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Disease-Associated SNP Variants of Vitamin D Receptor Exhibit Compromised Receptor Function and Genome Bookmarking Properties

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Abstract

Mitosis is vital for cell renewal and involves dynamic chromatin organization and nuclear architectural alternations. Regardless of these changes, some epigenetic marks/factors are inheritable throughout cell division. Over the years, it has been found that certain transcription factors remain bound to chromatin during the transcriptionally silent mitotic phase suggesting their potential role in transmitting regulatory information trans-generationally. This phenomenon is referred to as '*genome bookmarking*.' In recent findings, a few Nuclear Receptors (NRs) have been reported to be associated with mitotic chromatin (constitutive, ligand-dependent, or partner-mediated manner). Recent studies from our lab have shown that disease-associated polymorphic variants of NRs severely impair the genome bookmarking phenomenon exhibited by the receptor. Vitamin D Receptor (VDR), a member of the NR superfamily, has both calcemic and non-calcemic functions, including but not limited to cell proliferation and differentiation, immune modulation, reproduction, and metabolism. Thus, its abnormal function can lead to diseases like osteoarthritis, bone disorders, cancer, HVDRR, diabetes, etc. According to a study from our laboratory, VDR participates in the transmission of cellular traits to progeny cells by constitutively interacting with mitotic chromatin. Additionally, it promotes the interaction of its heterodimeric partner RXR with mitotic chromatin. Furthermore, in another recent study, we evaluated the mechanism involved in the malfunctioning of disease-associated VDR-SNP variants at multiple regulatory levels. This study revealed that the '*genome bookmarking*' property of VDR is severely impaired in several variants, both with and without its cognate ligand. Moreover, partner-mediated mitotic chromatin interaction of VDR-SNP variants was examined, with the results suggesting that partner RXR cannot rescue compromised or lost mitotic chromatin interaction. Based on these findings, small molecules termed '*tweaker-ligands*' that can reorient aberrant receptor conformation towards the normal functional output could be designed or repurposed for disease management.

Keywords: *Genome Bookmarking*, Hereditary Vitamin D-Resistant Rickets (HVDRR), Nuclear Receptor, Single Nucleotide Polymorphism (SNP), Tweaker Ligand, Vitamin D Receptor

1. Introduction

Mitosis is a vital process of cell division, involving chromatin reorganization and nuclear architectural alternations[1]. During cell division, chromosomes are segregated and evenly distributed between two identical daughter cells. This event ensures that both daughter cells receive a copy of the genetic material present in the progenitor, which is crucial in maintaining epigenetic integrity. This guarantees that both daughter cells obtain an identical copy of the genetic material present in the progenitor cell. In interphase, transcription occurs at multiple gene loci[2]. However, when a cell enters mitosis, specific changes occur, including increased phosphorylation of histones which leads to chromatin condensation. This condensation is central to the displacement of transcription factors, thereby preventing the nuclear transcription machinery from accessing the DNA and shutting off transcription completely[3]. However, a few key transcription factors have been

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observed to be associated with mitotic chromatin that remains bound to the target gene throughout the cell cycle. This may be an inherent cellular strategy to assist in the rapid reactivation of transcription machinery upon exit from mitosis. Recently, a few Nuclear Receptors (NRs) have been identified to be associated with mitotic chromatin. Pregnane X Receptor (PXR) was the first one to be reported as a mitotic bookmarking factor that shows a constitutive association with mitotic chromatin[4]. Soon, our laboratory also observed mitotic chromatin association of homodimeric receptors, Androgen Receptor (AR), and Estrogen Receptor (ER), albeit in a ligand-dependent manner[5,6].

Other general transcription factors such as osteoblast lineage-specific Runx2, pluripotency regulators Oct4 and Sox2, notch effector protein RBPJ, hematopoietic regulator GATA essential for cell-fate decision, etc., were also reported as genome bookmarking factors[7-9]. Despite extreme compaction of chromatin, some accessible sites provide a platform for specific transcription factors, for the rapid reactivation of genes[6,10,11]. This retention of regulatory proteins and some associated factors during the transcriptionally silent mitotic phase is referred to as *'genome bookmarking'*[12-14]. In post-mitotic cell development, epigenetic bookmarks regulate the expression of early genes that direct cell growth and lineage maintenance.

