The role of Alpha-1-acid glycoprotein 2 protein and the underlying *Orosomucoid 2* gene in different diseases

ABSTRACT

Alpha-1-Acid glycoprotein 2 (AGP2) or *orosomucoid* 2 (*ORM2*) is a major plasma protein. AGP2 is a member of the immunocalin family, a lipocalin subfamily expressed mainly in hepatocytes and also in astrocytes and endothelial cells. The detailed physiological role of this protein has not yet been known, but some physiological activates have been related to this protein, including drug binding and immunomodulating effects. As it belongs to the positive acute phase proteins (APP), AGP2 increases during acute phase reactions and under certain pathological conditions such as unspecific inflammatory stimulation. AGP2 play a critical role in many diseases throughout its critical biological pathways. This literature review evaluates and discusses the contribution of AGP2 in different diseases, including several types of cancer, Rheumatoid Arthritis and Coronavirus Disease 2019 (COVID-19).

Keywords: alpha-1-Acid glycoprotein 2; *Orosomucoid 2*; Inflammation; Microglia Activation; Glycosylation Modifications; Capillary Permeability; COVID-19; Cancer

1. INTRODUCTION

Alpha-1-acid glycoprotein (AGP), or *Orosomucoid (ORM*), is one of the leading and vital plasma proteins. It has been more than 70 years since discovering this glycosylated single chain protein and its isoforms. Since then, thousands of publications have been published investigating its structure and functions. AGP is considered a transport protein with

immunomodulation roles. However, the molecular function of AGP is still not fully understood. AGP have two main isoforms, AGP1 and AGP2. Most of the published studies were about AGP in the big picture as protein family and their general functions. This study aims to overview AGP2 and focuses on its physiological roles in different diseases. According to our awareness, no review article published focusing on the *ORM2* gene and its AGP2 protein yet.

2. STUDY DESIGN

Information about the protein and the underlying gene in the literature were obtained from virtual databases such as NCBI, Medline, and PubMed. Articles were chosen according to selected keywords from 1950 until 2021. Keywords of protein/gene were looked at which either scientific names or scientific symbols. The preferred name of this protein is alpha-1-acid glycoprotein 2, and the other names are AGP2 and AAGP2. The gene's scientific terms are; *orosomucoid 2* whereas scientific symbols for this gene are *ORM2*; *AGP2*; *AGP-B*; *AGP-B*'.

3. *Orosomucoid 2* (*ORM2*)

3.1 The structure of *ORM2* gene and its protein

3.1.1 *ORM* genes

ORM gene is synthesized in leukocytes but mainly in the liver. Moreover, Orosomucoid is composed of two variants: *ORM1* and *ORM2*, both genes are positioned on the long arm of chromosome 9 region 3 bands 2 (9q32) with 6 exons **(Figure 1)**

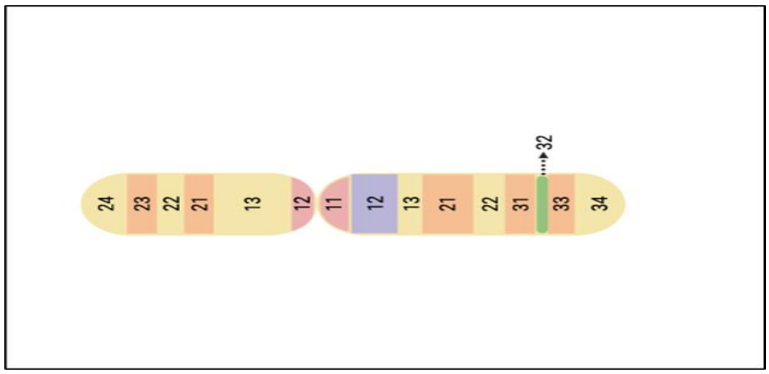

Figure 1: *ORM* genes are located on the long arm of chromosome 9 region 3 bands 2 (9q32) green.

Moreover, *ORM1* encoded by *AGP-A*, with three common alleles (*AGP S, AGP F1*, and *AGP F2*) generally referred to as F1*S variants, while *ORM2* encoded by *AGP-B; AGP-B'*, also referred to as the A variant. *AGP-B and AGP-B'* genes are identical due to a recent duplication (Luo, Lei, Sun, Liu, & Su, 2015). They differ by some features, such as the number of base pairs, as shown in **Table 1**. However, *ORM1* has 764 base pairs while *ORM2* has 759 base pairs with 201 amino acids linear for both. *ORM* is mainly found as a combination of these variants where the F1*S and A variants molar ratio in the blood from 2 to 3:1, and it changes depending on the acute phase reactions. However, the *ORM1* concentration is five times higher than *ORM2*. *ORM1* is the predominant subtype and the major isoform in circulation, while *ORM2* is the major isoform in the brain.

Table 1*:* The similarities and differences between *ORM1* and *ORM2*

	ORM 1	*ORM 2*
Encoded by	AGP-A. codes three variants (AGP F1, AGP F2, and AGP S)	AGP-B, AGP-B'

Synthesized by	Liver cells	
Base pair (bp)	764 bp	759 bp
Number of amino acids (aa)	201 aa linear	
Constitutive level	*ORM1* is fivefold higher than *ORM2*	
Distribution	The major isoform in circulation.	The major isoform in the brain

3.1.2 AGP2 protein

AGP2 (alpha-1-acid glycoprotein 2) is an acute-phase protein produced mostly by the liver, and also excreted in other extrahepatic tissues (Luo et al., 2015). AGP2 molecular weight is 23.6 kDa. Its carbohydrate content is high, 45% of the total protein mass, representing one of the most extensively glycosylated proteins with an isoelectric point (pI) of 5.03 (Fournier, Medjoubi-N, & Porquet, 2000). AGP2 considers a member of the immunocalin family and a lipocalin subfamily (Janciauskiene, Wrenger, & Welte, 2013). The precursor product of AGP genes is a single polypeptide chain of 201 residues, and a secretory N-terminal signal peptide of 18 residues is cleaved off, resulting in a protein with a single polypeptide chain of 183 amino acids (Luo et al., 2015; Taguchi, Nishi, Chuang, Maruyama, & Otagiri, 2013).

The difference between AGP variants (AGP1 and AGP2) is 22 amino acid substitutions out of a total of 183 amino acids (Fournier et al., 2000). X-ray diffraction was used to identify the crystal structure of AGP2 with a resolution of 2.1.Å. As shown in **Figure 2**, AGP2 is composed of four α-helices in addition to an eight-stranded β –barrel (Nishi et al., 2011). These eight β -strands represents a typical lipocalin fold forming the common drug-binding pocket feature in the lipocalin family and flanked by an α-helix (Schönfeld, Ravelli, Mueller, & Skerra, 2008). Two lobes make up the AGP2 binding pocket, I and II, and its entrance is positioned at the open end of the β -barrel, unlike AGP1 where the pocket is deep, wide and contains three lobes (I–III). This indicates that the AGP2 variant binding pocket is narrower

than the AGP1 variant, making AGP2 a distinctive ligand selectivity feature (Nishi et al., 2011).In addition, AGP2 displays two disulfide bounds that are located between cysteines 5-147 and 72-164 (Ceciliani & Pocacqua, 2007). AGP2 carries five sites of N-glycosylation attached to asparagine residues at Asn15, Asn38, Asn54, Asn75 and Asn85 (C. L. Fernandes, Ligabue-Braun, & Verli, 2015). The structural heterogeneity of AGP2 is mostly due to these glycans, depending on the branching degree and the terminating sugars attached (Ceciliani & Pocacqua, 2007). AGP2 can show various levels of branching such as bi-, tri- and tetra-antennary along with great diversity of the terminating sugars like sialic acid and fucose (Taguchi et al., 2013).

