Effect of caffeine on genes expressions of developing retinas in the chick model

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Abstract: It has been reported that overconsumption of caffeine during pregnancy leads to a deleterious effect within the nervous tissues during embryonic development. In this study, we further extrapolated the effect of caffeine in the developing retinas, which is known to be one of the most sensitive tissues in chick embryos. Morphological changes of retinal thickness and organization of neuroretinal epithelium were monitored using three gene markers, Atoh7, FoxN4, and Lim1. Upon treating with a single dose of caffeine (15 µmol at embryonic day 1 [E1]), relative thicknesses of developing retinas (particularly of E7 and E9) were significantly altered. Among the three genes studied, the expression pattern of Atoh7 was notably altered while those of FoxN4, and Lim1 mRNA showed only a slight change in these developing retinas. Quantitative polymerase chain reaction results supported the most notable changes of Atoh7 but not FoxN4, and Lim1 gene in the developing retinas, particularly at E7. The effect of caffeine towards other organs during development should be extrapolated and the awareness of its intensive consumption should be raised.

Key words: Caffeine, Chick embryo, Retina, Epithelium, Retinal ganglion cells

Introduction

Retinogenesis is a complex event that is involved in cellular organization in the definitive period which has been well studied in vertebrates, including chick and mouse models. Based on the periods of differentiation, retina progenitor cells (RPCs) have been divided into 2 subgroups, early-born group (retinal ganglion cell [RGC], horizontal cell [HC], and amacrine cell [AC]) and late-born group (cone photoreceptor, bipolar cell, rod photoreceptor, and Müller cell). The molecular controls of retinogenesis have been extensively documented, and many well-characterized molecules have been anticipated as the universal biomarkers for the key steps of retinal development [1, 2]. Our focus in this study is on the early-born population and their markers since they are the targets for many teratogens.

One of the common teratogens is caffeine, a white crystalline xanthine alkaloid, which exerts both positive and negative effects on the central nervous system (CNS). Its beneficial effects on recovering signs and symptoms of the CNS disorders have been demonstrated in the cases of Parkinson’s disease, Alzheimer’s disease, and glaucoma [3-5]. Alternatively, caffeine consumption in pregnant women has raised concern as a result of its increasing evidence in its interruption of organogenesis during the early development of embryos. The adverse effect of caffeine has been reported in many highly suspectable organs including heart, skeleton, gonad, neural tissue; brain, spinal cord, and retina [6-12]. Due to the structural homology of caffein and adenosine...
nucleoside, caffeine is known to interact with adenosine receptors (ARs) which antagonistically modulates an inhibition of phosphodiesterase enzyme and the release of intracellular calcium ions through the G-protein and cyclic adenosine monophosphate (cAMP)-independent signaling cascade [3, 13]. Effect of caffeine during retinal development has also been reported to be involved in 2 types of ARs, AR₁ and AR₂α, expressed in the inner and outer plexiform layers (IPL and OPL) from E10 until E17 of chick retinas [11]. The exposure to caffeine subsequently leads to down-regulation of both AR₁ and AR₂α in the developing retinas [14]. Apart from ARs, there are also a number of caffeine-responsive receptors such as GABA A-, ryanodine-, glycine-, gustatory-, and dopaminergic- receptors that are found in Drosophila [15-18]. Interestingly, dopaminergic receptors (DRs) are also detected in the early chick developing retinas, D1A and D1B depending on developmental ages [19]. These DRs also share a common pathway with ARs leading to an increase in the level of cAMP [20]. It has been reported that at early development of E9 (without the AR expression), caffeine affects retinal morphology causing a reduction of retinal thickness [20]. We thus further extrapolated here the possible selective susceptibility of caffeine towards any given types of neuroretinal cells in the early-born population of developing retina.

