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SHORT COMMUNICATION

Partial and whole gene deletion mutations of the *GCK* and *HNF1A* genes in maturity-onset diabetes of the young

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Abstract

Aims/hypothesis Heterozygous mutations of glucokinase (*GCK*) and hepatocyte nuclear factor-1 alpha (*HNF1A*; also known as hepatic transcription factor 1 [*TCF1*]) genes are the most common cause of MODY. Genomic deletions of the *HNF1B* (also known as *TCF2*) gene have recently been shown to account for one third of mutations causing renal cysts and diabetes syndrome. We investigated the prevalence of partial and whole gene deletions in UK patients meeting clinical criteria for GCK or HNF-1α/-4α MODY and in whom no mutation had been identified by sequence analysis.

Methods A multiplex ligation-dependent probe amplification (MLPA) assay was developed using synthetic oligonucleotide probes for 30 exons of the *GCK*, *HNF1A* and *HNF4A* genes.

Results Partial or whole gene deletions were identified in 1/29 (3.5%) probands using the *GCK* MLPA assay and 4/60 (6.7%) of probands using the *HNF1A/-4A* MLPA assay. Four different deletions were detected: *GCK* exon 2, *HNF1A* exon 1, *HNF1A* exons 2 to 10 and *HNF1A* exons 1 to 10. An additional Danish pedigree with evidence of linkage to *HNF1A* had a deletion of exons 2 to 10. Testing other family members confirmed co-segregation of the deletion mutations with diabetes in the pedigrees.

Conclusions/interpretation Large deletions encompassing whole exons can cause GCK or HNF-1α MODY and will

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not be detected by sequencing. Gene dosage assays, such as MLPA, are a useful adjunct to sequence analysis when a diagnosis of MODY is strongly suspected.

Keywords Deletion mutation · *GCK* · *HNF1A* · *HNF4A* · *HNF1B* · HNF-1α · HNF-4α · Maturity-onset diabetes of the young · MODY · *TCF1*

Abbreviations

GCK	glucokinase
HNF-1α	hepatocyte nuclear factor-1 alpha
HNF-4α	hepatocyte nuclear factor-4 alpha
MIRb	medium interspaced repeat
MLPA	multiplex ligation-dependent probe amplification
NAHR	non-allelic homologous recombination
RCAD	renal cysts and diabetes
SNP	single nucleotide polymorphism

Introduction

Heterozygous loss-of-function mutations in the glucokinase (*GCK*) and hepatocyte nuclear factor-1 alpha (*HNF1A*; also known as hepatic transcription factor 1 [*TCF1*]) genes are the most common cause of monogenic diabetes in the majority of populations studied and account for ~80% of UK patients with a genetic diagnosis of monogenic diabetes [1]. They result in distinct phenotypes; *GCK* mutations cause mild fasting hyperglycaemia (usually 5.5–8 mmol/l) from birth, whereas *HNF1A* mutations cause a progressive form of hyperglycaemia with diabetes usually diagnosed in adolescence/early adulthood [2]. Hepatocyte nuclear factor 4 alpha (*HNF4A*) mutations are rarer than *HNF1A* mutations but result in a similar diabetic phenotype [3]. A molecular genetic diagnosis is important for optimal management, as patients with *HNF1A or -4A* mutations are sensitive to low-dose sulfonylureas and those with *GCK* mutations rarely require pharmacological treatment [4].

The gold standard method for mutation screening is sequence analysis of the coding regions and conserved splice sites. However, this will not detect heterozygous deletion mutations encompassing one or more exons of the gene, since normal sequence will be generated from amplification of the non-mutated allele. In 2005, a large genomic rearrangement was shown to be the most common mutation causing the renal cysts and diabetes (RCAD) syndrome [5]. This rearrangement involves the deletion of at least 1.2 Mb that include the *HNF1B* gene (also known as *TCF2*) and may result from non-allelic homologous recombination (NAHR) mediated by segmental duplications. We have developed a gene dosage assay using the multiplex ligation-dependent probe amplification (MLPA) technique and synthetic probes for the nine exons of the *HNF1B* gene; with this we confirmed that whole gene deletions account for approximately one third of *HNF1B* mutations causing RCAD [6].

