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# Related Genes and Treatments of Transient and Permanent Diabetes Mellitus

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**Abstract:** NDM (neonatal diabetes mellitus) is a rare genetic disease, which happens in the 6 months of life. Some patients with NDM heal on their own, but the other patients will show the symptoms of NDM for their whole life. Based on the presence of the remission phase, NDM is divided into two categories: TNDM (transient neonatal diabetes mellitus) and PNDM (permanent neonatal diabetes mellitus). As a genetic disease, the mutations in different genes lead to different symptoms and the presence of the remission phase. The mutation in INS gene will cause PNDM, and the mutations on KCNJ11 and ABCC8 genes and the abnormalities of chromosome 6q24 will lead to TNDM. Previous articles have always given information about diseases rather scatteredly: they only studied only mutated gene in an article. Unlike them, this article will discuss and analyze these three types of mutation and both PNDM and TNDM in one article. It will give the pathologic mechanism of the diseases, and, for different mutated genes, three treatments are given in this article at the same time. The compilation shows more clearly about the different symptoms corresponding to the different mutated genes and the mechanism of the disease.

**Key words:** NDM, genetic mutations, insulin, sulfonylurea, CRISPR/CAS9.

## 1. Introduction

Diabetes happens when plethoric of glucose exists in human blood. Glucose is like fuel, provided energy and heat that is necessary for the human body [1]. People eat to ingest glucose, and glucose enters human blood and is absorbed by cells [1]. This process happens with the precondition of insulin which is generated by beta cells in the pancreas and exists in human blood [2]. If there is excess glucose in the blood, insulin would be produced and bind to the insulin receptors on cell membranes [2]. These receptors trigger the opening of the pathway and let the glucose enter the cells, then lower the glucose level in the blood [3]. When there is a dearth of insulin or no insulin in the blood, glucose will never enter cells, and the glucose level will be high in the blood (Siddiqui, A). Then, diabetes happens. In the US, the estimated diabetes prevalence reaches 24.7 million, which is 7.6% of the total population [4].

There are three major types of diabetes: type 1 diabetes, type 2 diabetes, and gestational diabetes [5]. Type 1 diabetes is a genetic disease: For most patients who get type 1 diabetes, caused by the autoimmune system disorder related to gene mutation, their antibodies kill the beta cells [6]. They have symptoms of hyperglycemia, decreasing beta cells, weight loss, feeling thirst, and lethargy [7].

A large amount of people is affected by type 2 diabetes, regardless of their locations, ages, and ethic. Around the world, about 463 million adults (20 to 79 age group) and 136 million people (above 65 years old) are diagnosed with type 2 diabetes [8]. Unlike type 1 diabetes, type 2 diabetes is caused by the decreasing level of insulin in the human body. When the beta cells lose their function or some organs resist insulin, this insulin deficiency happens [9]. The symptoms of type 2 diabetes are similar to the symptoms of type 1 diabetes, including weight loss, hyper feeding, feeling thirst, and hyperglycemia [10].

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Gestational diabetes occurs during pregnancy. The etiology of gestational diabetes is that pregnant women have weight increasing and produce lots of hormones, which are the main factors that cause gestational diabetes, and they need more insulin than no pregnant women [11]. Although the pancreas secrets more insulin in this period to meet the insulin requirement, it still is not enough, then, diabetes happens [11]. Type 1 diabetes, type 2 diabetes, and gestational diabetes are the three major type of diabetes, but there are other types of diabetes existing, such as the NDM (neonatal diabetes mellitus).

As a rare genetic disease, although there are not as many cases as the type 1 diabetes and type 2 diabetes, modern medicine has had deep understanding for this disease. The most important genes responsible for NDM have been identified. Although the complete eradication remains an open question, the pathology of common genetic mutations has been mastered, and we have already had the corresponding treatment plan, such as the sulfonylurea therapy for KCNJ11 and ABCC8 genes mutations and insulin therapy for abnormalities of 6q24. Also, more and more new ways are used to treat NDM, for example, treating diabetes with CRISPR/CAS9 technology.

