Transient Neonatal Diabetes, ZFP57, and Hypomethylation of Multiple Imprinted Loci

A detailed follow-up

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OBJECTIVE—Transient neonatal diabetes mellitus 1 (TNDM1) is the most common cause of diabetes presenting at birth. Approximately 5% of the cases are due to recessive ZFP57 mutations, causing hypomethylation at the TNDM locus and other imprinted loci (HIL). This has consequences for patient care because it has impact on the phenotype and recurrence risk for families. We have determined the genotype, phenotype, and epigenotype of the first 10 families to alert health professionals to this newly described genetic subgroup of diabetes.

RESEARCH DESIGN AND METHODS—The 10 families (14 homozygous/compound heterozygous individuals) with ZFP57 mutations were ascertained through TNDM1 diagnostic testing. ZFP57 was sequenced in probands and their relatives, and the methylation levels at multiple maternally and paternally imprinted loci were determined. Medical and family histories were obtained, and clinical examination was performed.

RESULTS—The key clinical features in probands were transient neonatal diabetes, intrauterine growth retardation, macroglossia, heart defects, and developmental delay. However, the finding of two homozygous relatives without diabetes and normal intelligence showed that the phenotype could be very variable. The epigenotype always included total loss of methylation at the TNDM1 locus and reproductive combinations of differential hypomethylation at other maternally imprinted loci, including tissue mosaicism.

CONCLUSIONS—There is yet no clear genotype–epigenotype–phenotype correlation to explain the variable clinical presentation, and this results in difficulties predicting the prognosis of affected individuals. However, many cases have a more severe phenotype than seen in other causes of TNDM1. Further cases and global epigenetic testing are needed to clarify this.

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Imprinted genes are characterized by a parent of origin expression pattern controlled by epigenetic modifications, including DNA methylation within differentially methylated regions (DMRs). The normal methylation pattern of DMRs is established in the germlines and maintained during embryogenesis. Imprinted genes are crucial in normal growth and development. Currently, 73 human imprinted genes are known (http://igc.otago.ac.nz). Aberrant methylation of these DMRs results in imprinting disorders such as transient neonatal diabetes mellitus 1 (TNDM1; MIM 601410), Prader-Willi syndrome (MIM 176270), Angelman syndrome (MIM 105830), Beckwith-Wiedemann syndrome (BWS; MIM 130650), and Silver-Russell syndrome (SRS; MIM 180860). However, the complex mechanisms underpinning establishment and maintenance of primary DMRs are not fully understood.

Until very recently, our knowledge was limited to imprinting disorders in which a single locus was exposed to abnormal methylation. It is now clear that some patients have methylation aberrations affecting multiple imprinted loci, which we termed hypomethylation of multiple imprinted loci (HIL) (1–3). This phenomenon is heterogeneous, and there are likely genetic and environmental causes.

We described the first heritable global imprinting disorder compatible with life...
in patients presenting with TNDM1 due to HIL. The patients have total loss of methylation (LOM) at the maternally methylated TNDM1 DMR (6q24) (4). In addition, they have a mosaic pattern of hypomethylation affecting other maternally methylated loci, primarily PEG3/ZIM2 (19q13.4) and GRB10 (7p12.2). Homozygous or compound heterozygous DNA sequence mutations in the ZFP57 were identified in more than half of these patients (5). The probands with ZFP57 mutations have a more complex TNDM1 phenotype compared with patients with TNDM due to other causes. The finding of heritable mosaic epigenetic aberrations associated with ZFP57 mutations indicates that the gene may be involved in maintenance of DNA methylation at imprinted regions during the early multicellular stages of human development. In mouse, zfp57 has a clear role in methylation maintenance at imprinted loci in both the germline and postfertilization but is also implicated in methylation establishment in the developing oocyte (6).

ZFP57 is localized at chromosome 6p22.1 spanning an 8.6-kb genomic region. The gene is organized in six exons encoding the ZFP57 protein consisting of 516 amino acids (Fig. 1). The ZFP57 protein is a Krüppel-associated box domain (KRAB) zinc finger protein with a KRAB A and KRAB B domain encoded by exons 4 and 5, respectively. The seven zinc fingers of the C2H2 type are encoded by exon 6.

