Improving molecular diagnosis in epilepsy by a dedicated high-throughput sequencing platform

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Running Title: Molecular diagnosis in epilepsy by target NGS

Supplementary information

Supplementary Methods

Statistical test

To assess the significance of the difference in the number of variants between patient and control cohorts we applied the Wilcoxon-Mann-Whitney (WMW) non-parametric statistical test.

The choice of this statistical test is justified by i) the assumption of independence of all observations (number of variants) in both the two cohorts, ii) the discrete and ordinal nature of the variables, which hampers using parametric tests, like Student's T.

We performed the WMW test using R v.2.15.1 typing the following command:

wilcox.test(Cases,Controls,correct=TRUE,conf.level=0.95)

When Cases and Controls are two arrays reporting the number of variants for patients and controls respectively, a continuity correction factor was applied and the significance level was set to 0,05. The test was performed both two-sides and one-side, before and after discrete filtering, respectively.

Because of the discrete nature of our observations we applied ties correction for standard deviation (s1.1) to ensure that the presence of *ex-aequo* observations was not affecting results.

$$Z = \frac{(T \pm 0{,}5) - \mu_T}{\sigma_T}, \qquad \text{(s1.1)}$$

$$\mu_T = \frac{N_1 \cdot (N_1 + N_2 + 1)}{2},$$

$$\sigma_T = \sqrt{\frac{N_1 \cdot N_2}{N \cdot (N-1)} \cdot \left(\frac{N^3 - N}{12} - \sum_{j=1}^{g} \frac{t_j^3 - t_j}{12} \right)} \quad .$$

- N_1, N_2 are the number of patients and controls respectively
- $N = N_1 + N_2$
- g is the number of ties
- t is the number of observations with the same rank within each tie

Supplementary Tables

Table 1S List of 67 genes included in the second version of custom epilepsy platform. The genes in bold are those in common with Lemke's platform.

Epilepsy Platform		
ALDH7A1	**ARHGEF9**	**ARX**
CCM2	CDKL5	CHRNA2
CHRNA4	CHRNA7	**CHRNB2**
CLN8	CNTNAP2	**CSTB**
DCX	DYRK1A	*EHMT1*
EPM2A	**FLNA**	**FOXG1**
GABRA1	**GABRD**	**GABRG2**
GPR98	**GRIN2A**	**GRIN2B**
KCNJ10	**KCNMA1**	**KCNQ2**
KCNQ3	**KCTD7**	KRIT1
LGI1	MAGI2	**MECP2**
MEF2C	**NHLRC1**	**OPHN1**
PAFAH1B1	**PCDH19**	PDCD10
PDYN	PLCB1	***PNKP***
PNPO	**POLG**	**PRICKLE1**
RELN	*ROGDI*	**SCARB2**
SCN1A	**SCN1B**	**SCN2A**
SCN9A	SHANK3	**SLC25A22**
SLC2A1	**SLC9A6**	***SPTAN1***
SRPX2	**STXBP1**	SYN1
TBC1D24	TCF4	**TSC1**
TSC2	**TUBA1A**	**TUBB2B**
UBE3A		

Table 2S Number of variants -single nucleotide variation (SNVs) and small indels- identified in patients and controls at different filtering steps. Each cell indicates the number of calls excluding synonymous SNVs (*a*) and eliminating those variants reported in dbSNP135, ESP with a frequency higher than 1% and already reported in-house exomes database (b). Taking in account only variants predicted to be deleterious at least by one prediction tool we considerably reduce the number of candidate mutations (*c*).

Variants Called						
Filter	Patient cohort			Control cohort		
	Total	Exon/ss	Intron / UTR	Total	Exon/ss	Intron / UTR
a) Total variants	1036	81	955	851	47	804
b) dbSNP135*, ESP, in house db	100	57	43	55	25	30
c) Predicted damaging	62	53	9	22	17	5

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List of references for each gene included in our platform.

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