HUTCHINSON-GILFORD PROGERIA SYNDROME (HGPS) WITH MITOCHONDRIAL DNA (MTDNA) HV1 CONTROL REGION MUTATIONS

Gazi Nurun Nahar Sultana¹, Atiqur Rahman M², Shahinuzzaman ADA³, Abdullah ABM⁴, Proyash Roy⁵, Rokeya Begum⁶

¹Centre for Advanced Research in Sciences, University of Dhaka, Dhaka-1000, Bangladesh
²Department of Microbiology, University of Dhaka, Dhaka-1000, Bangladesh.
³Pharmaceutical Sciences Research Division, Bangladesh Council of Scientific and Industrial Research, [BCSIR], Dhaka-1205, Bangladesh.
⁴Department of Medicine, Bangabandhu Sheikh Mujib Medical University, Dhaka-1000, Bangladesh
⁵Department of Genetic Engineering and Biotechnology, University of Dhaka, Dhaka-1000, Bangladesh
⁶Centre for Advanced Research in Sciences, University of Dhaka, Dhaka-1000, Bangladesh

Received 19-12-2013; Revised 18-01-2014; Accepted 13-02-2014

*Corresponding Author: Gazi Nurun Nahar Sultana, PhD Centre for Advanced Research in Sciences University of Dhaka
Tell: +880-2-9661920-59 (ext. 4628) Fax: +880-2-8615583 Email: nngazi@du.ac.bd, nngazi@gmail.com.

ABSTRACT

Hutchinson-Gilford Progeria syndrome (HGPS) is a rare (one in 4 - 8 million) genetic disorder among infants, characterized with premature aging. Nuclear encoded lamin A gene (LMNA) forms heterodimers with their splice variant lamin C to create filamentous structures present in nuclear lamina. Early studies revealed that mutations in Lamin A genes are responsible for HGPS. Mitochondrion, the key component of cellular metabolism is also an alleged candidate to have greater level of mutagenesis with aging and several disease conditions including cancer. In this study, on the first ever Progeria male patient identified in Bangladesh, we analyzed the mitochondrial DNA genome by direct sequencing with AB3130 Genetic Analyzer and have evaluated the genetic characteristic of (HGPS). We report some novel mtDNA anomalies, none of which are observed in patient's maternal relatives. We hereby, report four mutations (16475 G→A, 16488 A→T, 16496 C→T, and 16505 G→T) in the light strand (L-strand) control region of mitochondria. Apart from these, several other mutations (T34A/T34T heterozygous, GG59AA, A97G, A287G, T334C, C335T, G340C,) were observed in the control region (D-loop) of mitochondria. The D-loop region of mitochondria functions as control region of regulation for both mtDNA replication and transcription. In this region both cis acting mitochondrial encoded protein and trans-acting nuclear encoded proteins play indispensable roles to maintain the regular homeostasis between replication and transcription of mtDNA. Therefore, these mutations identified in this region are expected to pave the pathway of destabilizing mtDNA maintenance and trigger towards apoptosis and aging.

Keywords: Progeria, Lamin A, mtDNA genome, D-Loop, Case study, Bangladesh.

INTRODUCTION

Hutchinson-Gilford progeria syndrome (HGPS) is a rare disease, characterized with premature and accelerated aging, generally leading to death at approximately 13 years of age. Thus it is different from natural forms of aging albeit been regarded as a model of aging. To date mutations in LMNA and ZMPSTE24 genes have been found in patients with HGPS. Mitochondrion, the cellular power house, have been implicated in several disease conditions with mutations in the Mitochondrial DNA including both cancer and aging. Yet no previous studies on mitochondrial DNA of progeria patients were performed. The rarity of 1 in 8 million affected cases also makes it difficult to be studied extensively. Children with progeria are genetically predisposed to premature, progressive heart disease. Death occurs almost exclusively due to widespread heart disease, which is the major cause of death globally. We know that mitochondrial DNA (mtDNA) mutations accumulate with aging in several tissues in mammals. During the process of ageing, abundance morphology, and functional properties of mitochondria decay in several tissues including skeletal and cardiac muscle. Most molecular damage is reversible through repair or molecular turnover mechanisms but mtDNA changes are
irreversible due to lack of their repair mechanism. As a result accumulation of mutations ultimately leads to permanent mitochondrial dysfunction.

