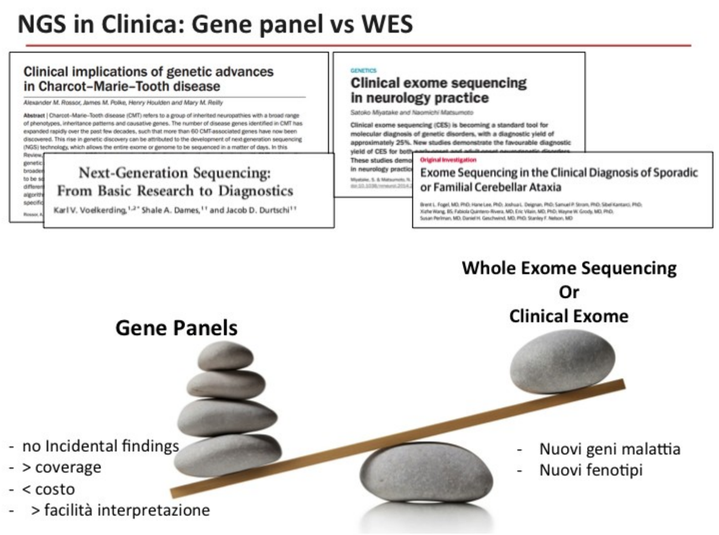
Discussion and conclusions

In order to extend the genetic analysis in a wide spectrum of diseases related to epilepsy, we are currently planning a second panel design using Nextera Rapid Capture technology. This panel contains also genes involved in other diseases presenting an epileptic phenotype such as Sialidosis, Lafora, Unverricht-Lundborg, Kufs, Niemann Pick and Gaucher. The platform will include the analysis of 93 genes. Our encouraging preliminary results suggest the usefulness of a NGS approach for large scale genetic investigation to increase the diagnostic score in the context of epilepsies.

