Rapid targeting and isolation of the β-like globin gene cluster fragment from AA, AS and SS genotypes using BamHI restriction enzyme

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The β-globin gene cluster contains the β-globin gene on which the substitution of the 17th nucleotide gives rise to sickle cell anemia and other β-globin gene variants with varying severity. Recent therapy measures focus on understanding the structure and mechanism of expression of the β-globin gene. In this study, we attempted to isolate a gene fragment containing the complete set of the human β-globin gene cluster from AA, AS and SS blood types, using the restriction enzyme BamHI which has a recognition site within the 19-kb 3’ cluster region downstream the β-globin gene cluster. DNA fragment size of 69.65 kb was generated by the enzyme from the genome of AS and SS blood types while the fragment generated from the AA blood type was 70.28 kb. Fragments generated using Bam HI was all comparable and close to the documented size of the β-globin gene cluster. In addition to the 69.65 kb fragment generated from the DNA from SS blood type, a novel 5.73 kb fragment was also visualized which was absent in the lanes containing restricted DNA from the AA blood type.

Key words: BamHI, beta-globin, genotype, isolation.

INTRODUCTION

The β-globin gene cluster lies on the short arm of chromosome 11 within a 70 kb region and contains 5 coordinately regulated genes arranged in the sequence of their expression (5’-ε-Gy-Ay-δ-β-3’), and is flanked 6 to 18 kb upstream by a regulatory region which contains a series of DNAse I hypersensitive sites known as the locus control region (Langdon and Kaufman, 1998). The β-globin gene cluster has a well studied restriction map, and some restriction enzymes; including; Hind II, Taq I, Pst I, Hinf I and BamHI have been shown to possess recognition sequences within and immediately outside the gene locus (Aliyu et al., 2008). Several hemoglobin variants have arisen over the years as a result of mutations in the beta globin gene cluster (Smith et al., 1998). One of the most significant of these variants is β⁸ mutation which results from a substitution of adenine by thymine on the 17th nucleotide of the β-globin gene in the β-globin gene cluster.

Genotype is known to be the major risk factor in the severity of sickle cell anemia (Smith et al., 1998). Carriers of the β⁸ gene are known to lead normal lives and exhibit little or no anemia (Smith et al., 1998). Homozygous for the β⁸ usually exhibit chronic anemia and other symptoms known to be associated with sickle cell anemia. Sub-Saharan Africa has the highest burden of sickle cell anemia with Nigeria alone accounting for over two thirds of the sickle cell anemia population in Africa (Ashley-Koch et al., 2000). Studies on the influence of genotype on the expression of the β-globin gene cluster are scanty. Understanding mechanisms of β-globin gene expression, and similarly diagnosis of the disease requires an efficient and rapid method for the preparation of the β-globin gene cluster. In order to understand variations in the β-globin gene cluster, it is also necessary to be able to successfully prepare the β-globin gene cluster both rapidly and
precisely.

Most methods of isolation of the β-globin gene cluster rely on primer specific PCR-amplification of the β-globin gene cluster from genomic DNA (Sarasawathy et al., 2010; Cao et al., 2004; Langdon and Kaufman, 1998). Conventional β-globin gene isolation requires the use of primer sequences complementary to the gene (Kaufman et al., 1999; Kaufman et al., 1998; Smith et al., 1998) and in the case of Detloff et al. (1994) PCR was also used to screen for the targeting of the β-globin gene.

In this research, we attempted to isolate and compare within genotypes the β-globin gene cluster from humans using BamHI restriction endonuclease with recognition site within the 19-kb 3’ cluster region downstream the β-globin gene cluster.

MATERIALS AND METHODS

Sample selection

Peripheral blood was obtained from adult volunteers who had previously signed consent forms approved by the Ahmadu Bello University Health Service Unit. 4 ml Blood samples were obtained from volunteers with AA, AS and SS genotypes and used for genomic DNA preparation. None of the volunteers of blood samples were undergoing treatment for any disease at the time of this study.

Genomic DNA preparation

DNA was prepared from 0.5 ml whole blood by an adaptation of the method of DNA isolation as described (Roe et al. 1995). Briefly, whole blood was collected in EDTA vacutainer tubes containing 50 mM EDTA, cells were washed with 1x SSC pH 7.6 and subsequently pelleted at 16, 000 rpm. The cells were then lysed with 10% SDS at 70°C for 20 min in the presence of 0.2 M sodium acetate pH 5.4. The mixture was then partitioned with phenol chloroform iso-amyl alcohol (8:7:1) mixture at 16,000 rpm for 1 min. DNA was then recovered from the aqueous phase by precipitation with cold absolute propanol at -20°C for 15 min and centrifugation at 16,000 rpm for 2 min. DNA precipitate was washed in 75% propanol and reconstituted in 10:1 TE buffer pH 7.6.

DNA quantification and purity assessment

Quantity of genomic DNA was assessed by taking absorbance at 260 nm, and the purity of genomic DNA was calculated using the ratio of DNA absorbance at 260 and 280 nm. Purity of 1.6 to 1.8 was taken as optimal purity.

Restriction digestion of genomic DNA

BamHI restriction enzyme digestion was carried out at 37°C for 24 h in a total volume of 50 μl containing 2 μg of human genomic DNA and 50 units of BamHI restriction enzyme, 100 mM Tris-HCl pH 8.0, 100 mM MgCl₂, 100 mM NaCl, and 100 mM β-mercaptoethanol.

