Patient recruitment strategy for family studies investigating novel genetic causes of maturity-onset diabetes of the young

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Abstract

A small but significant minority of patients with diabetes have a defect in a single gene that is responsible for their diabetes. Determining the genetic subtype of their diabetes not only offers insights into the pathophysiology of diabetes, but also helps clinicians to determine the most appropriate treatment.

Maturity-onset diabetes of the young (MODY) is an autosomal dominant subtype of diabetes. Mutations in the glucokinase *(GCK)* gene result in mildly elevated fasting hyperglycaemia (>5.5mmol/L) throughout life. Our aim was to recruit families with this phenotype who do not have a *GCK* gene mutation in order to identify a novel genetic cause for their diabetes.

The UK MODY database, which details all subjects who have undergone a genetic test for MODY, was used as a resource to identify patients with a phenotype suggestive of GCK-MODY in whom no *GCK* mutation had been found. Interested families were visited in their homes and blood was taken for biochemical analysis and DNA extraction. Anthropometric measurements were recorded on all recruited family members.

Twelve probands were identified but two were excluded as their phenotype was no longer consistent with GCK-MODY. Four were already consented for research and the remaining six were contacted through the MODY link nurses. To date, six multi-generation families have been collected.

Our novel recruitment strategy, involving the UK MODY database in combination with a research nurse and a nationwide team of MODY link nurses, has proved successful in identifying a small subset of families who are a resource for genetic studies. Our unique approach provides a recruitment protocol that can be adapted for other genetic studies. Copyright © 2005 FEND.

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Key words

genetics of diabetes; maturity-onset diabetes of the young; glucokinase; patient recruitment

Background

Over the last 20 years our understanding of the molecular genetics of diabetes has increased dramatically. One of the areas of research, which has been the most fruitful, has been the dissection of subtypes of diabetes, which have an autosomal dominant mode of inheritance and a young age of onset. Maturity-onset diabetes of the young (MODY) is a monogenic subtype of diabetes. The term 'monogenic' is used to describe a condition, which is caused by a single gene defect within a family. Currently six MODY genes have been described but they only account for around 90% of the MODY cases in the UK so this strongly suggests that there are further MODY genes to identify.[1] MODY is characterised by three main features: a young age of onset (typically but not exclusively less than 25 years of age in at least one family member), non-insulin dependence and an autosomal dominant inheritance (diabetes is passed on from an affected individual in one generation to the next and all children with an affected parent have a 50% chance of inheriting the condition).[2] The specific genetic subtypes of MODY have distinct clinical characteristics (for details of the common UK subtypes see Table 1). The discovery of the genes involved in these subtypes of diabetes has led to a better understanding of the pathophysiology behind the diabetes and enabled clinicians to select the most appropriate treatment options for these patients.[3,4]

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Table 1. The most common subtypes of MODY in the UK; their clinical phenotype and treatment options

MODY subtype	% of UK MODY families	Phenotype	Recommended initial treatment
Glucokinase	Approx 20%	Persistent raised FPG (5.5–8mmol/L) from birth Little rise (typically <3.5mmol/L) in OGTT Not usually obese Microvascular complications rare	**None**
HNF-1α	Approx 65%	Typically develop 12–30yrs FPG may be normal Large rise (usually >5mmol/L) in OGTT Worsening glycaemia with age Low renal threshold for glucose Not obese	**Diet followed by low dose sulphonylureas**
HNF-4α	Approx 5%	Typically develop 12–30yrs FPG may be normal Large rise >5mmol/L in OGTT Worsening glycaemia with age Not obese	**Diet followed by sulphonylureas**

The Molecular Genetics Laboratory at the Royal Devon & Exeter Hospital, UK, has provided molecular genetic testing for MODY since 1995. Testing was provided on a research basis until 2000 when a UK diagnostic service was introduced which has enabled clinicians to request an appropriate diagnostic genetic test for patients with suspected MODY. Genetic testing is useful because it can confirm the diagnosis, guide clinicians regarding the most appropriate treatment option, help to define the prognosis and also help with family counselling as other family members can be offered genetic testing.[2] A specific example of where genetic testing is useful would be in differentiating between children who have type 1 diabetes and those with MODY.[5] These conditions have different treatment options and prognoses in addition to implications for other family members.

