**Lvrn expression is not critical for mouse placentation**

Tomohiro TOBITA\(^1,2\), Daiji KIYOZUMI\(^1,3\), Masanaga MUTO\(^1\), Taichi NODA\(^1\) and Masahito IKAWA\(^1,4\)

\(^1\)Research Institute for Microbial Diseases, Osaka University, Osaka 565-0871, Japan
\(^2\)Graduate School of Medicine, Osaka University, Osaka 565-0871, Japan
\(^3\)Immunology Frontier Research Center, Osaka University, Osaka 565-0871, Japan
\(^4\)The Institute of Medical Science, The University of Tokyo, Tokyo 108-8639, Japan

Abstract. Preeclampsia is a systemic disease caused by abnormal placentation that affects both mother and fetus. It was reported that Laeverin (LVRN, also known as Aminopeptidase Q) was up-regulated in the placenta of preeclamptic patients. However, physiological and pathological functions of LVRN remained to be unknown. Here we characterized Lvrn function during placentation in mice. RT-PCR showed that Lvrn is expressed in both fetus and placenta during embryogenesis, and several adult tissues. When we overexpressed Lvrn in a placenta-specific manner using lentiviral vectors, we did not see any defects in both placentae and fetuses. The mice carrying Lvrn overexpressing placenta did not show any preeclampsia-like symptoms such as maternal high blood pressure and fetal growth restriction. We next ablated Lvrn by CRISPR/Cas9-mediated genome editing to see physiological function. In Lvrn ablated mice, maternal blood pressure during pregnancy was not affected, and both placentas and fetuses grew normally. Collectively, these results suggest that, LVRN is irrelevant to preeclampsia and dispensable for normal placentation and embryonic development in mice. 

**Key words:** Knockout, Lentivirus, Trophoblast

The placenta is an essential organ for fetal development through nutrient transport, gas exchange, and hormone secretion. Abnormalities in placental formation and function trigger pregnancy disorders such as preeclampsia (PE) and threatens both maternal and fetal lives [1–4]. PE occurs in 3–5% of all pregnant women and is characterized by new onset of maternal high blood pressure after 20 weeks of gestation together with other systemic symptoms such as renal dysfunction, or retarded fetal growth [5,6]. Insufficient trophoblast cell invasion, accompanying oxidative stress and endothelial dysfunction were regarded as the cause of PE [7,8].

Global gene expression analysis revealed that numerous genes were dysregulated in the PE placenta (reviewed in [9]). Laeverin (LVRN, also known as Aminopeptidase Q), which encodes a type-II transmembrane M1 aminopeptidase, is one gene that is upregulated in the extravillous trophoblast from PE placentas (up-regulated 10.0 fold compared to control [10], up-regulated 2.4 fold compared to control [11]). In human, LVRN is specifically expressed in extravillous trophoblast [12]. From *in vitro* experiments using primary human extravillous trophoblast and the BeWo cell line, LVRN appears to be involved in trophoblast invasion [13]. However, the relationship between *LVRN* expression levels and pathogenesis of PE is still unknown.

Here we examined physiological and pathological functions of LVRN by lentiviral vector mediated placenta-specific overexpression [14, 15] and CRISPR/Cas9 mediated gene knockout [16] in mice.

**Materials and Methods**

Animals

All animal experiments were approved by the Institutional Animal Care and Use Committee of the Research Institute for Microbial Diseases, Osaka University (H30-01-0). The *Lvrn* mutant mouse line B6D2-Lvrn<sup>em1Osb</sup> will be available to the scientific community through RIKEN BRC (http://muse.brc.riken.jp/en/).

RT-PCR

cDNAs were synthesized from various tissues using Trizol and SuperScript IV (Thermo Fisher Scientific, Waltham, MA, USA). cDNAs synthesized from 10 ng of total RNA were used for RT-PCR as templates using KOD Fx neo (TOYOBO, Osaka, Japan) with the following primers: forward; 5'-CGCAATGAGCTGACATGGAC-3' and reverse; 5'-CAGGCACTAGAAGCTTTCAC-3' for *Lvrn*, forward; 5'-CATCCCAGAGACCTTATGCA -3' and reverse; 5'-ATGGGACCCGAGATCC-3' for *Actb*. The amplification cycles were 94 degrees for 30 sec, 65 degrees for 30 sec, and 72 degrees for 30 sec for 40 cycles. The expected amplicon sizes for *Lvrn* and *Actb* are 216 bp and 171 bp, respectively.

