# A novel mutation in the transmembrane 6 domain of GABBR2 leads to a Rett-like phenotype

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**A novel mutation in the TM6 domain of GABBR2 leads to a Rett-like phenotype**

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**Running head:** A novel GABBR2 mutation in the TM6 domain

Dear Editor,

We read with great interest the recent article published by Yoo et al.[1] reporting four additional RETT-like (RTT) patients with the recurring A567T *GABBR2* mutation[2]. More interestingly, they showed, with *in vitro* and *in vivo* functional studies, that the severity of the phenotype caused by *GABBR2* mutations was directly linked to their impact on GABA signaling activity, this latter being more reduced with the two missense mutations, S695I and I705N associated with epileptic encephalopathy (EE)[1,3]. They hypothesized that variants position in different transmembrane (TM) domains of GABBR2, TM6 for S695I and I705N, and TM3 for A567T, could determine the phenotypic expression. This hypothesis was recently reinforced with the report of a novel *GABBR2* mutation also in TM6 and associated with infantile epileptic spasms[4].

We present a novel *de novo* heterozygous *GABBR2* mutation, A707T (Fig 1 A), identified by Whole Exome Sequencing also located in TM6 of GABBR2 (Fig 1B) but associated with a RTT phenotype. The carrier, a 12 year-old girl, had profound intellectual disability, hand stereotypies, sleep and breathing disturbances but no history of seizures. This mutation, predicted pathogenic by *in silico* analyses, lies in a region crucial for GPCR activation and positive allosteric modulation[5]. To assess its impact on GABA signaling activity, we co-expressed the two $GABA_B$ subunits with the chimeric G-protein $G\alpha qi_9$ in HEK-293 cells and measured the accumulation of inositol phosphate (IP-1) induced by the ligand GABA. We showed that the signaling activity of our A707T mutant is weakly induced by the agonist compared to that of the wild-type (Fig 1C), and this without altering GABBR2 cell surface expression (Fig 1D). The same results were observed with the A567T and I705N mutants (Fig 1C). Moreover, the four mutants tested have a basal activity stronger than that of the wild-type which might explain why the mutated receptor cannot be stimulated efficiently by

GABA (Fig 1C,1E). This basal activity is reversed by the competitive antagonist CGP54626, except for the mutant S695I which is already fully active in the absence of GABA and do not respond to GABA (Fig 1C, E) in accordance with Yoo et al. data[1]. To conclude, our results show that *GABBR2* mutations located within TM6 can also be associated with a Rett-like phenotype. The novel mutation described here, A707T, also exerts a deleterious effect on GABAB receptor activity. This deleterious effect could result from a constitutive activity of the mutated $GABA_B$ receptor highlighting a novel putative pathogenic mechanism for *GABBR2* variants.

**Acknowledgement**

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**Author Contributions**

MLV, MJ, LX, PR, FL and AT contributed to the conception and design of the study. MLV, MJ, LX, SB, ASD, SA, CB, AD, RR, SB, PR, FL and AT contributed to the acquisition and analysis of data. All authors contributed equally to drafting the text and preparing the figures.

**Potential Conflicts of Interest**

Nothing to report.

