Distinct Molecular and Morphogenetic Properties of Mutations in the Human HNF1β Gene That Lead to Defective Kidney Development

SILVIA BOHN,* HEIKE THOMAS,* GÜLÜZAR TURAN,* SIAN ELLARD,† CORALIE BINGHAM,† ANDREW T. HATTERSLEY,† and GERHART U. RYFFEL,*

*Institut für Zellbiologie, Universitätssklinikum Essen, Essen, Germany; and †Diabetes and Vascular Medicine, Peninsula Medical School, Exeter, Devon, United Kingdom.

Abstract. The homeobox transcription factor hepatocyte nuclear factor 1β (HNF1β) is a tissue-specific regulator that plays an essential role in early vertebrate development. In humans, heterozygous mutations in the HNF1β gene are associated with young-onset diabetes as well as a variety of disorders of renal development with cysts as the most consistent feature. This report compares and classifies nine different HNF1β mutations that lead in humans to distinct renal diseases, including solitary function- ing kidney, renal dysplasia, glomerulocystic kidney disease, and oligomeganephronia. Analysis of these mutants in vitro identifies mutants that either retain or lack DNA binding. Investigation of the transactivation potential in transfected cell lines reveals a strict correlation between DNA binding and transactivation. Introduction of these mutants into developing Xenopus embryos shows that these mutants interfere with pronephros development, the first kidney form in amphibian. Whereas three mutants lead in Xenopus to a reduction or agenesis of the pronephric tubules and the anterior part of the duct, six mutants generate an enlargement of the pronephric structures. The differential morphogenetic potential in the developing embryo does not strictly correlate with the properties observed in vitro or in transfected cell lines. This suggests that the functional test in the developing embryo defines features of the HNF1β protein that cannot be assessed in cell cultures. The distinct properties observed in the various HNF1β mutants may guide the classification of the phenotypes observed in patients with a mutated HNF1β gene.

The tissue-specific transcription factor hepatocyte nuclear factor 1β (HNF1β) is expressed in epithelial cells of many organs, including the pancreas and the kidney (1). Heterozygous mutations in the human HNF1β gene cause a subtype of maturity-onset diabetes of the young (MODY) designated MODY5. MODY is a dominantly inherited form of non–insulin-dependent diabetes with onset usually under the age of 25 yr (2). In addition, mutated HNF1β alleles are associated with a variety of disorders of renal development, including solitary functioning kidney (3), renal dysplasia (3,4), glomerulocystic kidney disease (GCKD) (5), and oligomeganephronia (6). The most consistent clinical feature of the renal phenotype is renal cysts. The presence of an HNF1β mutation in association with renal cysts and diabetes has been termed the renal cysts and diabetes (RCAD) syndrome (7,8).

HNF1β-deficient mouse embryos die at embryonic day 7.5 as a result of a defective differentiation of the extra embryonic visceral endoderm (9,10). This early embryonic lethality has precluded the analysis of HNF1β function in mammalian kidney development.

In vertebrates, three distinct types of kidneys—the pronephros, the mesonephros, and the metanephros—are formed progressively during development. All three kidneys have the same basic functional unit, the nephron, and similar mechanisms and regulatory genes are involved in nephrogenesis (11,12). In amphibians, the pronephros is the functional kidney throughout larval development, and the frog Xenopus laevis is most attractive to analyze the molecular and cellular events in pronephros formation (13,14). Using the Xenopus system, we have shown that the introduction of a mutated human HNF1β transcription factor into the developing frog embryo leads to partial or even complete agenesis of the pronephric tubules and duct (15).

HNF1β is a member of the homeodomain family and contains an N-terminal dimerization domain, a homeobox and a POU A domain involved in DNA binding, and a transactivation domain at the C-terminus (1). At the end of 2001, we were aware of 11 distinct mutations in the human HNF1β gene that are distributed over the entire open reading frame (2–6,8,16–18). In this report, we compare different HNF1β mutations, which seem to lead in humans to distinct renal diseases. By analyzing the molecular properties in vitro, in transfected cell cultures as well as in injected Xenopus embryos, we can classify the various HNF1β mutants.
Materials and Methods