This article will launch around NDM, talks about the major three genes that lead to the disease, and give the corresponding treatments for each of them. Different from the previous articles, this article does not study only one mutated gene that may cause the disease but focus on three of the most common mutated genes. Some of the mutations may lead to TNDM, the other may cause PNDM. In reporting each gene individually, the article will include the following aspects: (1) the mechanism and function of the normal gene; (2) the etiology of NDM in the case of this gene mutation; (3) symptoms of the NDM caused by this mutation, including the classical symptoms in people with diabetes and the specific symptoms produced by the mutation in this gene; (4) Current research progress and unsolved problems. Finally, the Treatment part will give the mechanism of the specific therapy and the successful cases.

## 2. NDM

NDM is a form of diabetes. It does not happen frequently and occurs in 1 in 300,000-400,000 births [12]. NDM is a genetic disease caused by a single mutation. The mutations in different genes show different phenotypes, but, basically, there are three major phenotypes: (1) The beta cells act abnormally on producing insulin, such as KCNJ11, ABCC8, and 6q24 genes mutation; (2) Some mutations cause the destruction of beta cells, such as INS and EIF2AK3 genes mutation; (3) Some mutations cause the pancreas to fail to develop, such as PTF1A gene mutation [13].

NDM can be diagnosed in the first few days of life, but the duration of treatments is uncertain, which means some patients cost several months to cure the disease and some patients spend their whole life on treating [14]. Depending on the length of curing time, recent studies have categorized NDM into two main general groups: TNDM (transient neonatal diabetes mellitus) and PNDM (permanent neonatal diabetes mellitus), and the former one happens more commonly (70% of all NDM patients) [14]. The symptoms of TNDM and PNDM are similar, and most are classical symptoms of diabetes: abnormal low weight of the newborns, hyperglycemia, polyuria, and dehydration [15]. TNDM begins in the first six months of life and recover before 18 months of life with no applying any treatment, but these recovered patients, after remission, are more likely to get type 2 diabetes in adolescence [16]; the starting time for PNDM is same as TNDM, but the only difference for them is that these patients have insulin dependence for their whole life [12].

Diagnosis of NDM is not easy. Firstly, the high glucose level is common in newborns in the first three to five days of their life, and this is the time that NDM happens. That is why distinguishing NDM from

normal infants in this period is not clear [17]. Since one symptom of NDM is abnormal weight loss, newborns who simply lose weight but do not have diabetes may also be misdiagnosed as having diabetes [15]. In clinical diagnosis, infants should first accept a blood glucose level test and do further genetic testing if their blood glucose level is larger than 200 gm/dL [13]. Secondly, distinguishing between TNDM and PNDM is the following problem. With similar symptoms, just testing the glucose level is not enough, and genetic testing is necessary. The mutation on different genes leads to different types of NDM: the phenotype of the mutation on the 6q24 gene corresponds only to TNDM, and the mutation on the GATA6 gene can only lead to PNDM [17].

NDM shows various phenotypes, corresponding to the mutations in various genes. Among them, the most common mutations are in KCNJ11, ABCC8, 6q24, and INS gene mutations [18].

## 3. Gene Mutation and Their Phenotypes

Among all types of gene mutations, KCNJ11, ABCC8, INS, and 6q24 mutations are four of the most common factors that can cause the NDM. Table 1 below shows these four genes and their locations, features, treatments, and the type of NDM they will lead to.

### *3.1 KCNJ11 and ABCC8*

The combination of a mutation in KCNJ11 and ABCC8 genes is a common mutation of neonatal diabetes, more than 50% of all cases that are dragonized as NDM [17]. These mutations can cause both TNDM and PNDM and lead to hyperglycemia, dehydration, and developmental delay [19].