The MODY link nurse project is a Department of Health funded initiative in the UK which is exploring ways in which genetic testing can be integrated into diabetes care.[6] The project involves 17 diabetes specialist nurses (DSNs) who are seconded to the project for 3.5 hours per week. They receive ongoing training in Exeter to learn about the genetics of diabetes, genetic counselling and genetic testing. The MODY link nurses disseminate this new genetic information to health care professionals in their allocated areas, assess patients with a possible diagnosis of MODY and provide support for known MODY families. The 17 nurses are each based in different locations throughout England and Scotland, as shown in Figure 1. Their role is to increase and update the knowledge of diabetes teams within their allocated area about the different types of MODY and the diagnostic genetic tests that are available. The nurses also assist in identifying families who are likely to have MODY and, with the support of the Exeter team, will recommend a diagnostic genetic test. In addition, they are also able to discuss the implications of the genetic test with the families and guide the follow up of other family members once the results have been received.

Mutations in the glucokinase *(GCK)* gene are responsible for one of the six known subtypes of MODY.[7] Patients with this genetic subtype are characterised by persistent stable elevated fasting plasma glucose (FPG) values (>5.5 mmol/L but <8mmol/L) and a small two-hour increment on an oral glucose tolerance test (typically <3.5mmol/L). Patients with GCK-MODY have a defect in insulin secretion from birth but are often asymptomatic and therefore are only identified during routine testing (e.g. in pregnancy). The aim of our current study was to recruit families with this phenotype, who do not have a mutation in the *GCK* gene, for further study with a view to identifying a novel genetic cause for their diabetes.

Materials and methods

Identifying patients for recruitment

The study was carried out with multicentre research ethics approval. The strategy employed for patient recruitment involved access to the UK MODY database which details all subjects in the UK who have under-

gone a genetic test for MODY, through either research studies or the service. To identify potential families for recruitment for this study, the database was searched for subjects who had undergone a genetic test for GCK-MODY but in whom no mutation had been identified. In order to identify the patients who had a phenotype which was most similar to those with GCK-MODY, and who were most likely to have a genetic rather than environmental reason for their elevated FPG, we selected patients who met the following criteria: (1) following a genetic test for GCK-MODY no mutation in the *GCK* gene was identified; and (2) the patient was diagnosed under the age of 15 years (this reduced the possibility that their elevated FPG was due to environmental factors such as BMI or due to a decline in beta-cell function with age). From those who met the above criteria, we selected patients with FPG levels of >5.5mmol/L, and, where data were available, a two-hour increment on an OGTT of <3.5mmol/L. In order to comply with data protection, the MODY link nurses were utilised as a network of health care professionals to approach potential families where informed consent had not already been given for this research. This was done either through direct contact with the family or through contact with the referring clinician. Interested families were then approached by letter. A patient information sheet was enclosed to explain the outline of the study and why we were interested in recruiting them and other members of their family. The letter was followed up a week later with a phone call. During the initial phone conversation it was explained that we needed to recruit as many family members as possible as large families are required for genetic linkage studies. We also explained that it would be necessary to test all family members as affection status would be assigned on the basis of an elevated FPG level (>5.5mmol/L). Once it was established that a family was keen to participate, information was taken over the phone to allow a detailed family tree to be drawn along with the relevant clinical details and geographical location of family members. This was especially important when a large extended family was to meet in one home. Participants were asked to fast from 10pm the previous evening and a letter confirming the appointment was sent to each family member. Figure 2 illustrates an overview of the recruitment strategy.

Figure 1. Map of the United Kingdom illustrating the location of the 17 MODY link nurses funded by the Department of Health/Scottish Executive

Collecting samples, anthropometric measurements and family histories

On meeting family members time was allowed for answering any questions they had regarding the research, as well as confirming clinical details and information on family structure to aid the drawing of a family tree.

All participants were consented prior to giving fasting blood samples. For children (under the age of 16 years at recruitment) consent was obtained from their parents or guardian and the children were given a verbal explanation of the study. As having a blood sample taken can be uncomfortable for children, we offered them the alternative of collecting buccal cells using a mouthbrush which was gently brushed inside the mouth for DNA extraction.

