Review

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The many roles of the Alzheimer-associated gene *PM20D1*

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Abstract

PM20D1 is a little studied enzyme until recently, belonging to the mammalian M20 peptidase family, which catalyzes both the synthesis and hydrolysis of N-acyl amino acids (NAAs). NAAs are bioactive lipids biosynthesized from free fatty acids and free amino acids. These molecules have been associated with many biological functions; however, most of the biochemical mechanisms have not yet been described. The best-known biochemical mechanism is the one involved in thermogenesis, which also has implications for reactive oxygen species levels and cell preservation. In the last few years, genetic variation in *PM20D1*, as well as changes in its methylation and expression levels, have been reported to be associated with several disease phenotypes, including Alzheimer's disease. In this review, we explore the current knowledge regarding the *PM20D1* gene, including aspects such as its biology, potential functions, regulation of its expression, and role in different phenotypes such as Alzheimer's disease, obesity, Parkinson's disease, and several other disorders.

Keywords: N-acyl amino acid, neuropsychiatric disorders, M20 peptidase, mQTL, eQTL

INTRODUCTION

In the last decades, a large amount of evidence regarding genetic risk variants for most common disorders has been generated mostly through genome-wide association studies (GWAS)[1]. However, less attention has been focused on the changes in methylation and expression associated with different phenotypes. This has changed in recent years with the increased use of whole-genome methylation and RNA sequencing techniques[2,3] (in addition to the long-existing expression microarray analyses). Some of the challenges faced by studies dealing with methylation and expression include their usually dramatically smaller sample sizes compared to GWAS, and that they often focus on either rather than both methylation and expression at the same time.

Such methylation studies have cast a spotlight on an until then little-studied gene, *PM20D1*, which encodes an enzyme belonging to the M20 peptidase family[4]. There is growing evidence that differential methylation in this gene is associated with several heterogeneous disease phenotypes [Table 1]. Additionally, it was discovered that genetic variants close to but not inside the gene can influence methylation levels at the gene's promoter[5-8], and this methylation correlates with expression[6]. It has been proposed that these variants had not been previously found in GWAS because the *PM20D1* region is not well represented and is in low linkage disequilibrium with the SNPs included in the common microarrays used in GWAS[6].

In this review, we explore the current knowledge regarding the *PM20D1* gene. First, we focus on what is known about its biology, potential functions, and regulation of its expression. We then review the evidence supporting the involvement of *PM20D1* in different phenotypes such as Alzheimer's disease (AD), obesity, Parkinson's disease (PD), and several other disorders.

BIOLOGY

PM20D1 belongs to the mammalian M20 peptidase family[4]. The main function of this secreted enzyme is the synthesis and hydrolysis of N-acyl amino acids (NAAs)[9,10] by catalyzing the biosynthesis of NAAs from free fatty acids and free amino acids, as well as the reverse hydrolysis reaction[9]. This gene is expressed in several tissues such as the liver, bladder, brain, large intestine, pancreas, kidney, and heart of mice[11]. In humans, it shows a notably high expression in pancreas and skin, but is also expressed in many other tissues[12]. In the case of the brain, expression occurs across all brain regions[12,13] and cell types[14,15]. This peptidase circulates through the bloodstream in tight association with low- and high-density lipoproteins. These lipoproteins work as coactivators in PM20D1 activity and as biosynthesis sites of the NAAs[16].

NAAs are bioactive lipids composed of a fatty acyl chain linked to an amino acid by an amide bond[17]. NAAs circulate through the bloodstream, with albumin as a physiologic N-acyl amino acid carrier which confers resistance to hydrolytic degradation by spatially segregating N-acyl amino acids away from their site of biosynthesis. Albumin also helps to maintain equilibrium by acting as a buffer between bound inactive and free active NAAs[16]. Many NAAs identified in mammals have putative roles associated with different physiological processes. Some of the biological activities associated with NAAs are vasodilation[18], neuroprotection[19], and pain sensation[20,21]. However, many of the biochemical mechanisms that explain the role of the NAAs are still unknown[22].