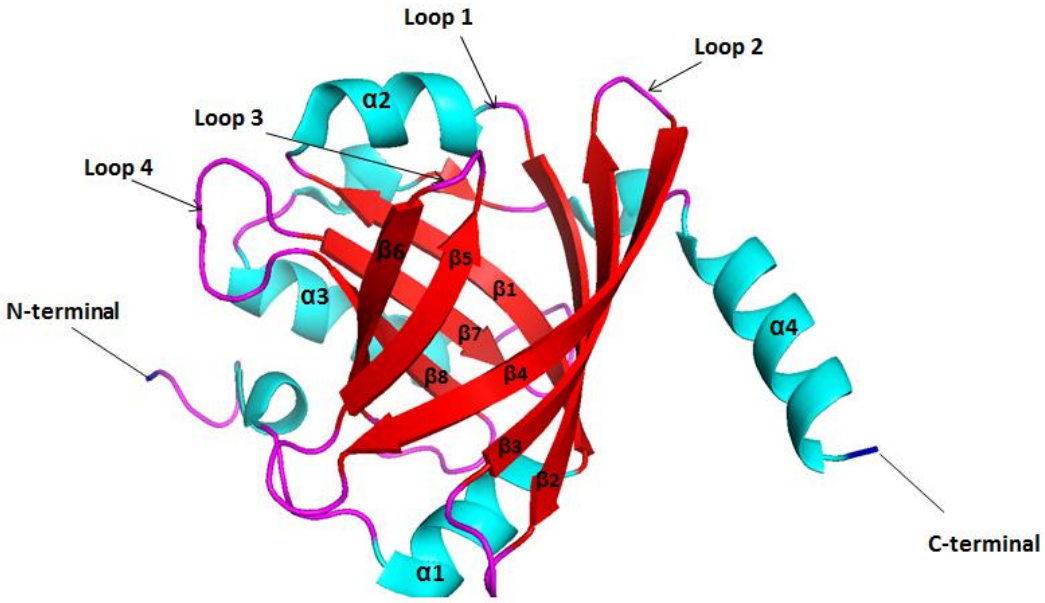

Figure 2: AGP2 structure (3APX) originally published by (Nishi et al., 2011). The 3D structure consists of N-terminal, C-terminal, eight β-strands (red), and four α-helices (light blue) connected by four loops (light magenta).

3.2 Functions of AGP2

AGP2 is a member of the immunocalin family, a lipocalin subfamily. Immunocalin functions as immunomodulatory and anti-inflammation, where the lipocalin can bind and transport a range of hydrophobic ligand and drugs (Jo et al., 2017; Taguchi et al., 2013). Although the biological function of AGP2 is still poorly understood, AGP2 has been shown to have a key role in drug binding and immunomodulating effects, as well as a variety of biochemical processes with physiological implications (S. A. Smith & Waters, 2019). Furthermore, AGP2 is considered as the most critical drug-binding and drug-transporting protein along with Albumin and lipoproteins, which directly affects pharmacodynamics and pharmacokinetics (S. A. Smith & Waters, 2019). Changes in AGP2 plasma levels during the acute-phase reactions (APR) and other pathological conditions, were reported to alter the concentration of free drugs without affecting the total drug concentration (Fournier et al., 2000). Depending on AGP physical-chemical properties, due to the presence of neuraminic acid residues on the glycoprotein's surface, this protein binds mostly to basic and neutral medicines from both endogenous and exogenous sources (Luo et al., 2015). Drug binding properties have been reported to be affected by the differences between AGP variants, where drugs such as binedaline, dipyridamole and warfarin were shown to bind more strongly to AGP1. While other drugs such as imipramine, methadone and disopyridamole show a higher affinity for AGP2 (S. A. Smith & Waters, 2019). The different binding selectivity between the two variants could be explained due to the different conformation of the binding sites; binding pocket lobes I and II are common in both variants, while a unique lobe III is only found in AGP1 (Baldassarre & Tanfani, 2013). As a result, AGP2 has a higher drug binding selectivity than AGP1. Therefore, AGP drug binding selectivity depends on AGP2 selectivity since AGP1 binds a wider range of drugs (Taguchi et al., 2013).

On the other hand, many drugs such as propranolol, progesterone and chlorpromazine bind equally to both variants due to their similarity. These drugs are highly hydrophobic and

neutral drugs that bind to the hydrophobic lobe I that are present in AGP1 as well as AGP2 (Baldassarre & Tanfani, 2013). Several factors influence AGP binding capacity, including concentration and protein conformational change, temperature, pH, the concentration and polarity of the ligand, the relative abundance of the AGP variants, and other amino acid residues that exist near AGP hydrophobic domains (Fournier et al., 2000). One of the unique properties found in AGP2 while binding to drugs is preventing the transferring of drugs from the bloodstream to the brain, limiting the effecting on the central nervous system due to therapeutic effects of drugs (Jolliet-Riant, Boukef, Duche, Simon, & Tillement, 1998).

3.3 Timeline of the *ORM*

ORM was first discovered by scientist K. Schmid in 1950, later Schmid clarified the α1-Acid Glycoprotein amino acid sequence (linear) in 1973 (K Schmid, 1950; Karl Schmid et al., 1973). Previous genetic evidence of a second *orosomucoid* structural locus (*ORM2*) in human blood (plasma) was discovered in 1987(Kamboh & Ferrell, 1987). Rojo-Domnguez and Hernández-Arana calculated the first 3D modeling of human 1-acid glycoprotein in 1993. Furthermore, the crystal structure of the recombinant F1*S variant (AGP1) of α1-acid glycoprotein had been determined in 2008 by using UV radiation-damage-induced phasing (UV RIP) (Schönfeld et al., 2008). Then, in 2011, a second crystal structure was determined using X-ray diffraction as the A variant (AGP2) (Nishi et al., 2011), identifying variations in drug-binding selectivity between two AGP genetic variants. In order to determine the *ORM* biological function, a previous study in 1977 by Chiu et al., indicated the interactions of the immune system with α1-acid glycoprotein specially on lymphocytes (Chiu, Mortensen, Osmand, & Gewurz, 1977). However, many studies indicated the connection of AGP with many different diseases. For instance, in Neoplastic Disease patients, an abnormal expression of *ORM* was detected (Rudman, Treadwell, Vogler, Howard, & Hollins, 1972), and a high

concentration of AGP plasma was detected in tumors such as lung cancer, ovarian cancer, and breast cancer (Duché et al., 2000). *ORM1* and *ORM2* plasma levels were found to be higher in varying severity COVID-19 situations in a recent investigation (Shu et al., 2020). The Summarized timeline of *ORM* events and its association with some diseases were represented in **Figure 3**.

