Journal of
Steroids & Hormonal Science

Clave et al. J Steroids Horm Sci 2015, 6:3
DOI: 10.4172/2157-7536.1000164

Review Article Open Access

Genomic Alterations in Ethanol-Exposed Trophoblast Cell Lines Induced by Chronic Ethanol Treatment

Sergi Clave[1,2,7], Xavier Joya[1,2], Anna Puiggros[3], Marta Bódalo[4], Judith Salat-Batlle[1,2], Marta Salido[3], Blanca Espinet[3], Óscar Garcia-Algar[1,2,5,6,*] and Oriol Vall[1,2,5,6]

[1]*Grup de Recerca Infància i Entorn (GRIE), Institut Hospital del Mar d'Investigacions Mèdiques (IMIM), Barcelona, Spain*
[2]*Red de Salud Materno-Infantil y del Desarrollo (SAMID), Programa RETIC, Fondos FEDER, Instituto Carlos III, Madrid, Spain*
[3]*Laboratori de Citogenètica Molecular. Servei de Patologia, Hospital del Mar, Barcelona, Spain*
[4]*Servei d'Anàlisis de Microarrays, Institut Hospital del Mar d'Investigacions Mèdiques (IMIM), Barcelona, Spain*
[5]*Departament Pediatria, Obstetricia i Ginecologia, i Medicina Preventiva, Universitat Autònoma Barcelona (UAB), Bellaterra, Spain*
[6]*Servei de Pediatria, Parc de Salut Mar, Barcelona, Spain*
[7]*Laboratori de Citogenètica Molecular. Servei de Patologia, Hospital del Mar, Barcelona, Spain*

Abstract

Background: Ethanol consumption during pregnancy results in a broad spectrum of damage, but the knowledge of its mechanism is lacking.

Objective: The aim of this study is to determine ethanol-caused genomic alterations in placental cell lines after a repeated ethanol treatment in order to describe new genomic targets of cell damage.

Methods: A model of sustained exposure to standard doses of ethanol on two *in vitro* human choriocarcinoma cell lines, JEG-3 and BeWo, was used. Chromosomic abnormalities and copy number alterations (CNAs) were assessed by G-Banding cytogenetics and oligonucleotide Single Nucleotide Polymorphism-Array analysis (CytoScan, Affymetrix).

Results: Chromosomal abnormalities did not change despite ethanol exposure except for the presence of a derivative chromosome 4 [add(4)(p14)] in exposed BeWo cells. Regarding SNP-Array analysis, a total of 21 CNAs were found to be caused by ethanol exposure, 16 in JEG-3 cell line and 5 in BeWo cell line, which were not found in controls. There was no coincidence between JEG-3 and BeWo regions affected by ethanol.

Conclusion: Trophoblast cell lines exposed repetitively to ethanol presented genomic instability resulting in CNAs. However, no region has been equally altered in both models to consider it an ethanol exposure target area. So, further studies involving different models and approaches that target gene regulation are required.

Keywords: Target; Ethanol; Placenta; Cytogenetics; Array; CNAs

Introduction

One of the health consequences of alcohol consumption during pregnancy is foetal alcohol syndrome (FAS) [1]. The prevalence of FAS is between 1.3 and 4.6 births per 1,000 [2], while the combined prevalence of FAS and alcohol-related neurodevelopmental disorders (ARND) is estimated to be as high as 9.1 per 1,000 [3]. This is due to the appearance of various permanent birth defects caused by the mother's consumption of alcohol during pregnancy, called foetal alcohol spectrum disorder (FASD) [4].

For the majority of FASD cases, strategies for damage-diagnosis are lacking and there are not biomarkers that offer a reliable information about the injury in the foetus [5]. Among that, selective biological markers for intrauterine alcohol damage promise to lead to interventional strategies targeted to these spectrum of undiagnosed cases [6].

Over the past decade, studies in zygotic and dizygotic twins provided the first evidence for the involvement of genetic factors in damage risk for FASD [7]. Since that, evidence has been accumulating and models for genomic dysregulation have emerged [8]. The consequences of prenatal ethanol exposure (PEE) observed in infants could be attributable, in part, to the damage exerted to the cells and, as consequence, this cell system losses partially its function [9]. Upon delivery, placenta is the most accessible fetal-maternal tissue and carries valuable information about the pregnancy including adverse effects on maternal and/or fetal physiology [5,10]. Ethanol produces some of fetal abnormalities via actions on the placenta. These alterations are: transport of nutrients, hormone/growth factor production and its deregulation [11-13]. Altogether, ethanol shifts the trophoblast from a state of proliferation to one of cell cycle arrest or differentiation, the mechanism of these changes is not understood.

