Review Article

REVIEW: ASSOCIATION BETWEEN DNA METHYLATION OF CYP GENE FAMILY IN VITAMIN D SIGNALLING PATHWAY AND TYPE 2 DIABETES

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ARTICLE INFO	ABSTRACT
	Diabetes is a major public health problem which occurs both due to environmental and genetic predisposition. The vitamin D deficiency is one of the factor causing type 2 diabetes and increasing its complications. Vitamin D regulates Ca^{2+} in humans and CYP2R1 gene methylation decreases vitamin D level. Ca^{2+} plays a pivotal role intracellular signal in the regulation of insulin secretion from pancreatic β-cells. So, deficiency of vitamin D due to CYP2R1 gene methylation affects Ca^{2+} secretion which further affects insulin level. This fluctuation in insulin level due to CYP2R1 gene methylation might results in type 2 diabetes, although this statement needs further verification via experimental methods.

INTRODUCTION

According to the report of WHO the global prevalence (age standardize) of diabetes has nearly doubled since 1980, rising from 4.7% to 8.5% in adult population in the year 2014 and 1.2 million deaths in year 2012 (WHO Report, 2016). Obesity and T2DM are major public health problem in recent world. Epidemiological data show that between 60% to 70% of T2DM patients are overweight or obese (Stumvoll *et al*., 2005). Similarly, for India this increase is estimated to increase from 69.1 million people in 2015 to 87 million in 2030 (Snehalatha and Ramachnadaran, 2009; International Diabetes Foundation). India is one of the 6 countries of the IDF SEA region. 415 million people have diabetes in the world and 78 million people in the SEA Region; by 2040 this will rise to 140 million (International Diabetes Foundation). The more detailed study on the prevalence and effect of T2DM in various parts and sector of India is done by Vipin Gupta in 2012 (Gupta, 2012). Moreover, Ford *et al*. suggest that one kilogram of weight gain increases the risk of T2DM development by 4.5% to 9% (Ford *et al*., 1997). It is found that the concern of T2DM is evoked by genetic and environmental factors. The increase is explosive in the prevalence of T2DM in past several decades.

Since the first genome-wide association study on T2DM in 2007, there is a lot progress made by researcher in the field of T2DM (Sladek *et al*. 2007). It has been also found that the epigenetics is the one of the most influential factor for T2DM (Kwak & Park, 2016). DNA methylation has been observed in the pathogenesis of a variety of biological processes and is affected by the environmental factors as well as aging (Florath *et al*., 2016; Maier *et al*., 2002). The mid to moderate Vitamin D deficiency is also identified as a risk factor for type 2 diabetes, due to its protective role against development of T2DM and its complications (Dhas *et al*., 2016). Expression of the vitamin D degrading and metabolizing enzyme is regulated through binding of $1,25$ -D_3-liganded VDR to vitamin D responsive elements ($VDRE_s$). However, the major regulators of $1,25$ -D_3 levels and signalling CYP2R1, CYP24A1, CYP27B1 and VDR, "the vitamin D tool" genes, are prone to epigenetic regulation. CpG islands span the promoters of CYP2R1, CYP24A1 and VDR, while a CpG island is located within the CYP27B1 gene locus. Therefore, DNA methylation and histone modification in these regions can change the chromatin state from an open to closed conformation and lead to transcriptional repression of these genes (Fetahu *et al*., 2014). CYP2R1 showed higher affinity and specificity for vitamin D than CYP27A1 (Cheng *et al*., 2003; Shinkyo *et al*.,

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2004). A genetic mutation of CYP2R1 will cause vitamin D deficiency (Cheng *et al*., 2004). The baseline methylation levels of CYP2R1 and CYP24A1 shows an inverse correlation with vitamin D response (Zhou *et al*., 2013). Observational studies to date have revealed inconsistent findings with respect to the relationship between vitamin D status and insulin resistance (Zhao *et al*., 2010; Kayaniyl *et al*., 2010; Rajakumar *et al*., 2012; de las heras, 2013). The studies have shown that the improvement in vitamin D status have reduce the risk for insulin resistance and herewith may contribute to the primary prevention of T2D and CVDs (Pham *et al*., 2015).

