Most mutations identified in the individuals were also found somatically mutated in a
Molecular diagnosis via whole exome sequencing has revealed that heterozygous
Most of the identified mutations localize in a region that has been shown to participate
Emmanuel Ozoruonye and Stephen Owens from the Neurobehavior Core facility at
increased or decreased - will affect neuronal activity.
• ophthalmological defects
• seizures
activation of Phospholipase C
• Activation of MAPK and Pi3K pathways
-GNB1 mutations in
- developmental delay,
- motor deficits and very low threshold to minimal clonic forebrain seizures, compared to WT littermates but all their electroconvulsive threshold in the 6 Hz psychomotor seizures model is unchanged.

Gnb1α subunits, but decrease it

Gnb1 α-associated GPCR signaling.

Gnb1/GFAP/Dapi MAP2/Gnb1/Dapi

Vertical screen holding

Non-induced

Figure 5. Intracellular signaling pathway activation is affected by the K78R mutation. a) Western Blot of P7 neurons expressing either GFP alone, or GFP-tagged partner proteins transfected following

CatWalk XT (Noldus Information Technology) is a modern automated test for gait

Figure 4. Gnb1 protein with the glutamic acid residue on position 52 (E52) provides stronger expression after neuronal maturation at DIV14. Data from 10 different animals reared and normalized to WT. All Gnb1K78R mice tend to die more during the development period, and high death rate of about 53% of the mice period compared to WT. All Gnb1K78R mice show increased mean duration of bursts and increased inter-burst intervals. At DIV14, Gnb1K78R show increased mean frequency of spikes in bursts, which correlates with the very long burst duration observed.

A: Acetylcholine

Mutations in Gnb1 affect interaction and activation of downstream effectors

Figure 4. Gnb1 interaction with downstream effectors is affected by Gnb1 mutations. a) Representative western blot of Gnb1α subunit from hippocampal slice. b) Quantification of interaction between Gnb1α-WT, Gnb1αK78R or Gnb1αD76G and the c-fos antibody. c) Quantification of Gnb1α-WT, Gnb1αK78R, Gnb1αD76G or Gnb1α-R52G and Jnk1/2 antibody. d) Gnb1α-K78R and D76G variants increase G

Figure 3. NEA reveals increased burst duration accompanied by decreased number of bursts in Gnb1K78R mice. Measurements from WT and Gnb1K78R mice at DIV14, showing that these major pathways may contribute to the phenotype. The K78R variant shows stronger expression in GABAergic neurons.

Mutations

Gnb1

GNB1 mutations in are associated with a neuropsychiatric disorder characterized by the following main features:
- developmental delay,
- hypotonia,
- ophthalmological defects

• In vivo assessment and characterization
• Screen candidate drugs
• Clamp on brain activity

Acknowledgements

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The mouse model K78R was generated by Victor Lin at the Transgenic Mouse Core facility at Columbia University Medical Center

General Approach

The Gnb1P57R mouse model phenocopies aspects of the human disease

Figure 2. Normal expression and localization of Gnb1P57R in mouse cerebral cortex. a) Western blot of Gnb1P57R subunit from hippocampal slice. b) Quantification of Gnb1P57R subunit from hippocampal slice. c) Gnb1P57R expressing interferon-like protein than WT neurons (bottom right panel). d) Western blot of Gnb1P57R subunit from hippocampal slice. e) Immunofluorescence for Gnb1P57R + MAP2 in hippocampal slice. f) Gnb1P57R shows increased mean duration of bursts and increased inter-burst intervals. At DIV14, Gnb1P57R show increased mean frequency of spikes in bursts, which correlates with the very long burst duration observed.

Summary

• Intergenic Gnb1K78R mice show >55% at P6, while homozygous Gnb1K78R mice show 100% death at P14. Gnb1K78R mice exhibit developmental delay, motor deficits and very low threshold to minimal clonic forebrain seizures.

• GIP expression and localization is not affected by the K78R mutation. GIP is expressed in the somatic region of both glutamergic and GABAergic neurons, with a stronger expression in GABAergic neurons.

• Gnb1K78R mutants burst less than WT mice, but for significantly longer period of times. The concurrent increase in inter-burst intervals suggests a longer recovery period from the bursts.

• KT11 and DTG1 variants increase GIP with GABA, but decrease it with other GABA types, suggesting these two GIP receptors may play a role in GABAergic neurons. Localization of GIP with GABA in WT and K78R mice reveals that these major pathways may contribute to the phenotype. The K78R variant shows stronger expression in GABAergic neurons, while DTG1 show increased interaction. The differential effects of mutations on certain interactions may contribute to the phenotype variability observed in affected individuals.

• Gnb1P57R variants show defective activation of NEA pathway, following GIPR activation, while MRAP-1 and RAP2A pathways are not affected. This suggests that GIP can regulate NEA pathway through its direct interaction with Gnb1K78R (as seen on GPCR assay).

Methods

• Phenotypic tests:
• Pups were weighed and tested on 7 days for developmental milestones and assessment.

• Secure susceptibility was determined using electroconvulsive threshold (ECT) tests, where mice were exposed to a single electroconvulsive stimulus and the magnitude of the motor response was measured as the low current in milliampere (mA) that elicited a full extension reflex of the hindlimb and forelimb flexion. In vivo assessment and characterization

• CatWalk (Noldus Information Technology) is a modern automated test for gait function and locomotion. It consists of an infrared-surfaced walkway (130 x 10 cm) and a high-speed camera undersurface. Mice are habituated for three days before the experiment. Light is reduced and influences the animal (footprint) when downward pressure is applied. Mice are allowed to traverse the walkway with time as needed to obtain at least 3 brain readings (without stopping or hallucinations). Parameters statistically calculated by the software include stride length, width, time of support, distance between (contralateral) paws, cadence, and average speed.

• The open field is a general test for locomotor activity. Each mouse is gently placed in the center of the open field (64 x 64 x 64 cm). All movements are recorded for 10 minutes. A von Frey hair stimulus was applied to the tail and paws to determine the threshold for nociception.

• Assessment & characterization

• Clamp on brain activity

• Intracellular signaling pathway activation is affected by the K78R mutation. a) Schematic of GIP signaling in neurons. b) Intracellular signaling pathway activation is affected by K78R mutation. mTOR pathway through its direct interaction with Gnb1K78R (as seen on GPCR assay).

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