Plasmid Constructions

The expression vector encoding human HNF1β wild-type, P328L329del CCTCT, and R137-K161del have been described (15). The additional HNF1β mutations were made by site-directed mutagenesis (Quick Change site-directed mutagenesis kit; Stratagene, La Jolla, CA) on the myc-Rc/CMVHNF1\(\beta\) construct using the following primer pairs: S151P, 5'-CGCACCTCCCCCGACATC-3', 5'-GATGCTGGGGAGGTGGG-3'; E101X, 5'-CCTACAACCTAGGAGGCG-3'; 5'-CCGCTCTCTCTATTTGGAGG-3'; R177X, 5'-CAGAAAGC-\(\alpha\)ATGAGAGATC-3'; P328L329delCCTCT, and R137-K161del have been described (15). The expression vector encoding human HNF1\(\beta\) mutant together with 100 pg of capped GFP mRNA were injected. The transactivation activity was measured after 20 h using the luciferase reporter assay system (Promega, Madison, WI) and a Lumat LB 9501 luminometer (Berthold, Wilbad, Germany). Immunofluorescence was performed as described previously (19). Nuclear extracts of cultured HeLa cells and gel shift experiments were performed as described (20,21).

Cell Culture, Transfection, Luciferase Assay, Immunofluorescence, and Gel Shift Experiments

HeLa and HEK293 cells were cultured at 37°C in Dulbecco’s modified Eagle’s medium supplemented with penicillin (100 U/ml), streptomycin (100 µg/ml), and 10% heat-inactivated FCS. The cells were transfected using 1.3 µg of reporter gene, 0.3 µg of expression vector, and 6 µl of lipofectamine (Invitrogen, Carlsbad, CA) in a 3.3-cm cell culture dish. The final DNA concentration was equalized by the addition of Rc/CMV vector. The transactivation activity was measured after 20 h using the luciferase reporter assay system (Promega, Madison, WI) and a Lumat LB 9501 luminometer (Berthold, Wilbad, Germany). Immunofluorescence was performed as described previously (19). Nuclear extracts of cultured HeLa cells and gel shift experiments were performed as described (20,21).

Embryos, Microinjection of Synthetic mRNA, and Immunohistochemistry

In vitro fertilization and culture of Xenopus laevis embryos were performed as described (22). The expression vectors encoding mutated HNF1\(\beta\) were digested with SmaI and used as a template for in vitro transcription driven by the T7 RNA polymerase. The green fluorescence protein (GFP) encoding expression vector pCSGFP2 (23) was linearized with PvuII and in vitro transcribed using SP6 RNA polymerase. A total of 250 pg of capped mRNA encoding an HNF1\(\beta\) mutant together with 100 pg of capped GFP mRNA were injected into one blastomere of two-cell-stage embryos. At the swimming larval stage 45 (24), the animals were fixed in MEMFA (0.1 M MOPS [pH 7.4], 2 mM EGTA, 1 mM MgSO4, 3.7% formaldehyde) and subsequently dehydrated in methanol and stored at -20°C.

For whole-mount immunostaining, the embryos were rehydrated with PBS and blocked with PBS and 0.1% Triton X (PBT)/20% goat serum for 1 h at room temperature. Hybridoma supernatant of the monoclonal antibodies 3G8 or 4A6 (provided by Dr. E. A. Oliver-Jones, Warwick University, Coventry, UK) was diluted 1:2 with PBT/20% goat serum and incubated overnight at 4°C. After washing five times with PBT at room temperature, incubation with a 1:1000 diluted Cy3-conjugated anti-mouse antibody was done overnight. Embryos were washed five times with PBT at room temperature and analyzed by fluorescence microscopy.

Statistical Analyses

The difference between injected and noninjected sides was evaluated by measuring the area through the widest part of the immunostained pronephros containing the pronephric tubules and anterior part of the pronephric duct. The measurements were done with the computer program Kappa Image Base Meteo (opto-electronics GmbH, Gleichen, Germany), and the noninjected side was used as a reference for each animal. No size difference was set as 100. A Mann-Whitney U test was used to calculate the significance of the phenotype (P value) obtained from each mutant in comparison with the GFP control injection.

Results

Selection of the HNF1\(\beta\) Mutants

At the beginning of our research project, 11 distinct mutations in the human HNF1\(\beta\) gene had been identified in exon as well as in the intron sequences close to exon-intron boundaries. We limited our analysis to mutations localized in the exon sequences, because the two mutations in intron 2 (17,18) influence splicing in an unpredictable way.