Vitamin D Signalling in Humans

In humans, vitamin D is produced mainly in the skin during exposure to solar ultraviolet blue (UVB) radiation (270-300 nm) (Jones, Strugnell & DeLuca, 1998). UVB radiation converts 7-dehydrocholesterol (7-DHC) in the skin to pre-vitamin D3, which immediately undergoes a thermal isomerization to vitamin D3. Dietary sources provide two forms of vitamin D: Vitamin D2 (ergocalciferol) derived from invertebrates (plants and fungi) and vitamin D3 (cholecalciferol) derived from animal sources. The hepatic enzyme 25-hydroxylase (CYP2R1) converts vitamin D to 25-hydroxyl vitamin D (25(OH)D). This major circulating form of vitamin D in the blood is a member of the cytochrome P450 superfamily of enzymes. To become biologically active, 25(OH)D is converted to 1,25-dihydroxyvitamin D (1,25(OH)2D), which is tightly regulated by calcium and phosphate concentrations through a negative feedback mechanism mediated by parathyroid hormone (PTH) (Henry, 2011). This occurs mainly in the kidneys, but also in other tissues expressing the enzyme 25(OH)D-1a-hydroxylase (CYP27B1). The biological effect of vitamin D is mediated when 1,25(OH)2D binds to the vitamin D receptor (VDR). To prevent excessive vitamin D signalling in the target organs, 1,25(OH)2D limits its own activity by inducing 24-hydroxylase (CYP24A1) converting 1,25(OH)2D to the biologically inactive water-soluble calcitroic acid which is excreted in the

In the blood circulation, most of vitamin D, 25(OH)D and 1,25(OH)2D are bound to DBP but also a small fraction is bound to albumin or exists in free form (Powe *et al*., 2013; Johnsen MS *et al*., 2014). DBP-bound 25(OH)D is the preferred biomarker of vitamin D status compared to 1,25(OH)2D which have a short half-life of 10-20 h (Carter, 2011). The transcriptional effects of vitamin D are regulated by 1,25(OH)2D binding to nuclear vitamin D receptors (VDR), which then undergoes Heterodimerization with the retinoid-X receptor (RXR) (Fig. 1) which binds to vitamin D response elements (VDRE) in the regulatory element region of vitamin D target genes. By candidate gene analysis, five vitamin D modulating genes have identified, including GC, CYP24A1, CYP2R1, CYP27B1 and VDR (Ahn *et al*., 2010). Approximately 25% of the inter-individual variability in plasma 25(OH)D concentrations can be explained by external factors such as diet, regular use of vitamin D supplements and exposure to sunlight (dependent on season and latitude) (Burgaz *et al*., 2007; shea MK 2009).