Blood samples for FPG, HbA_{1c}, fasting lipids and DNA extraction

were taken using a butterfly and vacutainer system. For viability/diagnostic purposes the fasting plasma sample was taken first; this was immediately stored on ice until the remaining samples had been taken. Before leaving the house this sample was spun in a portable centrifuge Thermo Medilite 6 and plasma decanted into a separate tube and stored on ice. This was assayed next morning in the local laboratory. The HbA_{1c} was assayed by the nurse using a DCA 2000+ Analyzer. The remaining samples for DNA and lipids were stored to be assayed at a later date.

A full medical history was recorded on a clinical research form; this included illnesses, present medications, pregnancies, clarification of family relationships, birth weight and gestation. Anthropometric measurements including weight, height, waist and hip were taken. Three blood pressure measurements were recorded using the Omron 705 CP machine, (Whites Medical, Rugby, UK).

Except for children, each member of the family was screened in a separate room to ensure privacy.

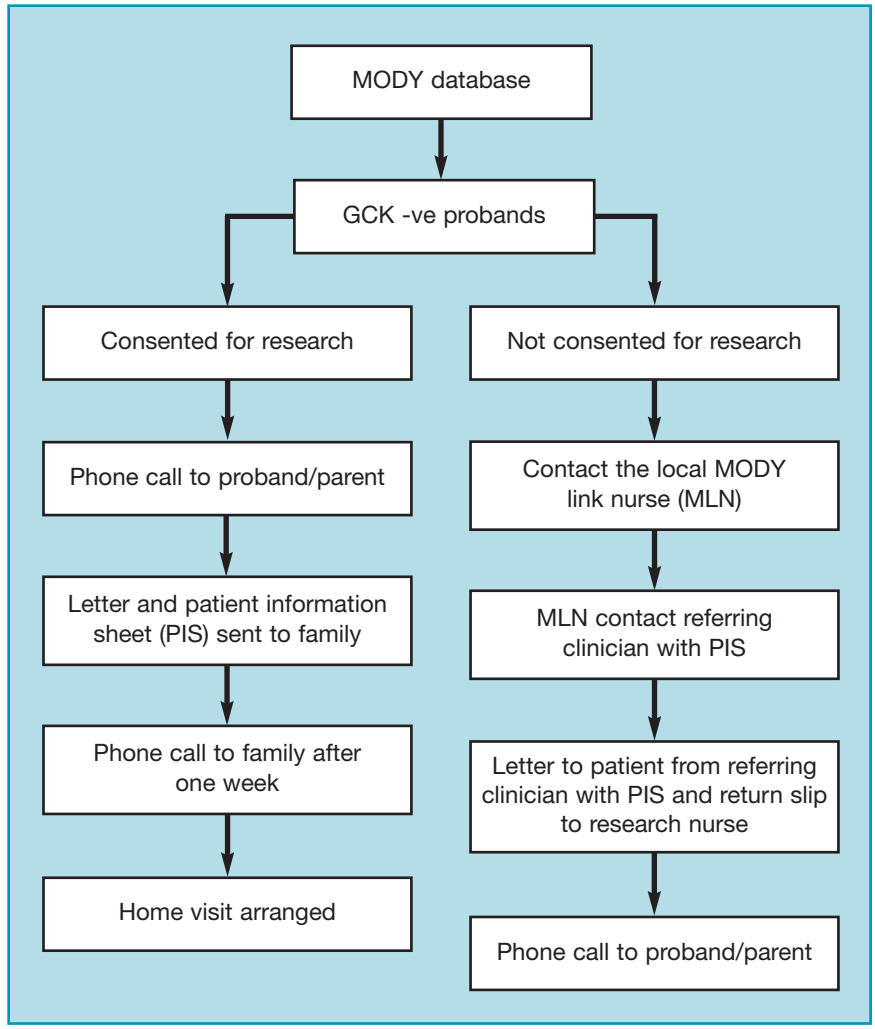

Figure 2. Flowchart illustrating the patient recruitment strategy employed in this study