During early retinal development, the orchestrated molecular controls to synthesize cellular organization in developing retinas has been well documented [1, 2]. In this study, we focused on the 3 marker genes which are spatiotemporal expressed in the 3 population of neuroepithelial cells, Atoh7 (for RGC), FoxN4 (for AC and HC), and Lim1 (for HC). Firstly, Atoh7 (atonal bHLH transcription factor 7) is a transcription factor in retinogenesis which plays an essential role in RGC differentiation [20]. In mouse embryos, the expression of Atoh7 is initially detected at E12 in RPC for specification and is gradually declined during the postnatal age [21-23]. Secondly, FoxN4 (Forkhead box N4) is a transcription factor in neurogenesis that exists in humans, mouse, chicken, xenopus, and zebrafish [24]. The expression of FoxN4 is involved in the genesis of early-born retinal cells including AC and HC which is a further required specifier for cell specialization [25-27]. Lastly, we focused on the specific regulator of FoxN4-lineage, Lim1 (Lim homeobox 1) which is involved in the development of female reproductive tract and head. In order to differentiate into HC, Lim1 is required as a downstream of FoxN4 to promote HC differentiation [27, 28]. In this study, we injected a single dose of caffeine at a concentration of 15 μmol/egg into the E1 embryo and observed the structural and genetic changes at E3, E5, E7, and E9. Alterations of the universal retinal genetic markers (Atoh7, FoxN4, and Lim1) are evident by in situ hybridization and real-time polymerase chain reaction (PCR) and the possible disturbance of caffeine towards differentiation and migration of the three populations of developing retino-neuronal cells including RGC, HC, and AC is discussed.

Materials and Methods

Chick embryos and caffeine administration

Fertilized chick eggs, Gallus gallus were obtained from Department of Animal Science, Faculty of Agriculture, Kasetsart University, Thailand. Handling of these embryos followed the guidelines of the Animal Care Committee, Faculty of Science, Bangkok (protocol no. MUSC61-029-431). The eggs were incubated at 38°C with a relative humidity of 60% on the rotatory plate. To administer the caffeine, a small window was created on the eggshell at the blunt end (air chamber). Thereafter, caffeine solution at a concentration of 15 μmol/egg was injected into a yolk sac of E1 fertilized eggs and the window was sealed with parafilm. Control eggs were injected with 0.9% normal saline solution with the same volume. All administered and control embryos were then cultured in the 38°C incubator.

Histological section preparation and image analysis

Administered chick embryos at E3–E9 were harvested by cracking the shell and dissected free from yolk matter. The eyes of embryos were carefully removed from the heads and fixed in 4% paraformaldehyde in 0.12 M phosphate buffer for either 6 hours (E3) or overnight (E5–E9) at 4°C. For paraffin embedding, the tissue was further dehydrated in the increasing percentage of ethanol, infiltrated in xylene, and finally embedded in paraffin. The 5 μm-thick sections were cut and routinely processed for hematoxylin and eosin staining. The images were acquired by a Nikon E600 light microscope (Nikon Instruments Inc., NY, USA) and Panoramic Digital slide Scanner (3D-Histech; 3DHISTECH Kft., Budapest, Hungary). They were further processed and analyzed by Adobe Photoshop CS6 software (Peachpit Press, Berkeley, CA, USA) to measure the retinal thickness. The measurement of retinal thickness was conducted at the retinal area which is opposite the mid-center point of the posterior lens.
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For cryo-sectioning, they were transferred into 10% sucrose in phosphate buffer overnight at 4°C and further submersed and embedded in gelatin-sucrose (14% gelatin, 0.1% agarose, 10% sucrose solution) and kept at -80°C. Sections were cut by a Leica RM2235 rotary cryo-microtome at -20°C (Leica, Buffalo Grove, IL, USA) at a thickness of 25 μm and adhered onto Matsunami platinum microscope slides (Matsunami Glass, Bellingham, WA, USA). All images were acquired, processed, and analyzed as mentioned above.

