

RESEARCH PAPER

Investigation of the effect of pd1.1, pd1.5, and pd1.9 mutations on pd1 gene function with a system biology approach

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Highlights

- Programmed cell death 1 gene plays a key role in development of some disorders such as various cancers.
- The pd1.1 variation may change the gene expression of pd1 by altering the transcription factor binding sites of the promoter region.
- The pd1.5 gene mutation as a non-synonymous variation may alter the function and structure of the pd1.

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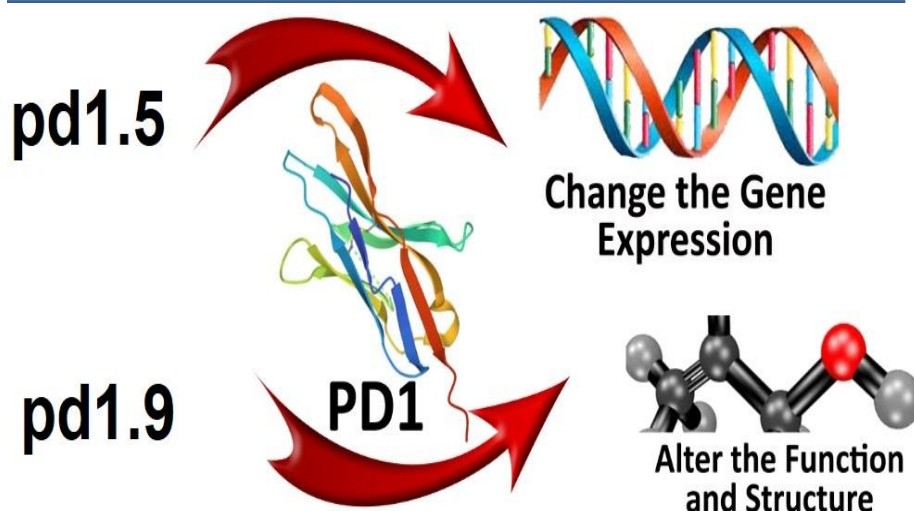
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Graphical Abstract



Programmed cell death 1 (pd1) is an immune-inhibitory receptor that is expressed in activated T cells. This gene may play a role in inhibition of actual anti-microbial and anti-tumor immunity. In human, this gene is located on the long arm of chromosome 2. This gene has many variations that three of which are more common. This study was aimed to investigate the influence of pd1.1, pd1.5, and pd1.9 variations on function of pd1 gene based on a bioinformatics approach. In this study, the EPD and PROMO webserver was used to evaluate the pd1.1 variation as a promoter mutation. It has been detected in the promoter region by EPD, while the transcription factor arrangement was evaluated by the PROMO server. But, some bioinformatics tools such as ProtScale – ExPASy and Ramachandran plot assay web servers were used to evaluate the effects of coding polymorphisms. Obtained data from EPD showed that the promoter of pd1 contains 60 nucleotides. Evaluation of upstream of pd1 revealed that the number of transcription factors could alter the pd1.1 variation. With regard to the pd1.5 polymorphisms, the result showed that it is considered as a synonymous variation, but the pd1.9 was known as a nonsynonymous mutation. Thus, the pd1.9 could alter the hydrophobicity and Ramachandran plots of PD1. The pd1.5 mutation may impact the expression of the pd1 gene because it changes the transcription factor arrangement on the upstream of pd1. Also, the pd1.9 substitution could alter the hydrophobicity and Ramachandran plots of protein.

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Introduction

To recognize a functional gene that stimulates programmed cell death, a research team (1) examined two cell lines: LyD9, a cell line that is a precursor to blood cells and develops apoptosis due to IL-3 deficiency. The second cell line was 2B4.11, a T-cell hybridoma and apoptotic by induction with ionomycin and phorbol myristate acetate. Since apoptosis required protein and RNA synthesis in both cells, reduction hybridization was used to detect genes essential for apoptosis (2). A cDNA library was prepared by resting cell LyD9 mRNA, and then pd1 cDNA was detected by specific probes (1). The amino acid sequence inferred from the pd1 gene showed that this protein is a type I transmembrane with a domain of IgV in the extracellular section. However, subsequent studies did not confirm the direct involvement of the pd1 gene in apoptosis (3). After that, the role of pd1 remained unknown (4). Subsequent studies have shown that mice with a defect in the pd1 gene (Pdccl1^{-/-}) develop lupus autoimmune disease (5, 6).