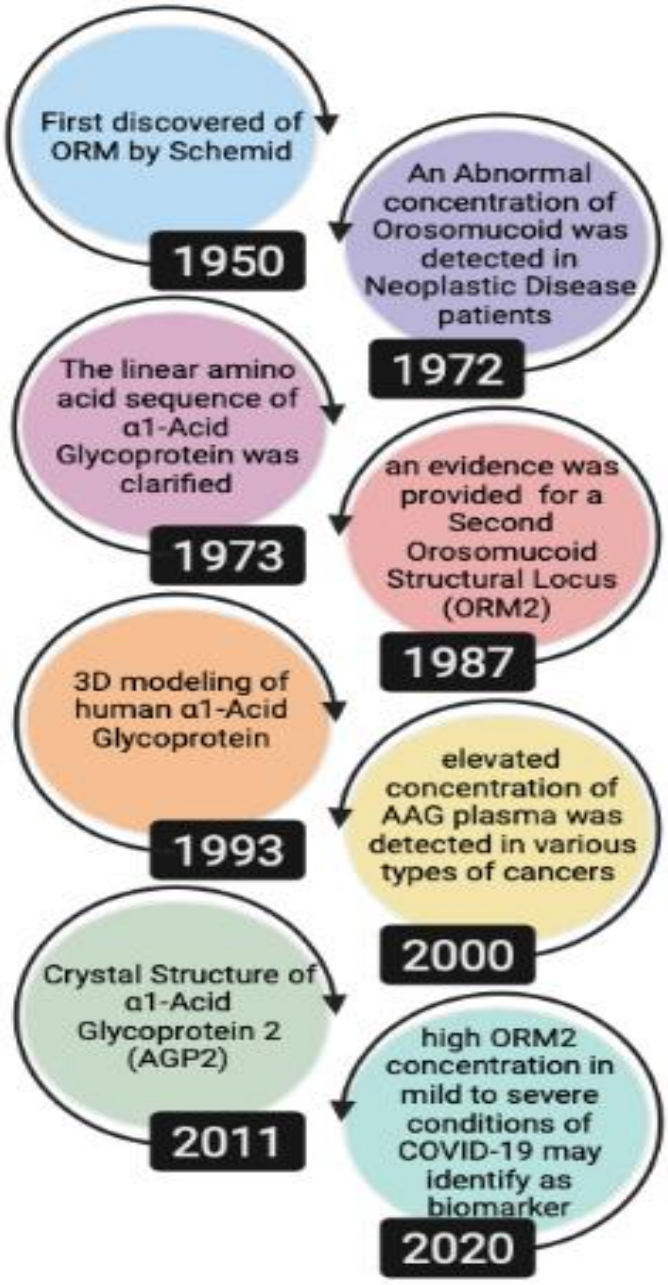

Figure 3: Timeline of *ORM* events and discoveries and the association with some diseases.

4. The role of AGP2 in different mechanisms

AGP2 is involved in both pro- and anti-inflammatory modulation along with being an immunomodulating molecule (Fournier et al., 2000; S. A. Smith & Waters, 2019). AGP2 has an inhibitory effect on platelet aggregation, lymphocyte proliferation and neutrophil chemotaxis via unknown mechanisms (Luo et al., 2015). In addition, AGP2 reduces cytokine secretion as a protection mechanism in a way that has not been fully elucidated (Fang et al., 2015). AGP2, on the other hand, is involved in monocyte activation and the production of pro-inflammatory cytokines such as IL-6 and IL-12 by monocytes (Ceciliani & Pocacqua, 2007). In a study about AGP2 role in neuroinflammation, AGP2 was found to play an anti-inflammatory role in neuroinflammation. Astrocytes express AGP2 in the hippocampus and during neuroinflammation, AGP2 inhibits microglial migration and accumulation in the inflammatory site. In a way to decrease neuroinflammation throughout reducing the inflammatory cell activation and migration. Along with reducing the mRNA expression of IL-1β, IL-6, and TNF-α, resulted from neuroinflammation. Furthermore, AGP2 knockdown in neuroinflammation led to increase proinflammatory mediators' expression of IL-1β, IL-6, and TNF-α, and enhance microglial activation in the hippocampus (Jo et al., 2017).

The hepatic production of many plasma proteins could be changed due to systemic injury such as inflammation called acute-phase reactants. Several pathogenic situations and stimulating agents, such as infection, inflammation, tumor, surgery, tissue injury, sepsis, and necrosis, cause AGP concentrations to rise tenfold (Luo et al., 2015). In 2005, a proteomic analysis detected an increase in AGP2 in cerebrospinal fluid from chronic fatigue syndrome patients (Baraniuk et al., 2005). *ORM* gene and its protein have been used as a biomarker for many diseases as well as cancer. Moreover, *ORM* levels are used as a biomarker for an

autoimmune disorder such as Rheumatoid arthritis (RA) which is an inflammatory joint disease (Brink, Lundquist, Alexeyenko, Lejon, & Rantapää-Dahlqvist, 2019), and also for early detection of type 2 diabetes nephropathy (Zhou et al., 2020). Besides, *ORM* could be used as a prognostic factor for the liver cancer (Zhu et al., 2020).

Because of its ability to bind directly with endothelial cell pores and reduce plasma water filtration into the surrounding tissues, AGP2 is vital in the capillary barrier. Many studies showed that AGP2 is important for capillary charge selectivity, which may play a role in conserving capillary permeability and organ perfusions (Luo et al., 2015; Sörensson, Matejka, Ohlson, & Haraldsson, 1999). AGP2 is thought to be clinically helpful in avoiding microvascular permeability increases in a variety of disorders, including Alzheimer's and stroke. Also, a study found that AGP2 could enhance brain endothelial function in the rat by reinforcing the monolayer integrity (Luo et al., 2015; S. Zhang & Mark, 2012). **Table 2** summarized several studies of AGP2 role in different biological mechanisms and pathways.

Table 2: Biological pathways of AGP2

References	Biological Effects	Type of Research	Mode of action
(Hughes & Watson, 2012; Stein et al., 2004).	Active suppression of inflammation during mammary gland involution.	AGP2 role in mouse mammary gland involution.	AGP2 act as anti-inflammatory factors, where it can modulate the immune system activity in a negative way and protect the cells from endotoxic shock.
(Spiller et al., 2012).	Neutrophil migration inhibition.	The role of AGP2 in polymicrobial sepsis susceptible diabetic mice.	AGP2 works as immunosuppressing by inhibiting neutrophil migration to the infection sites.
(S. Zhang & Mark, 2012).	Modulation of microvessel permeability.	AGP2 effects on the microvasculature of the brain.	AGP2 enhances blood–brain barrier (BBB) functional integrity by increasing the expression of TJ proteins (ccluding and ZO-1).
(Fang et al., 2015).	Tumor suppressor in hepatocellular carcinoma.	The function of AGP2 in hepatocellular carcinoma.	Inducting of AGP2 expression throughout the CCAAT/enhancer binding protein (C/EBP) leads to repressing HCC cell migration and invasion.
(Jo et al., 2017).	Modulating microglial activation and migration during neuroinflammation.	The expression and the function of AGP2 in the brain.	AGP2 inhibits microglial migration and activation by blocking the interaction of C-C chemokine ligand 4 (CCL4) with C-C chemokine receptor type 5.
(Zhu et al., 2020)	Prognostic factor associated with cancer-promoting pathways.	The expression, significance, and assessment of the AGP2 prognostic value in liver cancer.	The expression of AGP2 was significantly decreased in liver tumor tissues and was positively related to cancer-promoting pathways.
(Rojas-Colón et al., 2021).	Involved in neuronal survival, and memory.	The AGP2 function during lipopolysaccharide (LPS)-induced brain inflammation.	AGP2 reverses LPS-induced hippocampal-dependent spatial memory impairments after the α7 acetylcholine receptor treatment.