In this context, here we address the congruity of another classical nuclear receptor, Vitamin D Receptor (VDR), in *genome bookmarking* and its role in lineage commitment, cell growth, and cell identity. VDR is a primary regulator of calcium and phosphate homeostasis required for skeleton and bone mineralization. In addition, VDR has been linked to various non-calcaemic functions such as cellular growth and differentiation, immune system regulation, reproductive processes, metabolism, and cardiovascular health. It requires heterodimeric partner RXR (Retinoid X Receptor) to arbitrate the cellular actions of hormonally active vitamin D, i.e., 1α,25 dihydroxy vitamin D3[15-18]. Recently, a study from our laboratory has confirmed that VDR exhibits constitutive mitotic chromatin association throughout the cell division[14]. In addition, it was also reported that VDR promotes mitotic chromatin association of its heterodimeric partner RXR. However, further studies are required to explore the detailed molecular mechanisms behind the bookmarking phenomenon.

Polymorphism is referred to as the frequent occurrence (>1%) of two or more discontinuous genotypes or alleles at a specific chromosome region in a population[19]. These polymorphisms can occur in both the coding and non-coding regions of the gene, affecting the receptor's functionality. Most of the studied polymorphisms of VDR are SNPs (Single Nucleotide Polymorphisms) defined as the substitution of a single nucleotide for another at the polymorphic site[20]. Due to the putative involvement of VDR in a wide range of physiological processes, SNPs can result in receptor dysfunctioning/loss of function, eventually leading to the onset of diseases such as osteoarthritis, cancer, diabetes, tuberculosis, cardiovascular diseases, PCOS, and HVDRR (Hereditary Vitamin D-Resistant Rickets). We have recently observed that VDR-SNPs specific to HVDRR (a rare genetic disorder) are the causative reason for generalized resistance to vitamin D3[21]. Using point and deletion mutations in the VDR gene we have studied several HVDRR-associated VDR-SNP variants for their subcellular dynamics, transcriptional functions, '*genome bookmarking*', heterodimeric interactions with RXR, and receptor stability. This article emphasizes the most recent finding from our laboratory which establishes the link between mitotic chromatin association of VDR with HVDRR-related pathogenesis[21].

2. SNP Variants of VDR and Disease Association

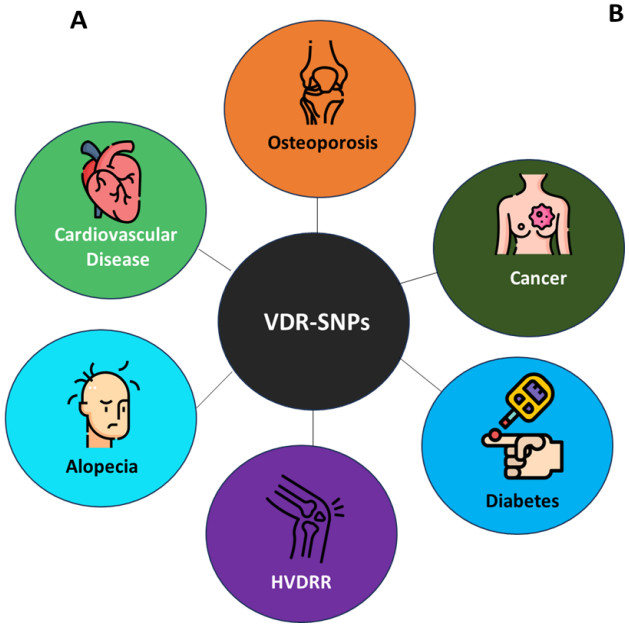
SNPs are the most prevalent form of genetic variation among human populations[22]. An SNP explicitly refers to an alteration in a single nucleotide within the DNA. Not all SNPs are associated with the disease. However, SNPs that are primarily located within a gene or in a regulatory region can disturb the gene function and directly play a role in the disease[23] NRs, as transcription factors, play a significant role in regulating gene expression, cellular signaling, and metabolism[24]. Therefore, SNPs within NR genes may inflict functional changes, affecting the receptor's ability to bind DNA, interact with co-regulators, or modulation of gene expression, and thus alter the receptor's physiological functions. According to the studies, several gene polymorphisms of NR have been found to be associated with diverse diseases[25-27]. Homodimeric receptor ERα harbouring SNPs (rs2234693) is a pathogenic factor for Systemic Lupus Erythematosus

(SLE) disease severity[28]. Similarly, polymorphism in other NRs, such as Heterodimeric receptor THR and orphan receptor HNF-4α, are reported in the risk of hypertension/systolic BP and Crohn's disease, respectively[29-30].