In 1998, Nishimura et al. (5) reported a Pdccl1^{-/-} mouse model in which the exons encoding the PD1 transmembrane region were replaced by the neomycin resistance gene (3). However, previous analyzes have shown that pd1 is expressed in active B and T cells. However, Pdccl1^{-/-} mice did not show any severe immune phenotype. Subsequent complex analyzes showed that pd1 modulated the immune response in a negative direction. However, the exact mechanism for regulating pd1 remained unknown due to the lack of its ligands. In vivo studies in mice with defective pd1 or pd-l1 genes have shown that pd1/pd-l1 interaction is a negative regulator of the immune response. The pd-l1 expression was demonstrated in human ovarian tumor cells. Experiments show that pd-l1 stimulates T cells to produce IL-10 (7). In contrast, another experiment showed that PD-L reduces PD-L1 T cell proliferation, IL-10, and IFN- γ (8). The pd-l1 is found in a variety of cancer cell lines—treatment of cancer cell lines with IFN- γ increases pd-l1 expression (9). Immunohistochemical analysis showed that pd-l1 is found in most human cancers (9). High expression of pd-l1 in most cancer tissues showed that pd-l1/pd-1 interaction could invade cancer cells (10). In vivo studies have shown that blocking pd-l1 can increase immune cell memory. The pd-l1-expressing myeloma does not grow in mice with a pd1 gene defect but grows in healthy mice (10, 11).

The pd1 gene is located on the long arm of chromosome 2 (2q37.3). This gene has five exons. Single nucleotide polymorphisms (SNPs) of this gene are very high, three used in this project. This study aimed to investigate the effect of pd1.1 (rs36084323), pd1.5 (rs2227981), and pd1.9 (rs2227982) polymorphisms on the function of the pd1 gene with a bioinformatics approach.

Materials and Methods

Selection of genetic variations

Based on previous literature and considering the importance of single nucleotide polymorphisms of the pd1 gene in the incidence and progression of various cancers, these variants were included in our study (rs36084323, rs2227981, and rs2227982). The position of each of them on the genome was determined. For this purpose, the polymorphism location on different regions of the gene was obtained from the National Center for Biotechnology Information database, and here it was determined whether these polymorphisms are exon, intron, downstream, or upstream variations. It was then determined which adenine nucleotides, guanine, cytosine, and thymine were located at each polymorphic site.

Pd1.1 genetic variation

This polymorphism was identified as an upstream transcript variant. Thus this gene can affect gene expression as a promoter variety. Therefore, the servers used were online websites for evaluating gene expression. For example, the EPD server (<https://epd.epfl.ch//index.php>) was used to determine the exact position and sequence of the pd1 gene promoter. Then, the exact position of pd1.1 polymorphism about the promoter was obtained. The list of transcription factors that bind to the polymorphic region before and after the mutation was then determined by PROMO software.

Evaluation of *pd1.5* and *pd1.9* genes mutations as a coding variation

The variation *pd1.5* (rs2227981) and *pd1.9* (rs2227982) were determined as synonymous and nonsynonymous coding sequence variation. The *pd1.5* mutation could be used to analyze the protein structure, but since it is a silent mutation, it cannot mainly affect the enzyme's function. So authors of this study, cannot do more assessment on this mutation. However, the *pd1.9* SNP is a missense variant that could affect the protein function and structure. For this purpose, the exact location of this SNP was determined on the protein sequence of *pd1*. Then the ExPasy webserver was used to determine the effects of rs2227982 on the structure of the protein. Some protein properties such as hydrophobicity index and Ramachandran plots of protein were assessed before and after mutation by online web servers.

Results

Effects of *pd1.1* gene variation on the promoter region of *pd1*

The promoter region of the *pd1* gene was deduced from the EPD database. This promoter consisted of 60 nucleotides. The exact sequence of the *pd1* promoter was as follows: gcgggcaccctccctcaacctgacctgggacagttccctccgctcaCCTCCGCCTGA. The detailed characteristics of this promoter are summarized in Figure 1. The obtained data from the PROMO web server revealed that the polymorphic region's transcription factor pattern is different for C and T phenotypes. As shown in Figure 2, the transcription factor for the C genotype is 22 whereas this ratio for the T genotype is 25 TFs. Therefore, this could influence gene transcription.

General information	
Promoter ID:	PDCD1_1
Promoter type:	multiple
Organism:	Homo sapiens (Human)
Gene Symbol:	PDCD1
Description of the gene:	programmed cell death 1
Sequence:	gcgggcaccctccctcaacctgacctgggacagttccctccgctcaCCTCCGCCTGA
Position in the genome:	Chromosome [NC_000002.12]; Strand [-]; Position [241858889]
RefSeq:	NM_005018
NCBI Gene:	PDCD1
GeneCards:	PDCD1

Figure 1. Some properties of the *pd1* gene. This gene also is known as PDCD1, and its full name is programmed cell death. The promoter sequence of this gene is also illustrated in the figure.