In this study we designed an MLPA assay to detect partial or whole gene deletions of the *GCK*, *HNF1A* and *HNF4A* genes using synthetic oligonucleotide probes for 30 coding exons; to incorporate a positive control DNA, we included probes for the *HNF1B* gene. We selected UK patients with a phenotype consistent with a *GCK* (n=31) or *HNF1A/-4A* mutation (n=64) and in whom no mutation was found by sequence analysis. A Danish family showing linkage to *HNF1A* but no mutation was also tested. We report deletion mutations of the *GCK* or *HNF1A* genes in 18 individuals from six families.

Methods

Participants Clinical referrals for MODY genetic testing to the Exeter laboratory (Department of Molecular Genetics, Royal Devon & Exeter NHS Foundation Trust, UK) between 1997 and 2006 were selected on the basis of their phenotype and negative molecular genetic test results after sequence analysis of the *GCK or HNF1A* and *HNF4A* genes. The *GCK* cohort included 31 probands with a fasting glucose between 5.5 and 8 mmol/l and an OGTT showing a 2 h increment of ≤4.6 mmol/l. The *HNF1A/-4A* cohort included 64 probands from families in which at least two generations were affected with diabetes and at least one person had been diagnosed before 25 years of age. Patients with pancreatic autoantibodies were not excluded from testing. A Danish MODY family with an autosomal dominant form of diabetes, affecting seven individuals before the age of 25 years, that had been shown to be linked to the *HNF1A* locus (logarithm of the odds [LOD] score=3.31 at a recombination fraction of 0.0 using marker D12S346), was also studied. Using denaturing HPLC and direct sequencing, we excluded mutations in the exons and promoter regions of the *HNF4A*, *GCK*, *HNF1A*, insulin promoter factor 1 (*IPF1*, also known as pancreatic and duodenal homeobox 1 [*PDX1*]) and neurogenic differentiation (*NEUROD1*) genes. Informed consent was obtained from all participants and the study was conducted in agreement with the declaration of Helsinki as revised in 2000.

Molecular genetics methods See Electronic supplementary material (ESM).

Results

Multiplex ligation-dependent probe amplification dosage assays were designed for the *GCK* and *HNF1A/-4A* genes and validated using DNA from a patient with an *HNF1B* gene deletion (p.Met1_Trp557del).

Three different heterozygous *HNF1A* deletion mutations were detected by MLPA. Two probands had heterozygous deletions of exons 2 to 10 (p.Gln109_Gln631del); in both, heterozygosity for exon 1 was confirmed by the presence of the heterozygous single nucleotide polymorphism (SNP) I27L (rs1169288). Conversely two probands with deletions of exon 1 (p.Met1_Gln109del) were heterozygous for multiple SNPs in exons 2 to 10 but none in exon 1. No heterozygous SNPs were present in the patient with the whole gene deletion (p.Met1_Gln631del). Analysis of microsatellite markers surrounding *HNF1A* showed heterozygosity in affected individuals from families DUK1416 (D12S1349, located 904 kb from the end of exon 1), DUK1526 (D12S2073, located 143 kb from the start of exon 2) and DUK1674 (D12S1721, located 740 kb from the start of exon 2). Hence the deletion mutation breakpoints for these families must be located within a 1.63 Mb region on chromosome 12q24.31.

We identified one deletion encompassing *GCK* exon 2 (p.Val16_Glu70del; Fig. 1). In order to map the breakpoints, additional synthetic MLPA probes were designed at 10 kb intervals between exons 1a and 3 (a distance of ~37 kb). Analysis of gene dosage across this region reduced the minimal deleted region to ~7 kb. Sequence analysis of a long-range PCR product (Fig. 1e) showed a heterozygous deletion of 2138 nucleotides (c.46-1758_208+217del), including exon 2 (Fig. 1f). This out-of-frame deletion is predicted to result in a premature termination codon and hence loss of function. The Repeat Masker program (www.repeatmasker.org) identified a medium interspaced repeat (MIRb) adjacent to the 5’ deletion breakpoint, while the 3’ breakpoint lies adjacent to an AluSx element.