5. AGP2 Involvement in Diseases

Many studies found and observed a relationship between AGP2 concentration and diseases. Upregulation or downregulation of *ORM2* or AGP2 could be used as a biomarker, prognostic factor for cancer, or therapeutic response prediction for many diseases, while the specific function of AGP2 is not recognized yet. These studies may indicate *ORM2* involvements and role in diseases, see **Table 3.**

5.1 AGP2 contributions in various diseases

AGP2 mainly contributes to diseases throughout five modes of action; by inhibiting microglia activation, inflammation, glycosyl pattern modifications, capillary permeability and platelet and neutrophil aggregation inhibitory activity as summarized in **Figure 4.**

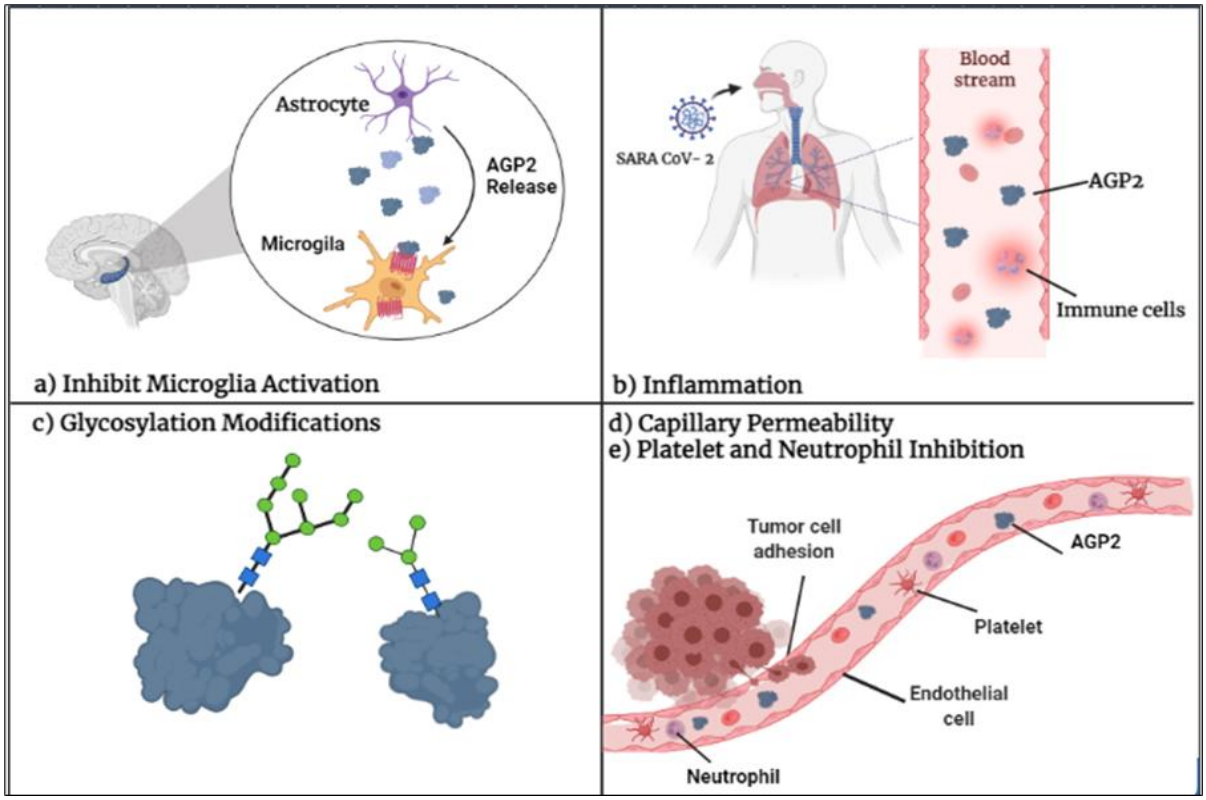

Figure 4: Alpha-1-Acid Glycoprotein 2 (AGP2) modes of action in various diseases. a) Inhibit microglia activation, b) Inflammation, c) Glycosylation modifications and d) Capillary permeability e) Platelet and neutrophil inhibition.

Table 3: The role of AGP2 in different disorders

References	Disorders	Expression levels	Study Type	Study Contribution
(Nagalla et al., 2007).	Down Syndrome (DS)	Up regulated	Down syndrome potential serum protein biomarkers.	The expression of AGP2 was changed on the surface of T cells in DS, indication of AGP2 function in DS.
(Fratini et al., 2012).	Sporadic Creutzfeldt-Jakob disease (CJD)	Up regulated	Plasma biomarkers in sporadic CJD.	Increases AGP2 plasma levels are suggesting a generalized systemic inflammation in sporadic CJD.
(Gao, Zhang, Whang, & Zheng, 2014)	Colorectal Cancer (CRC)	Up regulated	AGP2 role in stage II colorectal cancer.	AGP2 have an independent prognostic impact in stage II CRC, as its higher plasma levels were encouraging cancer invasion and correlated with the survival rates in CRC patients.
(Xi Zhang et al., 2015).	Congenital heart diseases	Up regulated	AGP2 alteration in Ventricular septal defect (VSD).	AGP2 altered levels were a response to inflammation in VSD cases, which linked with the increased pulmonary flow
(Fang et al., 2015).	Hepatocellular Carcinomas (HCC)	Down regulated	AGP2 function in hepatocellular carcinoma.	AGP2 expression was adversely associated with intrahepatic metastasis and histological grade in HCC tissue.
(Yang et al., 2016).	Autism	Up regulated	Potential urine biomarkers of autism.	AGP2 was found increased in autistic individuals compered to controls, as it was stated to be linked to autism.
(Baraniuk et al., 2005; Yang Sun, Zhang, & Liu, 2016).	Chronic Fatigue Syndrome (CFS)	Up regulated	The use of AGP2 as a valuable biomarkers for CFS.	AGP2 significantly increased in CFS, where its elevation levels were a characteristic change in cerebrospinal fluid in CFS.
(Suresh et al., 2016).	Idiopathic Nephrotic Syndrome (INS)	Up regulated	Identify urinary biomarkers in children diagnosed with INS.	AGP2 is a biomarker for monitoring the disease progression, since it was associated with focal and segmental sclerosis in children with INS.
(M. Fernandes & Husi, 2016).	Fabry disease (FD)	Up regulated	FD pathophysiology.	AGP2 was differentially expressed in FD, and was related to the acute inflammatory response and platelet degranulation.

References	Disorders	Expression levels	Study Type	Study Contribution
(Rucksaken et al., 2017).	Cholangiocarcinoma (CCA)	Up regulated	Evaluating plasma AGP2 as a potential biomarker for CCA.	AGP2 is associated with Opisthorchis viverrini infection via inflammation. Bile duct inflammation, including cholangitis and CCA result in AGP2 overexpression.
(Calamia et al., 2018)	Osteoarthritis (OA)	Down regulated	Candidate protein biomarkers in OA for prediction of the therapeutic response.	AGP2 serum concentration is useful for the therapeutic response prediction to chondroitin sulfate/glucosamine hydrochloride with an accuracy of 84,3% in OA patients.
(Kustán et al., 2018).	Psoriasis	Up regulated	The urinary AGP2 levels in psoriatic patients.	AGP2 is inflammatory marker in psoriasis. Where, urinary AGP2 was higher in psoriasis and significantly higher in the severe cases.
(Lin et al., 2018).	Hepatitis B virus-associated acute liver failure (HBV-ALF)	Down regulated	The molecular mechanism of HBV-ALF.	*ORM2* gene with immune response pathway; has a role in HBV-ALF pathogenesis and could be used as therapeutic targets for HBV-ALF.
(Brink et al., 2019).	Rheumatoid Arthritis (RA)	Up regulated	RA molecular processes and candidate proteins biomarkers.	Elevated AGP2 levels were an indication of the ongoing inflammation which is uniquely found in RA patients.
(Wan et al., 2019).	Ischemic Stroke	Up regulated	AGP2 role in the ischemic stroke.	AGP2 have been found to play a protective role in ischemic stroke.
(Zhu et al., 2020).	Liver Cancer	Down regulated	AGP2 expression and function in liver cancer.	AGP2 downregulation in liver tumors contributes to the liver cancer progression.
(Zhou et al., 2020).	Diabetic Nephropathy (DN)	Up regulated	Urinary AGP2 as a diagnostic of diabetic nephropathy.	Urine AGP2 levels were a positivity associated with DN progression and increased in the early stage which correlated with DN occurrence and development.
(Shu et al., 2020).	Coronavirus Disease 2019 (COVID-19)	Up regulated	COVID-19 biomarkers and their contribution to the disease pathogenesis.	AGP2 increased levels were associated with more severe COVID-19 conditions, and its plasma alterations were related to immune responses and platelet degranulation.

