

# The emerging role of the inwardly rectifying K<sup>+</sup> channels in autism spectrum disorders and epilepsy

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## Abstract

Autism is a complex behavioral disorder that develops prior to age three years and is distinguished by high heritability. Many genes predisposing to autism spectrum disorders (ASDs) have been identified. These findings have demonstrated that ASDs are etiologically heterogeneous; although, the mutations underlying ASDs are identifiable only in a minority of patients. Indeed, the causes of ASDs are unknown in more than 70% of patients. Recently, we have described two unrelated families whose affected individuals display a characteristic triad of symptoms of autism; such as impairments in social interaction, impairments in communication, restricted interests and repetitive behavior. They also displayed other symptoms commonly observed in autistic individuals; such as gait imbalance, clumsiness, mental retardation and epilepsy. The genetic analysis of these families resulted in the identification of new heterozygous point mutations in the *KCNJ10* gene that encodes the inwardly-rectifying K<sup>+</sup> channel Kir4.1 expressed predominantly, but not exclusively, in astrocytes. Functionally, the mutated channels exhibited a phenotype consistent with *gain-of-function* defects. These new findings highlight the emerging role of inwardly-rectifying K<sup>+</sup> channels and astrocyte dysfunction in autism spectrum disorders associated with epilepsy.

## Keywords

Autism, epilepsy, potassium channels, *KCNJ10*, Kir4.1 and Kir5.1

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## Introduction

Autism spectrum disorders (ASDs) are amongst the most common neuropsychiatric diseases worldwide and their prevalence shows no discrimination in terms of ethnicity, family income or educational levels. The prevalence rates had been estimated at 1% - 2.6% of the world population; however, a carefully executed study published recently<sup>1</sup> presented evidence for surprisingly high rates of these disorders (3.74% in males and 1.47% in females). ASDs are highly heritable and the prevalence in males appears to be three to four times that of females. Since the description of this disease by *Leo Kanner* and *Hans Asperger* almost 70 years ago, it is now well established that ASDs represent a heterogeneous group of disorders in terms of etiology, biology and phenotype. Generally, such complex disorders develop prior to age three years and are characterized by impairments in social interaction and communication, together with repetitive and stereotypic behaviors. Tantrums are the emotional outbursts most often associated with autistic children.

Generally, the onset of autism is gradual; however, approximately 30% have a "regressive" onset. Fifty to seventy percent of children with autism show intellectual disability on nonverbal IQ testing. About 25% of children (who fit the diagnostic criteria for ASDs at age two or three years) later in life begin to talk and communicate, and by the age of 6-7 years blend, to varying degrees, into the regular school population. The remaining 75% continue to experience lifelong disability requiring intensive parental, school, and societal support. For each autistic individual, a cost of \$3.66 million is estimated over a lifetime, taking into account medical care, extra education and lack of economic productivity. The causes of autism can be divided into "idiopathic," which comprises the majority of cases (>70%), and "secondary," in which an environmental agent, chromosome abnormality, or single-gene disorder can be identified. Recent studies suggest that several hundreds of loci are likely to contribute to the complex genetic heterogeneity of this group of disorders. A number of prenatal or postnatal conditions, chromosomal abnormalities (deletions of chromosomes, copy-number variations) and single gene disorders (*e.g.*, Fragile X Syndrome and Tuberous Sclerosis) have been linked to ASD. However, with the exception of Rett's Syndrome which is primarily due to mutations in the methyl-CpG-binding protein 2 (*MECP2*) gene, the etiology and neurobiological mechanisms underlying ASDs remain largely unclear. Several other lines of evidence indicate that genetic components are involved in susceptibility to ASDs, such as the much higher concordance rates of ASD in monozygotic twins (92%) than dizygotic twins (10%). Moreover, parents and siblings of affected children often show much milder, subclinical manifestations of autism, the "*broad autism*

