ABSTRACT

The identification and characterization of FGFR2 isoforms in the mid-face of Crouzon syndrome mouse model

Jamie Ong

Master of Science
Discipline of Pediatric Dentistry, Faculty of Dentistry
University of Toronto
2013

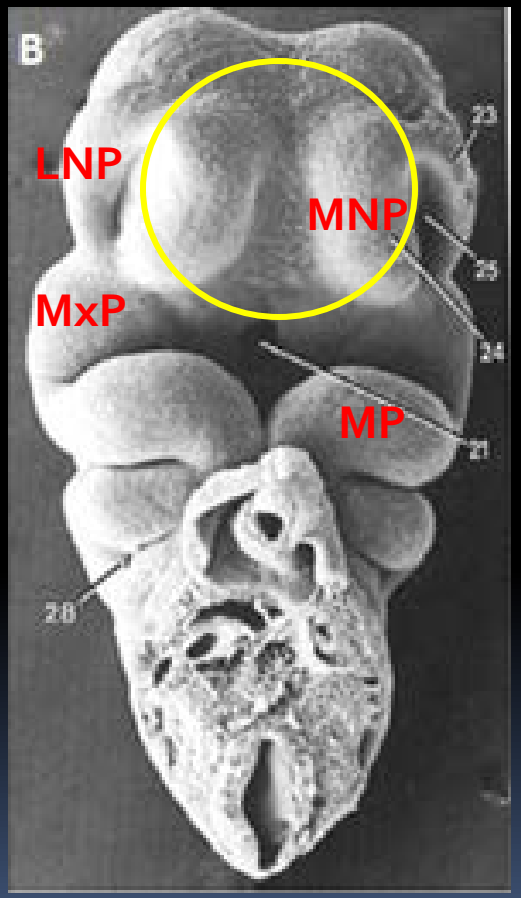
Fibroblast Growth Factors (Fgf) and their receptors (*Fgfrs*) have been found to be involved in the development of almost all structures in the craniofacial region. The mouse Fgfr2 gene contains 19 exons and several alternatively spliced variants of Fgfr2 have been identified in literature. These splice variants possess different ligand-receptor binding and tissue specificities resulting in their distinct functions during development. More recently, the presence of an additional band has been demonstrated only in the $Fgfr2^{W290R}$ mutant, a mouse model of the human Crouzon syndrome, on western blot analyses. **Objective:** The aim of this study is to determine whether the $Fgfr2^{W290R}$ mutant results in the generation of additional isoforms. **Methods:** The craniofacial region of E12.5 mouse embryos was dissected and RNAs from the tissues were extracted and reversed transcribed. Isolated cDNA from wildtype, homozygous and heterozygous tissues were then subjected to RT-PCR analyses using exon-specific primers and PCR amplified products were verified by gel electrophoresis. **Results:** The pattern and sizes of the amplified fragments appear to be identical in all three genotypes. **Conclusion:** RT-PCR analyses suggest the absence of mutant-specific isoforms in the $Fgfr2^{W290R}$ mutant. Given these results, it is likely that the additional bands observed in western blot analyses are a result of post-translational changes, most likely representing cleavage products of Fgfr2 rather than spliced variants.

