The potential consequences of bidirectional promoter methylation on *GLA* and *HNRNPH2* expression in Fabry disease phenotypes in a family of patients carrying a *GLA* deletion variant

MOHAMMED A. AL-OBAIDE, IBTISAM I. AL-OBAIDI and TETYANA L. VASYLYEVA

Department of Pediatrics, School of Medicine, Texas Tech University Health Sciences Center, Amarillo, TX 79106, USA

Abstract. Fabry disease (FD) is a rare inherited disease characterized by a wide range of symptoms attributed to *GLA* mutations resulting in defective α-galactosidase A (α-Gal A) and accumulation of glycosphingolipids. The *GLA* locus is paired in a divergent manner with the heterogeneous nuclear ribonucleoprotein *HNRNPH2* locus mapped in the *RPL36A-HNRNPH2* readthrough locus. As a follow-up to our recent finding of the co-regulation of *GLA* and *HNRNPH2* via a bidirectional promoter (BDP) in normal kidney and skin cells, the potential accumulative influence of BDP methylation and *GLA* mutation on the severity of FD in patients from the same family, two males and two females carrying a *GLA* deletion mutation, c.1033_1034delTC (p.Ser345Argfs) was addressed in the present study. The molecular analyses of the FD patients compared with the control revealed that the expression of *GLA* was significantly low (P<0.05), and *HNRNPH2* showed a tendency of low expression (P=0.1) when BDP methylation was elevated in FD patients, compared with low BDP methylation and high *GLA* expression (P<0.05), and a high trend of *HNRNPH2* expression in normal individuals. The accumulative effects of the mutation and BDP methylation with the severity of the disease were observed in three patients. One male FD patient, a member of the FD family diagnosed with progressive loss of kidney function, hypertension, and eventually a stroke, and the lowest level of α-Gal A enzyme activity showed the highest BDP DNA methylation level. It is concluded that the DNA methylation of *GLA-HNRNPH2* BDP may serve a role in diagnosing and treating FD.

Introduction

Fabry disease (FD) is a rare familial sex X-linked disorder attributed to *GLA* mutations. The disease is a progressive severe genetic condition that worsens over time and is characterized by various symptoms (1,2). Genetic and Rare Diseases Information Center (GARD) reported 76 symptoms that patients with this disease may have (rarediseases.info.nih. gov). The FD symptoms may develop during childhood (classic type) or middle adulthood (atypical type); males tend to have more severe symptoms (1). The *GLA* mutations can cause total or partial decreased activity of α-galactosidase A (α-Gal A) and accumulation of glycosphingolipids, globotriaosylceramide (Gb3/GL-3), and globotriaosylsphingosine (lyso-Gb3) in various cells and organs including the skin, eyes, kidneys, heart, brain, and peripheral nervous system (3,4). The therapeutic approach for FD is enzyme replacement therapy (ERT); this treatment is used to substitute the missing or an altered partially functional α-Gal A (5-8). Additionally, pharmacological chaperone (PC) 1-deoxygalactonojirimycin is used for the treatment of amenable α-Gal A missense mutations (9-11). However, ERT and PC cannot treat all FD symptoms and may cause adverse side effects; thus, persistent symptoms in patients reduce their quality of life (12). Consequently, it is an open question whether FD clinical manifestations are solely the result of α-Gal A malfunction. The *GLA* gene is located at chrX: 101397791-101408013, mapped at the reverse strand of the *RPL36A-HNRNPH2* readthrough locus (chrX: 101390890-101414140) that appears on the forward strand of the complete genomic region NC_000023.11. The *RPL36A-HNRNPH2* readthrough locus is composed of *RPL36A* and *HNRNPH2* genes mapped at chrX: 101390890-101396154 and chrX: 101408133-101414140, respectively. Ensembl (https://useast.ensembl.org/index.html) and ClinVar-NCBI (https://www.ncbi.nlm.nih.gov/clinvar/) databases showed that *GLA*, *HNRNPH2*, and *RPL36A* genes are mapped in the *RPL36A-HNRNPH2* readthrough genomic region in humans and are likely involved in FD and other genetic conditions (Table SI).

In our prior research (12), human cells (adult epidermal keratinocytes, renal glomerular endothelial cells, renal epithelial cells, and 293 T cells) were used to show the function of the bidirectional promoter (BDP) in the expression of *GLA* and *HNRNPH2* loci, which are paired in a divergent manner. One of the primary BDP features is the presence of a susceptible CpG Island (CGI) to DNA methylation (12). The promoters' methylation is associated with various diseases, and the level of CGI methylation is a further factor in the severity of the

Correspondence to: Professor Tetyana L. Vasylyeva, Department of Pediatrics, School of Medicine, Texas Tech University Health Sciences Center, 1400 S Coulter Street, Amarillo, TX 79106, USA
E-mail: tetyana.vasylyeva@ttuhsc.edu

disease (12-14). In the current study, peripheral blood from FD patients from the same family carrying a *GLA* deletion mutation was used to investigate the possible accumulative effects of *GLA* deletion mutation with BDP methylation levels on the severity of the disease in FD patients and on the *GLA* and *HNRNPH2* expression. Blood was chosen to avoid the use of more invasive tissues, such as skin or kidney biopsy.

Materials and methods

Participants. The control group included four healthy individuals, two females (C1 and C2) and two males (C3 and C4). The FD group included two females (FD1 and FD2) and two males (FD3 and FD4) from the same family (Table I). The healthy and FD patients' participants age ranges were 28-45 and 18-39 years respectively. The mean age of the healthy and FD participants was 34.3±7.5 and 31.5±9.3 years respectively. The inclusion criteria for participants were written informed consent, collection of venous blood, understanding and agreeing to comply with the planned study procedures, and for FD patients they had to be part of the family being studied and had to be diagnosed with FD. The females could not be or trying to get pregnant during the duration of the study. Exclusion criteria included being unable or unwilling to provide consent to participate in this study. The healthy subjects, two males and two females were recruited for the study from the outpatient clinic of the Texas Tech University Health Sciences Center (Amarillo). The healthy subjects were referred by primary care physicians and were not on any medications and had no significant medical problems. The healthy and FD participants agreed to the use of their samples and data in scientific research. The blood samples were collected between May 2016-May 2018. The severity of FD in the four patients was measured using the Mainz Severity Score Index (MSSI) and the Fabry Outcome Survey adaptation of the Mainz Severity Score Index (FOS-MSSI) (15).

