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Bone Morphogenetic Protein (BMP-2/BMP-7) Heterodimer and BMPR1A, BMPR2 Polymorphism in Simple Fractures among Sudanese Patients

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Abstract

BACKGROUND: Bone morphogenetic proteins are responsible for activating mesenchymal stem cells into osteocytes. This effect is signaled by serine-threonine kinase receptors called bone morphogenetic protein receptors. BMPR1A and BMPR2 polymorphisms were not reported to be associated with bone healing process.

AIM: The objective of this study was to investigate BMP-2/-7 heterodimer and BMPR1A/BMPR2 polymorphism with fracture healing progress.

SUBJECTS AND METHODS: This is a patients-control study conducted in selected hospitals in Khartoum, Sudan (Thirty patients and thirty controls). Blood samples taken from patients and healthy controls. Patients were followed by clinical examination until the point of functional recovery. Quantitative ELISA and protein-pull down assay done to BMP-2 and BMP-7. Genomic DNA extraction and PCR/RFP and sequencing done to BMPR1A and BMPR2 target sequences.

RESULTS: Patients and controls were matching in age and gender. Functional outcome regained after 4.1 months ± 2.6. BMP2/7 complex levels were 288.75pg/mL ± 266.8 and 532.23 pg/mL ± 582.5 in patients and control, respectively (p = 0.021). BMPR2 exhibited single nucleotide polymorphism among all participants, while there was 25% and 22% had variant [A] BMPR1A, 75% and 78% [T] variant BMPR1A in patients and control, respectively.

CONCLUSION: Significant change in plasma BMP-2/-7 heterodimer concentration was observed after trauma but no significant correlation between BMPR1A and BMPR2 polymorphism with fracture healing.

Introduction

Bone morphogenetic proteins (BMPs) are responsible for bone development and fracture healing process [1], [2]. BMPs have specific serine-threonine kinase membrane bound receptor. They are named bone morphogenetic receptor I and bone morphogenetic receptor II (BMPR-Ia, and BMPR-II). BMPR-Ia is more sensitive to BMP-2, while BMPR-II is necessary for the phosphorylation in BMPR-Ia [3]. A previous study confirmed through crystallography, the existence of BMP-2 homodimer binding to BMPR1a, in addition, the previous studies reported the osteogenic effect of BMP2/BMP4 and BMP-2/BMP-7 heterodimer on stimulation of BMPR1A [4], [5]. Recent study reported that BMPs heterodimer is more potent than homodimers in rats [6]. However, whether it is responsible for faster fracture healing which has yet to be revealed.

In general, BMPs bind to BMPR1, and the latter recruit BMPR2 which phosphorylate the cytoplasmic glycine-serine rich domain within BMPR1; this process increases BMPs affinity to BMPR1, that is, BMPR2 which is 530 amino acids long protein regulates BMPs bonding to BMPR1 [5], [7]. BMPs usually bind in higher affinity to BMPR1 than BMPR2; however, BMPs bind in higher affinity to a BMPR1 and BMPR2 heterodimer [8]. BMPR2 binds to all BMPs; in contrast, synonyms to BMPR1 is activin-like kinase (ALK), and it has many types; however, BMP-2 binds strongly to ALK-3 (BMPR1A), while BMP-7 binds to ALK-2 (BMPR1A)-overexpression of BMPR1A would stimulate bone formation without presence of BMP, which makes it the most important receptor [7].

BMPR2 mutation was associated with pulmonary hypertension [9], while for transforming growth factor β1 (TGF-β1) works on transforming growth factor β-receptors (TGF-β type-1 and TGF-β type-2) [10]. Interestingly, TGF has two receptors; TGFR1 and TGFR2-and binding of TGF to TGFR1 recruits TGFR2 which phosphorylates the glycine-serine rich domain within TGFRI to activate it-all that is similar to BMP receptors, as well [11].

