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Supplemental information

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GABBR1 Monoallelic *De Novo* Variants Linked to Neurodevelopmental Delay and Epilepsy

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Supplementary Data

WES METHODOLOGY

Patient 1

Exome sequencing was performed on the proband and the parents at Genesupport. The capture kit Twist Core Exome and Spike-In were used to capture and enrich the coding regions as well as the splice sites, and the sequencer Illumina NovaSeq 6000 was used as the sequencing platform tool. The sequence were aligned to the human genome reference GRCh37/hg19 using Sentieon (version 201911) and GATK. Finally, using the system Saphethor, the bioninformatic analysis was realized in trio first on a panel of 2113 genes implicated in neurodevelopmental disorders, and then on the totality of the genes covered by the capture kit (8907 genes). The evaluation of the variants was performed using numerous databases including gnomAD (version2.1.1), BRAVO, ClinVar (Version05-Oct-2020), LOVD, local datababses and in silico predictors. Finally, GERP was utilized to determine the conservation of the nucleotides. Confirmation of the variant and family segregation were performed via PCR and Sanger sequencing.

Patient 2

Genomic DNA extracted from leukocytes of the proband and the parents was used for whole-exome sequencing. Exome enrichment was performed on individually barcoded samples using SeqCap EZ MedExome Probes and SeqCap EZ Mitochondrial Genome Design probe (Roche) and sequencing was performed on Novaseq 6000 platform (Illumina) with 100bp paired-end reads. Reads were aligned to the hg19 reference genome using Novoalign version 3.02.13 (Novocraft) with default parameters.

After genome alignment, conversion of SAM format to BAM and duplicate removal was performed using Picard Tools (2.20.8). The Genome Analysis Toolkit, GATK (3.8) (McKenna et al., 2010) was used for local realignment around indels, base recalibration, variant recalibration, and variant calling. Variants were annotated using the GEMINI framework (Paila et al., 2013) and filtered based on the population frequencies using several public databases and an in-house database of population-specific variants. Identification of candidate variants was performed for autosomal dominant (de novo variants) and autosomal recessive inheritance patterns. Variants were further prioritized according to the functional impact and conservation score. Sanger sequencing confirmed the presence of the candidate.

McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, et al. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res 2010; 20: 1297-303.

Paila U, Chapman BA, Kirchner R, Quinlan AR. GEMINI: integrative exploration of genetic variation and genome annotations. PLoS Comput Biol 2013; 9: e1003153.

Patient 3

The exome capture was carried out with BGI Exome kit capture (59M) and the library was then sequenced on a BGISEQ-500, paired-end 100bp. Analysis of the raw data was performed using the software Varfeed (Limbus, Rostock) and the variants were annotated and prioritized using the software Varvis (Limbus, Rostock). All potential protein-influencing variants were prioritized with regard to their pathogenicity and clinical relevance according to all possible inheritance modes. On an exploratory base, CNV analysis of down to single exon deletion was performed. Coverage of more than 20x was been achieved in more than 95 % of target sequences in all family members.

Patient 4

Clinical exome sequencing was performed at GeneDx according to previously described methods (Retterer et al., 2016). Briefly, genomic DNA was obtained from the proband and parents, exonic regions and nearby splice junctions were captured and sequenced with 100bp or larger paired-end reads. Alignment was done with human genome build GRCh37/UCSC hg19, and Xome Analyzer, a custom analysis tool, was used for analysis. All potentially pathogenic variants were confirmed via capillary sequencing or another appropriate method.

Retterer K, Juusola J, Cho MT, Vitazka P, Millan F, Gibellini F, et al., Clinical application of whole-exome sequencing across clinical indications. Genet Med. 2016 Jul;18(7):696-704.

