Review

Chromosome 6q24-related diabetes mellitus

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Abstract. Chromosome 6q24-related diabetes mellitus is the most common cause of transient neonatal diabetes (TNDM), accounting for approximately two-thirds of all TNDM cases. Patients with 6q24-TNDM develop insulin-requiring diabetes soon after birth, followed by the gradual improvement and eventual remission of the disorder by 18 mo of age. The most important clinical feature of affected patients is a small-for-gestational age (SGA) birth weight, which reflects the lack of insulin in utero. It is believed that 6q24-TNDM is caused by the overexpression of the paternal allele of the imprinted locus in chromosome 6q24, which contains only two expressed genes, PLAGL1 and HYMAI. Identified mechanisms include: (1) duplication of the paternal allele, (2) paternal uniparental disomy, and (3) hypomethylation of the maternal allele. Many patients with TNDM relapse after puberty. Relapsed 6q24-related diabetes is no longer transient and typically occurs in non-obese patients who are autoantibody negative. Thus, these patients possess features indistinguishable from those of maturity-onset diabetes of the young (MODY). Conversely, it has been shown that not all patients with 6q24-related diabetes have a history of TNDM. 6q24-related diabetes should therefore be considered as one of the differential diagnoses for patients with MODY-like diabetes, especially when they are SGA at birth.

Key words: 6q24, imprinting, diabetes, neonate

Introduction

Chromosome 6q24-related diabetes mellitus (hereafter referred to simply as 6q24-related diabetes) was first reported by Temple et al. in 1995, who found that a paternal uniparental disomy (pUPD) of chromosome 6 is frequently present in patients with transient neonatal diabetes (TNDM), and suggested the presence of an imprinted gene in chromosome 6 (1). Subsequently, this hypothesis was validated by several observations (2), and it is now known that abnormalities in the imprinted locus on chromosome 6q24 are the most common cause of TNDM (3–5). The molecular and pathophysiological background for the development of diabetes has also been partially elucidated (3, 4), however, there are still several unanswered questions regarding 6q24-related diabetes.
diabetes. In this review, we attempt to outline both the current understanding of this clinical entity, and the ongoing efforts for improving our understanding of this condition and the clinical management of the patients.

**Clinical Presentation of 6q24-related TNDM (6q24-TNDM)**

Neonatal diabetes mellitus (NDM) is currently defined as diabetes that develops before 6 mo of age (6). NDM can be classified into two different subtypes: transient NDM (TNDM), which reaches remission by 18 mo of age, and permanent NDM (PNDM), which is more lasting. The most common cause of TNDM is an abnormality of the imprinted locus on chromosome 6q24, which accounts for two-thirds of all TNDM cases. Other causes include an activating mutation in one of the genes coding for the ATP-sensitive potassium channel, \( KCNJ11 \) or \( ABCC8 \), which accounts for most of the remaining cases of TNDM (6). However, the incidence of 6q24-related TNDM is still quite low, estimated at 1 in 200,000–400,000 births (5).

Almost all patients with 6q24-TNDM are born small-for-gestational age (SGA) with a means-adjusted birth weight of ~2.5 SDS (standard deviation score), or 1,930 g at 39 wk of gestation (5), which most likely reflects an insulin deficiency in utero. Since they are already insulin-deficient in utero, patients with 6q24-TNDM usually develop hyperglycemia soon after birth (mode: day 1) (5). In some patients, however, hyperglycemia is noticed a few days after birth.

At its onset, many patients experience hyperglycemic dehydration, which requires insulin treatment, although diabetic ketoacidosis is uncommon. In addition to diabetes, these patients often have several associated conditions, including macroglossia, umbilical hernia, central nervous system anomalies, renal anomalies, developmental delay, congenital heart diseases, limb anomalies, or hypothyroidism (5). These features are more common in patients with pUPD or maternal hypomethylation, and are less frequent in patients with paternal duplication, although the severity of diabetes does not differ between subtypes (5).

TNDM gradually recedes and reaches remission by 18 mo of age (median: 3 mo) (5). Following remission, patients generally remain euglycemic with normal glucose tolerance (7), and they also show catch-up growth and remission of short stature by 2 yr of age (8). However, it has been reported that some of the patients experience episodic hyperglycemia after remission (3), and that others experience diazoxide-responsive hyperinsulinemic hypoglycemia that could last for up to 2–3 yr (9).