Some NAAs have been described as thermogenic, for example, N-acyl-phenylalanines and N-acyl-leucines, which regulate energy metabolism[9,10,23]. The levels of these NAAs are physiologically increased after cold exposure and cause an uncoupling of mitochondrial respiration in different peripheral tissues by directly interacting with mitochondrial proteins[10,24,25]. Mitochondrial uncoupling occurs when ATP is not produced through electron transport[26], but redox energy is released in the form of heat, since protons are lost

Table 1. Phenotypes with reported differential methylation between cases and controls in human tissues and variants associated with *PM20D1* promoter methylation

Phenotype	Methylation status in cases	Tissue	mQTL variants*	References
Alzheimer	Hypermethylation (in advanced disease)	Brain prefrontal cortex[6,7], immortalized B cells[6], peripheral blood[7,8]	rs1172198[5] rs708727[5-8] rs823082[5]	[6-8]
	Hypomethylation/overexpression (close to diagnosis and in early disease)	Postmortem brain[5], brain prefrontal cortex[6,7], immortalized B cells[6], peripheral blood[7,45]	rs823088[5] rs1361754[5] rs960603[5,6]	[5-7,45]
Obesity (BMI)	Hypomethylation	Adipose tissue[36], peripheral blood[52]	rs823080[36]	[36,52]
	Hypermethylation	Peripheral blood	-	[47]
Parkinson	Hypermethylation in one study, inconclusive evidence in the other	Peripheral blood	rs823114[83]	[82,83]
Asthma	Hypomethylation	Peripheral blood	-	[86]
Respiratory allergy	Hypermethylation	Peripheral blood and saliva	-	[87]
Food allergy	Hypomethylation	Peripheral blood	-	[88]
Psoriasis	Hypermethylation	Peripheral blood	-	[89]
Multiple sclerosis	Direction of change not stated	Peripheral blood	-	[90]
Child abuse	Hypermethylation	Peripheral blood	-	[91]
Familial hypercholesterolemia	Direction of change not stated	Peripheral blood	-	[94]
Stroke	Hypermethylation	Peripheral blood	-	[96]
Covid severity	Direction of change not stated	Peripheral blood	-	[98]
Lung cancer	Direction of change not stated	Lung, bronchus	-	[99]
Hepatocellular carcinoma	Hypermethylation	Liver	-	[100]
Acute myeloid leukemia	Hypermethylation	Bone marrow mesenchymal stem cells	-	[101]
Chronic postsurgical pain	Hypermethylation	Peripheral blood	rs4951261 rs960603 rs708723 rs823114 rs11240547 rs2793374	[102]

*Only variants significantly associated with *PM20D1* promoter methylation in studies that reported differential methylation of the promoter between cases and controls are listed.

through the inner mitochondrial membrane[27]. This uncoupling activity is mostly limited to NAAs with neutral amino acid head groups and desaturated fatty acyl chains of medium length[23]. In mice, NAAs produce more energy expenditure and improve glucose homeostasis[9].

This thermogenic mitochondrial respiration uncoupling mechanism, activated by PM20D1 through direct binding of NAAs to mitochondria, is an independent alternative to uncoupling protein 1 (UCP1)[9,25]. UCP1 can be found in brown and beige adipocytes and can dissipate energy in the form of heat[28]. In the alternative mechanism, *PM20D1* is expressed mainly from adipocytes which express *UCP1*, resulting in the generation of NAAs. These NAAs then promote respiration uncoupling both in the *UCP1/PM20D1*-expressing adipocytes and neighboring adipocytes lacking UCP1, which confirms that the two mechanisms are independent[9]. Furthermore, in vitro evidence exists that NAAs can induce uncoupled respiration in unrelated cell types that completely lack UCP1. Therefore, through this PM20D1-dependent thermogenic mechanism, respiration uncoupling (resulting in glucose degradation without production of ATP) can occur in cells that are not specialized in dissipating chemical energy as heat[9].

Some benefits associated with mitochondrial uncoupling are the reduction of the proton motive force (Δp), which causes a local decrease in oxygen concentration and a reduction of reactive oxygen species (ROS) products[29-31]. Δp plays a very important role in the entry of certain proteins and calcium into the mitochondria[29]. In addition, the activation of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (mPTP) involved in the initiation of apoptosis is reduced[29,32]. Additionally, mitochondrial uncoupling promotes neuronal survival since these cells are highly oxidative and generate high levels of ROS[33]. Therefore, *PM20D1* may be involved in a method of mitochondrial uncoupling (through NAAs) that modulates ROS levels and improves neuronal survival, potentially playing a role in neurodegenerative diseases[5].