Vitamin D Signalling Effects in T2DM

A growing number of studies have uncovered polymorphisms associated with vitamin D concentrations. Vitamin D exerts its activity through two mechanisms: the hormone signalling, in which the biologically active form reaches target cells through the bloodstream; and the autocrine/paracrine signalling, in which locally produced-vitamin D3 affects the surrounding cells (Lai & Fang, 2013). Calcium is essential for development and maintenance of bone, cellular processes and neuromuscular functions. Insulin-secreting β-cells in pancreatic islets of Langerhans store insulin in membrane-bound secretory granules, which are rapidly mobilized for exocytosis in response to nutrient or non-nutrient stimuli (Hutton, 1989; Rorsman, 1997). It has been known for many years that cell secretory granules also contain very high concentrations of divalent cations, particularly Ca^{2+} and Mg^{2+}(Hutton, 1989).
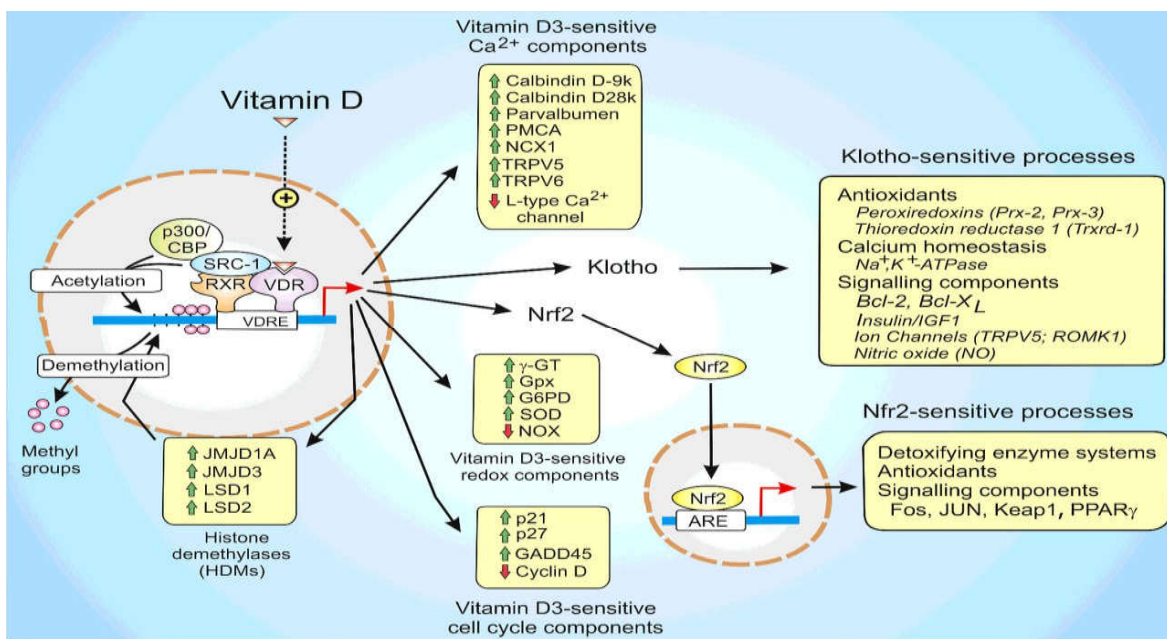

Figure 1 Vitamin D signalling and its secreted components. *(Source: - Berridge, 2015)*

In the pancreas, the CaSR (calcium sensing receptor) is involved in regulation of insulin secretion and Ca^{2+} is a pivotal intracellular signal in the regulation of insulin secretion from pancreatic β-cells (Squires *et al*., 2000). The promoter of the CaSR gene, a G-protein coupled receptor, harbours two VDREs suggesting that CaSR is a vitamin D target gene (canaff *et al*., 2002). The CaSR protein is found in abundance in cells of the parathyroid glands. The parathyroid glands produce and releases a hormone called parathyroid hormone that works to increase the level of calcium in the blood (U.S. NLM). At a sub stimulatory concentration of glucose (2 mmol/l), increasing extracellular $Ca2^+$ from 0.5 to 5.0 mmol/l caused an initial and rapid increase in insulin secretion from perifused islets, followed by a marked inhibition of secretion below basal levels. The inhibition of secretion was maintained when the extracellular Ca^{2+} concentration was increased to 10 mmol/l. Similar concentration-dependent inhibitory effects of extracellular Ca^{2+} were observed at a stimulatory concentration of glucose (20 mmol/l) (Squires *et al*., 2000). This type of regulation of Ca^{2+} levels and insulin secretion by Vitamin D can cause the diseases like T2DM. In cardiovascular system, vitamin D dependent stability of redox and Ca^{2+} signalling system plays an important role. Till date the link between the vitamin D and complications of type 2 diabetes is determined by very few studies. There may, however, be other important biological effects of Vitamin D and cholecalciferol, its active form 1,25-dihydroxycholecalciferol and ergocalciferol were found as a membrane antioxidant (Wesiman, 1993). Several evidences have shown that oxidative stress plays a key role in progression of chronic diseases such as diabetes (Maritim, Sanders & Watkins, 2003). T2DM have its association with the declining antioxidant capacity and increased oxidant capacity and increased production of ROS (Reactive Oxygen Species) through increased proteins, lipids and DNA oxidation products and advanced oxidation product (West, 2000; Schrauwen, 2004). The studies suggest that, deficiency of Vitamin D might hamper the Ca^{2+} regulation and causes increased oxidative stress which may in turns causes aging. The oxidative stress is cause or consequence of T2DM is still not completely determined. A plausible mechanism as suggested by Salpea *et al*. that the situation in the subjects who developed T2D may be that hyperglycaemia in the prediabetic state induces oxidative stress, which in turn causes oxidative telomeric DNA damage and consequent shortened telomeres, which eventually lead to premature senescence (Salpea *et al*., 2010).