Results

Searching the MODY database for patients who had undergone a molecular genetic test between 1995 and March 2003 identified a total of 1151 probands who had been tested. Of these 209 had undergone a genetic test for *GCK* and 147 did not have a mutation in the *GCK* gene. However, only 12 individuals (8%) of these probands fitted our stringent selection criteria (age of diagnosis <15 years, FPG >5.5mmol/L, negative for a *GCK* mutation, two-hour increment on an OGTT <3.5mmol/L). Two of these were excluded on the basis of their phenotypes which were no longer consistent with GCK-MODY upon follow up. One patient had subsequently been diagnosed with type 1 diabetes on the basis of positive auto-antibodies, and the second proband was subsequently identified as having familial hypercholesterolaemia in addition to their diabetes. Of the remaining 10 families, four had already been consented for research and therefore were contacted directly, and the other six were contacted through the MODY link nurses. The four families already consented for research all agreed to take part in the study and have been successfully recruited. Of the remaining six families who have been contacted through the MODY link nurses, to date we have recruited and collected samples on two families and of the remaining four families we are awaiting a reply from two. The pedigrees for the recruited families are illustrated in Figure 3. Two families did not respond to a letter from their referring clinician, and for the final family the referring clinician advised us that this particular family was difficult to trace and would be unsuitable for our study.

Discussion

One of the difficulties of genetic studies is identifying suitable families who meet the selection criteria. Since FPG increases with age due to a decline in beta-cell function in the normoglycaemic population, by

selecting children as our probands and basis for family recruitment we hope that we have identified individuals where there is a genetic explanation for their elevated FPG values. Since GCK-MODY is often asymptomatic, it is critical that all family members included in the genetic analysis have accurate biochemical measurements recorded, particularly FPG values, prior to affection status being assigned. We have identified several family members who were unaware of their impaired fasting glycaemia (IFG). Genetic studies rely on families and consequently their recruitment forms the basis for a successful project. Therefore, particular importance was given at recruitment to finding suitable families and approaching them in a correct manner: giving them adequate time to make an informed decision and to contact their extended families; being sensitive about contacting them at a convenient time; and, finally, being flexible in selecting a suitable time for visiting the family.
Not all families maintain contact with each other and one needs to be aware of delicate issues such as family relationships, divorce and family disagreements. Therefore, sensitivity and discretion are required when discussing family relationships.

We have made it our policy to write to each of our families after each visit thanking them for their time. All families are updated with progress of the overall study but they are not informed of their own specific results unless it would affect their health, e.g. IFG. In this case a letter is sent to their GP informing him or her of the patient's involvement in the study and their results.

In addition to making the initial contact with families who have not been consented for research, the MODY link nurses have provided an additional role in obtaining further clinical details on recruited families and providing assistance on home visits when samples from larger families are being collected. The network of MODY link nurses also facilitates the collection of samples from family members who do not live in close proximity to their family and cannot be present for the main home visit. The MODY link nurses are able to visit these individuals to collect samples and measurements – this avoids the necessity for the study research nurse to make home visits for one subject. The MODY link nurses have been instrumental in raising the awareness of the availability of genetic testing for diabetes in the UK. The additional knowledge that DSNs gain from the talks given by the MODY link nurses raises their awareness of possible patients in their care who might benefit from genetic testing. The increased number of referrals has consequently increased the number of families in the UK MODY database which has provided an improved resource for patient recruitment.

