family members and unrelated non-diabetic white controls was assessed by amplifying and directly sequencing the appropriate exon.

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Mutations in the hepatocyte nuclear factor-4α gene in maturity-onset diabetes of the young (MODY1)

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The disease maturity-onset diabetes of the young (MODY) is a genetically heterogeneous monogenic form of non-insulin-dependent (type 2) diabetes mellitus (NIDDM), characterized by early onset, usually before 25 years of age and often in adolescence or childhood, and by autosomal dominant inheritance[1]. It has been estimated that 2–5% of patients with NIDDM may have this form of diabetes mellitus[2,3]. Clinical studies have shown that prediabetic MODY subjects have normal insulin sensitivity but suffer from a defect in glucose-stimulated insulin secretion, suggesting that pancreatic β-cell dysfunction rather than insulin resistance is the primary defect in this disorder[4,5]. Linkage studies have localized the genes that are mutated in MODY on human chromosomes 20 (*MODY1*)[6], 7 (*MODY2*)[2] and 12 (*MODY3*)[7], with *MODY2* and *MODY3* being allelic with the genes encoding glucokinase[2], a key regulator of insulin secretion, and hepatocyte nuclear factor-1α (HNF-1α)[8], a transcription factor involved in tissue-specific regulation of liver genes but also expressed in pancreatic islets, insulinoma cells and other tissues. Here we show that *MODY1* is the gene encoding HNF-4α (gene symbol, *TCF14*), a member of the steroid/thyroid hormone receptor superfamily and an upstream regulator of HNF-1α expression[9–11].

The R-W pedigree (Fig. 1), which includes more than 360 members spanning 6 generations and 74 members with diabetes, including those with MODY, has been studied prospectively since 1958 (ref. 1). The members of this family are descendants of a couple that emigrated from East Prussia to Detroit, Michigan in 1861 with their four sons, three of whom were diabetic, and five daughters, one of whom was diabetic (refs 1 and 12, and S.S.F., unpublished results). Linkage studies have shown that the gene responsible for MODY in this family, *MODY1*, is tightly linked to markers in the chromosome 20 band 20q12–q13.1, with a multipoint lod score >14 in those branches of the family in which MODY is segregating[6,13]. The analysis of key recombinants in the R-W pedigree localized *MODY1* to a 13-cM interval (~7 megabases (Mb)) between markers *D20S169* and *D20S176*, an interval which also includes the gene encoding HNF-4α (ref. 14). The demonstration that mutations in the HNF-1α gene are the cause of the MODY3 form of NIDDM[8] prompted us to screen the HNF-4α gene for mutations in the R-W pedigree.

The 11 exons of the HNF-4α gene of two affected (V-20 and 22) and one unaffected (VI-9) subject from the R-W pedigree (Fig. 1) were amplified and the products of polymerase chain reaction (PCR) sequenced directly. The sequences were identical to one another and the complementary DNA[15], except for C → T substitutions in codons 130 (exon 4) and 268 (exon 7). The C → T substitution in codon 130 resulted in a Thr(ACT) → Ile(ATT) substitution and is a polymorphism (T/I130) with a frequency of the Ile allele in a group of 55 unrelated nondiabetic non-Hispanic white subjects of 5%. The C → T substitution in codon 268 generated a nonsense mutation CAG(Gln) → TAG(AM) (Q268X). In the R-W pedigree, Ile 130 and the amber mutation at codon 268 were present on the same allele.

The Q268X mutation created a digestion site for the restriction enzyme *Bfa*I; digestion of the normal allele generated fragments of 281 and 34 base pairs (bp), whereas the mutant allele generated fragments of 152, 129 and 34 bp. The Q268X mutation cosegregated with the at-risk haplotype and NIDDM in the R-W pedigree (Fig. 1) and was not observed on screening 108 healthy nondiabetic non-Hispanic white subjects (216 normal chromosomes). Seven subjects in the R-W pedigree who inherited the mutant allele (V-18, 37 and 48; VI-6, 11, 15 and 20) have normal glucose tolerance (Fig. 1). Five of these subjects (V-48, and VI-6, 11, 15 and 20) are less than 25 years old and thus are still within the age range when diabetes usually develops in at-risk individuals in this family. Of the others, subject V-18 is 44 years old and has had normal glucose levels in all oral glucose tolerance tests; and subject V-37, who is 36 years old, showed impaired glucose tolerance in one test and one diabetic response at 16–17 years old, but for the past 19 years each glucose tolerance test has been normal, even though she has a low insulin response to orally administered glucose. V-37 is lean and active, and showed increased sensitivity to insulin during a frequently sampled intravenous glucose tolerance test; during a prolonged low-dose glucose infusion, she became markedly hyperglycaemic[4,5]. Two subjects (V-1 and V-4) who have the mutation were considered to be non-diabetic on the basis of their medical history, but their