The list of mutants whose properties we have studied is shown in Figure 1. The missense mutation S151P has a change from serine to proline at amino acid position 151 encoding the POA domain (3). The two nonsense mutations E101X (5) and R177X (2) generate truncated proteins that lack the homeodomain and retain only a part of the POA domain. The five frameshift mutations have either an insertion such as A263insGG (16) and Y352insA (8) or a deletion such as P159delT (5), Q243delC (3), and P328L329delCCTCT (4). The mutations involve one or more nucleotides and lead either immediately or several codons downstream to a premature termination codon (PTC). All of these truncated proteins retain the complete dimerization domain but differ in the C-terminus. Whereas the PTC from mutant P159 occurs in the POA domain, the mutations A263 and Q243 are further downstream in the homeodomain. In contrast, the mutants P328L329 and Y352 contain the DNA binding domains and a part of the transactivation domain. The mutation R137-K161del (6) is an

![Figure 1](image-url)
in-frame deletion of 24 amino acids in the POU A domain and thus retains the full transactivation domain. To compare these mutants that contain distinct protein domains, we performed experiments in vitro and in vivo.

**Mutants Differ in Their Ability to Bind DNA and to Heterodimerize**

For allowing a comparison of the various HNF1β mutants, the wild-type human HNF1β cDNA was inserted into the expression vector myc-RC/CMV (15) containing a myc tag. From this wild-type expression vector, each mutant was generated by site-specific mutagenesis. To produce the mutated protein, we transfected HeLa cells with the corresponding expression vector and prepared nuclear extracts. To test the DNA-binding properties of these proteins, we used the nuclear extracts in gel retardation assays with an HP1 oligonucleotide containing an HNF1-binding site (25). Figure 2A demonstrates that the HNF1β wild-type (lane 2) and the Y352 (lane 16) mutant form a complex with the HP1 oligonucleotide, which is upshifted by the addition of a myc-tag specific antibody (lanes 3 and 17). As expected, the complex containing the truncated protein from mutant Y352 (lane 16) migrates faster than the one with full-length HNF1β (lane 2). In contrast, the mutants E101X, S151P, P159, R177X, Q243, and A263 (lanes 4 to 15) gave no retarded band indicating loss of DNA binding.

As each mutant contains the entire N-terminal dimerization domain, we probed the ability to form heterodimers with the wild-type protein by mixing the translation product of each mutant with the HNF1β wild-type protein. The mutant Y352 shows dimerization, because it forms a heteromeric complex with intermediate mobility (Figure 2B, lane 3). The same observation has previously been made for the P328L329 mutant (15) that also contains the entire domain for DNA binding. In contrast, none of the other mutants heterodimerizes with wild-type protein as assayed by gel retardation (data not shown).

**Mutants Differ in Their Transactivation Potential**

As the mutants differ in their domain structure, we investigated whether they have distinct transactivation potential. We transfected HeLa cells known to lack HNF1 proteins with a luciferase reporter construct containing four HNF1 binding sites in front of the thymidine kinase promoter (26). Transfection of saturating amounts of the expression vector encoding HNF1β wild type shows a 10-fold induction, whereas vectors with the mutants E101X, P159, R177X, Q243, A263, or S151P have lost their transactivation potential (Figure 3A). In contrast, the transactivation of the Y352 mutant is similar to the wild-type protein. However, transfecting low amounts of Y352 expression vector, the transactivation potential of the mutant is lower compared with the wild type, whereas at higher amounts, the mutant reaches the transactivation potential of the wild type (Figure 3B). We previously found a similar behavior for the mutant P328L329, although in this case, at high input, the mutant was even more active than the wild type, suggesting a gain of function mutation (15). To compare directly the transactivation potential between the two mutants, we transfected HeLa cells with increasing concentrations of either of these expression vectors. At each concentration, the mutant P328L329 revealed a higher induction than the Y352 mutant (Figure 3C). Therefore, Y352 has a lower transactivation potential and in contrast to P328L329 displays no gain of function mutation.

