

Myoclonic-Atonic Epilepsy Caused by a Novel de Novo Heterozygous Missense Variant in the *SLC6A1* Gene: Brief Discussion of the Literature and Detailed Case Description of a Severely Intellectually Disabled Adult Male Patient

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Introduction: Diagnostic exome sequencing has yielded over the past decades a great number of molecular diagnoses for genetic disorders in which both intellectual disability and epilepsy are present. One of these syndromes is myoclonic-atic epilepsy (MAE) that is caused by pathogenic variants in the *SLC6A1* gene located at 3p25.3. The most relevant clinical characteristics are intellectual disability, several forms of mostly treatment-resistant epilepsy starting at young age, serious disinhibitory behavioural problems, language impairment, higher pain tolerance, and symptoms from the autism spectrum, all in the absence of any consistent dysmorphism or malformation.

Methods: After an overview of the literature, here, the developmental trajectory of a 55-year-old severely intellectually disabled male with therapy-resistant epilepsy and aggressive outburst is reported in detail, in whom no etiological diagnosis had been performed. Next to genetic, neurological, and neuropsychiatric examination, psychological assessment with validated instruments was performed.

Results: Exome sequencing and targeted analysis of the patient and both his parents demonstrated a de novo missense variant in the *SLC6A1* gene which was never before described in the literature nor in control databases. The phenotypical presentation of the patient with treatment-resistant epilepsy, especially absences and myoclonic seizures, as well as sleep disturbances and autism, corresponds with a diagnosis of MAE.

Discussion: This case stresses that exome sequencing should be the first-tier diagnostic test for patients with unexplained neurodevelopmental disorders, regardless of their age, and that as yet the most suitable approach is the formation of an interdisciplinary team for treatment design and clinical management.

Keywords: myoclonic-atic epilepsy, *SLC6A1*, missense variant, intellectual disability, epilepsy, autism

Introduction

Over the past decade diagnostic exome sequencing has yielded a great number of molecular diagnoses for genetic disorders in which both intellectual disability and epilepsy are present.^{1,2} One of these syndromes is myoclonic-atic epilepsy (MAE; OMIM: #616421) that is caused by pathogenic variants in the *SLC6A1* gene (MIM: 137,165) located at 3p25.3. This gene encodes GAT-1 and is expressed in the neocortex and abundantly in the cerebellum.³ GAT-1 is one of the major transporters of gamma-aminobutyric acid (GABA) in the brain, responsible for the reuptake of GABA from the synapse and maintaining neurotransmitter homeostasis.⁴ Pathogenic variants in the *SLC6A1* gene lead to a disrupted

GABA homeostasis in the synaptic cleft resulting in a decreased clearance of GABA with subsequent enhancement of neuronal inhibition.^{5,6} This, in turn, has been associated with spike-wave EEG discharges which are a common phenomenon during absence seizures in patients with a *SLC6A1* pathogenic variant.^{1,4-8}

Already in 1970, Doose et al described a rare epilepsy syndrome called myoclonic astatic epilepsy that they postulated to have a genetic predisposition.⁹ In 2014, Dikow and co-workers described three young patients with overlapping proximal 3p25.3 microdeletions containing two genes, *SLC6A1* and *SLC6A11*, both encoding for major GABA transporters in the brain.¹⁰ They hypothesized that the loss of *SLC6A1* encoding GAT-1 was responsible for a consistent phenotype comprising intellectual disability, absent or poor speech, epilepsy, and stereotypic behaviour without distinct physical anomalies. Furthermore, likely pathogenic variants in *SLC6A1* were identified in 6 out of 160 patients with early-onset epilepsy with myoclonic atonic seizures.⁴ Subsequent studies identified individual patients with early-onset epilepsy and autism spectrum disorder with variants in *SLC6A1*.^{11,12}

A number of recent studies have demonstrated the diverse effects of different subtypes of pathogenic variants in the *SLC6A1* gene on the expression of functional GAT-1. Truncating variants (such as frame shifts and stop gains) likely result in lowering GAT-1 expression by haploinsufficiency, whereas a number of missense variants result in an even lower GAT-1 expression by a possible dominant-negative effect.¹³ These studies demonstrated retention and degradation of mutant GAT-1 in the endoplasmic reticulum (ER) resulting in a lower total expression of correctly folded and posttranslationally modified functional GAT-1 subunits.^{14,15}

