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원 저

대사 및 내분비 질환에 대한 광범위 신생아 선별 검사의 18년 추적 관찰

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18-year Follow-up of Extended Newborn Screening for Metabolic and Endocrine Disorders

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Purpose: To follow up Korean patients with metabolic and endocrine disorders ascertained by Korea Genetics Research Center, and assess the long-term effectiveness of extended newborn screening program in Korea.

Methods: From January 2000 to December 2017, tandem mass spectrometry and fluoroimmunoassay were employed in extended newborn screening (NBS). The NBS program obtained dried blood spots from 283,626 babies, 48 hours after birth, and screened for galactosemia, congenital hypothyroidism (CH), congenital adrenal hyperplasia (CAH), and 50 preventable inborn errors of amino acid, fatty acid, and organic acid metabolism.

Results: 28 cases of amino acid disorders, 75 cases of organic acid disorders, 27 cases of fatty acid disorders, 51 cases of urea cycle disorders, 127 cases of CH, 14 cases of CAH, and 15 cases of galactosemia were ascertained through NBS and subsequent confirmatory laboratory tests. Patients with amino acid metabolic disorders, galactosemia, CH, or CAH were more likely to have a better long-term outcome if detected early. Early management of MSUD led to much better outcome in over 90%. Despite early intervention, 32% of other organic acidemia cases still resulted in developmental delay and neurological problems. Fatty acid disorders showed varied results; those with EMA and MCAD had a good outcome, but those with VLCAD had serious neurological problems and considerably higher mortality. 75% with UCD experienced serious neurological complications and higher mortality.

Conclusion: The nation-wide NBS program must be accompanied by comprehensive long-term management and physician and family education of inborn errors of metabolism for a better outcome.

Key words: Newborn screening, Congenital adrenal hyperplasia, Congenital hypothyroidism, Galactosemia, Amino acid, Organic acid, Fatty acid disorders, Urea cycle disorder

Introduction

When bacterial inhibition assay was introduced in Korea in 1991 for the screening of congenital hypothyroidism and phenylketonuria (PKU), it was limited to low-income families. The Ministry of Health and Social Affairs adopted a nationwide service program for neonatal screening of phenylketonuria, galactosemia, maple syrup urine dis-

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ease, homocystinuria, histidinemia and congenital hypothyroidism for babies of low income pregnant women registered in health centers. Government decreased the test items from six to two (PKU and congenital hypothyroidism) in order increase the test numbers with the same budget from 1995. 78 laboratories wanted to participate for neonatal screening test in 1999. Government decided to screen for PKU, congenital hypothyroidism, maple syrup urine disease, homocystinuria, galactosemia and congenital adrenal hyperplasia from 2006. From 2014, thirteen laboratories had been participating. Interlaboratory quality control was started 6 times a year from 1994[1)].

The mass spectrometer separates and quantifies ions based on their mass/charge (m/z) ratios. The modern tandem mass spectrometer usually consists of two quadrupole mass spectrometers separated by a reaction chamber or collision cell. With appropriate internal standards, MS/MS permits rapid, sensitive and accurate measurement of many different types of metabolites with minimal sample preparations[2)].

Since the early 1990s, tandem mass spectrometry (MS/MS) had been exclusively used to identify and measure carnitine esters in blood and urine of children suspected of having inborn errors of metabolism[3)]. The MS/MS enabled a large expansion of potentially detectable congenital metabolic disorders that affect blood levels of organic acids[4)].

In 2000, our center started Korea's first pilot expanded newborn screening program with a MS/MS (Micromass, UK). Today, Korean government supports screening of six disorders: PKU, galctosemia, congenital hypothyroidism (CH), congenital adrenal hyperplasia (CAH), homocystinuria, and maple syrup urine disease (MSUD). Expanded newborn screening were not fully subsidized; parents paid the cost out-of-pocket. The Korean government has plans to subsidize the expanded newborn screening program from October, 2018.

We assessed the outcome of those detected by extended newborn screening in an 18-year-long study of Korean metabolic and endocrine disorder patients.