GENE EXPRESSION

Multiple recent studies have shown that *PM20D1* expression is, at least in part, genetically determined. Six SNPs (rs1172198, rs708727, rs823082, rs823088, rs1361754, and rs960603), which are located downstream of the gene, have been shown to be associated with both *PM20D1* methylation and expression levels and are thus considered methylation QTLs (mQTLs) as well as expression QTLs (eQTLs)[5-8,34]. These SNPs constitute a haplotype which plays a role in the methylation of the *PM20D1* promoter and its expression. As would be expected, the haplotype associated with higher methylation of the promoter is also associated with reduced expression of the gene. Sanchez-Mut *et al.* (2018) proposed that the genomic region where this haplotype is located acts as a regulatory region and induces repression through interaction with *PM20D1*'s promoter via a CTCF-mediated chromatin loop[6].

There are other genes on chromosome 1q in partial linkage disequilibrium with *PM20D1*. A study found a correlation between the genotype at these SNPs and expression in different tissues for other genes in the region besides *PM20D1*, specifically *NUCKS1*, *RAB7L1*, and *SLC41A1*[35]. Given that an association between the genotype at these SNPs (individually or in combination) and different disorders has been reported[5-8,36,37], the possibility of genotype-dependent expression for all four genes would raise the question of which gene is involved in a particular phenotype. To explore this, Sanchez-Mut *et al.* used a well-characterized sample of human brains to explore the DNA methylation levels in these four genes and found a strong correlation between genetic background (SNP genotypes) and CpG methylation only for *PM20D1*, as well as a slight correlation for *SLC41A1* for only two of the SNPs[5]. Then, they looked at expression levels and found a significant correlation between genetic background and expression levels only for the *PM20D1* gene, which they also observed in mice. They found a non-significant correlation trend between genotype and expression for *SLC41A1* in the human samples, in the same direction as in *PM20D1*. Therefore, in the brain, the genotype at these SNPs seems to strongly influence methylation and expression levels of *PM20D1* and possibly a lower degree for *SLC41A1*, but no effect on *NUCKS1* and *RAB7L1*.

However, *SLC41A1* and *PM20D1* are differentially regulated by AD-related stressors, with only *PM20D1* being upregulated by both amyloid-β and reactive oxygen species and only *PM20D1* being neuroprotective when overexpressed in cell and primary cultures[5]. Therefore, at least in the case of AD, the evidence suggests that, from this region, *PM20D1* is the main gene whose expression level is related to the phenotype.

From these and other studies, SNP rs708727 has emerged as the most significantly associated with *PM20D1* methylation and expression levels in the brain and other tissues. For the other SNPs that constitute a haplotype with rs708727, the level of association is less significant and varies between studies[5-8,35,38-41]. SNP rs708727 is a coding variant in the *SLC41A1* gene (not *PM20D1*) that results in the synonymous substitution p.Asn252Asn (NM_173854). The hypermethylation-associated allele is the A allele, with frequencies that vary between 0.3% in East Asians to over 44% in Finnish Europeans, according to gnomAD[42]. In the presence of the A allele, *PM20D1*'s promoter is hypermethylated, with the result that there is no

transcription. Methylation levels are much lower when the G allele is present, and transcription can occur[5-8] [Figure 1]. Therefore, the effect of outside factors on expression levels in the gene (e.g., oxidative damage-induced hypomethylation) can be seen mostly for chromosomes with the G allele[7].