Epigenetics of Humans Diseases

The term "epigenetics" was introduced in the 1940s by Waddington, as an attempt to define the role of genetics in developmental processes (Waddington, 1942- reprint). Epigenetics is a branch of biology which explores gene expression involving functional changes in chromatin structure and non-coding RNAs without change in nucleotide sequence of DNA. Epigenetics regulation mechanism includes modification of chromatin proteins, methylation of DNA and miRNA interference. Epigenetics mechanism regulates development and adaptation during the life of an organism and their alterations may result in various disorders such as cancer. Epigenetic marker is also found to be reversible, thus, it attracts many researchers to focus on epigenetic therapy. The interplay of DNA methylation and histone post-translational alteration, which cause as the result of regulatory proteins and non-coding RNAs, are key epigenetic players to rearrange chromatin into areas such as euchromatin, heterochromatin, and nuclear compartmentalization (Moosavi & Ardekani, 2016). The access of protein responsible for gene expression is determined by packing of chromatin, loose (euchromatin) or tight (heterochromatin). Euchromatin is a transcriptionally active form of chromatin, consists of histones H2A, H2B, H3 and H4 group into two H2A-H2B dimers and one H3-H4 tetramer to form nucleosomes, whereas, heterochromatin is an inactive form of chromosome arises as a result of histones interaction with H1 histones (linker Histone) (Russel, 2009). Epigenetics changes can be transferred to the next generation, because, they often happen during life time of an organism (Chandler, 2007). Initiation of DNA methylation at CpG sites is mediated by DNA methyltransferase enzymes such as DNMT1, DNMT3a and DNMT3b. CpG- rich regions are known as CpG islands (CGIs), defined as region of more than 200 bases with a G+C content of at least 50%. About 60% of human gene promoters are associated with CpG islands and are usually unmethylated in normal cell (Straussman, 2009). Most work in animals has focused on 5-methylcytosine in the CpG sequence context. Mammalian DNA methylation process is composed of two components, the DNA methyltransferase (DNMTs), which establish and maintain DNA methylation patterns, and the methyl-CpG binding proteins (MBDs), which reads methylation marks. DNA methylation represses transcription directly by inhibiting the binding of specific transcription factors, and indirectly, by recruiting MBDs and their associated repressive chromatin remodelling activities (Robertson, 2005). Inside the cells, S-adenosyl methionine (SAM) act, as an important methyl group donor and get converted to S-adenosylhomocysteine (SAH). In this sense folic acid and B12 play the determinant roles in re-methylation or the attraction of de-methylated from S-adenosyl methionine through passive and active mechanism (Kim YI, 2005).

Post-translation modification of core histone includes methylation, acetylation, phosphorylation, ubiquitination and sumoylation (Sharma; Kelly & Jones, 2010). Currently much attention is focused on core histones and methylation and acetylation of lysine and arginine. Enzymes responsible for modification of N-terminal tails of H2A, H2b, H3 and H4 are histone acetyltransferases (HATs), histone deacetyltransferases (HDACs), histone methyltransferases (HMTs) and histone demethylase (HDMs). Mass spectrometry of histone peptides in animals revealed 13 modification sites in histone H2A, 12 modification sites in histone H2B, 21 modification sites in histone H3, and 14 modification sites in histone H4 (Kovalchuk & Kovalchuk, 2012) (Table - 1). The effect of acetylation and methylation on the regulation was found to be dependent on the site of modification (Fig. 2). It was reported that acetylation of H3 histone at lysine-9/14 sites (H3KAc) and methylation of H3 histone at lysine-4 (H3K4me) were associated with activation of gene expression. Contrariwise, methylation of H3 histone at lysine-9 (H3K9me) or methylation of H3 histone at lysine-27 (H3K27me) was demonstrated to inhibit gene expression (Kasinska*et al*., 2015). However, histone methylation occurs only on arginines and lysines. Arginines can be mono- or di-methylated whereas lysines can be mono-, di- or trimethylated (Kouzarides, 2007). Arginine methylation can be either symmetrical or asymmetrical. The amino-terminal portion of

the core histone proteins contains a flexible and highly basic tail region, which is conserved across various species and is subject to various post-translational modifications. The HATs (Histone acetyltransferase) utilize acetyl CoA as cofactor and catalyse the transfer of an acetyl group to the amino group of lysine side chains. In doing so, they neutralize the lysine's positive charge and this action has potential to weaken the interactions between histones and DNA and helps them in uncoiling. (Bannister & Kouzarides, 2011).