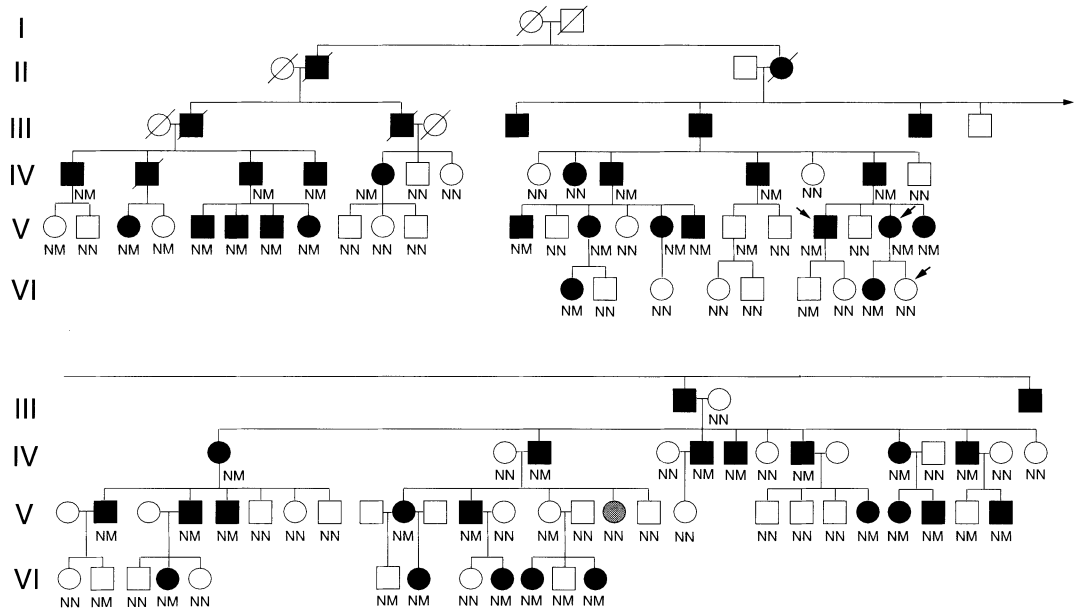

FIG. 1 The R-W pedigree, modified and abbreviated from ref. 14. Individuals with MODY/NIDDM are indicated by black symbols and non-diabetic individuals by white symbols. Subject V-39 has insulin-dependent diabetes mellitus. Arrows indicate the three subjects (two affected and one unaffected) who were screened for mutations in the HNF-4α gene. The HNF-4α genotype of each individual, if known, is indicated below the symbol: N, normal; M, Q268X mutation.

status needs to be evaluated by oral glucose tolerance testing. Our results indicate that the nonsense mutation in the HNF-4α gene in the R-W pedigree is highly but not completely penetrant, although the age of diabetes onset is variable.

In addition to subjects who inherited the Q268X mutation but are at present non-diabetic, there are subjects in the R-W pedigree who have NIDDM but did not inherit the Q268X mutation or at-risk haplotype. Subject IV-9 was diagnosed with NIDDM at 48 years of age and was hyperinsulinemic, a diagnosis consistent with late-onset NIDDM rather than MODY. We also tested her six children, one of whom had NIDDM and another impaired glucose tolerance, and all had two normal alleles (data not shown). Likewise, we tested 10 children of subject III-7, five of whom had NIDDM, and none had inherited the Q268X mutation, suggesting that the NIDDM in this branch of the R-W family is of a different aetiology. The five non-diabetic children of III-11 were also tested for the Q268X mutation and all were normal. The presence of both MODY and late-onset NIDDM in the R-W family has been noted previously[6]. The MODY phenotype results from a mutation in the HNF-4α gene. The cause(s) of the late-onset NIDDM is unknown. Finally, subject V-39, who has insulin-dependent diabetes mellitus, did not inherit the Q268X mutation, suggesting that mutations in the HNF-4α gene do not increase the risk of developing this form of diabetes mellitus.

