A Human Touch: Hominid-Specific LRRC37B Regulates Axon Initial Segment Excitability

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LRRC37B Is a Human Modifier of Voltage-Gated Sodium Channels and Axon Excitability in Cortical Neurons

Libé-Philippot B, Lejeune A, Wierda K, Louros N, Erkol E, Vlaeminck I, Beckers S, Gaspariunaite V, Bilheu A, Konstantoulea K, Nyitrai H, De Vleeschouwer M, Vennekens KM, Vidal N, Bird TW, Soto DC, Jaspers T, Dewilde M, Dennis MY, Rousseau F, Comoletti D, Schymkowitz J, Theys T, de Wit J, Vanderhaeghen P. *Cell*. 2023;186(26):5766-5783.e25. doi:10.1016/j.cell.2023.11.028. PMID: 38134874

The enhanced cognitive abilities characterizing the human species result from specialized features of neurons and circuits. Here, we report that the hominid-specific gene LRRC37B encodes a receptor expressed in human cortical pyramidal neurons (CPNs) and selectively localized to the axon initial segment (AIS), the subcellular compartment triggering action potentials. Ectopic expression of LRRC37B in mouse CPNs in vivo leads to reduced intrinsic excitability, a distinctive feature of some classes of human CPNs. Molecularly, LRRC37B binds to the secreted ligand FGF13A and to the voltage-gated sodium channel (Nav) b-subunit SCN1B. LRRC37B concentrates inhibitory effects of FGF13A on Nav channel function, thereby reducing excitability, specifically at the AIS level. Electrophysiological recordings in adult human cortical slices reveal lower neuronal excitability in human CPNs expressing LRRC37B. LRRC37B thus acts as a species-specific modifier of human neuron excitability, linking human genome and cell evolution, with important implications for human brain function and diseases.

Commentary

The recent manuscript by Libe-Philippot et al (2023)[1] is of great interest to the epilepsy community as it describes the function of the hominid-specific gene *LRRC37B*. This gene encodes a receptor enriched in cerebral cortex that localizes to the axon initial segment (AIS) and interacts indirectly with multiple epilepsy-linked proteins—including voltage-gated sodium channels—to regulate excitability of human neurons. This extends ongoing work from the Vanderhaegen group on the genomic basis of the evolutionary expansion and complexity of the human cerebral cortex via investigation of genes that have undergone human-specific duplication over evolutionary time.[2]

Differences between the human brain and that of nonhuman primates (and compared to animals commonly used in epilepsy research such as rodents) include size and complexity, particularly of the cerebral cortex, with a greater degree of gyrification and regional specialization thought to underlie higher-order cognitive processes such as language, understanding, and advanced motor function (eg, tool use). At the cellular level, differences are more subtle. The human neocortex is thicker ($\sim$3 mm) than that of nonhuman primates (1-2.5 mm) and rodents ($\sim$1 mm), and the dendritic arbors of human pyramidal cells are more complex and receive a higher density of synaptic contacts.[3,4] Comparative studies of neuronal function have necessarily been limited by a lack of access to healthy brain tissue, with much of the work in the field performed on *ex vivo* specimens resected during surgery for treatment-resistant epilepsy or brain tumor. There may be discrete neuronal cell types that are specific to, or more common in, human relative to other species (such as the double bouquet or "horse tail" cell, seen in humans and to a lesser extent in carnivores but not in rodents).[5] Yet, such work has suggested that the basic biophysical properties of human neurons are largely the same in mouse, rat, ferret, and so on.[6] Recent work suggests that dendrites of layer 5 pyramidal cells in human neocortex are less excitable than in rat,[7] while another report suggests that dendrites of human layer 2/3 neocortical pyramidal cells are more excitable than in rodent.[8]

Here, Libe-Philippot et al identify a novel duplication of *LRRC37B* in hominids (humans and chimpanzee). LRRC37B belongs to the LRR protein family which contain an extracellular leucine rich repeat domain with a high affinity for protein–protein interactions and important roles in cell communication in the developing nervous system, including in axon guidance, synapse formation, and myelination.[9] There are 4 *LRRC37* genes in humans. Libé-Philippot et al show that *LRRC37B* specifically first appears in the genomes of hominids

and is not present in other simians (Old and New World monkeys and other apes) or rodents, although orthologs exist in all species. By single-cell RNA sequencing analysis, *LRRC37B* is expressed in a subset of excitatory and inhibitory cells, concordant with publicly available RNAseq data from Allen Brain. In human brain tissue resected from patients who had undergone epilepsy surgery, LRRC37B localizes to the AIS of a subset of neocortical excitatory projection neurons, the proportion of which increases over development to nearly half of all excitatory projection neurons in adult human. Though chimpanzees also harbor the *LRRC37B* gene, there is little/no protein expression at the AIS, suggesting human specific regulation of LRRC37B expression and trafficking.