*phenotype*", further suggesting that ASDs can be, at least partially, linked to common genetic susceptibility factors. The high degree of clinical overlap between autism and epilepsy has led to the identification of a subgroup within the autistic spectrum, which has been labeled "*autism-epilepsy phenotype*". The risk of seizures in autism is reported to range between 5% and 46%, clearly exceeding that of the general population (0.5-1%). On the other hand, the prevalence of autism in epileptic population is reported at 32%, about 50 times higher than in the general population. Although the pathophysiological significance of this relationship is yet unsettled, it offers clues as to possible common genetic and molecular mechanisms responsible for both the seizures and the socio-cognitive and communicative dysfunction that define ASDs. Both an imbalance between the excitatory glutamatergic and inhibitory GABAergic systems, and variants in genes encoding for GABA receptor subunits, have been associated with autism susceptibility and seizures. A susceptibility locus for autism has also been mapped near a cluster of voltage-gated sodium channel genes (*SCN1A* and *SCN2A*), on chromosome 2, that are susceptibility genes for epilepsy (9). The identification of genes that appear etiologically relevant to both ASD and seizures could provide new insights for understanding the ASD-epilepsy relationship.

## Identification of new mutations in the Kir4.1 channels of children displaying autism, seizures and intellectual disability.

Inwardly-rectifying (Kir) potassium channels are expressed in a wide variety of excitable and non-excitable tissues throughout the body where they play a key role in the maintenance of the resting membrane potential and thereby the control of cellular excitability. Many Kir channels also play an important role in K<sup>+</sup> homeostasis by contributing to a wide range of different K<sup>+</sup> transport pathways. The Kir4.1 potassium channel was first cloned from the brain<sup>2</sup> where it is expressed predominantly, but not exclusively, in glial cells including both oligodendrocytes and astrocytes. Approximately 15 distinct Kir clones have been identified, forming seven major subfamilies: Kir1.0-Kir7.0. Our research group has established important biophysical properties and physiological roles for several Kir subfamilies members.<sup>3,4</sup> In particular, Kir5.1 does not produce functional K<sup>+</sup> channel activity when expressed by itself. Instead it selectively co-assembles with Kir4.1 and Kir4.2 forming heteromeric channels.<sup>2,5,6,7,8</sup> Heteromultimerisation between Kir4.1 and Kir5.1 produces an increase in single-channel conductance, an increase in rectification and very slow time-dependent activation at hyperpolarising potentials compared to homomeric Kir4.1<sup>5</sup>. Another key property

of heteromeric Kir4.1/Kir5.1 channels is their inhibition by hypercapnic acidosis. Recently, D'Adamo and *co-workers* showed that Kir5.1 subunits confer CO<sub>2</sub> sensitivity to locus coeruleus (LC) neurons.<sup>4</sup>

These studies also demonstrate that the ability of Kir subunits to heteromultimerise adds increased functional diversity to a limited number of gene products. Kir4.1 channels are encoded by the *KCNJ10* gene on chromosome 1q22. Two early studies indicated a linkage between missense variations in *KCNJ10* and seizure susceptibility in mice and in humans with temporal lobe and idiopathic generalized epilepsy.<sup>9,10</sup> However, these studies have not been supported by solid functional evidence. Recordings from surgical specimens of patients with intractable epilepsies have demonstrated a significant reduction of Kir conductance in astrocytes and an impaired ability for potassium clearance. Furthermore, conditional Kir4.1 knockout mice display premature lethality and stress-induced seizures<sup>11</sup>. Mutations in *KCNJ10* have been detected also in patients affected by seizures, ataxia, sensorineural deafness and tubulopathy (EAST syndrome<sup>12</sup>; SeSAME syndrome<sup>13</sup>). In vitro electrophysiological assays of some of the mutations detected in individuals with EAST/SeSAME syndrome, have demonstrated a large reduction of currents from mutant channels.<sup>14</sup> Overall, it appears that either the reduction or the absence of Kir4.1 causes epilepsy. The relationship between Kir channels and autism spectrum disorders is less straightforward. A linkage study on a Finnish extended pedigree has proposed *KCNJ10* as a candidate gene for ASD.<sup>15</sup> Furthermore, an *up-regulation* of Kir4.1 has been found in LC neurons of *MECP2*-null mice, an animal model of Rett syndrome.<sup>16</sup> This over-expression of Kir4.1 has been hypothesized to impair the neuromodulation actions of the LC, leading to the autonomic dysfunction and autistic behaviours seen in patients with Rett Syndrome.