Despite being a preliminary model, cell lines are important resources in order to characterize genomic alterations in pathological conditions [14]. Although G-banding techniques enable the identification of chromosomal aberrations (structural and numerical changes), remarkable alterations could remain unidentified in complex karyotypes [15]. The combined use with Single Nucleotide Polymorphism Array (SNP-Array) allows to give an average genomic profile of copy number gains and losses for all chromosomes [16].

Some studies have been published showing advances in genomic-based alterations in FASD cases [7,17,18] but these studies have not expanded the knowledge in relation to changes in copy number

***Corresponding author:** Oscar Garcia-Algar, Grup de Recerca Infància i Entorn (GRIE), Institut Mar d'Investigacions Mèdiques (IMIM), 08003 Barcelona, Spain, Tel: +34-932483551; Fax: +34-932483254; E-mail: 90458@hospitaldelmar.cat

Received August 19, 2015; **Accepted** December 07, 2015; **Published** December 10, 2015

Citation: Clave S, Joya X, Puiggros A, Bódalo M, Batlle JS, et al. (2015) Genomic Alterations in Ethanol-Exposed Trophoblast Cell Lines Induced by Chronic Ethanol Treatment. J Steroids Horm Sci 6: 164. doi:10.4172/2157-7536.1000164

alterations (CNAs) in placental cells. Herein we present the results of two different placental cell lines exposed chronically to ethanol and its related-genomic alterations with the aim to find out chromosomal regions that can be considered preliminary targets involved on the dysfunction observed in FASD.

Material and Methods

Cell culture

Human placental choriocarcinoma cell lines were purchased from the American Type Culture Collection (ATCC): JEG-3 (HTB-36; ATCC, Manassas, VA) and BeWo (CCL-98; ATCC). JEG-3 cells were maintained in Minimum Essential Media (MEM) supplemented with 10% (v/v) Foetal Bovine Serum (FBS), 20 mM L-glutamine, 10 mM sodium piruvate, 100 mg/mL streptomycin and 100 U/mL penicillin. BeWo cells were maintained in Ham's F-12 medium supplemented with 2 mM L-glutamine, 10 mM sodium piruvate, 100 mg/mL streptomycin and 100 U/mL penicillin; all get from Gibco, Montreal, CA. Cell cultures were maintained at 37°C in humidified 5% CO_2 atmosphere. These lines are the best characterized [9,19,20] and also allow chronic ethanol exposure during several hours as previously described [19,20].

Experimental design

We followed the experimental procedure to expose the *in vitro* models chronically to ethanol designed by van Steenwyk et al. with minor modifications: 600,000 cells per 50 cm^2 flask were seeded, with 5 mL of medium, until the cells were 80% confluent [21,22]. Ethanol-treated cells (50 mM) were cultured in an ethanol-saturated incubator as previously described [23]. This concentration are equivalent to the expected in the human placental tissue from moderate ethanol consumers in alcohol-depending consumers (386 mg/dl; approximately 85 mM) [24]. Control flasks were kept in ethanol-free media and subjected to media changes at the same time as ethanol exposed cells. The ethanol treated cells were maintained in a sealed vessel in which the atmosphere was saturated with ethanol in order to maintain the ethanol concentration at the level added to the medium.

G-banding cytogenetics

G-banding study was carried out on cell lines harvested when cell growth was subconfluent and actively dividing, adding KaryoMAX Colcemid Solution 10 μg/ml (Life Technologies, Rockville, MD, USA). After that, cells were treated with hypotonic solution (postassium chloride, 0.075 M) for 30 minutes at 37°C and were fixed in Carnoy solution. Chromosomes were banded using G-banding technique with Wright solution. All products get at Sigma-Aldrich, St. Louis, MO, USA. A minimum of twenty metaphases per cell line were studied in accordance with the the International Standing Committee on Human Cytogenetic Nomenclature (ISCN) 2013 [25].

