National and Kapodistrian University of Athens
School of Medicine
Department of Biological Chemistry

P41

Investigation of the potential link between mechanosensory proteins PC1/PC2 and Craniosynostosis

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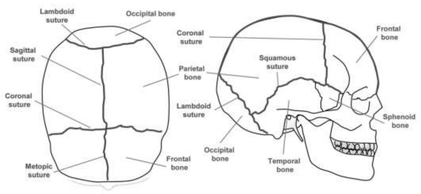
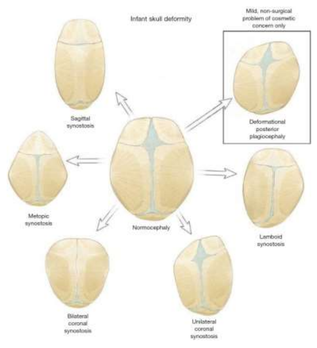
Introduction

• Skull development is a tightly regulated process that occurs along the osteogenic interfaces of the cranial sutures that allow rapid bone formation at the edges of the bone fronts **(Opperman LA, 2000)**

• **Premature closure of cranial sutures can result in pathological conditions such as Craniosynostosis**



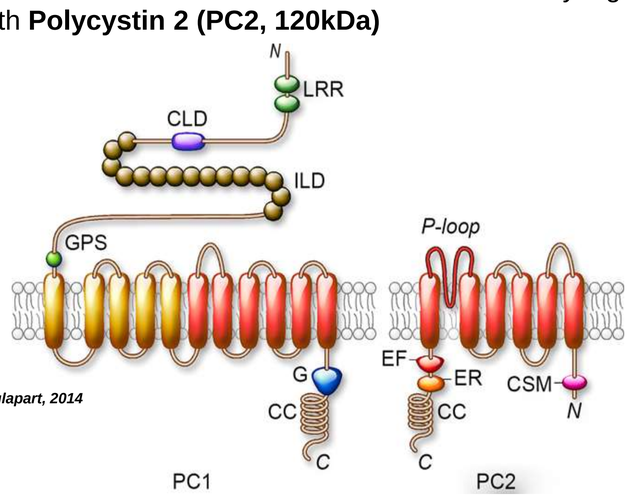
Senarath-Yapa K et al , 2012



Proctor MR , 2014

• **The mechanosensory proteins Polycystin 1 (PC1) and 2 (PC2) regulate skeletal development and potentially suture formation**

▪ **Polycystin-1 (PC1, 420 kDa)** spans the cell membrane, has a large extracellular domain and mediates mechanosensory signal together with **Polycystin 2 (PC2, 120kDa)**



Retailleau and Dulapart, 2014

• **PCs play a central role in cellular mechanosensation and mechanotransduction processes (Dalagiorgou G et al. 2013)**

• PC1 and PC2 was expressed in hPDL cells subjected to mechanical stretch for various time points **(Dalagiorgou G et al., 2013)**

• **PC1 modulates osteoblastic gene transcription and bone cell differentiation through the calcineurin/NFAT signaling pathway (Dalagiorgou G et al., 2013)**

• Mice subjected to midpalatal suture expansion in vivo, demonstrated that midpalatal force promoted cartilage formation **(Hou B et al., 2007)**

• **PC1-deficient mice present restricted growth effects at the skull base and in craniofacial sutures, without however knowledge of the underlying molecular mechanisms (Kolpakova-Hart E. et al., 2008)**

Aim of research

To investigate the role of PC1/PC2 in suture development and suture fusion

Methods

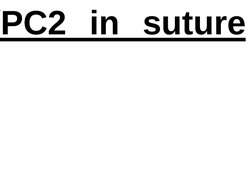
A. **Western blotting**: **A1)** Whole suture sagittal (SAG) bone tissue lysates were blocked and incubated with primary rabbit polyclonal antibodies for PC1 and PC2. **A2)** Similar procedures were followed for human samples

B1. Primary Sagittal Suture cultures: Suture derived mesenchymal cells were harvested from 9-day old Sprague Dawley rat:

1. SAG sutures with a bony margin on either side
2. Explants of SAG sutures were placed in100-mm tissue culture dishes with the endocranial surface flush to the plate
3. Explants were then cultured in standard growth Medium. It was replenished every 2 days-over the course of 1 week. Iin culture SAG-derived mesenchymal cells had migrated from tissue explants
4. At 7 days of primary culture, suture derived mesenchymal cells were passaged by trypsinization