Jamie Ong

IDENTIFICATION OF FGFR2 ISOFORMS IN THE MID-FACE OF WILDTYPE MICE AND FGFR2^{W290R} MUTANTS

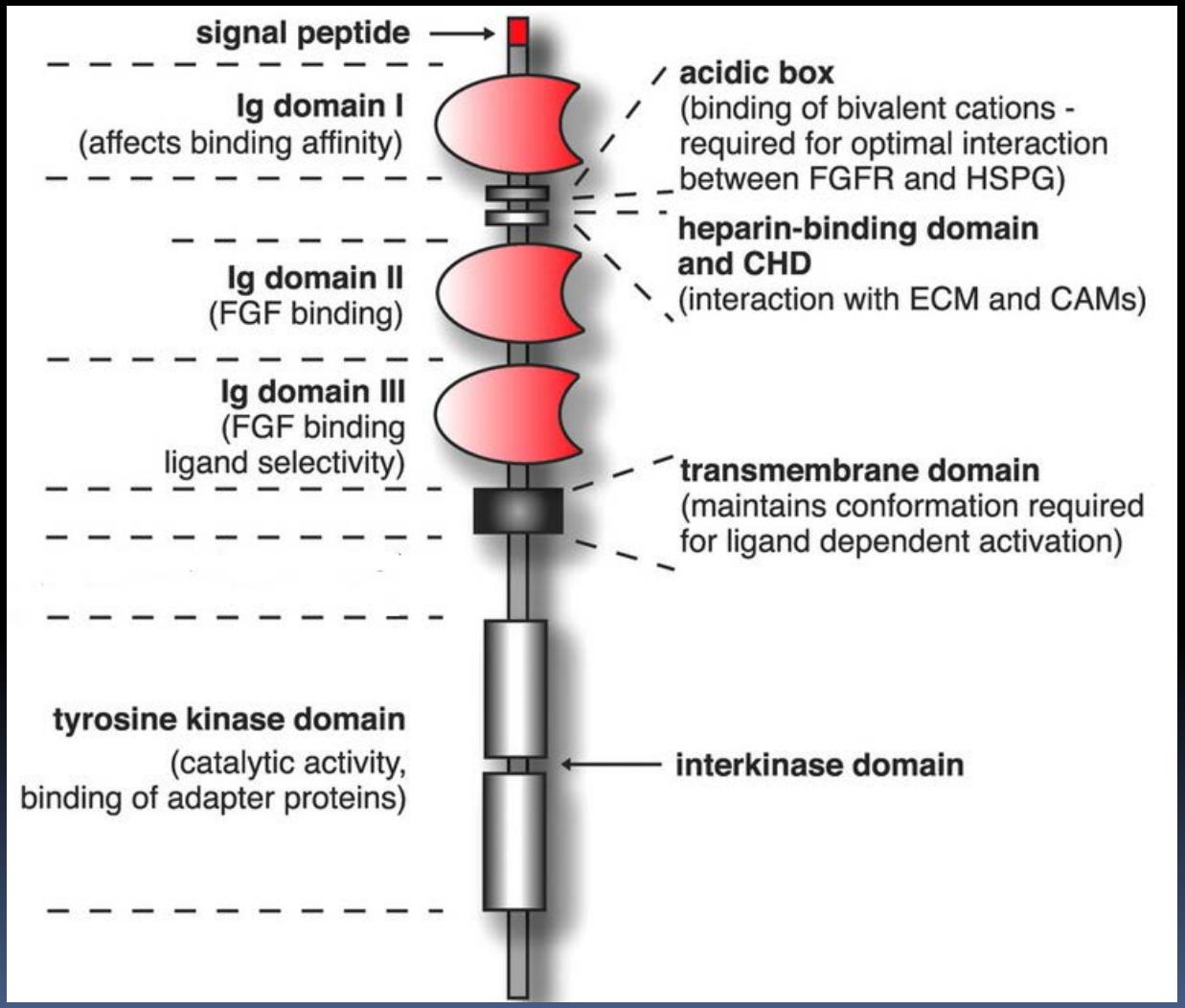
Background

- Facial primordia outgrowth depends on epithelial mesenchymal interactions
- 6th week of embryonic development
  - Frontonasal prominence
  - Medial nasal process (MNP)
  - Lateral nasal process (LNP)
  - Maxillary process (MxP)
  - Mandibular process (MP)



Kaufman MH, 1992

FGFR Protein



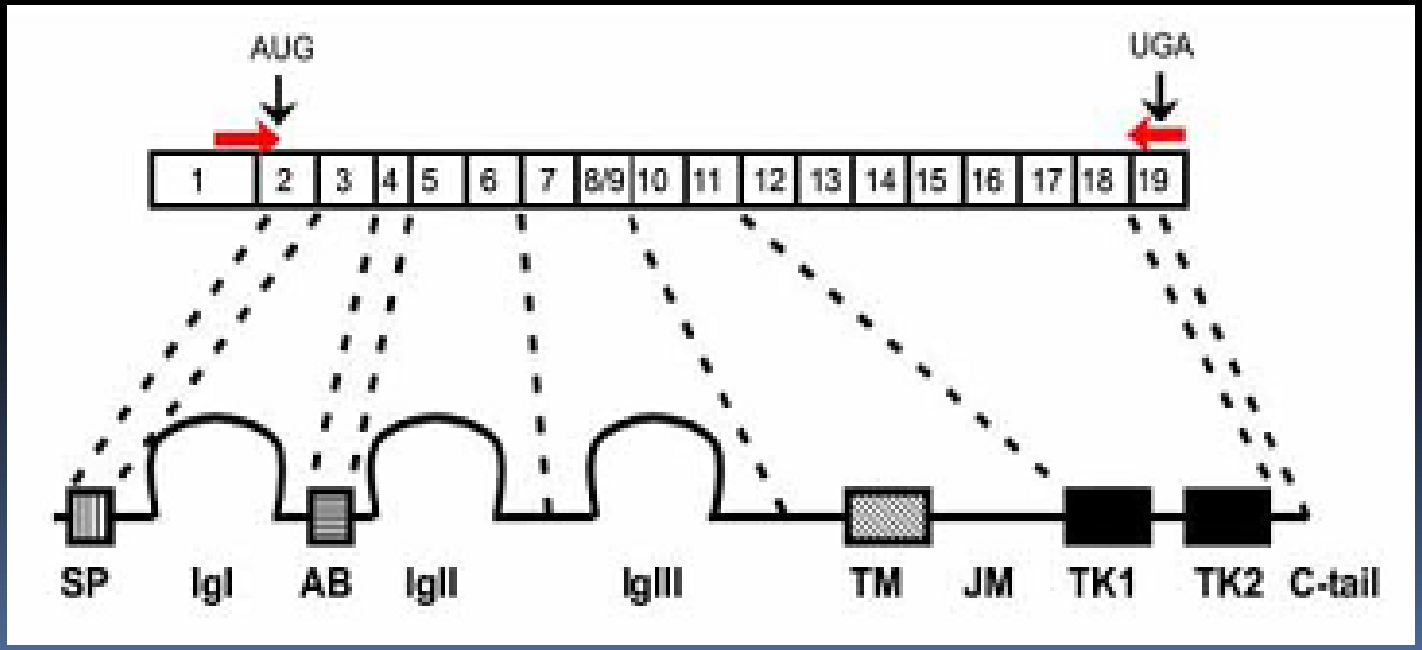
Böttcher & Niehrs, 2005

Background

- FGF signaling during osteogenesis and chondrogenesis
- FGFR family
  - Most common genetic mutations in syndromic craniosynostosis
- Fgfr2 mutations
  - Crouzon Syndrome, Pfeiffer syndrome, Apert syndrome and Jackson-Weiss syndrome

