Cumhuriyet Medical Journal, 44(2): 165-171, 2022
DOI: http://dx.doi.org/10.7197/cmj.1121531

Cumhuriyet Medical Journal

| cmj.cumhuriyet.edu.tr | Founded: 2004 Available online, ISSN:1305-0028 Publisher: Sivas Cumhuriyet Üniversitesi

Investigation of *CDKL5* Gene Mutations in Autistic Patients Accompanied with Intractable Seizures, Autistic Disorder and Seizure in Infancy and Early Childhood

Murat Erdoğan[1a]*, Hakan Gümüş[2b], Didem Behice Öztop[3c], Burhan Balta[1d], Keziban Korkmaz Bayram[4e], Munis Dündar[5f]

[1]Department of Medical Genetics, Kayseri State Hospital, Kayseri,Turkey. [2]Department of Pediatric Neurology, Erciyes University Faculty of Medicine, Kayseri, Turkey.[3]Ankara Universty School of Medicine, Department of Child and Adolescent Psychiatry, Turkey.[4]Department of Medical Genetics, Medical Faculty, Ankara Yıldırım Beyazıt University,Ankara, Turkey.[4]''Central Research Laboratory'' Research and Application Center, Ankara Yıldırım Beyazıt University,Ankara, Turkey. [5]Department of Medical Genetics, Faculty of Medicine, Erciyes University, Kayseri, Turkey

**Corresponding author*

Research Article

History

Received: 29/05/2022
Accepted: 18/06/2022

ABSTRACT

Cyclin-dependent kinase-like 5 (*CDKL5*, OMIM 300203), also known as STK9 (serine/threonine kinase 9), is a gene that is thought to play a role in the production of proteins involved in the normal development of the brain, although its function is not known exactly. It is located in the p22.13 region of X chromosome. Some of the mutations reported in this gene have been found to be associated with epilepsy characterized with progressive seizures, non-epileptic autism and mild epilepsy phenotypes in infancy and early childhood despite treatment with at least two antiepileptic drugs (AED). In this study, we evaluated the relationship between c.119C>T (A40V) rs122460159, c.215T>A/C (I72N, I72T) rs62641235, c.455G>T (C152P) rs122460157, c.525A>T (R175S) rs61749700, c.533G>A (G178D) rs267606715, c.539C>T (P180L) rs61749704, c.1330C>T (R444C) rs561753977 and c.2635_2636delCT (L879E) rs61753251 polymorphisms in *CDKL5* gene (NM_001323289) and intractable seizures and autism disorder. DNA extraction was performed after blood samples were collected. Identified mutations were analysed with Real-Time PCR method. The results obtained from the patient and control groups were compared. It was found that one female patient in the intractable seizure patient group carried the *CDKL5* gene c.525 A>T p.(R175S) mutation, while one female patient in the intractable seizure patient group carried the c.539 C>T p.(P180L) mutation. It is thought that *CDKL5* gene mutation research will be useful in the diagnosis of aetiology in new-borns that have intractable epilepsy despite AED treatment. The fact that CDKL5 mutant patients have autistic findings shows that this gene is among candidate genes for ASD, although no mutation was found in this patient group in our study.

Keywords: *CDKL5* gene, drug resistance epilepsy, genetic polymorphism, autistic disorder, RTPCR

İnfant ve Erken Çocukluk Döneminde Dirençli Nöbet, Otistik Bozukluk ve Nöbetin Eşlik Ettiği Otistik Hastalarda *CDKL5* Gen Mutasyonlarının Araştırılması