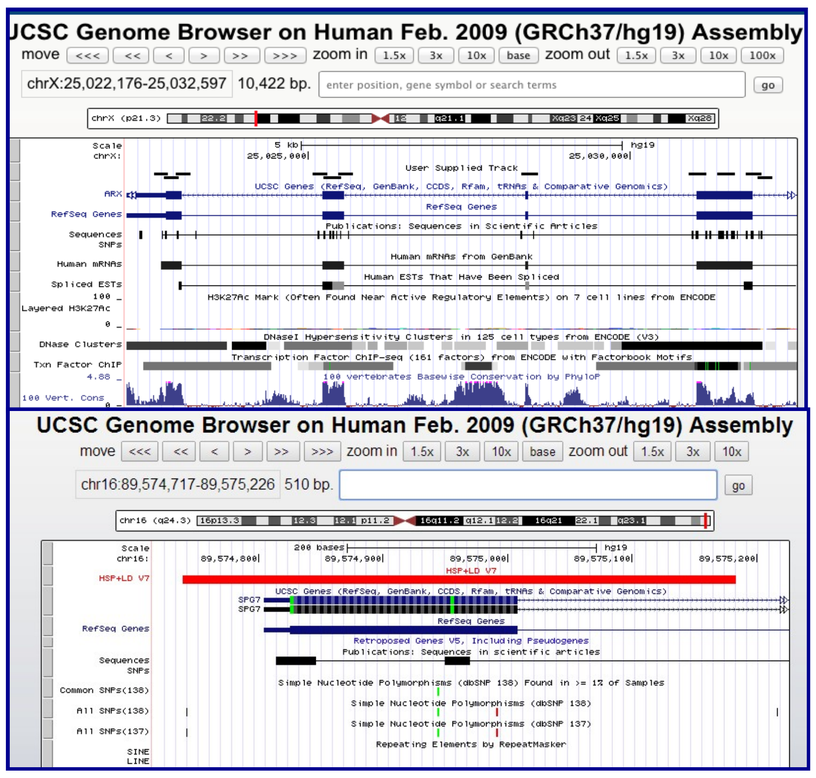


ENCEFALOPATIE EPILETTICHE:		
FORME TRATTABILI		
ALDH7A1-A16	5q23.2	Aldehyde Dehydrogenase 7 Family, Member A1
SLC2A1	1p34.2	Solute Carrier Family 2 (Facilitated Glucose Transporter), Member 1
PNPO	17q21.32	Pyridoxamine 5'-Phosphate Oxidase
PHGDH	1p12	Phosphoglycerate Dehydrogenase
SLC6A8	Xq28	Solute Carrier Family 6 (Neurotransmitter Transporter), Member 8
epilessie precoci/encefalopatie epilettiche		
KCNT1	9q34.3	Potassium Channel, Subfamily T, Member 1
ARX	Xp21.3	Aristaless Related Homeobox Mental Retardation, X-Linked
STXBP1	9q34.1	Syntaxin Binding Protein
CDKL5	Xp22.13	Cyclin-Dependent Kinase-Like 5
FOXG1	14q13	Forkhead Box G1
MECP2	Xq28	Methyl CpG Binding Protein 2 (Rett Syndrome)
UBE3A	15q11.2	Ubiquitin Protein Ligase E3A (Angelman)
KCNQ2	20q13.33	Potassium Voltage-Gated Channel, KQT-Like Subfamily, Member 2
KCNQ3	8q24	Potassium Voltage-Gated Channel, KQT-Like Subfamily, Member 2
GRIN2A	16p13.2	Glutamate Receptor, Ionotropic, N-Methyl D-Aspartate 2A
GRIN2B	12p12	Glutamate Receptor, Ionotropic, N-Methyl D-Aspartate 2B
GABRB3	15q12	Gamma-Aminobutyric Acid (GABA) A Receptor, Beta 3
HCN1	5p12	Hyperpolarization Activated Cyclic Nucleotide-Gated Potassium Channel
BENIGNE		
KCNQ2	20q13.33	Potassium Voltage-Gated Channel, KQT-Like Subfamily, Member 2
KCNQ3	8q24.22	Potassium Voltage-Gated Channel, KQT-Like Subfamily, Member 3
SCN2A	2q24.3	Sodium Channel, Voltage-Gated, Type II, Alpha Subunit
PRRT2	16p11.2	Proline-Rich Transmembrane Protein 2
ATP1A2-A12	1q23.2	ATPase, Na+/K+ Transporting, Alpha 2 Polypeptide
MIOCLONICA MALIGNA		
SYNGAP1	6p21.32	Synaptic Ras GTPase Activating Protein 1
CHD2	15q26.1	Chromodomain Helicase DNA Binding Protein 2
MIGRANTI		Forma clinica grave dell'infanzia
KCNT1	9q34.3	Potassium Channel, Subfamily T, Member 1