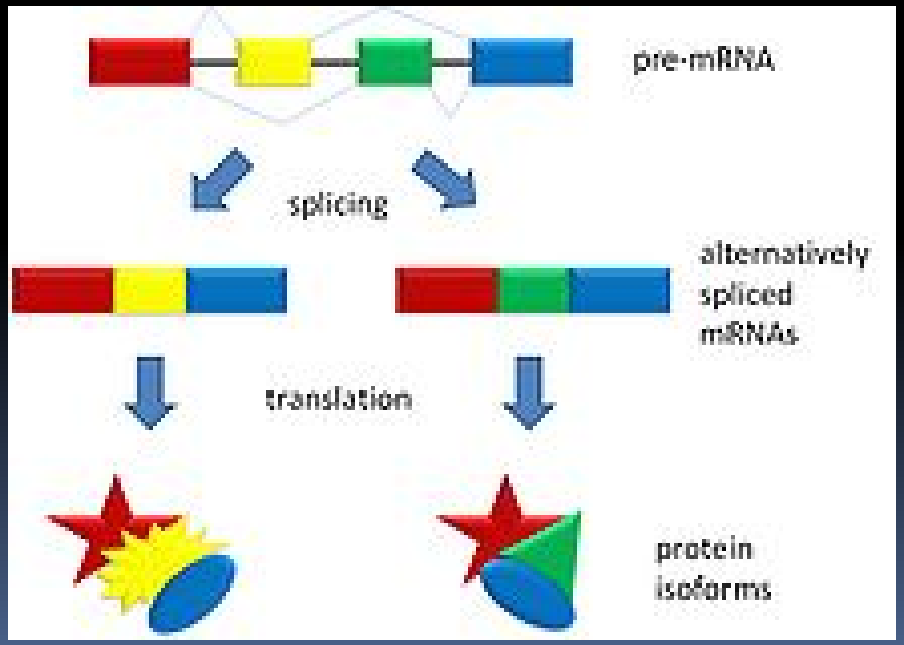
Genomic organization

- Mouse FGFR2 gene
  - 19 exons
  - 120kb segment of DNA



Alternative splicing

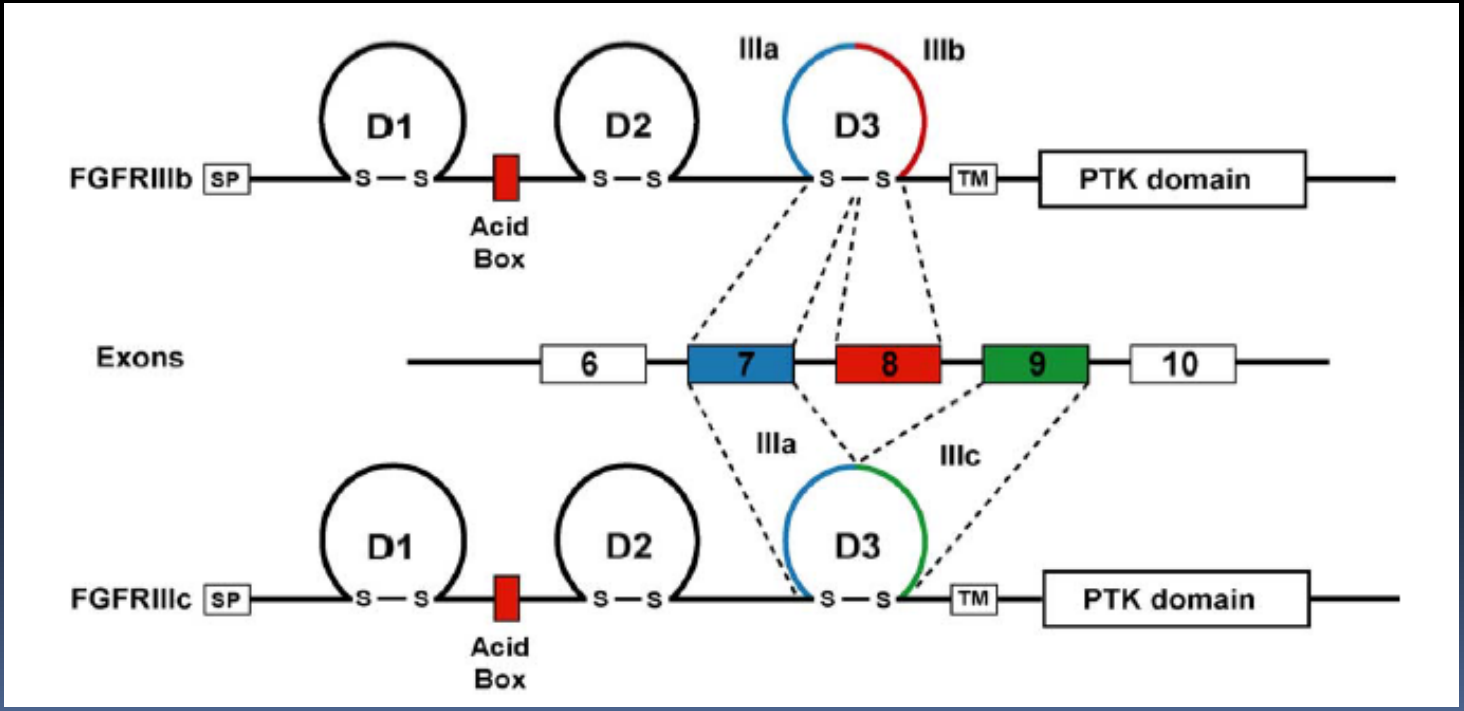
- Alternative mRNA splicing leads to various isoforms of FGFRs
  - 1 FGFR2 gene = multiple functionally distinct receptors



FGFR2 Isoforms

A. C-terminal half of IgIII:

- FGFR2 IIIb – epithelial cell specific
- FGFR2 IIIc – mesenchymal cell specific

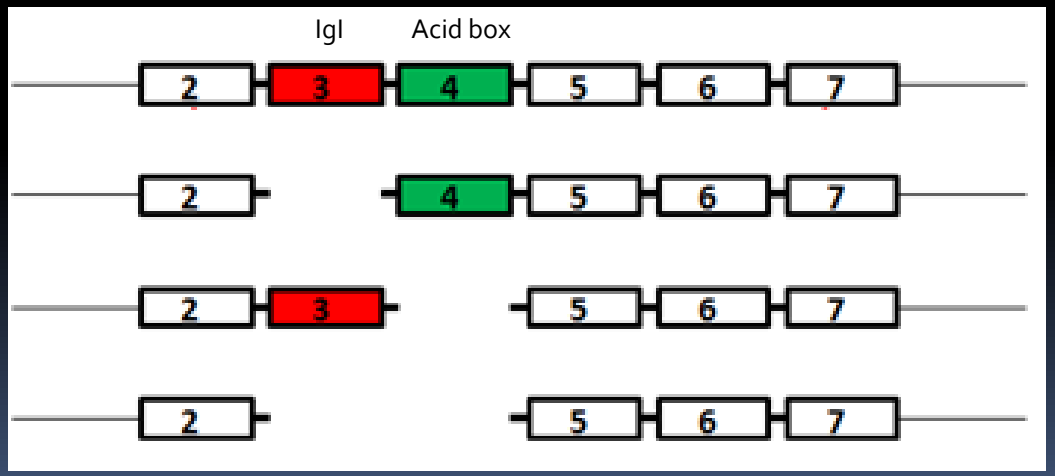


Eswarakumar et al., 2005

FGFR2 Isoforms

B. IgI domain & acid box

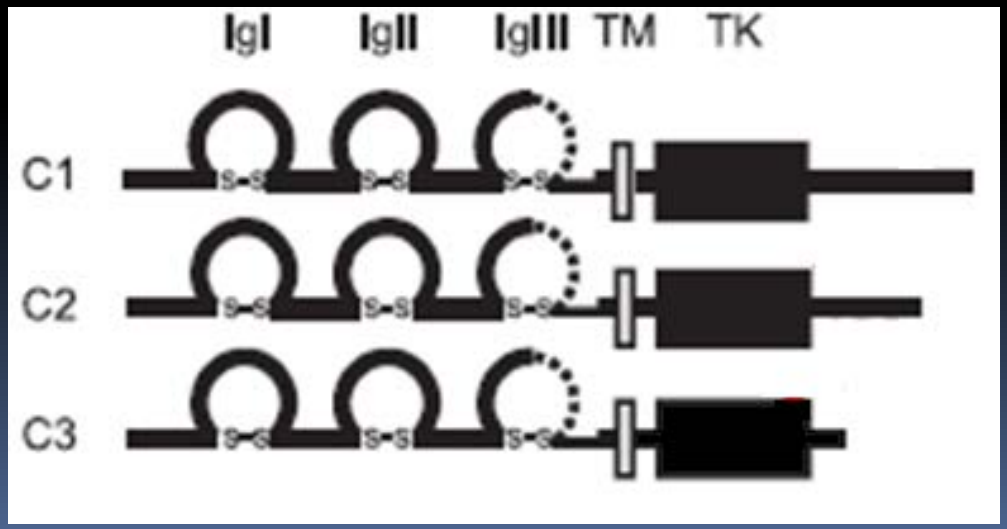
- - (IgI + AB) = increased ligand affinity
- - (IgI + AB) = autoinhibitory role?