Like other NRs, several VDR polymorphisms, such as Fok1, Apa1, Cdx2, Bsm1, and Taq1, have been reported in multiple diseases, including cancer, osteoporosis, diabetes, tuberculosis, cardiovascular disease, etc. (Figure 1)[31,32]. Over the years, several SNPs (variations/mutations) have been reported in the VDR gene, including, synonymous and non-synonymous polymorphism[33]. The synonymous SNPs are also known as silent variations or mutations, as there is no alteration in the amino acids due to the degeneracy of the genetic code[34]. However, when a codon encrypting a specific amino acid is substituted with a codon translating a different amino acid, it is known as a non-synonymous mutation (i.e., a missense mutation). Non-synonymous polymorphism also includes a nonsense mutation where one nucleotide substitution results in a premature stop codon that halts the translation process, producing a truncated polypeptide chain[35]. Epidemiological studies have implicated several missense and nonsense mutations of VDR as the cause of the rare autosomal disorder Hereditary Vitamin D-resistant Rickets (HVDRR)[36,37]. In clinical studies, it has been found that a patient with HVDRR and an atypical pattern of alopecia has a novel V26M polymorphism in the VDR DBD as the underlying molecular dysfunction[38]. A study on an Indian patient with vitamin D-dependent type II rickets has reported the occurrence of deletion mutation (c.716delA) in the VDR LBD, which caused a 50% reduction in receptor interaction with VDR response elements[39]. Although numerous VDR-SNPs are reported to cause HVDRR, the evidence of underlying molecular mechanisms and disease associations attributable to the receptor functions and prospective therapeutics are still fragmentary.

To understand the in-depth mechanism behind the HVDRR progression, first, we constructed different SNPs in the VDR gene using site-directed mutagenesis (Figure 2). These VDR-SNP variants were then analysed for their disease association by observing deviations in receptor behaviour at the level of i) subcellular receptor dynamics, ii) receptor transcriptional functions, iii) heterodimeric interactions with RXR, iv) receptor stability, and v) receptor-chromatin interaction or '*genome bookmarking*'.

A



B

Disease	SNP reference (ID)	SNP/Variation type
HVDRR	rs121909796	R274H, R324H
	rs121909801	R30X
	rs121909795	Q152X
	rs199583004	G1134T
	rs2229828	C444T
	rs11832059	T48G/C
Alopecia	rs7975232	Intron Variant
	rs2228570	T2G/C/A
Osteoporosis	rs2228570	A2C /G / T
	rs731236	A1056G/T
	rs1544410	Intron Variant
Cancer	rs731236	T1056C
	rs2228570	M1K/T/R
Diabetes	rs7975232	Intron Variant
	rs2228570	T152G, T2G/C/A
Cardiovascular disease	rs1544410	Intron Variant
	rs7975232	Intron Variant

Figure 1. The schematic representation of diseases associated with VDR-SNPs. (A) some of the diseases associated with polymorphism in the Vitamin D Receptor. (B) table represents the specific mutations in the VDR gene linked to diseases.

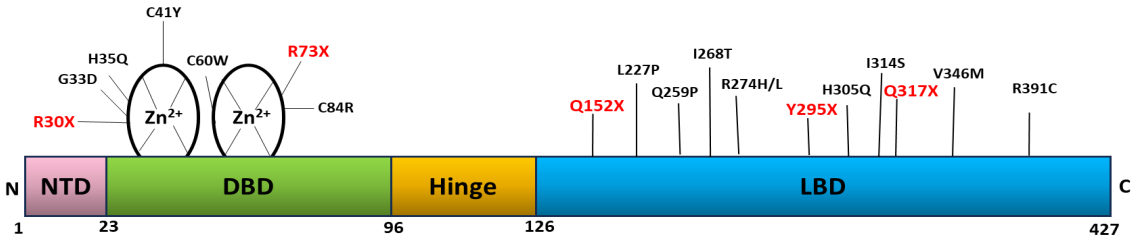


Figure 2. SNP variants of VDR associated with HVDRR disease. The figure illustrates the location of missense and nonsense SNP variants within the VDR gene. Missense SNP variants are indicated in black, while nonsense SNP variants are shown in red color. HVDRR: Hereditary Vitamin D-Resistant Rickets.