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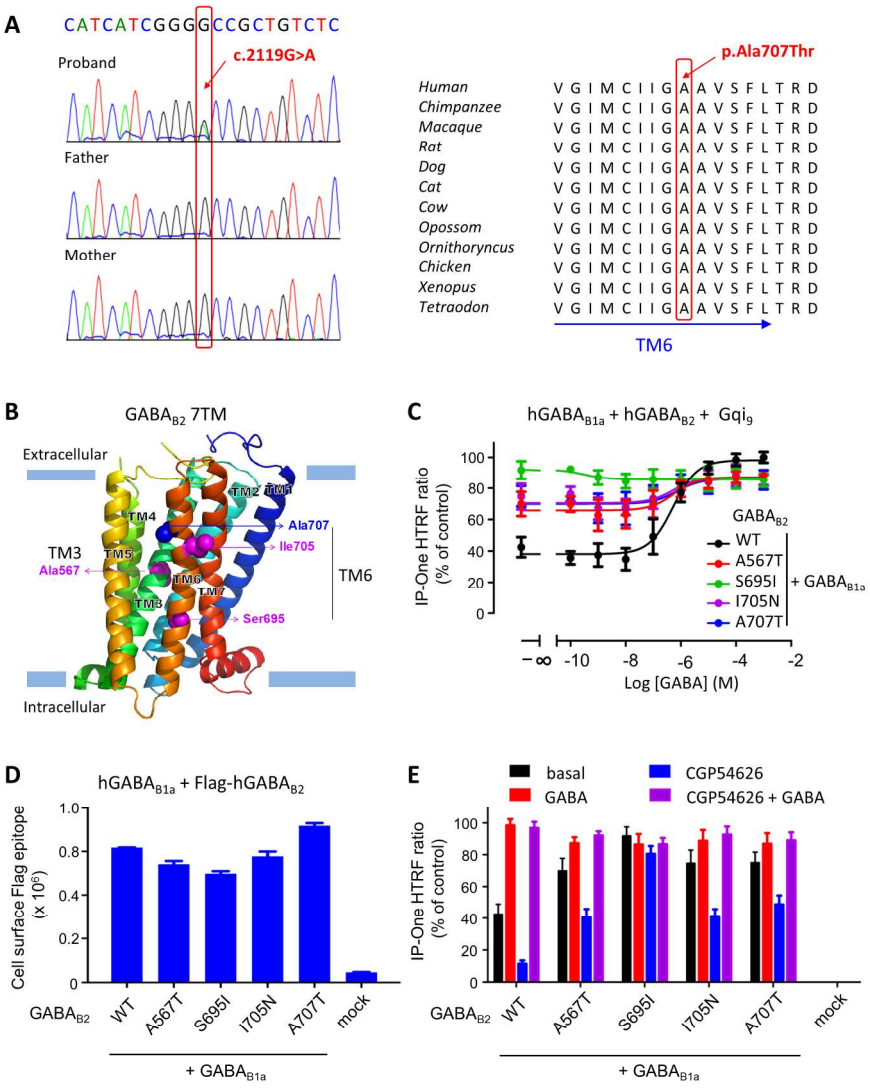
**Figure legend**

Figure 1: Identification and functional analysis of the A707T mutation

A) Sanger sequencing electrophoregrams showing the *GABBR2* heterozygous missense mutation c.2119G>A, p.(Ala707Thr) in the proband and wild-type sequences in her parents and amino-acid alignments showing high conservation of the residue 707 across multiple species. TM: Transmembrane domain.

B-E) Functional analysis of the $GABA_B$ receptor mutants. B) Structural model of the human $GABA_{B2}$ heptahelical transmembrane domain (7TM) with the mutated residues in RTT and EE patients indicated in Corey–Pauling–Koltun representation. All the amino acid residues affected by the human mutations are in transmembrane (TM) domains 3 and 6. C) Inositol-phosphate accumulation mediated by the wild-type and mutant Flag-tagged $GABA_{B2}$ co-expressed with the wild-type $GABA_{B1a}$ and the chimeric G protein subunit $G\alpha q_{i9}$ (a $G\alpha q$ protein in which the last nine C-terminal residues have been replaced by those from $G\alpha i_2$) which facilitates the coupling of Gi-coupled receptors to the phospholipase C signaling pathway (Monnier et al, 2011). Data are means ± SEM of at least three independent experiments. D) Cell surface levels of the Flag-tagged $GABA_{B2}$ mutants when co-expressed with the wild-type $GABA_{B1a}$. Amounts of Flag-tagged $GABA_{B2}$ mutants at the cell surface were quantified by ELISA in intact (i.e. non-permeabilized) cells using the Flag epitope at the extracellular N-terminus of the $GABA_{B2}$ subunit. Data are expressed as means ± SEM of triplicates from a typical experiment repeated at least three times. E) Inositol-phosphate accumulation mediated by the wild-type and mutant Flag-tagged $GABA_{B2}$ co-expressed with the wild-type $GABA_{B1a}$ and $G\alpha q_{i9}$, as in panel *B*. The $GABA_B$ receptor wild-type and $GABA_B$ mutants were incubated with 100 µM GABA, 10 µM CGP54626 or both. Data are means ± SEM of at least three independent experiments.

Monnier C., Tu H., Bourrier E., et al. (2011) Transactivation between two 7-TM domains: implication heterodimeric GABAB receptor activation. EMBO J. 30, 32-42.

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