As all HNF1β mutants retain the dimerization domain (Figure 1), we wondered whether the mutated factors interfere with

---

**Figure 2.** Gel shift assays with nuclear extract from transfected HeLa cells using the 32P-labeled HNF1-binding site as a probe. The cells were transfected with expression vector encoding wild-type or mutant HNF1β, and nuclear extracts were prepared after 24 h. (A) For visualizing the transfected HNF1β variants, the myc-specific antibody 9E10 was included in the reactions as indicated. As a control, nuclear extract from nontransfected cells was used (lane 1). For increasing the sensitivity, the autoradiogram has been overexposed, resulting in the diffuse bands. (B) Nuclear extracts of wild-type and mutant Y352 were mixed as indicated. The heterodimer is marked with an asterisk.
the activity of the wild-type protein. As HeLa cells are not of renal origin and thus may lack important cofactors such as DCoH (27), we used HEK293 cells, an embryonic human kidney cell line with properties of cells of the early “S-shape” (28) and expressing DCoH (our unpublished data). Figure 4 illustrates that a fivefold excess of expression vector encoding the mutant A263 is unable to interfere with the activity of the wild-type factor using a reporter gene with the natural P2 promoter of the human HNF4α gene containing one HNF1 binding site (29).

**Cellular Localization of the HNF1β Mutants**

To monitor whether the mutated HNF1β proteins show a distinct distribution within the cell, we determined the cellular localization by immunofluorescence in transfected HeLa cells using the antibody specific for the myc tag. The HNF1β wild-type as well as the mutant Q243, A263, and Y352 proteins are localized predominantly in the nucleus, whereas the proteins encoding mutant E101X, R177X, and P159 are mainly cytoplasmic (Figure 5). Therefore, we assume a nuclear localization signal at the N-terminus of the homeobox (between amino acid positions 177 and 243). The missense mutant S151P shows no preferential localization to the nucleus or to the cytoplasm.

**HNF1β Mutants Influence Pronephros Development in Xenopus laevis**

To analyze the effect of the different HNF1β mutations in a living organism, we injected RNA encoding HNF1β mutants

![Figure 3. Transactivation potential of wild-type (WT) and mutant HNF1β in transient transfection experiments. (A) The reporter construct syn(0)4 was co-transfected into HeLa cells with saturating amounts of expression vector (300 ng) encoding wild-type or mutated HNF1β. Fold induction refers to the activity without any HNF1β. (B, C) Saturation curves of wild-type HNF1β and the mutants Y352 and P328L329. Increasing amounts of expression vector were co-transfected with the reporter construct syn(0)4. The error bars indicate SD of six determinations.](image)

![Figure 4. Co-transfection of wild-type HNF1β and mutant A263. HEK293 cells were transfected with various amounts of expression vector together with the P2-285 HNF4α luciferase reporter containing the HNF1 binding site of the P2 promoter of the human HNF4α gene (29). Fold induction refers to the activity obtained by using the empty Rc/CMV expression vector. The error bars indicate SD of nine determinations.](image)

![Figure 5. Cellular localization of HNF1β mutants. Immunofluorescence of transfected HeLa cells with an expression vector encoding mutated HNF1β. The transfected myc-tagged proteins were visualized using the monoclonal myc 9E10 antibody and a Cy3-labeled secondary antibody. Bar = 5 μm.](image)
into one blastomer at the two-cell stage Xenopus embryo using RNA encoding GFP as a tracer. At the neurula stage, we selected and scored all normally developed animals with green fluorescence restricted to either of one side (Figure 6A), and at the swimming larval stage, the animals were fixed. To visualize the pronephric structures, we immunostained the larvae with two specific antibodies that stain the pronephric tubule and pronephric duct.

Injection of the frameshift mutation Y352 results in a reduced development of the pronephros on the injected side affecting the development of the pronephric tubules as well as the anterior region of the pronephric duct that coils underneath the convoluted tubules (Figure 6B). The posterior part of the duct is always present and not affected.

In contrast, introducing the mutant A263 leads to an enlargement of the developing pronephros on the injected side (Figure 6C). This enlargement can be seen best in the lateral view (Figure 6C, right side). To describe this enlargement more precisely, we stained the pronephros tubules and the anterior part of the pronephric duct with the monoclonal antibody 3G8 and 4A6, respectively. The enlargement of the pronephric tubules involves a more intensive coiling of a significantly longer tube (Figure 7A). Similarly, the anterior coiled pronephric duct is also longer (Figure 7B). The length increase was 53% on the injected side, and this difference was statistically significant ($P < 0.0005$) using the Mann-Whitney $U$ test. However, there are no abnormal structures, including cysts. This is in contrast to the mutants leading to a reduced pronephros that may generate a cyst as remaining structure (15).

Investigating the morphogenetic potential of the mutants S151P, E101X, R177X, P159, and Q243 in Xenopus embryos, we also observed an enlargement of the pronephric tubules and the anterior pronephric duct on the injected side (Figure 8) as observed by injecting the mutant A263.