The mutations in the KCNJ11 gene and the ABCC8 gene affect the KATP channel directly. ATP-sensitive potassium (KATP) channel located on the beta cell membrane is composed of two kinds of proteins: KIR6 proteins made by KCNJ11 gene and SUR1 proteins made by ABCC8 gene [20]. In the KATP channel, KIR6 proteins and SUR1 proteins correspond to each other: when binding to ATP, the channel closes, controlled by the Kir6 protein; when the voltage increases, the channel is activated [21]. Normally, when ATP in the beta-cell combines with the KATP channel, the channel opens and depolarizes the cell. This voltage change leads to the openness of the calcium channel which is also controlled by the changing voltage. Then, $Ca^{2+}$ enters the cell and promotes the secretion of insulin [22].

For the KCNJ11 gene mutation, this mutation allows the channel to remain open even after binding to ATP, so the cell membrane cannot be depolarized and $Ca^{2+}$ ions are not allowed to enter the cell. Thus, the beta-cell will not release insulin. Or, when the ABCC8 gene mutation happens, the KATP channel keeps closing. Then, the cell depolarization will never stop, and the dysregulated beta-cell releases excess insulin [22]. Either the deficit insulin or the excess insulin causes NDM.

Normally, the increase of ATP produced by glucose metabolism closes the KATP channel, depolarizes the cell membrane, and generates insulin. The mutations

**Table 1 Genes that will be discussed in this article, their symptoms, and possible treatments [17].**

| Gene | Location | PNDM or TNDM | Feature | Treatments |
|---|---|---|---|---|
| KCNJ11 | Chromosome 11q15 | Either | Low birthweight Developmental delay, Seizures (DEND syndrome), May have other neurological feature | Insulin, Sulfonylurea |
| ABCC8 | Chromosome 11q15 | Either | Low birthweight | Insulin, Sulfonylurea |
| INS | Short arm of chromosome 11 | PNDM | Low birthweight | Insulin, CRISPR/CAS9 |
| PLAGL1 | Long arm of chromosome 6 | TNDM | Low birthweight, possible IUGR, Diagnosed earlier than channel | Insulin, Sulfonylurea |

of no matter KCNJ11 or ABCC8 gene make the channel keep opening, so the depolarization of the cell membrane cannot happen. Then, the beta cells cannot release insulin.

*3.2 INS*

The beta cells cannot generate insulin directly, but produces preproinsulin first, transforms the preproinsulin to proinsulin, and forms insulin [23]. Because INS gene is translated to preproinsulin, which is the beginning of the whole process, the mutation in INS gene destructs the insulin secretion seriously: its mutation decreases 80% of the insulin level [23]. Then, preproinsulin passes through ER (endoplasmic reticulum) and forms proinsulin.

The mutation in INS gene has two categories: recessive mutation and dominant mutation, and both of them can cause diabetes. Recessive mutation causes NDM. According to Garin's experiment, the genetic pedigree of 15 families with recessive INS mutation was given. For each family, the parents were the recessive mutated gene carriers, and the newborns were inherited NMD, no matter the gene is homozygous or heterozygous [24]. The offspring may carry TNDM or PNDM. Dominant mutation can also cause diabetes named MIDY (Mutant INS-gene-induced Diabetes of Youth) [23]. The etiology of this disease is that the mutant INS gene is translated to misfolding proinsulin. This insulin accumulates in endoplasmic reticulum membrane and forms ER stress which leads to cell death [23].

*3.3 Chromosome 6q24 Abnormalities*

The abnormality associated with chromosome 6q24 is the most common cause of TNDM, which makes up 70% of all TNDM cases, and is diagnosed 1 in 200,000 to 400,000 births [25]. Despite the fact that abnormalities of chromosome 6q24 have been identified, it is still not clear which genes cause TNDM. One gene that probably can cause the disease is the PLAGL1 gene [26]. Based on existing cases, a hypothesis about chromosome 6 abnormalities causing diabetes has been suggested: Paternal UPD (uniparental disomy) [26]. Combined with the imprinted effect, the mechanism of TNDM can be explained. Fig. 1 illustrates the difference between the normal chromosome 6q24 and abnormal chromosome 6q24 affected by UPD.