This level of regulation of *PM20D1* expression has been described as an on-off switch that acts in all human tissues. Additionally, specifically in adipocytes, a variant near the gene is involved in expression regulation by the peroxisome proliferator-activated receptor γ (PPARγ) transcription factor[36] (see the section on obesity).
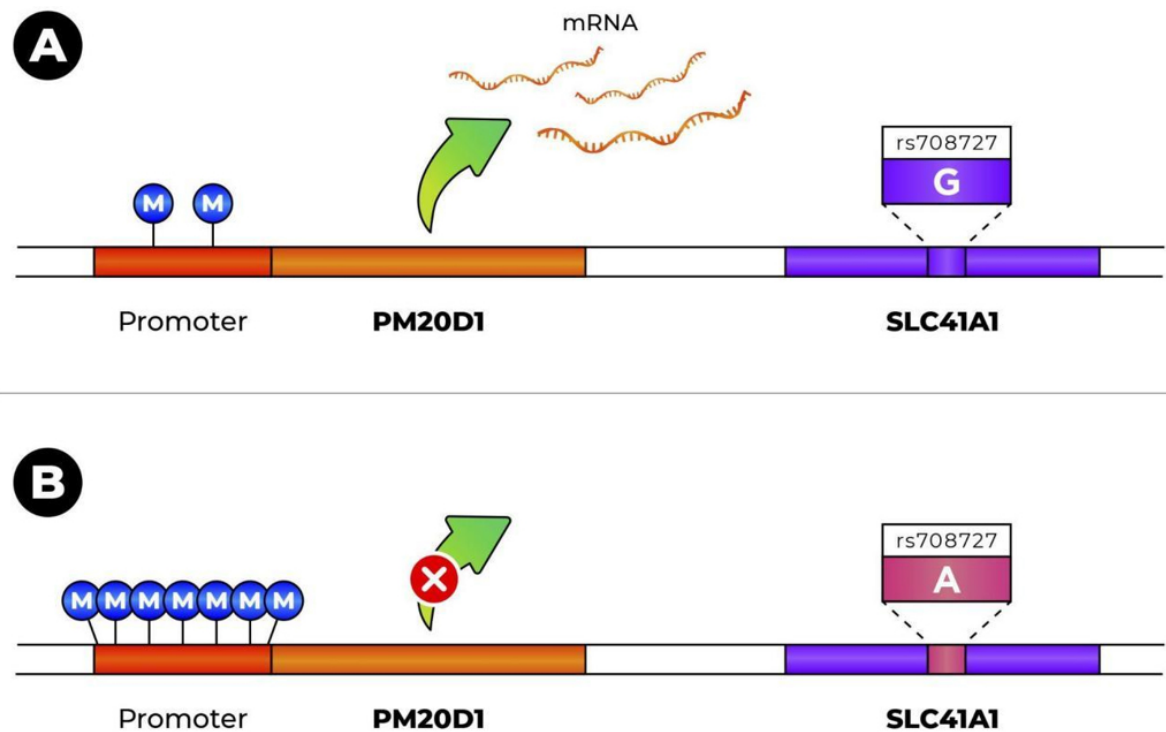
The role of rs708727 as an mQTL appears to be consistent across populations of different ancestries. Differential methylation in *PM20D1*'s promoter that has been reported among Caucasian American, African- American, and Han Chinese American individuals can be explained by differences in allele frequencies at the rs708727 locus[42,43]. Additionally, the association between rs708727 genotype and *PM20D1* methylation has also been reported in a sample of Costa Rican women[8].

ALZHEIMER'S

In 2018, in a very thorough study, Sánchez-Mut *et al.* reported for the first time an association of methylation status of the *PM20D1* promoter with Alzheimer's disease[6]. They found the promoter to be consistently hypermethylated in individuals with advanced-stage AD. The gene's promoter had been previously shown to be differentially methylated between human populations of different ethnic origins[43]. The authors proceeded to identify several SNPs that correlated in an allele-dose-dependent manner with *PM20D1* methylation; rs708727 has been found in later studies to show the most significant association and acts as an mQTL[7,8] (as described in the previous section). Moreover, they found that *PM20D1* expression was inversely correlated with the methylation of its promoter. Variant rs708727 and several SNPs in linkage disequilibrium with it have been previously described as eQTLs for *PM20D1*[34]. Additionally, using peripheral blood, another recent study detected an association of a differentially methylated region in *PM20D1* with the rate of cognitive decline in AD, as well as with the transition from cognitively healthy to the presence of cognitive impairment[44].

There is also functional evidence linking *PM20D1* to AD. In cell culture, *PM20D1* expression increased after treatment with neurotoxic insults related to AD, such as reactive oxygen species (ROS) and amyloid-β[5,6]. Additionally, in a mouse model with AD-related pathologies (APP/PS1), *PM20D1* expression was higher in the frontal cortex at symptomatic stages than in pre-symptomatic stages and control mice[6]. Finally, in vitro overexpression of *PM20D1* has been shown to decrease ROS-induced cell death and levels of amyloid-β in vitro, as well as reduce the amount of amyloid plaque and improve cognitive performance in mice[5,6]. These results suggest a neuroprotective role of *PM20D1,* but at first glance seem to be in contradiction with the hypermethylation (and presumed reduced expression) reported in individuals with advanced AD[6,8].

