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by Eva Chundrayetti

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Research Article

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Eva Chundrayetti[1]*, Indra Ihsan[1], Amel Yanis[2]

ABSTRACT

Aim: Down Syndrome (DS) or trysomy 21 is a genetic abnormality caused when 21st chromosome fails to separate during meiotic division. People with this syndrome are known to have mild-to-severe mental retardation. This syndrome is known for mild-to-severe mental retardation. One of the pivotal genes is DyrkIA, which some of its polymorphism, SNP rs2154545, may cause phenotypical changes in patient. This study is to prove that SNP rs2154545 polymorphism is occurred in DyrkIA gene and to analyze its relationship with mental retardation in DS. **Materials and Methods:** This is a cross-sectional observational study from 39 subjects' blood samples. All subjects come from special school, Kodya, Padang, clinically proven to have DS and confirm that they have trisomy in 21st chromosome by karyotyping analysis. **Results:** Their DNA samples were analyzed using restriction fragment length polymorphism (RFLP), sequenced, and its contigs were qualitatively analyzed. Multiple alignment and BLAST-NCBI were used to confirm the restriction result. DyrkIA gene rs2154545 polymorphism was observed in the form of homozygote mutant allele (AA) in 5.1% of all samples and 51.3% were mutant heterozygote allele (GA). The rest were wild type (43.6%). Statistical analysis was performed, but there was no significant relationship ($P > 0.05$) between gene polymorphism and mental retardation. **Conclusion:** DyrkIA gene rs2154545 polymorphism was proven to exist in some DS patients, but there was no significant relationship between polymorphism with every level of mental retardation (mild, borderline, moderate, and severe).

KEY WORDS: Down syndrome, DyrkIA gene rs2154545 polymorphism, Mental retardation

BACKGROUND

Down syndrome (DS) is a major pediatric issue due to its tremendous impact to children growth and development. Intelligent development is the most inhibited factor and causes hindrance in everyday life. DS patients need continuous help and counseling from family, community, and country. DS is caused by human chromosome abnormality and responsible to mental retardation, hypotonia, facial dysmorphic, faster Alzheimer onset, and other behavior disruptions.[1,2]

DS incident in the USA is predicted to occur once in every 800–1000 birth, while there is no definitive number in Indonesia yet. However, a research by Indonesia University predicted that at least 300.000 DS case occurred every year.[3] Based on the data from Dr. M. Djamil Hospital, Padang, there were 95 DS cases that occurred between 2009 and 2012 and 112 DS cases from 2013 to 2016.[4]

DS or trisomy 21 is mainly caused by meiotic non-disjunction (95.4% of all observed cases), Robertsonian translocation (2.7%), and mosaic non-disjunction (0.7%) and 2% of all observed cases are caused by rearrangement of 21st chromosome's genetic material with other acrocentric chromosomes such as 14th chromosome. 21st chromosome has 200–400 genes. Etiology and pathology study of DS is focused on the extra copy region from the proximal position of 21st chromosome, mainly at 21q22.3.[5]

Distal region of 21st chromosome's long arm, about 10 Mb in length, is known as DS critical region (DSCR). Some of the genes in DSCR is known to have role

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[1]Division of Children Health Sciences, Dr. M. Djamil Hospital, Faculty of Medicine, Andalas University, Jalan Perintis Kemerdekaan No 94, Padang 25127, West Sumatra, Sumatera, Indonesia, [2]Division of Mental Health Sciences, Dr. M. Djamil Hospital, Faculty of Medicine, Andalas University, Jalan Perintis Kemerdekaan No 94, Padang 25127, West Sumatra, Sumatera, Indonesia

***Corresponding author:** Eva Chundrayetti, Division of Children Health, Dr. M. Djamil Hospital, Faculty of Medicine, Andalas University, Jalan Perintis Kemerdekaan No. 94, Padang 25127, PO BOX 49, West Sumatra, Indonesia. Fax: +6275132838, HP: 08126621695. E-mail: echundrayetti@yahoo.com

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DS pathogenesis such as DyrkIA, which code superoxide dismutase 1 enzyme; cystathionine beta-synthase; and glycinamide ribonucleotide synthase-aminoimidazole ribonucleotide synthase-glycinamide formyltransferase.[6,7] Gene duplication in DSCR may give several depictions in main phenotypic abnormalities of DS. This evidence gives a significant contribution in DS pathogenesis, including phenotype abnormalities such as morphology figure, hypotonia, and mental retardation.