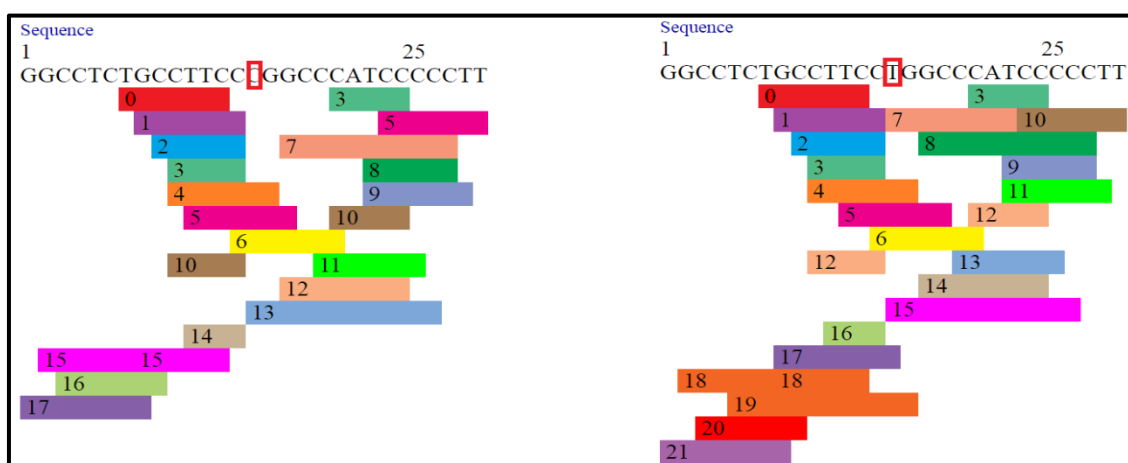


Figure 2. PROMO results, the transcription factor for the C genotype is 22, whereas this ratio for the C genotype is 25; The colored boxes show a different transcription factor.

Assessment of two *pd1.5* and *pd1.9* genes variation

Both *pd1.5* and *pd1.9* polymorphisms are in the coding sequence of *pd1*. The *pd1.5* variation could be utilized to examine the protein structure, but since it is a synonymous mutation, it cannot generally influence the enzyme function. So authors of this study could not perform more evaluation on this variation. However, the *pd1.9* variation is located on the coding sequence of *pd1* as a nonsynonymous mutation. The entire protein sequence of *pd1* was deduced from NCBI. This variation results in alanine to valine substitution at codon 214. The data from ProtScale – ExPASy revealed that the hydrophobicity changed due to alanine to valine mutation. This change can be due to the change like the amino acids because the two amino acids, alanine, and valine belong to two different families.

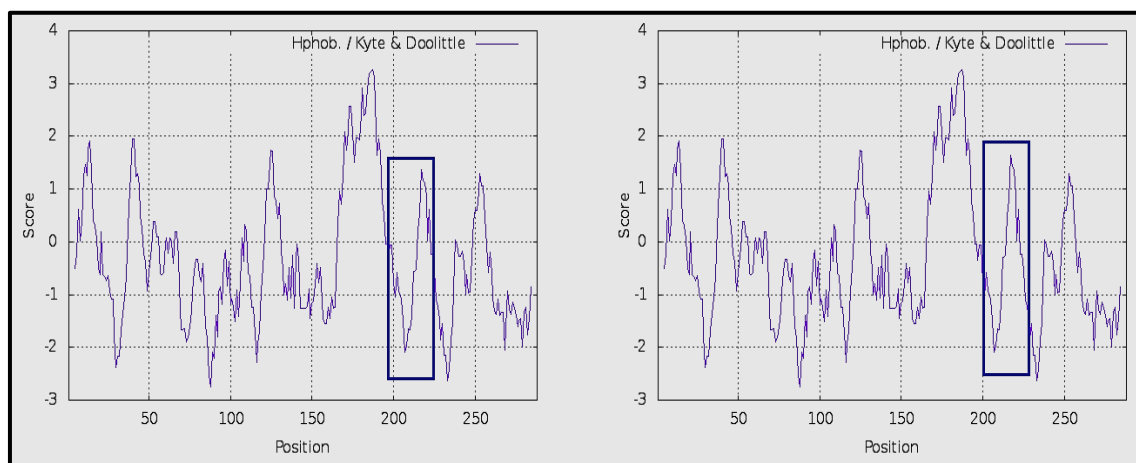


Figure 3. Hydrophobicity plot. This chart shows that the Ala214Val mutation may alter the hydrophobicity around the mentioned variation (It has been shown in blue boxes).