Süreç

Geliş: 29/05/2022
Kabul: 18/06/2022

ÖZ

Cyclin-dependent kinase-like 5 (CDKL5, OMIM 300203), STK9 (serine/threonine kinase 9) olarak da bilinen, işlevi tam olarak bilinmemekle birlikte beynin normal gelişiminde görev alan proteinlerin yapımında rol oynadığı düşünülen bir gendir. X kromozomunun p22.13 bölgesinde yerleşim gösterir. Bu gende bildirilen mutasyonların bir kısmı infantil dönemde ve erken çocuklukta en az iki antiepileptik ilaç (AEİ) tedavisine rağmen ilerleyen nöbetlerle karakterize epilepsi, epilepsisiz otizm ve ılımlı epilepsi fenotipleri ile ilişkili bulunmuştur. Bu çalışmada CDKL5 geninde 119C>T (A40V) rs122460159, 215T>A/C (I72N, I72T) rs62641235, 455G>T (C152P) rs122460157, 525A>T (R175S) rs61749700, 533G>A (G178D) rs267606715, 539C>T (P180L) rs61749704, 1330C>T (R444C) rs561753977 ve 2635_2636delCT (L879E) rs61753251 polimorfizmleri ile dirençli nöbet ve otistik bozukluk arasındaki ilişkiyi değerlendirdik. Kan örnekleri toplandıktan sonra, DNA eldesi yapıldı. Belirlenen mutasyonlar, gerçek zamanlı (Real-Time) PZR yöntemi ile araştırıldı. Hasta ve kontrol grubundan elde edilen sonuçlar karşılaştırıldı. CDKL5 geni 525 A>T (R175S), mutasyonunu dirençli nöbet hasta grubunda 1 kız çocuğunun, 539 C>T (P180L) mutasyonunu ise yine dirençli nöbet hasta grubunda bir kız çocuğunun heterozigot taşıdığı bulunmuştur. AEİ tedavisine rağmen dirençli epilepsili yenidoğanlarda CDKL5 geni mutasyon araştırmasının etyolojiye yönelik tanıda faydalı olacağı düşünülmektedir. CDKL5 mutant hastalarda otistik bulgulara rastlanması, çalışmamızda bu grup hastalarda mutasyon tespit edilmemiş olmasına rağmen hala OSB için bu geni hala aday genler arasında göstermektedir.

Anahtar sözcükler: *CDKL5* geni, ilaca dirençli epilepsi, genetik polimorfizm, otistik bozukluk, RTPZR

[a] drmerdogan40@gmail.com https://orcid.org/0000-0001-8768-4457 [b] hakgumus33@yahoo.com https://orcid.org/0000-0001-5896-074X [c] dboztop@gmail.com https://orcid.org/0000-0003-3189-2112
[d] burhan.balta.md@gmail.com https://orcid.org/0000-0003-2672-2493 [e] Drkorkmazbayram@gmail.com https://orcid.org/0000-0002-1228-1298 [f] dundar@erciyes.edu.tr https://orcid.org/0000-0003-0969-4611

How to Cite: Erdoğan M, Gümüş H, Öztop DB, Balta B, Bayram KK, Dündar M (2022), Investigation of *CDKL5* Gene Mutations in Autistic Patients Accompanied with Intractable Seizures, Autistic Disorder and Seizure in Infancy and Early Childhood, Cumhuriyet Medical Journal, June 2022, 44 (2): 165-171

Introduction

Epilepsy is the most common, non-contagious, neurological condition which causes significant disability and mortality in all age groups. It is defined as a condition characterized by two or more idiopathic epileptic seizures at any time [1,2]

No neurological disorder was found in approximately 50% of the patients. Epilepsies that occur without any known pathology in the central nervous system are called primary/idiopathic epilepsies. If there is an identifiable factor in the aetiology, they are called secondary/symptomatic epilepsy. Genetic causes have come to the fore in recent years in the primary group. These diseases constitute at least 1% of all epilepsies [3].

Despite antiepileptic treatment, approximately 30% of patients are resistant to treatment. Some genetic causes in the idiopathic group may cause intractable epilepsy in the patient. These genes can be listed as *ARX* [4,5], *SCN1A* [6], *PCDH19* [7], *STXBP1* [8], *LIS1* and *DCX* [9] and *CDKL5* [10,11]