Materials and Methods

283,626 Korean newborns, born between January 2000 and December 2017, participated in our extended newborn screening program (NBS). The NBS program obtained dried blood spots (DBS) from babies at least 48 hours after birth. Electrospray ionization (ESI) MS/MS (Micromass Quattro LC®, UK) and fluoroimmunoassay (Perkin Elmer Wallac DELFIA®, Finland) were used to screen 50 manageable diseases that include hypothyroidism, congenital adrenal hyperplasia, biotinidase deficiency, galactosemia, and inborn errors of amino acid, fatty acid, and organic acid metabolism. For MS/MS, acylcarnitines and amino acids were extracted from DBS and butylated with stable isotope internal standards, and were introduced into the inlet of MS/MS via a high-performance liquid chromatography. Following positive results from the repeat screening, the diagnosis was confirmed by urine organic acid (gas chromatography-mass spectrometry), plasma/urine amino acid (ion-exchange chromatography), enzyme/cofactor or orotic acid studies.

Diagnostic process included clinical and family history, biochemical tests (NH_3, lactic acid, blood gas, urine ketones, blood glucose, urine analysis and indicator tests), genetic counseling and subsequent molecular tests.

Results

Of 283,626 babies screened between January 2000 and December 2017, 207,047 (73%) underwent expanded newborn screening (NBS) (Table 1). 181 out of 207,047 were confirmed to have metabolic disorders; the detection rate was 1/1,144. 28 had amino acid metabolic disorders (detection rate of 1/7,395). 75 had organic acid metabolic disorders (detection rate of 1/2,761). 27 had a fatty acid metabolic disorder (detection rate of 1/7,668). 51 had urea cycle disorders (detection rate of 1/4,060), which include citrullinemia type I (1/12,940), ornithine transcarbamoylase deficiency (OTC)/carbamyl phosphate synthetase (CPS; 1/10,897), citrin deficiency (1/15,927), hyperammonemia hyperornithinemia hyperhomocitrullinemia (HHH; 1/103,524), and argininosuccinicaciduria (ASA; 1/207,047) (Table 2).

One in 261 were positive for T4 and TSH; the positive rate in the repeat screening was 1/2,233. CAH had a high false positive rate; 1 in 417 were positive in the initial screening whereas the repeat screening found 1/20,259 to be positive. Galactose had similar results; 1/505 were positive in the screening and the repeat screening yielded 1/18,908 positive rate. 3 cases with elevated levels of galactose had been diagnosed with portosystemic shunt due to hemangioma, portosystemic shunt without portal vein, and omphalocele. Biotinidase deficiency was extremely rare in Korea; 1/94,542 had a positive screening result, and repeat screening returned only one positive result in 1/141,813 (Table 3).

Overall NBS positive rate in the initial screening was 3,527/283,626 positive (approximately 1/80).

Table 1. The Positive Results of Newborn Screening, First Screening Results and Second Repeated Screening Rates

Year	Total	Hypothyroidism		CAH		Galactose		Tandem MS		Biotinidase	
		First positive	Second positive	First positive	Second positive	First positive	Second positive	First positive	Second positive	First positive	Second positive
2000	3,185	7	4	7	0	0	0	163	6	2	1
2001	7,895	23	3	13	1	13	1	32	6	0	0
2002	8,936	17	2	29	0	15	0	35	7	0	0
2003	72,056	266	22	132	0	116	0	134	6	0	0
2004	72,138	231	17	125	0	117	0	167	9	0	0
2005	71,157	134	21	137	0	120	4	155	8	0	0
2006	8,962	25	2	26	0	11	0	43	14	1	1
2007	7,605	29	6	24	0	14	0	34	17	0	0
2008	5,970	31	4	21	0	12	1	26	14	0	0
2009	3,010	23	7	33	3	15	2	73	18	0	0
2010	3,632	28	6	10	0	11	3	28	12	0	0
2011	2,185	30	5	30	1	15	1	20	19	0	0
2012	3,245	79	11	51	5	41	2	94	7	0	0
2013	2,207	36	4	15	0	31	0	70	12	0	0
2014	3,029	34	5	7	2	2	0	53	7	0	0
2015	2,746	39	2	12	1	15	1	43	7	0	0
2016	2,501	32	1	7	0	4	0	15	8	0	0
2017	3,167	21	5	1	1	10	0	12	4	0	0
Total	283,626/ Tandem (73%) 207,047	1,085	127	680	14	562	15	1,197	181	3	2
Frequency		1/261	1/2,233	1/417	1/20,259	1/505	1/18,908	1/173	1/1,144	1/94,542	1/141,813

Nearly one in ten (336/3,527) showed a positive result in the initial NBS and again in the repeated screening. Three babies' NBS results led to ascertainment of 3 affected mothers even though the babies were not affected.