In the meantime, a mechanistic model has been proposed for the role of *PM20D1* in AD[5-7], which explains the apparent contradiction. In individuals with hypermethylated *PM20D1* (induced in part by the A allele rs708727), there is no transcription and therefore no *PM20D1*-mediated-protection against damage. On the other hand, in individuals without the methylation-inducing allele in rs708727 (i.e., individuals with the GG genotype), expression is increased in the presence of AD-related stress in order to reduce ROS-induced cell death, reduce Aβ levels, and prevent cognitive damage. Studies with longitudinal data have shown that hypomethylation occurs before the symptomatic onset of the disease, and therefore pre-diagnosis[7,45], potentially to increase gene expression and generate protection from damage. Then, a gradual increase in methylation is seen during disease progression in individuals with AD, leading to a decrease in gene

Figure 1. Effect of the rs708727 genotype on promoter methylation and expression level of *PM20D1*: (A) presence of G allele induces *PM20D1* promoter hypomethylation and allows transcription; and (B) presence of A allele induces *PM20D1* promoter hypermethylation and prevents its transcription.
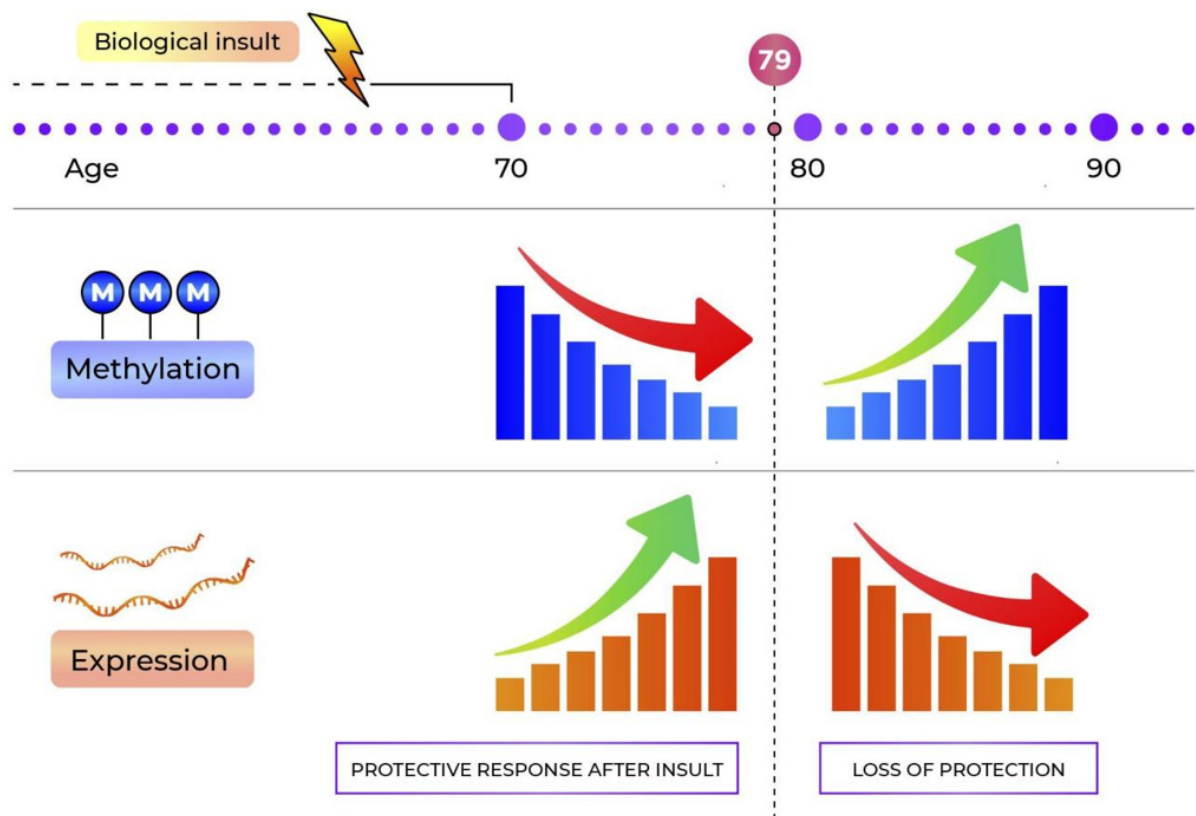
expression [Figure 2]. This explains the previously reported hypermethylation in advanced AD[6,8]. In one study, thanks to the prospective follow-up of individuals who converted from not affected to presenting AD, the authors identified the turning point in methylation level at 78-79 years of age[7]. They also found a higher risk for hypermethylation of the *PM20D1* promoter for females compared to male individuals.