In a review by Johannesen et al, 34 patients were described in whom the most relevant clinical characteristics were intellectual disability, epilepsy and language impairment. None of the patients showed consistent dysmorphisms or malformations.⁶ In 91% of the patients, epilepsy was present with most common seizure types absence and myoclonic or myoclonic-atonic seizures and a mean onset at about 3.7 years. In half of the patients behavioural problems were present, in particular aggressive behaviour, hand stereotypies, and symptoms from the attention-deficit-hyperactivity and/or autism spectrum. In 16 out of 34 patients the diagnostic criteria for MAE were fulfilled. In the majority of patients missense variants in *SLC6A1* were found. In a second review by Kahen and co-workers, the clinical phenotypes of 28 patients with pathogenic variants in *SLC6A1* were summarized.¹⁶ Also in this group of patients epilepsy was common (82%), especially absence, and atonic and myoclonic seizures. The frequency of absence seizures ranged widely, but this type of seizure was present daily in all patients, whereas the frequency of myoclonic seizures (52% of the patients) ranged from daily to monthly. In 61%, atonic or drop attack seizures were reported, while generalized tonic-clonic seizures were observed in only 14%. In addition to epilepsy, developmental delay, autism spectrum disorder, language impairment, movement disorders like tremor and dystonic movements, sleep disturbances, and high pain tolerance were frequently present. Like in the review by Johannesen et al, also here most variants were de novo missense variants.⁶ Kahen and co-workers concluded that pathogenic variants in *SLC6A1* are associated with a specific behavioural phenotype characterized by hypotonia, intellectual disability, language disorder (in particular speech delay), seizures, symptoms from the autism spectrum, aggressive behaviours with irritability and hyperactivity, high pain tolerance, sleep disturbances, and movement disorders.¹⁶ A similar description of the behavioural phenotype was reported by Fischer et al.¹⁷ With respect to treatment of epileptic phenomena, the research groups of both Johannesen and Kahen reported that valproic acid was most commonly used and effective in about half of the patients. Finally, Goodspeed et al reported about a cohort of 116 patients with *SLC6A1* variants recruited from several sources. Inclusion age ranged from 1 to 15 years except for 5 patients with an age between 17 and 28 years.¹⁸ Also in this cohort most variants were de novo missense variants. Concerning the behavioural phenotype, no differences were mentioned as compared to the descriptions by the research groups of Johannesen, Kahen, and Fischer.^{6,16,17}

We here report in detail the developmental trajectory of a 55-year-old severely intellectually disabled male with therapy-resistant epilepsy who was referred for the assessment of etiological diagnosis and treatment of persistent severe challenging behaviours.

Patient and Methods

Ethical Aspects

Assessments were performed at the Raphael Institute location Bredablick, centre for people with intellectual disabilities, Middenbeemster, the Netherlands, under supervision of consultants from the Dutch Centre for Consultation and Expertise,

Utrecht, the Netherlands. Both parents gave written informed consent for publication of the case history of their son (signed consent form dated August 2022 is provided to the Editorial Board). Institutional approval was not required, not only since both parents gave written informed consent but also because José Zuijdam (second author) and Anneke Scheick (third author) are the treating physician and psychologist of the patient, respectively. Moreover, the responsible manager of the Raphael Institute attended all multidisciplinary meetings and fully agreed with publication of the patient's case history.

Case Description

The patient is the second child of non-consanguineous healthy parents. He has a one year older brother and a two years younger brother, both healthy. Pregnancy and birth were unremarkable. His birth weight was 4000 g. Physical examination did not reveal any remarkable dysmorphisms or somatic anomalies. Apart from disorders within the mood spectrum, there is no family load with intellectual disability, epilepsy, neuropsychiatric disorders, or serious somatic diseases. During his first years there were no feeding problems or notable abnormalities in development or behavioural repertoire. At the age of four he was involved in a car accident that, however, only led to a broken clavicle and brain concussion without any brain damage. At the same age, learning problems and delayed development of speech and motor skills became gradually obvious, and four years later he was admitted for observation at the former Dutch institute for children with developmental delay "Ederhorst". Because of his restless and insecure behaviour, brief eye contact when playing games, as well as his speech perseverance, psychological tests were performed to establish his level of general intelligence. According to the at that time used Terman Stanford Achievement Test, a total IQ of 52 was established, whereas with the Leiter nonverbal test of intelligence and cognitive abilities a total IQ of 58 was found. In addition, clear autistic traits were observed like lack of contact with peers, perseverations, echolalia, repetitive behaviours, and problems in handling environmental changes.

During this observation period, regular occurrence of absences was noticed for the first time that resolved under treatment with three times daily 250 mg ethosuximide. At the age of 9, the neurologist advised to stop treatment with ethosuximide because the patient was more than one year free of absences. Thereafter, the patient followed special education until his twenties and was briefly employed in daycare activities. However, at the age of 20, tonic-clonic and focal epileptic seizures occurred for the first time, and subsequently treatment with twice daily 300 mg valproic acid was started that was later replaced by three times daily 200 mg carbamazepine. For behavioural control 5 mg periciazine daily was added. Because of increase of both absences and tonic-clonic seizures, despite adding twice daily 50 mg lamotrigine, as well as intensification of challenging behaviours, one year later, the patient was referred to SEIN specialized institute for epilepsy, for re-evaluation of the anti-epileptic treatment regimen. There, both tonic-clonic and focal seizures with decreased awareness and automatisms such as grimacing, drumming with the fingers, spout movements of the lips, wandering around and urine incontinence were observed. EEG-registration showed series of spike-wave complexes across the midline frontally with a maximum over the left hemisphere. CT-scanning of the brain revealed no abnormalities.