Expression levels of BMPR2 were observed to increase with expression of BMP-2, BMP-4, and BMP-7 during early onset of fracture [12]. Signal transduction from L45 loop region in Kinase domain in BMPR1 is necessary for phosphorylation [8].

With respect to bioactivity, it was reported that BMPs heterodimers are more potent than homodimers [13]. While the effect of some BMPs is variables, they tend to share some functions, for example, BMP-2, BMP-6, and BMP-9 which were responsible for transforming mesenchymal stem cells into osteoprogenitor cells, while BMP-2, BMP-4, BMP-7, and BMP-9 were responsible for changing osteoprogenitor cells into osteoblasts – then, all BMPs except BMP-3 trigger osteocytes formation from osteoblasts [13]. Not only does BMP-2 and BMP-7 had their osteogenic stimulation and suppressing myocytes formation from mesenchymal stem cells but also TGF-β1 was found to carry out the same process by activating BMPR1A, similarly [11]. This means that both BMPs and TGF-β1 not only share a common function but also a common signal transduction pathway.

Mutation in BMPR1A DFQ (107–109) REL profoundly lowers its affinity to BMP-2 [14], [15]. Yet, it is not known if such mutation exists in the human variant.

The objective of this study was to investigate the existence of BMP heterodimer and BMPR1A, BMPR2 polymorphism.

Materials and Methods

This is a patients-control study conducted in the five main hospitals providing orthopedic surgery care in Khartoum state in Sudan. We conducted this study during the period from January 2018 to January 2019. Inclusion criteria were as follows: All healthy individuals with no comorbidities in the patients group and control group and males and females from 18 to 65 years old. In the patients' group, including subjects with closed long bone fractures, while exclusion criteria were for those with comorbidities or bone disease.

Thirty patients (20 males and ten females) and 30 controls (20 males and ten females) consented to participate in the study. After taking demographic and anthropometric measurements and clinical examination, blood samples were taken (6 mL) into heparin and EDTA Vacutainers. Blood samples from the patient's group were collected at different intervals from fractures. Plasma was separated and stored at −80°C freezer to be used for protein pull-down assay.

Protein-protein interaction was tested through protein-pull down assay approach previously reported method [16]. Using ELISA kits for BMP-2 and BMP-7 from R&D systems (R&D systems MA, USA) - following manufacturer's protocol [17].

Human genomic DNA was obtained from whole blood using Quick Blood Genomic DNA Extraction Kit (DSBIO, China) – adhering to the manufacturer's protocol [18]. Polymerase chain reaction (PCR) of BMPR1a using primers reported earlier [19]. Primers previously reported were used in the PCR of BMPR2 exon 11 [20]. Expected product size for BMPR1a gene is 514 bp, and for BMPR2 target 310 bp. PCR amplicons were digested, and restriction fragment polymorphism was performed using TspR1, Bae1, HpyCH4V, and BsfR1 (NEB, USA). PCR products were sent to BGI (Hong Kong) for DNA sequencing.

Long bone fracture healing follow-up: A series of clinical examinations and radiographic images were conducted at specified follow-up sessions. Specialized physicians assessed physical and functional recovery. Follow-up of patients up to point of regaining functional recovery by physical component score [21], that is, for the upper limb function in daily activities such as combing, bathing, lifting, and taking care of hygiene, while for the lower limb, standing, walking unassisted, climbing stairs, and squatting.

Data analysis

We used Statistical Package for the Social Sciences (SPSS) version 25 for windows. Date was expressed as mean (m) and standard deviation (SD). Correlation (Pearson and Spearman), Chi-square, and unpaired t-test were performed. Odd ratio (OR) was performed using MedCalc for Windows, version 15.0 (MedCalc Software, Ostend, Belgium), $p < 0.05$ was considered statistically significant.