T1 and T2 diabetes and its complications (Fernandez-Valverde *et al.*, 2011).

Epigenetics of Vitamin D Signalling Pathway and T2DM

It has been earlier mentioned in this review that the major regulators of 1,25 -D_3 levels and signalling CYP2R1, CYP24A1, CYP27B1 and VDR, "the vitamin D tool or biomarkers" genes, are prone to epigenetic regulation. VDR, CYP2R1 and CYP24A1 each have CpG islands including their promotor regions, and CYP27B1 has a CpG island in the gene
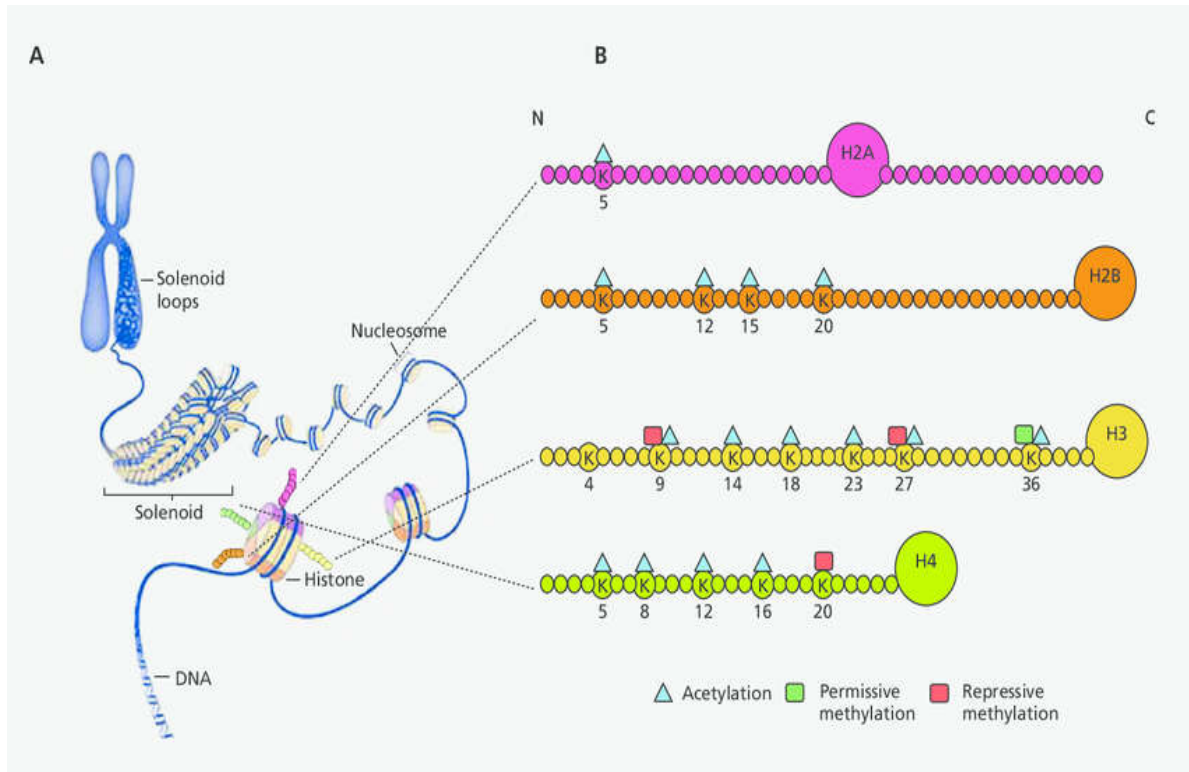

Figure 2 Chromatin structure and histone modifications at N-terminal Histone tails.

(Source – Bagot et al., 2014)

Table 1 Types of histone modifications in Humans.

