Centre de recherche
CHU Sainte-Justine
Le centre hospitalier universitaire mère-enfant

Université de Montréal

PROGRAMME DE STAGES D'ÉTÉ
Initiation à la recherche biomédicale
au Centre de recherche du CHU Sainte-Justine
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# A novel skeletal dysplasia: from gene mutation to disease mechanism

**Numéro de l'offre de stage : No. 19**

**Équipe de recherche**

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**Coordonnées**

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**Programmes d'études ciblés**

Biochimie, Biologie Moléculaire, Sciences Biomédicales

**Description du projet**

Spondyloepimetaphyseal dysplasia (SEMD) is a rare inherited bone disorder characterized by developmental changes in the growth plate, long bones and vertebrae. We have identified biallelic mutations in *ERI1* in seven families with either syndactyly, other digital anomalies and cardiac malformations or SEMD. ERI1 a key regulatory protein for RNA maintenance, encodes an evolutionarily conserved 3'-5' exoribonuclease the binds to 3' end of replication-dependent histone mRNAs and degrades them in the 3' to 5' direction, participates in 5.8S rRNA 3' end processing and degradation of microRNA degradation. We will study how mutations in this exoribonuclease might affect its activity and lead to SEMD. This project comprises of five aims:

1. Assess the impact of *ERI1* mutations on the expression levels of histone mRNAs (in synchronized cells) and 5.8S rRNA by qRT-PCR in fibroblast.

2. Determine the impact of *ERI1* mutations on 3' processing of histone mRNA and 5.8S rRNA by 3' RNA ligase-mediated rapid amplification of cDNA ends (3' RLM RACE) followed by cloning and Sanger sequencing.
3. Determine the effect of *ERI1* mutations on microRNAs and their targets by RNAseq.
4. Assess the enzyme activity of ERI1 by in vitro assay.
5. Generate a mouse model of ERI1-related SEMD to determine how missense mutations affect chondrocytes by the CRISPR-Cas9 system.

The experiments described above will allow us to confirm whether the mutations we found in different patients have a deleterious role on the ERI1 function and are the cause of the SEMD phenotype with syndactyly in patients and will thus allow for proper counseling of the families. Further, it will elucidate a new class of genetic diseases, those caused by abnormal 3' RNA processing, and potentially in protein translation.

## Rôle du stagiaire

To fulfill the aims of the project, the student would be expected to carry out various experiments based on his/her skill. Experiments include RNA isolation, qRT-PCR, 3' RACE, cloning, sequencing, recombinant ERI1 production in bacteria, protein purification, RNAseq data analysis, histomorphometry, immunohistochemistry, polysome profiling and pSILAC.

## Mots clés

Spondyloepimetaphyseal dysplasia; exoribonuclease 1 (ERI1); CRISPR-Cas9 system; 3' RNA ligase-mediated rapid amplification of cDNA ends (3' RLM RACE); RNAseq