

REVIEW ARTICLE

Neurological manifestations, thrombosis, and cancer in Arg393His antithrombin Hanoi

Khue Vu Nguyen^{1,2,3,4*} 

¹School of Medical Imaging, Jiangsu Medical College, Yancheng, Jiangsu, China

²Medical Imaging Institute of Jiangsu Medical College, Yancheng, Jiangsu, China

³Center for Molecular Biophysics, CNRS Orleans, Orleans, Loiret, France

⁴School of Medicine, Departments of Medicine and Pediatrics, Biochemical Genetics and Metabolism, The Mitochondrial and Metabolic Disease Center, University of California, San Diego, California, United States of America

Abstract

Antithrombin (AT) deficiency is associated with the highest risk of thrombotic events in both veins and arteries, which underlie strokes, heart attacks (myocardial infarction), peripheral artery disease, and even certain cancers. This review provides (i) a family case describing a “two-way association” between cancer—including kidney cancer, melanoma—and thrombosis such as stroke (encephalomalacia/gliosis) and deep venous thrombosis arising from a heterozygous Arg393His mutation in AT-Hanoi; (ii) a discussion concerning the multifaceted roles of AT beyond its anticoagulant function, including its involvement in host defense, inflammation, anti-angiogenesis and tumorigenesis, establishing AT as a pleiotropic protein; and (iii) perspectives exploring the potential implication of the β -amyloid precursor protein gene in the development of neurological features, thrombotic events and cancer, followed by a discussion on different types of immunotherapy used for the treatment of cancer, such as chimeric antigen receptor T-cell therapy and immune checkpoint inhibitor, as well as on the potential application of sonogenetics for non-invasive therapy of human diseases. This Arg393His point mutation in AT-Hanoi could be selected as a model of a rare hereditary blood-clotting disorder for the development of valuable drugs to modulate the coagulation cascade and thereby prevent the consequences of blood-clotting disorders that are partly responsible for neurological and cardiovascular diseases, as well as cancer.

*Corresponding author:

Khue Vu Nguyen
(khuenguyen52@yahoo.com)

Citation: Nguyen KV. Neurological manifestations, thrombosis, and cancer in Arg393His antithrombin Hanoi. *Brain & Heart*. 2026;4(3):025450071.
doi: 10.36922/BH025450071

Received: November 4, 2025

Revised: January 14, 2026

Accepted: February 11, 2026

Published online: April 8, 2026

Copyright: © 2026 Author(s). This is an Open-Access article distributed under the terms of the Creative Commons Attribution License, permitting distribution, and reproduction in any medium, provided the original work is properly cited.

Publisher's Note: AccScience Publishing remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Keywords: Arg393His antithrombin Hanoi; *BRAF* mutation; β -amyloid precursor protein; CAR T-cell therapy; Expression vectors via glycosylphosphatidylinositol; Immune checkpoint inhibitor; Melanoma; Sonogenetics

1. Introduction

A neurological disorder is any disorder of the nervous system and may present with different symptoms such as seizures, stroke, or dementia. Neurological disorders are the second leading cause of death worldwide, after cardiovascular diseases. According to a report on disability-adjusted life years related to neurological disorders, there was an 18% increase over the past 31 years, rising from 375 million years of healthy life lost in 1990

to 443 million in 2021. The most prevalent neurological disorders include stroke, neonatal encephalopathy (brain injury), migraine, Alzheimer's disease and other dementias, diabetic neuropathy (nerve damage), meningitis, epilepsy, neurological complications from preterm birth, autism spectrum disorder, and nervous system cancer. Over 80% of neurological deaths occur in low- and middle-income countries.¹⁻³ The most common presentation of cerebrovascular disease is cerebrovascular accident (CVA stroke), including ischemic stroke or mini-stroke (cell death resulting from a thrombosis, in which a blood clot forms inside a blood vessel), and hemorrhagic stroke (bleeding into or around the brain resulting from rupture of a cerebral blood vessel).^{4,5} Notably, inherited thrombophilias, including deficiencies of protein C, protein S, and antithrombin (AT), as well as factor V Leiden mutation and prothrombin G20210A mutations, predispose affected individuals to thrombophilia (a tendency to develop thrombosis) with venous thromboembolism (VTE), including deep venous thrombosis (DVT; a blood clot in a deep vein, most commonly in the lower limbs such as the femoral vein), and pulmonary embolism (PE; a blood clot lodged in the pulmonary vasculature). Portal/mesenteric vein thrombosis (PVT/MVT) and CVA have also been reported.⁶