For drawing the Ramachandran plot, at first, the protein sequence of *pd1* was deduced from NCBI, and then the location of mutation was determined on this sequence. Two sequences containing the mentioned SNPs were employed in the Phyre2 server. Therefore, the PDB structure was prepared for these two isoforms. Then these structures were analyzed by <https://swift.cmbi.umcn.nl/servers/html/ramaplot.html> webserver. The obtained data from the current study, showed that this polymorphism could change the Ramachandran plot (data was not shown).

Discussion

This study evaluated the effects of *pd1.1*, *pd1.5*, and *pd1.9* mutations on *pd1* gene function. The results showed that the *pd1.1*, an upstream variation, may influence the expression of *pd1* by changing the transcription binding sites for various TFs. However, two *pd1.5* and *pd1.9* mutations are located on the coding sequence of *pd1* and may impact the protein structure and function. It is found found that *pd1.5* variation is a synonymous mutation that could not mainly influence the structure of *pd1*. However, the *pd1.9* is a missense mutation that influences the hydrophobicity and Ramachandran plots of *pd1*. Therefore, two *pd1.1* and *pd1.9* mutations could be functional SNPs.

PD1 protein is a membrane protein containing 268 amino acids and is a member of the CD28/CTLA-4 family (T-cell regulators). It has an extracellular IgV domain with a transmembrane region that is accompanied by an intracellular tail. The intracellular tail has two tyrosine phosphorylation sites in a YXXL/I motif, so PD1 is considered a negative regulator of TCR. Its extracellular domain lacks the MYPPPY motif (an essential sequence for the binding of CTLA and CD28 bound to B7-1 and B7-2) (3, 12, 13). Recent data suggest that C-terminal tyrosine is located in an ITSM motif. The interaction of PD1 and BCR results in rapid phosphorylation of the Src homologous domain comprising tyrosine phosphatase 2 (SHP-2), which leads to dephosphorylation of key BCR

signals, including Iga/b and Syk. This activates PI3 K, PLC γ 2, and ERK and, as a result, increases intracellular calcium (14, 15).

The PD1 receptor was found in thymocytes and the transition phase from DN (CD4⁻ CD8⁻) to DP (CD4⁺ CD8⁺) (16, 17) and mature B cells and T cells (9, 18). The pd1 is also expressed in macrophages (17). Studies in mice with a defect in the pd1 gene have shown that the molecule acts as a reverse manager of immune responses. The mice's lack of pd1 leads to arthritis, glomerulonephritis, and cardiomyopathy (5, 6). Two types of ligands have been identified for pd1: pd-l1 (B7-H1) and pd-l2 (B7-DC) (8, 19). PD1 with PD-L1 or PD-L2 negatively regulates cytokine production and T cell proliferation (20, 21).

The pd-l1 and pd-l2 mRNA expression is found in a wide variety of human and mouse tissues such as the heart, placenta, pancreas, lymph nodes, spleen, and thymus (22). However, it is not expressed in brain and kidney tissues. The level of expression in some tissues is so limited that it indicates the possibility of post-transcriptional adjustment.

The expression levels of pd-l1 and pd-l2 in antigen-presenting cells (APCs) have been measured in various studies. Resting B cells and monocytes do not express pd-l1 and pd-l2 (23, 24), while pd-l2 is expressed only in inflammatory macrophages (25). Dendritic cells (DCs) express pd-l1 and pd-l2 only when stimulated (23, 26). Published data on the expression of pd-l1 and T cells, in addition to the Pd-1 receptor, could express the related ligands, especially following TCR stimulation, even in the absence of CD28 stimulation. The pd-l2 is not expressed in neutral T cells, and the current outcomes on the possibility of increasing their expression if the cell is stimulated are controversial (1, 9, 20, 27, 28). Expression of pd-l1 and pd-l2 on activated T cells reinforces the hypothesis that T:T interaction via pd-1/pd-l1 may limit TCR signaling afterward triggering. However, there is little evidence of this. The effects of genetic modification on human phenotypes, abnormalities, and diseases have been reported in many reports (29, 30).

Conclusion

In this study, the effects of three common mutations of pd1 have wisely been assessed to describe further its role in the risk of some disorders, especially in cancers. Based on obtained findings, the pd1.5 mutation as an upstream mutation could influence the expression properties of the pd1 gene. Because the mentioned polymorphism alters the transcription factors plan on the promoter region of pd1. Also, the pd1.9 mutation is an exonic substitution and could influence protein function and structure because the mentioned variation could change the hydrophobicity and Ramachandran plots of protein. Therefore, the mentioned mutations could be considered as genetic biomarkers for disease susceptibility. However, further experimental studies such as in vitro examinations are required to obtain more accurate outcomes.

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