**In situ hybridization**

Riboprobes of 400 to 500 nucleotides were designed and cloned in pBluescript II SK+ plasmids (General Biosystem, Durham, NC, USA) for constructing *Atoh7*, *FoxN4*, and *Lim1* probes. Antisense probes were synthesized by *in vitro* transcription using T3 RNA polymerase (Roche, Basel, Switzerland) and DIG-RNA labeling mix (Roche). They were precipitated with cold ethanol and 4M LiCl overnight at -20°C and recovered in diethylpyrocarbonate-treated distilled water.

**Quantitative real-time PCR**

Caffeine treated- and controlled- chick retinas were isolated, and the retinal pigment epithelium and lens were carefully removed. Isolated retinas were then subjected to a total RNA extraction using a Trizol reagent (Life Technology, Carlsbad, CA, USA) and Direct-zol RNA Mini Prep (ZYMO Research, Tustin, CA, USA) and resuspended in a DNase/RNase-free water. Approximately, 1 μg of RNA was reversely transcribed by an iScript Reverse Transcription Supermix for RT-qPCR (Bio-Rad, Hercules, CA, USA). Complementary DNA (cDNA) was amplified using a Luna Universal qPCR master mix (BioLabs, Ipswich, MA, USA) containing 10 μM primers (Table 1) and 0.5 μg of cDNA, in a total volume of 10 μl. Thermal cycle conditions were 95°C for 1 minute, 53.9°C for 30 seconds and 72°C for 30 seconds run at 44 cycles. The data were presented as mean±standard error of the mean. The differences between groups were analyzed by one-way ANOVA followed by the Bonferroni post-test by using a Graphpad Prism 9 software (Graphpad Inc.). A *P*-value <0.05 was considered to be statistically significant.

### Table 1. Primers that are used for real-time polymerase chain reaction

| Gene     | Accession number | Primer sequences                         | Product size (bp) |
|----------|------------------|------------------------------------------|-------------------|
| Atoh7    | NM_204668.1      | GAGAATGATTAACCTTCACTGTGAAC               | 99                |
|          |                  | GCTGTGCAAAAGGATCACTGTCTG                 |                   |
| FoxN4    | NM_001083359.1   | AGCACCTGGAGCAATGATTC                    | 131               |
|          |                  | CTTGAAACTAGGGAGGAGGC                    |                   |
| Lim1     | NM_205413.1      | GGGGGCTGGTCGCCAGGGCAT                   | 140               |
|          |                  | TTTTCTGTATGATATAGA                      |                   |
| Gapdh    | NM_204305.1      | ATGATCCCTTCTAGTGATCTG                   | 106               |
|          |                  | ATCACAAGTTTCCCCGTTC                      |                   |

Reference:

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Results

Comparative structural changes between caffeine-treated and control chick retinas

The histological structures and their morphometric parameters (thickness and cellular organization) between caffeine-treated and control chick embryos (E3–E9) were compared and shown in Table 2. The measured thicknesses of retinas in both groups were 47.49±4.62 (n=8) vs. 62.18±10.53 for E3 (n=5); 70.85±9.78 vs. 80.38±6.50 for E5 (n=6); 203.27±18.21 vs. 140.09±9.24** for E7 (n=6); and 254.81±27.52 vs. 215.30±23.83 for E9 (n=7). Interestingly, significant differences in the epithelial thickness were noted in E7 (P<0.01, double asterisk) and E9 (P<0.05, asterisk) groups. The cellular organization appeared rather similar in both groups (Fig. 1). In E3 to E7, stacking of cells forming neuroblastic layer (NBL) towards presumptive ganglion cell layer (pGCL) in the innermost layer was well ob-

Table 2. Thickness and cellular organization in control and caffeine-treated retinas

| Embryonic stage | Average of retinal thickness (μm) | Number of cell layers (layer) |
|-----------------|----------------------------------|------------------------------|
|                 | Control                          | Caffeine-treated             | Control                          | Caffeine-treated |
| E3              | 47.49±4.62                       | 62.18±10.53                  | 1 (NBL)                          | 1 (NBL)          |
| E5              | 70.85±9.78                       | 80.38±6.50                   | 1 (NBL)                          | 1 (NBL)          |
| E7              | 203.27±18.21                     | 140.09±9.24**                | 2 (pGCL, NBL)                    | 2 (pGCL, NBL)    |
| E9              | 254.81±27.52                     | 215.30±23.83*                | 5 (GCL, IPL, INL, OPL, ONL)      | 5 (GCL, IPL, INL, OPL, ONL) |

Values are presented as mean±standard error of the mean.