Autistic disorder (AD) is one of the childhood neuropsychiatric disorders [12]. Familial, environmental, biochemical, endocrine, immunological, and genetic factors are the leading factors in its aetiology. Chromosomal anomalies, gene copy number variants (CNVs) and single gene diseases are known genetic origin causes of ASD. *CDKL5* gene is involved in the construction of a protein required for normal brain development; this protein is thought to be involved in the regulation of some genes although its function has not been fully explained. It is expressed in all tissues, especially the brain (predominantly in neuronal nuclei and dendrites), thymus and testis. The protein encoded by this gene acts as a kinase that changes the activities of other proteins by adding oxygen and phosphate atoms at certain positions. It has not been found yet which proteins are targeted by this protein. It is localized in cells, especially the nucleus. *CDKL5* protein has roles in cell proliferation, neural migration, axonal growth, dendritic morphogenesis and synapse development and function in the adult brain. [13]. It is located in the p22.13 region of X chromosome. It encodes a phosphorylated protein of 1,022-1,030 size and 118 kD weight. It consists of 20 exons with a base length of 228,047 between 18.443.703 and 18.671.749 [14]. Subsequent studies have reported that it has 23 exons and the first 3 exons (1, 1a and 1b) are untranslated regions and probably represent the starting area of 2 transcriptions [15]. N-terminal catalytic area starts in exon 2 and the long C-terminal may have a regulating role. [16] It is associated with conditions called early infantile epileptic encephalopathy-2 (EIEE-2) and X-linked infantile spasm (ISSX-2). While pathogenic mutations are mostly concentrated in the N-terminal region of the gene, they have frequently been found in C-terminal region in cases of intractable seizures.

This study aims to investigate the variants of *CDKL5* gene determined by literature research by using Real-Time PCR method in 3 patient groups, those with intractable epilepsy in infancy, those with AD and those AD in addition to seizures, and healthy individuals in the same age range.

Materials and Methods

40 male and 27 female patients who were diagnosed with intractable epilepsy according to electroencephalogram (EEG) and seizure type results at Erciyes University Faculty of Medicine Paediatric Neurology Outpatient Clinic and who were also diagnosed with AD according to DSM-IV at Department of Child Mental Health in addition to epilepsy between December 2011 and March 2012. Control group consists of 13 females and 10 males with a similar average age who were admitted to Erciyes University Faculty of Medicine Department of Medical Genetics and who were not diagnosed with epilepsy or AD. A voluntary consent form was filled in for each member of the patient and the control group to document that they participated in the study voluntarily. This project has been approved by Ethics Committee of Erciyes University in accordance with the Declaration of Helsinki as revised in 2000. (Date 02/08/2011 and number 2011/433)

DNA Extraction

High Pure PCR Template Preparation Kit (Roche Diagnostics GmbH, Mannheim, Germany) was used to extract genomic DNA from blood and DNA extraction was performed. The purity and the concentration of isolated DNAs were calculated with spectrophotometric measurements in Nanodrop 2000 device. The purity of nucleic acids can be found by the ratio of A260/A280 and a ratio of ~1,8 was used in our study.

Analysis of CDKL5 Gene mutations with RT-PCR method

Primers used in the amplification of regions selected with RT-PCR method and probes-mutant and wild type- (TIB MOLBIOL GmbH, Berlin, Germany) used to determine the base exchange were designed from DNAs extracted from blood. Real time PCR was performed by using LightCycler® 480 (Roche Diagnostics GmbH, Mannheim, Germany). The probes were marked with LightCycler Red 640 at 5' end. Initially, denaturation and PCR processes were performed, and short DNA sequences called amplicon were obtained. These sequences were then denaturated again and the temperature was lowered. At this stage, the probe

binds to the target sequence and while the temperature is increased again, the mutation is detected by melting curve analysis according to presence or absence of mutation. The processes of denaturation, binding and elongation were performed, respectively in the analysis. The increase in the number of cycles (a total of 45 cycles) increases the number of products logarithmically.

Pearson Chi square analysis was used to compare genotype and phenotype differences. Analysis was conducted using R 3.1.1 (http://www.r-project.org) by considering a p value less than 0.05 as statistically significant.

Results

Mean age of the patient group diagnosed with intractable seizure consisting of 26 cases, 11 males and 15 females, was 5,1±3,8; mean age of the patient group diagnosed with AD consisting of 19 cases, 16 males and 3 females, was 6,8±2,6 and mean age of the patient group diagnosed with AD and epilepsy consisting of 26 cases, 11 males and 15 females, was 7,02±3,3. Mean age of the control group consisting of 23 healthy individuals, 13 females and 10 males, who were admitted to the department of medical genetics was 3,7±1,8.

