Evolutionary aspect of BMP15 function in folliculogenesis

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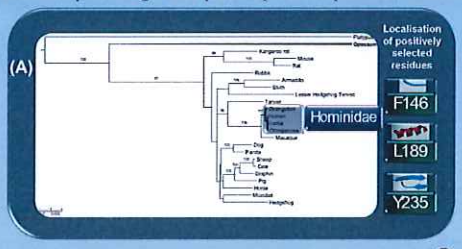
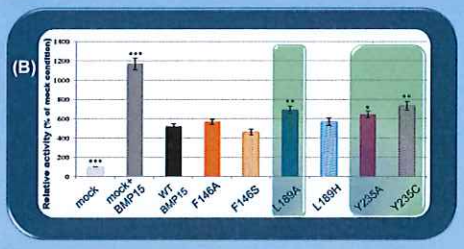
Background:

Bone Morphogenetic Protein 15 (BMP15) is a TGFβ-like oocyte-derived growth factor involved in ovarian folliculogenesis as a critical regulator of many granulosa cell processes and ovulation rate. Alterations of the BMP15 gene have been found associated with different ovarian phenotypic effects depending on the species, from sterility to poly-ovulation in sheep, slight subfertility in mouse or associated with primary ovarian insufficiency (POI) in women. We undertook phylogenetic analysis on several TGFβ/BMP family members expressed by the ovary and showed that BMP15 has a very strong divergence and a rapid evolution compared to others. We then used different approaches to investigate the functional evolution of BMP15 among mono and poly ovulating species.

Positive selection

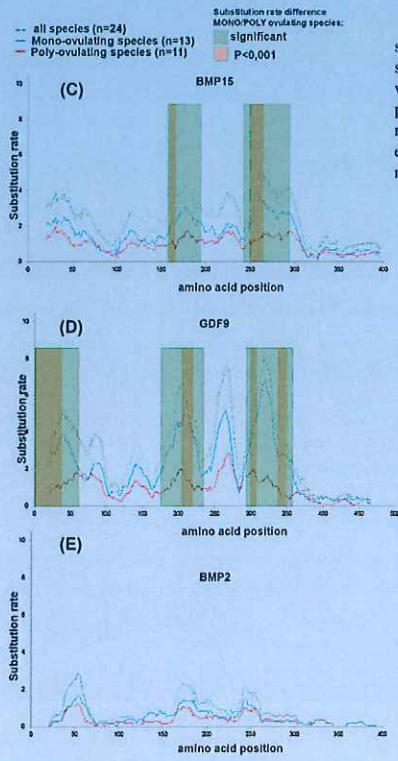
We analyzed 24 BMP15 mammal sequences by branch-site models from PAML packages and detected signals of positive selection in the hominidae clade corresponding to F146, L189 and Y235 residues in human BMP15 (A). The biological importance of these residues was tested functionally after site directed-mutagenesis in a COV434 cells luciferase assay. By replacing the positively selected amino acid either by alanine or the most represented residue in other studied species, only L189A, Y235A and Y235C mutants showed a significant increase of BMP15 signaling when compared to wild type (B). Interestingly, the Y235C mutation was previously identified in association with POI in women. It seems that the Y235 and L189 positions are important to limit the BMP15 activity in hominidae preventing a anticipated depletion of primordial follicular pool observed in POI patients.

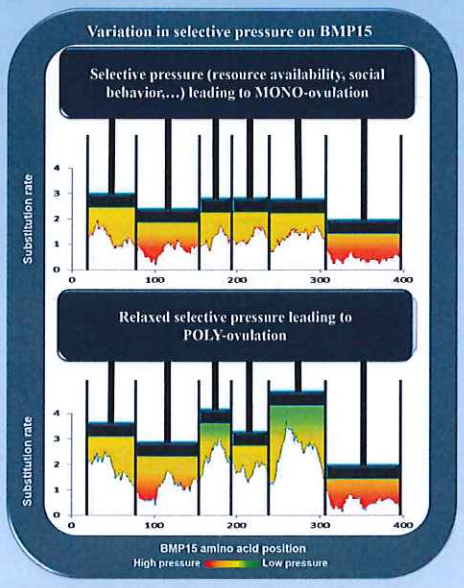


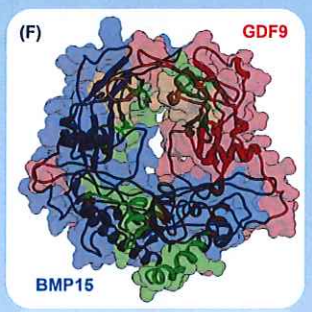


Substitution rates

We used parsimony algorithm to evaluate the amino-acids substitution rate of some proteins implicated in the ovarian function regarding the mono or poly-ovulating status of the species. On a panel of 24 mammals (13 mono-ovulating, 11 poly-ovulating), we demonstrated a better conservation of some areas specifically in BMP15 (C) and its paralog GDF9 (D) within mono-ovulating species. No such difference in the substitution rate regarding ovulation status was observed in the 9 other proteins tested (E, BMP2 as example). The *in silico* 3D structure prediction of BMP15 and GDF9 based on TGFβ1 model hypothesized the implication of these areas in dimer formation and stability (F).

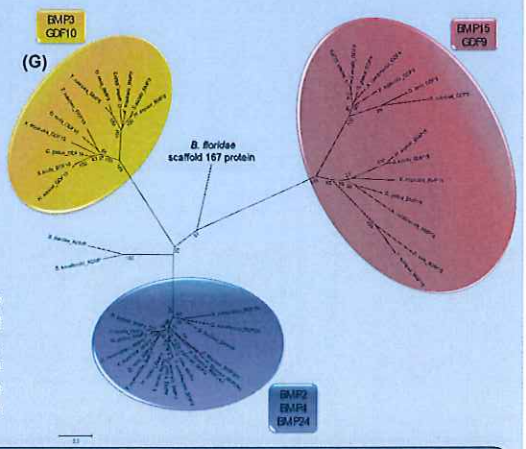






BMP15 evolutionary origin

A maximum likelihood phylogenetic study of BMP15 related proteins in deuterostomes revealed that *BMP15* and *GDF9* genes diverged from the same ancestral gene along with *BMP3* and *GDF10*, two other paralogous genes (G). This is confirmed by a closely related sequence in *Branchiostoma floridae* included in a bloc of synteny shared with human *BMP3*, *GDF10*, *BMP15* and *GDF9*. A substitution rate analysis for all branches of the phylogenetic tree revealed a progressive increase, from acorn worm to eutherian, in the conservation of an area known to be implicated in the expression of the BMP15 mature protein (not shown). All together, these data probably reflect a progressive acquisition/improvement of BMP15/GDF9 specific function in ovarian folliculogenesis in mammals.



In conclusion, this study evidences that BMP15 was submitted to a positive selection pressure in the hominidae clade. Some residues under positive selection like Y235 are of great importance for the normal function of the protein and thus for female fertility. We also demonstrate that BMP15 and GDF9 proteins are highly conserved within the mono-ovulating species in spite of the global high substitution rates in mammals. We propose that the high variations observed in specific areas of BMP15 and GDF9 of poly-ovulating species probably disturb the dimer formation and stability allowing poly-ovulation to occur.