Dual-specificity tyrosine phosphorylation-regulated kinase (Dyrk) is a homolog from Drosophila cerebellum which is responsible for neurogenesis. Dyrk has two specificities to tyrosine kinase and serine/threonine kinase and regulated by phosphorylated tyrosine.[8]

Latest research from DyrkIA gene showed that it located in long arm chromosome 21q22.3 and overexpressed in DS, playing a key role in brain degeneration and fast onset neurodegeneration, nerve fiber loss, and dementia in DS patient. Based on the identification of hundreds of genes that reformed by the DyrkIA overexpression, expression of cytosolic, cytoskeletal, and nuclear protein, including transcription factor and phosphorylation, it can be inferred that DyrkIA overexpression is the central of the multiple pathway deregulation in brain development and aging with structural and functional damage that leads to mental retardation and dementia. DrykIA overexpression in DS contributes directly in fast onset neurofibril degeneration by TAU hyperphosphorylation and indirectly by alternative splicing factor phosphorylation which imbalances the 3R-TAU and 4R-TAU.[9]

DrykIA is located in 21q22.3 chromosome arm. This location is 5.4 Mb in size and contains 30 genes based on the phenotype and genotype correlation in partial trisomy 21 case study. Similar correlation study in partial monosomy 21 revealed the location of DSCR. DSCR is 1.2 Mb in size and contains 10 genes, including DyrkIA.[10]

Another study showed that MBN or DyrkIA has role in central nervous system development, neuron proliferation, neurogenesis, neuron differentiation, cell death, and synapse plasticity.[11] Its overexpression in DS fetus brain supports the hypothesis of MNB's or DyrkIA's role in neuron development which bases the cognitive deficiency in DS patient.[12]

Some studies showed that DS is related to weaker short-term verbal memory capability and long-term explicit memory. However, DS infant usually shows latency in the early raw motoric mastery as the result of hypotonia.[13]

Mental retardation is hardly observed in infant younger than 3-year-old as the assessment instrument for this age range is not well correlated with the later IQ test. Formal IQ test in school age is considered trustable and reflects the children's ability in long term.[14]

Recent studies observed that DyrkIA is responsible in development interference and fast onset brain degeneration which causes the intelligence disruption as reflected in IQ level. This study is performed to elucidate the DyrkIA gene polymorphism SNP rs2154545 its relationship to intelligence level in DS.

The objectives of this study are to prove that SNP rs2154545 polymorphism is occurred in DyrkIA gene and to analyze its relationship with mental retardation in DS.

MATERIALS AND METHODS

This study was conducted to 39 students of special school, Kodya, Padang, who fulfilled the inclusion and exclusion criteria. As much as, 4 cc blood sample was taken from cubital vein for cytogenetic analysis and DNA extraction. Polymerase chain reaction (PCR) and sequencing were performed in Biomedical Laboratory in Medical Faculty, Andalas University, Padang and Macrogen, South Korea. IQ testing was performed by professional psychologist.

DNA Extraction (Genomic DNA Purification)

Genomic DNA was isolated from 300 µl blood sample using Genomic DNA Mini Kit (blood/cultured cell) GB100 (Geneaid). DNA isolation was performed by the following kit manual procedure in every step: Sample preparation, cell lysis, DNA binding, washing and rinsing, and DNA elution.