Normally, kids inherit two copies of genetic material from two parents. However, uniparental disomy is a genetic disease that both two copies of the offspring come from one parent, father, or mother [27]. In this case, both alleles on chromosome 6 are inherited from the father, meaning the patient has two copies of genetic material from the father and no copies from the mother. Fig. 1 shows the comparison of the normal chromosome (right) and the chromosome that has UPD (left). This abnormality leads to the over-expression of the paternal gene, the phenotype known as TNDM [27]. The PLAGL1 gene has two promoters, one is imprinted and the other is not, and only the imprinted promoter can express during development. In the first stage of TNDM, the imprinted promoter works, so the patient with this gene shows the over-expression of PLAGL1. After that, the newborn turns to use the non-imprinted promotor, so remission happens. Nevertheless, the imprinted promotor still affects the tissue, so type 2 diabetes relapses several years later [27]. For example, the researchers traced the DNA of a child with TNDM. In addition to the typical symptoms, the patient showed microcephaly and disturbance of intelligence.

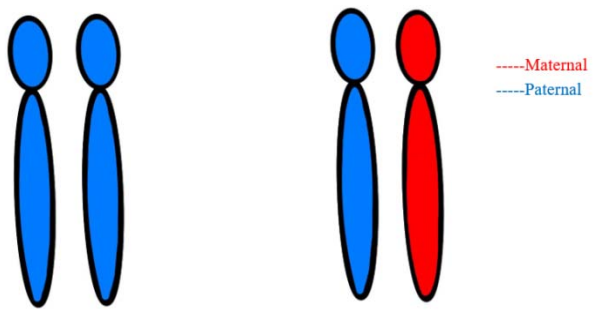


**Fig. 1 The abnormal chromosome 6q24 (on the left) and the normal chromosome 6q24 (on the right).**

After investigation, his father has a slight developmental delay, but his paternal grandmother is normal. There were two pairs of alleles on the long arm of chromosome 6, including two from the father. That is why he has both TNDM and mental retardation [27].

In spite of the mutation in both the combination of KCNJ11 and ABCC8 genes and chromosome 6q24 cause TNDM, the clinical presentations of them are different, and that is why these two mutations can be distinguished before doing genetic testing. (1) Age to be diagnosed. Since the abnormal insulin secretion of 6q24 TNDM happens in utero before birth, the characteristics of hyperglycemia can be diagnosed at birth, 0 to 4 weeks. However, the diagnosed age of KCNJ11 and ABCC8 mutated TNDM is after 4 weeks. (2) Average remission time. For abnormal 6q24 TNDM, on average, patients start remission at the age of 4 weeks and recur the diabetes symptoms at 16 years old. For mutated KCNJ11 and ABCC8 TNDM, the remission begins at a week and starts to relapse at 4.7 years old, on average, which means that the 6q24 TNDM has a longer remission time than the mutated KCNJ11 and ABCC8 TNDM. (3) Birth weight. The average birth weight for 6q24 TNDM patients is 1,950 g and 1,570 g for mutated KCNJ11 and ABCC8 TNDM patients [28].

## 4. Treatments

### *4.1 Sulfonylurea Therapy*

One treatment for NDM caused by KCNJ11 and ABCC8 mutations is sulfonylurea therapy. Since the mutations of KCNJ11 and ABCC8 genes keep the KATP channel open, insulin cannot be secreted and hyperglycemia happens. The mechanism of this therapy is that sulfonylurea helps close the channel without binding to ATP and promote the beta-cell to release insulin [19].

Recent studies have found that oral sulfonylurea is a short-term, more efficient way to treat KCNJ11 mutations, although neonatal diabetes can also be ameliorated by insulin therapy. In Pearson et al.'s study, after receiving an adequate does of sulfonylurea, 45 of 49 patients with NDM were able to stop taking insulin. The glycated hemoglobin levels decreased significantly after switching from insulin to sulfonylurea. Among them, 27 patients were insulin-independent for more than one year, and one patient even kept a glycated hemoglobin level of 5.7 percent for two years. Only five patients had unsuccessful switching [29]. Sulfonylurea therapy also does not have several side effects. Despite the patients who had unsuccessful switching experienced transitory diarrhea, children aged from 1.6 to 12.4 are not affected if the detrimental growth after taking this treatment. However, compared to channels, which carry KCMJ11 mutations, prevent more than 75 percent of KATP current, the sulfonylurea tolbutamide is less effective for other mutation such as Q52R, I296L, and L164P [29]. Thus, identifying various mutated genes should be identified before treating, and the clinical response of patients can be predicted.