Antibodies

A polyclonal antibody against mouse LVRN (NM_083284) was raised in rabbit by immunizing with the synthetic peptide CKNLQNKKRIARVVEWRKNT (amino acids 972–991) conjugated...
with keyhole limpet hemocyanin. Antiserum was purified by affinity chromatography with Sulfolink coupling gel (Thermo Fisher Scientific) conjugated with antigenic peptide. A rabbit monoclonal antibody against mouse GAPDH (14C10) was purchased from CST (Cell Signaling Technology, Danver, MA, USA). A mouse monoclonal antibody against beta-actin (AC-15) was from Abcam (Cambridge, UK). A rat monoclonal antibody against EGFP (K2) was generously gifted from S.C. Fujita at Mitsubishi Institute of Life Sciences, Tokyo, Japan. A rat monoclonal antibody TROMA-1 (MABT239) was purchased from Merck Millipore (Darmstadt, Germany). Alexa Fluor 546-conjugated goat anti-rabbit IgG antibody (A11006) and Alexa Fluor 488-conjugated goat anti-rat IgG antibody (A11071) were purchased from Thermo Fisher Scientific. A goat polyclonal antibody against rabbit IgG conjugated with horseradish peroxidase (111-035-003) and a goat polyclonal antibody against mouse IgG conjugated with horseradish peroxidase (115-035-003) were both from Jackson Immunoresearch (West Grove, PA, USA).

**Preparation of lentiviral vectors**

The HIV-1-based, self-inactivating lentiviral vectors were prepared as described previously [14]. Mouse Lvrn cDNA was amplified from E18.5 placental cDNAs with the following primers: forward; 5'-CCCCGCCTAGGGCGGCGATGAGGCCTGCTTCACCCTC-3' and reverse; 5'-CCCCGTCACTTCTACGTTTGTCTGGCTCAGCTC-3'. A 3.0 kb Lvrn fragment was cloned into pLV-CAG 1.2 using NheI and Sall sites to generate pLV-Lvrn. Lentiviral vectors were prepared as described previously [14]. In brief, the pLV-Egfp and pLV-Lvrn plasmids were transfected to 293T cells together with pMDL, pRev, and pVSV-G by the calcium phosphate method. Lentiviral vectors were harvested 2 days after transfection, and concentrated 1,000-fold by ultracentrifugation (first centrifuge; 19,400 rpm, 120 min, second centrifuge; 21,000 rpm, 90 min). After resuspension of precipitates with Hanks Balanced Salt Solution buffer, the concentration of LV-Lvrn was determined by measuring p24 gag antigens with an Enzyme-Linked Immunosorbent assay (ELISA) kit (Zeptometrix, Buffalo, NY, USA).

**Lentiviral transduction of mouse blastocysts**

Blastocysts collected from B6D2F1 females (SLC) were treated with acidic Tyrode solution (Sigma-Aldrich, St. Louis, MO, USA) to remove the zona pellucida. The zona pellucida-free blastocysts were incubated for 5 hours with LV-Lvrn or LV-Egfp lentiviral vectors at a concentration of 2.0 × 10^5 or 8.0 × 10^4 ng/ml of p24 diluted in KSOM medium. The transduced blastocysts were implanted into the uterus of pseudopregnant E2.5 ICR female mice. Fifteen blastocysts were implanted into each horn of the uterus. Placenta-specific gene transduction was confirmed by genomic PCR with the following primer pairs: forward; 5'-GGGAAGTTATTTATGATGTG-3' and reverse; 5'-ACCATGGTGAGCAAGGGCGAG-3'. The resulting pX330-Lvrn plasmid was injected into one pronucleus of B6D2F1 × B6D2F1 fertilized eggs as previously described [16]. Injected eggs were cultured in KSOM medium overnight and transferred into oviducts of pseudopregnant ICR females. The resulting pups were genotyped by genomic PCR with the primers: forward; 5'-AGTCGCTTGGGCTCTGCTAGAGGAG-3' and reverse; 5'-GTGAGCGCAGCTGCCACATACAGG-3' and direct DNA sequencing.