In mice, expression of zfp57 has been demonstrated in embryonic stem cells (expression levels declining with cell differentiation), testes, and ovary and in rat in the nervous system (7–9). Recent studies indicate that zfp57, via interaction with its cofactor KRAB-associated protein 1, recruits DNA methyltransferases and is required for the maintenance of methylation of DNA imprints, as well as of histones in embryonic stem cells (10,11). HIL also occurs in some patients with BWS associated with hypomethylation of KCNQ1OT1 DMR (11p15.5) (12–15) and SRS due to hypomethylation of H19 DMR (11p15.5) (14,16). In some of these patients, the hypomethylation can involve paternally or maternally methylated DMRs, in contrast to ZFP57-related TNDM1 HIL patients, in whom the hypomethylation occurs only at maternally imprinted loci. However, no underlying genetic defect for BWS or SRS HIL cases is so far known, except for a single case with a homozygous sequence mutation in the NLRP2 gene in the mother of two siblings with BWS and hypomethylation at KCNQ1OT1 DMR, in which one of the patients also had hypomethylation at PEG1/MEST DMR (7q32) (17). We and others (18,19) have sequenced ZFP57 in 27 BWS patients with hypomethylation of KCNQ1OT1 DMR and 30 SRS patients with hypomethylation at H19 DMR, respectively, and could not find disease-associated alterations, but these studies did not necessarily include cases with HIL.

In this study, we present the detailed genotype, phenotype, and epigenotype of the first 10 families with ZFP57-related TNDM1 HIL and discuss these findings in the context of the current knowledge of the molecular mechanisms involved.

**RESEARCH DESIGN AND METHODS** —The study was conducted in accordance with the Helsinki Declaration. Informed consent was obtained from all participants (parental consent obtained for children).

**Clinical investigations**
Clinical features of families 1–7 were previously described in brief (5), and family 1 was described extensively (3). Updated and more detailed medical and family histories were obtained for families 1–3, 6, and 7; in families 1, 2, 3, 6, and 7, additional members were examined. Families 8–10 have not been reported previously.

**Laboratory investigations**
ZFP57 sequencing and methylation studies were carried out on genomic DNA extracted from peripheral blood leukocytes.
Abnormal methylation results were confirmed with pyrosequencing of bisulfite-induced polymorphisms. In one patient (II-3, family 1), methylation studies were carried out on DNA from multiple tissues (cultured cells from the chorionic villus sampling at gestational age 11 + 2 weeks, postpartum placental tissue, umbilical cord and peripheral blood leukocytes, and skin biopsies from surgically removed sixth fingers). For the families 1–7, laboratory results for the probands and parents were published previously (5).

**ZFP57 analyses**

ZFP57 sequence alterations were investigated by direct sequencing of two PCR amplification products, which included exon 1 (49 bp) and exons 2–6 (4.8 kb), respectively. Exons 2–6 were amplified together in order to overcome amplification problems due to exon 6, which contained the repetitive zinc finger sequence. The primers were: 5'-CAGAGGAGTGGGGACAACAT-3' and 5'-CTAGCGCTACTTGGGACCAG-3' for exon 1 and 5'-CCCAGGCTGGTGTTGTTACT-3' and 5'-ATGCTCACTGCCTCCTTTGT-3' for exons 2–6. PCR was carried out using a slightly optimized protocol described previously (5). Shortly, DNA was amplified using Phusion high-fidelity polymerase (New England Biolabs) and the GC buffer provided with the addition of DMSO to a final concentration of 3%. The PCR conditions were: initial denaturation at 98°C (3 min), 29 cycles of 98°C (10 s), 50°C (10 s), and 72°C (1 min 45 s), and final extension at 72°C (5 min).

**Methylation-specific PCR and pyrosequencing**

Methylation status was targeted to specific imprinted loci: TNDM1, GRB10, PEGI/MEST, KCNQ1OT1, H19, DLK1 (14q32), SNRPN (15q12), PEG3/ZIM2, and NESPAS/GNAS-ASI (20q13.2) DMRs using methylation-specific PCR and pyrosequencing when necessary, as previously described (5,20).

**Ethical approval**

Family 1 was recruited as part of a Danish Imprinting and Methylation study, ethical approval number H-D-2008–079 and Danish Data Protection Agency number 2008-41–2565. Families 2–10 were recruited as part of the U.K. clinical local research network study, Imprinting Disorders Finding Out Why, ethics approval number 07/H0502/85.

