Gene 519 (2013) 169–172

Contents lists available at SciVerse ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Short Communication

Identification of the first deletion–insertion involving the complete structure of *GAA* gene and part of *CCDC40* gene mediated by an *Alu* element

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ARTICLE INFO

Article history:
Accepted 28 January 2013
Available online 9 February 2013

Keywords:
GAA
Deletion
CCDC40
Pompe disease
MLPA

ABSTRACT

Pompe disease is an uncommon autosomal recessive glycogen storage disorder caused by deficiency of acid α-glucosidase. Classic infantile form triggers severe cardiomyopathy, hypotonia, and respiratory failure, leading to death within the first two years of life. The majority of patients with Pompe disease have been reported to have point mutations in the *GAA* gene. We report the first complex deletion–insertion encompassing the complete structure of *GAA* gene and a large fragment of the gene *CCDC40* in a patient with very severe form of Pompe disease. Sequencing analysis of breakpoints allowed us to determine the potential implication of an *Alu* repeat in the pathogenic mechanism. We suggest that molecular strategy of Pompe disease should include systematic analysis of large rearrangements.

1. Introduction

Pompe disease (glycogen storage disease (GSD) type II; MIM 232300) is an uncommon autosomal recessive disorder, produced by deficiency of acid α-glucosidase (GAA, acid maltase, EC 3.2.1.3), which breaks down lysosomal glycogen (Hers, 1963).

Progressive accumulation of glycogen in cardiac, respiratory, and skeletal muscular fibers produces severe infantile form, which leads to death within the first year of life. The combined incidence of all clinical forms of Pompe disease is estimated to be 1/40.000 (Ausems et al., 1999).

Genetic background of Pompe disease lies on α-glucosidase gene. This gene spans from position 78.075.355 to 78.093.678 (GRCh37/hg19) on the long arm of chromosome 17 and comprises 20 exons (Höfsloot et al., 1990). Last update of Pompe Center mutations database contains about 300 entries (http://www.pompecenter.nl). Large deletions or duplications are 5–13% of the identified mutations; some of them involving substantial fragments of the gene (Tinkle and Leslie, 2007).

We report the first complex deletion–insertion involving the complete structure of *GAA* gene and a large fragment of the gene *CCDC40* in a patient with a severe form of GSD type II.

2. Materials and methods

2.1. Case report

The index case was the first child of unrelated Caucasians parents of Spanish origin. He was born at term, following a normal pregnancy and delivery. At birth, the weight was 2,710 grams (10th percentile). Both parents were completely normal and there was no family history of interest. During neonatal period the patient suffered a choking episode that needed clinical help. At the age of 6 months he presented weakness, hypotonia, hepatomegaly and hypertrophic cardiomyopathy. GAA activity in dry blood spot suggested GSD type II. At the age of 17 months, the patient died.

2.2. DNA amplification and sequencing

Genomic DNA from the patient and his parents was isolated from peripheral blood samples using standard procedures, after obtaining informed consent. All exons of *GAA* gene as well as flanking intronic fragments were amplified using self-designed oligonucleotides (available upon request) and submitted for sequencing of both strands. Briefly, direct sequencing of PCR products was performed using Big Dye Terminator cycle sequencing kits on an ABI PRISM 3100 genetic analyzer (Applied Biosystems, CA, USA).

Abbreviations: *MLPA*, Multiplex ligation-dependent probe amplification; Array CGH, array-comparative genomic hybridization; PCR, polimerase chain reaction; GAA, glucosidase, alpha, acid; *CCDC40*, coiled-coil domain containing 40; CDH4, cadherin-4; Kbp, kilobase pairs.

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http://dx.doi.org/10.1016/j.gene.2013.01.051

2.3. MLPA assay

There is no commercial MLPA available for this gene. To design our procedure we followed recommendations from MRC-Holland web site (MRC-Holland, Amsterdam The Netherlands).

The homemade *GAA* probes set consisted of 10 genomic regions corresponding to the following structures of the gene: Exons 1, 2, 4, 5, 6, 10, 14, 18 and 20. Gene sequences are designated according to RefSeq: NM_000152.

Each target sequence was verified by BLAST with human reference genome sequence, to avoid potential duplicity. Universal primer sequences were identical to those in the commercial MLPA kits (MRC Holland, Amsterdam, The Netherlands) (Schouten et al., 2002).

Fluorescent labeled MLPA products were analyzed using an ABI 3100 capillary sequencer (Applied Biosystems, CA, USA). Quantitative data from every peak size were normalized according to a set of 5 control samples.

2.4. Array CGH

MetabolArray® has been previously developed in our laboratory (Desviat et al., 2011), covering *GAA* region with 1 oligonucleotide each 80 bp, allowing accurate determination of chromosomal breakpoints. Array experiments were performed as recommended by the manufacturer (Agilent Technologies, Santa Clara, CA, USA). The analysis and visualization of MetabolArray® data was performed using Agilent Genomic WorkBench 6.5. The algorithm used was Aberration Detection Method 2 (ADM-2).