HNF-4α is a member of the steroid/thyroid hormone receptor superfamily and is most highly expressed in liver, kidney and intestine[9,16]. It is also expressed in pancreatic islets and insulinoma cells (ref. 17, and K.Y., unpublished results). HNF-4α is a key regulator of hepatic gene expression and is a major activator of HNF-1α, which in turn activates the expression of a large number of liver-specific genes, including those involved in glucose, cholesterol and fatty acid metabolism[9,10]. Its expression in kidney, intestine and pancreatic islets implies that it is important in tissue-specific regulation of gene expression in these tissues as well, although its specific function in non-hepatic tissues has not been addressed. Homozygous loss of functional HNF-4α protein causes embryonic lethality characterized by defects in gastrulation, underscoring the key role played by this transcription factor in development and differentiation[18]. The phenotype of the heterozygous animals was not described and further studies are necessary to determine whether they represent a mouse model of MODY.

The importance of HNF-4α in regulating hepatic gene expression is well established[9,10], but the role it plays with HNF-1α, the *MODY3* product and a downstream target of HNF-4α, in regulating gene expression in the insulin-secreting pancreatic β-cell is still unclear, although HNF-1α is known to be a weak transactivator of the insulin gene[19]. The mechanism by which mutations in HNF-4α result in an autosomal dominant form of NIDDM characterized by pancreatic β-cell dysfunction is not known. The nonsense mutation in HNF-4α found in the R-W family is predicted to result in the synthesis of a protein of 267 amino acids with an intact DNA-binding domain. However, it is missing the regions involved in dimerization and transcriptional activation in other members of the steroid/thyroid hormone superfamily[20–23] and so is probably unable to dimerize or bind to its recognition site and activate transcription. We propose that the dominant inheritance is due to a reduction in the amount of HNF-4α *per se*, rather than to a dominant-negative mechanism. A decrease in functional HNF-4α appears to have a critical effect on β-cell function, perhaps because of decreased HNF-1α expression; mutations in this gene also lead to MODY[8]. Prediabetic subjects with mutations in either the HNF-4α or HNF-1α genes exhibit similar abnormalities in glucose-stimulated insulin secretion, with normal insulin secretion rates at lower glucose concentrations but lower-than-normal rates as the glucose concentration increases[5,24], a result consistent with HNF-4α and HNF-1α affecting a common pathway in the pancreatic β-cell. The absence of overt hepatic, renal or gastrointestinal dysfunction in affected members of the R-W pedigree suggests that the levels of HNF-4α in these tissues, although possibly lower than normal, are sufficient to ensure normal function, or that alternative pathways are sufficient for expression of key genes. However, detailed investigations of hepatic glucose production and metabolism have not been made in subjects from the R-W pedigree and it is possible that subtle alterations in these processes may be operating.

The demonstration that MODY can result from mutations in the HNF-1α and HNF-4α genes suggests that this form of NIDDM is primarily a disorder of abnormal gene expression. Genes encoding other proteins in the HNF-1α/HNF-4α regulatory cascade[10] should be considered as candidates for other forms of MODY and/or late-onset NIDDM. The role of HNF-4α in the development of the more common late-onset NIDDM is unknown. There is no evidence for linkage of markers flanking the HNF-4α gene with late-onset NIDDM in Mexican Americans[25] or Japanese (ref. 26, and N. Iwasaki and G.I.B., unpublished results), implying that mutations in the HNF-4α gene are unlikely to be a significant genetic factor contributing to the development of late-onset NIDDM. However, acquired defects in HNF-4α expression may contribute, at least in part, to the β-cell

dysfunction that characterizes late-onset NIDDM[27], especially if it is central in regulating gene expression in the pancreatic β-cell, as suggested by the association of mutations in the HNF-4α gene with MODY. Furthermore, the similarity between HNF-4α and ligand-dependent transcription factors raises the possibility that HNF-4α and the genes it regulates respond to an unidentified ligand. The identification of this ligand could lead to new treatments for diabetes. □