References	Disorders	Expression levels	Study Type	Study Contribution
(Yue Sun et al., 2020)	Adult-Onset Still's Disease (AOSD)	Up regulated	Potential biomarkers for AOSD diagnosis.	AGP2 might be biomarker for AOSD diagnosis. Since it's increased levels were positively correlated with the disorder activity.
(Eidet et al., 2021).	Bacterial Keratitis	Up regulated	The upregulated tear proteins in bacterial keratitis.	AGP2 was upregulated tear protein in bacterial keratitis patients, expressed by local corneal leukocytes.
(Naskar et al., 2021).	Parkinson's disease (PD)	Down regulated	PD biomarkers predicting cognitive impairment.	AGP2 was altered and associated with defense/ immune protein activity in PD with cognitive impairment.
(Mao et al., 2021).	Coronavirus Disease 2019 (COVID-19)	Down regulated	The biological states of recovered COVID-19 patients.	AGP2 is used as potential marker for monitoring the inflammation condition in COVID-19 recovered patients.

5.1.1 Inhibit Microglia Activation

Autism, Parkinson's disease, and Alzheimer's disease (AD) are diseases that a strong neuroinflammatory component was proven. AGP2 expression in the brain was being studied in order to better understand its involvement in neuroinflammation. In this study**,** Lipopolysaccharide (LPS) -induced neuroinflammation model was created (Jo et al., 2017). LPS is a gram-negative bacteria endotoxin known to incite inflammation by encouraging the cells to secret proinflammatory mediators. Therefore, LPS models were used to illustrate the neuroinflammatory mechanism in neuroinflammation and neurodegenerative diseases (Batista, Gomes, Candelario-Jalil, Fiebich, & De Oliveira, 2019). Systemic injection of LPS was used to induce the neuroinflammation condition in the brain. After the intraperitoneal injection of LPS, the mRNA expression of the AGP proteins family was examined. It revealed that AGP2 expression, not the other isoform, was strongly produced in the brain within 24 h after LPS injection. AGP2 mRNA showed a higher level in the hippocampus compared to other brain regions. The study also reported that AGP2 is mainly expressed in the brain throughout astrocytes cells. On the other hand, a reverse process was created for knockdown AGP2 mRNA level in the brain using lentiviral shRNA. The LPS induction, along with AGP2 knockdown resulted in enhancing many proinflammatory cytokines expression (IL-1β, TNF-α, and IL-6). Conforming AGP2 anti-inflammatory role in neuroinflammation and its role of being used to diagnose, prevent, or treat neurological and brain disorders (Jo et al., 2017). COVID-19 disease could be one of them, since the SARS-CoV-2 virus is linked with neurological alterations in COVID-19 cases (De Felice, Tovar-Moll, Moll, Munoz, & Ferreira, 2020).

5.1.2 Inflammation

Inflammation is widely recognized to play a critical function in cancer progress and it's a hallmark of cancer. Inflammation plays a role in all stages of cancer, including tumor development, progression, malignant conversion, invasion, and metastasis. Interestingly, cancer-related inflammation is found to pave the way for cancer, in fact, it represents 15%–20% of all cancer cases, where incident such as infection, chronic inflammation, or autoimmunity were reported to exist in the exact location for long time before tumor initiation (Greten & Grivennikov, 2019). Therefore, many proteins were chosen to be potential biomarkers for cancer due to their involvement in inflammation reactions. And for that, AGP2 is considered as an essential acute phase plasma protein in inflammation and could play vital roles in monitoring the tumor development and progression indicting/distinguishing cancer in different stages. AGP2 is mainly produced from hepatocytes upon stimulation by the immune cell–derived cytokines activated by the inflammation and tumorigenesis. AGP2 level raised at 21 days before the tumor start in cholangiocarcinoma (CCA) and induced by the inflammation resulting from Opisthorchis viverrini infection. Where, O. viverrini infection promotes inflammation-mediated tumorigenesis. Consequently, overexpression of AGP2 indicates the early stage of CCA and its increased levels that correlate positively with the tumor progression (Rucksaken et al., 2013). Moreover, AGP2 expression has been positively associated with the development of several kinds of cancer such as colorectal and lung cancer (Asao et al., 2013; Gao et al., 2014; Xuhua Zhang et al., 2012).

On the other hand, AGP2 upregulation upon inflammation promotes different types of diseases including rheumatoid arthritis (RA), Sporadic Creutzfeldt-Jakob disease (CJD), Congenital heart diseases, Psoriasis and Coronavirus Disease 2019 (COVID-19) regardless of the disease genesis. Coronavirus Disease 2019 (COVID-19) is a pandemic infectious disease rapidly spreading worldwide triggered by the infection of the SARS-CoV-2 virus. Although

COVID-19 is regarded as one of the worst epidemics in history, with over two million confirmed deaths and millions of cases. However, COVID-19 pathogenesis is still poorly understood. Extensive efforts have been done investigating its development and understanding pathogenesis along with identifying potential biomarkers. Furthermore, a study about the host plasma proteins alterations that could involve in the COVID-19 pathogenesis, revealed that AGP2 was significantly increased in COVID-19 patients and showed a specific upregulated pattern with the development of the disease severity indicating in the more severe conditions (Allard et al., 2020; Shu et al., 2020). The increasing of AGP2 levels in plasma are responding to the early phases of immune responses to virus infection. Acute inflammation is related with COVID-19 in severe cases and positively correlates with the disease severity (Mao et al., 2021). In addition to platelet degranulation and metabolism, a study also identified pathways related to COVID-19 pathogenesis (Shu et al., 2020). Moreover, AGP2 was connected to fibrinogen chains which could be associated with a severe infection in fatal COVID-19 conditions (Rezaei-Tavirani et al., 2021). AGP2 plasma levels would be used as a marker of the status of SARS-CoV-2 virus infections since its levels were decreased in COVID-19 recovered cases. This report described the clinical characteristics of 3 months COVID-19 recovered patients, which shows that recovered patients are still under the condition of inflammation (Mao et al., 2021).

5.1.3 Glycosylation Modifications

In addition to AGP2 concentrations and plasma levels, the glycosylation alterations are also considered as a diagnosis approach for physiological and pathophysiological conditions. AGP2 undergo three types of changes in glycosylation, they are sialytion, fucosylation and the degree of branching. Modifications in glycosylation are correspond to different diseases and disease stages and progression (Keser et al., 2021). Multiple glycoforms were identified

on AGP2 for instance, 51 glycoforms were found at Asn93. These glycoforms indicate that AGP2 has the ability to modify its N-glycosylation pattern responding to physiological changes or stimulations (Chen et al., 2021). Thus, AGP2 functions in drug binding and immunomodulation are highly dependent on AGP2 carbohydrate composition. Therefore, AGP2 glycosylation is an alternative biomarker target (Ceciliani & Pocacqua, 2007). For example, AGP contribute to Alzheimer's Disease through aberrant glycosylation (Quaranta et al., 2019). The glycosylation alterations of AGP are linked to inflammatory processes, specifically neuroinflammation associated with AD as previously mentioned. In cancer patients, fucosylated glycans are novel tumor markers, the elevation of fucosylated glycans in tri- and tetra-antennary structures were found in cancer cases (Asao et al., 2013). Moreover, the glycosylation changes of AGP help distinguishing hepatocellular carcinoma from cirrhosis; AGP sialylation and fucosylation were diverse in the hepatocellular carcinoma, liver cirrhosis and also in the control group. The concentrations of sialylation and fucosylation were high in HCC and cirrhosis but low in controls, indicating the critical role of sialylation and fucosylation that may play in HCC and cirrhosis. (D. Zhang et al., 2017). N-acetylgalactosamine, fucose, and galactose may be employed as markers of breast carcinoma progression (K. D. Smith, Behan, Matthews-Smith, & Magliocco, 2012).