Rs2154545 (G>A) Genotyping

SNP Rs1254545 DyrkIA (NCBI accession number: NG_009366.1) genotyping was performed with restriction fragment length polymorphism (RFLP)-PCR method. PCR reagents consist of: (1) 0.2 µM deoxynucleoside triphosphates, (2) 100 ng genomic DNA, (3) 1.25 U HotStarTaq DNA polymerase with its buffer, (4) 0.5 µM forward primer rs2154545-F (5'-GGATTCTGTAGTGGATATGTAGTGG-3'), and (5) 0.5 µM reverse primer rs2154545-R (5'ACCCTGTTCCACGCTCAAAA-3'). All reagents made up to 25 µl total volume [Figure 1].

PCR was performed in these settings: (1) Initial denaturation at 95°C for 5 min, (2) followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 53°C for 30 s, and elongation at 72°C for 30 s; lastly, (3) PCR was terminated with final elongation at 72°C for 10 min. PCR product was examined in 1.5% agarose gel electrophoresis with DNA GelRed stain in GelDoc. Rs2154545 PCR product was 456 bp.

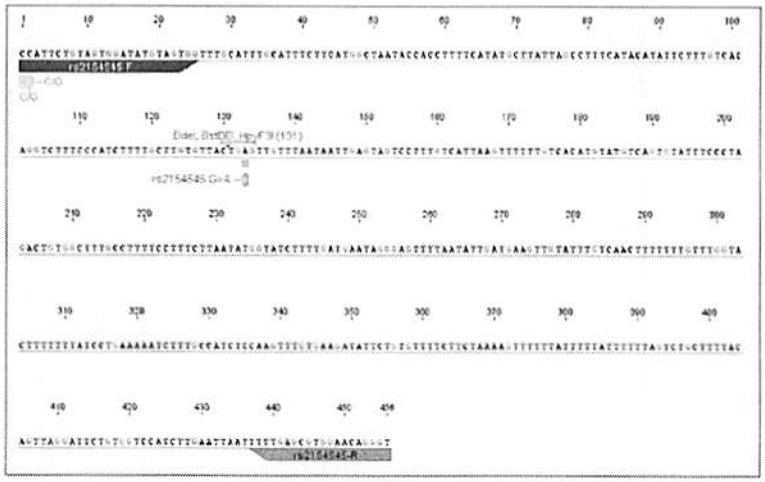

Figure 1: Restriction fragment length polymorphism -PCR map of DYRKIA (NCBI accession number: NG_009366.1) for rs2154545 (G>A) mutant allele with DdeI restriction site

PCR product was cut by 1 U DdeI (restriction site = C^TNAG) restriction enzyme at 65°C for 2 h. After that, restriction result was examined in 1.5% agarose gel electrophoresis with GelRed DNA stain in GelDoc. Individuals with wild type rs2154545 genotype (GG allele) produced 456 bp DNA band. Meanwhile, mutant heterozygote rs2154545 genotype (GA allele) produced 456 bp, 326 bp, and 130 bp band. Mutant homozygote rs2154545 genotype (AA allele) produced 326 bp and 130 bp band.

RESULTS

Every subject in this study had trisomy 21, confirmed by karyotyping analysis. From 39 subjects, 25 (64.1%) of them were male, aged equal or >10-year-old (71.8%), born from 35 years old or more mother (74.4%), and have mild-borderline mental retardation (63.1) [Table 1].

Table 1: Study subjects characteristics

Characteristic	f=39 (%)
Gender	
*Male trisomy	25 (64.1)
*Female trisomy	14 (35.9)
Age	
<10 years old	11 (28.2)
≥10 years old	28 (71.8)
Mother's age	
≤35 years old	10 (25.6)
35 years old	29 (74.4)
Nutrition status	
*Underweight	13 (43.6)
*Norm weight	18 (30.8)
*Overweight	8 (25.6)

Karyotyping Analysis

Blood samples were collected from all subjects and placed in heparin-coated vacutainer (green cap vacutainer) and then cultured with 3–4 days incubation period. After that, the cells were harvested and harvested products were dropped to a glass slide and left for 1–3 days. After that, banding procedure was implemented. Banding result was analyzed and six metaphase cells were selected for karyotyping. 47 chromosomes, XY, trisomy 21 (male) and 47 chromosomes, XX, trisomy 21 (female) were acquired [Figure 2].