Isolation of generated fragments

Agarose gel electrophoresis was used to separate fragments generated. 91.2 ng of restricted DNA was electrophoresed along-side a 5 to 50 kb DNA size marker on 0.8% agarose gel. The electrophoresis was carried out at 100 to 120 mA for 30 min. Resolved fragments were visualized under a UV-transilluminator and the fragment pattern photographed with a digital Polaroid camera.

RESULTS

Yield of genomic DNA from 0.5 ml whole blood

The mean yield of genomic DNA obtained from 0.5 ml of whole blood differed between genotypes as shown in Figure 1. The highest mean quantity obtained was 7.06 μg obtained from whole blood of the SS genotype and the lowest was 5.80 μg obtained from the AA genotype.

Purity of genomic DNA from 0.5 ml whole blood

All the samples produced genomic DNA of acceptable purity as shown in Figure 2.

Digestion with Bam HI generated the β-like globin gene cluster

In order to isolate the entire β-globin gene cluster we subjected the *2µg of human genomic DNA to 50 units of Bam HI. A characteristic 69.65 kb DNA fragment was visualized from the genome of the SS and AS genotype, while the fragment visualized by the genome of the AA genotype used in this study was 70.28 kb the fragment pattern is shown in Figure 3. In addition, a novel 5.75 kb was visualized in from the SS genome.

DISCUSSION

The β-globin gene is a well studied gene and had been well characterized with well defined restriction sites (Langdon and Kaufman, 1998). Restriction enzymes with recognition sequences outside the β-globin gene clusters include; Hind II which has a recognition site 5’ upstream the ε-globin gene of the β-globin gene cluster, and the BamHI restriction enzyme which has a recognition site within the 19-kb 3’ cluster region downstream the β-globin gene of the β-globin gene cluster. In addition to the recognition site 5’ of the beta globin gene, Hind II is also known to have recognition sites within the β-globin gene cluster (Langdon and Kaufman, 1998). The major reason for the choice of BamHI is to produce a gene fragment which would contain the entire β-globin gene cluster and the locus control region of the gene.

Similar fragment sizes were generated for both the AA, AS and SS genotypes digested with BamHI all close to the documented size of the β-globin gene cluster (Langdon and Kaufman, 1998). The fragments generated from the sickle cell patient and AS was 69.65 kb, while
Figure 1. Mean yield of genomic DNA from the three genotypes. DNA was quantified by reading absorbance of the samples at 260 nm. The mean of three readings of three independent isolation events was plotted against the corresponding genotype as shown above. A significantly higher yield of genomic DNA was obtained from the SS genotype \( P < 0.05 \).

Figure 2. Mean purity of isolated genomic DNA for the three genotypes. The purity was calculated using the ratio of absorbance at 260 and 280 nm of the DNA samples. The mean was computed from the calculated purity of each isolation event.

The fragment generated from the AA patient corresponded to a fragment size of 70.28 kb. These observed differences could be attributable to the polymorphic differences although the presence of the same sized fragment from the AS and SS genotype suggests a significant characteristic peculiar to AS and SS genotypes which is similar to the findings in the study by Lin et al. (2004) and Fullerton et al. (2000). Similarly, 70.28 kb fragment generated was similar in all the AA patients used for this study this could be clinically significant because it offers a rapid and precise way to molecularly determine the presence of the \( \beta^S \) gene. The presence of the 5.73 kb observed in the lanes with genomes of the AS and SS genotypes digested with BamHI, suggests the presence of a control sequence upstream the \( \beta^S \) gene. The 5.73 kb fragment could also be of diagnostic significance as the fragment was not visible in the lanes containing AA digested with BamHI restriction enzyme and could also suggest a polymorphic region present in the AS and SS patient used for the study but absent in the AA patient as previously demonstrated (Currat et al., 2001; Fullerton et al., 2000; Chang et al., 1983).

The difference in the fragment sizes of the \( \beta \)-like globin cluster could reveal conservation in certain sequences among the genotypes although further studies would be required to confirm this.
Comparative studies of several human populations have concentrated on the diversity associated with \( \beta^A \) chromosomes. Several mutations associated with haemoglobinopathies have also been documented in the \( \beta \)-globin gene cluster (Weatherall, 2010; Strouboulis et al., 1992). The \( \beta^S \) (sickle cell) mutation is very common in countries where \textit{Plasmodium falciparum}-mediated malaria is endemic (Currat et al., 2001; Orkin et al., 1993; Antonarakis et al., 1984; Pagnier et al., 1984). This study demonstrated a rapid and precise method to isolate the \( \beta \)-globin gene circumventing the rigors of PCR and expense of primers which are common problems which plague the application of molecular methods to clinical diagnosis in third world countries although more studies is required to completely sequence the gene fragments isolated.

To our knowledge, this is the first report of the isolation of the beta-like globin gene cluster among the different genotypes in Nigeria which is known to have the highest occurrence of the \( \beta^S \) gene.

The quantity of DNA obtained from 0.5 ml of blood using this protocol varied clearly between genotypes, the highest quantity of DNA obtained using this method was from the SS genotype. In comparison of the mean quantities of DNA obtained from the different genotypes, the amount of DNA obtained from AA blood samples were the lowest using this method. This could be due to the fact that the AA genotype exhibit the lowest turnover rate for RBCs as compared to AS and SS (Orkin et al., 1983), and the higher content of immature RBC content in AS and SS content as compared to AA patients could be responsible for the higher DNA content as it has been reported that immature RBCs contain nucleus and as such would also contain DNA.

REFERENCES