FGFR2 Isoforms

C. Length of C-terminal region

- C1
- C2 = 34 amino acids shorter than C1
- C3 = 19 amino acids shorter than C2
- Transforming activity: C3 > C1/C2

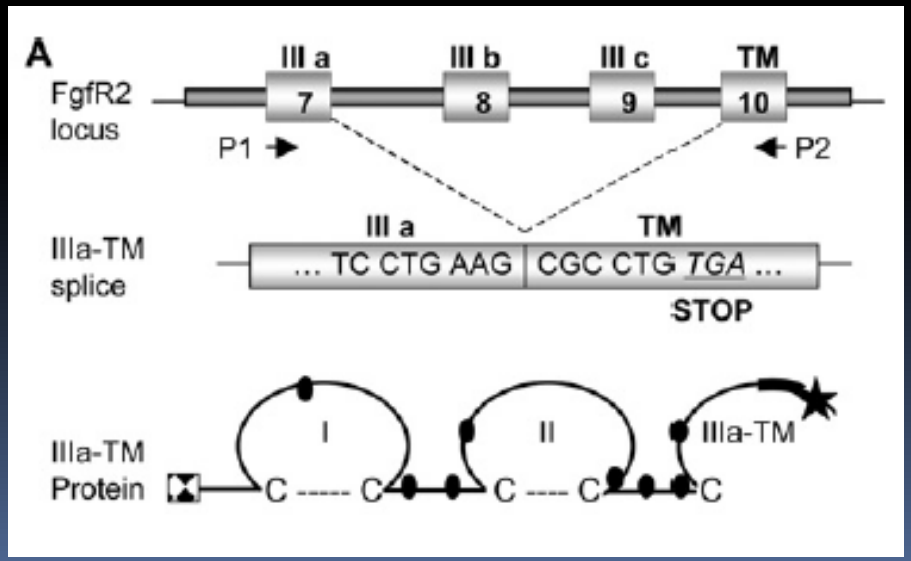


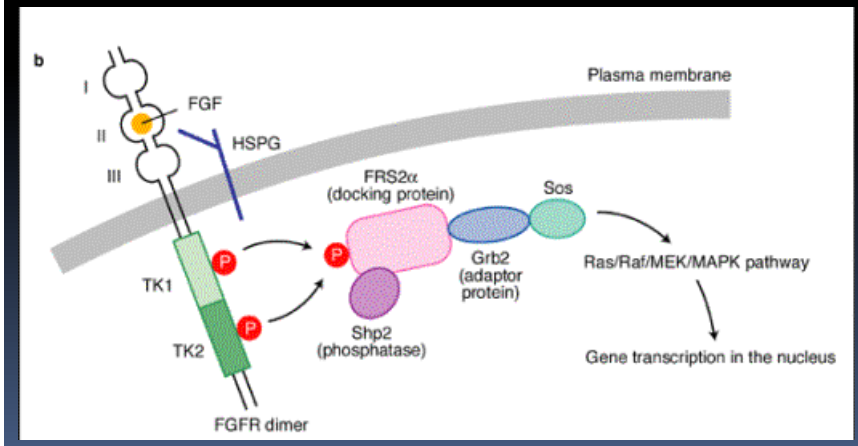
Cha et al., 2009

FGFR2 Isoforms

D. Soluble FGFR isoforms

- Lacks the TM domain
- Aberrant splicing of exon 7 into exon 10 → premature termination codon containing transcript

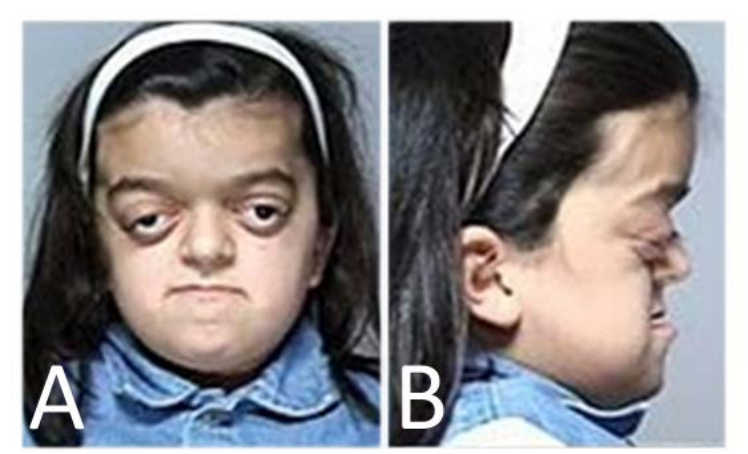


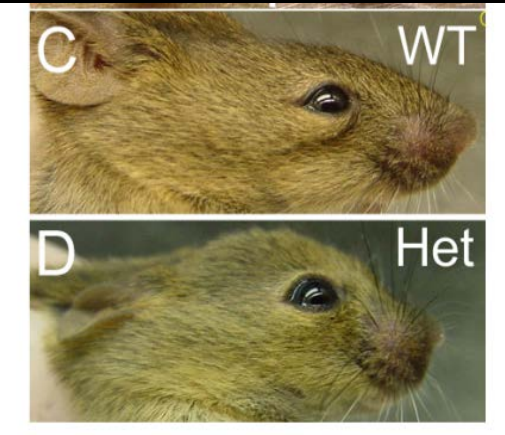


Wheldon et al., 2011

$Fgfr2^{W290R}$ Mouse Model of Crouzon syndrome

- Phenotypic features
  - Short snout
    dome-shaped skull
  - Retruded mid-face
  - Variable septal deviation
  - Decreased skull length
  - Dome-shaped skull
  - Malocclusion





Mai et al, 2010

Hypothesis

1. FGFR2 isoforms and their ligands have distinct temporal, spatial and quantitative expression in the midfacial region of WT mice at critical stages of mid-facial development; and

2. The W290R mutation in FGFR2 results in the production of alternatively spliced variants of FGFR2.

Aims

1. To characterize the temporal, spatial and quantitative expression of specific isoforms of FGFR2 in four distinct areas of the developing mid-face of wildtype (WT) mice at critical stages of midfacial development (E9.5, E10.5, E12.5).