Information on the specific contribution of mtDNA instability to human aging can be inferred through the analysis of disorders associated with increased mtDNA mutation or deletion frequency. Tissues most affected by disorders associated with inherited mtDNA mutations are the same tissues markedly affected by normal aging; these include the heart, brain, skeletal muscle, kidney and the endocrine system. Disorders associated with increased levels of mtDNA mutations generally fall into two classes: those associated with specific, maternally-inherited mtDNA mutations; and, those associated with mutations in nucleus-encoded genes important for maintaining the fidelity of mtDNA replication and mtDNA stability. Because disorders in the latter category result in random accumulation of many different mtDNA mutations and deletions, they may better represent the potential consequences of age-related mtDNA mutation accumulation in humans. With this view, the whole mtDNA of the patient along with his mother and brother were sequenced. Out of nine novel mutations observed in the family, six were confined to Progeria patient.

MATERIALS AND METHODS

Case Presentation

Only one progeria patient was found in Bangladesh, his medical history and interview was taken in 2011. He is a 12-year-old boy from a low-income family background of Bangladeshi origin. His appearance significantly reflects progeria characteristics. He has enlarged head, shrinks skin and protruded eyes. He is only 3.6 ft height and 32 kg of body weight. At the age of 13 years he looks more than 50 years old person. No molecular study was recorded. Therefore, we investigated the molecular causative gene in mitochondrial genome for this patient. Finally blood sample was collected for the study of his mitochondrial genome. As a control, blood samples were also collected from the mother (43 yrs) and the brother (10 yrs) of the patient who represent normal and healthy features.

Clinical symptoms

Patient's medical history recorded progressive heart disease, which is number one cause of death globally. He has been admitted to hospital with stomach pain but finally he was diagnosed with multiple clinical complications including hernia. He had an operation for hernia and release with follow up treatment.

DNA isolation, PCR and Sequencing

DNA was isolated by standard proteinase K treatment followed by phenol/ chloroform/ isoamyl alcohol extraction from patient and control. DNA was precipitated with 0.3 M sodium acetate (pH 5.2) in 70% ethanol at -20°C overnight and resuspended in Tris-EDTA (TE) buffer (pH 8.0). DNA quantification was performed by taking absorbance at 260 nm and visualized by 0.8% agarose gel electrophoresis. Complete mitochondrial genome was amplified using twenty four sets of primers and the resultant amplicons were checked in 2% agarose gel electrophoresis. Distinct PCR bands were observed for different primers amplifying non-coding D-loop and coding ND3, ND4, and Cytochrome b genes, ATP6, ATP8, COX I-V along with diluted 1 Kb+ DNA ladder. 20 µl PCR reaction contained 10-20 ng DNA and 0.5 µM primers, 0.2 mM each of deoxynucleotide triphosphate (dNTP), 1U of TaqMan™ DNA Polymerase (Applied Biosystem, USA) and 2.5 mM MgCl2. The PCR amplification of specific regions of mtDNA was performed on the basis of following cycling conditions: initial denaturing at 95°C for 5 min followed by 94°C for 30 sec, 58°C for 30 sec, and 72°C for 2 min for 35 cycles and final extension step at 72°C for 7 min. In Sequencing PCR, the ABI-prism Big Dye Terminator v3.1 containing ampliTaq polymerase, dye terminators (fluorescent label), deoxynucleotide triphosphate, magnesium chloride, was used for direct sequencing of PCR product for specific primers (forward/ reverse primer). The sequencing PCR was performed on the basis of following cycling conditions: initial denaturing at 95°C for 1 min followed by 94°C for 10 sec, 55°C for 30 sec, and 60°C for 4 min for 35 cycles.

MtDNA sequence analysis

The purified sequencing PCR products were analyzed by electrophoresis in the ABI-Prism 3130 Genetic Analyzer (Applied Biosystems, USA). The complete mtDNA sequence patterns were observed and edited by using Mac-based software (Auto Assembler V 3.0) and BioEdit Sequence Alignment Editor V 7.0.9.0 (http://www.mbio.ncsu.edu/bioedit/bioedit.html). The sequences were aligned by using bl2seq tool of NCBI (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and compared with the revised Cambridge Reference Sequence, rCRS (NCBI Reference Sequence: NC_012920.1). MtDNA polymorphisms were compared with the mitochondrial genome database of world population by using Mitomap (www.mitomap.org).