The detection rate for the *GCK* cohort was 1/29 (3.5%; two samples failed) and 4/60 (6.7%; four failures) for the *HNF1A/-4A* cohort. An *HNF1A* deletion mutation was also present in the Danish family with evidence of linkage to

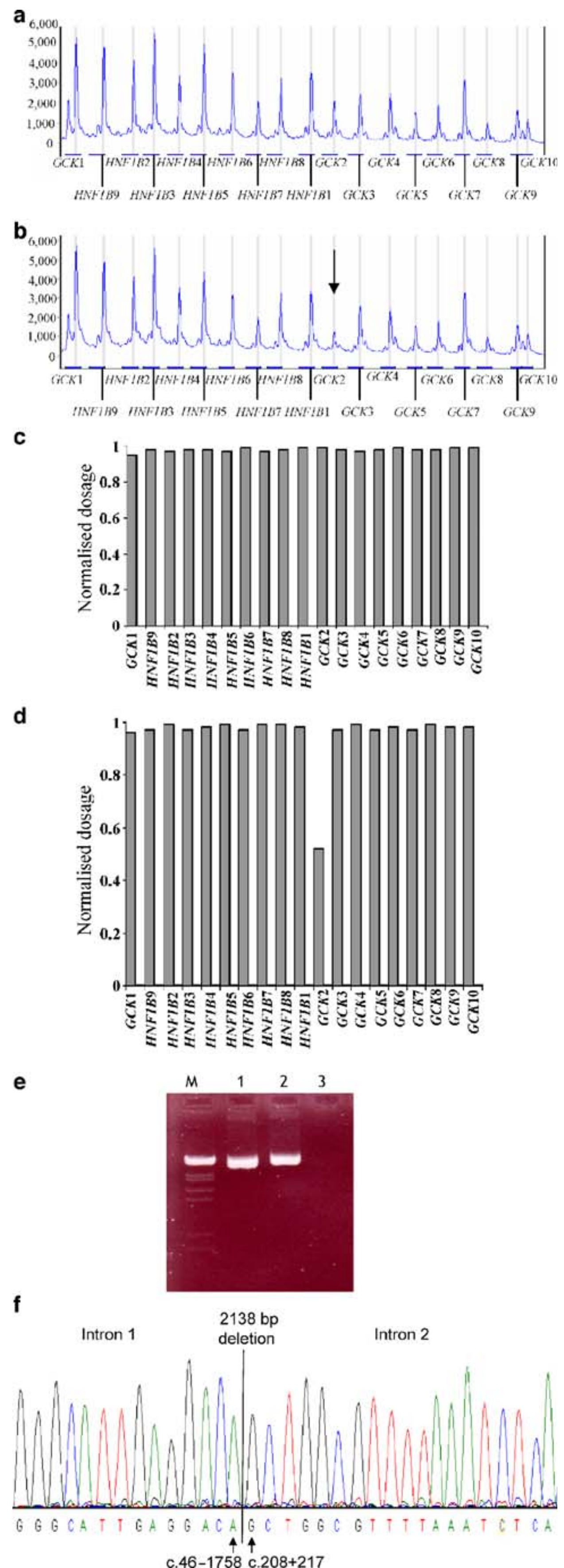

Fig. 1 Detection of exon 2 *GCK* gene deletion by MLPA. **a** Capillary electrophoresis analysis of MLPA products from normal control and **b** patient DUK1508 (*GCK* exon 2 deletion). Arrow highlights reduced *GCK* exon 2 peak height. **c** Graphical representation of *GCK* probes normalised to controls in the normal control and **d** patient (with a *GCK* exon 2 deletion). **e** Gel electrophoresis of long-range PCR products using primers located in intron 1 and intron 2 for the patient (lane 1), normal control (lane 2) and negative control (lane 3). The largest band for the size standard (M) is 7 kb. f Sequence analysis of long-range PCR product showing the breakpoint at c.46-1758 and c.208+217

this gene. Testing of 12 additional affected members from five families showed that in all families the deletion mutations co-segregated with early-onset diabetes (Fig. 2).