5.1.4 Capillary Permeability

In tumor metastasis, tumor cell adhesion to the microvascular wall is a critical step, which is part of tumor invasion. The interaction between tumor cells and blood host cells such as platelets, endothelial cells, and immune cells is what causes tumor cell adhesion (Plantureux et al., 2018). The endothelium regulates tumor cell extravasation by assisting the adhesive between tumor cells and the vascular wall (Stroka & Konstantopoulos, 2014). Microvascular endothelial cells produce AGP that expressed in hepatocytes and upon released into the

circulation, AGP2 performed its function in maintaining normal permselectivity of the capillary walls (Luo et al., 2015; Sörensson et al., 1999). AGP was discovered to has anti-inflammatory effects upon the endothelial cells by decreasing microvascular permeability and reducing the tumor cell adhesion (Cai, Fan, Zeng, Zhang, & Fu, 2012; Sörensson, Ohlson, Björnson, & Haraldsson, 2000). AGP2 inhibits endothelial cell capillary-like tube formation seen through reduced cell adhesion and reversibly decreases cell migration. As a result, AGP2 has shown anti-angiogenic effects that is detected in the lung microvascular endothelia (Miranda-Ribera, Passaniti, Ceciliani, & Goldblum, 2014). Tumor angiogenesis includes tube formation of endothelial cells for metastatic tumor cells to proliferation and migration.

5.1.5 Platelet and Neutrophil Aggregation Inhibitory Activity

Tumors have a unique mechanism referred to as tumor cell induced platelet aggregation, where primary tumor tend to influence the platelets production, activation, and aggregation. Platelet activation is important for tumor growth and metastatic progression. Tumor cells themselves have the ability for platelet activation, activated platelets interact with neutrophils actively participate to cancer-associated thrombosis and directly relate with metastatic potential. And in return, platelet participates in tumor invasion, via enhancing tumor metastasis by boosting tumor cell adherence to the vessel wall. Thus, the use of drugs targeting platelets activation could reduce cancer-associated thrombosis and tumor progression (Konstantopoulos & Thomas, 2009; Plantureux et al., 2018). Alternatively,, neutrophils are suggested to be essential players in tumor development, where an increased level of neutrophils in blood is associated with advanced cancer. Tumor-associated neutrophils (TANs) have been found to have a role in various stages of tumor growth, including anti and pro-tumor functions (Uribe-Querol & Rosales, 2015; Wu & Zhang, 2020). In consequence, AGP2 has previously been suggested to inhibit platelet aggregation as well

as inhibition of neutrophil aggregation and migration (Luo et al., 2015). This observation shows that AGP2 may have essential function in tumor metastasis and progression in many ways. AGP2 was found to be adversely associated with intrahepatic metastasis and to be often downregulated in hepatocellular carcinoma (HCC) tissues, despite the fact that it is normally extensively expressed in liver tissues. AGP2 also decreased cell migration, invasion, and metastasis in HCC cells in vitro and in vivo, acting as a tumor suppressor (Fang et al., 2015).

5.1.6 Participate in the Therapy

AGP2 is an important pharmacokinetic factor in clinical treatment since it is one of the most significant drug-binding proteins in plasma. Because AGP2 plasma levels were altered following therapy, they are employed as a diagnostic and predictive for clinical treatment (Fournier et al., 2000). Due to decreasing levels in individuals receiving both chemotherapy and hormone treatment, AGP is used as a medication response indicator in cancer patients (Al-jabbar, Hameed, & Mehdi, 2021). AGP2 is used as a biomarker for prognostic evaluation of stage II colorectal cancer (CRC). Its plasma levels help to evaluate the stage II CRC patient’s prognosis so the possible effectiveness of adjuvant therapy could be chosen (Gao et al., 2014). For example, AGP2 levels were decreased significantly and connected with the response of interferon (IFN) treatment in liver cancer. Also, AGP2 downregulation in liver tumors was used as a marker for liver cancer therapy it is considered as an efficient predictor for immune therapy to cure liver cancer (Zhu et al., 2020). In osteoarthritis disease, AGP2 was used as a predictor for drug response in knee osteoarthritis. AGP2 was able to predict patients' response to chondroitin sulfate/glucosamine hydrochloride with an accuracy of 84,3%. Where AGP2 levels in responder’s patients were significantly decreased compering to non-responders (Calamia et al., 2018). In rare genetic cases as in Fabry disease, AGP2 was

downregulated in patients undergoing enzyme replacement therapy (M. Fernandes & Husi, 2016).

Conclusion and Further Study

ORM2 is an acute-phage gene, and 1-acid glycoprotein 2 (AGP2) is one of the key acute-phase proteins, with ORM2 expression and AGP2 plasma levels changing in response to various pathophysiological situations and disorders. However, AGP2 has a role in many illnesses, but few studies described how AGP2 participated in the disease's pathogenesis. Therefore, more genetic and physiological studies are needed to help in identifying AGP2 biological pathways since their molecular functions are not completely understood.

Competing interests

There are no conflicting interests declared by the authors.

References

Al-jabbar, S. A., Hameed, R. M., & Mehdi, W. A. (2021). The Intrasubject Variability in the Acute Phase Protein (Orosomucoid) Levels and their Influenced by Breast Malignancy. *Indian Journal of Forensic Medicine & Toxicology, 15*(3).

Allard, L., Ouedraogo, E., Molleville, J., Bihan, H., Giroux-Leprieur, B., Sutton, A., . . . Deutsch, D. (2020). Malnutrition: percentage and association with prognosis in patients hospitalized for coronavirus disease 2019. *Nutrients, 12*(12), 3679.

Asao, T., Yazawa, S., Nishimura, T., Hayashi, T., Shimaoka, H., Saniabadi, A. R., & Kuwano, H. (2013). Development of a novel system for mass spectrometric analysis of cancer-associated fucosylation in plasma α1-acid glycoprotein. *BioMed research international, 2013*.

Baldassarre, M., & Tanfani, F. (2013). Structural and functional characterization of human α1-acid glycoprotein.

Baraniuk, J. N., Casado, B., Maibach, H., Clauw, D. J., Pannell, L. K., & Hess, S. (2005). A chronic fatigue syndrome–related proteome in human cerebrospinal fluid. *BMC neurology, 5*(1), 1-19.

Batista, C. R. A., Gomes, G. F., Candelario-Jalil, E., Fiebich, B. L., & De Oliveira, A. C. P. (2019). Lipopolysaccharide-induced neuroinflammation as a bridge to understand neurodegeneration. *International journal of molecular sciences, 20*(9), 2293.

Brink, M., Lundquist, A., Alexeyenko, A., Lejon, K., & Rantapää-Dahlqvist, S. (2019). Protein profiling and network enrichment analysis in individuals before and after the onset of rheumatoid arthritis. *Arthritis research & therapy, 21*(1), 1-14.

Cai, B., Fan, J., Zeng, M., Zhang, L., & Fu, B. M. (2012). Adhesion of malignant mammary tumor cells MDA-MB-231 to microvessel wall increases microvascular permeability via degradation of endothelial surface glycocalyx. *Journal of Applied Physiology, 113*(7), 1141-1153.

Calamia, V., Picchi, F., Rego-Pérez, I., Camacho, M., González, L., Fernández-Puente, P., . . . Blanco, F. (2018). Orosomucoid 2 serves for predicting but not for monitoring drug response in patients with knee osteoarthritis treated with chondroitin sulfate/glucosamine hydrochloride. *Osteoarthritis and Cartilage, 26*, S192-S193.

Ceciliani, F., & Pocacqua, V. (2007). The acute phase protein α1-acid glycoprotein: a model for altered glycosylation during diseases. *Current Protein and Peptide Science, 8*(1), 91-108.