Results

Two-thirds of the participants were male, while one-third were female. Both patients and control groups matched in terms of age and gender (Table 1). The plasma concentration of BMP2/BMP-7 complex was 288.75 pg/mL ± 266.8 in patients and 532.23 pg/mL ± 582.5 in controls. The patients group mean plasma concentration was significantly lower than in controls ($p = 0.021$). No significant difference observed in the physical component score between patients and control (OR = 1.87, $p = 0.27$). BMPR2 PCR product was further digested by restriction enzymes TspR1 and HpcCH4V, leaving equal products size 100–200kb. PCR products sequencing was done to both patients and control groups; in BMPR1A sequencing (A5659T) was observed (Figure 1), while in BMPR2 sequencing (G1472A) was observed (Figure 2). BMPR1A had 2 variants in the patients group; [A] variant was 25% of subjects, while [T] variant was 75%, while the control group had similar distribution as well; [A] variant 22%,

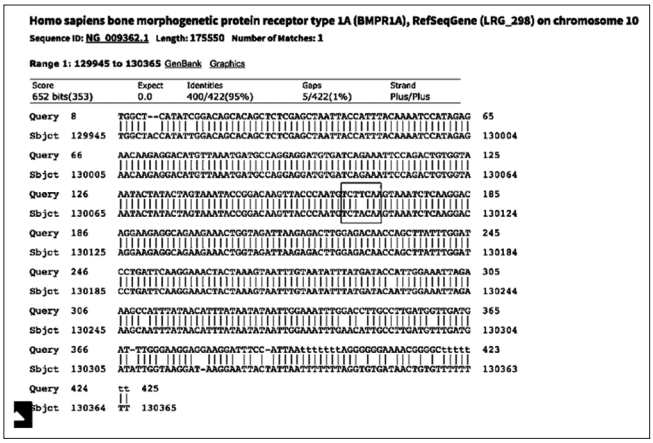

Figure 1: BMPR1A DNA sequence BLAST. Sequence alignment showing A5659T variant

while [T] variant 78% (Table 2). No significant difference was observed between subjects with [T] variant and [A] variant (OR = 1.28, p = 0.79). On the other hand, BMPR2 was 100% carrying the [G] variant in both patients and control groups. No significant difference was observed in BMPR1A variants and BMP heterodimer concentration or fracture healing time (Table 2).

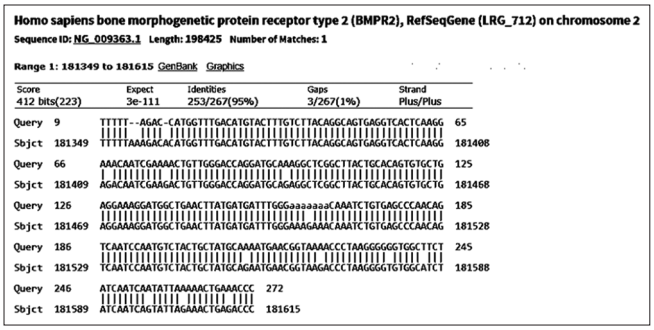

Figure 2: BMPR2 DNA sequence BLAST. Sequence alignment showing A variant (G1472A)

Discussion

The patients and controls in this study were well matched in age and gender. Knowing that, BMP-7 created a heterodimer with BMP-2-corroborated by recent *in vitro* studies [22]. The heterodimer concentration was much greater than the homodimer; implying the possible role of BMP-2/7 in fracture healing – coinciding with previous reports that heterodimers are more potent than homodimers [6]. In this study, there was a significant drop in the BMP heterodimer concentration, which could have a role in the pathophysiology of fracture healing. On sequencing of BMPR1A, it was observed that [T] variant exists abundant than [A] variant (75% and 78%) (25% and 22%) in patients and control (Table 1) – which contradicts with previously reported in the study conducted in Poland, where [A] allele was more than 90% [19]. That is expected since [A] variant was

Table 1: Demographic data, BMPR1A variants, and its correlation with BMP-2/7 complex, and Healing time

Demographics	Patients	Control	Statistics
Age (years) (m ± SD)	36.8 ± 15.5	31.2 ± 9.3	t = 1.6 p = 0.96
Gender			
Males	20	20	
Females	10	10	
BMP-2/7 complex (m ± SD)	288.75pg/mL ± 266.8	532.23 pg/mL ± 582.5	p = 0.021

associated with renal anomalies, and all participants in this study did not have any known comorbidities.

