# Development of type 2 diabetes caused by a deficiency of a $tRNA^{lys}$ modification

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**Abstract**

Genetic variations in the *cdk5 regulator associated protein 1-like 1 (cdkal1)* gene have been identified in whole-genome association studies as a risk factor for the development of type 2 diabetes (T2D). A recently study showed that Cdkal1 was a mammalian methythiotransferase, which specifically synthesizes 2-methylthio-$N^6$-threonylcarbamoyladenosine ($ms^2t^6A$) at position 37 of $tRNA^{lys}(UUU)$. The $ms^2t^6A$ modification in $tRNA^{lys}(UUU)$ was important for the accurate decoding of its cognate codon. In pancreatic β-cell-specific Cdkal1 knockout (Cdkal1 KO) mice, a deficiency of $ms^2t^6A$ caused the mistranslation of a Lys codon in proinsulin, resulting in improper processing. The mice showed a decrease in insulin secretion and glucose intolerance. In addition, the mistranslation contributed to the expression of the endoplasmic reticulum (ER) stress response in Cdkal1-deficient β-cells. Furthermore, Cdkal1 KO mice were hypersensitive to high-fat diet-induced glucose intolerance as well as the ER stress response. These findings might potentially explain the molecular pathogenesis of T2D in patients carrying *Cdkal1* variations.

**Cdkal1 as a risk factor for T2D**

Recent advances in whole-genome association studies (GWASs) have resulted in the identification of a number of genes associated with T2D. Among these risk genes, *cdkal1* is one of the most reproducible across different ethnic populations. To date, five single nucleotide polymorphisms (SNPs) in intron 5 of *cdkal1* (rs4712523, rs10946398, rs7754840, rs7756992 and rs9465871) have been shown to significantly influence the risk of T2D. Subsequent follow-up studies have found that the SNPs in *cdkal1* are associated with decreased insulin secretion, but not peripheral insulin sensitivity. For example, homozygous carriers of the risk allele rs7756992 have 22% lower insulin secretion than non-risk allele carriers. Among nondiabetic offspring of T2D patients, carriers of GC and CC genotypes of rs7754840 had 11 and 24% lower first-phase insulin release than carriers of the GG genotype. At present, the biological function of the intronic SNPs in *cdkal1* is largely unknown. Recent studies have found that SNPs in *transcription factor 7-like 2 (tcf7l2)*, another T2D risk gene, were associated with a distinct alternative splicing pattern of *tcf7l2* mRNA. Thus, it is

conceivable that the SNPs in *cdkal1* also regulate the expression pattern of *cdkal1* mRNA, which would ultimately affect insulin secretion in pancreatic β-cells.

**Methylthiolation of tRNA$^{lys}$(UUU) by Cdkal1**

Cdkal1 shares considerable domain and amino acid homology with Cdk5 regulator subunit-associated protein (Cdk5Rap1). Because Cdk5 is implicated in insulin secretion in pancreatic β-cells, Cdkal1 may regulate insulin secretion through interaction with Cdk5. However, unlike Cdk5Rap1, Cdkal1 is not involved in Cdk5-dependent signaling.

Cdkal1 contains several typical domains conserved in the methylthiotransferase (MTTase) family; the UPF0004, radical S-adenosyl-L-methionine (SAM) and TRAM domain. Enzymes in the MTTase family catalyze chemically challenging reactions, involving a C-H to C-$SCH_3$ conversion, through a radical. The UPF0004 domain and SAM domain utilize [4Fe-4S] clusters to generate the highly reactive 5'-deoxyadenosyl radical, which

is necessary for C-$SCH_3$ to form. In addition to the conserved domains, Cdkal1 contains a unique hydrophobic domain at the C-terminus, which allows the protein to localize to the endoplasmic reticulum (ER) membrane.

Methythiolation ($ms^2$) by the MTTase family has been observed in tRNA and ribosomal protein. For example, MiaB, a bacterial protein, catalyzes the methylthiolation of $N^6$-isopentenyladenosine ($i^6A$) to generate 2-methyl-thio-$N^6$-isopentenyladenosine ($ms^2i^6A$) at position 37 ($A^{37}$), 3'-adjacent to the anticodon in some tRNAs. In amino acid sequence, MiaB shares high homology with the bacterial protein YqeV and its mammalian homologue Cdkal1. Both YqeV and Cdkal1 specifically catalyze the methylthiolation of $N^6$-threonyl-carbamoyladenosine ($t^6A$) to synthesize 2-methylthio-$N^6$-threonyl-carbamoyladenosine ($ms^2t^6A$) at $A^{37}$ in $tRNA^{Lys}$(UUU).

