Expression of the FAM19A chemokine family members in the developing *Xenopus* brain

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Directed by Professor Hosung Jung

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This certifies that the Master's Thesis of
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우여곡절 끝에 졸업하게 되어 감회가 새롭습니다. 막상 졸업할 때가 되니 시원하면서도 입학 했을 때 마음처럼 조금 더 열심히 할 수 있지 않았을까 하는 후회도 듭니다. 아쉬운 마음도 있지만 석사과정동안 많이 배우고 조금이나마 성숙하고 성장할 수 있어 감사한 시간이었습니다. 이후 어떤 길을 가게 되더라도 이곳에서 지낸 시간들이 많은 도움이 될 것 같습니다.

그동안 많이 부족했던 저를 이끌어 주신 지도 교수님이신 정호성 교수님과 학위 논문을 준비하는 과정에서 많은 조언을 해 주신 복진웅, 김철훈 교수님께 깊이 감사드립니다. 실험실에 있는 동안 여러모로 항상 도움 주신 옥지연선배, 정재인선배, 김은진 선배, 홍재역 학생, 먼저 졸업한 장정임 학생, 덕분에 무사히 졸업 할 수 있게 된 것 같아 감사드립니다.

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나은정 씀

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Expression of the FAM19A chemokine family members in the developing *Xenopus* brain

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(Directed by Professor Hosung Jung)

Family with sequence similarity 19 member A subunits' genes are composed of five highly homologous genes which encode small secreted proteins. These proteins are also known as FAM19A1, FAM19A2, FAM19A3, FAM19A4, and FAM19A5. They are highly conservative in vertebrates and mainly expressed in the brain and they are related to MIP-1alpha, a member of the CC-chemokine family. These proteins are postulated to function as brain-specific chemokines or neurokines that act as regulators of immune and nervous cells yet it is not well known about their receptors and exact functions. Using the developing brain of *Xenopus tropicalis* as a model. I am investigating their expression stages and patterns during the development of the central nervous system

Key words: chemokine, neurokine, TAFA, brain patterning, development

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I. INTRODUCTION

Chemokines are small, structurally related proteins. They play a major role as regulating processes in host defense mechanisms such as leukocyte degranulation, integrin activation, leukocyte chemotaxis, and angiogenesis[1–4]. They have been highly conserved during evolution. Their amino acid sequences have high similarities across the vertebrates. Over 40 chemokines have been identified in humans, and they can be categorized into four major families, C family CC family, CXC, and CX3C family, according to the pattern of conserved cysteine residues which form intrachain disulfide bond[3].

In 2004, Y. Tom Tang identified a cluster of five highly homologous novel genes which named the TAFA family. Their domain organization appears to be evolutionarily related to small CC chemokine family. Those genes are mainly expressed in the central nervous system and they show different expression patterns in human brain[3]. Those TAFA family is currently registered in HUGO gene nomenclature committee (HGNC) as family with sequence similarity 19 member A1, C-C motif chemokine like (FAM19A1), family with sequence similarity 19 member A2, C-C motif chemokine like (FAM19A2), family with

sequence similarity 19 member A3, C-C motif chemokine like (FAM19A3), family with sequence similarity 19 member A4, C-C motif chemokine like (FAM19A4), and family with sequence similarity 19 member A5, C-C motif chemokine like (FAM19A5). Human FAM19A1 coded 133 amino acid protein, which has 20 amino acid as signal peptide. FAM19A2 coded 131 amino acid protein, which has 31 amino acid as signal peptide. FAM19A3 coded 133 amino acid, which has 31 amino acid as signal peptide. FAM19A4 coded 140 amino acid protein which has 36 amino acid as signal peptide. And FAM19A5 coded 125 amino acid protein which has 26 signal peptides. FAM19A chemokines family members are postulated to function as brain-specific chemokines or neurokines that act as regulators of immune and nervous cells yet it is not well known about their receptors and exact functions.

II. MATERIALS AND METHODS

1. *Xenopus tropicalis* embryos

Xenopus eggs were fertilized in vitro and grown in 0.1X modified Barth's saline (MBS). The jelly coat was removed by 2% L-cysteine in 1X MBS for 5 minutes in room temperature, and washed in 0.1X MBS. Xenopus embryonic stages are described as (Nieuwkoop and Faber, 1994). There are seven major stages which are cleavage; stage2 to 6, blastula; stage7 to 9, gastrula; stage 10 to 12, neurula; stage 13 to 21, early tailbud; stage22 to 28, late tailbud; stage 29 to 44, and tadpole; stage45 to 50.

2. RNA isolation and RT-PCR of FAM19A members

For expression analysis, I isolated RNA from stage 7, 13, 24, 33, 40 *Xenopus* embryos, and adult brain tissue and made cDNA. I used those cDNA as template and conduct RT-PCR using specific primers (Table 1) based on the sequences available in Genbank. The PCR condition were 94℃ (30 sec.), annealing at 58℃ (45 sec.), and extension at 70℃ (60 sec.) for 42 cycles.

3. Cloning of Xenopus FAM19A members

Xenopus FAM19A members were amplified by PCR from *Xenopus tropicalis* adult brain cDNA using specific primers (Table 1). The PCR condition were 94℃ (30 sec.), annealing at 58℃ (45 sec.), and extension at 70℃ (60 sec.) for 42 cycles. The resulting PCR products contain CDS. The PCR products were purified, cloned into pGEM T easy vector (Promega, Madison, Wisconsin, United States) and sequenced. Those constructs were referred pGEMT-FAM19A1, pGEMT-FAM19A2, pGEMT-FAM19A3, pGEMT-FAM19A4, and pGEMT-FAM19A5.

4. Wholemount *in situ* hybridization

At stage 24, 33, 44 embryos were fixed in 4% paraformaldehyde with DEPC water. Antisense riboprobes were in vitro transcribed using T7 RNA polymerase (Roche, Basel, Switzerland) and Sp6 RNA polymerase (Roche, Basel, Switzerland) and digoxigenin labeling mix (Roche, Basel, Switzerland) and templated cDNA encoding FAM19A1 (pGEMT-FAM19A1), FAM19A2 (pGEMT-FAM19A2), FAM19A3 (pGEMT-FAM19A3), FAM19A4 (pGEMT-FAM19A4), FAM19A5 (pGEMT-FAM19A5), Pax2 (pBSIISK+Pax2), and Pax6 (pCS2R-Pax6) (Table 2). Wholemount *in situ* hybridization was performed as describe before (Harland, 1991) with slight modification. Embryos are bleached with hydroperoxide. Hybridization performed with 5μg/ml riboprobes for over 18 hours at 60℃ hybridization chamber. Hybridized probes were visualized using anti-digoxigenin antibody conjugated to alkaline phosphatase (Roche, Basel, Switzerland) and BM purple (Roche, Basel, Switzerland).

Table 1. **Primer design of FAM19A members.**

Gene		Primer sequences
FAM19A1	**5'**	5'-TTAGGCCTATGTACGGTGTCATAAAGGCACG-3'
	3'	5'-TTGAGCTCGGAGATTATTAGGATCTCGGGTGAAT-3'
FAM19A2	**5'**	5'-TTTGGATTCATGATGAATAAAAGATATCTGCAGAAAG-3'
	3'	5'-TTGAGCTCCTACCGTGTTACCTTTGTTGTTTTC-3'
FAM19A3	**5'**	5'-TTTGGATTCATGGGTAGAAGAGATATACAAAGCATCA-3'
	3'	5'-TTGAGCTCCTATCGGGTGACCTTTGTTGTTT-3'
FAM19A4	**5'**	5'-TTTGGATTCATGCTAATCCCCCACCATG-3'
	3'	5'-TTTGAATTCCTACCGTGTTACCTTGGTCGTTT-3'
FAM19A5	**5'**	5'-TTTGGATTCATGCAGCTCTTGAAAGCTTTAT-3'
	3'	5'-TTTGAATTCTCAGGAGACTGTTGTTGTTTTT-3'

Table 2. DNA Construct information.