The metabolic disorders detected by MS/MS were 28 amino acid disorders, 75 organic acid disorders, and 51 urea cycle disorders. The most common amino acid disorders detected by NBS was transient tyrosinemia 1/2,149 (132/283,626). The incidences of classical PKU, hyperphenylalanemia, and atypical PKU were 1.4/100,000, 1/100,000, and 0.7/100,000, respectively. Hypermethioninemia had an incidence rate of 2/100,000 while homocystinuria was found in 1.7/100,000.

In order of the frequency, the most common organic acid disorders were PPA (7.4/100,000), MMA (5.6/100,000), 3-MCCD (5.6/100,000; 13 newborns and 3 mothers), MSUD (4.5/100,000), and IVA (1.4/100,000). Urea cycle disorders detected by NBS were OTC/CPS (6.6/100,000), citrullinemia (5.6/100,000), and citrin deficiency (4.5/100,000). Fatty acid disorders, MCADD, EMA, VLCAD had incidence rates of 4.2, 3.1, and 1.8 out of 100,000, respectively. Endocrine disorders showed much higher frequencies: hypothyroidism (1/2,233) and CAH (1/20,259).

Discussion

NBS programs are now well-established in many developed countries[5,6]. A typical service model comprises of a central laboratory performing all the tests and screening a sufficient number of babies to obtain the economies of scale and make adequate numbers of diagnoses to maintain laboratory expertise and follow-up protocols.

In most countries, there is a move to greater uniformity of NBS panels, protocols, and clear guidelines for evaluating proposals for new tests. The USA is probably the most advanced in this aspect with comprehensive policies published by the American College of Medical Genetics and Genomics[7].

In Korea, the first newborn screening program started in 1991. It was free but offered only to low-income families. In 1997, the Ministry of Health and Social Affairs decided to provide nationwide screening. Between 1991 and 2005, the NBS, which had an emphasis on hypothyroidism, detected 718 CH (1/5,164) and 86 PKU (1/43,114) patients out of 3,707,773 newborns screened[8].

In a 10-year (2006-2015) multi-center report, voluntary NBS using MS/MS found 261 cases of

Table 3. Metabolic and Endocrine Diseases through Newborn Screening Program

Hypothyroidism	127/283,626 (1/2,233)
Congenital adrenal hyperplasia (CAH)	14/283,626 (1/20,259)
Galactosemia	15/283,626 (1/18,908)
Portacaval shunt	3
Biotinidase deficiency	2/283,626 (1/141,813)

Table 2. Metabolic Disorders Detected by Tandem MS

Metabolic Disorders Detected by Tandem MS (181/207,047)	
Amino acid disorders (28)	PKU (4), HyperPhe (3), Atypical PKU (2), Methioninemia (6), Homocystinuria (5), Prolinemia (1), Tyrosinemia: transient (132), type I (1), type II (1), type III (2), Amino acid transport disorder (LPI) (3)
Organic acid disorders (75)	PPA (21), MMA (16), MSUD (13), IVA (4), 3-MCCD (13+3 mothers), GA-1(2), 2-methylbutyrylglycinuria (2), Malonic Acidemia (1)
Urea cycle disorders (51)	Citrullinemia (16), OTC/CPS (19), ASA (1), Citrin Deficiency (13), HHH (2)
Fatty acid disorders (27)	EMA (9), MCADD (12), VLCAD (5), Primary Carnitine Deficiency (1)

confirmed metabolic disorders out of 3,445,238. The detection rate for the metabolic disorders by MS/MS was 1/13,205[9]. In another report covering 3 years (2012-2014), a commercial laboratory screened 119,948. They had 6,681 positive results in the initial screening; of these, 713 cases were also positive in the subsequent screening[10]. In our experience, of 283,626 babies screened between January 2000 and December 2017, 73% (207,047) opted for expanded NBS. 181 out of 207,047 were confirmed to have metabolic disorders; the overall detection rate was 1/1,144.