DNA Extraction (Genomic DNA Purification)

DNA extraction was carried out by following the instruction manual from Genomic DNA Mini Kit (Geneaid) and examined by gel electrophoresis. Every sample produced DNA band [Figure 3]. This implies that the genomic DNA was successfully isolated.

PCR Genotyping rs2154545 (G>A) Result

After PCR and restriction enzyme treatment, DS32, DS15, DS39, and DS31 DNA samples were separated into three DNA bands: 456 bp, 326 bp, and 130 bp, which imply that the GA allele (mutant heterozygote) was present in those subject. DS40 DNA was separated to two bands: 326 bp and 130 bp, which implies the AA allele (mutant homozygote). Meanwhile, DS26, DS27, and DS11 only produce one band (456 bp) which implies GG wild-type allele [Figures 4 and 5].

Sequencing

PCR product from a few samples was purified and sent to Macrogen, South Korea, for sequencing. Below is the sequencing result of DS22 [Figures 6 and 7].

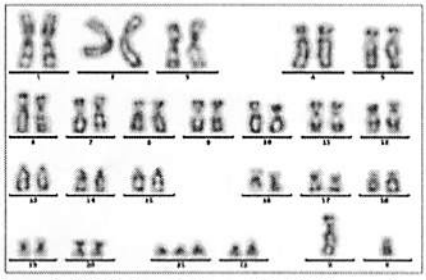

Figure 2: DS 01 karyotyping result with three copies of 21st chromosome and XY sex chromosome which implies that DS01 patient is a male

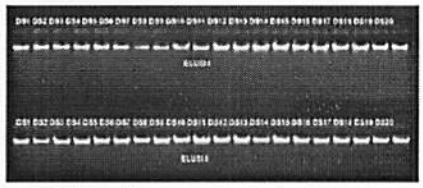

Figure 3: Electrophoresis result of isolated DNA from DS1 to DS20 patients

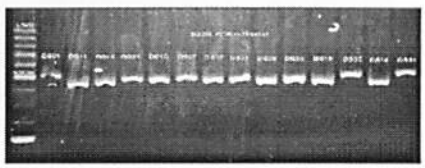

Figure 4: Electropherogram of Down syndrome subject's rs2154545 polymerase chain reaction (PCR). Rs2154545 (G>A) PCR product was 456 bp band

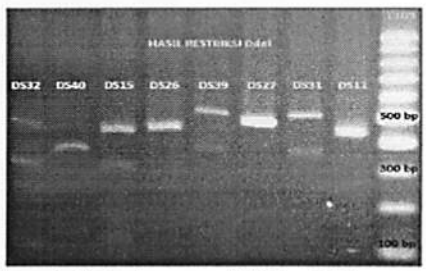

Figure 5: Electropherogram of DdeI restriction enzyme cutoff Down syndrome subject's rs2154545. Rs2154545 (G>A) polymerase chain reaction product was 456 bp band

Table 2: DyrkIA gene rs2154545 polymorphism

rs2154545 polymorphism	f (%)
Wild type (GG allele)	17 (43.6)
Mutant homozygote (AA allele)	2 (5.1)
Mutant heterozygote (GA allele)	20 (51.3)

Multiple Alignment Result

From all 39 subjects, as much as, 17 subjects (43.6%) had wild-type allele (GG), 2 subjects (5.1%) had mutant homozygote (AA allele), and 20 subjects (51.3%) had mutant heterozygote (GA allele) [Table 2]. Subjects with homozygote mutant allele (AA) were equally distributed in mild-borderline and moderate-severe mental retardation but statistically insignificant ($P > 0.05$) [Table 3].