Construct name	Antibiotic resistance	Insert size	Antisense cut	Sense cut	orientation
pGEMT-FAM19A1	Amp	645bp	StuI	XbaI	T7 sense
pGEMT-FAM19A2	Amp	399	NcoI	XbaI	T7 sense
pGEMT-FAM19A3	Amp	402	NcoI	XbaI	T7 sense
pGEMT-FAM19A4	Amp	624	NcoI	ecoRI	T7 sense
pGEMT-FAM19A5	Amp	477	SpeI	ecoRI	Sp6 sense
pGEMT-FAM19A5S	Amp	228	SpeI	ecoRI	Sp6 sense
pBSIISK+Pax2	Amp	423	XhoI	NotI-HF	T7 sense
pCS2R-Pax6	Amp	1275	BglII-HF	XhoI	Sp6 sense

III. RESULTS

1. Sequences analysis of Xenopus FAM19A members

PCR product of FAM19A chemokine family members were amplified from adult *Xenopus* brain cDNA. Sequence analysis (Fig. 1) indicates that at the amino acid level, FAM19A chemokine family members share over 55% identity expect signal peptide with others. Also, all five members have CC cysteine residue motif like other CC chemokine family.

Based on PCR product sequencing result, I analysis FAM19A members mRNA gene structure and their proteins. *Xenopus* FAM19A1 mRNA have 5 exons and it contains 444bp CDS. Its protein is 147 amino acid which contain TAFA superfamily region; 55 to 142 (Fig. 2A and B). *Xenopus* FAM19A2 mRNA have 4 exons and it contains 396bp CDS. Its protein is 131 amino acid which contain TAFA superfamily region; 42 to 128 (Fig. 3A and B). *Xenopus* FAM19A3 mRNA have 4 exons and it contains 441bp CDS. Its protein is 146 amino acid which contain TAFA superfamily region; 44 to 130 (Fig. 4A and B). *Xenopus* FAM19A4 mRNA have 6 exons and it contains 624bp CDS. Its protein is 207 amino acid which contain TAFA superfamily region; 117 to 204 (Fig. 5A and B). and *Xenopus* FAM19A5 mRNA have 4 exons and it contains 384bp CDS. Its protein is 127 amino acid which contain TAFA superfamily region; 35 to 123 (Fig. 6A and B).

FAM19A members are highly conservative in vertebrates. Sequence analysis (Fig. 2C) indicates that at the amino acid level, *Xenopus* FAM19A1 shares 83% identity with human FAM19A1, 82% identity with mouse FAM19A1, and 76% identity with chicken FAM19A1. Sequence analysis of FAM19A2 (Fig. 3C) indicates that *Xenopus* FAM192 shares 90% identity with human FAM19A2, 71% identity with

mouse FAM19A2, and 94% identity with chicken FAM19A2. Sequence analysis of FAM19A3 (Fig. 4C) indicates that *Xenopus* FAM193 shares 86% identity with human FAM19A3, 85% identity with mouse FAM19A3, and 93% identity with chicken FAM19A3. Sequence analysis of FAM19A4 (Fig. 5C) indicates that *Xenopus* FAM194 shares 62% identity with human FAM19A4, 67% identity with mouse FAM19A4, and 70% identity with chicken FAM19A4. And, sequence analysis of FAM19A5 (Fig. 6C) indicates that *Xenopus* FAM195 shares 93% identity with human FAM19A5, and 98% identity with mouse FAM19A5. All five proteins are preserved CC cysteine residue motif at same location among the species.

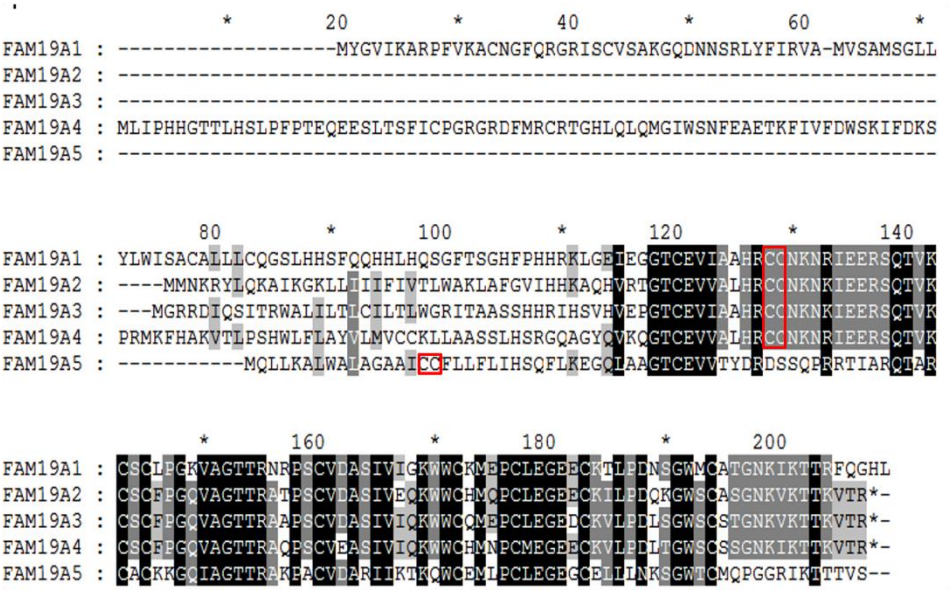

Figure 1. *Xenopus* FAM19A members show high amino acid similarity. Amino acid sequences of *Xenopus* FAM19A members were aligned using GeneDoc. Conserved amino acids in all five members or in at least two members are highlighted in black and grey. The conserved cysteine residues, signature motif of CC chemokine, are marked as red boxes.

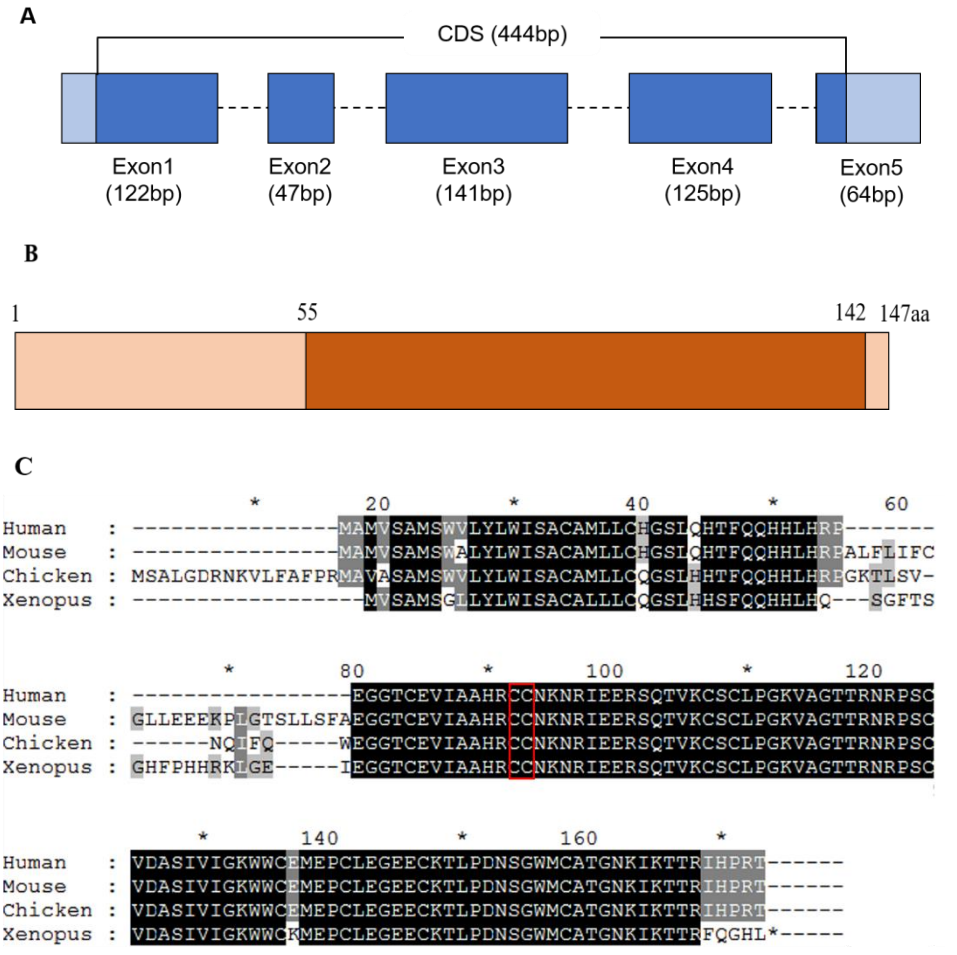

Figure 2. Sequence analysis of *Xenopus* FAM19A1. **(A)** Fam19A1 mRNA structure analysis generated by comparing with genomic DNA and mRNA sequences. Dark blue box means coding DNA sequence (CDS). **(B)** Based on mRNA CDS sequence, its translated protein is known. TAFA superfamily region is showed as dark brown box. **(C)** Deduced amino acid sequences from human, mouse, chicken and *Xenopus* FAM19A1 were aligned using GeneDoc. Conserved amino acid in all four species or in at least two species are highlighted in black and grey. The conserved cysteine residues, signature motif of CC chemokine, are marked as red box.