NBL, neuroblastic layer; pGCL, presumptive ganglion cell layer; GCL, ganglion cell layer; IPL, inner plexiform layer; INL, inner nuclear layer; OPL, outer plexiform layer; ONL, outer nuclear layer.

Significant differences are denoted by *P<0.05 and **P<0.01.

Fig. 1. Histology of developing retinas of control and caffeine-treated embryos at the stages of E3–E9. The paraffin sections were stained with hematoxylin and cosin and the images were acquired at low magnification (bar 200 μm, A–H) and high magnification (bar 100 μm, I–P) to demonstrate cellular organization in retinas. NBL, neuroblastic layer; pGCL, presumptive ganglion cell layer; GCL, ganglion cell layer; IPL, inner plexiform layer; INL, inner nuclear layer; OPL, outer plexiform layer; ONL, outer nuclear layer. *Delaminated IPL.
served in both control (Fig. 1A–C, I–K) and caffeine-treated retinas (Fig. 1E–G, M–O). At E9 where all definitive epithelial layers including outer nuclear layer (ONL), OPL, inner nuclear layer (INL), IPL, and GCL were clearly identified in the normal developing retina (Fig. 1L), they were also well-identified in caffeine-treated animals (Fig. 1P). Particularly, the IPL (located between GCL and INL, representing the first delamination process of GCL between the two nuclear layers) could still be clearly visible in both groups (asterisk in Fig. 1L, P).

**Caffeine altered expressions of neuronal gene markers in retinas**

Among the three markers studied, expression of *Atoh7* mRNA appeared to be significantly altered in all aged embryos upon being treated with caffeine. The expression of
**Atoh7** was detected at the outermost layer of the developing retina (the site where the RPC-proliferative cells were localized) in the E3-control embryos (Fig. 2A). Similarly, its expression was also detectable at the outermost layer of the E3-caffeine treated embryos (Fig. 2E). At E5 of the control group, the expression of **Atoh7** was additionally detected in the innermost layer or pGCL where RGC cell population was localized (asterisk in Fig. 2B) apart from that of the outermost layer. In contrast, **Atoh7** remained slightly detected in the outermost layer while it was non-detectable in the pGCL layer of the caffeine-treated E5 group (Fig. 2F). At E7 and E9 of the control animals, similar expression pattern of **Atoh7** was observed as with E5-control where the staining was found in GCL, but not in outermost NBL. In the same vein, the expression of **Atoh7** was also observed in the GCL and outermost layer of NBL in the E7 and ONL in the E9 caffeine-treated embryos (Fig. 2G, H).

Two other markers that were studied herein were **FoxN4** and **Lim1** which have been used to differentiate AC and HC lineages. Localization of these two mRNA seemed to be minimally altered after the animals were subjected to caffeine treatment. As shown in Fig. 2L-X, the expressions of **FoxN4** and **Lim1** mRNA were still silent in E3 control embryos both in control and caffeine injected embryos. At E5, **FoxN4** was expressed in the innermost layer of pGCL and **Lim1** was found to be more specific to the inner layer of pGCL in the control animals (Fig. 2J, R). The staining pattern of both **FoxN4** and **Lim1** appeared to be scattered within this NBL layer in the caffeine-treated animals (Fig. 2N, V). In the control group of E7, localization of **FoxN4** was relatively different from that of **Lim1**, in which **FoxN4** was not detected in the retina while **Lim1** was observed at the vicinity of pGCL, an anticipated localization of the migratory HCs in the control group (Fig. 2S) as well as in ONL and GCL at E9 (Fig. 2T). In comparison to the caffeine-treated group, the expression of **FoxN4** was detected as faint staining in the middle layer of INL (Fig. 2P), while the expression of **Lim1** had no notable change on the animals treated with caffeine (Fig. 2X).