Discussion

We report six families with a partial or whole gene deletion of *GCK* (n=1) or *HNF1A* (n=5). The deletion mutations showed co-segregation with early-onset diabetes within the pedigrees and are predicted to result in loss of function. The absence of heterozygous SNPs from the deleted region reduces the likelihood of a false positive result. The identification of the first *HNF1A* whole gene deletion provides conclusive evidence for haploinsufficiency as a mutational mechanism for HNF-1α MODY.

Partial and/or whole gene deletions were identified in 5/89 probands selected using clinical criteria for GCK or HNF-1α/-4α MODY (6% positive). The pick-up rates for sequence-based diagnostic molecular genetic testing in the UK are ~40% for *GCK*, ~30% for *HNF1A* and ~15% for *HNF4A* mutations respectively (K. Colclough, S. Ellard

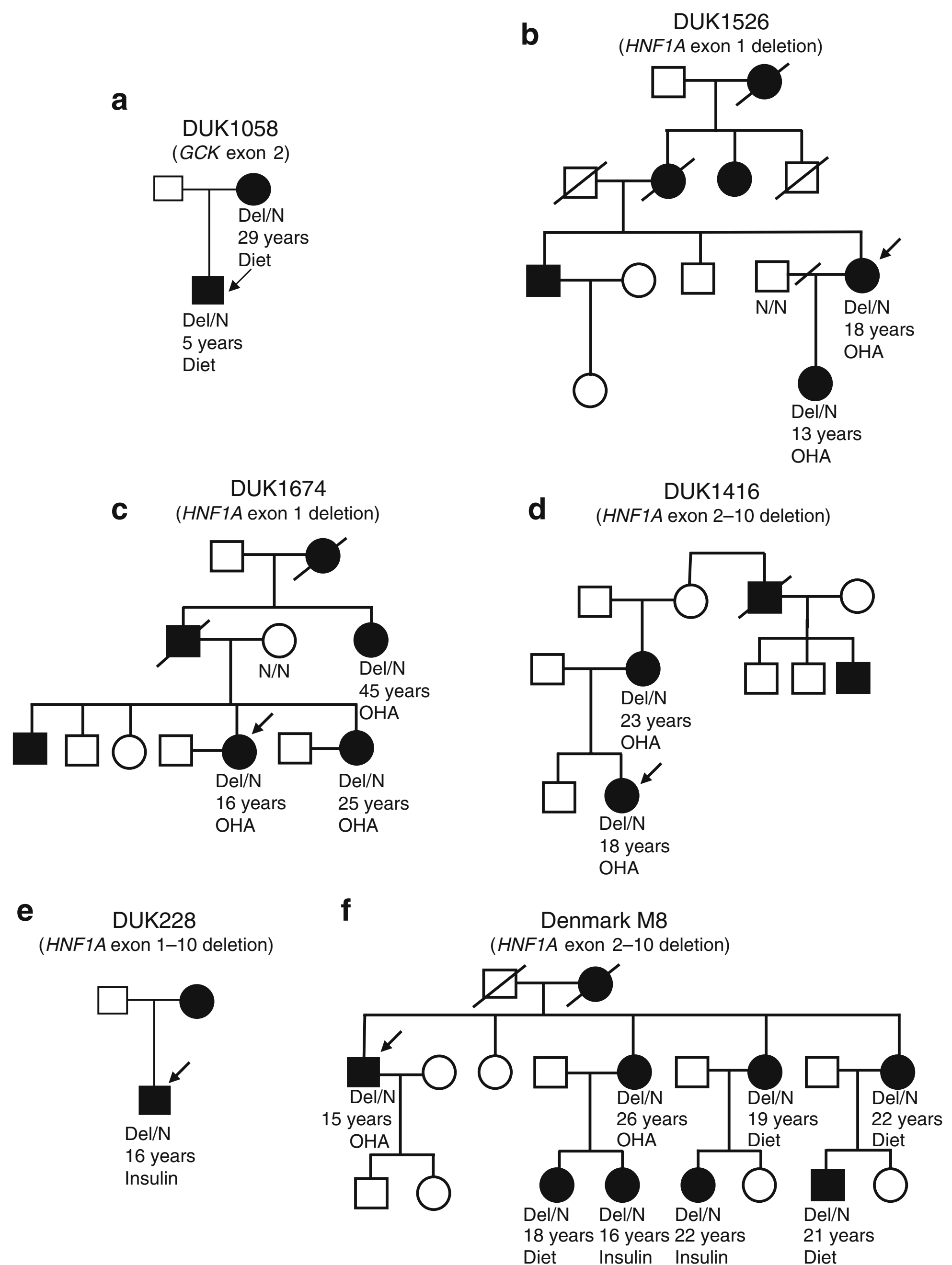

Fig. 2 Partial pedigrees for patients DUK1058 (**a**), DUK1526 (**b**), DUK1674 (**c**), DUK1416 (**d**), DUK228 (**e**) and Denmark M8 (**f**). Deletion mutation carriers (Del/N) are shown, with age at diagnosis and current treatment. Filled symbols indicate affected individuals and probands are shown by an arrow. OHA, oral hypoglycaemic agent

and A. T. Hattersley, unpublished data). Extrapolation from these data suggests that partial and/or whole gene deletions may represent up to 3% of all mutations. The inclusion of a dosage test for *HNF1A* and *GCK* mutations would result in only a small increase in the proportion of positive tests, but since mutations in these genes account for the majority of monogenic diabetes (~80% of UK cases) [1], a significant number of additional patients might be identified. The benefits of a genetic diagnosis include the possibility of transferring from insulin injections to sulfonylurea tablets for HNF-1α MODY [7], stopping insulin and oral medication treatment for GCK MODY [4] and the availability of accurate predictive testing for family members.