Genomics databases. The sequences of the *GLA*, *HNRNPH2*, and *RPL36A* and the predicted sequence of the BDP for the divergently paired genes *GLA* and *HNRNPH2* were searched in NCBI-Gene (https://www.ncbi.nlm.nih.gov/gene/), UCSC genome browser (https://genome.ucsc.edu/), and the Ensembl genomics databases (https://useast.ensembl.org/index.html). Genomic tools in the databases were used to retrieve the sequences and identify the forward and reverse strands. The BLAT tool (https://genome.ucsc.edu/cgi-bin/hgBlat?command=start) was used to verify map position and the EMBOSS Programs (EMBL-EBI) (https://www.ebi.ac.uk/Tools/emboss/) were used, EMBOSS cpgplot to discover CpG Islands (https://www.ebi.ac.uk/Tools/seqstats/emboss_cpgplot/) and EMBOSS matcher (https://www.ebi.ac.uk/Tools/psa/emboss_matcher/) and EMBOSS needle (https://www.ebi.ac.uk/Tools/psa/emboss_needle/) to perform DNA alignment analysis.

RNA and DNA extraction from blood and RT-qPCR. The methods used were based on our previous study (12). Briefly, an RNA/DNA purification kit (cat. no. 48700; Norgen Biotek Corp.), was used to extract RNA and DNA from blood cells. RT-qPCR was performed in triplicate using a Taq Universal

SYBR Green One-Step Kit (Bio-Rad Laboratories, Inc.) and quantified using a Bio-Rad iCylcer iQ system and the included software (Bio-Rad Laboratories, Inc.). The Bio-Rad thermocycling protocol was optimized per experimental requirements using the designed primers for *GLA* and *HNRNPH2* and the reference gene *HPRT1* (12). IDT tool (idtdna.com) was used to design the specific primers for *GLA* and *HNRNPH2* expression. The designed primer sets were: *HNRNPH2* forward, 5'-AGTAGTTCTGGTCGTCGTCTA-3' and reverse, 5'-ACACACCAACCTCTAACGATAC-3'; and *GLA* forward, 5'-AGGTTACCCGCGGAAATTTAT-3' and reverse, 5'-GAAACGAGGGCCAGGAAG-3'. Normalization and analysis for the target genes were performed using *HPRT1* as a reference gene (forward primer, 5'-TGAGGATTTGGAAAGGGTGT-3' and reverse, 5'-GAGCACACAGAGGGCTACAA-3'). Relative expression measurements were performed according to the $2^{-\Delta\Delta Cq}$ method (16,17).

Methylation status of CGI. The CGI (323 bp) methylation of *GLA-HNRNPH2* BDP was tested using bisulfite DNA treatment and MSP analysis as described by Al-Obaide *et al* (12). Purified genomic DNA (100 ng) from blood was treated using the Methylamp DNA Modification kit (Epigentek Group Inc.), and the converted DNA was cleaned, captured, and eluted using R6 (Modified DNA Elution) solution and an F-Spin column. Eluted DNA was analyzed using the iTaq universal SYBR-Green reaction mix (Bio-Rad Laboratories, Inc.). The methylated primer sets were designed using the MethPrimer tool (urogene.org/methprimer/). The MSP primers for methylated and unmethylated regions of the BDP 323 bp CGI-2 were as follows: M pair (forward, 5'-TTTTTTTAAACGGTTATAGCGAGAC-3' and reverse, 5'-CTTAATTTACCAAATAACCCGTA-3'); and U pair (forward, 5'-TTTTTTAAATGGTTATAGTGAGATGG-3' and reverse, 5'-AATACAACACCTTAATAATCCCAAA-3'). The percentage methylation was calculated as: $100/[1+2\Delta Cq$ (meth-unmeth)]. ΔCq (meth-unmeth) was calculated by subtracting the Cq values of methylated CGI signals from the Cq values of the unmethylated CGI signal (13,14).

Detection of the GLA deletion variant. The genetic analysis to identify the *GLA* mutation was performed by Duke University Health System/BGL-Genzyme Fabry Testing (Durham). The patient's genomic DNA from peripheral blood was amplified by PCR, followed by Sanger DNA sequencing of the coding region of the *GLA* gene and flanking intronic sequences, with a minimum of 20 bp of the *GLA* gene.

α-GAL test. The α-GAL test was performed by Quest diagnostics (Amarillo, TX, USA) using Flow Injection Analysis on a Tandem Mass Spectrometry to verify abnormal serum α-GAL results in male patients with a clinical presentation suggestive of FD (testdirectory.questdiagnostics.com/test/test-detail/37621/alpha-galactosidase-leukocytes?cc=MASTER).

Statistical analysis. Microsoft Excel 365 (Microsoft Corporation) was used for sorting the data and for analysis. Data are presented as the mean ± SD. GraphPad Prism version 7.01 (GraphPad Software, Inc.) was used for statistical analysis of the various parameters reported in this study. A Student's

Table I. List of the FD patients and their clinicopathological criteria.