DISCUSSION

This study had 39 subjects who were DS patients with trisomy 21 (46XX + 21 and 46XY + 21) caused by fertilization of gamete cell with extra copy of 21st chromosome with normal gamete cell and produce zygote with 47 chromosomes. This abnormality occurred in 90–95% of all observed cases (Khalil, 2006; Descartes, 2007). 25 subjects (65.1%) were male and 14 subjects were female (35.9%). This finding is in accordance with Johnson's result which more male DS patient was found.[12]

Mental retardation prevalence in DS patient is around 2–3% of all cases. Most of them (85%) have IQ retardation in mild level, while 0.3–0.5% have severe mental retardation.[13] Mild mental retardation is likely to occur 7–10 times than moderate and severe mental retardation.[14] Although physical appearance of DS is easily identified, mental retardation is the most consistent identifier in DS, whom likely has IQ 51 in average.[15] This study found 64.1% of patients with mild-borderline mental retardation and 35.9% with moderate-severe mental retardation.

This study found 16 male DS patients with mild-borderline mental retardation and nine males with moderate-severe. It also found 10 female DS patients with mild-borderline mental retardation and four males with moderate-severe. Statistical analysis showed that there was no significant relationship between mental retardation levels with gender ($P > 0.05$).

As much as, six under 10-year-old subjects suffered mild-borderline mental retardation and five subjects in the same age group had moderate-severe mental retardation. 20 subjects, older than 10 years old, had mild-borderline mental retardation, while eight subjects in the same age group had moderate-severe mental retardation. Statistical analysis did not reveal any significant relationship between age and mental retardation level ($P > 0.05$).

Based on the study conducted in 2000, mental retardation patients were found in 12 patients every 1000 school-age children or one in every 83 children.[16] Mental retardation is mainly occurring in older patients. This finding is consistent with this study finding, which 11 subjects (28.2%) in <10-year-

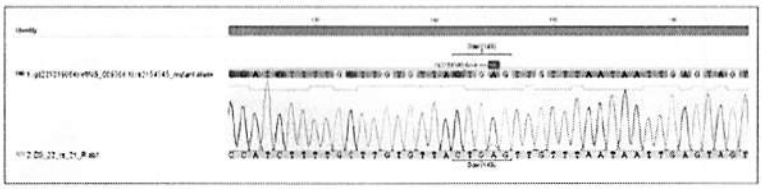

Figure 6: Contig process of sequencing product of Down syndrome (DS) 22 DNA sample with forward primer for rs2154545 (G>A) SNP. Sequencing result indicated that DS22 is a mutant heterozygote (GA allele) which in agreement with the restriction fragment length polymorphism result

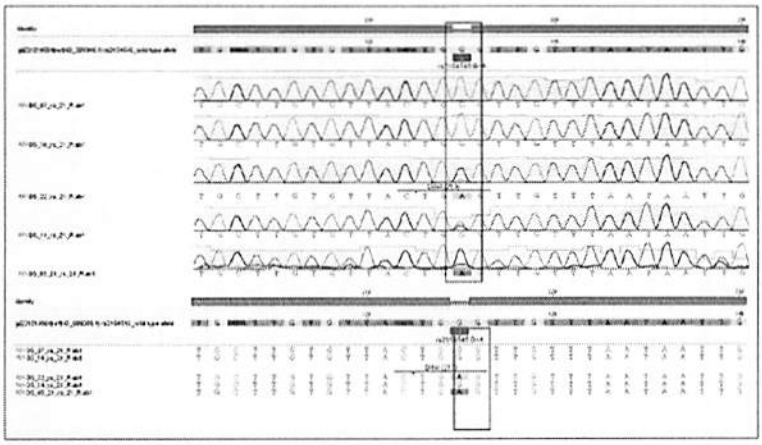

Figure 7: Multiple alignment from five contigs of sequenced samples. Multiple alignment showed that Down syndrome (DS) 22 and DS 05 have SNP polymorphism rs2154545 in the DyrkIA exon sequence as indicated by nucleotide substitution from G to A in DS 22 and DS 05