Histone Type	Histone Modifications
H2A	H2AK5ac, H2AK9ac, H2AZ
H2B	H2BK120ac, H2BK12ac, H2BK20ac, H2BK5ac, H2BK5me1, UbH2B
H3	H3K14ac, H3K18ac, H3K23ac, H3K27ac, H3K27me1, H3K27me2, H3K27me3, H3K36ac, H3K36me1, H3K36me3, H3K4ac, H3K4me1, H3K4me2, H3K4me3, H3K79me1, H3K79me2, H3K79me3, H3K9ac, H3K9me1, H3K9me2, H3K9me3, H3R2me1, H3R2me2, H3ac
H4	H4K12ac, H4K16ac, H4K20me1, H4K20me3, H4K5ac, H4K8ac, H4K91ac, H4Kac, H4R3me2, H4ac

Source – (Kovalchuk&kovalchuk (chap.5), 2012)

Another mechanism of epigenetics regulation is gene silencing through single-stranded non-coding RNAs. miRNAs are small molecules, approximately 19 to 33 nucleotides in length, derived from endogenous transcripts generating a hairpin structure, that interact with regions of mRNA. Translation inhibition occurs when miRNA and mRNA pairs, as a result of degradation of mRNA and/or destabilization of mRNA structure (Humphreys, 2005). In last five years It is also found that miRNAs are involved in inflammatory processes contributing to the development of type 2 diabetes mellitus (Hamar, 2012). A comprehensive review has recently summarized miRNAs in β-cell biology, insulin resistance, and

body (Fetahu *et al.*, 2014). Differential methylation of these islands in promotor regions of genes may modulate gene expression, with hypermethylation often associated with decreased expression, and hypo-methylation associated with increased expression (Deaton, 2011). GWAS using DNA from blood leukocytes on 22 African American male youths Zhu and colleagues identified a small differential methylation in CYP2R1 and CYP24A1 between those with severe vitamin D deficiency, compared to vitamin D sufficient individuals (Zhu *et al.*, 2013). A study by Beckett *et al.*, in 2016 has confirmed that there is an inverse association between CYP2R1 methylation status and plasma 25(OH)D and confirmed the

lack of association for CYP27B1 (Beckett *et al.*, 2016). Inverse or negative association between CYP2R1 methylation and plasma 25(OH) D depicts that's the methylation of CYP2R1 region lowers down the secretion of plasma 25(OH)D which causes Vitamin D deficiency. CYP2R1 gene is located on chromosome 11p15.2, is the primarily enzyme responsible for the hydroxylation of vitamin D to 25(OH)D. The CYP2R1 gene is composed of 16,599 bases and 501 amino acids (Genecards.org).

An observational study form the Nurses Health Study includes 83,779 women > 20 years of age found to have increased risk of type 2 diabetes in those with low level of vitamin D (Pittas *et al.*, 2006). A hospital-based study of 50 diabetic patients of both the sexes (31 males and 19 females) was conducted by (Siddiqui *et al.*, in 2016), in which they found that 25(OH) vitamin D level was negatively correlated with HbA1c in diabetic patients. This suggests that the decrease in vitamin D status is related to increase in insulin level in the body in T2DM or we can also say that, hyperglycemia is linked with poor vitamin D status and the effects of this deficiency during type 2 diabetes seem to have negative consequences on insulin resistance and glucose homeostasis. A possible reason of vitamin D deficiency mediated hyperglycemia in T2DM patients is explained on the basis of crucial role of vitamin D in maintaining extracellular calcium concentrations and calcium influx into β-cells, which is necessary for insulin secretion and thereby glucose uptake in insulin-sensitive tissue (Huang *et al.*, 2002).

Summary and Future Directions

This review is based on the basic principles which tries to shows a direct association between T2DM and vitamin D. The DNA methylation of CYP2R1 gene and its association with vitamin D deficiency makes CYP2R1 most probable gene to study DNA methylation, in respect to T2DM. The Ca^{2+} is involved in the insulin secretion and Ca^{2+} concentration is regulated by vitamin D. So, *there is a chance that the DNA methylation of CYP2R1 gene region can destabilize calcium level by interrupting vitamin D secretion, which can further interrupt insulin secretion leading to T2DM.* This statement needs further verification via experimental methods to reach on a definite conclusion. There are also several other future paradigms in this research like association between Vitamin D deficiency-oxidative stress and T2DM, Vitamin D deficiency-Oxidative stress and premature senescence.

Conflict of Interest

The authors declare that there is no conflict of interest associated with this manuscript.

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