**Quantitative analysis of the marker gene expressions**

We also quantitated the expression levels of the three marker genes collected from E7 and E9 embryos (n=3 each) using real-time PCR analysis (Fig. 3). We had to confess that in our handling method, the retinas of E3 and E5 were too small to be collected and it was difficult obtaining enough RNA, therefore, only data of E7 and E9 were presented here. The results of qPCR well agreed with those of in situ hybridization, and **Atoh7** gene expression was mostly affected by caffeine treatment, while the other two markers showed minimal gene alterations. Specifically, the level of **Atoh7** gene expression was reduced by 85% (0.15±0.01 relative fold change) and 57% (0.43±0.03 relative fold change) in E7 and E9, respectively (P<0.05). The relative fold changes of **FoxN4** level at E7 and E9 were not significantly altered, with the values of 0.99±0.13 (or 1% reduction) for E7 and 0.64±0.13 (or 36% decrease) for E9. A similar trend of change was also observed for **Lim1** where the gene levels were 0.84±0.21 (16% reduction) for E7 and 0.99±0.21 (1% decrease) for E9.

**Discussion**

During retinogenesis, many teratogens are known to
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As many previous studies have reported that ARs were local-
ized in the retinal of chicken, rabbit, mice, rats, monkeys,
and human [15, 34-36]. However, it has also been reported
that the expression of both AR receptors in the developing
chick retinas starts to be observed at E12 to E15, the tim-
ing which does not well correspond with the expression
of the three marker genes studied herein. Other receptors
expressed during the early embryonic period and have been
reported to have interfered with caffeine should thus be
more considerable. As earlier mentioned, DRα is expressed
as early as E7 embryos, its interruption by caffeine antago-
nistic binding would interfere with developing genes or tran-
scription factors expressed in this given period [19]. The best
example earlier mentioned is the expression of Pax6 tran-
scription factor which is also known to be interrupted by
caffeine treatment [11]. In fact, Pax6 (as well as Sox2) are the
upstream regulators of Atoh7 that are expressed within RPC
population once the stem cells are committed to be GCL cell
lineage [37]. Therefore, interference of Atoh7 (particularly at
GCL; Fig. 2) and the reduced retinal thickness at E7 and E9
would thus well agree with this previous finding [33]. How-
ever, the non-responsiveness of FoxN4 and Lim1 mRNA (both
of which are the other downstream cascade of Pax6 that
further differentiate into AC and HC lineages) towards caf-
feine administration would present a further interesting is-
sue of whether the signal transductions of these lineages are
upregulated by a different type of caffeine-sensitive receptors
as well as regulated by different molecular cascades apart
from Pax6. Regardless of its molecular mechanism, one pos-
sible conclusion that could be drawn from our study is that
early exposure to caffeine during early development leads to
a structural change of the retina in which the thickness of
neuroepithelium and cellular organization is significantly
altered (Fig. 2). It is more likely that caffeine would cause a
delay or alteration of the organization pattern of the specific
population in the neuroepithelial cells (in our case, Atoh7-
expressing RGCs). More extensive investigation is thus
required to explore this complex involvement of caffeine
in retinogenesis. Nevertheless, healthcare issues should be
raised to sensitize consumers about the risk of caffeine that
could potentiate retinal hazards and thus newborns’ visual
ability.