**The physiological role of methylthiolation in tRNA**

The methylthiolation of $A^{37}$ in tRNA is critical for regulating decoding fidelity. For example, the $ms^2i^6A$ modification is important for preventing the misreading and

frame-shifting of cognate codons during translation in bacteria. Crystal structural analysis has revealed that the methylthiolation of $i^6A$ stabilizes the codon-anticodon interaction through cross-strand stacking with the base of the first nucleotide of the mRNA codon, which thus prevents mistranslation.

Because the chemical structure of $ms^2t^6A$ is highly similar to that of $ms^2i^6A$, it is conceivable that the $ms^2$ modification of $t^6A$ in $tRNA^{Lys}$(UUU) is also critical for preventing mistranslation of the Lys codon. By using a luciferase-based assay system, we have shown the $ms^2$ modification in $tRNA^{Lys}$(UUU) to be critical for preventing misreading of Lys. Firefly luciferase was utilized as a reporter for examining the decoding fidelity of the Lys codon, because previous studies have shown that tLys529 in firefly luciferase is essential for its activity. In *yqeV* -deficient cells of *Bacillus subtilis* (Δ*yqeV*), which lack the $ms^2$ modification in $tRNA^{Lys}$(UUU), we observed a significant decrease in firefly luciferase activity. Notably, enhancement of the translation rate resulted in a further decrease in luciferase activity. These results suggest that the $ms^2$ modification in $tRNA^{Lys}$(UUU) is important for preventing misreading of the Lys codon,

especially when the translation rate is high.

**Pancreatic β-cell dysfunction in mice lacking the $ms^2$ modification of $tRNA^{Lys}(UUU)$**

Precisely controlled proinsulin translation is critical for maintaining functional pancreatic β-cells. In *Akita* mice, mutation of the *proinsulin* gene directly causes a severe diabetic phenotype due to the presence of misfolded proinsulin. In transgenic mice in which Ser51 in eukaryotic initiation factor 2 alpha subunit (eIF2a) was mutated to alanine, moreover, abnormally enhanced translation caused β-cell failure due to heightened and unregulated proinsulin translation. Thus, abnormal translation of proinsulin subsequently evokes an ER stress response in β-cells, which ultimately disrupts the function of β-cells.

Human proinsulin as well as mouse Insulin I contain two Lys residues. One of them is located in the cleavage site between the C-peptide and the A chain. We hypothesized that the mistranslation of the critical Lys codon in proinsulin might inhibit the cleavage and generate misfolded proinsulin, which in turn might

impair the function of β-cells. We generated Cdkal1 KO mice, in which the $ms^2$ modification in $tRNA^{Lys}$(UUU) was ablated in β-cells. In the islets of Cdkal1 KO mice, a significant decrease in the incorporation of lysine into proinsulin as well as C-peptide production was observed. These observations suggest that a lack of the $ms^2$ modification in $tRNA^{Lys}$(UUU) in β-cells causes mistranslation of the Lys codon in proinsulin, resulting in the inhibition of subsequent processing. Furthermore, accumulation of mistranslated and misfolded proinsulin in Cdkal1 KO islets induced ER stress response as well as defective intracellular trafficking of secretory and plasma membrane proteins. Consequently, Cdkal1 KO mice exhibited glucose intolerance and a decrease in first-phase insulin secretion, which were also observed in Cdkal1 *null* mice. In addition, Cdkal1 KO mice fed a high-fat diet developed severe glucose intolerance due to a severe ER stress response in β-cells.

In summary, Cdkal1, a T2D risk factor, is a novel tRNA-modifying enzyme. The $ms^2$ modification of $tRNA^{Lys}$(UUU) by Cdkal1 is important for preventing

mistranslation of the Lys codon. A deficiency of the $ms^2$ modification in Cdkal1 KO mice caused a mistranslation of Lys in the *proinsulin* gene, which in turn induced an ER stress response and affected the function of β-cells. Cdkal1 KO mice showed glucose intolerance and decreased first-phase insulin secretion, which resemble the features of homozygous carriers of risk *cdkal1* SNPs. Taken together, the mistranslation in β-cells caused by a deficiency of the $ms^2$ modification of $tRNA^{Lys}$(UUU) may underline the molecular pathogenesis of T2D in patients carrying risk *cdkal1* SNPs. Consistent with this hypothesis, recent studies also found that proinsulin conversion was decreased in homozygous carriers of risk *cdkal1* SNPs. Futher studies in human samples are needed to examine whether the risk *cdkal1* SNPs are associated with the level of $ms^2$ modification.

Fig.1 Schematic model of regulation of **b**-cell function by Cdkal1. Cdkal1 methylthiolates tRNALys(UUU) at A37, which stabilizes the interaction between tRNALys(UUU) and its cognate codon AAG as well as AAA. Deficient of ms2t6A modification in tRNALys(UUU) by Cdkal1 deficiency causes mistranslation of Lys codon in Proinsulin, which induce ER stress response and reduce mature insulin content in **b**-cells. Dysfunction of **b**-cells by Cdkal1 deficiency would ultimately lead to the development of T2D.