The substantial variations in the detection rates across laboratories suggest multiple confounding factors. Newborn screening positive babies' parents often voluntarily request repeat newborn screening with another laboratory. A number of families with prior history of developmental delay, metabolic or other genetic diseases have directly requested newborn screening and confirmatory testing, skewing the frequency of inborn errors of metabolism. Furthermore, our laboratory has observed a sharp decline in the number of babies screened over the years as there have been noticeable drops in birth rates over the years in Korea [25], and larger corporate laboratories have entered the lucrative newborn screening market and competed aggressively for their market share.

The MS/MS is usually operated in a multiple reaction monitoring mode, in which selected metabolites are measured. In practice, not all metabolites detected in scanning mode make particularly useful NBS targets. For example, glycine has a limited value in diagnosing non-ketotic hyperglycinaemia[11]. OTC/CPS is characterized by extremely low levels of citrulline, and NBS often fail to detect these patients. OTC/CPS can not be ruled out by normal NBS results.

There were false positives generated by contamination of DBS. However, only a small number of contaminated samples were identified in our long-term study.

MS/MS is not able to reliably detect OTC and CPS because of low citrulline levels. In case of hyperammonemia with low citrulline, urea cycle disorders should be ruled out, even with normal newborn screening results. C3 carnitine has a relatively poor PPV for methylmalonic and propionic acidemias, partly due to the fact that babies with increased free carnitine have secondary increases in C2 carnitine and C3 carnitine[12]. To make differential diagnosis, urine organic acid analysis should be performed.

Tyrosine is a marker for tyrosinemia type I and II[13]. However, it is perhaps the most common metabolite with transient elevation for various reasons. In our study, only 4 cases of out 136 were confirmed to have tyrosinemia. For tyroninemia type I, succinylacetone study should be carried out. Levels of citrulline detected in NBS are not helpful in distinguishing which type of cirullinemia the patients have. Most newborns with mild elevation of citrulline are without symptoms[14,15,19]. For hydroxy-C5 holocarboxylase synthase and 3-methylcrotonyl-CoA carboxylase deficiency, most maternal deficiencies were ascertained by their babies' NBS[16-18]. Other supplementary tests would be alloisoleucine for maple syrup urine disease[20], and total homocysteine and methylmalonic acid for homocystinuria, methylmalonic acidurias and cobalamin defects[21]. Biotinidase deficiency testing is carried out in several countries[22-24]. However, in our experience, it is extremely rare in the Korean population (only two confirmed cases in 18 years).

Long-term outcome of patients with amino acid metabolic disorders, galactosemia, CH, or CAH were more likely to be better if detected early

through NBS and actively managed. Early management of maple syrup urine disease led to much better prognosis in over 90% while in 32% of organic acidemia (MMA, PPA, IVA, GA) cases, early intervention still resulted in developmental delay and neurological problems. Fatty acid disorders showed varied outcomes; those with EMA and MCAD had good prognosis, but those with VLCAD had serious neurological problems and considerably higher mortality. In spite of the NBS program, 75% with urea cycle disorders experienced serious neurological complications, and compared to other metabolic disorders, more patients died. Carbohydrate metabolic disorders had good outcome except one case with galactokinase deficiency.

Early diagnosis and management can prevent complications. A follow-up of newborn screening showed amino acid disorders had a better outcome by early detection and treatment, but the urea cycle disorders had neurologic complication in 75% of patients, even with early diagnosis and management. In organic acid disorder screening, MMA and PPA patients had 32% neurologic sequel. 90% of MSUD patients showed normal growth and development. Fatty acid disorders including EMA and MCADD had excellent outcome but VLCADD had worse prognosis (highest mortality rate). Most galactosemia patients were able to prevent cataracts by soy formula, but language developmental delay and ovarian failure were unavoidable.

Conclusion

The nation-wide NBS program must be accompanied by comprehensive long-term management and follow-up as well as physician education of inborn errors of metabolism for better outcome.

요 약

목적: 한국 유전학 연구소에서 실시한 광범위 신생아 스크리닝 검사(Newborn screening, NBS)로 진단된 선천성 대사질환 및 내분비질환을 가진 한국인 환아의 추적 관찰 및 장기적인 예후를 평가하기 위하여 본 연구를 시작하였다.