No statistically significant difference was found between patient groups in terms of familial epilepsy history in the comparison made according to Chi-square test (p=0,321). Abnormalities were detected in electrophysiological studies in all cases in DN patient group, in 21% of AD group and 95% of AD and seizure group. Statistically significant difference was found between groups according to Chi square test (p<0.001). In terms of drug therapy, no significant difference was found in DN and seizure and AD group with Chi-square test when compared in terms of polytherapy, in other words, the use of 2 or more AEDs p=0.06(p>0.05). In the analyses made in terms of the mental states of patients, no patients with mild intellectual disability were found in DN seizure group, while the rate of patients with severe intellectual disability was 77%. In the AD and seizure group, while there were no patients with normal mentality, the rate of patients with severe disability was approximately 70%. In the AD group, intelligence interaction levels were found to be close to each other. In the intractable seizure group, while MR images of 8 of the cases showed anomaly (such as increase in CSF distance, moderate dilation of the ventricles, thinning of the corpus callosum, atrophies secondary to epilepsy), MR images of 17 patients in the seizure +AD group were found to be normal p= 0,02 (p<0.05).

Genotyping and phenotyping of CDKL5 Mutations

Table 1 shows the genotyping results obtained from RT PCR and two patients in the intractable seizure group were found to be heterozygous for p.R175S and p.P180L variants. Genotypes were found to be normal in all of the participants included in the study in terms of other variants.

The first patient in whom we found a mutation is a 7-year-old female patient. She was admitted with myoclonic seizures when she was 15 days old. She had infantile spasm (IS) and epileptic encephalopathy (EE). Our second patient was a 1.5-year-old female patient. She was admitted with ISSX-2 phenotype when she was 15 days old. She had IS/EE clinic. Table 2 shows the clinical and laboratory findings of patients. In both of our patients, seizure onset was in the neonatal period. They had intractable seizures despite polytherapy. Both patients had autistic findings and while patient number 1 had RETT syndrome phenotype, patient number 2 had ISSX-2 phenotype. Both changes are located in the 8th exon within the catalytic region of the gene, and they are single point mutations. In the first, the adenine nucleotide at position 525 was replaced by thymine, while serine was formed instead of the amino acid arginine. The second change is the formation of leucine instead of proline as a result of the exchange of cytosine with thymine in position 539. These mutations are changes previously reported in literature and they are classified as pathogenic. Table 3 shows the relevant mutations and changes.

Table 1. Patient's genotype

	Control	Intractable seizures	Autistic Disorder + Seizured	Autistic Disorder
R175S				
Wild Type	23 (%100)	25 (%96)	22 (%100)	19 (%100)
Heterozygous	-	1 (%4)	-	-
P180L				
Wild Type	23 (%100)	25 (%96)	22 (%100)	19 (%100)
Heterozygous	-	1 (%4)	-	-

Table 2. Patient clinic and labrotory findings (mutant patient's)

Findings	Patient 1	Patient 2
Mutation	c.525A>T	c.539C>T
Phenotype	RETT Syndrome	ISSX-2
Gender	F	F
Age	7	1.5
OFC (at birth)	34 cm(50p)	NA
OFC (decreasing with age)	50 cm(50p)	1.5 y' da 43cm (<3p)
Seizure Age (Onset)	15th day after birth	15th day after birth
Infantile spasm/EE	+	+
Type of Seizure	Myoklonic	Tonic
EEG- on set	N/A	grossly irregular back ground rhythm with spikes, sharp waves
EEG- follow up	Spike wave and multiple spike wave activity originating from FT, FC, CP regions of both hemispheres and spreading to the whole hemisphere, presence of partial suppression patterns	Burst suppression pattern consistent with hypsarrhythmia
Interictal EEG	Normal	Normal
Theraphy	Polytheraphy	Polytheraphy
Autistic features	+	-
Mental retardation	+ (Severe)	+ (Severe)
Eye contact	-	-
Hand stereotype	+	+
Hypotonia	+	+
Growth retardation	+	+
Speech	-	-
MRI	Normal	Normal
Antiepileptic drug resistance	+	+
Regression	+	+
MECP2 mutation	N/A	-

F: Female, ISSX-2: Infantıle Spasm Syndrome, X-Lınked 2; OFC:occipitofrontal circumference, EE: Epileptic encephalopathy, N/A: Not Available

Table 3. Characteristics of mutations

	Patient 1	Patient 2
Gender	F	F
Mutation		
Localization	Exon 8	Exon 8
Type	Missense	Missense
Pathogenic/Nonpathogenic	Pathogenic	Pathogenic
Codon change	AGA-AGT	CCA-CTA
Amino acid replacement	Arg175Ser	Pro180Leu
Nucleotide replacement	c.525A>T	c.539C>T
Protein replacement	R175S	P180L
Region	Catalytic	Catalytic
Heredity	N/A	N/A