Oligonucleotide SNP-array

Genomic DNAs were extracted from cell cultures at time of harvesting the cells using the PureLink Genomic DNA Mini Kit (Life Technologies), according to manufacturer's protocol. Genome-wide high-resolution Single Nucleotide Polymorphisms (SNPs) array CytoScan HD (Affymetrix, Santa Clara, CA, USA) was used containing both SNPs and oligonucleotide probes. Procedures for DNA digestion, ligation, PCR amplification, fragmentation, labelling and hybridization with the arrays were performed according to the manufacture's protocols (Affymetrix). Copy Number Alterations (CNAs), mosaic/clonal status, and Loss of Heterozygosity (LOH) were analyzed using Chromosome Analyses Suite (ChAS) Software (Affymetrix).

Results

JEG-3

By karyotyping, chromosome copy number per metaphase was variable ranging to 71-73 chromosomes per cell, which hampered to characterize the karyotype compared with normal human cells. JEG-3 cell line showed a complex karyotype with many structural chromosomal aberrations (in both, control and exposed conditions) listed below: t(4;11)(p15q13), add(7)(p22), add(7)(q36), add(15)(p11), i(13)(q10), del(18)(q21), add(19)(p13) and two marker-chromosomes with material of unknown origin. No differences between exposed and non-exposed JEG-3 cells were found (Figure 1).

A total of 16 CNAs were identified by SNP-Array in ethanol exposed cells/nuclei: 11 losses and 5 gains (Table 1).

Finally, genes contained in these CNAs JEG-3 regions are: G-protein coupled receptors (*TACR1*, *ADRA2B* and *ADRA1A*), transcriptional regulators (*ID2*, *DNMT3A* and *EGR3*) and catalytic enzymes (*GAD1* and *BHMT*)..We also group these genes as belonging to main diseases: cancer (*ID2*, *EFEMP1*, *TACR1*, *IGFBP2*, *BHMT*, *SAMD5*, *EGR3* and *ARF6*), neurological diseases (*GAD1*, *EGR3*, *ADRA1A* and *ADNP*) and developmental diseases (*MAP2*, *SHH* and *OTX2*).

BeWo

In this cell line, chromosome copy number per metaphase was also variable, ranging to 63-80 chromosomes per cell. We found differences due to the ethanol input regarding karyotype. In non-exposed cells, we observed a deletion in the short arm of chromosome 4 [del(4)(p11)] but not in exposed cells, where a derivative chromosome with material added to 4p [add(4)(p14)] was considered (Figure 1). The remaining chromosomal aberrations were found in both conditions: add(1)(p36), der(1)(qter→q25::p36→qter), del(3)(p11), del(4)(p11), add(4)(p14), del(12)(q11), i(13)(q10), add(16)(q24), del(X)(p11) and 2 non-characterized marker chromosomes.

Only 5 altered regions were differently identified in ethanol-exposed cells by SNP-Array, one loss and four gains (Table 1).

Genes coding for receptors and transport proteins (*GABRA2*, *GABRA4*, *HTT* and *SLC1A1*) and transcriptional regulators (*RBPJ*, *PPARGC1A* and *SMARCA2*) were allocated in these CNAs BeWo regions. Classifying them according to related diseases, cancer (*RAC1*, *SMARCA2* and *JAK2*) and alcohol dependence (*GABRA2* and *MPDZ*) were the most relevant.

Discussion

Trophoblast cell lines exposed repetitively to ethanol developed several CNAs in comparison to trophoblast non-exposed to ethanol. In particular, only one structural change was found to be related with ethanol exposure in BeWo cell line: [add(4)(p14)]. Furthermore, this study can support the hypothesis that ethanol causes genomic damage but this damage occurs randomly and non-specifically.

Regarding CNAs associated with ethanol exposure, previous publications suggested that cell lines are in continuous adaptation to the environment due to their immortal capacity and that some genomic changes are likely resulting from *in vitro* evolution of the karyotype [26,27]. So, environmental factors such as ethanol exposure can cause clone selections. This reason could explain the no detection of common regions in both cell lines.