Recently we have reported a mutational screening of *KCNJ10* in 52 children with cryptogenic epilepsy, 14 of these also had a concurrent diagnosis of ASD.<sup>17</sup> We identified two heterozygous *KCNJ10* mutations p.R18Q in two identical twins and p.V84M in a 14-year-old child. Clinically, the two 8-year-old identical twins showed impaired social interaction, sleep difficulties, and hypotonia at 5 months. Two months later both exhibited epileptic spasms within the same 24 hour period. Other symptoms included clumsiness, absence of speech, severe disorder of social interaction, stereotypies, repetitive behaviors, symptoms of anxiety, depression, obsessive-compulsive disorder and intellectual disability (IQ: 58). The 14-year-old child showed normal psychomotor development until 12 months of age, when sleep disorder, poor social gaze, no response to name, absence of

language development, and withdrawal behaviors became evident. At 6 years, he experienced complex partial seizures.

The p.R18Q and p.V84M mutations that we have identified replaced amino acid residues that are highly conserved throughout evolution and were undetected in about 500 healthy chromosomes. Reduced penetrance was observed in three apparently asymptomatic mutation carriers and in one that was mildly affected. Incomplete penetrance is relatively common in “channelopathies”, particularly when they affect the central nervous system. The effects of mutations on channel activity were functionally assayed using a heterologous expression system. These studies have indicated that the molecular mechanism contributing to the disease relates to an increase in either surface-expression or conductance of the Kir4.1 channel.<sup>17</sup> Unlike previous syndromic associations of genetic variants in *KCNJ10*, the pure neuropsychiatric phenotype in our patients suggests that the new mutations affect K<sup>+</sup> homeostasis for the most part in the brain, acting through *gain-of-function* defects. These results provide novel insight into the etiology of autism/epilepsy phenotype, and a new direction for more effective therapeutic approaches.

#### Postulated contributions of Kir4.1 dysfunction to ASD and epilepsy

Kir4.1 channels are expressed predominantly in astrocytes but also in neurons. Astrocytes make up 90% of all human brain cells and each astrocyte controls the activity of many thousands of synapses (about 140 000 in the hippocampus). Astrocytic Kir4.1 channels help maintain the ionic and osmotic environment in the extracellular space, by promoting K<sup>+</sup> transport from regions of high [K<sup>+</sup>]<sub>o</sub>, which results from synaptic excitation, to those of low [K<sup>+</sup>]<sub>o</sub>. This polarized transport of K<sup>+</sup> in astrocytes is essential for normal neuronal activity and excitability and for synaptic functions. Defective astrocyte-mediated regulation of [K<sup>+</sup>]<sub>o</sub> in the brain presents an entirely original mechanistic hypothesis for association between the allelic variations we have identified in *KCNJ10* and ASD with seizure susceptibility and intellectual disability. Co-occurrence of epilepsy and ASD in patients harboring the *KCNJ10 gain-of-function* mutations suggests that dysfunction in the astrocytic-dependent K<sup>+</sup> buffering may be a common mechanism contributing to seizures as well as the core behavioral features of ASD.

Although controversial, it has been proposed that the *loss-of-function* of glial potassium conductance

would favor extracellular  $K^+$  accumulation, contributing to neuronal hyper-excitability and epilepsy. Kuffler and *co-workers* first demonstrated that depolarizations of glial cells, induced by nerve stimulations, were attributable to the high  $K^+$  permeability of these cells.<sup>18</sup> Glial membrane potential tightly follows  $[K^+]_o$  variations along the equilibrium potential calculated with the Nernst equation. The role of Kir4.1 in this process has been demonstrated by targeted ablation of the Kir4.1 gene (*Kcnj10*) in mice. In Kir4.1 knock out glial cells no membrane potential variations were observed during  $[K^+]_o$  increases induced by nerve stimulations.<sup>19</sup> These studies demonstrated the central role played by Kir4.1 channels in astrocyte-operated  $K^+$  buffering. Noteworthy, homozygous Kir4.1 knockout mice die within 2–3 weeks of birth showing severe motor impairment caused by dysmyelination and axonal degeneration.<sup>19</sup>