The results of this comprehensive analysis revealed that HVDRR-associated SNP variations influence the normal functioning of the receptor, which provides insight into the molecular basis of disease pathogenesis.

3. Disease Associated VDR-SNP Variants Exhibit Compromised Mitotic Chromatin Binding

As mentioned in the preceding sections, WT-VDR exhibits constitutive mitotic chromatin retention and promotes mitotic chromatin association of its heterodimeric partner RXR. Also, studies have revealed that the DBD region plays a crucial role in bookmarking by VDR[14]. However, the mechanistic intricacies remained elusive. Exploring inter- and intra-molecular interactions of VDR-SNPs will reveal the depths of hitherto subtle molecular mechanisms of genome bookmarking and its significance in disease prognosis. Both missense and nonsense SNP in VDR can exhibit aberrant receptor behavior, albeit the degree of severity may vary depending on the nature of amino acid substitution/ deletion and their location in receptor. There are SNPs in DBD that are essential for binding DNA response elements of target genes. In addition, there are SNPs in LBD which are essential for ligand binding and heterodimerization with RXR. We hypothesize that nonsense SNPs have a more severe impact than missense SNPs due to their ability to introduce premature stop codons, resulting in truncated protein and loss of critical functional domains. Subsequently, this disrupts protein folding, size, stability, and function, leading to a more pronounced impact on cellular processes. The SNP alteration also compromised VDR mitotic chromatin association, preventing the receptor from re-establishing lineage-specific gene and epigenetic modifications.

In our recent study, we observed that nonsense SNPs in VDR that truncate the receptor in middle of the DBD or LBD have compromised receptor chromatin association. SNP variants, i.e. R30X and R73X, truncate the receptor in the middle of the first and second zinc figures in DBD, respectively. These nonsense SNP variants were analysed for their bookmarking property, and it was found that both SNP variants remained excluded from mitotic chromatin. Even the presence of cognate ligand calcitriol, exhibited no influence on mitotic chromatin behaviour of R30X and R73X-VDR. Since these mutations cause premature truncations, LBD cannot be synthesized, and thus making the binding site inaccessible. In addition, these variants also failed to facilitate bookmarking in co-expressed RXR as LBD is requisite for VDR-RXR heterodimerization and DBD for chromatin association.

Other three VDR-SNP variants, Q152X, Y295X, and Q317X, truncate the receptor LBD at 152, 295, and 317 amino acid positions, respectively. Q152X variant demonstrates comparable mitotic chromatin association to the wild-type receptor, regardless of the presence or absence of its cognate ligand. However, it fails to promote mitotic chromatin interaction of heterodimeric partner RXR. According to reports, VDR interacts with RXR via dimerization domains spanning 317 to 396 amino acids in LBD[40]. Based on this fact, it can be deduced that Q152X is incompetent for promoting bookmarking of partner RXR, possessing only 151 amino acid length receptors. The other two variants, Y295X and Q317X, also failed to exhibit mitotic chromatin association alone, with cognate ligand and with co-expressed RXR. In most cases