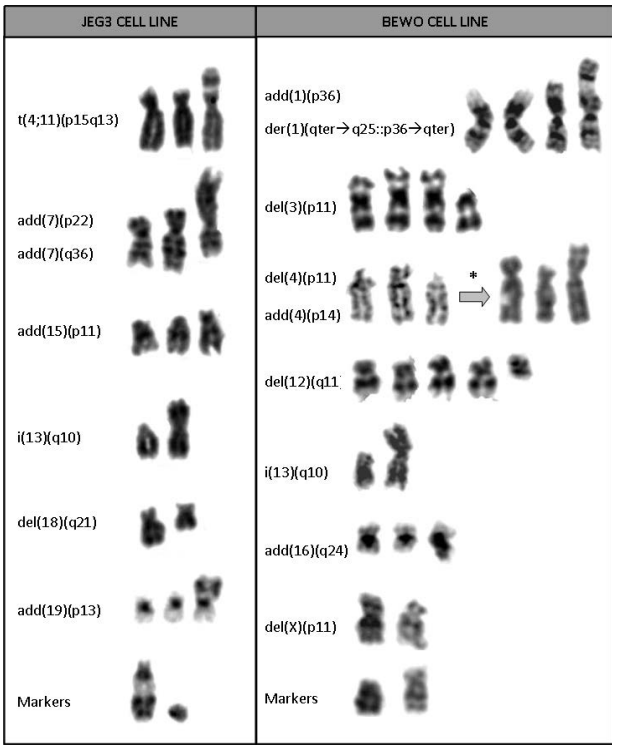

Figure 1: Representative images of JEG-3 and BeWo chromosomal structural aberrations in both, exposed and non-exposed conditions. *[del(4)(p11)] was found in controls and [add(4)(p14)] in ethanol-exposed condition, only in BeWo cell line. The remaining alterations were found invariable between both conditions in JEG-3 and BeWo cells lines.

JEG-3						
CNA TYPE	CHR.	START (BP)	END (BP)	START (BAND)	END (BAND)	SIZE (MB)
Loss	1	200873046	202737249	q32.1	q32.1	1864
Gain	1	203997398	204148044	q32.1	q32.1	151
Loss	2	12770	242783384	p25.3	q37.3	242771
Loss	4	63042492	63703335	q13.1	q13.1	661
Gain	5	51443863	53404950	q11.2	q11.2	1961
Loss	5	53452238	136639313	q11.2	q31.2	83187
Loss	5	175576586	177316728	q35.2	q35.2	1740
Gain	7	132417988	159119707	q32.3	q36.3	26702
Loss	8	158048	146295771	p23.3	q24.3	146138
Loss	8	67597724	70184331	q13.1	q13.2	2587
Gain	12	2961266	4667910	p13.33	p13.32	1707
Loss	14	20511672	107285437	q11.2	q32.33	86774
Loss	16	85880	897652	p13.3	p13.3	812
Gain	18	7079983	8192904	p11.31	p11.23	1113
Loss	18	18602260	20472563	q11.1	q11.2	1870
Loss	20	31382737	62915555	q11.21	q13.33	31533
BeWo						
CNA TYPE	CHR.	START (BP)	END (BP)	START (BAND)	END (BAND)	SIZE (MB)
Loss	3	84764277	85599114	p12.1	p12.1	835
Gain	4	68345	49093788	p16.3	p11	49025
Gain	7	3258340	4367700	p22.2	p22.2	1109
Gain	7	5002251	10508051	p22.1	p21.3	5506
Gain	9	203861	17076367	p24.3	p22.2	16873

Table 1: Copy number alterations (gains and losses) in ethanol-exposed conditions detected by oligonucleotide SNP-Array. These alterations listed below were only found in exposed cell lines.

However, the genes located in the CNA affected areas have been involved in several pathways related to FASD pathogenesis, such as nervous system development, growth restriction, as well as metabolic pathways such as glucocorticoid signaling and retinol, insulin and nitric oxide balanced levels [28-30]. Furthermore, it is important to note that in our study we found frequent association between genes of the CNAs areas and genes involved in cancer development like *TACR1*, *IGFBP2* and *RAC1* [31-33]. Also, the genes altered in both placental cell lines (*SMAD5*, *SHH* and *POMC*) have been previously associated with PEE [34-36].

It worth to be mentioned that genetic factors from the mother and from the foetus could contribute to develop FASD [17]. It is known that polymorphisms in alcohol metabolizing enzymes have a significant impact on the risk for FASD [37]. For example, variations in the alcohol dehydrogenase 1B (class I) gene (*ADH1B*) have been reported to confer either increased or decreased likelihood of developing FASD [38,39]. So, genomic predisposition is known to be present in ethanol exposed cases but no genome-wide studies have demonstrated specific chromosomal alterations that can be validated as candidate targets involved in placental damage after ethanol exposure.