F: Female **Arg**-R:Arginine **Ser**-S:Serine **Pro**-P:Proline **Leu**-L:Leucine **N/A**: Not Available

Discussion

In this study, child patients who were diagnosed with intractable seizures, autistic disorder, and seizure in addition to autistic disorder in infancy and early childhood and healthy controls were analysed in terms of specific variants of *CDKL5* gene. The most important finding of this study is that some mutations of the *CDKL5* gene were found to be positive in patients who previously had epileptic seizures, who had infantile spasm findings and/or findings that overlapped with the Rett Syndrome phenotype but were not defined as Rett Syndrome, which was in accordance with the literature. On the other hand, these mutations were not found in autistic disorder and autistic disorder +seizure group, unlike the hypothesis. While mutations were found in 2 patients in the intractable seizure group of 26 individuals, no mutations were identified in the other patient groups and the control group.

First studies on *CDKL5* gene started in 1998. In this year, Montini et al. identified a new gene consisting of 20 exons in the Xp22 region, called *STK9* (*serine/threonine kinase 9*), later known as *cyclin-dependent kinase-like 5* (*CDKL5*) .(14) In 2003, Kalscheuer et al. found for the first time in two female patients with X-linked infantile spasm (ISSX-2-X-linked West Syndrome), hypsarrhythmia and severe mental retardation that *STK9* gene was affected as a result of a balanced translocation between X chromosome and between chromosomes 6 and 7. [15] They had early onset IS, hypsarrhythmia on EEG, severe MR common findings and similarities to patients in whom we found mutations. Our cases were also sporadic, as in this study.

In a study they conducted on two female patients with Rett syndrome, Scala et al. found deletion type mutations that impair function in *CDKL5* gene of both patients. These findings showed for the first time that atypical Rett syndrome can also be caused by *CDKL5* inactivation [17]. The clinic, electrophysiological findings, seizure age and drug resistance of our patient number 1 were very similar to the patients in this study.

In 2004, Tao et al. detected the mutation we found in our patient number 2 in monozygotic twins. These were patients diagnosed with RETT syndrome. However, phenotypic features were more severe in one when compared with the other. Phenotypic heterogeneity is common in *CDKL5* mutations. This was explained with X chromosome inactivity (XCI) [18] Since no study was conducted on XCI in our cases, we do not have data related with this. However, the fact that no mutation was found in the sibling of our patient number 1 who had similar findings may not support this view.

The fact that our patients who were found to have mutation were in the Rett syndrome variant and ISSX-2 phenotype, as in patients in previous studies, is an important finding of our study in terms of being in parallel with the literature. *MECP2* mutations are rare in atypical or variant Rett patients. Studies conducted have shown that these are candidates for *CDKL5*.

In their study, Archer et al. found seizure onset age as the first six months predominantly (59/73 patients). [19] In our patient, it was found as 6 months (2,75-7,75) in drug resistant group. It was found as 15,5 (5,75-25,50) months in AD +seizure group. Grosso et al. found median age as 30 days and based on this result, they recommended for *CDKL5* mutant patients to be classified within newborn intractable seizures. [20] In parallel with this, the mutant cases in our study are characterized by seizures starting in the first 15 days of life.

As stated in different studies, *CDKL5* mutations were found to be much more frequent in female patients when compared with male patients. While 86.5% of the mutations found are in female patients, only 16.5% are in male patients [21]. In a study conducted by Nemos et al. on 177 patients, while mutation rate was found as about 8% in female patients, mutation was not found in any of the male patients. While the rate increased up to 28% in patients with early seizure and infantile spasm phenotype, this rate was 9% in our study. The reason for this can be the low number of patients and the scanned mutations.