**Measurement of blood pressure**

Blood pressure (BP) was measured by the tail-cuff method with BP98A (Softron, Tokyo, Japan) as described previously [22]. The pregnant mice were gently secured in a small net without anesthesia. After their behavior, heart rates, and blood pressures were stabilized, both systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured at least five times. The blood pressure of each embryonic day were also measured in at least five individual females. SBP and DBP data from pregnant mice carrying three to fourteen pups were used for further statistical analysis.

**Histology**

Female pregnant mice were sacrificed on the following days of pregnancy 14.5, and 18.5. Embryos and placentaes were collected and weighed. Placentas were fixed in 4% paraformaldehyde (Wako, Osaka, Japan) in PBS, embedded in paraffin, and sectioned at 8 μm thickness. Specimens were stained with Mayer hematoxylin solution (Wako) and Eosin solution (Wako). Specimens were mounted in Permound (Falma, Tokyo, Japan) and analyzed with a BZ-X710 microscope (Keyence, Osaka, Japan).

**Immunofluorescence**

Rehydrated paraffin sections were incubated with blocking solution (2% goat serum in PBS) at room temperature for 1 h. Specimens were incubated with 3.0 μg/ml of rabbit anti-LVRN antibody diluted in blocking solution at 4 degrees at overnight. After washing with PBS, specimens were incubated with Alexa Fluor 546-conjugated goat anti-rabbit IgG antibody (1/400 diluted) and 0.1 μg/ml of Hoechst 33342 in blocking solution at room temperature for 1.5 h. After
washing with PBS, specimens were sealed with 10% glycerol in PBS and analyzed with a BZ-X710 microscope.

**Statistical analysis**

All data are shown as the mean ± SD of at least three independent experiments. Statistical analyses were performed using Student’s t-test after the data were tested for normality of distribution. Values under 0.05 were regarded as significant.

**Results**

**Expression of Lvrn in mouse tissues**

We performed RT-PCR and found that Lvrn mRNA is expressed ubiquitously in adult mouse organs such as brain, skin, heart, kidney, testis and ovary (Fig. 1A). During gestation, Lvrn mRNA was detected in both fetus and placenta at all examined embryonic ages, (E) 8.5, 10.5, 12.5, 14.5, 16.5, and 18.5 (Fig. 1A). Ubiquitous expression in adult and continuous expression during embryonic development suggests that LVRN might play fundamental functions in vivo.

**Placenta-specific Lvrn overexpression in mice**

To examine the effects of Lvrn overexpression on placental formation and function, we utilized lentiviral vector (LV)-mediated placenta-specific gene expression. We prepared third generation lentiviral vectors expressing control EGFP or murine Lvrn under the strong ubiquitous CAG promoter [14] (Fig. 1B). We transduced blastocyst stage embryos by two different concentrations, 2.0 × 10³ p24-ng/ml and 8.0 × 10³ p24-ng/ml, after removal of the zona pellucida. The transduced blastocysts were transplanted into the uteri of pseudopregnant female mice.

Placenta-specific viral vector integration was examined by genomic PCR. The amplicons were only detected in placentae but not in fetuses from LV-transduced embryos (Fig. 1C). When we performed immunoblot analysis, EGFP protein was detected only in LV-Egfp transduced placentas (Fig. 1D). For LVRN, we detected very weak endogenous signals in fetuses and placentae from non-transduced samples (Fig. 1D). After LV-Lvrn transduction, we detected strong signals in the placentas dose-dependently, suggesting LVRN is overexpressed in these placentas (Fig. 1D). Trophoblast-specific overexpression of LVRN protein in placental tissue was also confirmed by immunohistochemistry with antibodies against LVRN and CK8 (trophoblast marker, Cytokeratin 8) (Fig. 1E). There were no obvious differences in trophoblast giant cell invasion.

**Pathological functions of LVRN in mouse placenta**

To assess the effects of placenta-specific overexpression of LVRN, we observed maternal blood pressure, fetal development, and placental histology. We obtained comparable numbers of healthy fetuses and placentas by Cesarian section at E18.5. No significant differences in the blood pressures were observed throughout gestation (Fig. 2A) and no significant differences in fetal and placental weight (Supplementary Table 1: online only, Fig. 2B). From HE staining, we could not detect any overt abnormalities in the placentas overexpressing LVRN (Fig. 2C). Immunohistochemical staining of CK8 revealed that there were no obvious differences in trophoblast giant cell invasion (Fig. 2D). These results suggest that placenta-specific Lvrn overexpression does not interfere with pregnancy in mice.