Chen, Z., Yu, Q., Yu, Q., Johnson, J., Shipman, R., Zhong, X., . . . Okonkwo, O. (2021). In-depth Site-specific Analysis of N-glycoproteome in Human Cerebrospinal Fluid and Glycosylation Landscape Changes in Alzheimer's Disease. *Molecular & Cellular Proteomics, 20*.

Chiu, K. M., Mortensen, R., Osmand, A., & Gewurz, H. (1977). Interactions of alpha1-acid glycoprotein with the immune system. I. Purification and effects upon lymphocyte responsiveness. *Immunology, 32*(6), 997.

De Felice, F. G., Tovar-Moll, F., Moll, J., Munoz, D. P., & Ferreira, S. T. (2020). Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the central nervous system. *Trends in neurosciences, 43*(6), 355-357.

Duché, J.-C., Urien, S., Simon, N., Malaurie, E., Monnet, I., & Barré, J. (2000). Expression of the genetic variants of human alpha-1-acid glycoprotein in cancer. *Clinical biochemistry, 33*(3), 197-202.

Eidet, J. R., Akopian, M., Olstad, O. K., Jørstad, Ø. K., Moe, M. C., Petrovski, G., & Pepaj, M. (2021). The acute phase response protein SERPINA3 is increased in tear fluid from the unaffected eyes of patients with unilateral acute anterior uveitis. *Journal of Ophthalmic Inflammation and Infection, 11*(1), 1-10.

Fang, T., Cui, M., Sun, J., Ge, C., Zhao, F., Zhang, L., . . . Jiang, G. (2015). Orosomucoid 2 inhibits tumor metastasis and is upregulated by CCAAT/enhancer binding protein β in hepatocellular carcinomas. *Oncotarget, 6*(18), 16106.

Fernandes, C. L., Ligabue-Braun, R., & Verli, H. (2015). Structural glycobiology of human α1-acid glycoprotein and its implications for pharmacokinetics and inflammation. *Glycobiology, 25*(10), 1125-1133.

Fernandes, M., & Husi, H. (2016). Integrative systems biology investigation of Fabry disease. *Diseases, 4*(4), 35.

Fournier, T., Medjoubi-N, N., & Porquet, D. (2000). Alpha-1-acid glycoprotein. *Biochimica et Biophysica Acta (BBA)-Protein Structure and Molecular Enzymology, 1482*(1-2), 157-171.

Fratini, F., Principe, S., Puopolo, M., Ladogana, A., Poleggi, A., Piscopo, P., . . . Confaloni, A. (2012). Increased levels of acute-phase inflammatory proteins in plasma of patients with sporadic CJD. *Neurology, 79*(10), 1012-1018.

Gao, F., Zhang, X., Whang, S., & Zheng, C. (2014). Prognostic impact of plasma ORM2 levels in patients with stage II colorectal cancer. *Annals of Clinical & Laboratory Science, 44*(4), 388-393.

Greten, F. R., & Grivennikov, S. I. (2019). Inflammation and cancer: triggers, mechanisms, and consequences. *Immunity, 51*(1), 27-41.

Hughes, K., & Watson, C. J. (2012). The role of Stat3 in mammary gland involution: cell death regulator and modulator of inflammation. *Hormone molecular biology and clinical investigation, 10*(1), 211-215.

Janciauskiene, S., Wrenger, S., & Welte, T. (2013). Immunoregulatory Properties of Acute Phase Proteins—Specific Focus on α1-Antitrypsin. *Acute Phase Proteins*, 1.

Jo, M., Kim, J.-H., Song, G. J., Seo, M., Hwang, E. M., & Suk, K. (2017). Astrocytic orosomucoid-2 modulates microglial activation and neuroinflammation. *Journal of Neuroscience, 37*(11), 2878-2894.

Jolliet-Riant, P., Boukef, M., Duche, J., Simon, N., & Tillement, J. (1998). The genetic variant A of human alpha 1-acid glycoprotein limits the blood to brain transfer of drugs it binds. *Life sciences, 62*(14), PL219-PL226.

Kamboh, M., & Ferrell, R. (1987). Genetic studies of low-abundance human plasma proteins. VI. Polymorphism of hemopexin. *American journal of human genetics, 41*(4), 645.

Keser, T., Tijardović, M., Gornik, I., Lukić, E., Lauc, G., Gornik, O., & Novokmet, M. (2021). High-throughput and site-specific N-glycosylation analysis of human alpha-1-acid glycoprotein offers a great potential for new biomarker discovery. *Molecular & Cellular Proteomics, 20*.

Konstantopoulos, K., & Thomas, S. N. (2009). Cancer cells in transit: the vascular interactions of tumor cells. *Annual review of biomedical engineering, 11*, 177-202.

Kustán, P., Kőszegi, T., Miseta, A., Péter, I., Ajtay, Z., Kiss, I., & Németh, B. (2018). Urinary orosomucoid a potential marker of inflammation in psoriasis. *International journal of medical sciences, 15*(11), 1113.

Lin, H., Zhang, Q., Li, X., Wu, Y., Liu, Y., & Hu, Y. (2018). Identification of key candidate genes and pathways in hepatitis B virus-associated acute liver failure by bioinformatical analysis. *Medicine, 97*(5).

Luo, Z., Lei, H., Sun, Y., Liu, X., & Su, D.-F. (2015). Orosomucoid, an acute response protein with multiple modulating activities. *Journal of physiology and biochemistry, 71*(2), 329-340.

Mao, K., Tan, Q., Ma, Y., Wang, S., Zhong, H., Liao, Y., . . . Tan, X. (2021). Proteomics of extracellular vesicles in plasma reveals the characteristics and residual traces of COVID-19 patients without underlying diseases after 3 months of recovery. *Cell death & disease, 12*(6), 1-18.

Miranda-Ribera, A., Passaniti, A., Ceciliani, F., & Goldblum, S. E. (2014). α1-Acid glycoprotein disrupts capillary-like tube formation of human lung microvascular endothelia. *Experimental lung research, 40*(10), 507-519.

Nagalla, S. R., Canick, J. A., Jacob, T., Schneider, K. A., Reddy, A. P., Thomas, A., . . . Lambert-Messerlian, G. M. (2007). Proteomic analysis of maternal serum in down syndrome: identification of novel protein biomarkers. *Journal of proteome research, 6*(4), 1245-1257.

Naskar, A., Stezin, A., Dharmappa, A., Hegde, S., Philip, M., Kamble, N., . . . Yadav, R. (2021). Fibrinogen and Complement Factor H are promising CSF protein biomarker (s) for Parkinson's disease with cognitive impairment-A Proteomics and ELISA based study. *bioRxiv*.

Nishi, K., Ono, T., Nakamura, T., Fukunaga, N., Izumi, M., Watanabe, H., . . . Curry, S. (2011). Structural insights into differences in drug-binding selectivity between two forms of human α1-acid glycoprotein genetic variants, the A and F1* S forms. *Journal of Biological Chemistry, 286*(16), 14427-14434.

Plantureux, L., Mège, D., Crescence, L., Dignat-George, F., Dubois, C., & Panicot-Dubois, L. (2018). Impacts of cancer on platelet production, activation and education and mechanisms of cancer-associated thrombosis. *Cancers, 10*(11), 441.

Quaranta, A., Karlsson, I., Ndreu, L., Marini, F., Ingelsson, M., & Thorsén, G. (2019). Glycosylation profiling of selected proteins in cerebrospinal fluid from Alzheimer's disease and healthy subjects. *Analytical Methods, 11*(26), 3331-3340.

Rezaei-Tavirani, M., Nejad, M. R., Arjmand, B., Tavirani, S. R., Razzaghi, M., & Mansouri, V. (2021). Fibrinogen Dysregulation is a Prominent Process in Fatal Conditions of COVID-19 Infection; a Proteomic Analysis. *Archives of Academic Emergency Medicine, 9*(1).