When the epilepsy rates of *CDKL5* mutant patients are examined, intractable seizures have a rate of 75% and progressively decreasing seizures have a rate of 25%. [10] The mutant patients in our study were patients whose seizures recurred despite polytherapy, which was in line with the literature. Prognosis is negative in such patients. Seizures definitely recur after at the end of the stage called honeymoon period with treatment. Although it has been stated that it is not possible to speak of a specific diagnostic EEG finding, hypsarrhythmia and burst pattern findings are remarkable in our patient number 2. Similarly, no structural anomaly in the brain specific to *CDKL5* mutant patients was described and imaging was found to be normal in both of our patients. In the group of Rett syndrome variant, infantile spasm, and AED resistant patient group whose *CDKL5* mutation was investigated in literature, [10] approximately 85% autistic findings were found. While the presence of autism findings suggests that *CDKL5* mutations can have a role in the aetiology of autism, no mutations were found in any of the patients in autistic findings patient group. The Insufficient number of our patients can be considered as one of the causes of this. Age, cognitive level, and language development are

factors that increase risks. In our study, there was no history of EEG anomaly in 4 cases, despite the presence of EEG anomaly. On the other hand, a study conducted showed EEG anomaly in half of the AD patients who did not have seizures with long term EEG records. [22]

Our study aims to contribute to the relationships between *CDKL5* mutations and epilepsy cases that start and progress in infantile period and intractable epilepsy cases despite AED treatment, Rett syndrome variants and infantile spasm cases. Although *CDKL5* mutations have been reported in literature in these patient groups, there is not much information about *CDKL5* mutation in autistic disorder (AD), AD +epilepsy patient groups. The results found in this study show that no mutations were observed in these two groups. However, since *CDKL5* mutant patients may show autistic findings, this gene may be a candidate gene for ASDs. For this, it is recommended to perform mutation analyzes with a larger patient population and more advanced methods in ASD groups.

References

1. Hauser W, Kurland LT. The epidemiology of epilepsy in Rochester, Minnesota, 1935 through 1967. Epilepsia 1975; 16:1-66.

2. Commission on Classification and Terminology of the International League Against Epilepsy (ILAE). Guidelines for epidemiologic studies on epilepsy. Commission on Epidemiology and Prognosis. Epilepsia 1993; 34: 592-96.

3. Tripathi M and Jain S. Genetics of epilepsy. Current Scıence, vol. 82, no. 6, 25 March 2002

4. Guerrini R, Moro F, Kato M, Barkovich AJ, Shiihara T, McShane MA, Hurst J, Loi M, Tohyama J, Norci V, Hayasaka K, Kang UJ, Das S, Dobyns WB. Expansion of the first PolyA tract of ARX causes infantile spasms and status dystonicus. Neurology 2007 Jul 31; 69: 427– 33.

5. Kato M, Saitoh S, Kamei A, Shiraishi H, Ueda Y, Akasaka M, Tohyama J, Akasaka N, Hayasaka K. A longer polyalanine expansion mutation in the ARX gene causes early infantile epileptic encephalopathy with suppression burst pattern (Ohtahara syndrome). Am J Hum Genet 2007 Aug; 81: 361–66.

6. Marini C, Mei D, Temudo T, Ferrari AT, Buti D, Dravet C, Dias AI, Moreira A, Calado E, Seri S, Neville B, Narbona J, Reid E, Michelucci R, Sicca F, Cross HJ, Guerrini R. Idiopathic epilepsies with seizures precipitated by fever and SCN1A abnormalities. Epilepsia 2007 Sep; 48: 1678– 85.

7.Depienne C, Bouteiller D, Keren B, Cheuret E, Poirier K, Trouillard O, Benyahia B, Quelin C, Carpentier W, Julia S, Afenjar A, Gautier A, Rivier F, Meyer S, Berquin P, Hélias M, Py I, Rivera S, Bahi-Buisson N, Gourfinkel-An I, Cazeneuve C, Ruberg M, Brice A, Nabbout R, Leguern E. Sporadic infantile epileptic encephalopathy caused by mutations in PCDH19 resembles Dravet syndrome but mainly affects females. PLoS Genet. 2009 Feb; 5(2): e1000381

8.Saitsu H, Kato M, T Mizuguchi T, Hamada K, Osaka H, Tohyama J, Uruno K, Kumada S, Nishiyama K, Nishimura A, Okada I, Yoshimura Y, Hirai S, Kumada T, Hayasaka K, Fukuda A, Ogata K, Matsumoto N. De novo mutations in the gene encoding STXBP1 (MUNC18-1) cause early infantile epileptic encephalopathy. Nat Genet 2008 June; 40: 782–88.