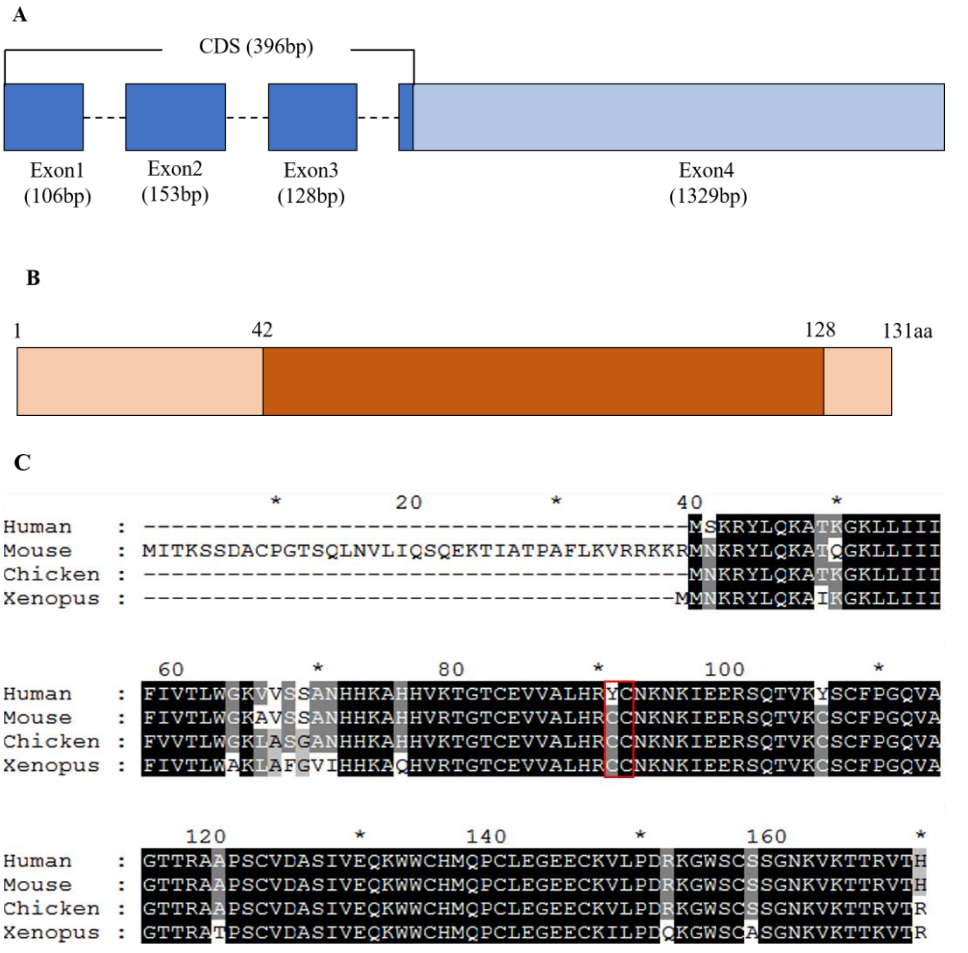

Figure 3. Sequence analysis of *Xenopus* FAM19A2. (A) Fam19A2 mRNA structure analysis generated by comparing with genomic DNA and mRNA sequences. Dark blue box means coding DNA sequence (CDS). **(B)** Based on mRNA CDS sequence, its translated protein is known. TAFA superfamily region is showed as dark brown box. **(C)** Deduced amino acid sequences from human, mouse, chicken and *Xenopus* FAM19A1 were aligned using GeneDoc. Conserved amino acid in all four species or in at least two species are highlighted in black and grey. The conserved cysteine residues, signature motif of CC chemokine, are marked as red box.

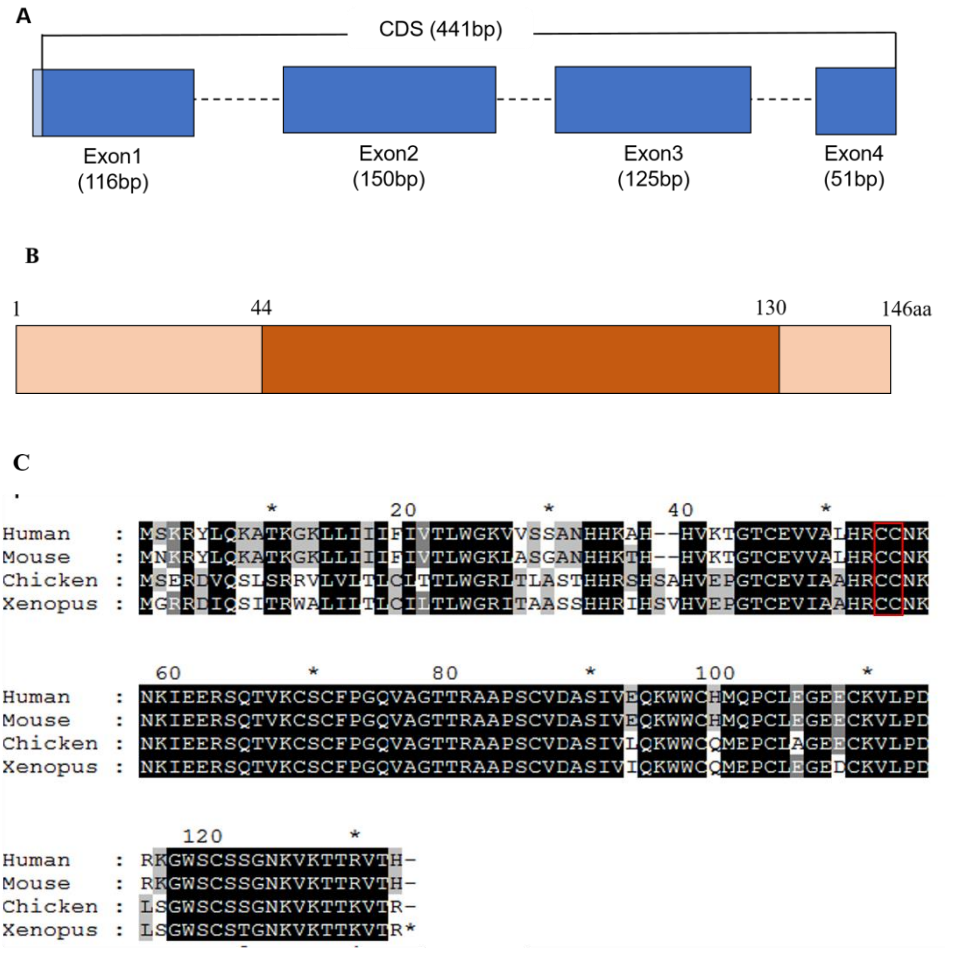

Figure 4. Sequence analysis of *Xenopus* FAM19A3. (A) Fam19A3 mRNA structure analysis generated by comparing with genomic DNA and mRNA sequences. Dark blue box means coding DNA sequence (CDS). **(B)** Based on mRNA CDS sequence, its translated protein is known. TAFA superfamily region is showed as dark brown box. **(C)** Deduced amino acid sequences from human, mouse, chicken and *Xenopus* FAM19A1 were aligned using GeneDoc. Conserved amino acid in all four species or in at least two species are highlighted in black and grey. The conserved cysteine residues, signature motif of CC chemokine, are marked as red box.