Our experience has illustrated that families respond better to

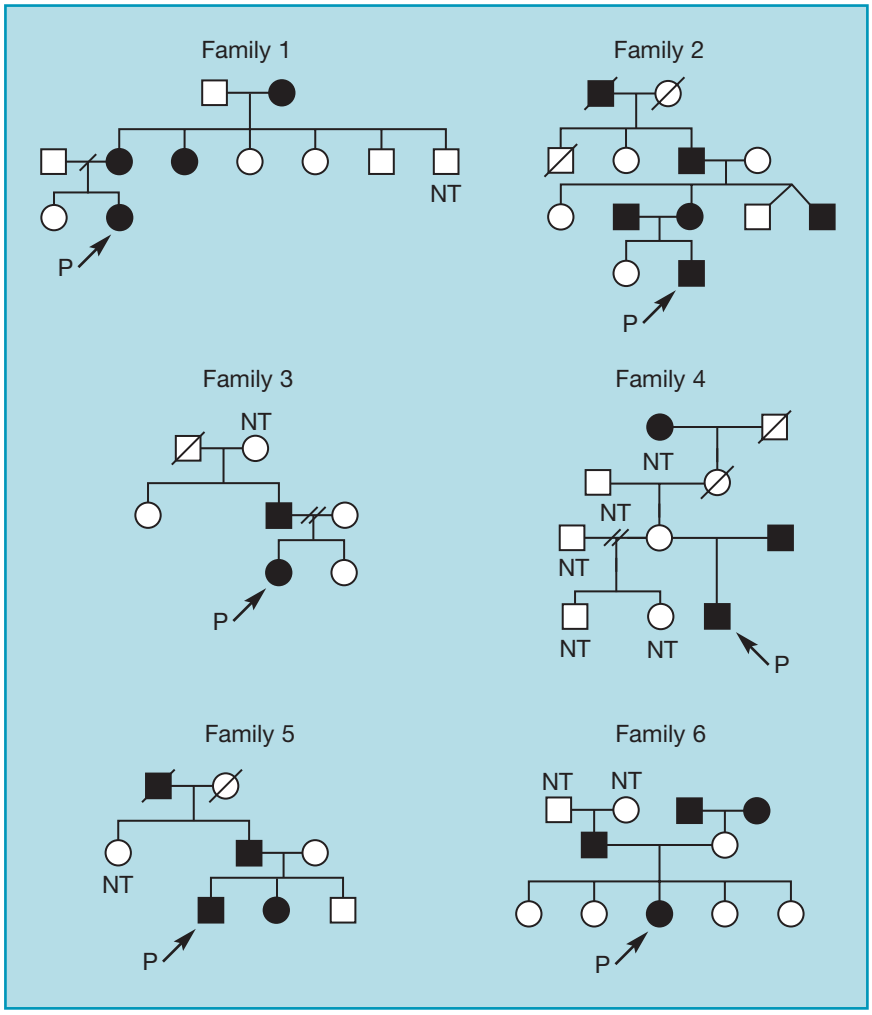

Figure 3. Pedigrees for recruited families. Circles represent females, squares represent males. Filled (black) circles and squares represent persons with elevated fasting plasma glucose (>5.5mmol/L). NT denotes that the subject has not been tested. P and an arrow denote the proband in each family (the first affected member recruited for this study)

direct contact by telephone rather than by letter. This is evident by the 100% success rate with recruitment from families contacted directly, compared to the response from those who are informed about the study only by letter. However, the combined approach of using a national database with a team of trained and experienced MODY link nurses has proved effective in establishing a collection of families suitable for genetic studies. Our study has shown that DSNs who have received additional training in the genetics of diabetes are a valuable tool not only in selecting appropriate families for recruitment, but also in collecting essential background information and samples which are necessary for determining the precise phenotype. We hope that this approach will continue to prove effective in extending our current cohort and also for future genetic studies investigating novel causes of diabetes.

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Eurowatch

Patient mobility across the EU: issues and challenges

John Bowis, MEP

Most of us are aware of the long-established E111, a certificate that can be taken when travelling, studying, being posted or seeking employment outside one's own country and within the European Union. This allows access to unplanned medical care on the same basis as local people and entitles the holder to have any costs reimbursed by their home country. The E111 and other current paper forms are being replaced by the European health insurance card – the so-called Health Passport.

In recent years, patients have also sought medical treatment in other countries for non-emergencies. Far less well known has been the E112 certificate, which authorises visits to other member states for planned treatment. E112 authorisation has been rarely given in the past. More usually, in recent times there have been referrals of groups of patients under bilateral contracts, as part of waiting time initiatives. Now the debate has been moved on and more power placed in the hands of patients themselves. There has been a series of court rulings by the European Court of Justice on patients' rights to receive planned treatment in other EU member states in situations where they cannot be treated in their home country within an acceptable time limit.

These patient mobility rights, stemming from the European Treaty's enshrined principle of freedom of movement with the EU, are