9. Guerrini R, Parrini E. Neuronal migration disorders. Neurobiol Dis. 2010 May; 38(2): 154–66.

10. Bahi-Buisson N, Nectoux J, Rosas-Vargas H, Milh M, Boddaert N, Girard B, Cances C, Ville D, Afenjar A, Rio M, Héron D, N'guyen MA, Arzimanoglou A, Philippe, Jonveaux CP, JChelly J, Bienvenu T. Key clinical features to identify girls with CDKL5 mutations. Brain 2008 Oct;131(Pt10) 2647–61.

11. Mari F, Azimonti S, Bertani I, Bolognese F, Colombo E, Caselli R, Scala E, Longo I, Grosso S, Pescucci C, Ariani F, Hayek G, Balestri P, Bergo A, Badaracco G, Zappella M, Broccoli V, Renieri A, Kilstrup-Nielsen C, Landsberger N. CDKL5 belongs to the same molecular pathway of MeCP2 and it is responsible for the early-onset seizure variant of Rett syndrome. Hum Mol Genet 2005 Jul 15; (14) 14: 1935–46.

12. Allik H, Larsson JO, Smedje H (2006). Sleep patterns of school-age children with Asperger syndrome or high-functioning autism. J Autism Dev Disord 36: 585-95.

13. Olson HE, Demarest ST, Pestana-Knight EM, Swanson LC, Iqbal S, Lal D, Leonard H, Cross JH, Devinsky O, Benke TA. Cyclin-dependent kinase-like 5 (CDKL5) deficiency disorder: clinical review. Pediatr Neurol. 2019 August; 97: 18–25.

14. Montini E, Andolfi G, Caruso A, Buchner G, Walpole SM, Mariani M, Consalez G, Trump D, Ballabio A, Franco B. Identification and Characterization of a Novel Serine–Threonine Kinase Gene from the Xp22 Region. Genomics 1998 Aug 1; 51: 427–33

15. Kalscheuer VM, Tao J, Donnelly A, Hollway G, Schwinger E, Kübart S, Menzel C, Hoeltzenbein M, Tommerup N, Eyre H, Harbord M, Haan E, Sutherland GR, Ropers HH, Gécz J. Disruption of the serine/threonine kinase 9 gene causes severe X-linked infantile spasms and mental retardation. Am J Hum Genet 2003 Jun; 72 (6): 1401–11.

16. Hector RD, Dando O, Landsberger N, Kilstrup-Nielsen C, Kind PC, Bailey MES, Cobb SR. Characterisation of CDKL5 Transcript Isoforms in Human and Mouse. PLoS One. 2016; 11(6): e0157758.

17.Scala E, Ariani F, Mari F, Caselli R, Pescucci C, Longo I, Meloni I, Giachino D, Bruttini M, Hayek G, Zappella M, Renieri A. CDKL5/STK9 is mutated in Rett syndrome variant with infantile spasms. J Med Genet 2005 Feb; 42(2): 103–7

18.Tao J, Van Esch H, Hagedorn-Greiwe M, Hoffmann K, Moser B, Raynaud M, Sperner J, Fryns JP, Schwinger E, GécJz, Ropers HH, Kalscheuer VM. Mutations in the X-linked cyclindependent kinase-like 5 (CDKL5/STK9) gene are associated with severe neurodevelopmental retardation. Am J Hum Genet 2004 Dec75(6): 1149–54.

19.Archer HL, Evans J, Edwards S, Colley J, Newbury-Ecob R, O'Callaghan F, Huyton M, O'Regan M,Tolmie J, Sampson J, Clarke A, Osborne J. CDKL5 mutations cause infantile spasms, early onset seizures, and severe mental retardation in female patients. J Med Genet 2006 Sep; 43(9): 729–34.

20.Grosso S, Brogna A, Bazzotti S, Renieri A, Morgese G, Balestri P. Seizures and electroencephalographic findings in CDKL5 mutations: Case report and review. Brain & Development 29 (2007): 239–42

21.Nemos C, Lambert L, Giuliano F, Doray B, Roubertie A, Goldenberg S, Delobel B, Layet V, N'guyen MA, Saunier A, Verneau F, Jonveaux P, Philippe C. Mutational spectrum of CDKL5 in early-onset encephalopathies: a study of a large collection of French patients and review of the literature. Clin Genet 2009; 76: 357-71.

22. Tuchman R, Rapin I. Epilepsy in autism, Lancet Neurol. 2002 Oct;1(6): 352–8