Cancer is considered the second leading cause of mortality worldwide, after cardiovascular diseases.⁷ More than 35 million new cancer cases are predicted by 2050.⁸ Cancer is a major risk factor for VTE and arterial thromboembolism (ATE), particularly VTE.⁹ The relationship between cancer and thrombosis dates back to 1865, when Dr. Armand Trousseau described an association between gastric cancer and VTE.¹⁰ Since this initial report, numerous studies have demonstrated a two-way clinical association between cancer and thrombosis, mainly involving thrombotic complications such as VTE in the cancer population. Cancer not only induces dysregulation of the hemostatic system, but components of the hemostatic system are also actively involved in cancer pathogenesis.^{9,11-14} However, there is limited evidence regarding the risk of occult cancer (cancer in which the primary tumor site cannot be identified) among patients with idiopathic (unprovoked, clot occurring without an obvious cause) and secondary VTE (provoked, with a clear trigger such as surgery, hospitalization, pregnancy, estrogen therapy, reduced mobility for up to three days, or genetic conditions).¹⁵ To date, available data are derived mainly from population-based cohort studies.¹⁶⁻¹⁸ However, these studies have limitations, including the inability to establish causality and uncertainty regarding cancer stage at diagnosis. In addition, an interconnection between cancer and neurodegenerative diseases has been

reported.^{19,20} To explore external and internal factors contributing to the onset of cancer and dementia, several analytical methods have been developed, including atomic force microscopy¹⁹ and positron emission tomography (PET) imaging.²⁰ However, understanding and identifying the molecular mechanisms underlying these diseases as biomarkers remains challenging because of high levels of polymorphism and nanoscale dimensions of potential biomarkers.

Among blood-clotting disorders, AT deficiency—although rare in the general population, with a prevalence of heterozygous AT deficiency ranging from 0.02% to 0.2%²¹—confers one of the highest risks of VTE among hereditary thrombophilias. Clinical manifestations vary, but the first thrombotic events usually occur before the age of 40, with DVT and PE being the most common presentations. Although less frequent, thrombosis can also occur in cerebral veins (VTE), leading to CVA, as well as in the liver and intestines (PVT and MVT), kidneys (renal vein thrombosis), and arteries (ATE), which underlie heart attacks (myocardial infarction) and strokes, and peripheral artery disease.^{22,23} However, the relationship between AT deficiency and cancer-associated thrombosis remains unclear. Cornelia *et al.*²⁴ reported no association across most cancer types, except for brain tumors.

This review provides (i) a family case describing thrombosis, including stroke (encephalomalacia/gliosis), DVT, together with a “two-way association” between cancer, such as kidney cancer, melanoma, and thrombosis from a heterozygous Arg393His mutation in AT-Hanoi; (ii) a discussion of AT functions beyond anticoagulation, including signaling roles in host defense, inflammation, anti-tumorigenic, and anti-angiogenesis, thereby defining AT as a pleiotropic protein; and (iii) perspectives on the constructions of expression vectors via a glycosylphosphatidylinositol (GPI) anchor to study intermolecular interactions, which may be useful for exploring the potential implication of the β -amyloid precursor protein (*APP*) gene in the development of neurological features, thrombotic events and cancer. In addition, the review discusses different types of cancer immunotherapy, such as chimeric antigen receptor T-cell therapy (CAR T-cell therapy), immune checkpoint inhibitors (ICIs), as well as the potential application of sonogenetics for non-invasive therapy of human diseases.