Our findings coincide with previously reported variation in BMPR2 (G1472A) [20]. This variant could imply an adverse outcome if such individuals would develop pulmonary hypertension in the future. This point mutation existing in the kinase domain could also affect the main function of BMPR2, which is phosphorylation of BMPR1A to increase its osteogenic effect [23]. This polymorphism was digested by TspR1 and HpcCH4V; leaving equal products size 100–200kb; which is the first to be noted since it was not displayed at the New England Biolabs database.

Table 2: BMPR1A variants and their correlations with plasma BMP-2/7 complex concentration and fracture healing time

BMPR1A variant	A	T	Statistics
Percentage (%)	25%	75%	
BMP-2/7 complex (m ± SD)	369 pg/ml ± 383	134 pg/ml ± 65	t = 1.0 p = 0.329
Fracture healing time (m ± SD)	2.7 months ± 1.3	3 months ± 1	t = 0.36 p = 0.725

This variant (A5659T) also means that there is overexpression of BMPR1A as well reported in the same study [19]. It is possible that fracture healing despite low BMPs could be due to BMPR1A overexpression, confirmed by previous study [7]. This overexpression could also explain the low BMP-2, BMP-7, and BMP-10 in the blood by negative feedback. On the other hand, such mutation in the kinase domain of BMPR1A might affect the activation of smad and overall osteogenesis; causing slower healing rates as observed on the Sudanese subjects under study.

Even though the overall healing rates among subjects under study was slow, there was no significant association between BMP receptors polymorphism and the fracture healing time (Table 1); eliminating such polymorphism from being a direct contributor to the healing mechanism of fracture healing. Perhaps in the future, an animal trial to assess such polymorphism on fracture healing would confirm such claims.

Conclusion

BMP2/7 heterodimer exist among Sudanese subjects higher than BMP-2 or BMP-7 monomers. The majority of participants had BMPR1A [T] allele. All participants had BMPR2 [G] variant. BMPR1A and BMPR2 polymorphism had no association with fracture healing outcome.

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Declarations

Authorship

The authors confirm that all authors have made substantial contributions to all the following:

- The conception and design of the study, or acquisition of data, or analysis and interpretation of data
- Drafting the article or revising it critically for important intellectual content
- Final approval of the version to w submitted
- Sound scientific research practice.

The authors further confirm that:

- The manuscript (including related data, figures, and tables) has not been previously published by any of the authors nor under consideration elsewhere
- No data has been fabricated or manipulated (including images) to support the conclusions
- This submission does not represent a part of single study that has been split up into several parts to increase the quantity of submissions and submitted to various journals or to one journal over time (e.g., “salami-publishing”).

Plagiarism

- The authors confirm that the work submitted is original and does not transgress the plagiarism policy of the journal
- No data, text, or theories by others are presented as if they were the author’s own
- Proper acknowledgements of other’s work have been given (this includes material that is closely copied, summarized, and/or paraphrased), quotation marks are used for verbatim copying of material
- Permissions have been secured for material that is copyrighted.

Compliance with ethical guidelines

“The author/s declare that this submission is in accordance with the principles laid down by the Responsible Research Publication Position Statements as developed at the 2nd World Conference on Research Integrity in Singapore, 2010.”

“Before commencement of the study ethical approval was obtained from the following ethical review board: *Provide name and reference number.”*

“All procedures were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008.”

“Informed written consent was obtained from all patients for being included in the study.”

Ethical clearance was obtained from the University of Khartoum Research Board (ref. no. 2/2017).

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