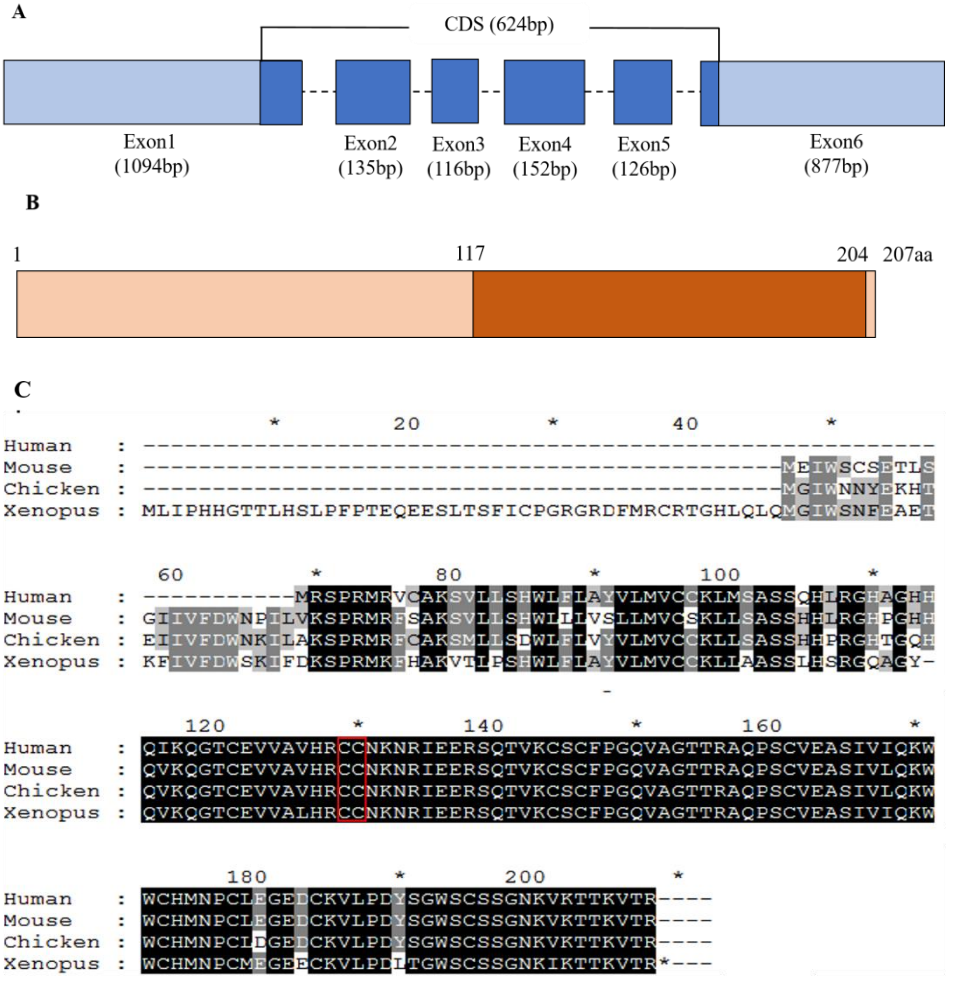

Figure 5. Sequence analysis of *Xenopus* FAM19A4. (A) Fam19A4 mRNA structure analysis generated by comparing with genomic DNA and mRNA sequences. Dark blue box means coding DNA sequence (CDS). **(B)** Based on mRNA CDS sequence, its translated protein is known. TAFA superfamily region is showed as dark brown box. **(C)** Deduced amino acid sequences from human, mouse, chicken and *Xenopus* FAM19A1 were aligned using GeneDoc. Conserved amino acid in all four species or in at least two species are highlighted in black and grey. The conserved cysteine residues, signature motif of CC chemokine, are marked as red box.

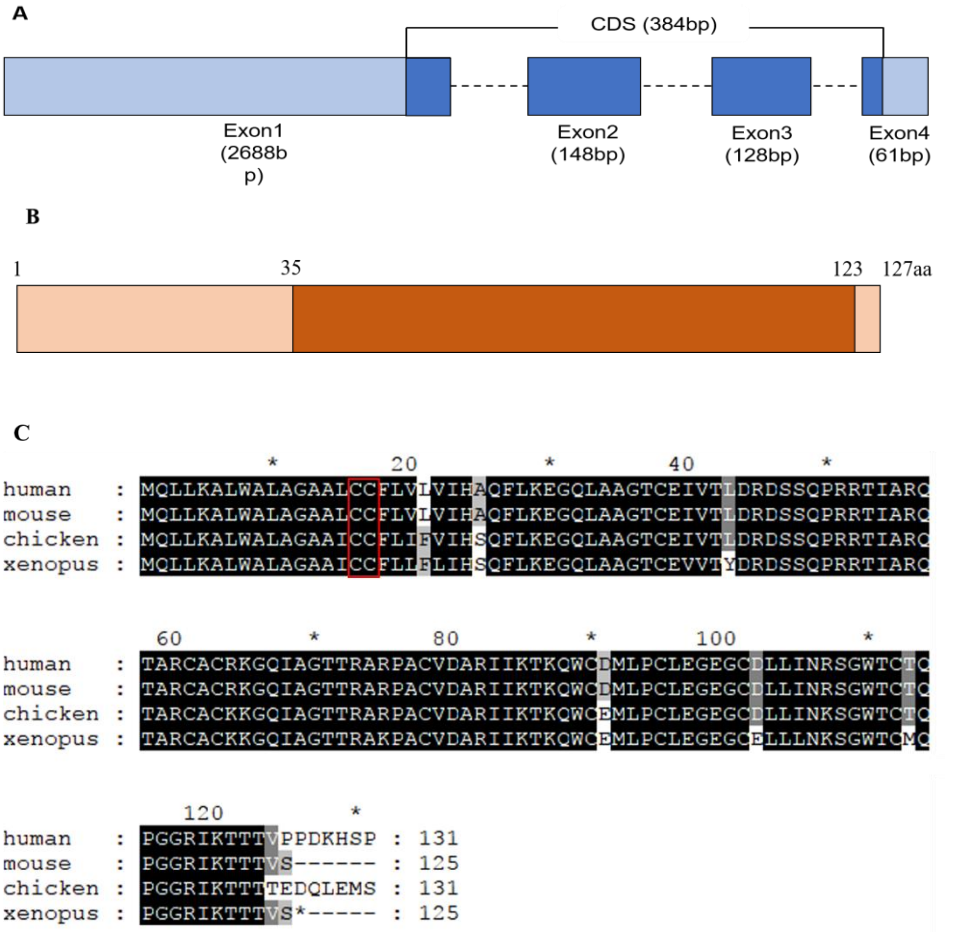

Figure 6. Sequence analysis of *Xenopus* FAM19A5. (A) Fam19A5 mRNA structure analysis generated by comparing with genomic DNA and mRNA sequences. Dark blue box means coding DNA sequence (CDS). **(B)** Based on mRNA CDS sequence, its translated protein is known. TAFA superfamily region is showed as dark brown box. **(C)** Deduced amino acid sequences from human, mouse and *Xenopus* FAM19A1 were aligned using GeneDoc. Conserved amino acid in all three species or in at least two species are highlighted in black and grey. The conserved cysteine residues, signature motif of CC chemokine, are marked as red box.

2. Temporal expression analysis of Xenopus FAM19A members

FAM19A members are mainly expressed in the brain(3) but it is not well known when they expressed during the development. Using RNA sequencing database(5) conduct gene expression analysis. *Xenopus* FAM19A1 mRNA sequencing data was not reported yet. According to *Xenopus* FAM19A2 mRNA sequencing data, *Xenopus* FAM19A2 transcripts are first detected from stage 22 and persist throughout development (Fig. 7). *Xenopus* FAM19A3 transcripts are first detected from stage 20 and persist throughout development (Fig. 8). *Xenopus* FAM19A4 transcripts are first detected from stage 12 and persist throughout development (Fig. 9). And *Xenopus* FAM19A5 transcripts are first detected from stage 8 and persist throughout development (Fig. 10). Based on gene analysis, I picked the six stages in development, which are stage 7; blastula, stage 13; neurula, stage 24; early tailbud, stage33; late tailbud, stage 40; late tailbud, and adult brain tissue from adult *Xenopus tropicalis*, to confirm expression with RT-PCR. The result (Fig. 11) shows that *Xenopus* FAM19A1 mRNAs start to express at stage 7, *Xenopus* FAM19A2 mRNAs start to express at stage 40, *Xenopus* FAM19A3 mRNAs start to express at stage 33, *Xenopus* FAM19A4 mRNAs start to express at stage 40, and *Xenopus* FAM19A5 mRNAs start to express at stage 13.