2. Updated case history of the proband and family with Arg393His antithrombin Hanoi

Herein, we present an updated version of the previous report describing a heterozygous point mutation Arg393His (R393H), located in exon 7 of AT, named

AT-Hanoi after the city in Viet Nam where the proband (II-2) was born. This mutation is associated with the highest risk of thrombotic events.²⁵ In addition to a CVA with encephalomalacia/gliosis at the age of 23 (with no specific treatments) (Figure 1), DVT of the leg, and kidney cancer at the age of 42 were observed in the proband (II-2).²⁵ He is currently 73 years old and in very good health. He remains under long-term treatment with warfarin (3 mg/day) to prevent blood clots, maintaining an international normalized ratio between 2 and 3, as well as atorvastatin (10 mg/day) to prevent hypercholesterolemia and atherosclerosis. He is free of cancer, having undergone surgical removal of the affected kidney followed by more than 31 years of surveillance, which has revealed no detectable cancer recurrence. Otherwise, age, race, and sex are not considered confounding factors in this case of thrombosis. The most important recent event in this family concerns the son (III-4), aged 39 years, of the proband (II-2). He developed a cutaneous melanoma originating from a mole on the back, which subsequently spread to the lymph nodes (stage III) (Figure 2). Following surgical removal

of the mole and three cancer-affected lymph nodes, he was treated with ICI (pembrolizumab) administered by intravenous infusion, during which common adverse reactions such as eczema and poliosis were observed. After three cycles of ICI therapy over a two-month period, he subsequently received combination treatment with dabrafenib (Tafinlar) targeting the proto-oncogene B-Raf gene (*BRAF* V600E (T1799A) mutation²⁶ and trametinib (Mekinist) (an inhibitor of mitogen-activated protein kinase enzymes) for one year to prevent melanoma recurrence. Notably, somatic missense mutations in the *BRAF* gene are found in approximately 66% of malignant melanomas and at a lower frequency across a wide range of other human cancers.²⁶ He is currently undergoing follow-up care with PET scanning to monitor for cancer recurrence or metastasis.

3. Characteristics of human AT

In addition to its anticoagulant function,²⁵ AT also possesses potent anti-inflammatory, anti-angiogenic, and anti-tumorigenic activities.