Figure S1

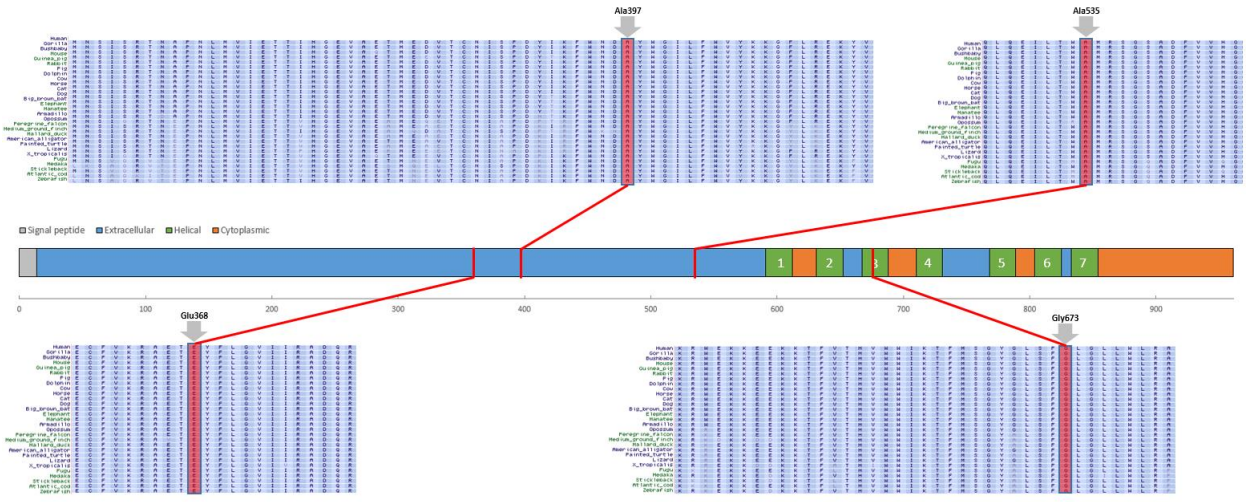

Figure S1. Evolutionary conservation of the four GABBR1 *de novo* variants. The cartoon of the primary structure of the GABBR1 protein is shown in the middle (the scale below depicts the number of aminoacids). The 7 transmembrane domains are numbered and shown in green. The aminoacids

shown with arrows are mutated in the patients studied. The species shown are depicted in the left of each panel.

Table S1. Parameters of GABA concentration-response curves fitted to the raw data in Fig. 1e

	WT	Glu368Asp	WT+Glu368Asp		*WT & Glu368Asp*	*WT & WT+Glu368Asp*	*Glu368Asp & WT+Glu368Asp*
basal	0.17	-0.02	0.05	*Kruskal-Wallis (p<0.0001) & Dunn's*	*p<0.0001*	*p=0.3938*	*p=0.2597*
Emax	0.96	0.50	1.02	*ANOVA (p<0.0001)/Games-Howell's*	*p<0.0001*	*p=0.5754*	*p<0.0001*
EC50 [µM]	1.12	91.88	1.88	*ANOVA (p<0.0001)/Holm-Sidak's*	*p<0.0001*	*p<0.0001*	*p<0.0001*
n	15	13	4				
	WT	Ala397Val	WT+Ala397Val		*WT & Ala397Val*	*WT & WT+Ala397Val*	*Ala397Val & WT+Ala397Val*
basal	0.17	0.17	0.27	*ANOVA (p=0.2954)/Tukey's*	*p=0.9996*	*p=0.3001*	*p=0.2995*
Emax	0.96	0.55	1.08	*ANOVA (p=0.0003)/Holm-Sidak's*	*p<0.0001*	*p=3702*	*p=0.0153*
EC50 [µM]	1.12	0.61	0.82	*ANOVA (p=0.0003)/Tukey's*	*p=0.0002*	*p=0.2026*	*p=0.4708*
n	15	17	4				
	WT	Ala535Thr	WT+Ala535Thr		*WT & Ala535Thr*	*WT & WT+Ala535Thr*	*Ala535Thr & WT+Ala535Thr*

basal	0.17	0.12	0.17	*Kruskal-Wallis (p=0.3985) & Dunn's*	*p=0.6735*	*p>0.9999*	*p>0.9999*
Emax	0.96	0.60	1.07	*ANOVA (p=0.0004)/Games-Howell's*	*p=0.0085*	*p=0.3528*	*p=0038*
EC50 [µM]	1.12	0.80	0.94	*ANOVA (p=0.0275)/Tukey's*	*p=0.0208*	*p=0.5664*	*p=0.7254*
n	15	17	4				
	WT	Gly673Asp	WT+Gly673Asp		*WT & Gly673Asp*	*WT & WT+Gly673Asp*	*Gly673Asp & WT+Gly673Asp*
basal	0.02	-0.01	0.01	*t-test*		*p=0.4471*	
Emax	0.97	0	1.04	*t-test with Welch's correction*		*p=0.5784*	
EC50 [µM]	3.35	-	3.04	*t-test with Welch's correction*		*p=0.5830*	
n	12	10	16				

Table S2. IC50 values for CGP54626 dose response curves fitted to the raw data in Fig. 1f

IC50 [µM]	WT	Glu368Asp	Ala397Val	Ala535Thr
@ 10 µM GABA (n=3*)	0.01	0.01	0.02	0.02
@100 µM GABA (n=6)	0.24	0.01	0.50	0.41
@100 µM GABA t-test WT vs.		*0.0002*	*0.0027*	*0.0116*
		Welch's correction		

*Glu368Asp: n=2 (ambiguous fit)