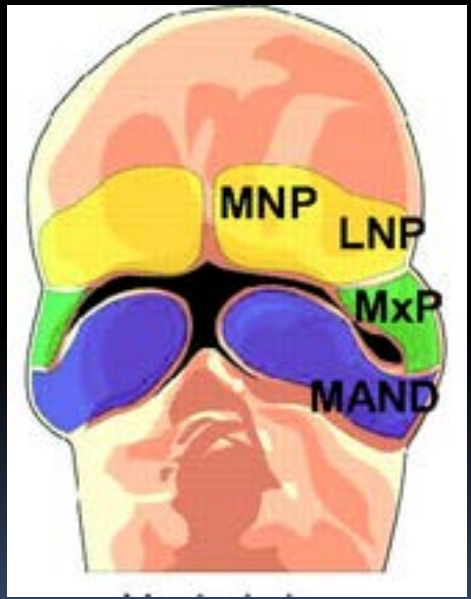
2. To determine whether the W290R mutation of FGFR2 results in the generation of additional isoforms.

Significance

- Increasing evidence that some tissue-specific effects of FGFR2 mediated by FGFR2 isoforms
- Characterization of expression patterns of FGFR2 isoforms in different regions of the face never been reported in literature
- The proposed experiment will provide insight into potential tissue-specific biological processes unique to the mid-facial region

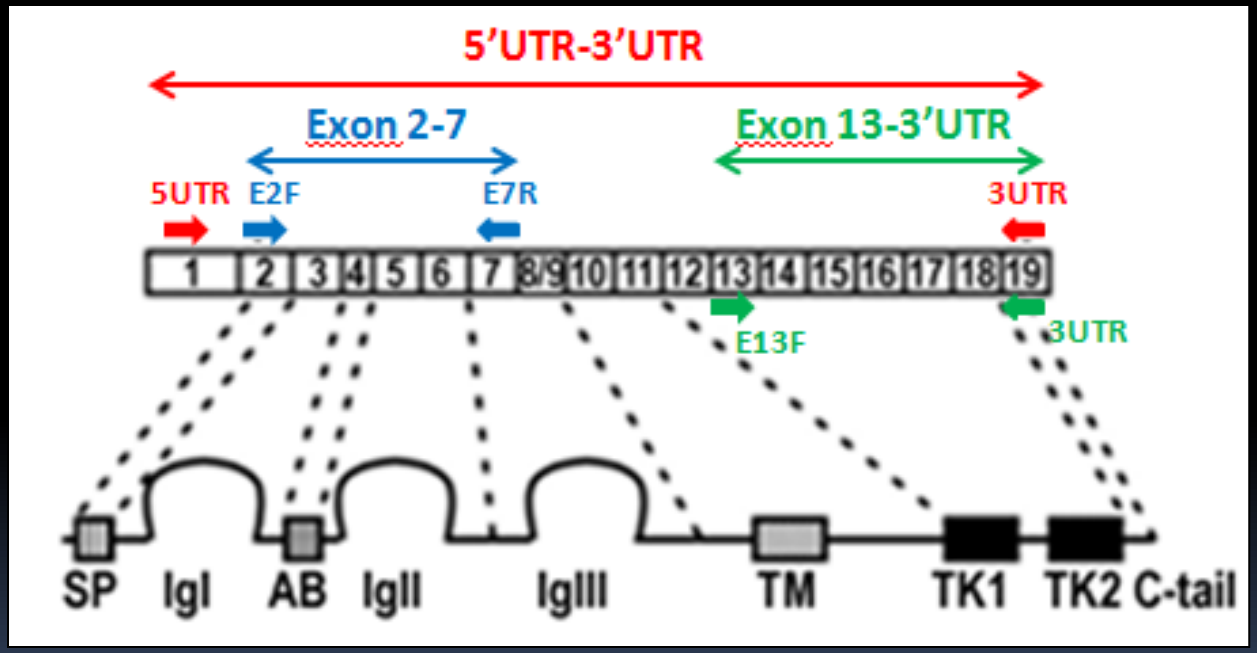
Materials & Methods

- AIM 1: *To characterize the temporal and quantitative expression of specific isoforms of FGFR2 in four distinct areas of the developing mid-face of wildtype (WT) mice at critical stages of midfacial development (E9.5, E10.5, E12.5).*
  - Dissection: LNP, MNP, MxP, MN of E9.5, E10.5, E12.5 embryos
  - RNA extraction: RNA harvested and reversed transcribed
  - PCR amplification of isolated cDNA