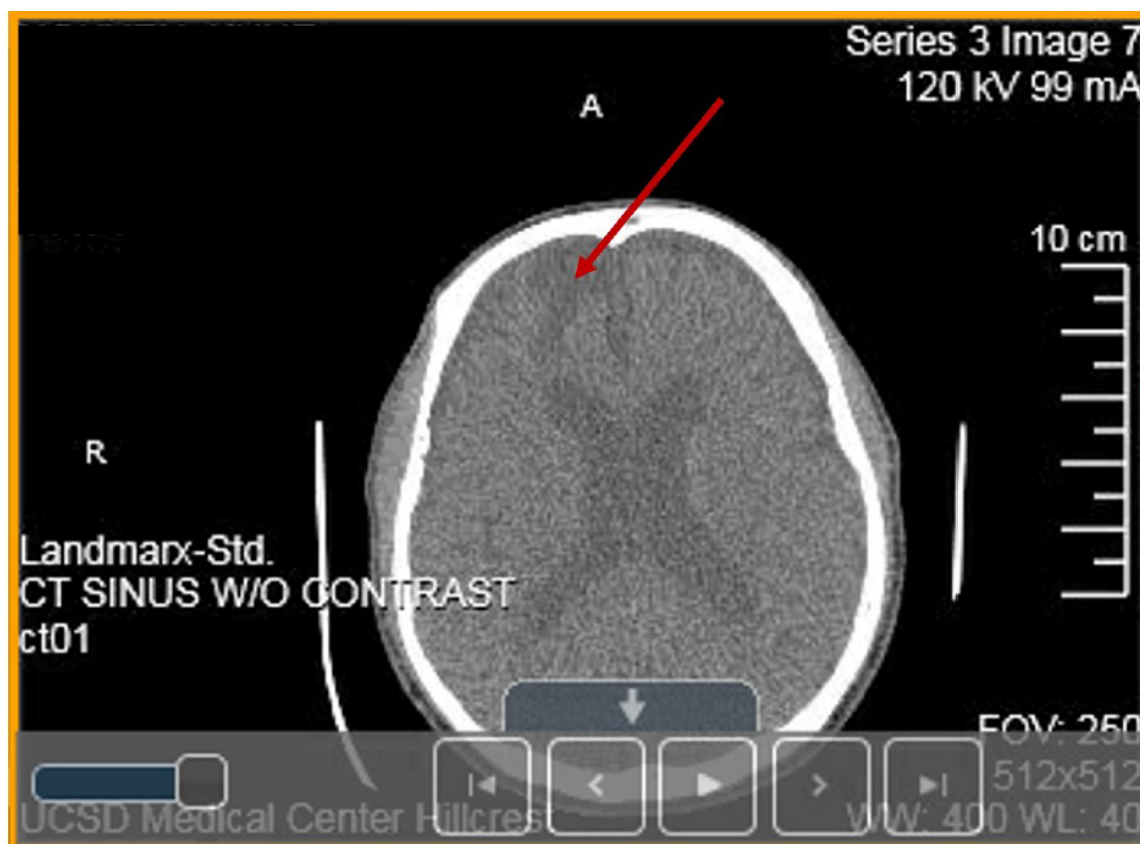


Figure 1. Non-contrast cerebral computed tomography scans of the paranasal sinuses, acquired with 0.625 mm axial slices, and reformatted in the coronal and sagittal planes. Limited portions of the brain are visualized, showing encephalomalacia/gliosis in the anterior right frontal lobe, suggestive of sequelae from prior trauma (dark area at the top-left corner, red arrow). Image adopted from a study published by Nguyen.²⁵

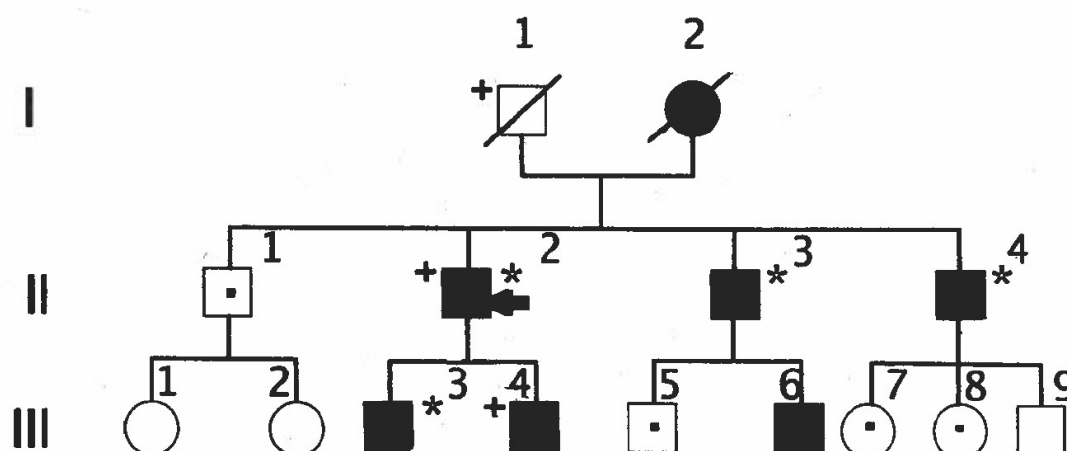


Figure 2. Pedigree of the family. The proband (II-2) is indicated by an arrow.

Notes: * denotes thrombosis; + denotes cancer; solid symbols denote presence of heterozygous Arg393His point mutation; dotted symbols denote absence of heterozygous Arg393His point mutation; open symbols denote not investigated; dashed symbols denote deceased; Male □; Female ○.

3.1. Anticoagulation

For details, please refer to Nguyen's review.²⁵ The basic concepts are summarized below.

3.1.1. Gene structure

Human AT is a heparin-binding serpin (heparin cofactor) and a serine protease inhibitor. It is encoded by the serpin peptidase inhibitor, clade C, member 1 gene, located on chromosome 1 (q23–25), and consists of seven exons. AT is a glycoprotein synthesized in the liver, with a molecular weight of 58,200 daltons and a length of 432 amino acids. It has four possible glycosylation sites at asparagine (Asn) residues 96, 135, 155, and 192.