The role of methylation patterns in *PM20D1*, both in the absence of AD symptoms and throughout disease progression, merits further investigation. The triggers involved in the switch from hypo- to hypermethylation have not been elucidated. For example, it has been suggested that changes in the expression levels of epigenetic regulator *MeCP2* could play a role in *PM20D1* repression in AD[46]. Importantly, there is evidence of a strong correlation between blood and brain methylation levels in the gene[7,8], which will greatly facilitate the study of gene expression in larger populations.

OBESITY

PM20D1 has been pointed out as a strong candidate to combat obesity by inducing UCP1-independent adaptive thermogenesis. It was previously postulated that, similar to other metalloproteinases, it could be associated with obesity in rodent models[47]. Several lines of evidence provide support in favor of this hypothesis. By increasing the levels of the enzyme PM20D1 in the blood of mice, their respiration as well as the concentration of NAAs are increased. In addition, through a direct supply of NAAs, an improvement in glucose homeostasis and an increase in mitochondrial energy expenditure are observed[9]. Additionally, it has been reported that obese mice treated with PM20D1 on a high-fat diet had significantly less body weight gain (9%-10%) after 40 days. The weight difference was exclusively due to a 30% reduction in lean mass, and an increase in O_2 and CO_2 volumes was also observed[48]. In light of these results, PM20D1 or its products

Figure 2. Model of the fluctuation in *PM20D1* methylation and expression in the years leading up to a diagnosis of Alzheimer's disorder.

have been proposed as potential therapeutic agents against obesity[49].

Mouse *PM20D1* shares 71% sequence identity and 86% similarity with that of humans[9]. In 2019, two peroxisome proliferator-activated receptor γ (PPARγ) binding sites were identified near the *PM20D1* gene transcription start site in human adipocytes but not in mice. PPARγ activation of *PM20D1* in adipocytes differs between individuals due to a single genetic difference at rs6667995, where the alternative allele (C) disrupts a PPARγ binding motif[36].

Studies in humans have generated results that seem to be in contrast to what has been observed in mice. A recent human study found a significant increase in the serum concentration of PM20D1 and two different NAAs (C18:1-Leu and C18:1-Phe) in individuals presenting overweight or obesity. Serum concentration was positively correlated with body weight, BMI, waist circumference, and waist-hip ratio, as well as parameters related to glucose dysregulation and insulin resistance. Adjusting for age and BMI, the data suggest an association with the development of insulin resistance and glucose dysregulation. A significant increase in serum PM20D1 concentration was also observed with the presence of an increasing number of metabolic syndrome components[50]. Several different explanations have been proposed for the increased PM20D1 and NAA levels in obesity: (1) it is a result of an increase in adipose tissue, because adipocytes are one of the major expression sites for *PM20D1*; (2) it is a defense mechanism to prevent progression of obesity through UCP1-independent thermogenesis (which would be in line with the overexpression in early cognitive impairment seen for AD); and (3) it reflects PM20D1 or NAA resistance in the body[50].

Several SNPs in the haplotype associated with *PM20D1* expression levels show significant genome-wide association with BMI[36,51], while weaker associations with type 2 diabetes and HDL cholesterol levels have been reported as well[36]. The direction of these associations is consistent with expectations: variants associated with lower BMI are also associated with lower diabetes risk and higher HDL cholesterol. For the SNPs in the haplotype, the strength of association with *PM20D1* expression correlates very strongly with the strength of association with BMI/obesity. However, and again, in contrast to what would be expected from results in mice studies, the haplotype associated with absent *PM20D1* expression is the one associated with lower BMI[36]. Consistent with this result, hypomethylation (and presumably increased expression) of *PM20D1* has been reported in a group of women with obesity compared to controls[52]. There is also one report of hypermethylation in individuals with obesity[47].

The evidence from GWAS suggests that levels of *PM20D1* expression (which are at least in part genetically determined) are associated with BMI and the risk of obesity. However, independent studies have found that NAA levels do not change significantly in knockout mice for *PM20D1* or humans homozygous for the haplotype associated with silenced *PM20D1* expression[10,36]. This suggests that other enzymes generate and regulate levels of NAAs, and that *PM20D1*'s effect on the body weight phenotype could involve a mechanism different from NAA regulation.