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Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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References

1. Reinhardt R, Centanin L, Tavhelidse T, Inoue D, Wittbrodt B, Concordet JP, Martinez-Morales JR, Wittbrodt J, Sox2, Tlx, Gli3, and Her9 converge on Rx2 to define retinal stem cells in vivo. EMBO J 2015;34:1572-88.
2. Xiang M. Intrinsic control of mammalian retinogenesis. Cell Mol Life Sci 2013;70:2519-32.
3. Ribeiro JA, Sebastião AM. Caffeine and adenosine. J Alzheimers Dis 2010;20(Suppl 1):S3-15.
4. Espinosa J, Rocha A, Nunes F, Costa MS, Schein V, Kazlauckas V, Kalinine E, Souza DO, Cunha RA, Porciúncula LO. Caffeine consumption prevents memory impairment, neuronal damage, and adenosine A2A receptors upregulation in the hippocampus of a rat model of sporadic dementia. J Alzheimers Dis 2013;34:509-18.
5. Madeira MH, Elvas F, Boia R, Gonçalves FQ, Cunha RA, Ambrosio AF, Santiago AR. Adenosine A2AR blockade prevents neuroinflammation-induced death of retinal ganglion cells caused by elevated pressure. J Neuroinflammation 2015;12:115.
6. Rana N, Moond M, Marthi A, Bapatla S, Sarvepalli T, Chatti K, Challa AK. Caffeine-induced effects on heart rate in zebrafish embryos and possible mechanisms of action: an effective system for experiments in chemical biology. Zebrasfish 2010;7:69-81.
7. Hawkins JA, Hu N, Clark EB. Effect of caffeine on cardiovascular function in the stage 24 chick embryo. Dev Pharmacol Ther 1984;7:334-43.
8. Ma ZL, Qin Y, Wang G, Li XD, He RR, Chuai M, Kurihara H, Yang X. Exploring the caffeine-induced teratogenicity on neurodevelopment using early chick embryo. PLoS One 2012;7:e34278.
9. Park M, Choi Y, Choi H, Yim JY, Roh J. High doses of caffeine during the periubertal period in the rat impair the growth and function of the testis. Int J Endocrinol 2015;2015:368475.
10. Dorostghoal M, Erfani Majd N, Nooraei P. Maternal caffeine consumption has irreversible effects on reproductive parameters and fertility in male offspring rats. Clin Exp Reprod Med 2012;39:144-52.
11. Brito R, Pereira-Figueiredo D, Socolato R, Paes-de-Carvalho R, Calaza KC. Caffeine exposure alters adenosine system and neurochemical markers during retinal development. J Neurochem 2016;138:557-70.
12. Sheth S, Brito R, Mukherjea D, Rybak LP, Ramkumar V. Adenosine receptors: expression, function and regulation. Int J Mol Sci 2014;15:2024-52.
13. de Carvalho RP, Braas KM, Adler R, Snyder SH. Developmental regulation of adenosine A1 receptors, uptake sites and endogenous adenosine in the chick retina. Brain Res Dev Brain Res 1992;70:87-95.
14. Mustard JA. The buzz on caffeine in invertebrates: effects on behavior and molecular mechanisms. Cell Mol Life Sci 2014;71:1375-82.
15. Lee Y, Moon SJ, Montell C. Multiple gustatory receptors required for the caffeine response in Drosophila. Proc Natl Acad Sci U S A 2009;106:4495-500.
16. Moon SJ, Köttgen M, Jiao Y, Xu H, Montell C. A taste receptor required for the caffeine response in vivo. Curr Biol 2006;16:1812-7.
17. Nall AH, Shakhmantsir I, Cichewicz K, Birman S, Hirsh J, Sehgal A. Caffeine promotes wakefulness via dopamine signaling in Drosophila. Sci Rep 2016;6:20938.
18. Soares HC, de Melo Reis RA, De Mello FG, Ventura AL, Kurtebach E. Differential expression of D(1A) and D(1B) dopamine receptor mRNAs in the developing avian retina. J Neurochem 2000;75:1071-5.
19. Neve KA, Seamans JK, Trantham-Davidson H. Dopamine receptor signaling. J Recept Signal Transduct Res 2004;24:165-205.
20. Ma ZL, Wang G, Cheng X, Chuai M, Kurihara H, Lee KK, Yang X. Excess caffeine exposure impairs eye development during chick embryogenesis. J Cell Mol Med 2014;18:1134-43.
21. Brown NL, Patel S, Brzezinski J, Glaser T. Math5 is required for retinal ganglion cell and optic nerve formation. Development 2001;128:2497-508.
22. Yang Z, Ding K, Pan L, Deng M, Gan L. Math5 determines the competence state of retinal ganglion cell progenitors. Dev Biol
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23. Le TT, Wroblewski E, Patel S, Riesenber AN, Brown NL. Math5 is required for both early retinal neuron differentiation and cell cycle progression. Dev Biol 2006;295:764-78.