2.5. Junction fragment analysis

To precisely determine the sequences of breakpoints, we designed the oligonucleotides GAGCTCAGGAGGAAGACGGAT and TTGTCACTA CTACGTCCACCT, located outside the deleted region, according to array CGH results. These primers are 40.294 nucleotides far from each other in the normal structure of the chromosome. PCR amplification rendered one specific band of about 1.500 bp in the deleted allele. Sequencing analysis of the fragment was performed by standard procedures.

Junction fragments were analyzed with BLASTN application (http://www.ncbi.nlm.nih.gov/BLAST), University of California Santa Cruz Genome Browser (http://genome.ucsc.edu/) and RepeatMasker application (http://www.repeatmasker.org/).

3. Results

Complete sequencing analysis of *GAA* gene from the patient allowed us to identify in exon 5 the missense mutation c. 925 G>A (p.Gly309Arg). Chromatogram showed that this mutation could be in apparent homozygosity. Sequencing analysis of exon 5 was carried out in samples from both parents. We found that only the mother was

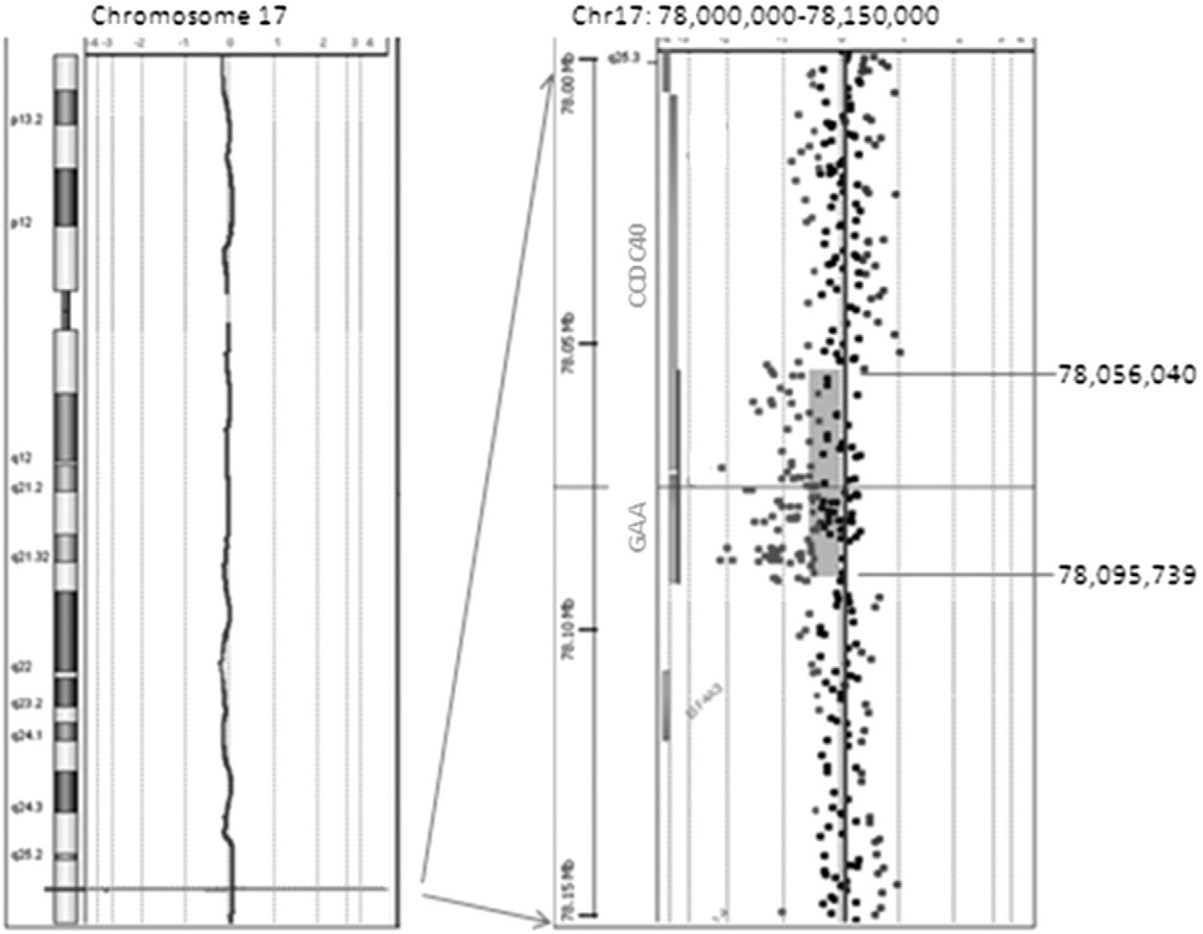

Fig. 1. Array CGH results obtained from Pompe patient. The left panel shows the whole chromosome view of data from Chromosome 17. The right panel is an expansion of the fragment containing 150 Kb. including GAA and CCDC40 structure. The right panel shows the heterozygous deletion. Potential breakpoints are indicated (from ~78,056,040 to ~78,095,739 (Chromosome 17: NCBI Build 37, USCS hg19, Feb. 2009).