It is worth noting that a significant fraction of the patients with diabetes relapse after puberty (median age: 14 yr) (8). Relapsed diabetes is permanent and is characterized by diminished insulin secretion without obesity and the absence of autoantibodies, which mimics maturity-onset diabetes of the young (MODY). A relapse rate of ~50% has previously been reported (3), but a recent report suggests that the rate could be as high as 80% (10). The age of relapse can be as early as 4 yr (8), and cases of relapse during pregnancy have been reported (3).

**Causes of 6q24-related Diabetes**

It has been established that 6q24-TNDM is caused by an excess expression of the paternal allele of the imprinted locus on chromosome 6 (3, 4). The imprinted locus has now been narrowed down to a 70-kb region on chromosome 6q24 (11), which contains only two genes, \( PLAGL1 \) (pleiomorphic adenoma gene-like 1, also known as \( ZAC1 \)) and \( HYMAI \) (hydatidiform mole associated and imprinted). In most tissues, both genes are expressed from the paternal allele using the imprinted P1 promoter, although biallelic expression from the unmethylated P2 promoter, approximately 55 kb upstream of P1, has been shown in leucocytes, the liver, and
the spleen (12). The imprinting is supported by a differentially methylated region (DMR) of approximately 1 kb at the common 5' end of both genes (3) (Fig. 1). Normally, the DMR is unmethylated in the paternal allele and methylated in the maternal allele, leading to the expression of these genes from the paternal allele. The overexpression of the paternal allele is caused by one of three mechanisms: (a) duplication of the paternal alleles (33%), (b) pUPD (41%), or (c) hypomethylation of the maternal allele (26%) (5).

Duplication of the paternal allele could be de novo or inherited as a dominant trait, and could also be a part of complex chromosomal rearrangements. pUPD also generally occurs de novo, and the disomy is either segmental or whole-chromosomal. In addition to TNDM, patients with pUPD could show symptoms of chromosome 6-related recessive disorders if they were originally heterozygous carriers of these mutations. Hypomethylation of the maternal allele could be an isolated event or part of hypomethylation of multiple imprinted loci (5). Approximately half of the cases with multiple locus hypomethylation are caused by biallelic recessive mutations in ZFP57 at chromosome 6p22.1, which controls the methylation status of multiple imprinted loci (5, 13). In the remaining 50%, the causes of multiple locus hypomethylation are unknown.

**Structure of the 6q24 Imprinted Loci**

Figure 1 shows the structure of the 6q24 imprinted locus. Of the two genes in this locus, PLAGL1 encodes a C2H2-type zinc-finger protein, which is involved in cell cycle arrest and is absent from a number of solid tumors, including those of breast and ovarian cancer (14, 15). At present, for several reasons, PLAGL1 is believed to play a major role in the development of 6q24-TNDM. In contrast, HYMAI does not code for a protein and its role in the development of TNDM is uncertain.

**Causes of Hyperglycemia**

The clinical course of 6q24-TNDM is partially replicated by transgenic (Tg) mice harboring a genomic fragment spanning the 6q24 imprinted locus (16). From the histological data of Tg mice (16), as well as other data obtained by postmortem pathology (17), functional tests on TNDM patients
(18), and the results of overexpression of the PLAGL1 cDNA in β cell lines (19), it is believed that there are multiple causes of hyperglycemia in patients. In utero and in the postnatal TNMD period, patients show hypoplasia of pancreatic β cells (16, 17), and are deficient in their insulin secretory capacity (18). The postnatal period is characterized by the rapid increase in the β cell mass (16), followed by a normalization in the insulin secretory capacity. A relapse of diabetes is associated with the reemergence of defective insulin secretion. In addition, the overexpression of PLAGL1 cDNA in the INS1 β cell line is associated with the suppression of glucose-induced insulin secretion (GSIS) (19). Since PLAGL1 is known to inhibit the cell cycle, its overexpression in β cells could be the cause of reduced β cell mass in utero. PLAGL1 has also been shown to inhibit the expression of PAC1 and RASGRF1, and activate the expression of PPARγ, SOCS3, and TCF4, possibly causing abnormal GSIS in the patients (20). Interestingly, while the GSIS pathway is inhibited by these alterations, G-protein coupled pathways of insulin secretion, such as the incretin pathway, appear to be intact in the PLAGL1 overexpressing cells, and in the mouse model of TNMD type 1, a GLP1 analog (liraglutide) relieved hyperglycemia (21). The mechanism leading to the rapid increase in the β cell mass in the early postnatal period and that leading to the relapse of β cell failure later in life, however, are not known.