**RESULTS**

**Molecular genetics**

In 14 individuals from 10 TNDM1 HIL families, ZFP57 sequence mutations were identified on both alleles. The mutations in nine individuals from families 1–7 were previously published (5). In this study, we included additional family members (families 1–7) and three new families (families 8–10) (Fig. 2). Two families have two homozygous affected individuals (families 1 and 6), and in family 2, two relatives with no TNDM1 history are homozygotes. Six families (families 1–6) are consanguineous. In eight families (families 1–6, 8, and 10), the affected individuals are homozygous, and in families 7 and 9, the probands are compound-heterozygous. The 10 different sequence changes include 5 missense mutations (families 4, 5/7, 6, 9, and 10), 4 frame-shift mutations (families 2, 3, 7, and 8/9), and a nonsense mutation (family 1). Two mutations were observed twice: p.R228H in families 5 and 7 and p.L133HfsX49 in families 8 and 9 (Supplementary Table 1) (21). The missense
mutations in families 4, 5/7, 6, and 10 affect conserved residues, and the first three involve histidine, which is a metal ion-binding amino acid. The missense mutation in family 9 affects a nonconserved amino acid. Amino acid changes in zinc finger domains or KRAB domains are predicted to affect DNA binding of the ZFP57 protein and its possible function, respectively. In nine families (families 1 and 3–10), the ZFP57 mutations are in exon 6 and in family 2 in exon 5 (Fig. 1).

**Methylation studies**

The individuals with homozygous and compound heterozygous ZFP57 mutations have mosaic patterns of hypomethylation regarding both the number and combination of affected imprinted loci and the extent of hypomethylation in each locus (Supplementary Table 1). The epigenetic signature of ZFP57 homozygous and compound heterozygous individuals includes total LOM of TNDM1 DMR, partial hypomethylation of PEG3 DMR, and partial or complete hypomethylation of GRB10 DMR. Additionally, some individuals have partial hypomethylation of the other one to three loci, PEG1, KCNQ1OT1, and NESPAS/GNAS-AS1 DMRs. There is no obvious correlation between methylation levels of affected loci and the type of ZFP57 mutation. Intrafamilial divergences exist regarding the number of affected loci (families 1 and 6) and the extent of hypomethylation at the individual locus (family 2) (Supplementary Table 1). This remains unexplained. We found no aberrant methylation at the maternally methylated SNRPN DMR or at the paternally methylated H19 or DLK1 DMRs.

**Clinical features**

The key clinical features of the 12 ZFP57 homozygous and compound heterozygous TNDM1 HIL individuals are neonatal diabetes mellitus (NDM), intrauterine growth retardation (IUGR), macrosomia, and developmental delay (Table 1). Detailed clinical features of each of these plus the two asymptomatic ZFP57 homozygous relatives with HIL are shown in Supplementary Table 2. The 12 affected individuals were ascertained through TNDM, which was indistinguishable from other causes. Three out of four patients over the age of 4 years had diabetes relapse (Table 1). Ten out of the 12 affected individuals had IUGR with a birth weight less than or equal to the second percentile, and in the two remaining affected individuals, birth weight was less than the 25th percentile (Table 1). In six out of seven children, postnatal growth showed rapid catchup. Additionally, the affected individuals showed various combinations of developmental delay and/or congenital anomalies. Recurrent features in the affected individuals were: macroglossia, umbilical hernia, congenital heart disease, ear lobe abnormalities, hemihypertrophy of extremities, central nervous system structural abnormalities and developmental delay, or pectus carinatum (Supplementary Table 2).

**Clinical variability**

ZFP57-related HIL shows inter- and intrafamilial clinical variability. One of the most intriguing variations is the nonoccurrence of diabetes (Supplementary Table 2) in two homozygous individuals, I-2 and II-1 in family 2, who are the father and sister of the homozygous proband II-3. All three have total LOM of TNDM1 DMR (Supplementary Table 1); however, both I-2 and II-1 are healthy with no signs of diabetes and are developmentally normal. I-2 is 39 years of age and has normal glucose tolerance testing. Likewise, there is no history of diabetes in II-1, though she did have a low birth weight and postnatal growth catchup. There is previous evidence from families with inherited duplications of 6q24 that relatives at genetic high risk do not always present with TNDM1 (4,22,23), and there are reports of cases with paternal uniparental disomy of chromosome 6 (UPD6) in older patients, who have no history of TNDM1 (24). Presumably, our two healthy ZFP57-related HIL individuals are at risk for developing nonsulin-dependent diabetes later in life as individuals with 6q24 duplications and no history of NDM developed diabetes later in life (4,22,23).

The clinical phenotype was particularly severe in two affected individuals (II-2 in family 1 and II-1 in family 3) who had severe developmental delay and structural brain abnormalities involving an absent/hypoplastic corpus callosum. In family 1, the homozygous sister, II-1, has a much milder phenotype than II-2 who was severely ill and died at 11 months (Supplementary Table 2). Both families 1 and 3 are consanguineous, thus possibly other deleterious recessive genes may contribute to the phenotypes.