방법: 2000년 1월부터 2017년 12월까지 태어난 283,626명의 신생아를 대상으로 하였으며 출생 48시간 이후에 발뒤꿈치, 혹은 정맥혈액을 채취하여 특수여과지에 묻혀 건조시켰다. 건조 혈액여지를(Dried blood spot, DBS) 이용하여 탠덤 질량 분석법과 형광 면역측정법을 사용하여 광범위 신생아 스크리닝 검사(NBS)를 실시하였다. 신생아 스크리닝 선별검사 프로그램은 갈락토오스 혈증, 선천성 갑상선 기능 저하(Congenital hypothyroidism, CH), 선천성 부신 과형성증(Congenital adrenal hyperplasia, CAH), 아미노산, 지방산 및 유기산 대사질환등 예방 가능한 질환 50여종을 선별하여 검사를 시행하였다.

결과: 광범위 신생아 스크리닝 검사(Extended NBS)를 통해 아미노산 대사질환 28예, 유기산 대사질환 75예, 지방산 대사질환 27예, 요소회로 대사질환 51예, CH 127예, CAH 14예, 갈락토스혈증 15예가 선별하여 확진검사로 진단되었다. 아미노산 대사 장애, 갈락토스혈증, CH, CAH 환자는 조기에 발견 치료 할 경우 예후가 더 좋았다. 단풍당뇨(MSUD) 환아에서는 조기진단 치료로 90% 이상이 정상 성장 발달을 보였다. 그러나 유기산 혈증 환아에서는 32%에서 발달 지연 및 신경학적 휴유증이 관찰되었다. 지방산 대사 질환에서는 다양한 결과가 나타났다. 단쇄지방산(SCAD, EMA)와 중쇄지방산(MCA, MCAD) 환자는 예후가 좋았으나 초장쇄지방산(VLCAD) 환자는 대부분 심각한 신경학적 장애를 보이거나 사망하였다. 요소회로 대사질환(UCD) 환아는 조기진단과 치료에도 불구하고 75%가 심각한 신경학적 합병증과 높은 사망률을 경험했다.

결론: 전국적인 신생아 스크리닝(NBS) 프로그램은 국가적인 차원에서 전국민을 대상으로 포괄적인 검사, 관리, 치료가 필요하다. 이를 위하여 숙련된 의료진과 환아의 부모 혹은 관련된 가족에 대한 특수교육이 필요

하다.