To quantify the effect seen by expressing the mutated HNF1$\beta$ protein in Xenopus embryos, we compared the difference between injected and noninjected sides by measuring the stained area of the pronephric tubules and the anterior part of the duct. As a control, we used embryos injected with GFP RNA alone. The values are summarized in Figure 9 and were statistically analyzed using the Mann-Whitney $U$ test. By comparing the effect of each mutant with the GFP control, we deduced a significant difference between the mutants and GFP in all cases ($P < 0.01$; Figure 9). Whereas the mutant Y352 leads to a significant decrease in pronephros size, all of the other mutants lead to a significant enlargement.

Figure 6. Expression of the HNF1$\beta$ mutants in Xenopus embryos. (A) Dorsal view of a neurula stage larvae expressing green fluorescence protein (GFP) on the injected right side. (B and C) Whole-mount immunostaining with a mixture of the monoclonal antibodies for pronephric tubules (3G8) and duct (4A6) (37). A Cy3-conjugated secondary anti-mouse antibody was used to get red fluorescence. The injected sides are marked with an asterisk. Pronephric tubules (pt), coiled duct (cd), and duct (d) are indicated in the lateral views. Bar = 500 $\mu$m.

Figure 7. Whole-mount staining of the pronephric tubules and the anterior part of the pronephric duct. Larvae derived from A263-injected embryos were immunostained with the pronephric tubules-specific antibody 3G8 (A) or the duct-specific antibody 4A6 (B) and a Cy3-conjugated secondary antibody. A and B show each a lateral view from the left as well as from the right side, with dorsal side up. The injected side was on the left in these two animals and is marked with an asterisk. A shows pronephric tubules; in B, the coiled duct (cd) and duct (d) are indicated. Bar = 250 $\mu$m.
Discussion

We have analyzed nine different mutations of the HNF1β gene that are associated with renal diseases in humans. These mutants can be categorized on the basis of their DNA binding, their functional activity in vitro, and their effect on pronephros development. On the basis of the position and type of mutation, one would predict distinct properties of the various mutated transcription factors (summarized in Figure 10). This prediction is fulfilled by our functional analysis of the DNA binding properties in gel retardation assays: All mutants that lack part or the entire domains involved in DNA binding (i.e., the POU A and homeodomain) fail to bind DNA. This is most evident for the nonsense mutants E101X and R177X, as well as the frameshift mutants P159delT, Q243delC, and A263insGG, but also for the mutant R137-K161del containing a 24–amino acid deletion in the POU A domain. Obviously, S151P, the only missense mutation in our collection, has also lost its ability to bind DNA, suggesting either that the serine residue at position 151 in the POU A domain is critical for DNA binding per se or that the change to a proline at this position destroys important structural features of the DNA binding domain. In contrast, the two frameshift mutants P328L329 and Y352 that both retain the POU A and homeobox domain (Figure 10) bind DNA as efficiently as the wild-type protein. By comparing the activity of wild-type HNF1β with the mutant Y352, the activity of the mutant is low at low input of transfected expression vector but reaches the activity of the wild type at saturating amounts (Figure 3B). This documents that this mutant has a distinct functional property compared with the wild-type protein as previously found for the mutant P328L329 (15). However, the mutant P328L329 shows at high input of expression vector an activity even higher than the wild type. As this gain of function effect is not found in the Y352 mutant (Figure 3C), we assume that the additional 27 amino acid residue generated by the frameshift of the mutation P328L329 has properties of an activation domain (Figure 10).

Comparing the various mutants, the presence of DNA binding is strictly correlated with the ability to dimerize and to transactivate (Figure 10). This finding is not surprising as DNA binding is a prerequisite in our dimerization assay in gel retardations and transactivation depends on the ability to interact with the HNF1 binding site in the reporter construct.

Our observation that none of the mutants shows any interference with the activity of the wild-type protein suggests that the dimerization domain present in the mutated proteins is not sufficient for interference in our test assays, thus preventing a dominant negative effect on the wild-type factor. Recently, data have been reported that A263 acts in a dominant negative manner in HepG2 and MIN6 cells (30) as well as in HeLa cells (31). This alternative finding may reflect that reporter genes with different promoters were used. However, a general and profound effect of any of these mutants on the wild-type protein in the organism is unlikely, as such an effect would knock out the activity of the wild-type HNF1β allele that
would possibly be lethal as shown by early embryonic developmental arrest in knockout mice (9,10).