Table 3: DyrkIA gene polymorphism rs2154545 relationship with Mental Retardation level

SNP rs2154545	Mental retardation				Total		
	Mild-Borderline		moderate-severe				
	f	%	f	%	f	%	p
Wild Type	9	52.9	8	47.1	17	100	
Homozygote	1	50	1	50	2	100	0.355
Heterozygote	15	74	5	25	20	100	
Total	25	64.1	14	35.9	39	100	

old age group had mental retardation. Meanwhile, more subject (28 subjects, 71.8%) older than 10 years old had mental retardation.

As much as, 29 subjects' mother (74.4%) gave birth at the age equal or older than 35 years old. According to the theory, DS cases were found once in every 700 births from 35 years old to older mother and the prevalence increases as the mother's age progresses.[17,18] Similar study in South Africa also found similar result, the prevalence of DS is increasing as the birth mother progresses in age, especially past 35 years old.

DS can also occur as the result of mutation. There are two hypotheses that explain the etiology of DS as the result of mutation, which are as follows: (1) Instability hypothesis that explain the loss of chromosome stability and (2) gene doses hypothesis that explain the overexpression of gene.

DyrkIA polymorphism SNP rs2154545 was found in this study. Wild type (GG allele) comprises about 17 samples (43.6%), mutant homozygote (AA allele) was two samples (5.1%), and 20 samples with heterozygote mutant (51.3%). In general, there were 22 rs2154545 SNP mutants (56.4%) of DyrkIA gene. This result implies that DyrkIA polymorphism has role in DS pathogenesis. DyrkIA overexpression has the potency to damage the brain and cause mental retardation.

In human, DyrkIA gene is located in HSA 21 loci, long arm of 21q22.3 chromosome, which is the critical site of DS and usually called DSCR. DSCR comprises 5.4 Mb and consists of 30 genes, which were found from phenotype-genotype correlation study in partial trisomy 21 patients.[19,20]

Some studies showed that DyrkIA gene has influence in central nervous system development, such as neuron proliferation, neurogenesis, differentiation, cell death, and synapse plasticity. This gene overexpression in the fetus brain with DS supports the hypothesis of DyrkIA role in neurodevelopment that bases the cognitive deficit in DS patients.[21]

SNP rs2154545 heterozygote allele was mainly found in DS patient with mild-borderline mental retardation (74.0%), while homozygote mutant was distributed equally in mild-borderline mental retardation and moderate-severe. However, there was no significant difference statistically.

Another study in senior age with Alzheimer subjects in Madrid, Spain, with other DyrkIA genetic variations did not find any significant result.[22] This study was conducted in Alzheimer patients in Spain with SNP polymorphism rs117001483, rs2835740, rs1137600, rs2154545, rs8132976, and rs2835762 in DyklA gene.

A study in Chinese population found the relationship between mild cognitive amnestic disturbances with SNP in three systems that related to Alzheimer pathogenesis. Those three systems were amyloid cascade pathway, Tau metabolism, and cholesterol.[23,24]

CONCLUSION

It can be concluded that DS has trisomy in 21st chromosome cytogenetically and there are observable polymorphism SNP rs2154545 and SNP rs8132976 in DryklA gene which express high amount of TAU protein with mild-borderline mental retardation as most occurred clinical manifestation.