B1

SAG primary culture



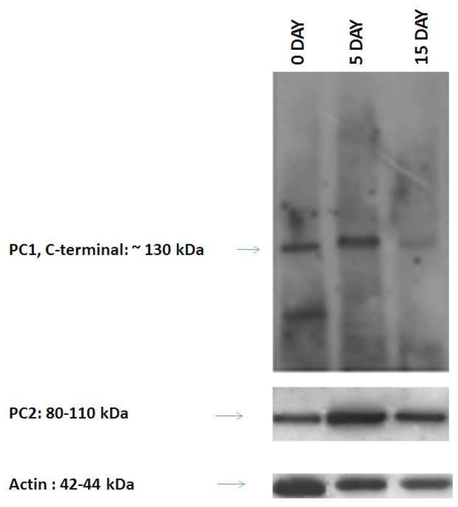
B2. RT-PCR/PCR: RNA was extracted from primary SAG suture cells, post-natal day 9. Expression of PC1, SOX9 and RUNX2 was observed

C. Immunohistochemistry: Paraffin-embedded sections of SAG suture bone tissue from 0, 5, 15 day-old Sprague Dawley rats and H/A staining, was used. Expression of PC1/PC2 was evaluated with primary rabbit polyclonal antibodies for PC1 and PC2

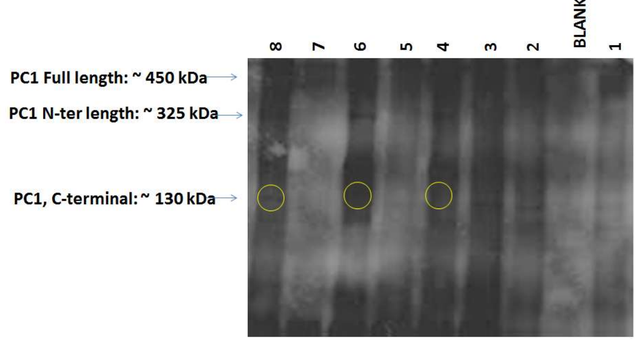
Results

A1) Western Immunoblotting revealed a differential expression pattern for PC1 and PC2 in SAG sutures at p1/p5/p15 days

Activated form of PC1 (cleaved C-terminal) and PC2 levels were elevated at postnatal day 5

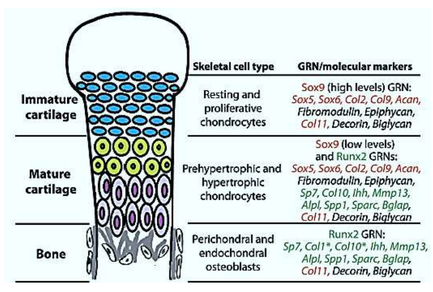


A2) PC1 expression in human craniosynostosis samples was detected in the area of synostotic sutures

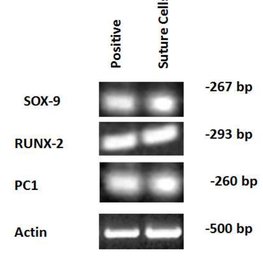


1: syndromic
2: Dolichocephaly
3: Dolichocephaly
4: periosteo-syndromic
5: syndromic
6: plagiocephaly
7: osteo-plagiocephaly
8: syndromic

B2) In primary suture SAG cell cultures PC1 presence was associated with an elevated expression of the osteoblastic marker RUNX2 and a lower expression of the chondrocytic marker SOX-9