24. Boije H, Edqvist PH, Hallböök F. Temporal and spatial expression of transcription factors FoxN4, Ptf1a, Prox1, Isl1 and Lim1 mRNA in the developing chick retina. Gene Expr Patterns 2008;8:117-23.

25. Edqvist PH. Neuronal development in the embryonic retina: focus on the characterization, generation and development of horizontal cell subtypes [PhD dissertation]. Uppsala: Acta Universitatis Upsaliensis; 2006.

26. Gouge A, Holt J, Hardy AP, Sowden JC, Smith HK. Foxn4—a new member of the forkhead gene family is expressed in the retina. Mech Dev 2001;107:203-6.

27. Li S, Mo Z, Yang X, Price SM, Shen MM, Xiang M. Foxn4 controls the genesis of amacrine and horizontal cells by retinal progenitors. Neuron 2004;43:795-807.

28. Dyer MA, Livesey FJ, Cepko CL, Oliver G. Prox1 function controls progenitor cell proliferation and horizontal cell genesis in the mammalian retina. Nat Genet 2003;34:53-8.

29. Marillat V, Sabatier C, Failli V, Matsunaga E, Sotelo C, Tessler-Lavigne M, Chédotal A. The slit receptor Rig-1/Robo3 controls midline crossing by hindbrain precerebellar neurons and axons. Neuron 2004;43:69-79.

30. Klebanoff MA, Levine RJ, Clemens JD, Wilkins DG. Maternal serum caffeine metabolites and small-for-gestational age birth. Am J Epidemiol 2002;155:32-7.

31. Bracken MB, Triche EW, Belanger K, Hellenbrand K, Leaderer BP. Association of maternal caffeine consumption with decrements in fetal growth. Am J Epidemiol 2003;157:456-66.

32. Bakker R, Steegers EA, Obradov A, Raat H, Hofman A, Jaddoe VW. Maternal caffeine intake from coffee and tea, fetal growth, and the risks of adverse birth outcomes: the generation R study. Am J Clin Nutr 2010;91:1691-8.

33. Momoi N, Tinney JP, Liu LJ, Elshershari H, Hoffmann PJ, Ralphe JC, Keller BB, Tobita K. Modest maternal caffeine exposure affects developing embryonic cardiovascular function and growth. Am J Physiol Heart Circ Physiol 2008;294:H2248-56.

34. Blazynski C, Perez MT. Adenosine in vertebrate retina: localization, receptor characterization, and function. Cell Mol Neurobiol 1991;11:463-84.

35. Braas KM, Zarbin MA, Snyder SH. Endogenous adenosine and adenosine receptors localized to ganglion cells of the retina. Proc Natl Acad Sci U S A 1987;84:3906-10.

36. Kvanta A, Seregard S, Sejersen S, Kull B, Fredholm BB. Localization of adenosine receptor messenger RNAs in the rat eye. Exp Eye Res 1997;65:595-602.

37. Nguyen-Ba-Charvet KT, Rebsam A. Neurogenesis and specification of retinal ganglion cells. Int J Mol Sci 2020;21:451.