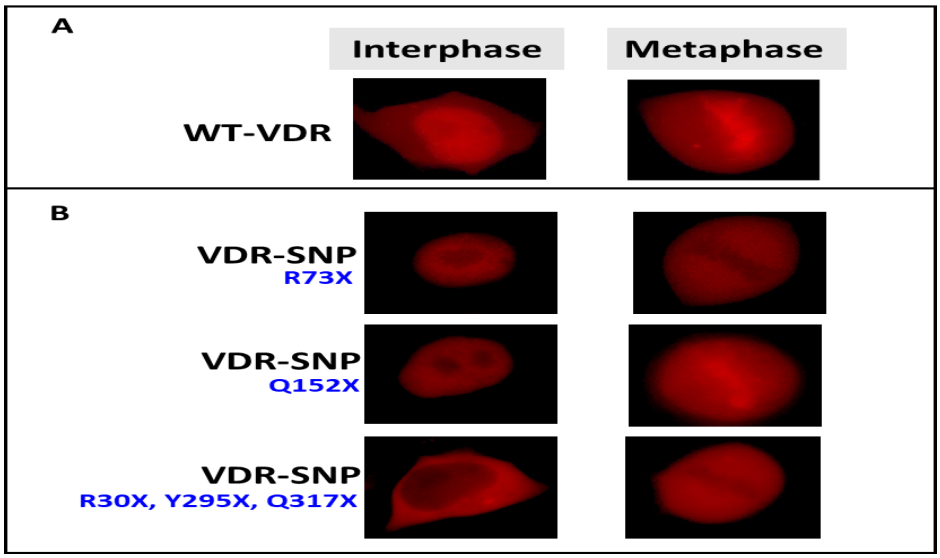


Figure 3. Representation of interphase and metaphase cells expressing WT-VDR and VDR-SNP variants. (A) WT-VDR exhibits partially cytoplasmic and predominantly nuclear subcellular localization during interphase and significant mitotic-chromatin association during metaphase. (B) VDR-SNP variants exhibit impaired subcellular localization (either nuclear or cytoplasmic shifted) and no mitotic chromatin binding (except Q152X).

Table 1. A comprehensive analysis of disease-associated VDR-SNP variant[21]

SNP/Wild-type-VDR	CDS mutation (Region)	Disease association	Transcriptional activity		Subcellular Localization				Mitotic Chromatin Binding			
			Alone	With RXR	Alone		With RXR		Alone		With RXR	
					V	L	V	L	V	L	V	L
WT-VDR	-	-	Basal activity	Increased activation	N>C	N	N>C	N	Yes	Yes	Yes	Yes
R30X	CGA-TGA (DBD)	HVDRR with Alopecia	Completely lost	No effect	C>N	C>N	C>N	C>N	No	No	No	No
R73X	CGA-TGA (DBD)	HVDRR with Alopecia	Completely lost	No effect	N	N	N	N	No	No	No	No
Q152X	CAG-TAG (LBD)	HVDRR with Alopecia	Reduced activity	No effect	N	N	N	N	Yes	Yes	No	No
Y295X	TAC-TAA (LBD)	HVDRR with Alopecia	Completely lost	No effect	C>N	C>N	C>N	C>N	No	No	No	No
Q317X	CAG-TAG (LBD)	HVDRR with Alopecia	Completely lost	No effect	C>N	C>N	C>N	C>N	No	No	No	No

(V: Vehicle, L: Ligand (calcitriol), DBD: DNA binding domain, LBD: Ligand binding domain, X represents termination codon.)

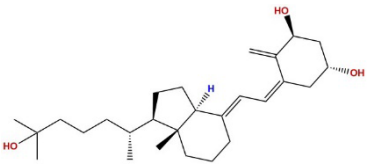
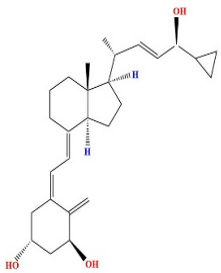
LBD truncation of VDR resulted in loss of transcription function and mitotic chromatin binding. This contrasts with AR, where deletion of LBD (ΔLBD-AR) exhibited constitutive activity and mitotic chromatin binding[41]. In summary, the findings of this study indicate that the '*genome bookmarking*' property of VDR is critically impaired by HVDRR-related nonsense SNPs (Figure 3)[21].