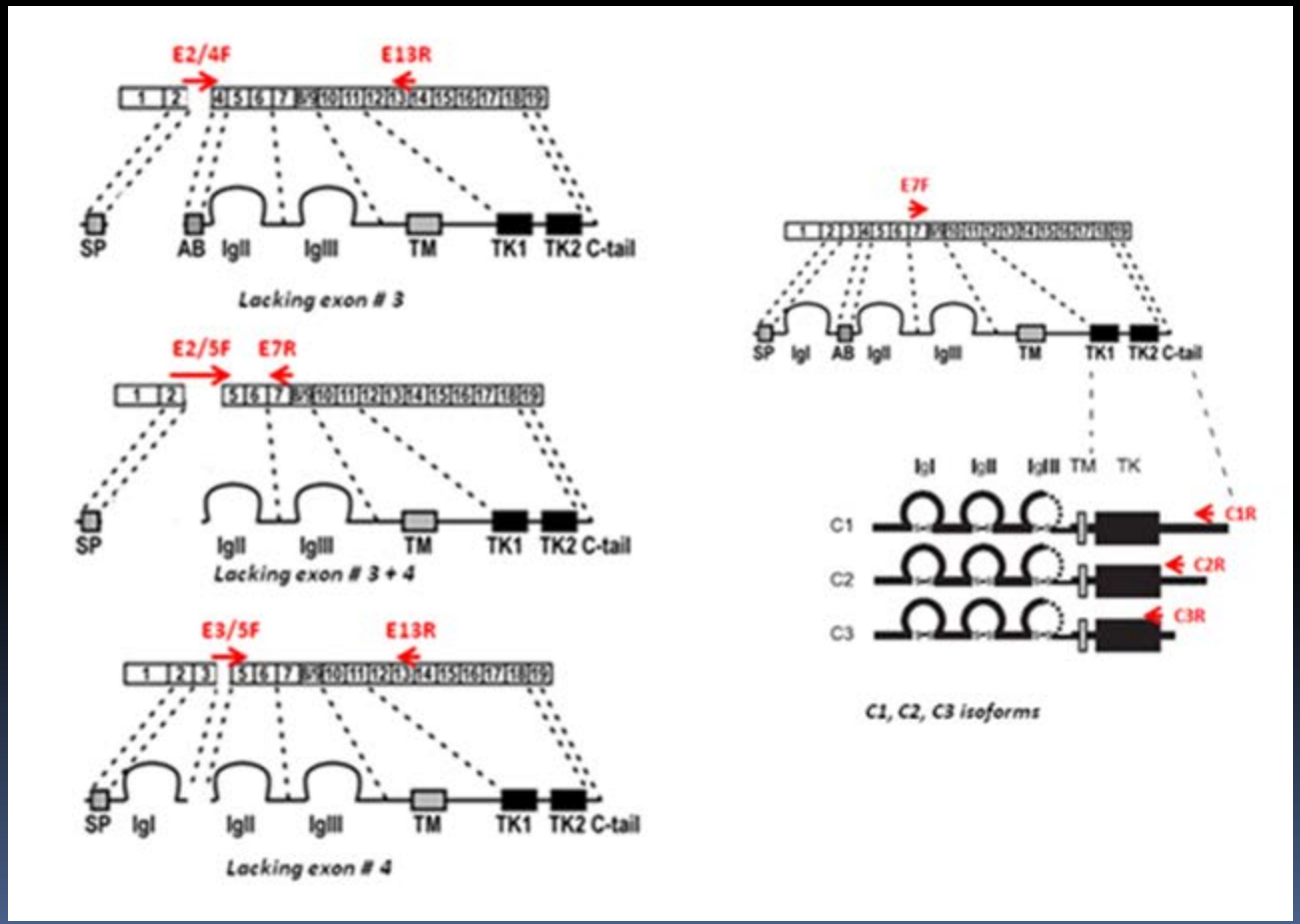
Materials & Methods

- PCR amplification



Materials & Methods

- Real time RT q-PCR

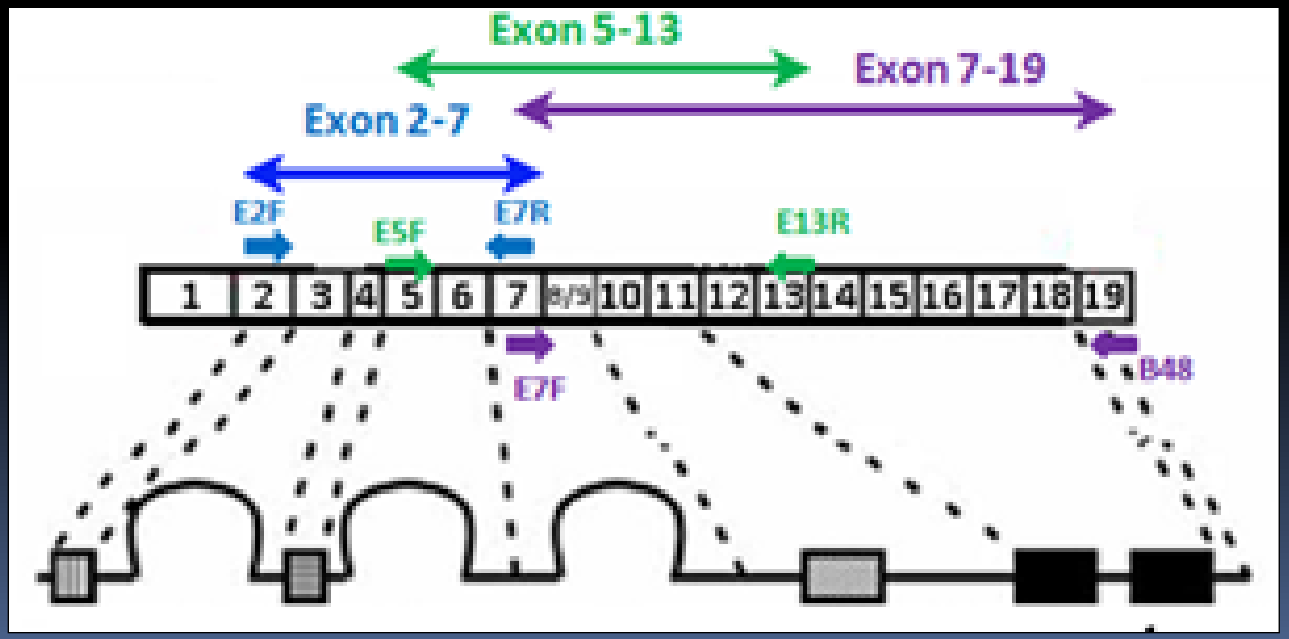


Materials & Methods

- AIM 2: *To determine whether the W290R mutation of FGFR2 results in the generation of additional isoforms*
  - Dissection: craniofacial tissues of E16.5 embryos
  - RNA extraction: RNA harvested and reversed transcribed
  - PCR amplification of isolated cDNA

Materials & Methods

- PCR amplification
  - Missing exons 3 and 8/9
  - Missing exons 3, 8/9 and part of 10
  - Missing exon 3, 8/9, 10 and 11