Because of progressive challenging behaviours with temper tantrums and aggressive outbursts, aged 20 years, the patient left his parents' home and was admitted to an institute for people with intellectual disabilities. After several transfers to different institutions due to habituation problems, at the age of 44 years, the patient was finally admitted to the present institute for people with intellectual disabilities where he participated in various simple daycare activities while mostly staying at his parents' home during the weekends. His behavioural repertoire showed again clear autistic traits like lack of eye contact, perseverations, echolalia, and compulsive behaviours. In addition, a higher pain tolerance and severe sleep problems were noticed. With the ESSEON scale a social-emotional developmental level of maximally 3 years was established.¹⁹

Throughout subsequent years, treatment with various anti-epileptic drugs like valproic acid, topiramate, levetiracetam, clobazam, lacosamide, and brivaracetam did not result in seizure-free episodes. During these years, several times episodes of anxieties, mood instability, and increase of compulsive and challenging behaviours occurred that were interpreted as part of a disorder within the depressive/compulsive spectrum for which different psychotropics were prescribed such as paroxetine, citalopram, lorazepam, and quetiapine, all, however, without any clinically relevant effect.

In the meantime, it has become obvious that the patient, apart from intellectual disability, displayed also an autistic and compulsive behavioural repertoire.

Since seizures especially absences with automatic behaviours persisted, aged 53 years, the patient was readmitted to the SEIN specialized institute for epilepsy where both tonic-clonic and focal seizures with reduced awareness, automatic movements with a duration of 5 to 15 minutes, and urine incontinence were observed. A diagnosis of focal epilepsy was made, and a combination of 800 mg carbamazepine (3.47 mg/L) and 200 mg lacosamide (3.4 mg/L) was prescribed. For behavioural control and sleep problems, treatment with 300 mg quetiapine was continued. Two years later the patient was hospitalized for ten days at the Academic Centre for Epileptology Kempenhaeghe for re-evaluation of his epilepsy. A 24-hour video-EEG registration disclosed during sleep frontal and sporadic frontopolar or frontotemporal epileptiform activities. The previously established diagnosis of focal frontal epilepsy from unknown etiology was confirmed, and it was advised to discontinue lacosamide gradually and to increase the dose of carbamazepine in case of re-occurrence of tonic-clonic seizures.

Since behavioural problems with disinhibited behaviours, sleep problems, and clinical signs of epilepsy (absences followed by automatisms and most probably also myoclonic seizures) persisted despite adjustments of daily guidance focused on his autistic behaviours, the first author of this paper was asked for consultation by the Centre of Consultation and Expertise.

Investigations

Somatic and neurological investigation at the age of 55 years disclosed no abnormalities, especially no dysmorphic signs. Height, weight, and head circumference were 180 cm, 77.3 kg, and 58.5 cm, respectively. Anti-epileptic treatment consisted of twice daily 500 mg carbamazepine only (plasma concentration at 1200 mg: 4.6 mg/L). For behavioural control and sleep disturbances 500 mg quetiapine was prescribed (plasma concentration at 300 mg: <14 µg/L), combined with three times daily 1 mg lorazepam. Relevant hematological (eg, white blood count and thrombocytes) and biochemical parameters (eg, vitamin status, thyroid and liver parameters, glucose, and lipid spectrum) were all normal. His behaviour showed clear autistic traits, and the staff members reported the regular occurrence of absences with automatisms and incidentally most probably also short myoclonic seizures.

As assessed with the Vineland Adaptive Behaviour Scale (VABS²⁰), mean developmental age scores on the domains of communication, daily activities, socialization, and motor skills were 22, 35, 14, and 38 months, respectively. Social-emotional development as measured by the Dutch scale for emotional development in people with intellectual disability (ESSEON-R²¹) corresponded with a developmental age of 2.5 years. According to the Social Responsibility Scale (SRZ²²) cognitive capacities corresponded with a developmental age of 2 years. With the revised scale for autism and related disorders (AVZ-R²³) a total score of 16 was established (scores between 10 and 19 are indicative for autism). In addition, the total score on the Autism Diagnostic Observation Schedule (ADOS-2²⁴) of 25 (cut-off: 9) corroborated a diagnosis of autism.

