Reviewer #1:
1. Why do you want to do mitochondrial genome mutation analysis instead of whole exon sequencing and so on?
Response: The genetic pattern of these two families is maternal inheritance, which means the mitochondrial DNA mutations is the molecular basis, we detected the mitochondrial genome mutation analysis and GJB2 gene mutation analysis.

2. Informed consent was given to 16 people, but there were no subjects in the text. Who were they? How many blood samples were taken? What were the results of 4268T>C validation in the family?
Response: We modified the sentence in the manuscript, as following: “Informed consent, blood samples, and clinical evaluations were obtained from all 36 subjects in these two families and 100 controls according to protocols approved by the Ethics Committee of Zhejiang University School of Medicine.” Moreover, we confirmed the results of 4268T>C validation in the family using the following criteria: (1) missense mutation; (2) conservation index (CI) from other 16 vertebrates>75%; (3) absence in the controls; (4) potential structural and functional alterations; (5) pedigree analysis.

3. Which family is the cell of origin III-3?
Response: I modified the cell of origin as following: “immortalized patient cell lines from the proband patient (HD223-III-3) bearing the 4268T>C mutation,”

4. There are 34 mutations detected, 33 known and 1 unknown (4268T>C). Why do you define the unknown as this family mutation?
Response: I added the criteria in the manuscript, as following: These variants were evaluated for the pathogenicity using the following criteria: (1) missense mutation; (2) conservation index (CI) from other 16 vertebrates>75%; (3) absence in the controls; (4) potential structural and functional alterations; (5) pedigree analysis.