Recently, it has been shown that isolated episodes of local neuronal hyperactivity trigger a large and synchronous calcium elevation in closely associated astrocytes. These activated astrocytes signal back to neurons, favoring the recruitment of neurons into a coherent activity that underlines the hypersynchronous ictal discharge.<sup>20</sup> The functional analyses carried out here suggest, as an alternative pathogenic mechanism, that an increased and faster influx of  $K^+$  into astrocytes through Kir4.1-containing channels during intense neuronal activity may lead to larger membrane depolarization and higher intracellular calcium elevations in these cells. Calcium elevations in astrocytes are associated with the release of gliotransmitters, such as glutamate and D-serine that trigger discharges in neurons, promoting local neuronal synchrony and epileptic activity. We are tempted to speculate that a recurrent neuron-astrocyte-neuron excitatory loop may develop at a restricted brain site, as a consequence of *gain-of-function* of Kir4.1 channels, and contribute to the initiation of seizures. The new mutations that we have identified in *KCNJ10* also produce *gain-of-function* of the heteromeric channels formed by Kir4.1 and Kir5.1 subunits.<sup>17</sup>

This channel type is highly expressed in astrocytes but also in LC neurons<sup>4</sup>. This suggests that such mutations may additionally alter the noradrenergic system of the brain. LC produces and transports all of the noradrenaline (NE) in the cerebral cortex and hippocampus and most of the NE in other parts of the neuraxis, including the cerebellum. Interestingly, astrocytes are the main cellular target of NE terminals in the brain which also form neuronal-glia-vascular associations.<sup>21</sup> Therefore, NE is considered one of the main players in the neuronal control of glial activation<sup>22</sup>. Aoki *and* Pickel demonstrated that astrocytic  $\alpha_2$  receptors are present near adrenergic nerve terminals, which rise from neurons originating in the LC.<sup>23</sup>

A developmental dysregulation of this LC-NE network has been implicated in the modulation of autistic behaviors in humans. In addition, the *up-regulation* of Kir4.1 channels, which has been found in LC neurons of *MECP2*-null mice, further supports a possible causative role of LC dysfunction in ASD.

40% to 60% of autistic children show some degree of mental retardation. Such defects appear closely tied to developmental events occurring later in childhood which depend on synaptic activity and activity-dependent changes that ultimately result in synaptic plasticity, learning and memory formation. LC plays crucial roles in learning and memory processes and Kir4.1 has been implicated in the modulation of synaptic strength.<sup>11</sup> Kir4.1 channel activity also shows a profound developmental regulation, which correlates with both cell differentiation and the developmental regulation of extracellular  $K^+$  dynamics, *in vivo*. Moreover, astrocyte-released neuroactive substances have been shown to affect neuronal excitability, excitatory and inhibitory synaptic transmission and plasticity, as well as synaptogenesis and neuronal wiring. Thus, either the possible effects of R18Q mutation in promoting channel trafficking or the increased single channel conductance of V84M may alter a number of different mechanisms related to  $K^+$  homeostasis, cell differentiation and synaptic plasticity and contribute to ASD and epilepsy insurgence. However, additional studies are necessary in order to understand how dysfunction of the *KCNJ10*-encoded potassium channel causes a complex neuropsychiatric disease such as ASD with seizures and intellectual disability. Our research groups, which are at the forefront in fighting these disorders, will try to elucidate these mechanisms in the near future.

### Concluding remarks

Solving the puzzle of autism is certainly an extremely difficult task. We have added a few pieces to this puzzle by identifying novel genetic defects in children affected by autism/epilepsy with intellectual disability. Despite the abundance of astrocytes in the CNS, to a certain extent they have been overlooked in the search for mechanisms underlying psychiatric disorders and epilepsy. Our evidence supports the hypothesis that astrocytes, as well as neurons, may play a role in at least some neuropsychiatric phenotypes. As a consequence, astrocytes may represent a crucial target for novel pharmacological control of abnormal electrical discharges and synaptic function in the CNS of children affected by such devastating disorders. Indeed, several tricyclic antidepressants, such as nortriptyline, have already been demonstrated to preferentially block the glial

Kir4.1 channel, suggesting that Kir4.1 also could be an important target offering new opportunities for ASD and epilepsy therapy. Without doubt, these studies are important in identifying signaling pathways and circuits involved in ASDs and in finding novel pharmacological interventions to ameliorate the symptoms. More broadly however, understanding these experiments of *Nature* helps us to understand the physiological workings of the human body.

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