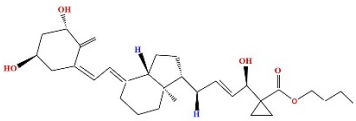
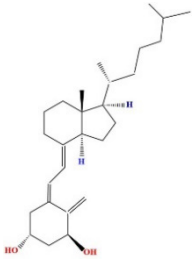
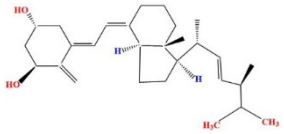
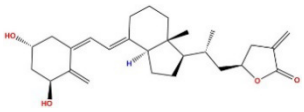
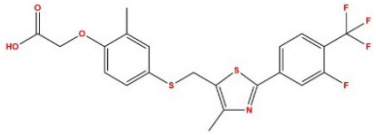
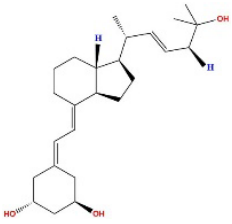
In addition to receptor mitotic chromatin association, the functional analysis was also performed to assess the transcriptional activity of VDR-SNP variants. Using promoter-reporter-based luciferase assay, we evaluated the impact of HVDRR-associated SNP variants for transcriptional activity. All VDR-SNP variants, except Q152X, showed a complete loss in activity and represented as a null type. Q152X demonstrated reduced transcriptional activity without treatment with cognate ligand[21]. However, no activity was observed with ligand and with co-transfected RXR (Table 1). Due to premature truncation, the altered conformational structure of the receptor was anticipated to be responsible for these functional losses.

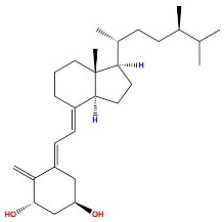
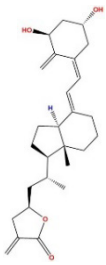
4. Prospect to Fine-Tune Receptor by Tweaker Ligands

Being ligand-modulated, members of the nuclear receptor superfamily can serve as druggable targets for the treatment of NR-mediated diseases. The 'receptor conformation' of VDR is central to the physiological functioning of this factor. It is conceivable that the receptor conformations can be fine-tuned between 'transcriptionally active' and 'transcriptionally inactive' states depending on the nature of the interacting ligands (agonist, antagonist, selective modulators, inverse agonist/antagonist). To support this fact, some studies have proposed vitamin D analogs as promising therapeutic candidates for the treatment of HVDRR[42-45]. For instance, in HVDRR, a vitamin D analog 20-epi-1,25(OH)2D3 is reported to stabilize the receptor variants, which in turn increases transcriptional activity[46]. Over the past few years, different vitamin D (calcitriol) analogs, based on their mode of actions, have been used in the treatments/management of diseases other than HVDRR (Table 2). Similarly, the concept and application

Table 2. Small molecule modulators of VDR and their potential role in disease management

Ligand/small molecule modulator	Structure	Mode of action	Related disease	Physiological outcome	Reference
Calcitriol		Agonist	Hypocalcaemia, osteoporosis	Disease improvement	47
Calcipotriol		Antagonist	Psoriasis	Disease improvement	48

ZK159222		Antagonist/ partial agonist	Anti-inflammatory property	Reduce macrophage-stimulated activation of NF-κB signalling	49
Alfacalcidol		Agonist	Arthritis, myelodysplastic syndrome	Disease improvement	50
Doxercalciferol		Agonist	Secondary hyper-parathyroidism	Suppress PTH level	51
TEI-9647		Antagonist	Paget's disease	Suppress osteoclasts and excessive bone resorption	52
GW0742		Antagonist	Psoriasis, benign prostate hyperplasia, autoimmune diseases, and osteoporosis	Inhibits the interaction between VDR	53
Paricalcitol		Agonist	2HPT	PTH suppression	54

Tacalcitol		Agonist	Psoriasis	Improvement in PASI score	55
TEI-9648		Antagonist	Cancer	Block HL-60 differentiation	56

of potential VDR small molecule modulators or 'tweaker ligands' can be designed using a similar approach to normalize HVDRR-specific receptor malfunctioning.

5. Conclusion

The present review sheds light on the impact of a single amino acid substitution in the structure of VDR, which can detrimentally influence the receptor's ability for '*genome bookmarking*.' Our findings highlight that SNPs residing both in the DNA-Binding Domain (DBD) and Ligand-Binding Domain (LBD) are critical for mitotic chromatin retention of VDR. Also, individuals harbouring these genetic alterations may impede the transmission of epigenetic information to their progeny cells. Understanding the interactions between receptors and chromatin, as well as the factors influencing genome bookmarking, holds the potential for pioneering therapeutic and diagnostic approaches. The outputs of this study could reveal molecular mechanisms of VDR and Vitamin D-associated malfunctioning. Furthermore, it is anticipated that polymorphism-specific small molecule modulators can be designed to tweak the receptor conformation toward normal functional output.

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