Patients	Sex/age, years	Symptoms	Family history	ERT	RRT	Notes
FD1	F/35	Arrhythmia/Bradycardia, Peripheral Neuropathy	Yes	Yes	No	ERT stopped
FD2	F/34	Arrhythmia/Bradycardia, Peripheral Neuropathy	Yes	Yes	No	ERT stopped
FD3	M/18	Multiple angiokeratomas, Peripheral Neuropathy	Yes	Yes	No	Died of an unrelated cause
FD4	M/39	Progressive loss of kidney function, ESRD Hypertension, Depression, stroke, angiokeratomas	Yes	Yes	Yes	Died of a from stroke

M, male; F, female; ERT, enzyme replacement therapy; RRT, renal replacement therapy; FD, Fabry disease; ESRD, end-stage renal disease.
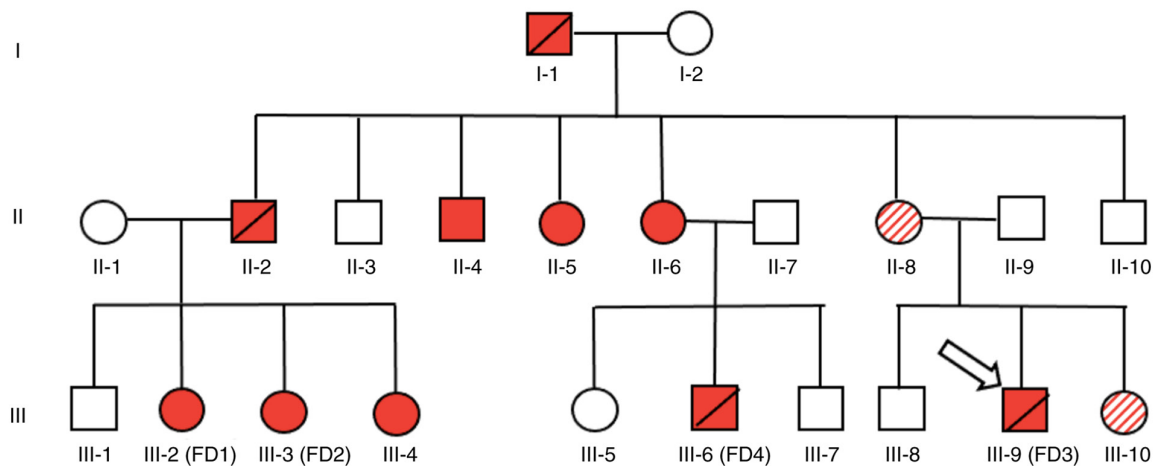

Figure 1. The pedigree of the FD family. Circle, female; square, male; white, not examined subjects or individuals who showed no symptoms; black diagonal line, FD deceased family member; red diagonal line, carrier; arrow, the first patient diagnosed in the study. FD1-FD4: patients participated in the study. FD, Fabry disease.

t-test or a one-way ANOVA with a post-hoc Tukey's multiple comparisons test was used to evaluate differences between the independent groups simultaneously and to test the statistical differences between every possible pair of all groups. P<0.05 was considered to indicate a statistically significant difference.

Results

Case presentation. Four members of the same family were diagnosed with FD (Table I); two males and two females were recruited for the study from the outpatient clinic of the Texas Tech University Health Sciences Center. The four patients showed variable MSSI and FOS-MSSI severity scores (Table SII). The FD family history is shown in the pedigree diagram (Fig. 1). Among the male members of the family, one, FD4 (Table I, Fig. 1) was diagnosed based on a very low level of the α-GAL enzyme (<1%), the test was

performed by Quest Diagnostics after presenting to our clinic with progressive loss of kidney function, depression, stroke, angiokeratomas, and hypertension. Additionally, FD3 was diagnosed with the typical distribution of angiokeratomas and peripheral neuropathy. Two female patients, who were sisters (FD1 and FD2) had arrhythmias, bradycardia, and peripheral neuropathy with no renal involvement. BGL-G enzyme Fabry Testing performed the genetic analysis that showed the presence of a heterozygote *GLA* variant c.1033_1034delTC, p.Ser345Argfs (accession ID: VCV000092538; www.ncbi.nlm.nih.gov/clinvar).

Fabrazyme (agalsidase β), an enzyme replacement therapy, was used to treat FD patients, which, although prevented the development of renal involvement in the male who was diagnosed early, did not lead to the resolution of all symptoms in the observed patients; the patients were followed-up for 5 years. Considering our previous finding (12), the potential
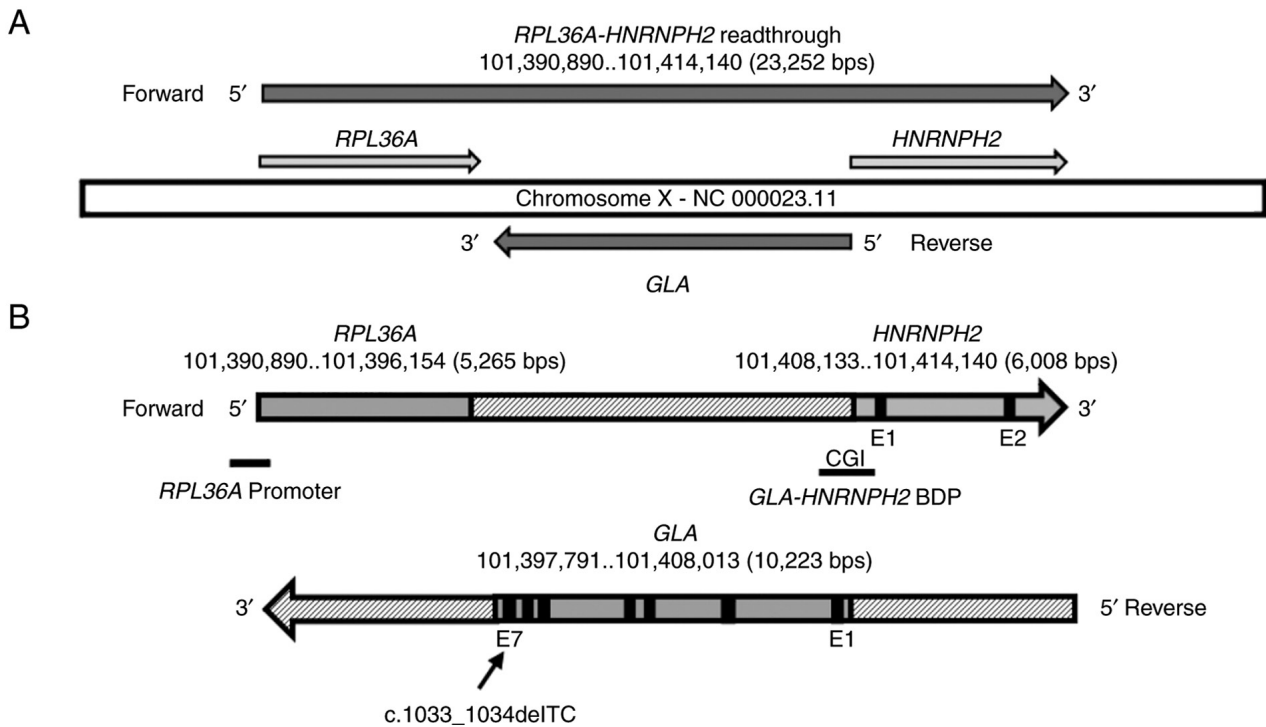
Figure 2. The *GLA* and *HNRNPH2* genes in the *RPL36A-HNRNPH2* readthrough region. (A) *GLA* and *HNRNPH2* loci are divergently paired, mapped at forward and reverse strands respectively. (B) The BDP shares a sequence of 991 nucleotides at 5' ends of *GLA* and *HNRNPH2* loci. CGI: CpG Island. BDP, bidirectional promoter.