During the RT-PCR analysis, I revealed a preciously unannotated splicing variant (Fig. 12). This shorter mRNA isoform (FAM19A5S) results from splicing of the second exon and encodes a shorter protein that lack 50 amino acids near the N-terminus. PCR product of FAM19A5S were purified and cloned into pGEM T easy (Progema) and sequenced. This construct referred pGEMT-FAM19A5S.

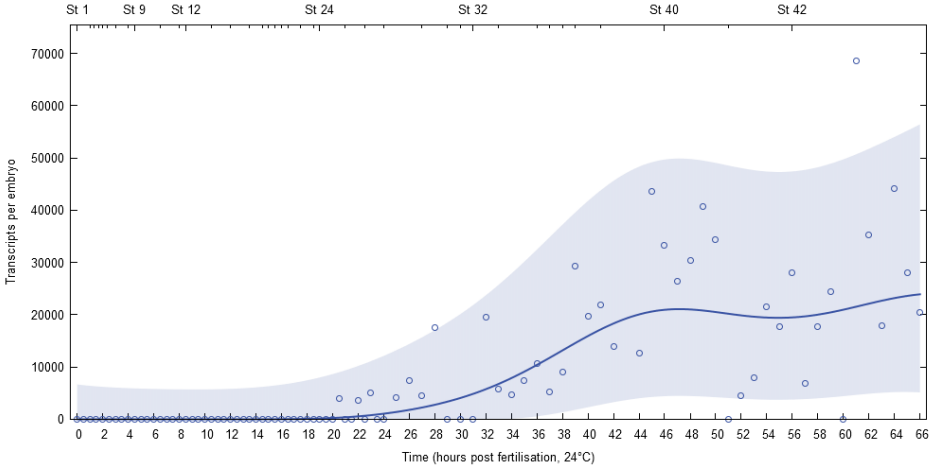

Figure 7. FAM19A2 gene expressed is developmentally regulated. Gene expression analysis. The above plots were generated using the previously published RNA-seq data (Owens et at., 2016). Y-axis is represented number of transcripts per embryo. X-axis is represented time and its correlated embryo stages at 24℃.

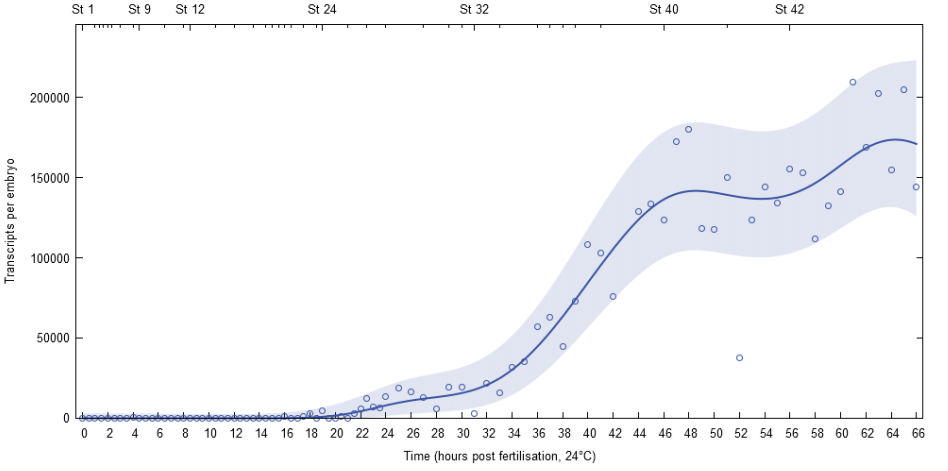

Figure 8. FAM19A3 gene expressed is developmentally regulated. Gene expression analysis. The above plots were generated using the previously published RNA-seq data (Owens et at., 2016). Y-axis is represented number of transcripts per embryo. X-axis is represented time and its correlated embryo stages at 24℃.

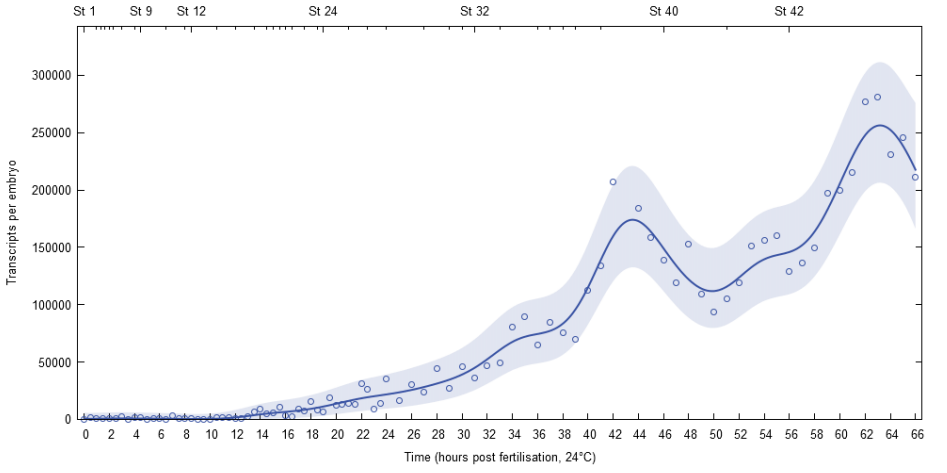

Figure 9. FAM19A4 gene expressed is developmentally regulated. Gene expression analysis. The above plots were generated using the previously published RNA-seq data (Owens et at., 2016). Y-axis is represented number of transcripts per embryo. X-axis is represented time and its correlated embryo stages at 24℃.

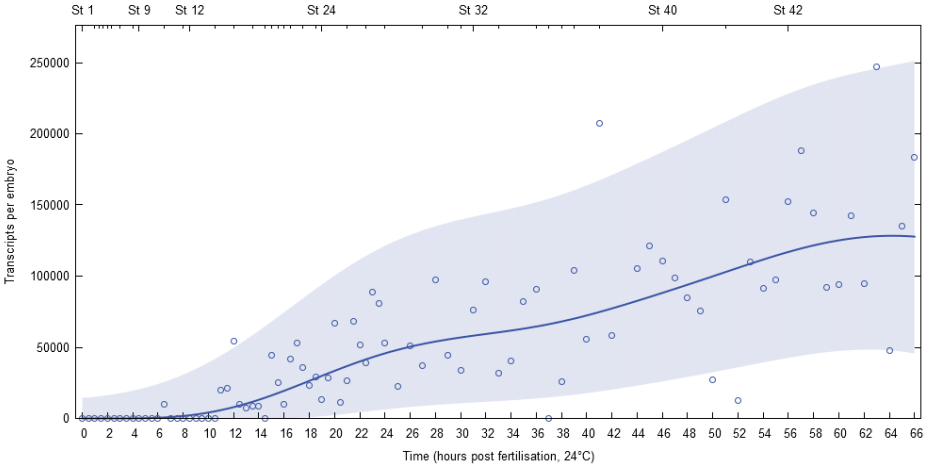

Figure 10. FAM19A5 gene expressed is developmentally regulated. Gene expression analysis. The above plots were generated using the previously published RNA-seq data (Owens et at., 2016). Y-axis is represented number of transcripts per embryo. X-axis is represented time and its correlated embryo stages at 24℃.