PARKINSON'S

The genes *PM20D1*, *SLC41A1*, *RAB29* (also called *RAB7L1*), *NUCKS1,* and *SLC5A3* are part of the PARK16 locus[53]. Several investigations have found an association between SNPs of the PARK16 locus and idiopathic Parkinson's disease (PD)[37,53-58]. Nevertheless, results have differed across studies and populations, with important variations in the SNPs significantly associated with PD and their allelic frequencies in the groups of cases and controls. The underlying mechanism and the PARK16 genes involved in the onset of PD are unclear, but putative mechanisms have been suggested for *SLC41A1*, *NUCKS,* and *RAB29*[59-62], including epistasis and allelic heterogeneity models[63,64].

Despite the lack of known mechanisms involving *PM20D1* in the pathogenesis of PD, it is well known that mitochondrial dysfunction and oxidative stress are present in many PD patients[65,66]. Mitochondrial dysfunction in PD patients is evidenced by mitochondrial complex I (MCI) deficiency in substantia nigra[67,68]; this region contains the dopaminergic neurons that are lost in PD. González-Rodríguez *et al.* (2021) recently showed that MCI dysfunction is enough to cause progressive, human-like parkinsonism in mice[69]. The partial inhibition of MCI by drugs increases ROS production and promotes cellular oxidative stress[70,71]. These processes participate in the damage of the dopaminergic neurons in PD[72]. In addition, the dopaminergic neurons may be under increased oxidative stress, since the oxidative metabolism of dopamine produces ROS[73,74]. Given the function of *PM20D1* and the role of NAAs as endogenous mitochondrial uncouplers, as well as their potential role against oxidative activity[5,75,76], it cannot be ruled out that *PM20D1* has a neuroprotective effect on the development of PD.

To the best of our knowledge, only two variants in *PM20D1* with significant associations have been reported. Intronic variant rs11240572 has been significantly[53,77-79] or close to significantly[80] associated with the PD phenotype; the minor allele (A) has been proposed to have a protective effect due to its higher frequency in controls than cases. In contrast, Deng *et al.* (2019) reported that Chinese PD patients with rs11240572-A presented a faster progression and greater deterioration of motor function than non-carriers[81]. The common coding variant rs1891460-C (p.Ile149Val) has been reported as nominally associated with a reduced PD risk in individuals of European and Ashkenazi Jewish ancestry[57]; there are no other studies that confirm this association. Few studies have sequenced *PM20D1* looking for variants in PD

patients[57,63], while introns and UTR regions have not been deeply explored. Therefore, the existence of unknown variants involved in PD cannot be ruled out.

There is one report of hypermethylation of *PM20D1* in PD[82]. Additionally, several studies have focused on genetic variants that correlate with the expression level of the gene. The rs708723-T variant in *RAB29* has been reported to be associated with Parkinson's disease and correlates with increased methylation of CpG sites in *PM20D1* in frontal cortex and cerebellar tissues, but also with the expression of *RAB29* and *NUCKS1*[56]. In the same tissues, the risk PD variant rs823118-T, located between *RAB29* and *NUCKS1*, increases *PM20D1* methylation and affects the expression of *RAB29* and *NUCKS1*[64]. Goldstein *et al.* (2021) found evidence suggesting that the risk non-coding variant rs823114-A, located upstream of the *NUCKS1* gene, has an eQTL and mQTL effect on *PM20D1* and other genes in the PARK16 locus[83]. Additionally, Cibulka *et al.* (2022) reported that minor allele A for rs708727 in *SLC41A1* is associated with PD in the Slovak population[37]. As mentioned in previous sections, the Alzheimer-associated rs708727 is an mQTL and eQTL for *PM20D1*, and the A allele is associated with higher methylation of the *PM20D1* promoter and absent expression[5-8] [Figure 1]. In addition, dementia is a common diagnosis in patients with PD, manifesting mainly in late stages of the disease[84,85]. This suggests that there may be common mechanisms in the development of PD and AD, including mechanisms of epigenetic regulation[37].

OTHER PHENOTYPES

As evidenced in the previous sections, *PM20D1* is closely related to AD, obesity, and PD; however, *PM20D1* has been associated with a wide variety of other phenotypes. Among those is asthma, where the hypomethylation of probe cg14893161 was initially shown in babies born to mothers with asthma and atopic mothers without asthma[86]. A direct association between hypermethylation of *PM20D1* and respiratory allergy, specifically for probe cg11965913, has also been identified; expression of *PM20D1* is regulated by the TLR5 pathway, which is closely related to allergic responses[87]. In addition to a respiratory allergic response, hypomethylation in the gene has also been associated with food allergies[88].