link between CGI methylation levels of the BDP and the expression of *GLA* and *HNRNPH2* was investigated in the FD family members with variable clinical manifestations and FD severity.

The genomic setting of TC deletion in the GLA locus. The *GLA* locus is mapped to the reverse strand of the *RPL36A-HNRNPH2* readthrough locus that appears on the forward strand of the complete genomic region NC_000023.11 (Fig. 2A). The c.1033_1034delTC deletion mutation is in *GLA* exon seven of the translated region (Fig. 2B and 3A). The position of the TC dinucleotide deletion is c.1033_1034delTC in the coding sequence at the last TC dinucleotide of the TC tri-dinucleotide repeat, TCTCTC, underlined in Fig. 3B. The consequence of TC deletion is a shift in the DNA sequence and the generation of a distorted reading frame of the coding sequence and the formation of three premature nonsense codons indicated by the circled portion of the sequence in Fig. 3C, compared with one natural termination codon, TAA in the normal *GLA* sequence (Fig. 3B). The deletion mutation results in an amino acid serine (S) (Fig. 4A), encoded by the triplet TCA (Fig. 3B), along the α-Gal A polypeptide sequence, being altered to a new sequence starting with arginine (R) (Fig. 4B), encoded by the triplet AGG (Fig. 3C). The new amino acid sequence is terminated by a premature nonsense codon, TAA (END) (Fig. 3C and 4B). In addition to the NCBI-ClinVar database (Table II), peer-reviewed studies (18-23) have also reported the deletion variant, c.1033_1034delTC, in FD patients previously.

Methylation analysis of the GLA- HNRNPH2 BDP. The *GLA* and *HNRNPH2* loci are located within the readthrough locus *RPL36A-HNRNPH2*, the *GLA* locus is at the reverse strand, whereas the *HNRNPH2* locus appears at the forward strand (Fig. 2A). Our previous study showed one of three identified BDP CpG islands, CGI-2 composed of 323 bp and mapped along the BDP sequence, was methylated in four normal human cell types (12). Using the same Methylation-Specific PCR (MSP) protocol and the same primers reported in our previous study (12), the methylation status of the BDP CGI-2 at position 241-563 (Fig. S1) in DNA isolated from blood samples of FD patients compared with normal individuals was evaluated. The DNA methylation analysis showed variable levels of methylation in BDP in the tested blood samples; DNA methylation was elevated in both the male and female patients compared with methylation in the normal group (Fig. 5A-1 and B-1, P<0.05).