One clear limitation in the present prospective study is that the data obtained are not homogeneous and are considered preliminary especially for describing new biomarkers. Further investigations to better understand the effects of ethanol also using other models and human cells are needed.

In summary, trophoblast cell lines chronically exposed to ethanol presented genomic instability resulting in chromosomal alterations. Despite that, as no genetic aberrations are commonly found in our *in vitro* models, we are not able to define any candidate damage-targets for a dysfunction of the placenta. Future work should be done with more replicates and also considering other models or even human samples.

References

1. Clarren SK, Smith DW (1978) The fetal alcohol syndrome. N Engl J Med 298: 1063-1067.
2. Sampson PD, Streissguth AP, Bookstein FL, Little RE, Clarren SK, et al. (1997) Incidence of fetal alcohol syndrome and prevalence of alcohol-related neurodevelopmental disorder. Teratology 56: 317-326.
3. Streissguth AP, O'Malley K (2000) Neuropsychiatric implications and long-term consequences of fetal alcohol spectrum disorders. Semin Clin Neuropsychiatry 5: 177-90.
4. May PA, Gossage JP (2001) Estimating the prevalence of fetal alcohol syndrome. A summary. Alcohol Res Health 25: 159-167.
5. Joya X, Friguls B, Ortigosa S, Papaseit E, Martínez SE, et al. (2012) Determination of maternal-fetal biomarkers of prenatal exposure to ethanol: a review. J Pharm Biomed Anal 69: 209-22.
6. Miranda RC, Kable J, Reynolds JN, Valenzuela CF (2013) A report on the Fetal Alcohol Spectrum Disorders Study Group meeting of 2012, theme title, „Biomarkers for FASD". Alcohol 47: 583-587.
7. Warren KR, Li TK (2005) Genetic polymorphisms: impact on the risk of fetal alcohol spectrum disorders. Birth Defects Res A Clin Mol Teratol 73: 195-203.
8. Kleiber ML, Diehl EJ, Laufer BI, Mantha K, Chokroborty-Hoque A, et al. (2014) Long-term genomic and epigenomic dysregulation as a consequence of prenatal alcohol exposure: a model for fetal alcohol spectrum disorders. Front Genet 5: 161.
9. Lui S, Jones RL, Robinson NJ, Greenwood SL, Aplin JD, et al. (2014) Detrimental effects of ethanol and its metabolite acetaldehyde, on first trimester

human placental cell turnover and function. PLoS One 9: e87328.