**Clinical features of heterozygotes**

Twenty-five individuals with heterozygous ZFP57 mutations were identified in the 10 ZFP57-related TNDM1 HIL families. We have some clinical information on 15. None of these had TNDM1, and to the best of our knowledge, 12 of the 15 had a normal phenotype and development. Three heterozygous individuals (I-2 and II-3 in family 1 and II-1 in family 7) had symptoms needing further investigation: I-2 in family 1 developed gestational diabetes mellitus in her third pregnancy. We are only aware of two other heterozygotes tested for diabetes: II-3 in family 1 had blood glucose measured neonatally, and I-2 in family 10 had oral glucose tolerance tests due to diabetes in several relatives. All measurements were normal. II-3 in family 1 had bilateral postaxial polydactyly like her homozygous sister, II-1, did. Otherwise, II-3 has a normal phenotype and normal development by the age of 5 months. Her methylation status was normal (including skin fibroblasts from the bilateral sixth digits; data not shown), and her placenta was extensively examined with normal results (clinical and histological examination and immunochemical staining for p57kip2 protein). No other family members had polydactyly, and it seems likely to be unrelated. In the nonconsanguineous family 7, II-1 shared a neurologic phenotype with progressive contractures with his compound heterozygous brother, II-3. However, II-1 also had severe developmental delay and facial dysmorphic features possibly related to prematurity and not seen in II-3. The reason for the neurologic findings remains unexplained despite extensive investigations. The phenotype of II-3 is described in Supplementary Table 2. With the limited knowledge available, we conclude that the few symptoms observed in the heterozygotes are likely due to other causes.

**CONCLUSIONS**—In general, ZFP57-related TNDM1 HIL individuals seem to have a more complex phenotype compared with patients with isolated LOM of TNDM1 DMR (Table 1), and there are many possible explanations.

However, for HIL patients ascertained through other imprinting syndromes, there are conflicting reports of the phenotypic consequences. In some patients ascertained with BWS (12,14,15) or SRS (14), no additional clinical features related to HIL compared with single-locus hypomethylation were noted. For example, there was no clinical history of TNDM1 in the BWS (13) or SRS (14) HIL patients who had total or partial
Table 1—Comparison of phenotypic features in ZFP57-related TNDM1 HIL individuals (ZFP57mut/mut) and individuals with isolated 6q24 TNDM1 of unknown cause and UDP6 or duplication 6q24

| Features                                      | ZFP57mut/mut individuals | TNDM1: isolated LOM 6q24* |
|-----------------------------------------------|--------------------------|---------------------------|
|                                               | Affected individuals     | Nonaffected individuals   | LOM 6q24* |
|                                               | n/p %                    | n/p %                     | n/p %     |
| Multiple hypomethylation                      | 12/12 100               | 2/2 100                   | 0/12 0    |
| TNDM1                                         | 12/12† 100†             | 0/2 0                     | 12/12 100 |
| IUGR (below 2nd percentile)                   | 10/12 83                | 1/2‡ 50                   | 7/11 64   |
| IUGR (below 25th percentile)                  | 12/12 100               | 1/2‡ 50                   | 9/11 82   |
| Macroglia                                     | 8/11 73                 | nk —                      | 5/10 50   |
| Developmental delay                           | 5/11 45                 | 0/2 0                     | 0/8 0     |
| Congenital heart disease                      | 4/11 36                 | 0/2 0                     | 0/10 0    |
| Umbilical hernia                              | 3/11 27                 | 0/2 0                     | 0/11 0    |
| One or more other minor congenital abnormality | 8/11 73                 | 0/2 0                     | 3/11 27   |
| Epilepsy                                      | 2/10 20                 | 0/2 0                     | 0/11 0    |
| Visual impairment                             | 2/10 20                 | 1/2‡ 50                   | 0/8 0     |
| Hearing impairment                            | 1/10 10                 | 0/2 0                     | 0/8 0     |
| Brain imaging abnormality                     | 2 structural, brain     | — None imaged              | —         |
| abnormalities, 1 normal CT, 11 not imaged     |                          |                           |           |
| Age at presentation of diabetes, average (days) | 20.2 (n = 12)           | n/a                       | 9.7 (n = 10) |
| Age at presentation of diabetes, median (days)| 14.5 (n = 12)           | n/a                       | 4 (n = 10) |
| Age of recovery, average (months)             | 5.4 (n = 10)            | n/a                       | 4 (n = 6) |
| Age of recovery, median (months)              | 4.3 (n = 10)            | n/a                       | 2 (n = 6) |
| Recurrence of diabetes, patients age >4 years (persons) | 3/4 75                  | n/a                       | 3/5 60    |
| Age of recurrence of diabetes (average years) | 8 (2 y 8 m–11 y) (n = 3) | n/a                       | 13.7 (11–16 y) (n = 3) |