**Generation of Lvrn-KO mice**

To further elucidate LVRN function in vivo, we next generated Lvrn-KO mice using CRISPR/Cas9. We designed single-guide RNA targeting 20 nts immediately downstream of the translational initiation site (Fig. 3A). There were no off-target sites identified that matched 12 nts at the 3' end plus the PAM sequence. We injected 129 B6D2F1 × B6D2F1 zygotes with pX330 plasmid expressing the Lvrn targeting sgRNA and Cas9 simultaneously. Among the 14 pups obtained, we found a pup carrying a 62 bp deletion at the targeted site (Supplementary Table 2: online only, Fig. 3A). Germline transmission of the mutation was determined by genomic PCR (Fig. 3B). We did not see any signal in both fetus and placenta with anti-LVRN (C-terminus) immunoblot analysis, indicating the LVRN was ablated in Lvrnem1/em1 mutant mice (Fig. 3C).

**Physiological functions of LVRN in mouse placenta**

To assess the effects of Lvrn disruption, we mated Lvrnem1 females with Lvrnem1 males and observed fetal development, maternal blood pressure, and placental histology. We obtained healthy fetuses in Mendelian ratios (+/+ : +/em1 : em1/em1 = 19 : 40 : 23 from 10 litters, Supplementary Table 3: online only) with no significant differences in maternal blood pressure (Fig. 4A) throughout gestation, fetal and placental weight among the groups (Fig. 4B). To eliminate the contribution of maternal LVRN, we mated Lvrnem1/mem1 females with Lvrnem1/em1 males. From these homozygous crosses, we did not see any defects in fetal and placental development (Fig. 4B). Histological analysis did not show any overt abnormalities (Fig. 4C) and trophoblast invasion into maternal spiral artery in LvrnKO placentas (Fig. 4D). It should be noted that no obvious differences were observed at E14.5 (Supplementary Fig. 1: online only). These results suggest that LVRN is not required for embryonic development, placental formation and function in mice.

**Discussion**

Preeclampsia is a systemic disease caused by abnormal placentation and affects both mother and fetus. Epidemiological studies have suggested the biological molecules contributing to placental vasculogenesis and controlling maternal blood pressure as the causes or exacerbating factors in preeclampsia (e.g., soluble Fms-like tyrosine kinase (sFLT1) [17], soluble Endoglin (sENG) [18], transcriptional factors such as STOX1 [19], and protein peptidases (ADAMs, MMPs) [20, 21]). Further, their physiological and pathological roles in preeclampsia have been elucidated using gene manipulated animals [22–25]. We have previously demonstrated that he LV-mediated placenta specific sFLT1 gene expression resulted in preeclampsia in mice [22]. In the present study, we selected Lvrn, a highly expressed gene in preeclamptic placentas [10, 11] and analyzed its role using the same LV-mediated placenta specific expression approach. However, we did not see any preeclamptic symptoms in the treated mice. We further determined the LVRN physiological functions by making CRISPR/Cas9 mediated KO mice. The gene deletion did not cause any defects in pregnancy. Although LVRN overexpression might be used as a biomarker in human, we conclude that the LVRN is
dispensable for placentation and placental functions in mice.

In human, LVRN is an active M1 aminopeptidase that degrades Kisspeptin-10 and promotes extravillous trophoblast invasion [26]. The consensus peptide recognition sequence of LVRN aminopeptidase is HXME. In rodents, Histidine has been substituted with Glycine and altered LVRN’s substrate specificity [27]. Moreover while human LVRN is specifically expressed in extravillous trophoblast [12], Lvrn mRNA was ubiquitously detected in mice (Fig. 1A). These differences may explain our negative results and will give us a lead to understand the physiological significances of LVRN in different species. The placenta-specific overexpression of human LVRN in mouse should also be tried as we generated preeclamptic model mice with human sFLT1.

Besides, differences in repertoires of aminopeptidases in human and mouse need to be considered. Human and mouse have 13 and 11 aminopeptidases, respectively. It is reported that the disruption of Enpep (aminopeptidase A) that degrades Angiotsensin-II resulted in elevated blood pressure baseline symptoms in mice [28]. Several
SNIPs have been found in ERAP2 (Endoplasmic reticulum aminopeptidase 2) gene in preeclamptic patients. Comparative studies on the different aminopeptidases would also be beneficial to elucidate the unique function of LVRN.