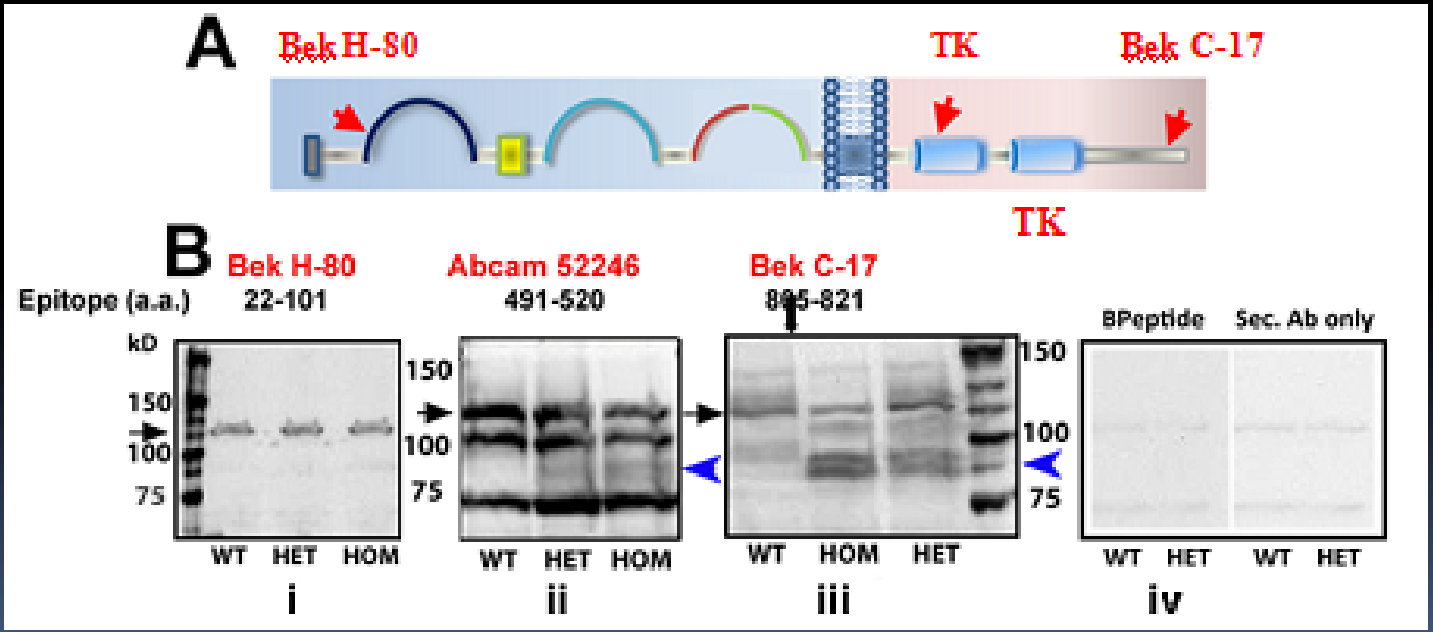


Analysis of data

- Gel electrophoresis
  - Observed vs. expected bands
- Relative quantitation
  - GADPH expression as internal standard for normalization
  - Relative difference in expression of genes of interest determined with the use of threshold cycle C_t

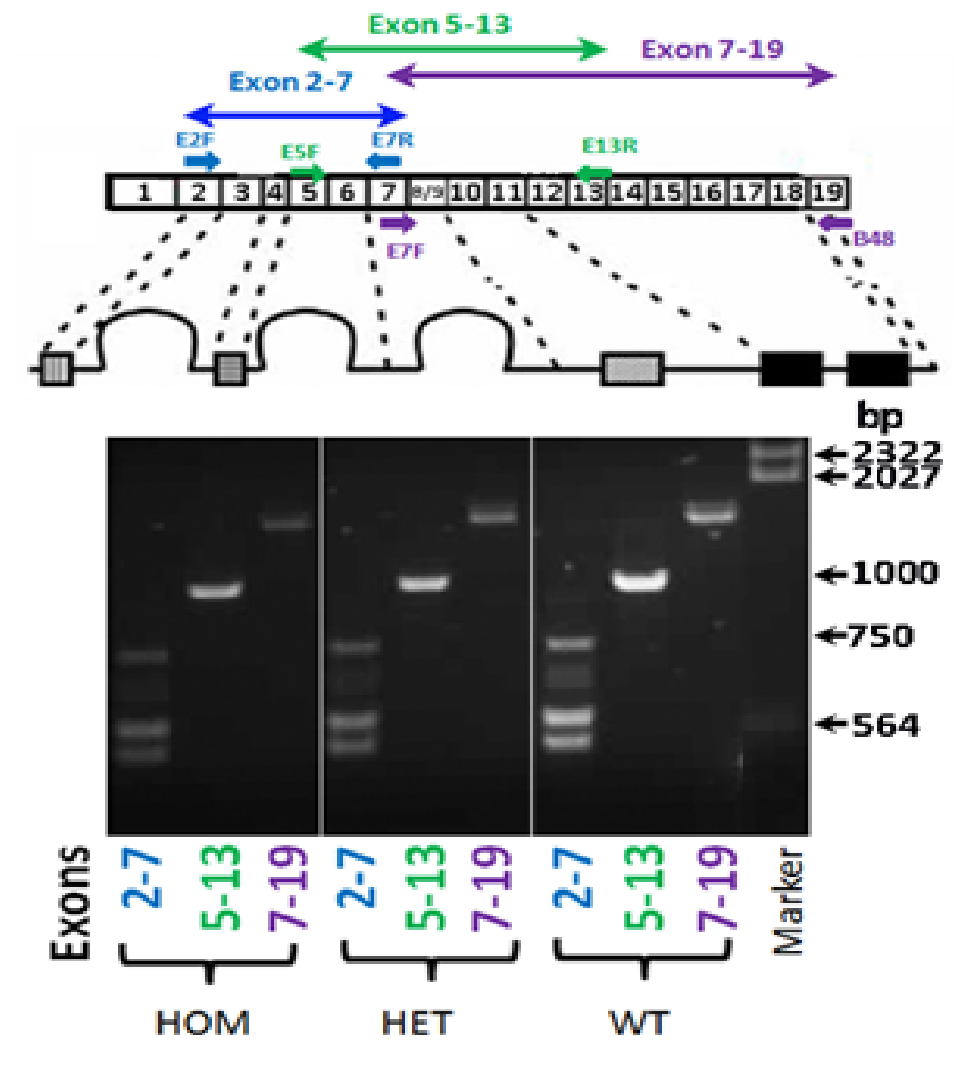
Preliminary data (Aim # 2)

- Western blot analyses using 3 antibodies against specific epitopes in FGFR2
- Additional bands (80-100kD) in HET and HOM samples