Diagnosis

It is not possible to differentiate 6q24-TNDM from other causes of NDM by simple laboratory analysis, or by the severity of hyperglycemia, although patients with 6q24-TNDM tend to be smaller for gestational age (5) compared with other NDM patients. The definite diagnosis is made by analyzing the copy number, parental origin, and methylation status of the 6q24 imprinted locus using specific laboratory tests, including methylation-specific PCR (22), comparable genomic hybridization (CGH) array, single-nucleotide polymorphism (SNP)/CGH array, or polymorphic marker analyses of the patients and their parents.

Treatment

During the neonatal period, the patients are treated with insulin until remission, using continuous intravenous or subcutaneous infusions. The insulin treatment regimen is not different from that for other NDM patients (23). After relapse, patients have traditionally been treated using insulin or sulfonylurea (24), since they are generally not obese and are insulin deficient. Sulfonylureas are effective to achieve an optimal glycemic control, sometimes for an extended period of time. However, we often observe a secondary failure of treatment with sulfonylurea similar to that seen for type 2 diabetes (personal observation). Since PLAGL1 overexpression does not interfere with the incretin pathway (21), combination therapy with a DPP4 inhibitor (alogliptin) and a β-glucosidase inhibitor (voglibose) has been attempted for an adult patient with relapsed 6q24-related diabetes (25). The treatment was effective in keeping the HbA1c at 6–6.5% for an extended period of time, without any dietary constraints or recommended exercise (25). However, after seven years of optimal control, the patient’s HbA1c begins to creep up to >7%, at which point the patient is given an additional treatment of glinide before meals which restored their optimal glycemic control (personal observation). Evidently, a formal clinical trial is needed to establish an optimal treatment regimen, and GLP1 analogs, which are expected to stimulate the proliferation of β cells, should be included in such clinical trials. These efforts have up until now been hampered by the rarity of this disorder, and national or international collaboration will be needed to resolve this matter.
**Recent Developments: 6q24-related Diabetes without TNDM**

As described above, relapsed 6q24-related diabetes clinically resembles MODY. Although dominantly inherited diabetes is relatively rare for 6q24-related diabetes, except when the patients have duplication of the paternal allele, *de novo* diabetes is also not uncommon among known MODY patients (26). Interestingly, there have been several reports of patients with 6q24-related diabetes who do not have a history of TNDM (18, 27, 28). Since it is not clear whether these patients did not have TNDM or whether they had relatively mild diabetes which went unrecognized during the neonatal period, we tested the possibility of the presence of 6q24-related diabetes among patients who had MODY-like diabetes and who really did not have a history of TNDM (29). Through methylation-specific PCR amplification of the 6q24 imprinted locus, we screened 113 patients who were referred to our institution for suspected MODY and tested negative for mutations in the common MODY genes (*GCK, HNF1A, HNF4A, and HNF1B*) and for the mitochondrial 3243A>G mutation. As a result, we identified three patients with pUPD of the 6q24 locus. The results were confirmed by a SNP/CGH array and a polymorphic marker analysis. All three patients were born SGA (1444–1646 g) and were hospitalized in neonatal intensive care units for approximately 40 days due to low birth weights and their associated morbidities. After a period of normal life, these patients developed diabetes between the ages of 9 and 14. We traced these patients using the neonatal clinical history of the corresponding hospitals where they were treated, and found that none suffered hyperglycemia during the neonatal period (29). The fact that these patients were born SGA suggests that they were insulin-deficient in utero, and somehow their diabetes came into remission before birth.

Three patients out of 113 is not a large part of the unknown MODY. However, out of these 113 patients, only 8 were born with a small birth weight for gestational age of < –2.5 SD, and among these patients with an SGA birth weight, 6q24-related diabetes was the second most common next to HNF1B-MODY. Therefore, for patients with MODY-like diabetes with a history of SGA birth weight, we should consider the possibility of 6q24-related diabetes.

**Conclusions**

Chromosome 6q24-related diabetes, which was first identified as the most common cause of transient neonatal diabetes, has now been recognized as a cause of MODY-like diabetes without a history of neonatal diabetes. The molecular basis for this imprinting disorder has been largely elucidated. However, the mechanisms behind the rapid postnatal expansion of β cells and those behind β cell failure later in life are still not well understood. Since many of the patients treated with sulfonylurea or DPP4 inhibitors were found to experience secondary treatment failure, further elucidation of these mechanisms might lead to the establishment of better treatments for this disorder.

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