10. Bearer CF (2001) Markers to detect drinking during pregnancy. Alcohol Res Health 25: 210-218.
11. Wimalasena J (1994) Ethanol has direct effects on human choriocarcinoma cell steroid hormone secretion. Alcohol Clin Exp Res 18: 369-374.
12. Karl PI, Fisher SE (1993) Ethanol alters hormone production in cultured human placental trophoblasts. Alcohol Clin Exp Res 17: 816-821.
13. Karl PI, Fisher SE (1994) Chronic ethanol exposure inhibits insulin and IGF-1 stimulated amino acid uptake in cultured human placental trophoblasts. Alcohol Clin Exp Res 18: 942-946.
14. Kleivi K, Teixeira MR, Eknaes M, Diep CB, Jakobsen KS, et al. (2004) Genome signatures of colon carcinoma cell lines. Cancer Genet Cytogenet 155: 119-131.
15. Caspersson T, Zech L, Johansson C, Modest EJ (1970) Identification of human chromosomes by DNA-binding fluorescent agents. Chromosoma 30: 215-227.
16. Kallioniemi A, Kallioniemi OP, Sudar D, Rutovitz D, Gray JW, et al. (1992) Comparative genomic hybridization for molecular cytogenetic analysis of solid tumors. Science 258: 818-821.
17. Ramsay M (2010) Genetic and epigenetic insights into fetal alcohol spectrum disorders. Genome Med 2: 27.
18. Mead EA, Sarkar DK (2014) Fetal alcohol spectrum disorders and their transmission through genetic and epigenetic mechanisms. Front Genet 5: 154.
19. Wimalasena J, Beams F, Caudle MR (1994) Ethanol modulates the hormone secretory responses induced by epidermal growth factor in choriocarcinoma cells. Alcohol Clin Exp Res 18: 1448-1455.
20. Joya X, Salat-Batlle J, Velezmoro-Jáuregui G, Clavé S, Garcia-Algar O, et al. (2015) Prenatal ethanol exposure and placental hCG and IGF2 expression. Placenta 36: 854-862.
21. van Steenwyk G, Janeczek P, Lewohl JM (2013)Differential Effects of Chronic and Chronic-Intermittent Ethanol Treatment and Its Withdrawal on the Expression of miRNAs. Brain Sci 3: 744-756.
22. Clave S, Joya X, Salat-Batlle J, Garcia-Algar O, Vall O (2014) Ethanol cytotoxic effect on trophoblast cells. Toxicol Lett 225: 216-221.
23. Eysseric H, Gonthier B, Soubeyran A, Bessard G, Saxod R, et al. (1997)There is not simple method to maintain a constant ethanol concentration in long-term cell culture: keys to a solution applied to the survey of astrocytic ethanol absorption. Alcohol 14: 111-115.
24. Jones AW, Hahn RG, Stalberg HP (1992) Pharmacokinetics of ethanol in plasma and whole blood: estimation of total body water by the dilution principle. Eur J Clin Pharmacol 42: 445-458.
25. Basel: Karger (2013) International Standing Committee on Human Cytogenetic Nomenclature. ISCN 2013 : an international system for human cytogenetic nomenclature (2013)
26. Poaty H, Coullin P, Peko JF, Dessen P, Diatta AL, et al. (2012) Genome-wide high-resolution aCGH analysis of gestational choriocarcinomas. PLoS One 7: e29426.
27. Bagley RG, Ren Y, Kurtzberg L, Weber W, Bangari D, et al. (2012) Human choriocarcinomas: placental growth factor-dependent preclinical tumor models. Int J Oncol 40: 479-486.
28. McCarthy N, Eberhart JK (2014) Gene-ethanol interactions underlying fetal alcohol spectrum disorders. Cell Mol Life Sci 71: 2699-2706.
29. Moonat S, Starkman BG, Sakharkar A, Pandey SC (2010) Neuroscience of alcoholism: molecular and cellular mechanisms. Cell Mol Life Sci 67: 73-88.
30. Guerri CA, Bazinet, Riley EP (2009) Foetal Alcohol Spectrum Disorders and alterations in brain and behaviour. Alcohol Alcohol 44: 108-114.
31. Rodriguez FD, Covenas R, (2011) Targeting opioid and neurokinin-1 receptors to treat alcoholism. Curr Med Chem 18: 4321-34.
32. Fatayerji N, Engelmann GL, Myers T, Handa RJ (1996) In utero exposure to ethanol alters mRNA for insulin-like growth factors and insulin-like growth factor-binding proteins in placenta and lung of fetal rats. Alcohol Clin Exp Res 20: 94-100.
33. Lindsley TA, Shah SN, Ruggiero EA (2011) Ethanol alters BDNF-induced Rho GTPase activation in axonal growth cones. Alcohol Clin Exp Res 35: 1321-1330.
34. McCarthy N, Wetherill L, Lovely CB, Swartz ME, Foroud TM, et al. (2013) Pdgfra protects against ethanol-induced craniofacial defects in a zebrafish model of FASD. Development 140: 3254-3265.
35. Zhang C, Frazier JM, Chen H, Liu Y, Lee JA, et al. (2014) Molecular and morphological changes in zebrafish following transient ethanol exposure during defined developmental stages. Neurotoxicol Teratol 44: 70-80.
36. Xia LP, Shen L, Kou H, Zhang BJ, Zhang L, et al. (2014) Prenatal ethanol exposure enhances the susceptibility to metabolic syndrome in offspring rats by HPA axis-associated neuroendocrine metabolic programming. Toxicol Lett 226: 98-105.
37. Reynolds JN, Weinberg J, Clarren S, Beaulieu C, Rasmussen C, et al. (2011) Fetal alcohol spectrum disorders: gene-environment interactions, predictive biomarkers, and the relationship between structural alterations in the brain and functional outcomes. Semin Pediatr Neurol 18: 49-55.
38. Jacobson SW, Carr LG, Croxford J, Sokol RJ, Li TK, et al. (2006) Protective effects of the alcohol dehydrogenase-ADH1B allele in children exposed to alcohol during pregnancy. J Pediatr 148: 30-37.
39. Dodge NC, Jacobson JL, Jacobson SW (2014) Protective effects of the alcohol dehydrogenase-ADH1B*3 allele on attention and behavior problems in adolescents exposed to alcohol during pregnancy. Neurotoxicol Teratol 41: 43-50.