Yamini, 2012

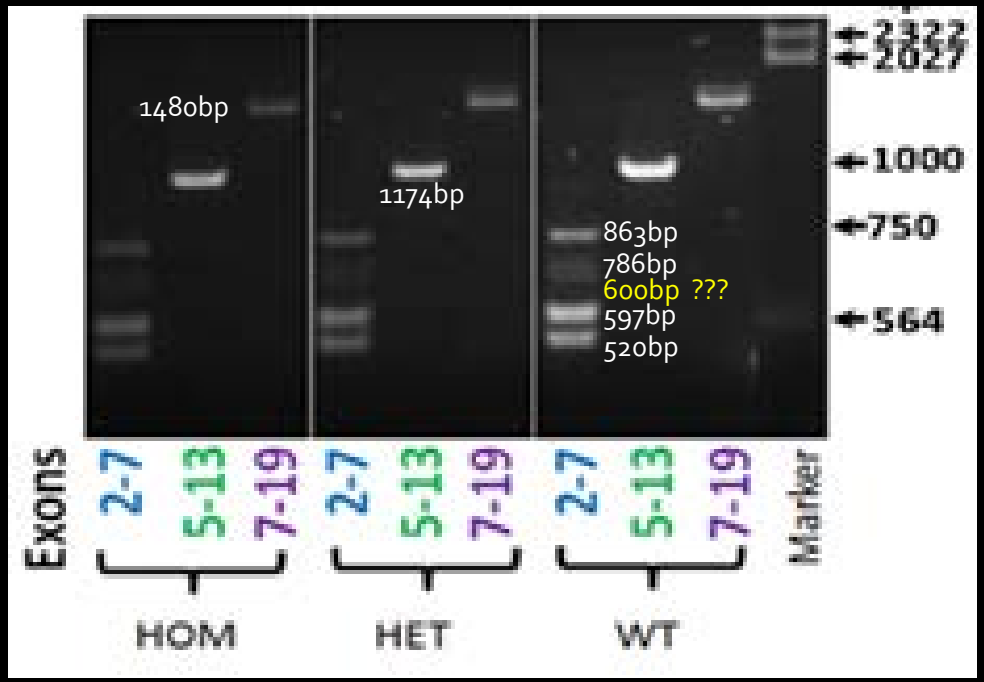
Results (Aim # 2)

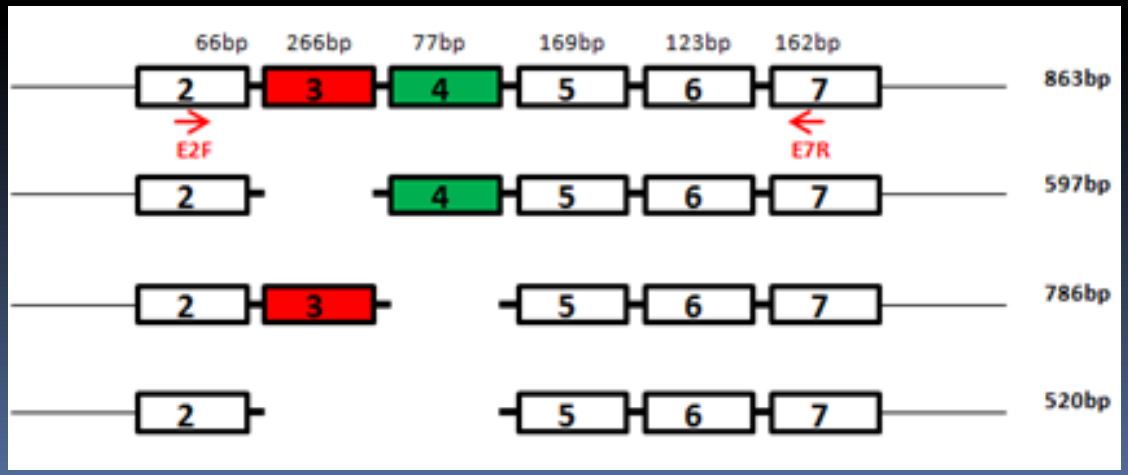


Pattern and size of fragments identical in all three genotypes

Absence of mutant specific isoforms

Results (Aim # 2)





Results (Aim # 2)

- Additional bands in HET and HOM samples = cleavage products?

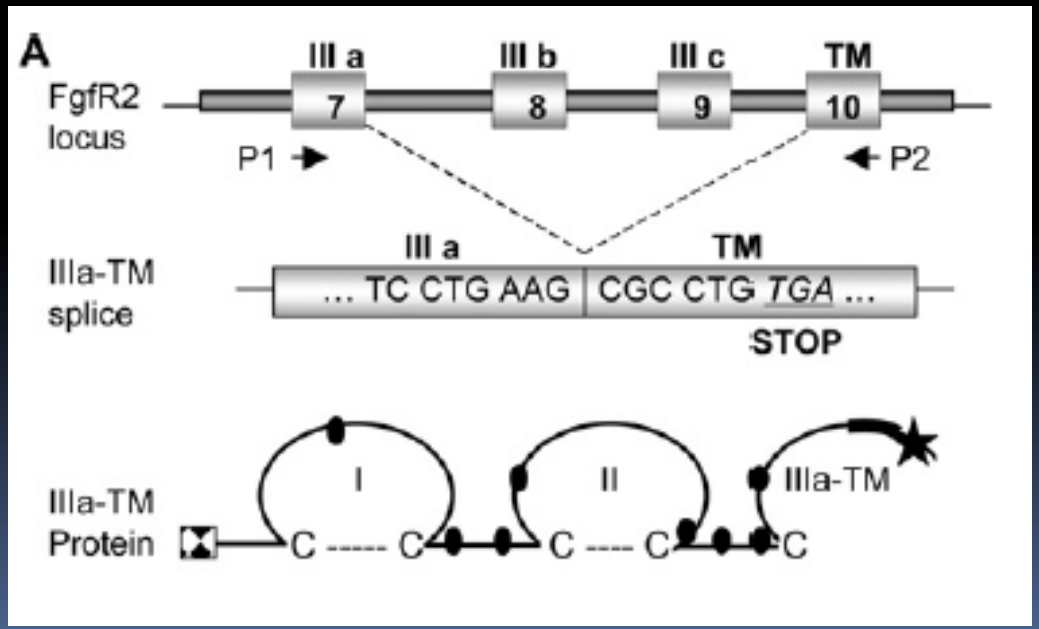
~~**Presence of alternatively spliced mRNA transcripts**~~

OR

Proteolytic cleavage of receptors

Results (Aim # 2)

- *Wheldon et al, 2011*
  - Soluble truncated FGFR2 molecule encoded by a premature termination codon containing transcript



Results (Aim # 2)

- *Pandit et al, 2002*
  - Transmembrane form of FGFR3 undergoes proteolytic cleavage to produce soluble extracellular form of FGFR3