CT, computed tomography; m, months; n/a, not applicable; nk, not known; n/p, number of observations for number of individuals; y, years; ZFP57mut/mut, Hil individuals with homo- or compound-heterozygous mutations of ZFP57 (n = 14 individuals). *Figures from Wessex Regional Genetics Laboratory, Salisbury Hospital, Salisbury, U.K., with the cutoff date 22 December 2010. †Include two children with NDM and no recovery yet. ‡Birth weight only known for one of the ZFP57mut/mut nonaffected individuals. §Ear lobe abnormality, asymmetry, pectus carinatum, polydactyly, and tracheomalacia. ||Two other affected individuals had a single seizure neonatally or in childhood. ¶One ZFP57mut/mut nonaffected individual had episodic diplopia in his late thirties.

LOM of TNDM1 DMR. However, the clinical presentation of BWS was modified in some BWS Hil patients (13), and two patients with SRS Hil due to LOM of H19 DMR had additional features including language delay (16). Furthermore, among 3 of 77 SRS patients with hypomethylation at both H19 DMR and KCNQ1OT1 DMR, 1 had an umbilical hernia that is not typical for SRS, but a common feature for BWS (25). Azzi et al. (14,26) suggested that in cases with comparable levels of hypomethylation at multiple loci, one locus may have an (epi)dominant effect over the other(s).

The clinical variability observed in this cohort may be explained by the loci involved in Hil. Apart from TNDM1 DMR, the other loci most consistently involved were GRB10 and PEG3. GRB10 is a negative regulator of IGF signaling and is imprinted in fetal skeletal muscle and other postnatal tissues. It has long been a candidate for SRS associated with UPD7 (27). Although the affected individuals in the ZFP57-related TNDM1 Hil cohort consistently had a low birth weight, all of them had weight catchup and normal last height measurements. As this pattern is also observed in most cases with TNDM1 due to isolated genetic aberrations at 6q24, this cannot be attributed to the changes at GRB10 DMR. Our knowledge is even less about the clinical impact of hypomethylation of KCNQ1OT1 DMR. Calcium metabolism was investigated in the two affected individuals with partial hypomethylation at NESPAS DMR (II-1 in family 1 and II-3 in family 7), which is part of the complex imprinted GNAS cluster and is associated with pseudohypoparathyroidism type IB (MIM 603233) (30). However, no consistent abnormality of calcium metabolism was demonstrated. It still remains a possibility that methylation abnormalities at other loci not tested are responsible for the clinical differences between patients.

Another possible mechanism for the extreme clinical variability is divergent extent of hypomethylation at the individual locus and the tissues involved (26). We found, however, no obvious
relationship between the methylation pattern and the phenotype. All of the homozygous individuals in family 2 with divergent penetrance of diabetes (I-2, II-1, and the proband II-3) had total LOM of TNDM1 DMR and partial LOM of PEG3 DMR. At GRB10 DMR, however, the proband (II-3) had total LOM, whereas the two healthy individuals (I-2 and II-1) only had partial hypomethylation. However, this potential association was not confirmed in other individuals (the probands in families 3–5, III-2 in family 6, and probands in families 8–10) with the same epigenetic signature and TNDM1 (Supplementary Tables 1 and 2).

In family 1, methylation analyses in II-1 and II-2 of blood leukocytes showed partial LOM of KCNQ1OT1 DMR but was normal at this locus in skin fibroblasts (3). However, this remains a possible reason for variability but is very difficult to test in humans.

There is evidence that in mice, zfp57 is a maternal-zygotic effect gene (6), and this may be impacting in humans. The maternal effect is described as the phenomenon in which a phenotype in the progeny is caused by a genetic alteration in the maternal genome rather than an alteration of its own (31,32). When zfp57 expression in mice was lost only in the zygote (zfp57<sup>−/−</sup> offspring from zfp57<sup>+/−</sup> mothers), partial hypomethylation in some embryonic maternally methylated and some paternally methylated DMRs and perinatal and neonatal lethality was observed in the majority of homozygous offspring. In contrast, in the zfp57<sup>−/−</sup> offspring from zfp57<sup>+/−</sup> mothers, there was highly penetrant midgestation lethality, and the developing embryo had complete LOM at the above-mentioned DMRs.