SCN2A	2q24.3	Sodium Channel, Voltage-Gated, Type II, Alpha Subunit
PLCB1	20p12.3	Phospholipase C, Beta 1
SCN1A	2q24.3	Sodium Channel, Voltage-Gated, Type I, Alpha Subunit
PROGRESSIVE		
CTSD	11p15.5	Cathepsin D Ceroid-Lipofuscinosis, Neuronal 10
POLG	15q26.1	Polymerase (DNA Directed), Gamma
TWINCKLE C10ORF2	10q24.31	Chromosome 10 Open Reading Frame 2
PPT1 (CLN1)	1p34.2	Palmitoyl-Protein Thioesterase 1
CONVULSIONI FEBBRILI		
SCN1A	2q24.3	Sodium Channel, Voltage-Gated, Type I, Alpha Subunit
SCN2A	2q24.3	Sodium Channel, Voltage-Gated, Type II, Alpha Subunit
SCN1B	19q13.12	Sodium Channel, Voltage-Gated, Type I, Beta Subunit
SCN8A	12q13.13	Sodium Channel, Voltage Gated, Type VIII, Alpha Subunit
GABRG2	5q34	Gamma-Aminobutyric Acid (GABA) A Receptor, Gamma 2
PCDH19	Xq22.1	Protocadherin 19
CHD2	15q26.1	Chromodomain Helicase DNA Binding Protein 2
CACNA1A	19p13.2	Calcium Channel, Voltage-Dependent, P/Q Type, Alpha 1A Subunit
DISORDINI MIGRAZIONE NEURONALE		
DCX	Xq23	doublecortin
PAFAH1B1/LIS1	17p13.3	Platelet-Activating Factor Acetylhydrolase 1b, Regulatory Subunit 1
FLNA	Xq28	Filamin A
TUBA1A	12q13.12	Tubulin, Alpha 1a
TUBB2B	6p25.2	Tubulin, Beta 2B Class IIb
TUBB3	16q24.3	Tubulin, Beta 3 Class III
TUBB8	10p15.3	Tubulin, Beta 8 Class VIII
DISPLASIA SOTTOCORTICALI		
HESX1	3p14.3	Homeobox 1
COL4A1	13q34	Collagen, Type IV, Alpha 1
COL4A2	13q34	Collagen, Type IV, Alpha 2
POLIMICROGIRIE BILATERALI FRONTO-TEMPORALI		
GPR56	16q13	G Protein-Coupled Receptor 56
POLIMICROGIRIE BILATERALI PERISILVANE		
SRPX2	Xq21.33-q23	Sushi-Repeat Containing Protein, X-Linked 2
EMX2	10q26.11	Empty Spiracles Homeobox 2
MICROCEFALIA ETEROTOPICA NODULARE PERIVENTRICOLARE		
ARHGEF2	1q21-q22	Rho/Rac Guanine Nucleotide Exchange Factor (GEF) 2
DISORDINI DI RELINA		
RELN	7q22	reelin
VLDLR	9p24	Very Low Density Lipoprotein Receptor





