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Brief report

A novel synonymous substitution in the GCK gene causes aberrant splicing in an Italian patient with GCK-MODY phenotype

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ABSTRACT

GCK gene analysis in an Italian MODY patient revealed a novel synonymous substitution in exon 4 (c.459T>G; p.Pro153Pro) resulting in an aberrant transcript lacking the last eight codons of the same exon. Our findings emphazise the importance of not underestimating synonymous variations when screening for disease-causing mutations.

1. Introduction

Maturity-onset diabetes of the young (MODY) is a genetically heterogeneous form of monogenic diabetes characterized by autosomal dominant inheritance, early onset (usually < 25 years of age) and beta-cell dysfunction [1,2].

Heterozygous loss-of-function mutations in the glucokinase gene (*GCK*) result in GCK-MODY or MODY2, one of the most common MODY subtypes [3,4].

So far, the mutational spectrum of GCK-MODY is made up of more than 600 different mutations [5], of which approximately 9% are splicing mutations including two synonymous substitutions potentially affecting pre-mRNA splicing [6,7].

Last decade developments in RNA processing analysis have shown that synonymous variations, historically classified as non-pathogenic, may interfere with correct pre-mRNA splicing and cause genetic disease [8]. Therefore, the

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characterization of such variations is of great importance for genetic counselling in clinical practice and should not be overlooked [9–11].

This report describes a novel synonymous *GCK* variant detected in a patient with clinical suspicion of GCK-MODY and its effects on gene splicing.

2. Subjects, materials and methods

A non-obese 15-year-old Italian boy with suspected GCK-MODY and his first-degree relatives (parents, brother and sister) were investigated. The proband fulfilled the recently published inclusion criteria for GCK-MODY [4]: (1) a 7-year history of mild fasting hyperglycaemia (6.4–7.3 mmol/l); (2) HbA1c between 6.2 and 6.8%; (3) 2-h post-challenge plasma glucose < 4.0 mmol/l; (4) no family history of diabetes (fasting plasma glucose < 5.5 mmol/l in each family member).

Genomic DNA was extracted from EDTA-anticoagulated peripheral blood using a standard salting-out procedure.

Exons 1a and 2–10 (beta-cell *GCK* isoform), flanking intronic regions and beta-cell promoter were PCR-amplified (primers and PCR conditions available upon request) and bidirectionally sequenced using BigDye Terminator v3.1 chemistry (Applied Biosystems, Foster City, CA, USA) after treatment with ExoSAP-IT (USB Corporation, Staufen, Germany).

The novel variant detected in the proband (c.459T>G) was analyzed in his first-degree relatives and in 105 unrelated normoglycaemic Euro–Caucasian control subjects by DNA sequencing and MspI-RFLP analysis, respectively.

Potential deleterious effects of the variant on pre-mRNA splicing were investigated by a combination of *in silico* and mRNA analyses.

In silico analysis was performed using the following three programs: CRYP-SKIP (http://cryp-skip.img.cas.cz/) [12], Human Splicing Finder (HSF; http://www.umd.be/HSF/) [13] and Splice Site Prediction by Neural Network (NNSPLICE; http://www.fruitfly.org/seq_tools/splice.html) [14].

Lymphoblastoid cell lines (LCLs) were established by EBV-transformation of peripheral blood lymphocytes (as ECACC, Porton Down, Salisbury, UK) from the proband, his parents and sister, and from two unrelated controls. Total RNA was extracted from LCLs using the TRIzol Reagent (Invitrogen, Carlsbad, CA, USA) and cDNA was synthesized using the SuperScript III First-Strand Synthesis System for RT-PCR (Invitrogen) according to the manufacturer's instructions. For each sample, at least three distinct cDNA preparations were amplified (for primers and PCR conditions see Supplementary Table 1) and sequenced as described above.

The evolutionary conservation of the 459T nucleotide was ascertained by multiz alignment in the UCSC Genome Browser (http://genome.ucsc.edu/cgi-bin/hgc?hgsid=169300447&o=44183869&t=44198887&g=multiz46way&i=multiz46way). Homology searches for the amino acids 154–161 were carried out by using PSI-BLAST (http://www.ebi.ac.uk/Tools/sss/psiblast/) and COBALT (http://www.ncbi.nlm.nih.gov/tools/cobalt/) programs.

3. Results

A novel heterozygous synonymous substitution in proband *GCK* exon 4, c.459T>G (p.Pro153Pro), was identified (Fig. 1). This variation was not found in any of the tested normoglycaemic individuals (first-degree relatives and unrelated controls).

Since the base change was located next to a previously described cryptic donor splice site [15], 25 bp upstream of the canonical donor splice site of intron 4 (Fig. 1), we hypothesized its involvement in pre-mRNA splicing. CRYP-SKIP, HSF and NNSPLICE splicing predictor programs showed that the cryptic splice site score of the mutant sequence was higher than the wild-type counterpart (Supplementary Table 2), suggesting a splicing alteration due to the 459G allele.

Sequence analysis of *GCK* transcripts from the proband LCL revealed three different homozygous sequences (Fig. 2). Transcript 1 corresponds with the expected PCR product containing the wild-type nucleotide (detected in PCR products

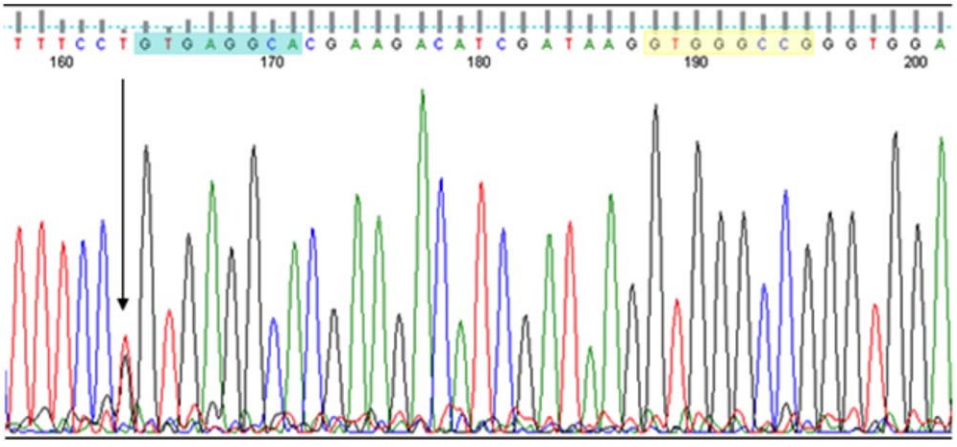

Fig. 1 – Electropherogram of proband genomic DNA showing the heterozygous c.459T>G variant (arrow). Canonical and cryptic donor splice sites are highlighted in yellow and in blue, respectively. Mutation nucleotide numbering is based on the GenBank reference sequence NM_000162.3 in accordance with the Human Genome Variation Society (HGVS) recommendations (http://www.hgvs.org/mutnomen/). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)