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References

1) Lee DH. The Past, Present, Future of Newborn Screening in Korea. Journal of the Korean Society of Inherited Metabolic Disease 2014;14(1):1-9.
2) Maher S, Jjunju F PM, Taylor S. Colloquium: 100 years of mass spectrometry: Perspectives and future trends. Rev. Mod. Phys. 87(1):113-135. 2015. Bibcode:2015RvMP...87..113M. doi:10.1103/RevModPhys.87.113.
3) Millington DS, Terada N, Kodo K, Chace DH. A review: carnitine and acylcarnitine analysis in the diagnosis of metabolic diseases: advantages of tandem mass spectrometry. In: Matsumoto I, editor. Advances in chemical diagnosis and treatment of metabolic disorders, Vol 1. New York: John Wiley & Sons, 1992: 59-71.
4) Chace DH, Kalas TA, Naylor EW. Use of tandem mass spectrometry for multianalyte screening of dried blood specimens from newborns. Clin Chem 2003; 49:1797-817. doi:10.1373/clinchem.2003.022178. PMID 14578311.
5) Jaques AM, Collins VR, Pitt J, Halliday JL. Coverage of the Victorian newborn screening programme in 2003: a retrospective population study. J Paediatr Child Health 2008;44:498-503.
6) Metz MP, Ranieri E, Gerace RL, Priest KR, Luke CG, Chan A. Newborn screening in South Australia: is it universal?. Med J Aust 2003;179:412-5.
7) Watson MS, Mann MY, Lloyd-Puryear MA, Rinaldo P, Howell RR. Newborn screening: toward a uniform screening panel and system. Genetics in Medicine 2006;8:1S-11S.
8) Choi TT, Lee DH. Results of Neonatal Screening Test and Prevalence at Birth of Phenylketonuria and Congenital Hypothyroidism for 15 Years in Korea. Journal of the Korean Society of Inherited Metabolic Disease 2006;6:24-31.
9) Lee B, Lee J, Lee J, Kim SY, Kim JW, Min WK, et al. 10-year Analysis of Inherited Metabolic Diseases Diagnosed with Tandem Mass Spectrometry. Journal of the Korean Society of Inherited Metabolic Disease 2017;17:77-84.
10) Cho SE, Park EJ, Seo DH, Lee IB, Lee HJ, Cho DY, et al. Neonatal Screening Tests for Inherited Metabolic Disorders using Tandem Mass Spectrometry: Experience of a Clinical Laboratory in Korea. Lab Med Online Vol. 5, No. 4: 196-203, October 2015 http://dx.doi.org/10.3343/lmo.2015.5.4.196.
11) Tan ES, Wiley V, Carpenter K, Wilcken B. Non-ketotic hyperglycinemia is usually not detectable by tandem mass spectrometry newborn screening. Mol Genet Metab 2007;90:446-8.
12) Lindner M, Ho S, Kolker S, Abdoh G, Hoffmann GF, Burgard P. Newborn screening for methylmalonic acidurias--optimization by statistical parameter combination. J Inherit Metab Dis 2008;31:379-85.
13) Magera MJ, Gunawardena ND, Hahn SH, Tortorelli S, Mitchell GA, Goodman SI, et al. Quantitative determination of succinylacetone in dried blood spots for newborn screening of tyrosinemia type I. Mol Genet Metab 2006;88:16-21.
14) Haberle J, Pauli S, Schmidt E, Schulze-Eilfing B, Berning C, Koch HG. Mild citrullinemia in Caucasians is an allelic variant of argininosuccinate synthetase deficiency (citrullinemia type 1). Mol Genet Metab 2003;80:302-6.
15) Waisbren SE, Levy HL, Noble M, Matern D, Gregersen N, Pasley K, et al. Short-chain acyl-CoA dehydrogenase (SCAD) deficiency: an examination of the medical and neurodevelopmental characteristics of 14 cases identified through newborn screening or clinical symptoms. Mol Gen Metab 2008;95:39-45.
16) Dantas MF, Suormala T, Randolph A, Coelho D, Fowler B, Valle D, et al. 3-Methylcrotonyl-CoA carboxylase deficiency: mutation analysis in 28 probands, 9 symptomatic and 19 detected by newborn screening. Hum Mutat 2005;26:164.
17) Stadler SC, Polanetz R, Maier EM, Heidenreich SC, Niederer B, Mayerhofer PU, et al. Newborn screening for 3-methylcrotonyl-CoA carboxylase deficiency: population heterogeneity of MCCA and MCCB mutations and impact on risk assessment. Hum Mutat 2006;27: 748-59.
18) Koeberl DD, Millington DS, Smith WE, Weavil SD, Muenzer J, McCandless SE, et al. Evaluation of 3-methylcrotonyl-CoA carboxylase deficiency detected by tandem mass spectrometry newborn screening. J Inherit Metab Dis 2003;26:25-35.
19) Campbell CD, Ganesh J, Ficicioglu C. Two newborns with nutritional vitamin B12 deficiency: challenges in newborn screening for vitamin B12 deficiency. Haematologica 2005;90(Suppl):ECR45.
20) Oglesbee D, Sanders KA, Lacey JM, Magera MJ, Ca-

setta B, Strauss KA, et al. Second-tier test for quantification of alloisoleucine and branched-chain amino acids in dried blood spots to improve newborn screening for maple syrup urine disease (MSUD). Clin Chem 2008;54:542-9.
21) Matern D, Tortorelli S, Oglesbee D, Gavrilov D, Rinaldo P. Reduction of the false-positive rate in newborn screening by implementation of MS/MS-based secondtier tests: the Mayo Clinic experience (2004-2007). J Inherit Metab Dis 2007;30:585-92.
22) Gonzalez EC, Marrero N, Frometa A, Herrera D, Castells Newborn Screening 68 I Clin Biochem Rev Vol 31 May 2010 E, Perez PL. Qualitative colorimetric ultramicroassay for the detection of biotinidase deficiency in newborns. Clin Chim Acta 2006;369:35-9.
23) Moslinger D, Stockler-Ipsiroglu S, Scheibenreiter S, Tiefenthaler M, Muhl A, Seidl R, et al. Clinical and neuropsychological outcome in 33 patients with biotinidase deficiency ascertained by nationwide newborn screening and family studies in Austria. Eur J Pediatr 2001;160:277-82.
24) Weber P, Scholl S, Baumgartner ER. Outcome in patients with profound biotinidase deficiency: relevance of newborn screening. Dev Med Child Neurol 2004;46:481-4.
25) Korean Statistical Information Service (KOSIS). http://kosis.kr/index/index.do.