Since no any genetic investigation had ever been made in order to elucidate the etiology of the intellectual disability accompanied by treatment-resistant epilepsy, whole-exome sequencing analysis was performed as described by Neveling and co-workers²⁵ that demonstrated heterozygous missense variants of unknown pathogenicity in the *TRIP12* gene [Chr2 (GRCh37):g.230663741G>A NM_001284214.1:c.3251C>T p.(Ser1084Leu)] and in the *SLC6A1* gene [Chr3(GRCh37):g.11070505T>G NM_003042.4:c.1163T>Gp.(Met388Arg)]. Subsequent exome sequencing and targeted analysis of the patient and both his parents showed that the *TRIP12* variant was also present in his healthy mother and could therefore be considered as a rare likely benign familial polymorphism. The missense variant in the *SLC6A1* gene, however, appeared to be de novo in the patient and was considered to be likely pathogenic. This missense variant in the *SLC6A1* gene has never before been described in the literature nor in control databases such as the Genome Aggregation Database (gnomAD²⁶). The de novo missense variant was considered to be likely causative for the severe intellectual disability, treatment-resistant epilepsy, as well as the clinical phenotype of the patient and corresponds with a diagnosis of myoclonic-atonic epilepsy (MAE).

Outcome and Follow-Up

After extensive multidisciplinary discussion of the etiological diagnosis, it was decided to focus individualized daily guidance as much as possible to the patient's autistic behaviour repertoire. This approach, however, did not result in a substantial and persistent reduction of challenging behaviours, in particular aggression and temper tantrums, nor in less sleep problems, both most probably due to the daily occurrence of epileptic phenomena, especially regular absences and myoclonic seizures. Although the effectiveness of the symptomatic psychopharmacotherapy with quetiapine and lorazepam was doubtful, it was decided to keep the dosage unchanged. Because of persistence of absence and myoclonic seizures and the possible association between epileptic activity and serious behavioural problems, it was recently advised to add valproic acid to carbamazepine. At present it is not known whether addition of valproic acid has resulted in a decrease of absences and myoclonic seizures and/or a reduction of aggressive behaviours.

Discussion

Here, for the first time a 55-year-old severely intellectually disabled male patient is described with a de novo heterozygous missense *SLC6A1* variant that, to the best of our knowledge, has never been published in the literature, nor is it included in the international databases or presented in our inhouse database. His main phenotypical characteristics include severe intellectual disability, a spectrum of treatment-resistant epilepsy syndromes (particularly absence and myoclonic seizures) with début at the age of four, autism, serious behavioural problems, especially aggressive outburst and temper tantrums, and sleep problems, all in the absence of any dysmorphic signs or somatic anomalies.

Despite treatment with various anti-epileptics, however, it appeared to be not possible to reach a seizure-free status. The earlier mentioned preclinical findings by the research groups of Mermer and Wang have led to promising results of experiments where the treatment of cells with pathogenic *SLC6A1* with 4-phenylbutyrate (PBA) showed a (partial) rescue of the functional GAT-1 defect. PBA appears to function as a chaperone that seems to partially overcome the ER retention and increases GAT-1 activity.^{13–15} A clinical trial (identifier: NCT04937062) is now initiated to study the effect of PBA in patients with pathogenic variants in the *SLC6A1* gene.²⁷

Although the behavioural phenotype of the patient is in full agreement with the etiological diagnosis of MAE, in the described patient, here, for the first time, the developmental trajectory of a patient with MAE until his sixth decade of life is described in detail. In addition, the results of extensive formal assessment of developmental issues, and cognitive and social-emotional levels of functioning are reported, whereas with the AVZ-R and the ADOS a diagnosis of autism was formally made. The observation that autism is a core symptom of MEA is supported by mouse models demonstrating that alterations in GABAergic enzyme systems lead to autism.^{28,29}

Conclusion

Genetic analysis with modern techniques and detailed individual phenotyping with objective methods is helpful for the interdisciplinary treatment and prognosis of patients with rare neurodevelopmental disorders. In the here presented adult patient it is illustrated that there is insufficient available information as yet to the most appropriate pharmacotherapy in patients with *SLC6A1* pathogenic variants, and treatment should therefore be in accordance with current strategies for the specific epileptic syndrome. As far as known from the existing literature, most successful results may be obtained with valproic acid, lamotrigine, and ethosuximide and perhaps also with other anti-epileptics that can modulate GABA concentrations such as vigabatrin and tiagabine. Possibly, the results of the proposed PBA study may further contribute to the putative pharmacological treatment strategies.

Meanwhile, the most suitable treatment approach is the formation of an interdisciplinary team for comprehensive clinical management. Furthermore, this case demonstrates that exome sequencing should be the first-tier diagnostic test for patients with unexplained neurodevelopmental disorders, irrespective of their age, as is also advocated by Srivastava and co-workers.³⁰

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Author Contributions

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

Disclosure

None of the authors report any conflict of interest for this work.

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