Expression of GLA and HNRNPH2 in Fabry patients and healthy individuals. The molecular events regulating the expression of the *GLA* and *HNRNPH2* transcripts are not well established. As a follow-up to our previous finding of *GLA-HNRNPH2* BDP and the observed methylation levels in normal kidney and skin cells (12), in this study, the potential impact of BDP methylation on *GLA* and *HNRNPH2* expression in four FD patients carrying the deletion mutation c.1033_1034delTC (p.Ser345Argfs) was determined. As shown in Fig. 5, the expression of *GLA* is significantly lower (P<0.05) and *HNRNPH2* showed a tendency of low expression (P=0.1)) in the FD patients when the levels of BDP methylation were high compared with high *GLA* expression when BDP methylation was low in normal individuals (P<0.05). The results showed potential accumulative effects of the *GLA* mutation c.1033_1034delTC and BDP methylation with the severity of disease in FD patients as discussed below. This association was
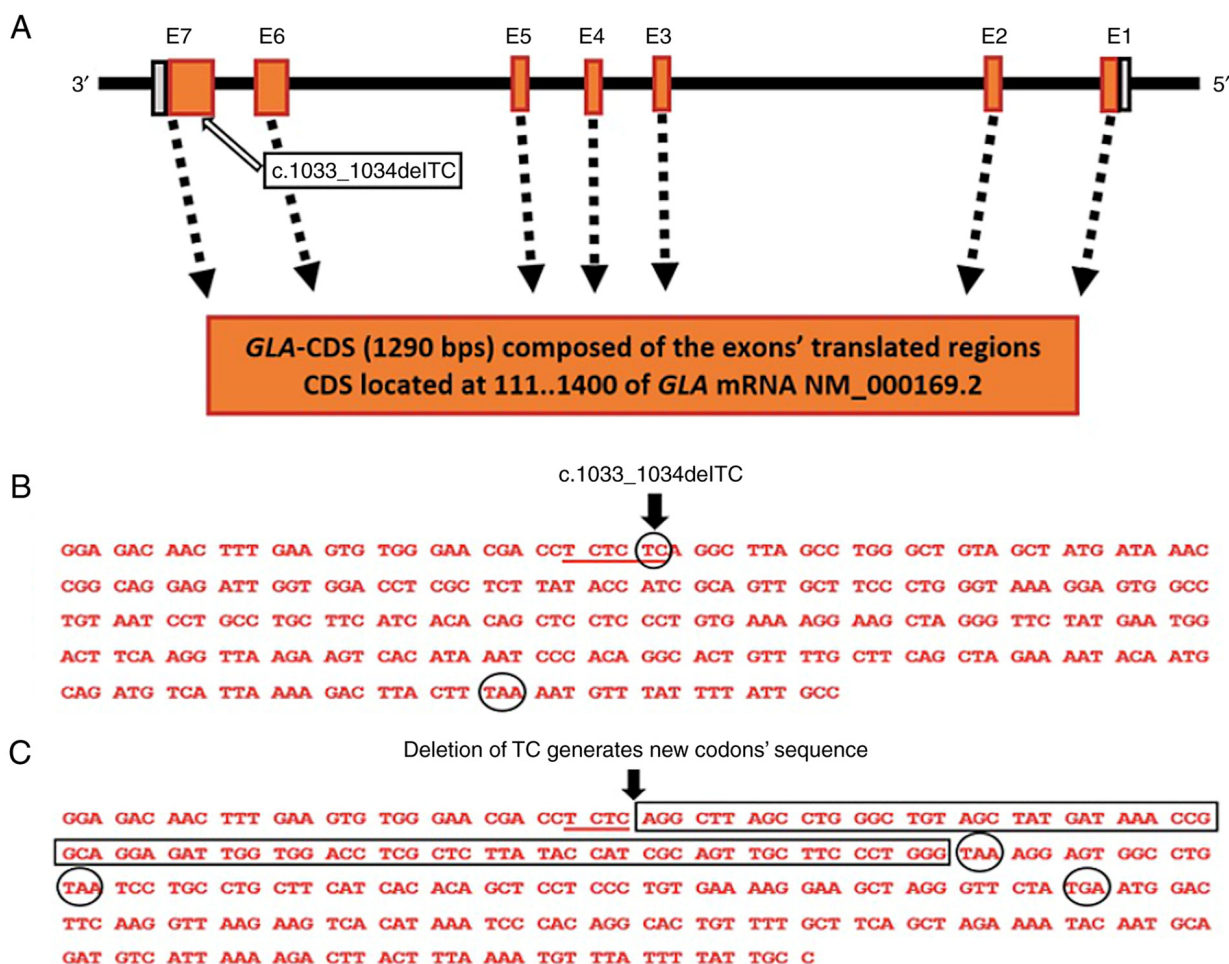
Figure 3. Genomic features of the c.1033_1034delTC mutation in the *GLA* locus. (A) The *GLA* transcript NM_000169.2 is composed of seven exons; the location of the deletion mutation is in the translated sequence of exon 7. (B) The position of the TC deletion (circled) in the normally translated sequence of *GLA*-exon 7. At the end of *GLA* exon 7 is the normal termination codon, TAA (circled). Underlined sequence shows the TC repeat. (C) A TC deletion generated a new sequence of codons for new amino acids that are shown in the box. The mutation generated a premature termination codon, TAA (circled), and two more termination codons were generated, TAA and TGA. Brown boxes, translated sequences of exons; gray boxes, un-translated sequences of exons 1 and 7.
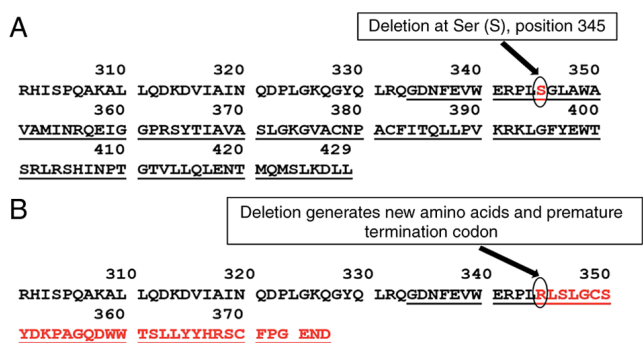

Figure 4. The generated amino acid sequence of the α-Gal A polypeptide at the site of the deletion mutation. (A) The affected amino acid Serine (S) at position 345 along the normal α-Gal A polypeptide sequence. (B) The deletion mutation generated an arginine (R), and the new amino acid sequence is terminated by a premature nonsense codon (END). α-Gal A, α-galactosidase A.

Discussion

The detailed genetic presentation has not yet been fully elucidated in FD, which is a clinically heterogeneous, slow, and progressive disease that can show >70 symptoms (rarediseases.info.nih.gov). Although FD is a life-threatening, multisystemic condition, and patients exhibit a wide range of clinical symptoms, the primary cause of the disease is attributed to pathogenic *GLA* mutations (1-5). The present study highlights the association of additional genetic factors in addition to *GLA* with FD and shows an indicator of suspicion that FD is caused solely by *GLA* mutations. Our previous study (12) and the current study show the potential of including study of *HNRNPH2* and the *GLA-HNRNPH2* BDP methylation status in the diagnosis, therapy, and development of the disease.

Although few studies have dealt with the role of methylation in FD, for a review see Di Risi *et al* (24); the present study provided further evidence on the potential role of DNA methylation involvement in the clinical manifestation of FD. The *GLA* mutations can cause total or partial decreased activity of α-Gal A and accumulation of glycosphingolipids (3,4). Intriguingly, the potential source of *GLA-HNRNPH2* BDP

clearly demonstrated in male patient FD4, a family member who was diagnosed with progressive loss of kidney function, depression, stroke, angiokeratomas, and hypertension. He had the highest BDP DNA methylation and lowest *GLA* and *HNRNPH2* expression levels (Fig. S2, Table SIII).