3.1.2. Allosteric activation

Human AT is the major plasma inhibitor of activated proteases in both the intrinsic and extrinsic coagulation pathways, including factor II (thrombin IIa), factor X (Xa), factor IX (IXa), and factor VII (VIIa). Protease inactivation occurs between arginine 393 (P1) and serine 394 (P1'), and is termed the reactive site loop (RSL) or reactive center loop (Figure 3). It has two specific binding sites: the RSL site binds proteases such as thrombin (Factor IIa), Factor Xa, and Factor IXa, and the other site binds heparin (a linear sulfated polysaccharide belonging to the glycosaminoglycan [GAG] family). Heparin alone has no direct anticoagulant effect. The native state of AT inactivates these proteases ineffectively (due to conformational inaccessibility of the P1–P1' bond). Inhibition is accelerated approximately 1,000-fold by the binding of heparin to arginine and lysine

residues in the D-helix motif located on the surface of AT that results in the conformational change (allosteric activation) of the P1–P1' loop and exposure of the P1–P1' reactive center, ultimately inhibiting blood coagulation. The minimum heparin structure required to induce the conformational change in AT is a specific pentasaccharide (H5) sequence, AGA*IA ($A_{NAc,6S}$ -GlcUA- $A_{NS,3,6S}$ -I_{2S}- $A_{NS,6S}$) containing 3-O sulfate, 3-OS, modification.²⁷ Binding of the negatively charged 3-OS sulfate of H5 sequence to the positively charged arginine and lysine residues in the D-helix motif induces a conformational change that activates AT for inhibiting proteases (Figure 4).^{28,29} Notably, the H5 sequence is sufficient to accelerate the inhibition of factor Xa, while the inhibition of thrombin requires a bridging contribution from heparin, resulting in the formation of a trimolecular complex: AT, thrombin, and the heparin species (Figure 4).^{28–30} This mechanism of allosteric activation of AT by heparin has been suggested to occur via heparan sulfate proteoglycan receptors (with GAGs containing the H5 sequence and 3-OS modification) present on the surface of endothelial cells lining the vessel wall.²⁸

3.1.3. Isoforms

There are different isoforms of AT,²⁵ including normal or native conformation state (native AT), latent state (L-AT), and prelatent AT, which represents an intermediate between native AT and L-AT. AT circulates initially in its native form. The transition from native AT to L-AT is irreversible and can be induced by heating at 50–60 °C; at physiological temperature (37 °C), approximately 10% of