### *4.2 Insulin Therapy*

For diabetes caused by the mutation in INS gene and the abnormalities of chromosome 6q24, insulin therapy should be applied. INS gene mutation leads to PNDM, and patients should use insulin sustainedly. Although insulin therapy cannot radically resolve the problem of dysfunction of beta-cell, some evidence has shown the aggressive treatment with insulin saving some beta-cell functions in some degree [17].

For TNDM cause by 6q24 abnormalities in different phases, the usage and dosage are also different. In the acute phase, the phase that shows NDM symptoms, IV insulin should be used as soon as hyperglycemia happens. Other than injecting insulin, oral should also be used. At the same time, patients should have daily monitor of their blood glucose level and adjust the dosage of used insulin at any time [17].

During the remission phase, patients can stop intaking insulin but should be aware of the recurrence of the diabetes. They need to measure their blood glucose level for every day and keep exercising. In the relapse phrase, insulin therapy still works. It has been shown by successful cases that sulphonylureas may work in this phrase (6q24 transient neonatal diabetes).

### *4.3 CRISPR/CAS9*

Clustered, regularly interspaced, short, palindromic repeats (CRISPR) system was an immune system in archaea and bacteria, and CRISPR-associated (CAS) protein is the center of the whole system. Cas9 protein is the one used in CRISPR/CAS9 genetic scissors. It is composed of an identical segment of DNA repeated several times and different spacer DNAs inserted between them. On one side of the CRISPR system, CAS genes are connected to the palindrome and create the CAS protein. When a new type of virus injects its DNA to the bacteria, the CAS protein breaks the virus' DNA, takes it, and copies it into the CRISPR system. The copied DNA becomes a new spacer DNA inserted into the palindrome and makes the bacteria have the ability to identify such viruses in the next time. It contains two domains: HNH endonuclease domain and RuvC nuclease domain. The mechanics of this technology is that scientists first utilize a specific CAS protein named Cas9 protein which is composed of two parts, tracer RNA and crRNA. This protein can identify the DNA segments corresponding to its crRNA in bacteria and cut them. Scientists replace the crRNA with DNA they want to change, so Cas9 protein identifies the corresponding DNA segment in a cell and cuts it [30].

Unlike type 1 and type 2 diabetes, polygenetic diseases, NDM is a monogenetic disease, which makes CRISPR/CAS9 treatment possible. Scientists found a mutated point on INS gene and tried to fix it in the stem cells [31]. In order to adapt more patients to this treatment, hPSCs (human pluripotent stem cells) are chosen as the target cells [32]. They corrected the mutation with ex vivo approach, according to the correct template, and made the stem cells differentiate outside the mice body. These corrected stem cells were differentiated to pancreatic endoderm and delivered back to the mice body. The mice show normal glucose regulation and secrete insulin normally [31].

## 5. Conclusion

The mutation and abnormalities of KCNJ11 gene and the ABCC8 gene, INS gene, and chromosome 6q24 are three of the most common factors that will cause NDM. Through the discussion of their functions, pathology, and symptoms, modern medicine has completed the fundamental discover of the disease. Based on the current information we should focus on several aspects in the future: (1) Case sharing. Since NDM is a rare disease, there are not enough cases to support a continuous multifaceted study. (2) The diagnosis of NDM. Since NDM happens in the neonatal period and the classical symptoms of it, such as the weight loss and insulin deficiency, also happen in normal newborns, diagnosing NDM quickly and accurately is a future direction of studying. (3) A radical cure for NDM. Current treatment methods are basically to alleviate and control the disease, there is no effective method to cure the disease. The application of CRISPR/CAS9 technique raises hopes of a cure for NDM.

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