Changes in the methylation levels of *PM20D1* have been reported in several additional phenotypes. In the case of psoriasis, an upregulation in gene methylation has been reported in affected individuals[89]. A differentially methylated region at *PM20D1* has also been identified for multiple sclerosis[90].

PM20D1 is also susceptible to epigenetic changes as a result of external factors, such as lifetime events and environmental conditions. In individuals who have suffered child abuse, there is evidence of hypermethylation of *PM20D1*[91]. *PM20D1* has also been involved in the response mechanism of cold exposure, in which there is an upregulation of *PM20D1* under conditions of constant exposure to cold[92].

There is increasing evidence that *PM20D1* plays a role in several metabolic conditions, such as obesity and diabetes (discussed above). Additionally, a suggestive pleiotropic association with polycystic ovary syndrome has been described for the gene; this is a metabolic condition closely related with obesity and diabetes[93]. For familial hypercholesterolemia, DMRs have been found within *PM20D1,* specifically in the cg14893161 probe[94]. On the other hand, decreased serum PM20D1 has been associated with severity in carotid atherosclerosis patients[95].

Furthermore, the very diverse phenotypes related to *PM20D1* include stroke, a condition that was determined to present hypermethylation within *PM20D1* independent of the body mass index[96]. A study of breast tissue from healthy women found differences in *PM20D1* methylation according to ethnic origin[97]. Additionally, in an epigenome-wide association study for COVID-19 severity, the *PM20D1* gene was found

to be differentially methylated[98].

PM20D1 has a potential role in cancer as well; differentially methylated regions at *PM20D1* have been reported for different types of cancer. As a first example, the methylation level of *PM20D1* (together with other genes) has been proposed to allow detection of the presence of lung cancer, as well as to characterize the type of tumor[99]. Additionally, hypermethylation at *PM20D1* sites has been found in hepatocellular carcinoma and acute myeloid leukemia, suggesting that the gene is downregulated or totally silenced[100,101].

Finally, it is important to highlight the role of *PM20D1* in neurological disorders. In addition to its involvement in AD and PD (as detailed in previous sections), hypermethylation of CpG sites in *PM20D1* (and presumably reduced expression) has been associated with an increased risk of suffering chronic postsurgical pain[102]. Additionally, it has been reported that *PM20D1* is differentially methylated between different types of epilepsy (focal vs. generalized epilepsy)[103].

In most cases, the evidence supporting the role of *PM20D1* in these disorders comes from a single report with a small sample size. Therefore, further studies are needed to confirm these associations, as well as to understand the mechanisms involved.

CONCLUSION

Evidence supporting the role of the *PM20D1* gene in several heterogeneous disorders has been steadily accumulating in recent years. Thus far, it appears that the main effect of this gene on phenotype comes from changes in its expression levels, rather than from genetic variants that affect its structure or function. However, genetic variants in the genomic region close to *PM20D1* do play a role in the regulation of the gene's expression levels, as has been shown by the multiple studies confirming the existence of these mQTLs and eQTLs. This stresses the importance of performing comprehensive studies that explore genetic variation, methylation, and expression at the same time. Although the direction of change in methylation or expression of *PM20D1* varies among disorders, many studies report hypermethylation or reduced expression of *PM20D1* (or a higher frequency of the reduced expression-associated haplotype) in affected individuals. This (and other functional evidence) supports the idea of the protective role of *PM20D1*, which is lost in affected individuals with silenced expression. *PM20D1* has been shown to activate mitochondrial uncoupling, which plays a role in response to oxidative stress. Oxidative stress is known to be involved in the development and progression of several *PM20D1*-associated disorders, including obesity, Alzheimer's disease, and Parkinson's disease, and could potentially be the common link among them. The exact biological mechanisms involved in each case await elucidation, which could potentially open up promising avenues for treatment.

DECLARATIONS

Authors' contributions
Contributed to the conception of the study: Raventós-Vorst H, Chavarría-Soley G
Drafting of the manuscript: Garro-Núñez D, Mora-Cubillo P, Fonseca-Bone S, Picado-Martínez MJ, Fonseca-Brenes M, Chavarría-Soley G
Supervision: Garro-Núñez D, Raventós-Vorst H, Chavarría-Soley G

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Ethical approval and consent to participate
Not applicable.

Consent for Publication
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