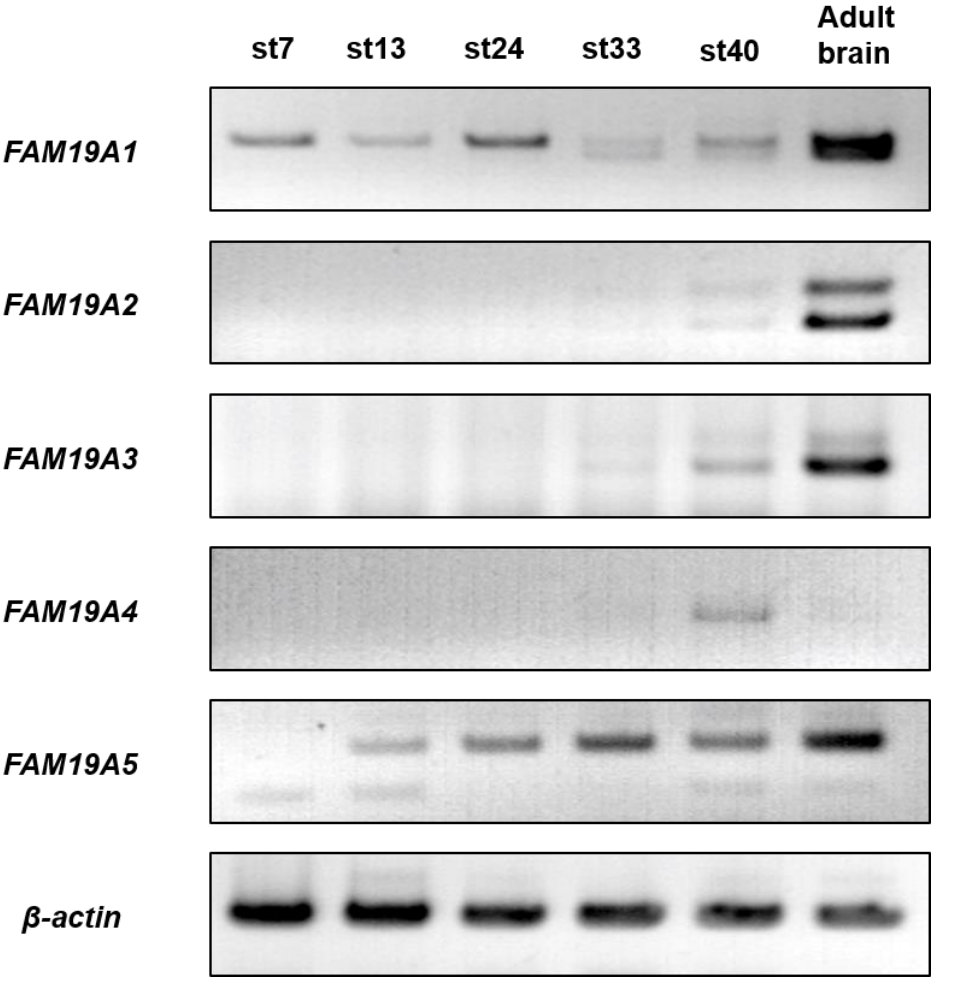

Figure 11. Overall temporal expression of Xenopus FAM19A members during development. RT-PCR analysis of the developmental expression of *Xenopus* FAMa19A members. Stages are according to Nieuwkoop and Faber (1967). β-actin is shown as a loading control.

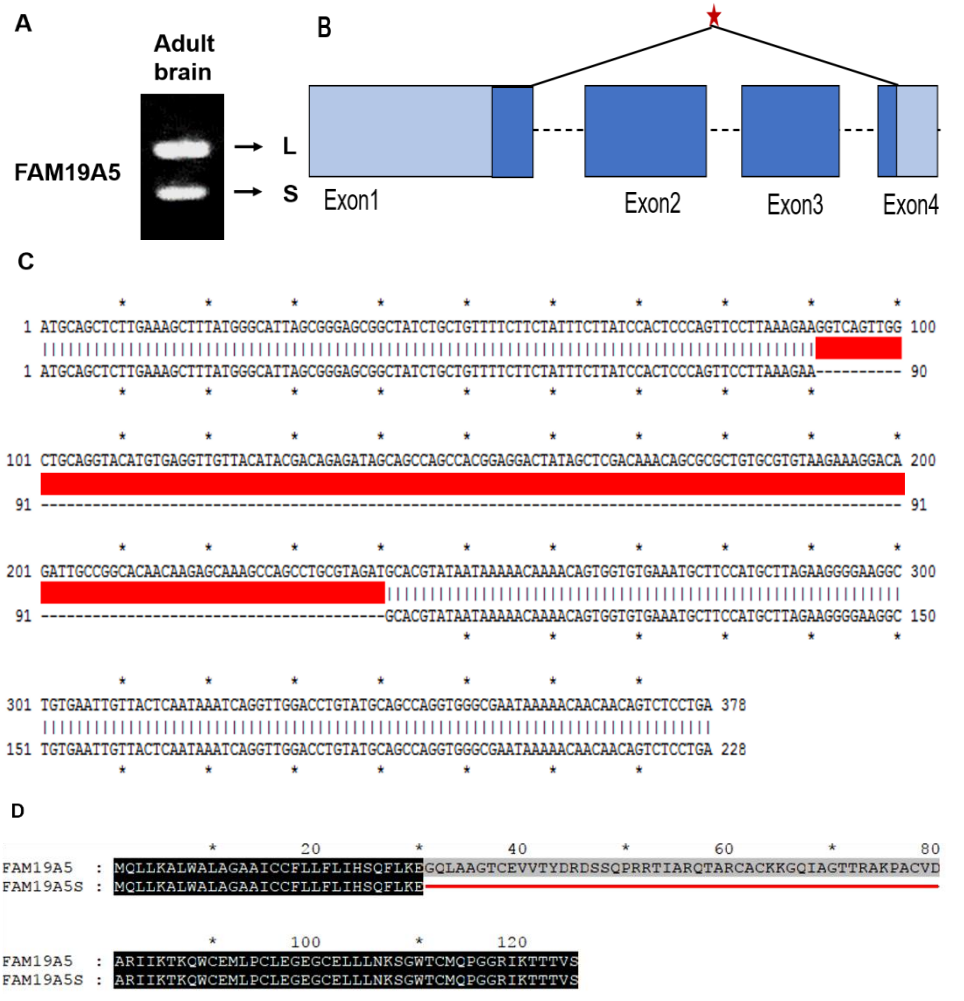

Figure 12. Identification of a novel FAM19A5 mRNA isoform encoding a protein isoform. **(A)** RT-PCR analysis revealed a previously unannotated splicing variant. **(B)** This shorter mRNA isoform (FAM19A5S) results from skipping of the second exon. **(C)** This isoform has deletion of 150 amino acid compare to FAM19A5. **(D)** This isoform encodes a shorter protein that lacks 50 amino acids near the N-terminus.

3. Spatial expressions analysis of Xenopus FAM19A members

To analyzed the expression of *Xenopus* FAM19A chemokine family members, Wholemount *in situ* hybridization was performed on embryos at stage 44. *Xenopus* FAM19A1 mRNAs are expressed at the telencephalon-diencephalon border, forebrain-midbrain border, and midbrain-hindbrain border (Fig. 13). *Xenopus* FAM19A2 mRNAs are weakly expressed in the diencephalon and at the forebrain-midbrain border (Fig. 14). *Xenopus* FAM19A3 mRNAs are expressed in the telencephalon, at the forebrain-midbrain border, and throughout the midbrain and the hindbrain (Fig. 15). *Xenopus* FAM19A4 mRNAs are weekly expressed in the telencephalon, at the forebrain-midbrain border and at the ciliary marginal zone (CMZ) of eye (Fig. 16). *Xenopus* FAM19A5 mRNAs are expressed in the telencephalon, at the midbrain boundary (Fig. 17). *Xenopus* FAM19A5S mRNAs are weekly expressed at the forebrain-midbrain border and telencephalon (Fig. 18). Each *Xenopus* FAM19A members' expression pattern is distinctive. However, they are commonly expressed at the forebrain-midbrain border. Their expression area in the brain are summarized in diagram (Fig. 19).

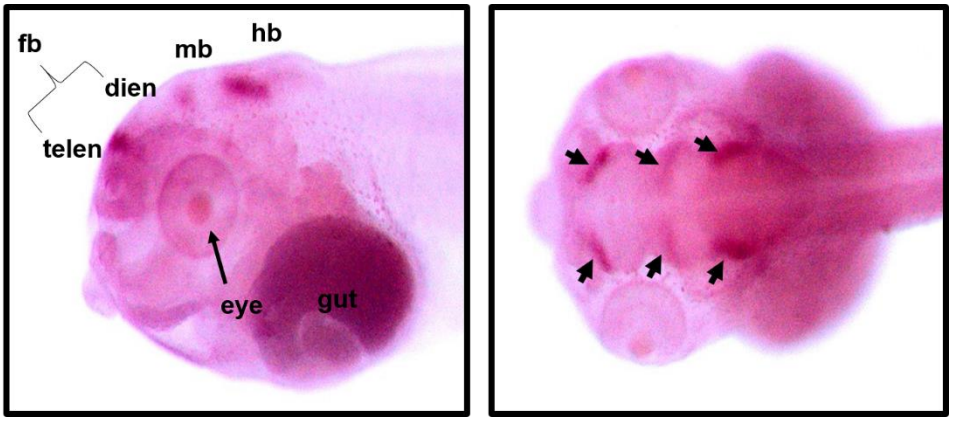

Figure 13. Expression of FAM19A1 by Wholemount *in situ* hybridization. FAM19A1 is expressed at the telencephalon-diencephalon border, forebrain-midbrain border, and midbrain-hindbrain border. Embryos which performed wholemount in situ hybridization are stage 44. fb; forebrain, mb; midbrain, hb; hindbrain, telen; telencephalon, and dien; diencephalon.

