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Research Letter to *American Journal of Medical Genetics Part A*

Autosomal recessive *SLC12A2*-syndrome

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To the Editors,

We recently described *de novo* heterozygous variants in *SLC12A2* as a cause of a neurodevelopmental disorder (OMIM 619083), a subset with variants in exon 21 causing autosomal dominant non-syndromal deafness (OMIM 619081). *SLC12A2* encodes NKCC1 (a sodium-, potassium-, chloride-co-transporter), which is widely expressed during human development. NKCC1 has been demonstrated to play a central role in neurogenesis and the excitatory-inhibitory GABA switch. Multiple splice isoforms of *SLC12A2* exist, with the exon 21 containing isoform the only transcript in the developing cochlea. This is proposed to explain why exon 21 variants cause non-syndromal deafness, while variants in other exons cause a more severe neurodevelopmental disorder. The report of a child with a multi-system neurodevelopmental disorder associated with homozygous deletion of *SLC12A2* (in the context of uniparental disomy for chromosome 5) lead to the proposal of an autosomal recessive condition with bi-allelic *SLC12A2* variants. Here we present further evidence for an autosomal recessive form of S*LC12A2* syndrome.

We identified bi-allelic loss of function variants in *SLC12A2* in a female neonate with a multi-system disorder. She was the second child of non-consanguineous parents. She was born at 40+2 weeks in good condition. At 1 month of age she was intubated for respiratory syncytial positive bronchiolitis. After discharge, she had a brief respiratory arrest requiring cardiopulmonary resuscitation at home. A barium swallow demonstrated gastro-oesophageal reflux. Laparotomy and division of adhesions was performed for mid-gut malrotation. Brain magnetic resonance imaging was normal. Audiometry demonstrated bilateral sensorineural hearing loss (BLSNHL). At the age of 1 year and 4 months she had global developmental delay. She was unable to sit or roll, but had developed a social smile. Seizures were not reported. She was fed by nasogastric tube. Recurrent episodes of *Staphylococcus aureus* parotiditis and pharyngitis occurred. Comparative Genomic Hybridisation (CGH) was normal.

Rapid neonatal trio exome sequencing demonstrated bi-allelic variants in *SLC12A2*. A paternally inherited stop variant (g. 5: 128114275 C>T) and maternally inherited splice donor variant (g. 5: 128138723 CAGTA>C). The impact of these variants on SLC12A2 transcript stability was evaluated. **Details of RNA work, ACMG classification**. No other likely pathogenic or pathogenic variants were identified.

There is emerging evidence for both dominant and recessive neurodevelopmental disorders associated with *SCL12A2* variants (Table 1 summarises reported recessive cases). A homozygous 22kB deletion of *SLC12A2* associated with uniparental disomy of chromosome 5 was reported in a child with BLSNHL, global developmental delay and gastrointestinal dysfunction. In 2 consanguineous Saudi Arabian families, identical bi-allelic *SLC12A2* splice variants were identified in 4 children with neurodevelopmental delay, failure to thrive and BLSNHL in some. No gastrointestinal abnormalities were reported. Two Swedish sisters with bi-allelic *SLC12A2* loss-of-function variants have been described. One died as a neonate with respiratory failure secondary to aspiration. At age 8, the surviving sister has severe developmental delay, gastrointestinal malrotation and dry respiratory mucosa. Both had *S. aureus* infections as neonates. Given the overlapping features between the reported cases, we propose that this confirms the existence of a recessive *SLC12A2* syndrome.

The presentation of bi-allelic *SLC12A2* variants reflects the known functions of NKCC1. NKCC1 regulates ion and water flow across secretory epithelia into the lumen of tubular organs. Deficiency of NKCC1 results in viscous mucus, with plugging of the respiratory tree, xerostomia and intestinal obstruction. It has been suggested that this is a similar mechanism and presentation to Cystic Fibrosis (CF). The known role of NKCC1 in neurogenesis is compatible with the severe neurodevelopmental disorder. The reports of recurrent *S. aureus* infections are intriguing. In a recent UK Biobank based Genome Wide Association Study (GWAS), the *SLC12A2* locus was associated with risk of soft tissue

infections. The mechanism underlying this is unclear. In *SLC12A2* null phagocytes, ingestion of an apoptotic corpse results in upregulation of pro-inflammatory and pro-oxidant genes. This suggests that *SLC12A2* deficiency can interfere with immune cell function. The presence and clinical significance of immune dysfunction in *SLC12A2* syndrome is unclear, but should be borne in mind.

The molecular explanation for why some genomic variants cause dominant and some cause recessive disease is not known. Based on clinical assessment, the individuals with *de novo* heterozygous variants are less severely affected than those with bi-allelic *SLC12A2* variants. This suggests that gene dosage may be contributing to phenotypic severity. All of the recessive *SLC12A2*-syndrome patients were associated with bi-allelic loss-of-function variants (stop variants, splice-site variants). There was no obvious phenotype in the heterozygous parents, though we cannot exclude subtle neurocognitive or hearing phenotypes. The majority of the *de novo* heterozygous *SCL12A2* syndrome patients have missense variants. The severe neurodevelopmental phenotype in these individuals with variants outside exon 21 might be due to a dominant negative effect of mutant NKCC1. This is entirely plausible given that NKCC1 functions as a dimer. The loss-of-function *SLC12A2* variants are unlikely to lead to production of a mutant protein, with altered structure, and so unlikely to have a dominant-negative effect.

We propose that current evidence supports the existence of both a dominant and an autosomal recessive form of *SLC12A2*-syndrome. We summarise the reported patients with bi-allelic *SLC12A2* variants in Table 1. This condition is likely to present in the neonatal period with hypotonia, poor feeding and respiratory distress. Bi-allelic *SLC12A2* variants should be considered in the differential diagnosis of critically ill neonates but would require exome sequencing for diagnosis.

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	Current case	Stodberg 1	Stodberg 2	Kilquist	Bilal Shamsh Family 1	Bilal Shamshi Family 1	Bilal Shamshi Family 2	Bilal Shamshi Family 2
Sex, age (year)	F, 1	F, RIP 22 days	F, 8	M, 5	M, 6	F, 9	F, 5	F, RIP 3 months
Variant		c.1431delT c.2006-1G>A	c.1431delT c.2006-1G>A	22 kb deletion, UPD chromosome 5	c.2617-2A>G	c.2617-2A>G	c.2617A>G	c.2617A>G
Microcephaly			-2.5 SD	<3SD	Yes	Yes	Yes	
Developmental delay	Unable to sit		Cannot walk No speech	Unable to sit No Speech	Sit support No Speech	Sit support No Speech	Sit support No Speech	
Respiratory	Mucous plugging	Apneas	Mucous plugging	Mucous plugging	Respiratory distress		Recurrent aspiration	Recurrent aspiration
Gastrointestinal	NGT fed, Midgut malrotation	Normal	Midgut malrotation	NGT fed, Midgut malrotation				
Xerostomia	+		+	+				
Seizures	No		No	No				
BLSNHL	+		+	+				
Brain MRI	Normal		Basal ganglia injury	Reduced cerebral volume	Reduced cerebral volume	White matter hyperintensity	Normal at 6 months	
Infection	*S. aureus*	*S. aureus*	*S. aureus*					

	parotitis	parotitis	parotitis + sepsis					