TruSeq Custom Amplicon design



PATIENT	gene	POSITION	PROTEIN
GLUT34	GRIN2A	NM_001134407.1:c.3118G>A	NP_001127879.1:p.Glu1040Lys
	SLC6A8	NM_005629.3:c.1885G>C	NP_005620.1:p.Val629Leu
GLUT94	GRIN2B	NM_000834.3:c.1544T>C	NP_000825.2:p.Ile515Thr
	UBE3A	NM_000462.3:c.2627A>G	NM_000462.3:c.2627A>G(p.=)
GLUT130	POLG (AR)	NM_002693.2:c.2159C>A	NP_002684.1:p.Thr720Asn
GLUT 145	FLNA	NM_001110556.1:c.4463A>G	NP_001104026.1:p.Gln1488Arg
	CHD2	NM_001271.3:c.3734+7A>G	
GLUT 164	PRRT2	NM_001256442.1:c.881C>T	NP_001243371.1:p.Ser294Phe
	PNPO	NM_018129.3:c.322C>T	NP_060599.1:p.Arg108Cys
	RELN	NM_005045.3:c.5717T>C	NP_005036.2:p.Ile1906Thr
GLUT 183	COL4A2	NM_001846.2:c.3326G>A	NP_001837.2:p.Arg1109Gln
H443	GPR56	NM_005682.5:c.1693C>T	NP_005673.3:p.Arg565Trp
H532	TUBB8	c.314A>G	p.His71Arg
	TUBB8	c.638A>G	p.Lys213Arg
H533	COL4A1	NM_001845.4:c.3712C>T	NP_001836.2:p.Arg1238Cys
	TUBB8	c.314A>G	p.His71Arg
	TUBB8	c.638A>G	p.Lys213Arg
H1235 Freri	TUBB8	NM_177987.2:c.166+4A>C	
H1622	CDKL5	NM_003159.2:c.400C>T	NP_003150.1:p.Arg134Ter
	PPT1	NM_000310.3:c.628G>C	NP_000301.1:p.Gly210Arg
H2370	MAGI2	NM_012301.3:c.393C>A	NP_036433.2:p.Asp131Glu
	RELN	NM_005045.3:c.6343G>A	NP_005036.2:p.Gly2115Ser
	PEX5L	NM_016559.2:c.758G>A	NP_057643.1:p.Ser253Asn
NMD360	GRIN2B	NM_000834.3:c.43G>A	NP_000825.2:p.Val15Met
NMD 378	FOXG1	NM_005249.4:c.738C>G	NP_005240.3:p.Tyr246Ter
NMD 394	COL4A1	NM_001845.4:c.2903G>A	NP_001836.2:p.Arg968Gln
	POLG	NM_002693.2:c.1760C>T	NP_002684.1:p.Pro587Leu
	POLG	NM_002693.2:c.752C>T	NP_002684.1:p.Thr251Ile
S1441	ATP1A3	NM_001256214.1:c.396C>T	NM_001256214.1:c.396C>T(p.=)
	GRIN2B	NM_000834.3:c.2038C>G	NP_000825.2:p.Pro680Ala
	KCNQ3	NM_004519.3:c.1720C>T	NP_004510.1:p.Pro574Ser
S1445	KCNQ2	NM_172107.2:c.928G>A	NP_742105.1:p.Gly310Ser
	COL4A1	NM_001845.4:c.4372C>T	NP_001836.2:p.Pro1458Ser
S1447	FLNA	NM_001110556.1:c.4589T>C	NP_001104026.1:p.Val1530Ala
	CDKL5	NM_003159.2:c.65G>T	NP_003150.1:p.Gly22Val
S1449	COL4A1	NM_001845.4:c.2903G>A	NP_001836.2:p.Arg968Gln
	COL4A2	NM_001846.2:c.1655C>A	NP_001837.2:p.Thr552Lys
	ATP1A3	NM_001256214.1:c.193-5C>G	
S 1487	SLC2A1	NM_006516.2:c.152G>A	NP_006507.2:p.Arg51His
	KCNQ3	NM_004519.3:c.1624G>A	NP_004510.1:p.Asp542Asn
	KCNQ3	NM_004519.3:c.1075G>T	NP_004510.1:p.Val359Leu
	GABRB3	NM_021912.4:c.933G>T	NP_068712.1:p.Met311Ile
S1560	SCN2A	NM_001040142.1:c.256A>G	NP_001035232.1:p.Ile86Val
S1635	GPR56	NM_005682.5:c.641C>T	NP_005673.3:p.Ser214Leu
S1636	GRIN2A	NM_001134407.1:c.3228C>A	NP_001127879.1:p.Asn1076Lys
S1652	MAGI2	NM_012301.3:c.2131C>A	NP_036433.2:p.Pro711Thr
GLUT57	PHGDH	NM_006623.3:c.425A>G	NP_006614.2:p.Glu142Gly
H3170	GRIN2A	NM_001134407.1:c.2765C>T	NP_001127879.1:p.Ala922Val
S1654	KCNE2	NM_172201.1:c.170T>C	NP_751951.1:p.Ile57Thr
S1687	COL4A2	NM_001846.2:c.17G>A	NP_001837.2:p.Arg6His
S1722	SCN2A	NM_001040142.1:c.3446G>C	NP_001035232.1:p.Gly1149Ala
S1723	RELN	NM_005045.3:c.5156C>T	NP_005036.2:p.Ser1719Leu
	GRIN2A	NM_001134407.1:c.2899G>C	NP_001127879.1:p.Val967Leu
S1724	HESX1	NM_003865.2:c.385G>A	NP_003856.1:p.Val129Ile
	HCN1	NM_021072.3:c.1240G>A	NP_066550.2:p.Val414Met
	COL4A1	NM_001845.4:c.1807C>T	NP_001836.2:p.Pro603Ser
	GPR56	NM_005682.5:c.844G>C	NP_005673.3:p.Gly282Arg
S1731	CHD2	NM_001271.3:c.3110_3111insGCG	NP_001262.3:p.Glu1037_Arg1038insArg
S1732	COL4A1	NM_001845.4:c.1420G>A	NP_001836.2:p.Gly474Arg
S1734	GRIN2A	NM_001134407.1:c.422C>T	NP_001127879.1:p.Thr141Met
	SYNGAP1	NM_006772.2:c.2195G>A	NP_006763.2:p.Arg732Lys
S1772	RELN	NM_005045.3:c.5618C>T	NP_005036.2:p.Thr1873Ile
S1773	KCNQ3	NM_004519.3:c.2146G>C	NP_004510.1:p.Asp716His
S1774	PEX5L	NM_016559.2:c.85C>T	NP_057643.1:p.Gln29Ter
S1775	SCN2A	NM_001040142.1:c.2119A>T	NP_001035232.1:p.Ile707Leu

RESULTS

Number of patients:	**50**
Patients with variants:	**41 (82%)**
Patients with mutations with pathological effect:	**12 (24%)**
Patients with variants of unknown significance (VUS)	**29 (58%)**
No variants	**9 (18%)**

- **Clearly pathogenic (10 mutations in 6 patients)**
- **Unknown significance (VUS) (31 variants in 22 patients)**
- **Unlikely to be pathogenic (2 variants in 2 patients)**
- **no variants in 10 patients**

Data analysis

Data validation

- Silico prediction: [SIFT; PolyPhen-2;PMUT; MutationTaster]
- Sanger sequencing validation
- Segregation study
- Italian control's screening

Classification

1 - Clearly not pathogenic
2 - Unlikely to be pathogenic
3 - Unknown significance (VUS)
4 - Likely to be pathogenic
5 - Clearly pathogenic

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