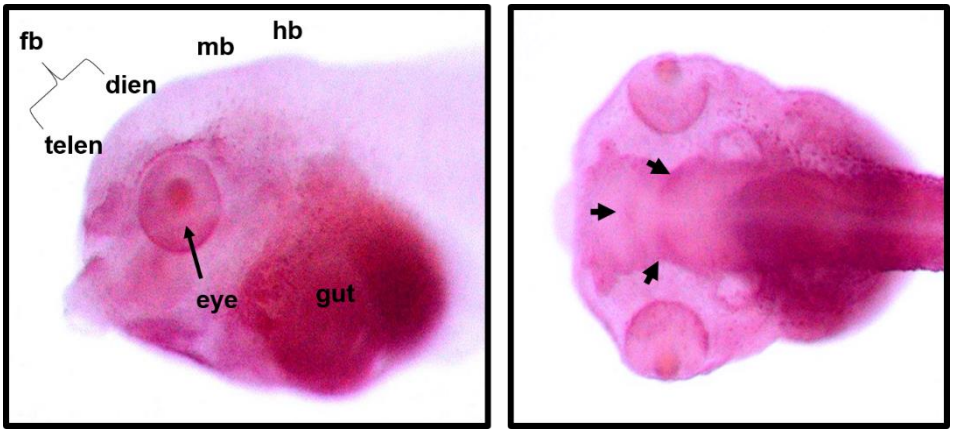

Figure 14. Expression of FAM19A2 by Wholemount *in situ* hybridization. FAM19A2 is weakly expressed in the diencephalon and at the forebrain-midbrain border Embryos which performed wholemount in situ hybridization are stage 44. fb; forebrain, mb; midbrain, hb; hindbrain, telen; telencephalon, and dien; diencephalon.

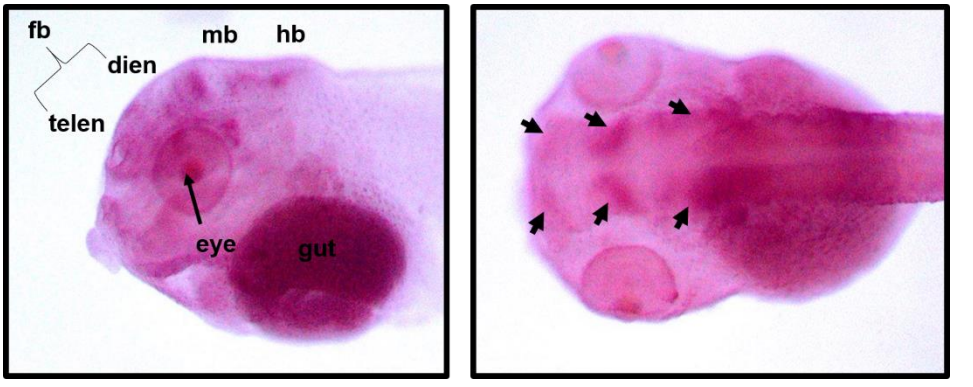

Figure 15. Expression of FAM19A3 by Wholemount *in situ* hybridization. FAM19A3 expressed in the telencephalon, at the forebrain-midbrain border, and throughout the midbrain and the hindbrain. Embryos which performed wholemount in situ hybridization are stage 44. fb; forebrain, mb; midbrain, hb; hindbrain, telen; telencephalon, and dien; diencephalon.

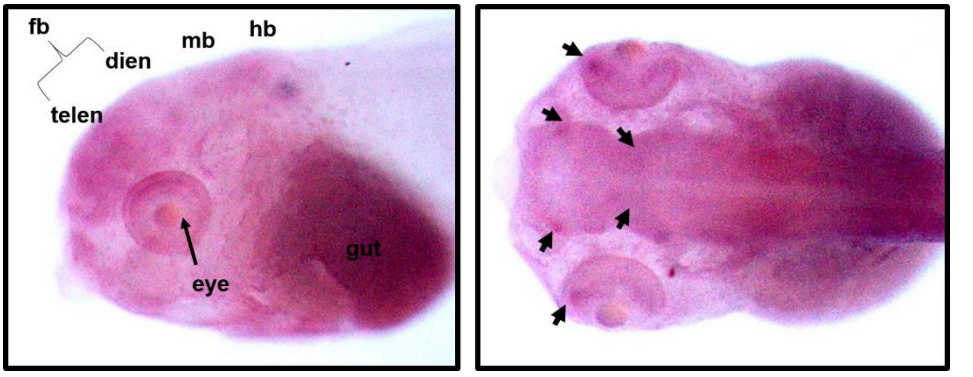

Figure 16. Expression of FAM19A4 by Wholemount *in situ* hybridization. FAM19A4 is expressed in the telencephalon, at the forebrain-midbrain border and at the ciliary marginal zone (CMZ) of eye. Embryos which performed wholemount in situ hybridization are stage 44. fb; forebrain, mb; midbrain, hb; hindbrain, telen; telencephalon, and dien; diencephalon.

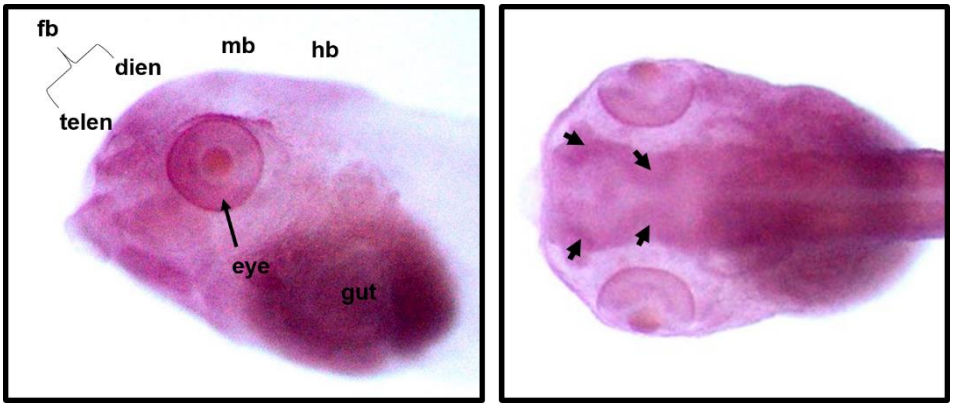

Figure 17. Expression of FAM19A5 by Wholemount *in situ* hybridization. FAM19A5 is expressed in the telencephalon, at the midbrain boundary. Embryos which performed wholemount in situ hybridization are stage 44. fb; forebrain, mb; midbrain, hb; hindbrain, telen; telencephalon, and dien; diencephalon.

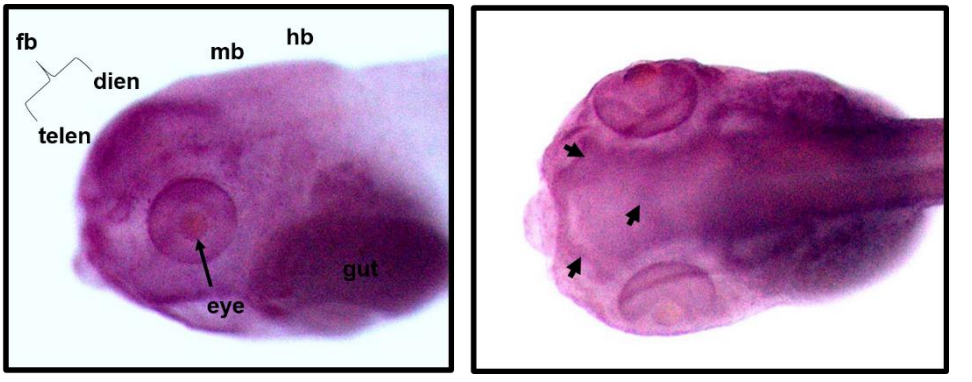

Figure 18. Expression of FAM19A5S by Wholemount *in situ* hybridization. FAM19A5S is weekly expressed at the forebrain-midbrain border and forebarin. Embryos which performed wholemount in situ hybridization are stage 44. fb; forebrain, mb; midbrain, hb; hindbrain, telen; telencephalon, and dien; diencephalon.