In conclusion, we show Lvrn, as a single factor, is irrelevant to preeclampsia and dispensable for normal placentation and embryonic development in mice. By taking advantages of LV vectors, one can introduce multiple genes in a single cell. The CRISPR/Cas9 approach also allows us to delete multiple genes from a single cell. These approaches will shed light on the combined effects of the family genes on placentation and placental functions.

Acknowledgments

We thank Ferheen Abbasi, Hirotaka Kato, Mayo Kodani, and Takaumi Matsumura for technical assistance in generating the Lvrn mutant mice, and Julio Manuel Castaneda for critical reading.

This work was supported by KAKENHI JPT15K06999 (to DK), JP18K14612 (to TN), JPA18J116750 (to TT), JP17H01394, JP25112007, AMED JP18gm501001, and Takeda Science Foundation (to MI).

References

1. Cross JC. Placental function in development and disease. Reprod Fertil Dev 2006; 18: 71–76. [Medline] [CrossRef]
2. Maltepe E, Fisher SJ. Placenta: the forgotten organ. Annu Rev Cell Dev Biol 2015; 31: 523–552. [Medline] [CrossRef]
3. Hod T, Cerdeira AS, Karumanchi SA. Molecular Mechanisms of Preeclampsia. Cold Spring Harb Perspect Biol 2015; 7: 531–540.
4. Sirerar M, Thadhani R, Karumanchi SA. Pathogenesis of preeclampsia. Curr Opin Nephrol Hypertens 2015; 24: 131–138. [Medline] [CrossRef]
5. Mol BWJ, Roberts CT, Thangaratinam S, Magee LA, de Groot CJM, Hofmeyr GJ. Pre-eclampsia. Lancet 2016; 387: 999–1011. [Medline] [CrossRef]
6. Chaiworapongsa T, Chaemsaithong P, Yeo L, Romero R. Pre-eclampsia part 1: current
Placental soluble FMS-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction in severe preeclampsia. Placenta 2012; 33(Suppl): S15–S122. [CrossRef]

Fisher SJ. Why is placentation abnormal in preeclampsia? Am J Obstet Gynecol 2015; 213(Suppl): SI15–SI122. [CrossRef]

Louwen F, Muschol-Steinmetz C, Reinhard J, Reutter A, Yuan J. A lesson for cancer research: placental microarray gene analysis in preeclampsia. Omicetarget 2012; 3: 759–773. [Medline] [CrossRef]

Sitras V, Paulsen RH, Gronaas H, Leivik J, Hansen TA, Vårtun A, Acharya G. Differential placental gene expression in severe preeclampsia. Placenta 2009; 30: 424–433. [Medline] [CrossRef]

Leavey K, Bainbridge SA, Cox BJ. Large scale aggregate microarray analysis reveals three distinct molecular subclasses of human preeclampsia. PLoS One 2015; 10: e0116508. [Medline] [CrossRef]

Fujisawa H, Higuchi T, Yamada S, Hirano T, Sato Y, Nishioka Y, Yoshio S, Tatsu, K, Ueda M, Maeda F, Fujii S. Human extravillous trophoblasts express latexerin, a novel protein that belongs to membrane-bound glutamin metallopeptidases. Biochem Biophys Res Commun 2004; 313: 962–968. [Medline] [CrossRef]

Horie A, Fujisawa H, Sato Y, Sugimaki M, Matsumoto H, Maruyama M, Konishi I, Hattori A. Latexerin/aminopeptidase Q induces trophoblast invasion during human early placentaion. Hum Reprod 2012; 27: 1267–1276. [Medline] [CrossRef]

Okada Y, Ueshin Y, Isotani A, Saito-Fujita T, Nakashima H, Kimura K, Mizoguchi A, Ob-Hora M, Mori Y, Ogata M, Oshima RG, Okabe M, Ikawa M. Complementation of placental defects and embryonic lethality by trophoblast-specific lentiviral gene transfer. Nat Biotechnol 2007; 25: 233–237. [Medline] [CrossRef]

Tobita T, Kiyozumi D, Ikawa M. Placenta-specific gene manipulation using lentiviral vector and its application. Placenta 2017; 59(Suppl 1): S37–S43. [Medline] [CrossRef]

Mashiko D, Fujihara Y, Sato T, Miyata H, Isotani A, Ikawa M. Generation of mutant mice by pronuclear injection of circular plasmid expressing Cas9 and single guided RNA. Sci Rep 2013; 3: 3355. [Medline] [CrossRef]