The analysis of molecular properties of the mutated HNF1β proteins in vitro or in cell cultures is too simplistic to evaluate the functional properties of the various mutants in an entire developing organism. Therefore, our analysis of the morphogenetic potential of the HNF1β mutants in the developing Xenopus is most meaningful. Using this approach, we succeeded to get two distinct groups of HNF1β mutants. The “enlargement” group consists of mutated factors leading to an enlargement of the pronephros, whereas the “reduction” group is characterized by a partial or complete agenesis of the pronephros. The enlargement group consists of the mutants S151P, E101X, R177X, P159, Q243, and A263 that all are characterized by a lack of DNA binding and thus have also no transactivation potential in transfected cells. On the basis of the induced phenotype in the developing Xenopus, these mutated factors that lack DNA binding cannot be inactive proteins but rather interact with some regulatory components. The reduction group consists of the mutants P328L329 and Y352 retaining the domains required for DNA binding but also the internal deletion mutant R137-K161 that has lost its DNA binding potential. Similar to the members of the enlargement group, the mutant R137-K161 gives a phenotype in the developing Xenopus system, although this mutant has no activity in the cell cultures (6).

The anatomy of the Xenopus pronephros is simple in comparison with the human metanephros, but the basic structures are similar and essentially the same regulators are expressed (11–14). From in situ hybridization, it is known that HNF1β is expressed in the pronephros anlage before any cellular differentiation (32) and thus is among the first regulators of nephrogenesis, including lim1 and Pax8 (13,14). It seems most likely that the different Xenopus phenotypes that we obtained by introducing HNF1β mutants will be reflected in different subtypes of renal diseases in humans. However, the clinical data in humans are limited, because a systematic analysis in the few patients (approximately 30 known to date) has not been performed. This is partly because in many patients, only radiologic imaging is available and there is no histology. In cases in which histology has been performed, small samples have been taken and these may not represent the entire organ. Furthermore, samples were analyzed from fetal tissues as well as from adults without knowing the progressive changes that occur throughout the disease. Further complications reside in the fact that each mutation was found in one family, and mutant carriers within a given family display considerable variation in renal dysfunction.

The two mutants E101X and P159 (5) were found in hypoplastic GCKD identified already 20 yr ago as a well-defined familial kidney disease (33). This might suggest that loss of function mutations of HNF1β generate this renal defect. However, recently in hypoplastic GCKD, the mutation P334fsinsC that retains all domains needed for DNA binding has been identified (34), thus refuting the hypothesis that hypoplastic GCKD depends on an HNF1β mutation with impaired DNA binding.

The two nonsense and the five frameshift HNF1β mutations lead to PTC, so the mutant mRNA transcripts may be subject to nonsense-mediated decay, resulting in a reduced level of HNF1β protein (35). Such haploinsufficiency with only the wild-type allele producing functional protein cannot be rigorously excluded. However, two mutations (R137-K161del and S151P) lack any PTC and therefore are not subject to nonsense-mediated decay. With these mutants, we observed in injected Xenopus the reduction as well as the enlargement
phenotype as with the other mutants. This is another strong argument for qualitatively different mutations in the human HNF1β gene.

Patients who carry the HNF1β mutants S151P, Q243, or R137-K161 have renal and genital tract malformations (3,6): In female patients, the uterus can be rudimentary with vaginal aplasia (Müllerian duct aplasia), or there may be a Müllerian duct fusion abnormality producing a bicornuate uterus or uterus didelphys. Potentially, genital tract malformation could also be explored in the *Xenopus* system. Our observation that the posterior part of the pronephric duct from which the genital tract arises is almost normal (see Figures 6, B and C, and 7B) may argue against this. However, in our approach, the human HNF1β mutant is introduced into *Xenopus* in a transient manner, as we inject the corresponding RNA that has a limited half-life in the developing embryo and thus cannot interfere in later stages of development. Therefore, the analysis of the morphogenetic potential on genital development requires a stable transgenic approach combined with the conditional use of recombinase action. This may be achieved in the near future, as we recently have established this technique in *Xenopus* (36).

### Acknowledgments

This work was supported by the Deutsche Forschungsgemeinschaft (Ry5/7-2), Diabetes UK, and the National Kidney Research Fund (grant TF13/2000). Dr. Hattersley is a Wellcome Trust Research Leave fellow.

The authors are grateful to E.A. Oliver-Jones for the antibodies 3G8 and 4A6.

### References