Table II. Previous submissions on the c.1033_1034delTC (p.Ser345Argfs) variant.[a]

Submitter and submission date	Clinical significance	Submission accession
GeneDx, Sep 21, 2015	Pathogenic	SCV000292562.9
Integrated Genetics/Laboratory Corporation of America, Jun 3, 2016	Pathogenic	SCV000695728.1
EGL Genetic Diagnostics, Eurofins Clinical Diagnostics, Apr 20, 2018	Pathogenic	SCV000110103.8

[a]Data in the Table adapted from ClinVar-NCBI (ncbi.nlm.nih.gov/clinvar/variation/92538/?new_evidence=false).
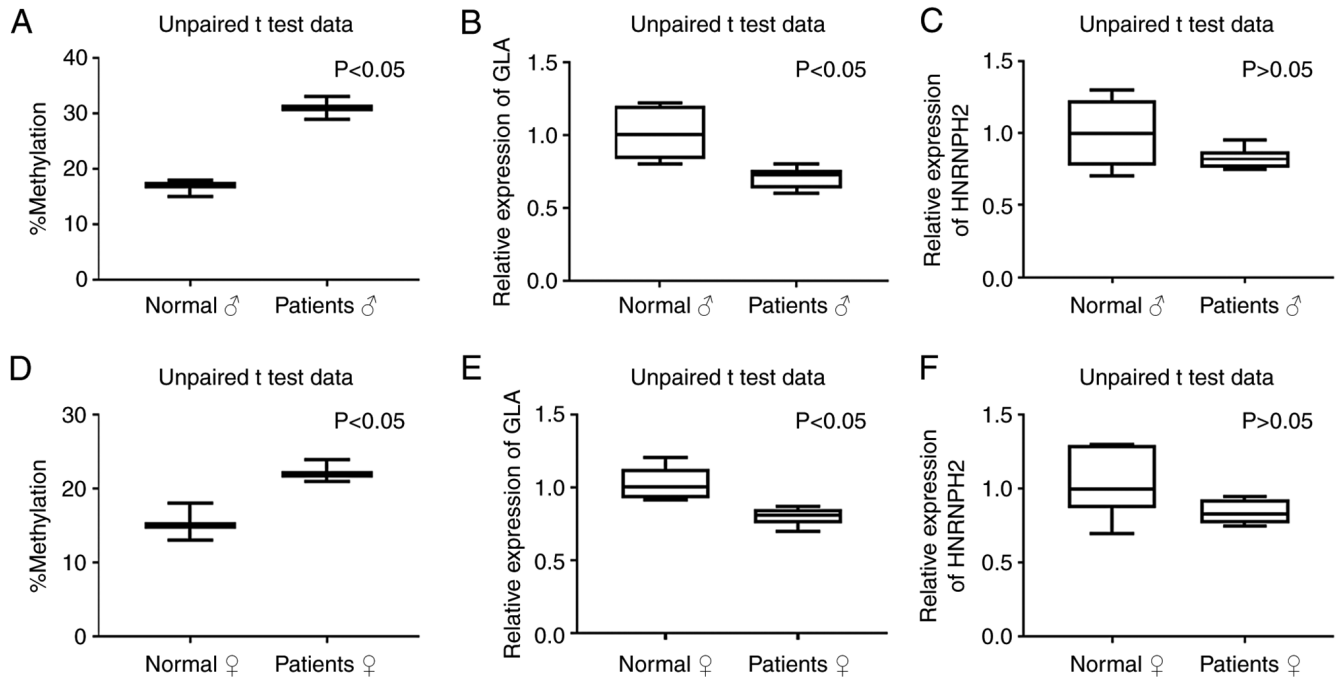

Figure 5. Association between BDP methylation and the expression of *GLA* and *HNRNPH2* in the normal individuals and FD patients. (A) DNA methylation of the BDP in the males in the normal group and FD group. (B and C) Expression of *GLA* and *HNRNPH2* in the males in the healthy group and FD group. (D) DNA methylation of the BDP in the females in the normal group and FD group. (E and F) Expression of *GLA* and *HNRNPH2* in the females in the healthy group and FD group. FD, Fabry disease patients; BDP, bidirectional promoter.

methylation is likely sphingolipids. Aside from their prominent roles as structural lipids, sphingolipids and their metabolizing enzymes are found in the nucleus and linked to chromatin remodeling and epigenetic regulation of gene expression (25). Furthermore, additional species of sphingolipids serve different functions, such as functioning as signaling molecules and can control gene expression via DNA methylation (26). A further point is that the FD patients in the present study were carriers of a TC deletion caused by c.1033_1034delTC in the *GLA* exon 7 near the 3'-UTR region, and such a deletion may influence the regulatory function of the 3'-UTR sequence. For example, methylation of the N(6) position of adenosine m(6)A is a posttranscriptional modification of RNA. It was found that m(6)A sites are enriched near stop codons and in 3' UTRs, and there is an association between m(6)A residues and microRNA-binding sites within 3' UTRs (27).

The majority of patients with FD may experience chronic or episodic pain, known as FD crises or acroparaesthesiae (12,28-30). The development of pain in FD is hypothesized to be primarily neuropathic; the suggested cause is serum and tissue accumulation of Gb3 and its influence on the peripheral nervous system, which may lead to cell swelling (31-34). Furthermore, a question has been raised on whether the *HNRNPH2* and the BDP methylation may play a role in diagnosing and treating chronic pain in FD patients and other related FD clinical symptoms. Earlier studies have demonstrated the association between alternative RNA splicing and pain (35,36). The products of HNRNP genes, including *HNRNPH2*, are RNA binding proteins that are associated with the mRNA splicing process (12). *HNRNPH1* and *HNRNPF* are post-transcriptional regulators of opioid receptor expression (37), and similar protein structures are produced by *HNRNPH2* and *HNRNPF* (38). This may suggest *HNRNPH2* involvement in the pain experienced by FD patients. BDP methylation may cause abnormalities in *HNRNPH2* expression and defects in mRNA splicing. A previous study suggested that DNA methylation not only affects gene expression but also regulates alternative splicing (39).

At present, little is known regarding the role of *RPL36A* in FD. The knockdown of *RPL36A*, the first gene in the *RPL36A-HNRNPH2* readthrough region, using a targeting siRNA showed a significant decrease not only in *RPL36A* expression but also in *GLA* expression (40). The *RPL36A* gene, also known as MIG6, encodes the ribosomal protein L36a, and over-expression of this protein is associated with cellular proliferation in hepatocellular carcinoma (41,42).