Family 6 may be an example for maternal-zygotic effect, in which a homozygous child (III-2) was born to a homozygous mother (II-1) who was herself born at term with TNDM1 (Supplementary Table 2). Besides TNDM1, III-2 only had minor malformations (a ventricular septal defect that closed spontaneously and a minor esophageal hernia). She had no evidence of a more severe phenotype, and neither was her degree of LOM higher when compared with other affected individuals (Supplementary Table 1). As zfp57<sup>−/−</sup> offspring of zfp57<sup>+/−</sup> female mice die in utero (6), the survival of III-2 may be explained by the nature of the ZFP57 missense mutation. Some residual activity of ZFP57 may be retained with a missense mutation, whereas in mice, both zfp57 alleles are knocked out. It is also possible that ZFP57-related HIL individuals comprise the milder end of a phenotypic spectrum, in which the full-blown form is prenatally lethal (4). Interestingly, family 2 is the only family in which the ZFP57 mutation itself; family 2 is the only family in which the ZFP57 mutation in this particular family affects the KRAB domain, which should be essential for the association of

Figure 3—ZFP57-related HIL is a small subgroup of NDM. ABCC8, gene coding for the Kir6.2 subunit of the ATP-sensitive potassium channel of the β-cells of the pancreas; KCNJ11, gene coding for the SUR1 subunit of the ATP-sensitive potassium channel of the β-cells of the pancreas; n, number of first members identified in each pedigree at Wessex Regional Genetics Laboratory, Salisbury Hospital, Salisbury, U.K., with the cutoff date 22 December 2010; 6q24, chromosome 6q24; Type 1, TNDM1; Type 2, TNDM2 (MIM 610374); Type 3, TNDM3 (MIM 610582); ZFP57 mut/mut, HIL individuals with homo- or compound-heterozygous mutations of ZFP57. Hypomethylation DMR HIL, n = 33 (four of these had insufficient sample to investigate more loci than TNDM locus).
KRAB-associated protein 1 with the DNA methyltransferases (11). Further functional investigations would be needed to verify this.

NDM can be divided into permanent NDM and TNDM (Fig. 3). More than 50% of NDM cases are transient (33). TNDM is a rare entity with an incidence of ~1:200,000 (34–36). Of these transient diabetic cases, 6q24 abnormalities account for 60–70% (33,37). Individuals with ZFP57-related TNDM1 HIL account for only a small fraction of all individuals with TNDM. As a diagnostic pathway and differential diagnosis, 6q24 testing is the first step for neonates (<3 weeks) presenting with diabetes and the second step (following sequencing the potassium channel genes KCNJ11 and ABCC8) in children >3 weeks (38). Three known genetic mechanisms of 6q24 TNDM1 exist: UPD6, paternal duplications of 6q24, and hypomethylation of TNDM1 DMR (4). In the latter group, the hypomethylation is isolated to TNDM1 DMR in 41% (4) (12 of 29 pedigrees, Fig. 3) or part of a more complex hypomethylation, TNDM1 HIL, in 59% (4) (17 of 29 pedigrees). Homozygous or compound-heterozygous ZFP57 mutations are found in 59% (10 of 17) of these TNDM1 HIL pedigrees. We recommend that all patients with TNDM1 due to LOM at TNDM1 DMR are tested for HIL and for mutations in ZFP57. The results may have consequences for reproductive risk, and, in families with ZFP57 mutations, prenatal genetic testing is possible. However, genetic counseling is difficult because the clinical phenotype is variable.

In conclusion, homozygous and compound-heterozygous mutations in ZFP57 cause a human imprinting syndrome as yet only ascertained through presentation with TNDM1. It causes an increased risk of low birth weight, macroglossia, and developmental delay; however, the phenotype is highly variable, ranging from death at 11 months to normal health in the fourth decade. We found no evidence of abnormal clinical phenotypes caused by ZFP57 heterozygosity. The findings in humans are less severe than those in the knockout mouse model, as reflected by the expanded phenotypic description of the first 10 families identified with this disorder, but there remain many challenges before our knowledge is secure enough to provide definite clinical advice for families. Patients with unusual imprinting syndromes are an important resource for novel biological insights into epigenetic mechanisms and the regulation of human gene expression.

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