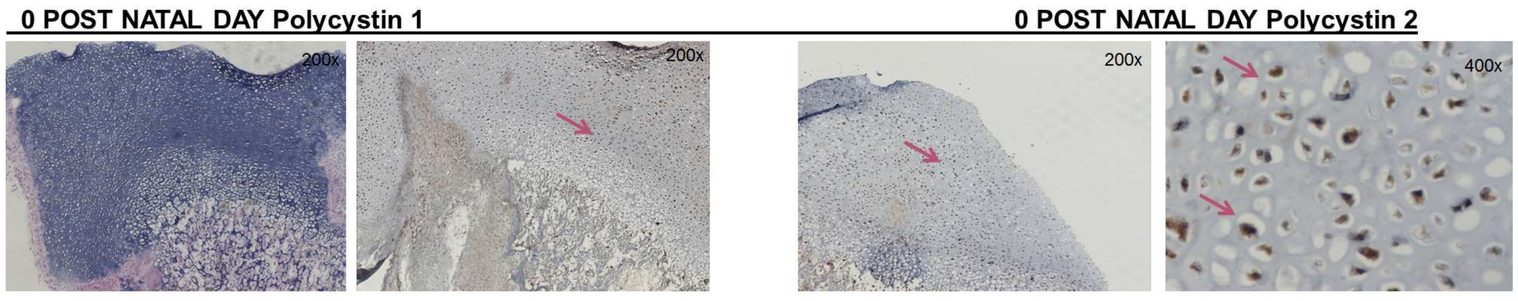


Gómez-Picos P and Eames BF,2015

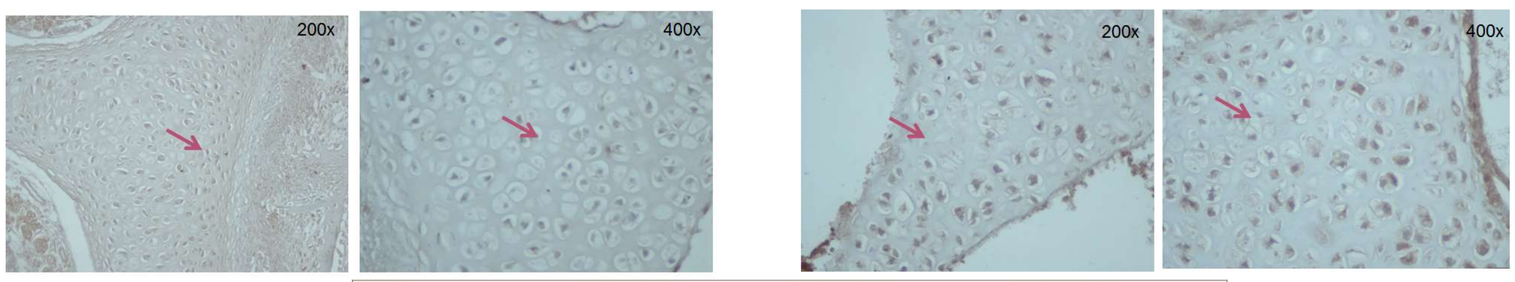


C) Immunohistochemical analysis showed nuclear expression of PC1/PC2 in SAG sutures:

0 POST NATAL DAY Polycystin 1 | **0 POST NATAL DAY Polycystin 2**



5 POST NATAL DAY Polycystin 1 | **5 POST NATAL DAY Polycystin 2**



PROTEIN	0 POST NATAL DAY			5 POST NATAL DAY			15 POST NATAL DAY											
EXPRESSION-LOCALIZATION	CYTOSOLIC			NUCLEAR			CYTOSOLIC			NUCLEAR			CYTOSOLIC			NUCLEAR		
%: CELL PERCENTAGE, I: intensity, H: H-score	%	I		%	I	H	%	I	H	%	I	H	%	I	H	%	I	H
PC1	70	++	140	70	++	140	40	+	40	50	+	50	0	+++	0	50	+++	150
PC2	0	+++	0	100	+++	300	40	-/+	20	40	-/+	20	65	+/++	97,5	65	+/++	97,5

Conclusions

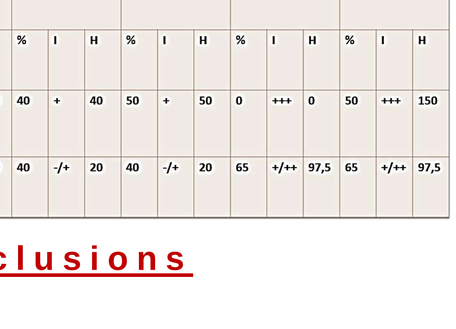
▪ **A differential expression pattern for PC1 and PC2 in SAG sutures at p1/ p5/ p15 days. An elevated PC1/PC2 expression at post-natal day 5 was observed. PC1 expression was also detected in human craniosynostosis samples.**

▪ **In primary suture SAG cell cultures, PC1 presence was associated with an elevated expression of the osteoblast marker RUNX2 and a lower expression of chondrocyte marker SOX-9.**

▪ **Nuclear localization of PC1/PC2 expression was observed in post-natal days 0 and 5 indicating their activation at these stages.**

Our data demonstrate that Polycystins are implicated in suture formation and growth, playing a potential role in premature obliteration of sutures that occur in pathological conditions such as Craniosynostosis.

Ongoing research will try to elucidate the potential cross-talk of PC1 signaling and Wnt-β catenin intracellular pathway in suture fusion processes:



Katsianou MA et al, BBA Clinical 2016

Conflict of Interest: **None declared**

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