# *IN-VIVO* GENETIC ABLATION OF METABOTROPIC GLUTAMATE RECEPTOR TYPE 5SLOWS DOWN DISEASE PROGRESSION IN THE SOD1G93A MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

**Carola Torazza[1], Tiziana Bonifacino[1], Marco Milanese[2], Francesca Provenzano[1], Aldamaria Puliti[3], Giambattista Bonanno[4]**

*[1]Department of Pharmacy, Unit of Pharmacology and Toxicology University of Genoa, Italy, Genoa - Italy, [2]Department of Pharmacy, Unit of Pharmacology and Toxicology University of Genoa, Italy; Center of Excellence for Biomedical Research, University of Genoa, Italy, Genoa - Italy, [3]Department of Neuroscience, Rehabilitation, Ophthalmology, Genetics and Maternal and Child Health, University of Genoa, Italy, Genoa - Italy, [4]Department of Pharmacy, Unit of Pharmacology and Toxicology University of Genoa, Italy; Center of Excellence for Biomedical Research, University of Genoa, Italy; IRCCS San Martino Polyclinic Hospital, Genoa Italy, Genoa - Italy*

**Introduction:** Amyotrophic lateral sclerosis (ALS) is a fatal neuromuscular disease in which cortical and spinal cord motor neurons (MNs) degenerate causing irreversible muscle wasting, weakness and spasticity. Despite the research efforts in the field, the mechanisms causing selective MN death are still largely unknown, thus prejudicing successful pharmacological treatments. Major causes of MN damage are effects downstream of the abnormal glutamate (Glu) neurotransmission. Glu exerts its actions through activation of ionotropic and metabotropic receptors. In particular, group I metabotropic Glu receptors (mGluRs), which includes mGluR1and mGluR5, have been shown to actively contribute to the excitotoxicity in ALS and represent druggable molecular targets. We previously demonstrated that halving mGluR1or mGluR5expression in the widely studied $SOD1^{G93A}$ mouse model of ALS had a positive impact on disease onset and survival as well as on cellular and biochemical parameters altered in ALS. While motor skills were ameliorated both in female and male $SOD1^{G93A}$ mice lacking mGluR1, unexpectedly, motor skills were improved in male $SOD1^{G93A}$ mice lacking mGluR5, only. To further validate the role of Group I mGluRs in ALS, we generated in this study mGluR1or mGluR5null mice expressing the $SOD1^{G93A}$ mutation.

**Materials and methods:** $SOD1^{G93A}$ male mice were crossed with $Grm1^{-/+}$ females to generate $SOD1^{G93A}Grm1^{-/+}$ double mutants carrying the $Grm1^{-/+}$ heterozygous mutation and the SOD1*G93A transgene. $SOD1^{G93A}Grm1^{-/+}$ double mutants from the initial crossing were again crossed with $Grm1^{-/+}$ mice to obtain $SOD1^{G93A}Grm1^{-/-}$ mice. The same procedure was applied crossing $SOD1^{G93A}$ male and $Grm5^{-/+}$ mice to obtain $SOD1^{G93A}Grm5^{-/-}$ mice. Body weight was measured before behavioural test sessions. Disease onset was defined retrospectively as the time when the body weight was significantly lower than that of control mice. Survival was identified as the time at which mice were unable to right themselves within 20s when placed on their side. Rotarod and balance beam tests were used to assay motor coordination; hind limb extension reflex and gait in an open field tests were used to assay motor skills; grip strength test for forelimbs and hanging wire test for hind limb grip endurance were used to assay muscle strength. Spinal cord sections from WT, $Grm5^{-/-}$, $SOD1^{G93A}$, and $SOD1^{G93A}Grm5^{-/-}$ mice were used to count MNs, after staining with haematoxylin and eosin, and to assess reactive astrocytes and microglia, by labelling glial fibrillary acidic protein (GFAP) and ionized calcium binding adaptor molecule 1(IBA1) as markers, respectively.

**Results:** $SOD1^{G93A}Grm1^{-/-}$ mice were characterized by reduced dimensions at birth, showed motor alteration typical of an ataxic phenotype during growth and survived even less than $SOD1^{G93A}$ mice. Therefore, we abandoned the studies with $SOD1^{G93A}Grm1^{-/-}$ mice. On the contrary, $SOD1^{G93A}Grm5^{-/-}$ mice exhibited a favourable phenotype. In fact, they exhibited delayed disease onset and prolonged survival, when compared to $SOD1^{G93A}$ mice. These effects were associated to enhanced number of preserved MNs and decreased astrocyte and microglia activation. Motor ability amelioration was more pronounced in male $SOD1^{G93A}Grm5^{-/-}$ mice than in male heterozygous $SOD1^{G93A}Grm5^{-/+}$ mice and, most important, it was present also in female mice.

**Discussion and conclusion:** All together our results showed that knocking out mGluR5had a very positive effect on the $SOD1^{G93A}$ mouse phenotype, which was more favourable than in heterozigous $SOD1^{G93A}Grm5^{-/+}$ mice. These results represent a proof of concept, supporting the assumption that dampening mGluR5activity has a positive impact in ALS, and make mGluR5a valuable target for promising pharmacological treatment of the disease.