REFERENCES

1. ACOG Committee on Practice Bulletins. ACOG practice bulletin No 77: Screening for fetal chromosomal abnormalities. Obstet Gynecol 2007;109:217-27.
2. Huret JL, Minor SL, Dorkeld F, Dessen P, Bernheim A. Atlas of genetics and cytogenetics in oncology and haematology, an interactive database. Nucleic Acids Res 2000;28:349-51.
3. American Academy of Pediatrics. Committee on Genetics. American academy of pediatrics: Health supervision for children with down syndrome. Pediatrics 2001;107:442-9.
4. Medical Record. Padang, West Sumatera. Indonesia: M. Djamil Hospital, 2009-2016.
5. American Association of Intellectual and Developmental Disabilities; Frequently Asked Questions on Intellectual Disability. Available from: http://www.aaidd.org. [Last retrieved on 2014 Aug 08].
6. Barkoukis A, Reiss NS, Dombeck M. Childhood Disorder: Mental Retardation. Available from: http://www.mentalhelp.net. [Last retrieved on 2014 Aug 08].
7. Capone GT. Down syndrome: Advances in molecular biology and the neurosciences. J Dev Behav Pediatr 2001;22:40-59.
8. Chang S, Wen S, Chen D, Jin P. Small regulatory RNAs in neurodevelopmental disorders. Hum Mol Genet 2009;18:R18-26.
9. Chen H, Woolley PV Jr. A developmental assessment chart for non-institutionalized down syndrome children. Growth 1978;42:157-65.
10. Choi JK. Hematopoietic disorders in down syndrome. Int J Clin Exp Pathol 2008;1:387-95.
11. Crane E, Morris JK. Changes in maternal age in England and wales implications for down syndrome. Downs Syndr Res Pract 2006;10:41-3.
12. Descartes MC. Cytogenetics. In: Kliegman RM, Jenson HB, Stanton BF, editors. Nelson Textbook of Pediatrics. 18th ed. Philadelphia, PA: Saunders Elsevier; 2007.
13. Dutta S, Gangopadhyay PK, Mukhopadhyay K. Molecular aspects of down syndrome. Indian Pediatr 2005;42:339-44.
14. Vázquez-Higuera JL, Sánchez-Juan P, Rodríguez-Rodríguez E, Mateo I, Pozueta A, Frank A, *et al.* DYRK1A genetic variants are not linked to Alzheimer's disease in a Spanish case-control cohort. BMC Med Genet 2009;10:129.
15. Johnson CP, Walker WO Jr. Mental retardation: Management and prognosis. Pediatr Rev 2006;27:249-55.
16. Khalil A. Screening for down syndrome. J Obstet Gynecol India 2006;56:205-11.
17. Kentrup H, Becker W, Heukelbach J, Wilmes A, Schürmann A, Huppertz C, *et al.* Dyrk, a dual specificity protein kinase with unique structural features whose activity is dependent on tyrosine residues between subdomains VII and VIII. J Biol Chem 1996;271:3488-95.
18. Kida E, Walus M, Jarzębek K, Palminiello S, Albertini G, Rabe A, *et al.* Form of dual-specificity tyrosine-(Y)-phosphorylation-regulated kinase 1A nonphosphorylated at tyrosine 145 and 147 is enriched in the nuclei of astroglial cells, adult hippocampal progenitors, and some cholinergic axon terminals. Neuroscience 2011;195:112-27.
19. Liu X, Yue C, Xu Z, Shu H, Pu M, Yu H, *et al.* Association study of candidate gene polymorphisms with amnestic mild cognitive impairment in a Chinese population. PLoS One 2012;7:e41198.
20. Morris JK, Mutton DE, Alberman E. Revised estimates of the maternal age specific live birth prevalence of down's syndrome. J Med Screen 2002;9:2-6.
21. Park J, Oh Y, Chung KC. Two key genes closely implicated with the neuropathological characteristics in down syndrome DYRK1A and RCAN1. BMB Rep 2009;42:6-15.
22. Rachidi M, Lopes C. Mental Retardation and Human Chromosome 21 Gene Overdosage: From Functional Genomic and Molecular Mechanisms Towards Prevention and Treatment of the Neuropathogenesis of Down Syndrome. Genomics, Proteomics, and the Nervous System. New York: Springer; 2011. p. 21-8.
23. Tejedor FJ, Hämmerle B. MNB/DYRK1A as a multiple regulator of neuronal development. FEBS J 2011;278:223-35.
24. Vasconcelos MM. Mental retardation. J Pediatr 2004;80:s71-82.

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