In the present study, we also sought to explain the heterozygous status of *GLA* variant c.1033_1034delTC, p.Ser345Argfs in the FD female patients. The inheritance of several X-linked conditions is not visibly dominant or recessive (43). In females, one altered copy of the gene usually leads to less severe health problems than those in affected males, or it may have no warning signs. In females a high FD penetrance was observed; at least 70% of females showed the clinical manifestations of the disease (44). Thus, it is suggested that when referring to females with FD, the term carrier should be avoided and replaced by the term heterozygotes (45).

Finally, although the results of the present study showed further evidence of the potential involvement of BDP methylation, in addition to the *GLA* gene and the *HNRNPH2* gene in FD severity, the precise mechanism that regulates the bidirectional transcription of *GLA* and *HNRNPH2* is yet to be fully understood. Additional studies using novel experimental and bioinformatics-based methods, including high-throughput approaches and data analysis by developing machine learning models for computational estimation of methylation profiling (46,47) are required for a better understanding of the architecture, cis-regulatory elements of *GLA* and *HNRNPH2* and the cumulative effects of *GLA* mutations and the *GLA-HNRNPH2* BDP methylation in FD.

Acknowledgements

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

TLV analyzed and interpreted the clinical patient data. MAAO and IIAO performed and interpreted the molecular analyses. TLV and MAAO confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Institutional Review Board of the Texas Tech University Health Science Center (Amarillo, USA).

Patient consent for publication

Written informed consent was obtained from the healthy individuals and patients, whom all agreed to the use of their blood samples for scientific research and for the publication of the anonymized data.

Competing interests

The authors declare that they have no competing interests.

References

1. Mehta A and Hughes DA: Fabry Disease. Adam MP, Mirzaa GM, Pagon RA, Wallace SE, Bean LJ, Gripp KW and Amemiya A (eds). GeneReviews® Seattle: University of Washington, Seattle, WA, 1993-2018, 2017.
2. Garman SC and Garboczi DN: The molecular defect leading to Fabry disease: Structure of human alpha-galactosidase. J Mol Biol 337: 319-335, 2004.
3. Zarate YA and Hopkin RJ: Fabry's disease. Lancet 372: 1427-1435, 2008.
4. Svarstad E and Marti HP: The changing landscape of Fabry disease. Clin J Am Soc Nephrol 15: 569-576, 2020.
5. Rozenfeld P and Neumann PM: Treatment of fabry disease: Current and emerging strategies. Curr Pharm Biotechnol 12: 916-922, 2011.
6. Lidove O, West ML, Pintos-Morell G, Reisin R, Nicholls K, Figuera LE, Parini R, Carvalho LR, Kampmann C, Pastores GM and Mehta A: Effects of enzyme replacement therapy in Fabry disease: A comprehensive review of the medical literature. Genet Med 12: 668-679, 2010.
7. Keating GM: Agalsidase alfa: A review of its use in the management of Fabry disease. BioDrugs 26: 335-354, 2012.
8. Thurberg BL, Rennke H, Colvin RB, Dikman S, Gordon RE, Collins AB, Desnick RJ and O'Callaghan M: Globotriaosylceramide accumulation in the Fabry kidney is cleared from multiple cell types after enzyme replacement therapy. Kidney Int 62: 1933-1946, 2002.
9. Khanna R, Soska R, Lun Y, Feng J, Frascella M, Young B, Brignol N, Pellegrino L, Sitaraman SA, Desnick RJ, et al: The pharmacological chaperone 1-deoxygalactonojirimycin reduces tissue globotriaosylceramide levels in a mouse model of Fabry disease. Mol Ther 18: 23-33, 2010.
10. Germain DP, Hughes DA, Nicholls K, Bichet DG, Giugliani R, Wilcox WR, Feliciani C, Shankar SP, Ezgu F, Amartino H, et al: Treatment of Fabry's disease with the pharmacologic chaperone migalastat. N Engl J Med 375: 545-555, 2016.
11. Modrego A, Amaranto M, Godino A, Mendoza R, Barra JL and Corchero JL: Human α-galactosidase A mutants: Priceless tools to develop novel therapies for Fabry disease. Int J Mol Sci 22: 6518, 2021.
12. Al-Obaide MA, Al-Obaidi II and Vasylyeva TL: Unexplored regulatory sequences of divergently paired GLA and HNRNPH2 loci pertinent to Fabry disease in human kidney and skin cells: Presence of an active bidirectional promoter. Exp Ther Med 21: 154, 2021.
13. Ng EK, Leung CP, Shin VY, Wong CL, Ma ES, Jin HC, Chu KM and Kwong A: Quantitative analysis and diagnostic significance of methylated SLC19A3 DNA in the plasma of breast and gastric cancer patients. PLoS One 6: e22233, 2011.
14. Shaker MM, Shalabi TA and Amr KS: Correlation of methylation status in MTHFR promoter region with recurrent pregnancy loss. J Genet Eng Biotechnol 19: 44, 2021.
15. Whybra C, Bähner F and Baron K: Measurement of disease severity and progression in Fabry disease. In: Fabry Disease: Perspectives from 5 Years of FOS. Mehta A, Beck M and Sunder-Plassmann G (eds). Chapter 32. Oxford: Oxford PharmaGenesis, 2006.
16. Livak KJ and Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408. 2001.
17. Schmittgen TD and Livak KJ: Analyzing real-time PCR data by the comparative C(T) method. Nat Protoc 3: 1101-1108, 2008.