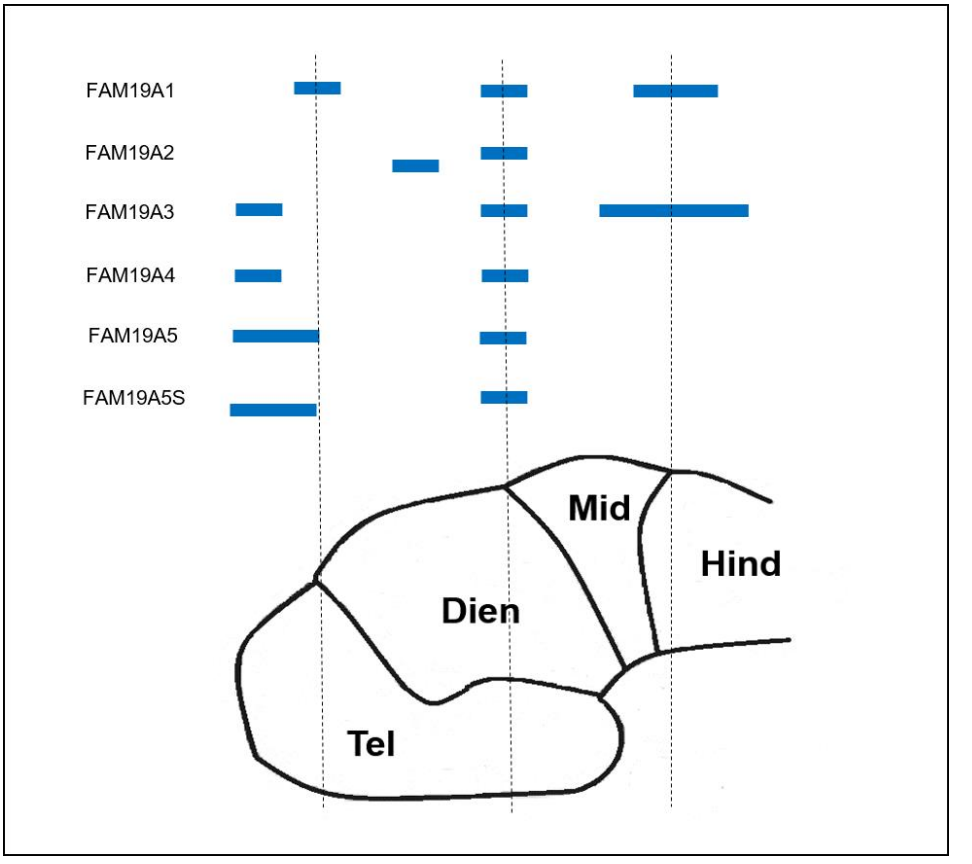

Figure 19. Summary of Expression pattern of *Xenopus* FAM19A members. Each FAM19A members' expression patterns are different but expression area in the brain could be catalogued. Expression area are marked as blue bar which are correlated with the *Xenopus* brain area. Embryos which performed wholemount in situ hybridization are stage 44. Tele; telencephalon, Dien; diencephalon, Mid; midbrain. Hind; Hindbrain.

IV. DISCUSSION

According to sequence analysis, *Xenopus* FAM19A members' proteins have TAFA superfamily region like human FAM19A proteins. Also, sequence analysis indicates that at the amino acid level, FAM19A five proteins are highly homologous and have CC cysteine residue motif like other CC chemokine family. and All five proteins are highly conservative in vertebrates and preserved CC cysteine residue motif at same location among the species.

From gene expression analysis and RT-PCR analysis I could verified expression stages of FAM19A members. FAM19A1 and FAM19A5 start to express in early stage of development like blastula; stage7 while other FAM19A members start to express comparatively in late stage like late tailbud; stage40.

During the RT-PCR analysis I revealed a previously unannotated splicing variant. This shorter mRNA isoform (FAM19A5S) results from skipping of the second exon. This isoform has deletion of 150 amino acid compare to FAM19A5. This isoform encodes a shorter protein that lacks 50 amino acids near the N-terminus. Expression pattern of FAM19A5S seems different with FAM19A5'. It means they could have different function. In further study, I could work what is the difference between FAM19A5 and FAM19A5 function.

FAM19A members have distinctive manner of expression pattern though all five genes are expressed at the border of forebrain-midbrain. Their function is not known but one possibility is that they have a role in segmentation or patterning of the brain area from expression patterns and another is that patterning or segmentation of brain effect to FAM19A members' expression level. Because of FAM19A proteins are secreted protein, it is hard to say what would be correct until their receptors are reveal. In further study, I could investigate their receptors and their role in the developing brain.

V. CONCLUSION

Family with sequence similarity 19 member A subunits' genes are composed of five highly homologous genes which encode small secreted proteins. These proteins are also known as FAM19A1, FAM19A2, FAM19A3, FAM19A4, and FAM19A5. They are highly conservative in vertebrates and mainly expressed in the brain and also, they are evolutionally related CC-chemokine family. These proteins are postulated to function as brain-specific chemokines or neurokines that act as regulators of immune and nervous cells yet it is not well known about their receptors and exact functions. In this thesis, firstly I show homologue among the FAM19A chemokine family members, and they are highly conserved in vertebrates using sequence analysis. Secondly, the developing brain of *Xenopus tropicalis* as a model, I am investigating their expression stages of FMA19A members with gene expression analysis and RT-PCR. FAM19A1 and FAM19A5 transcripts started to express in blastula, and also, FAM19A2, FAM19A3, and FAM19A4 transcripts started to express in late tailbud stage. Finally, I show FAM19A members' expression patterns in developing *Xenopus* brain. They have distinctive manner of expression pattern though all five genes are expressed at the border of forebrain-midbrain. Their function is not known but I could have expected that they have a role in segmentation or patterning of the brain area during the development.

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ABSTRACT (IN KOREAN)

발생중인 개구리 뇌에서 FAM19A chemokine family의 발현

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FAM19A family는 5개의 유전자 (FAM19A1, FAM19A2, FAM19A3, FAM19A4 그리고 FAM19A5)로 구성되어 있으며 주로 뇌에서 발현한다. 이들은 진화적으로 보존되어 있어 척추동물에서 발현하고 개구리의 전사체 분석 연구에 의하면 발생 중에도 발현하는 것으로 보인다. 이들 유전자들이 코딩하는 단백질들은 CC-chemokine family 와 계통 발생학적으로 비슷하여 chemokine 으로써의 기능을 예상할 수 있으나 이들이 리간드로 작용하는 수용체는 아직 알려지지 않았다. FAM19A family가 뇌에서 특정적으로 발현하기 때문에 뇌 특이적 chemokine으로써 중추신경계에서 면역 반응을 조율하는 역할을 할 가능성이 있고, neurokine으로 작용하여 신경세포의 생존과 분화에 영향을 미칠 가능성도 있다. 그러나 아직 이들이 어느 발생 단계에, 뇌의 어느 부위에 발현하며, 어떠한 기능을 하는지는 제대로 밝혀져 있지 않다. 앞서 말한 바와 같이 FAM19A family가 진화적으로 보존되어 있으며 발생과정 중에 발현하고 chemokine으로써 역할을 할 것으로 예상되지만, 아직 이들이 뇌 발생 중에 어떠한 역할을 하는지는 전혀 알려져 있지 않기 때문에 본 연구에서는 발생 단계별로 변화를 보기에 용이한 *Xenopus tropicalis*을 실험 동물로 FAM19A family의 발현 시점과 뇌에서의 발현 패턴을 확인하였다.

핵심 되는 말: chemokine, neurokine, TAFA, 뇌 세부구조 결정, 발생