Maynard SE, Min JY, Merch J, Lim KH, Li J, Mondal S, Libermann TA, Morgan JP, Sellke FW, Stillman JE, Epstein FH, Sukhatme VP, Karumanchi SA. Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfuncion, hypertension, and proteinuria in preeclampsia. J Clin Invest 200; 111: 649–658. [Medline] [CrossRef]

Levine RJ, Lam C, Qian C, Yu KF, Maynard SE, Sachs BP, Sibai BM, Epstein FH, Romero R, Thadhani R, Karumanchi SA, CPEP Study Group. Soluble endoglin and other circulating antiangiogenic factors in preeclampsia. N Engl J Med 2006; 355: 992–1005. [Medline] [CrossRef]

van Dijk M, Oudejans CBM. STOX1: Key player in trophoblast dysfunction underlying early onset preeclampsia with growth retardation. J Pregnancy 2011; 2011: 521826. [Medline] [CrossRef]

Espino Y, Sosa S, Flores-Plegio A, Espejo-Nuñez A, Medina-Bastidas D, Vadillo-Ortega F, Zaga-Clavellina V, Estrada-Gutierrez G. New insights into the role of matrix metalloproteinases in preeclampsia. Int J Mol Sci 2017; 18: 1–10. [Medline] [CrossRef]

Pollheimer J, Fock V, Koidler M. Review: the ADAM metalloproteinases - novel regulators of trophoblast invasion? Placenta 2014; 35(Suppl): S57–S63. [Medline] [CrossRef]

Kumasawa K, Ikawa M, Kidoya H, Hasawa H, Saito-Fujita T, Morioka Y, Takakura N, Kimura T, Okabe M. Pravastatin induces placental growth factor (PGF) and ameliorates preeclampsia in a mouse model. Proc Natl Acad Sci USA 2011; 108: 1451–1455. [Medline] [CrossRef]

Venkatesha S, Toporsian M, Lam C, Hanai J, Mammo T, Kim YM, Boulah Y, Lim KH, Yuan HT, Libermann TA, Stillman JE, Roberts D, D’Amore PA, Epstein FH, Sellke FW, Romero R, Sukhatme VP, Letarte M, Karumanchi SA. Soluble endoglin contributes to the pathogenesis of preeclampsia. Nat Med 2006; 12: 642–649. [Medline] [CrossRef]

Doridot L, Passet B, Méhat C, Rigourd V, Barbaux S, Ducat A, Mondou F, Vilotte M, Castille J, Breunier-Fouché M, Daniel N, le Provost F, Bauchet AL, Baudrie V, Hertig A, Buffat C, Simonzi C, Germain G, Villette JL, Vaiman D. Preeclampsia-like symptoms induced in mice by fetalplacental expression of STOX1 are reversed by aspirin treatment. Hypertension 2013; 61: 662–668. [Medline] [CrossRef]

Plaks V, Rinkenberger J, Dai J, Flannery M, Sund M, Kanasaki K, Ni W, Kalluri R, Werb Z. Matrix metalloproteinase-9 deficiency phenocopies features of preeclampsia and intrauterine growth restriction. Proc Natl Acad Sci USA 2013; 110: 11109–11114. [Medline] [CrossRef]

Fujisawa H, Matsumoto H, Sato Y, Horie A, Ono M, Nakamura M, Mizumoto Y, Kuguchi K, Fujisawa H, Hattori A, Maida Y, Daikoku T, Imakawa K, Arai Y. Factors regulating human extravillous trophoblast invasion: chemokine-peptidase and CD9–integrin systems. Curr Pharm Biotechnol 2018; 19: 764–770. [Medline] [CrossRef]

Maruyama M, Arisaka N, Goto Y, Ohsawa Y, Inoue H, Fujisawa H, Hattori A, Tsujimoto M. Histidine 379 of human latexerin/aminopeptidase Q, a nonconserved residue within the exopeptidase motif, defines its distinctive enzymatic properties. J Biol Chem 2009; 284: 34692–34702. [Medline] [CrossRef]

Mizutani S, Wright JW, Kobayashi H. Placental leucine aminopeptidase- and aminopeptidase A-deficient mice offer insight concerning the mechanisms underlying preterm labor and preeclampsia. J Biomed Biotechnol 2011; 2011: 286947. [Medline] [CrossRef]