Methods

Isolation and partial sequence of the human HNF-4α gene. Three PAC clones (114E13, 130B8 and 207N8) containing the human HNF-4α gene were isolated from a library (Genome Systems) by screening DNA pools by PCR with the primers HNF4-1 (5′-CACCTGGTGATCACGTGGTC-3′) and HNF4-2 (5′-GTAAGGCTCAAGTCATCTCC-3′). The partial sequence of the gene was determined after amplifying PAC 114E13 and genomic DNA with specific primers whose sequences were based on the human cDNA[15,28] and selected using the exon–intron organization of the mouse HNF-4α gene[29] as a guide. PCR products were sequenced using an AmpliTaq FS Dye Terminator Cycle Sequencing Kit (Applied Biosystems) and an ABI Prism 377 DNA Sequencer. This gene consists of 11 exons, with the introns being located in the same positions as in the mouse gene[29]. Alternative splicing generates a family of mRNAs, HNF-4α1, 2 and 4, the latter two of which contain inserts of 30 and 90 nucleotides, respectively[15,28,29]. The sequence of exon 1B, the exon encoding the insertion in HNF-4α4 mRNA, revealed an additional T between nucleotides 219 and 220 in both alleles of five unrelated individuals (10 chromosomes) not present in the cDNA sequence[28], which causes a frameshift and generates a protein of 98 amino acids whose function is unknown. The sequences of the exons and adjacent introns have been deposited in the GenBank database (accession numbers, U72959–U72969).

Screening of the HNF-4α gene for mutations. The eleven exons and flanking introns were amplified using PCR and specific primers (the primer sequences are described in Supplementary Information). PCR conditions were denaturation at 94 °C for 5 min followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 60 °C for 30 s, and extension at 72 °C for 30 s, with a final extension at 72 °C for 10 min. PCR products were purified using a Centricon-100 membrane (Amicon) and sequenced directly from both ends using an AmpliTaq Cycle Sequencing Kit and ABI Prism 377 DNA sequencer. The sequence of the Q268X mutation was confirmed by cloning the exon-7 PCR product into pGEM-T (Promega) and sequencing individual clones. The presence of the C → T mutation in codon 268 in members of the R-W pedigree and a group of unrelated non-diabetics was assessed by amplifying exon 7, digesting with *Bfa*I, and separation of the digested PCR product on a 3% agarose gel. The frequency of the T/I130 polymorphism was determined by amplification of exon 4 and digestion with *BstB*I, which does not cleave the Thr allele but cleaves the Ile allele into fragments of 184 and 87 bp.

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Role of learning in three-dimensional form perception

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One of the most remarkable characteristics of the human visual system is its ability to perceive specific three-dimensional forms in single two-dimensional contour images. This has often been attributed to a few general purpose and possibly innately specified shape biases[1–6], such as those favouring symmetry and other structural regularities (Fig. 1). An alternative approach proposed by the early empiricists[7–10] and since tested[11] suggests that this ability may also be acquired from visual experience, with the three-dimensional percept being the manifestation of a learned association between specific two-dimensional projections and the correlated three-dimensional structures. These studies of shape learning have been considered inconclusive, however, because their results can potentially be accounted for as cognitive decisions that might have little to do with shape perception *per se*. Here we present an experimental system that enables objective verification of the role of learning in shape perception by rendering the learning to be perceptually manifest. We show that the human visual system can learn associations between arbitrarily paired two-dimensional pictures and (projectionally consistent) three-dimensional structures. These results implicate high-level recognition processes in the task of shape perception.

Our experiment comprised a training and a test phase (Fig. 2). For use as stimuli, we generated random two-dimensional (2-D) line-drawings and assigned them arbitrary three-dimensional (3-D) structures. During the training phase, subjects were shown one such object rocking through an angle of ±20 degrees about a frontoparallel horizontal axis passing through its centroid (we shall refer to this as the 'training' object). This was to allow the subjects to observe the correlation between the object's mean-angle projection and its associated 3-D structure through the kinetic-depth effect (KDE)[12,13]. The test phase commenced five seconds after the training session and was intended to assess subjects' shape learning. Subjects were shown either the training object rocking back and forth, or another object that had the same mean-angle projection but a completely different 3-D structure (the 'test' object). They were asked to indicate whether the objects looked rigid or non-rigid. We expected that if the subjects had indeed learned an association between the training object's mean-angle projection and its 3-D structure, then, when presented with a test object having the same mean-angle projection, they would perceptually impose on it the learned 3-D structure and the observed motion pattern would be mapped onto this learned structure. If in fact the test object had a different 3-D structure, the sequence would appear to depict a non-rigid deformation. We