18. Ashley GA, Shabbeer J, Yasuda M, Eng CM and Desnick RJ: Fabry disease: Twenty novel alpha-galactosidase A mutations causing the classical phenotype. J Hum Genet 46: 192-196, 2001.
19. Germain D, Biasotto M, Tosi M, Meo T, Kahn A and Poenaru L: Fluorescence-assisted mismatch analysis (FAMA) for exhaustive screening of the alpha-galactosidase A gene and detection of carriers in Fabry disease. Hum Genet 98: 719-726, 1996.
20. Lukas J, Giese AK, Markoff A, Grittner U, Kolodny E, Mascher H, Lackner KJ, Meyer W, Wree P, Saviouk V and Rolfs A: Functional characterisation of alpha-galactosidase a mutation as a basis for a new classification system in fabry disease. PLoS Genet 9: e1003632, 2013.
21. Nakano S, Morizane Y, Makisaka N, Suzuki T, Togawa T, Tsukimura T, Kawashima I, Sakuraba H and Shibasaki F: Development of a highly sensitive immuno-PCR assay for the measurement of α-galactosidase A protein levels in serum and plasma. PLoS One 8: e78588, 2013.
22. Rodríguez-Marí A, Coll MJ and Chabás A: Molecular analysis in Fabry disease in Spain: Fifteen novel GLA mutations and identification of a homozygous female. Hum Mutat 22: 258, 2003.
23. Shabbeer J, Yasuda M, Benson SD and Desnick RJ: Fabry disease: Identification of 50 novel alpha-galactosidase A mutations causing the classic phenotype and three-dimensional structural analysis of 29 missense mutations. Hum Genomics 2: 297-309, 2006.
24. Di Risi T, Vinciguerra R, Cuomo M, Della Monica R, Riccio E, Cocozza S, Imbriaco M, Duro G, Pisani A and Chiariotti L: DNA methylation impact on Fabry disease. Clin Epigenetics 13: 24. 2021.
25. Spiegel S, Milstien S and Grant S: Endogenous modulators and pharmacological inhibitors of histone deacetylases in cancer therapy. Oncogene 31: 537-551, 2012.
26. Silva GD, Coeli-Lacchini FB and Leopoldino AM: How do sphingolipids play a role in epigenetic mechanisms and gene expression? Epigenomics 14: 219-222, 2021.
27. Meyer KD, Saletore Y, Zumbo P, Elemento O, Mason CE and Jaffrey SR: Comprehensive analysis of mRNA methylation reveals enrichment in 3' UTRs and near stop codons. Cell 149: 1635-1646. 2012.
28. Magg B, Riegler C, Wiedmann S, Heuschmann P, Sommer C and Üçeyler N: Self-administered version of the Fabry-associated pain questionnaire for adult patients. Orphanet J Rare Dis 10: 113, 2015.
29. Gibas AL, Klatt R, Johnson J, Clarke JT and Katz J: A survey of the pain experienced by males and females with Fabry disease. Pain Res Manag 11: 185-192, 2006.
30. Crosbie TW, Packman W and Packman S: Psychological aspects of patients with Fabry disease. J Inherit Metab Dis 32: 745-753, 2009.
31. Tabira T, Goto I, Kuroiwa Y and Kikuchi M: Neuropathological and biochemical studies in Fabry's disease. Acta Neuropathol 30: 345-354, 1974.
32. Gadoth N and Sandbank U: Involvement of dorsal root ganglia in Fabry's disease. J Med Genet 20: 309-312, 1983.
33. Miller JJ, Aoki K, Moehring F, Murphy CA, O'Hara CL, Tiemeyer M, Stucky CL and Dahms NM: Neuropathic pain in a Fabry disease rat model. JCI Insight 3: e99171, 2018.
34. Rajan JN, Ireland K, Johnson R and Stepien KM: Review of mechanisms, pharmacological management, psychosocial implications, and holistic treatment of pain in Fabry disease. J Clin Med 10: 4168, 2021.
35. Donaldson LF and Beazley-Long N: Alternative RNA splicing: Contribution to pain and potential therapeutic strategy. Drug Discov Today 21: 1787-1798, 2016.
36. de la Peña JB and Campbell ZT: RNA-binding proteins as targets for pain therapeutics. Neurobiol Pain 4: 2-7, 2018.
37. Song KY, Choi HS, Law PY, Wei LN and Loh HH: Post-transcriptional regulation of mu-opioid receptor: Role of the RNA-binding proteins heterogeneous nuclear ribonucleoprotein H1 and F. Cell Mol Life Sci 69: 599-610, 2012.
38. Alkan SA, Martincic K and Milcarek C: The hnRNPs F and H2 bind to similar sequences to influence gene expression. Biochem J 393: 361-371, 2006.
39. Lev Maor G, Yearim A and Ast G: The alternative role of DNA methylation in splicing regulation. Trends Genet 31: 274-280, 2015.
40. Al-Obaide MA and Vasylyeva TL: The Knockdown of RPL36A downregulates GLA expression associated with Fabry disease in vitro model. ASN ePosters: Abstract PO1600, 2020.
41. Kim JH, You KR, Kim IH, Cho BH, Kim CY and Kim DG: Over-expression of the ribosomal protein L36a gene is associated with cellular proliferation in hepatocellular carcinoma. Hepatology 39: 129-138, 2004.
42. Xu M, Wang Y, He HT and Yang Q: MiR-589-5p is a potential prognostic marker of hepatocellular carcinoma and regulates tumor cell growth by targeting MIG-6. Neoplasma 65: 753-761, 2018.
43. Happle R: X-chromosome inactivation: Role in skin disease expression. Acta Paediatr Suppl 95: 16-23, 2006.
44. Dobyns WB: The pattern of inheritance of X-linked traits is not dominant or recessive, just X-linked. Acta Paediatr Suppl 95: 11-15, 2006.
45. Rozenfeld PA: Fabry disease: Treatment and diagnosis. IUBMB Life 61: 1043-1050, 2009.
46. Weingarten-Gabbay S, Nir R, Lubliner S, Sharon E, Kalma Y, Weinberger A and Segal E: Systematic interrogation of human promoters. Genome Res 29: 171-183. 2019.
47. Fan S, Wang L, Liang L, Cao X, Tang J and Tian Q: The progress on the estimation of DNA methylation level and the detection of abnormal methylation. Quant Biol 10: 55-66, 2022.