In contrast to *HNF1B* deletion mutations, which almost exclusively affect the entire gene (two partial gene deletions vs 30 whole gene deletions reported) [5, 6, 8], *GCK* and *HNF1A* deletion mutations show greater diversity and partial gene deletions are more common. This observation is likely to reflect the underlying genomic architecture. The breakpoints of the 1.2 Mb minimal deleted region that encompass the *HNF1B* gene are not known, but a 17q12 duplication of 1.46 Mb that includes this region was found in a patient with idiopathic mental retardation [9]. The breakpoints of this duplication map to a pair of 66 kb segmental duplications that share 99.7% homology and implicate NAHR as the mechanism for the deletion and duplication events. The closest segmental duplications flanking *HNF1A* are DC2982 (distal) and DC2979 (proximal), separated by 11 Mb. Microsatellite analysis in three families suggested that the *HNF1A* deletions are located within a smaller region of 1.63 Mb and hence segmental duplications are unlikely to promote these rearrangements. Further work is required to sequence the breakpoints of the *HNF1A* deletions in order to determine: (1) if the four probands with exon 1 or exon 2 to 10 deletions have unique rearrangements; and (2) whether the deletion mutations result from NAHR between other repetitive sequences (e.g. Alu repeats) or non-homologous end-joining. The breakpoints for the *GCK* exon 2 deletion are adjacent to MIRb and AluSx repetitive elements, suggesting that this mutation may have arisen through NAHR.

In conclusion, we report that partial or whole gene deletions can cause GCK and HNF-1α MODY. These deletion mutations are not a common cause of monogenic diabetes, but may comprise up to 3% of all *GCK/HNF1A* mutations. Although whole gene deletions of *HNF1B* account for a much higher proportion (33%) of *HNF1B* mutations, *GCK* and *HNF1A* mutations are a more frequent cause of monogenic diabetes (80 vs 6% of UK cases, [1]). We therefore recommend that gene dosage analysis be incorporated into diagnostic molecular genetic testing for MODY in order to maximise the number of patients with monogenic diabetes who may benefit from a genetic diagnosis.

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Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

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