RESULTS

The results report some novel mutations in HVR-1 region. Apart from control region anomalies, we also found some mutations in coding regions but the amino acid changes are synonymous, which do not affect the protein synthesis. The four new novel mutations in HVR-1 regions have been confirmed by comparison with 48 control samples of our database as well with mother and brother of the patient. Also novelty of mutations was confirmed by comparison with MITOMAP data base. The list of the mutations found in patient is shown in Table 1 and selected chromatogram images are shown in Figure 1 and 2. From the sequencing of total mtDNA, we also confirmed the haplotype of the patient and relatives (Mother & Brother), all three have the same haplotype M37e with a sequence motif of (16111-16189-16223-16295), and further coding region sequence at A10398G-C10400TpC10556T confirmed the macro haplogroup M. Few known mutations were observed in COX-1 and COX-III. LMNA gene was also scanned for mutations and a heterozygous mutation in codon S573S was observed in exon 12 near a splice site. This was common in the patient’s brother also.
Table 1: List of novel mutations in Progeria patient and relatives

<table>
<thead>
<tr>
<th>Regions</th>
<th>Mutations</th>
<th>Patient</th>
<th>Mother</th>
<th>Brother</th>
</tr>
</thead>
<tbody>
<tr>
<td>HV II</td>
<td>G35A</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>HV II</td>
<td>G36A</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>HV II</td>
<td>GG59AA</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>HV II</td>
<td>A97G</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>HV II</td>
<td>A287G</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>HV II</td>
<td>T334C</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>HV II</td>
<td>C335T</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>HV II</td>
<td>G340C</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cytochrome c oxidase subunit III (COIII)</td>
<td>A9218G</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>NADH dehydrogenase subunit 4L</td>
<td>C10556T</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>HV I</td>
<td>16185 T-ins</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>HV I</td>
<td>16475 G-ins</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>HV I</td>
<td>16488 A-ins</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>HV I</td>
<td>16496 C-ins</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>HV I</td>
<td>16505 G-ins</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>LMNA exon 12</td>
<td>codon S573S</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Hutchinson-Gilford Progeria syndrome (HGPS) is a rare but progressive disorder that causes children to age rapidly, beginning from the first two years of their lives. Although they appear normal at birth, the signs and symptoms of aging, such as slow growth and hair loss begin to appear by the end of first year of their lives. The average life span is 13 years of these patients although may expand up to 20 years or more. Alike senility, in Progeria, heart problems or strokes are the most prominent cause of death.

Many theories of aging were presented before, although the underlying molecular mechanism is largely elusive. The mitochondrial theory of aging speculates mtDNA mutations are associated with age dependent decline of mitochondrial respiratory function. Many such mutations impair the respiratory chain and Oxidative phosphorylation systems. This further increases the production of ROS and subsequent 

**Figure 1: A97G Mutation**

**Figure 2: GG59AA Mutation**
damage of mtDNA and thereby impaire cellular functions to accelerate aging 3. The mammalian mitochondrial genome is closed circular double stranded DNA molecule of about 16.6 Kb. The strands of the DNA duplex are classified into Heavy strand and Light Strand based on its G+C composition. The D-loop region of mitochondria functions as control region of regulation for both mtDNA replication and transcription(15). In this region both cis acting mitochondrial encoded protein and trans-acting nuclear encoded proteins play indispensable roles to maintain the regular homeostasis between replication and transcription of mtDNA 16-17. This region has previously been implicated with other mutations in different disease conditions 3-6. Therefore, the novel mutations identified in this region is suspected to have some degrading role in destabilizing mtDNA maintenance and trigger towards apoptosis in HGPS. Absence of conclusive markers in LMNA gene study confirms also, that sole reason for HGPS in this case was not due to the mutation (shared between the patient and his brother) in LMNA gene but could be for some other chromosomal aberration that we could not have identified.

**CONCLUSION**

This study concluded that some novel and known mutations in mtDNA are large in numbers which enhanced the aging of HGPS patients. However, it is clear from this study that LMNA gene mutations is not sole responsible for Hutchinson-Gilford Progeria Syndrome (HGPS). Further investigations are required to clarify the precise mechanisms and whether other DNA repair mechanisms both mitochondrial and/or nuclear are also involved in HGPS.

**ACKNOWLEDGEMENT**

This work was supported from the Centre for Advanced Research in Sciences (CARS), University of Dhaka. The authors also thank to patient and his family for kind cooperation and donation of blood samples.

**REFERENCES**