To probe the function of LRRC37B, the researchers overexpressed a fluorescently-tagged version of the human gene in mice prenatally via *in utero* electroporation. The overexpressed protein localized to the AIS in mouse neurons. However, mouse neurons overexpressing LRRC37B had *decreased* excitability, with lower firing rates. Neurons exhibited higher rheobase, slower action potential rise time and prolonged spike width, decreased input resistance, and increased capacitance. The authors show a lower upstroke velocity (dV/dt) of the AIS component but not the soma component of the phase plot (voltage vs dV/dt), suggesting dysfunction of the AIS. There does not appear to be a change in the AIS length (based on staining with Ankyrin-G) or distance of the AIS from the soma. While the mechanism of the changes in input resistance and capacitance are unclear, these results suggest that LRRC37B "humanizes" the mouse neurons and decreases neuronal excitability via acting on the spike generating mechanism at/near the AIS. The authors then perform a side-by-side comparison of neurons in human neocortex that express LRRC37B and those that do not, identified via post-hoc immunostaining, and find that neurons expressing LRRC37B have lower excitability than LRRC37B-negative neighbors.

To further probe how LRRC37B might regulate spike generation, the authors uncovered a fascinating set of interactions that likely explain the effects of LRRC37B on neuronal excitability. LRRC37B has a high affinity for binding extracellular FGF13A, a secreted isoform of FGF13 (that one of the isoforms of FGF13 is secreted is also novel data). FGF13 has been previously known to bind to and regulate Nav1.6, the voltage-gated sodium channel α subunit encoded by the epilepsy-associated gene *SCN8A*. In the presence of (and only in the presence of) FGF13, LRRC37B coprecipitates with Nav1.6. However, LRRC37B also interacts with another regulator of Nav1.6, the epilepsy-linked sodium channel accessory β1 subunit (encoded by *SCN1B*). The presence of LRRC37B disrupts the interaction of β1 with Nav1.6, suggesting that it may act as a "switch" to modulate excitability at the AIS.

The direct contribution of the hominid-specific LRRC37B to seizure susceptibility is unknown, but localization of LRRC37B to the AIS is important due to the role of the AIS as a hub of neuronal excitability and potential role of AIS dysfunction in the pathophysiology of epilepsy.[10] LRRC37B interacts with at least 3 genes variants in which are known to cause a spectrum of epilepsies. LRRC37B is not itself a human disease gene (yet), although the probability of being loss-of-function intolerant (pLI) is 1, suggesting loss-of-function variants (which might be expected to increase excitability of excitatory projection neurons in neocortex) are not tolerated.

One limitation of the work is- that little direct evidence is presented to support the conclusion that sodium current density is decreased at the AIS. Similar results could be obtained due to changes in the exact location of sodium channels and/or the distance of the spike generating zone from the soma. It is somewhat curious that the authors do not report analysis of the voltage threshold for action potential generation. Direct recordings from the axon or potentially voltage imaging would have further enhanced the rigor of the data. That said, alterations in the amplitude of the initial deflection of the phase plot strongly suggest that something is happening at the AIS.

This study also raises additional questions. For example, the authors show LRRC37B expression in somatostatin-positive GABAergic interneurons, although the role of LRRC37B in these cells was not explored. It is also possible that expression of LRRC37B is modified by activity, or even upregulated in epilepsy as a homeostatic mechanism to decrease network excitability. Such investigations will be technically difficult due to the limitations inherent in studying a gene that is exclusively expressed in humans, but if we want to fully understand the role of genes such as LRRC37B in epilepsy, we will need the human touch, the most powerful force in the world (Gandhi).

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Declaration of Conflicting Interests

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