Rojas-Colón, L. A., Dash, P. K., Morales-Vías, F. A., Lebrón-Dávila, M., Ferchmin, P. A., Redell, J. B., . . . Vélez-Torres, W. I. (2021). 4R-cembranoid confers neuroprotection against LPS-induced hippocampal inflammation in mice. *Journal of neuroinflammation, 18*(1), 1-13.

Rucksaken, R., Charoensuk, L., Pinlaor, P., Pairojkul, C., Khuntikeo, N., & Pinlaor, S. (2017). Plasma orosomucoid 2 as a potential risk marker of cholangiocarcinoma. *Cancer Biomarkers, 18*(1), 27-34.

Rucksaken, R., Khoontawad, J., Roytrakul, S., Pinlaor, P., Hiraku, Y., Wongkham, C., . . . Pinlaor, S. (2013). Proteomic analysis to identify plasma orosomucoid 2 and kinesin 18A as potential biomarkers of cholangiocarcinoma. *Cancer Biomarkers, 12*(2), 81-95.

Rudman, D., Treadwell, P. E., Vogler, W. R., Howard, C. H., & Hollins, B. (1972). An abnormal orosomucoid in the plasma of patients with neoplastic disease. *Cancer research, 32*(9), 1951-1959.

Schmid, K. (1950). Preparation and properties of an acid glycoprotein prepared from human plasma. *Journal of the american chemical society, 72*(6), 2816-2816.

Schmid, K., Kaufmann, H., Isemura, S., Bauer, F., Emura, J., Motoyama, T., . . . Nanno, S. (1973). Structure of α1-acid glycoprotein. Complete amino acid sequence, multiple amino acid substitutions, and homology with the immunoglobulins. *Biochemistry, 12*(14), 2711-2724.

Schönfeld, D. L., Ravelli, R. B., Mueller, U., & Skerra, A. (2008). The 1.8-Å crystal structure of α1-acid glycoprotein (Orosomucoid) solved by UV RIP reveals the broad drug-binding activity of this human plasma lipocalin. *Journal of molecular biology, 384*(2), 393-405.

Shu, T., Ning, W., Wu, D., Xu, J., Han, Q., Huang, M., . . . Bie, Y. (2020). Plasma proteomics identify biomarkers and pathogenesis of COVID-19. *Immunity, 53*(5), 1108-1122. e1105.

Smith, K. D., Behan, J., Matthews-Smith, G., & Magliocco, A. M. (2012). Alpha-1-acid glycoprotein (AGP) as a potential biomarker for breast cancer. *Acute phase proteins, regulation and functions of acute phase proteins, 2012*, 201-222.

Smith, S. A., & Waters, N. J. (2019). Pharmacokinetic and pharmacodynamic considerations for drugs binding to alpha-1-acid glycoprotein. *Pharmaceutical research, 36*(2), 30.

Sörensson, J., Matejka, G. r. L., Ohlson, M., & Haraldsson, B. r. (1999). Human endothelial cells produce orosomucoid, an important component of the capillary barrier. *American Journal of Physiology-Heart and Circulatory Physiology, 276*(2), H530-H534.

Sörensson, J., Ohlson, M., Björnson, A., & Haraldsson, B. r. (2000). Orosomucoid has a cAMP-dependent effect on human endothelial cells and inhibits the action of histamine. *American Journal of Physiology-Heart and Circulatory Physiology, 278*(5), H1725-H1731.

Spiller, F., Carlos, D., Souto, F. O., De Freitas, A., Soares, F. S., Vieira, S. M., . . . Cunha, F. Q. (2012). α1-Acid glycoprotein decreases neutrophil migration and increases susceptibility to sepsis in diabetic mice. *Diabetes, 61*(6), 1584-1591.

Stein, T., Morris, J. S., Davies, C. R., Weber-Hall, S. J., Duffy, M.-A., Heath, V. J., . . . Gusterson, B. A. (2004). Involution of the mouse mammary gland is associated with an immune cascade and an acute-phase response, involving LBP, CD14 and STAT3. *Breast Cancer Research, 6*(2), 1-17.

Stroka, K. M., & Konstantopoulos, K. (2014). Physical biology in cancer. 4. Physical cues guide tumor cell adhesion and migration. *American Journal of Physiology-Cell Physiology, 306*(2), C98-C109.

Sun, Y., Wang, F., Zhou, Z., Teng, J., Su, Y., Chi, H., . . . Liu, T. (2020). Urinary Proteomics Identifying Novel Biomarkers for the Diagnosis of Adult-Onset Still's Disease. *Frontiers in immunology, 11*, 2112.

Sun, Y., Zhang, Z. X., & Liu, X. (2016). Orosomucoid (ORM) as a potential biomarker for the diagnosis of chronic fatigue syndrome (CFS). *CNS neuroscience & therapeutics, 22*(3), 251.

Suresh, C., Saha, A., Kaur, M., Kumar, R., Dubey, N., Basak, T., . . . Batra, V. V. (2016). Differentially expressed urinary biomarkers in children with idiopathic nephrotic syndrome. *Clinical and experimental nephrology, 20*(2), 273-283.

Taguchi, K., Nishi, K., Chuang, V. T. G., Maruyama, T., & Otagiri, M. (2013). *Molecular aspects of human alpha-1 acid glycoprotein—structure and function*: InTech.

Uribe-Querol, E., & Rosales, C. (2015). Neutrophils in cancer: two sides of the same coin. *Journal of immunology research, 2015*.

Wan, J. J., Wang, P. Y., Zhang, Y., Qin, Z., Sun, Y., Hu, B. H., . . . Liu, X. (2019). Role of acute-phase protein ORM in a mice model of ischemic stroke. *Journal of cellular physiology, 234*(11), 20533-20545.

Wu, L., & Zhang, X. H.-F. (2020). Tumor-Associated Neutrophils and Macrophages—Heterogenous but Not Chaotic. *Frontiers in immunology, 11*.

Yang, L., Rudser, K., Golnik, A., Wey, A., Higgins, L., & Gourley, G. (2016). Urine protein biomarker candidates for autism. *J Proteomics Bioinform, 14*, 004.

Zhang, D., Huang, J., Luo, D., Feng, X., Liu, Y., & Liu, Y. (2017). Glycosylation change of alpha-1-acid glycoprotein as a serum biomarker for hepatocellular carcinoma and cirrhosis. *Biomarkers in medicine, 11*(5), 423-430.

Zhang, S., & Mark, K. S. (2012). α1-Acid glycoprotein induced effects in rat brain microvessel endothelial cells. *Microvascular research, 84*(2), 161-168.

Zhang, X., Wang, K., Yang, Q., Wang, J., Xuan, C., Liu, X. C., . . . He, G. W. (2015). Acute phase proteins altered in the plasma of patients with congenital ventricular septal defect. *PROTEOMICS–Clinical Applications, 9*(11-12), 1087-1096.

Zhang, X., Xiao, Z., Liu, X., Du, L., Wang, L., Wang, S., . . . Zhang, X. (2012). The potential role of ORM2 in the development of colorectal cancer. *PLoS One, 7*(2).

Zhou, X.-h., Liu, S.-y., Feng, L., Yang, B., Li, Y.-f., Wang, X.-q., . . . Wang, H.-h. (2020). Urinary orosomucoid and retinol binding protein levels as early diagnostic markers for diabetic nephropathy.

Zhu, H.-Z., Zhou, W.-J., Wan, Y.-F., Ge, K., Lu, J., & Jia, C.-K. (2020). Downregulation of orosomucoid 2 acts as a prognostic factor associated with cancer-promoting pathways in liver cancer. *World journal of gastroenterology, 26*(8), 804.