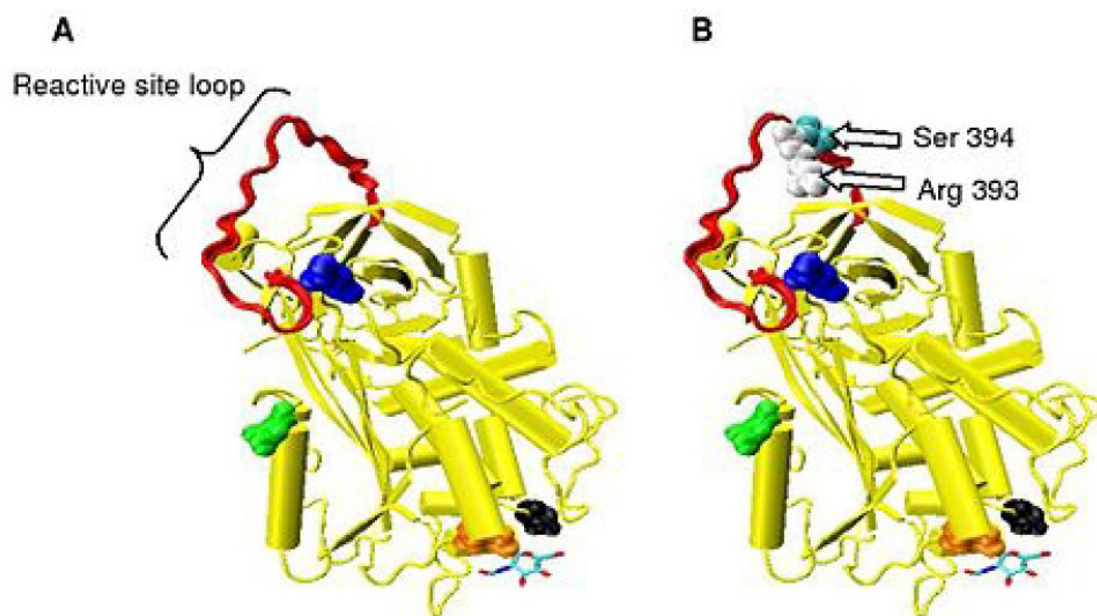


Figure 3. The reactive Arg393–Ser394 bond is located on an exposed loop at the surface of the molecule. This loop is called the reactive site loop or reactive center loop.

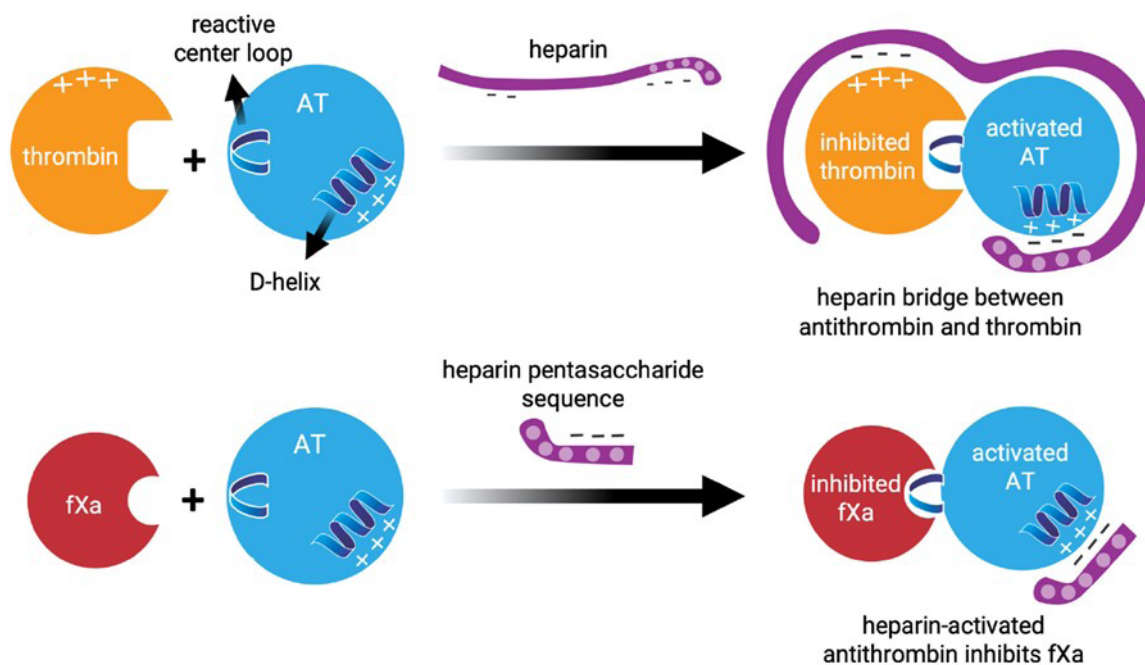


Figure 4. The anticoagulant effect of heparin occurs via the binding of antithrombin (AT) with thrombin and activated factor X (fXa). Image adopted from a study published by Rodgers and Mahajerin.²⁹

circulating AT in the blood is converted to L-AT over a 24-h period.

In plasma, AT circulates as two glycoforms: α -AT (90–95%) and β -AT (5–10%). β -AT binds heparin with a 5–10-fold higher affinity²⁸ because α -AT is fully glycosylated at all

four sites, whereas β -AT lacks glycosylation at Asn-135.²⁸ Although β -AT constitutes only a minor fraction (5–10%) of the total plasma AT, its higher heparin affinity suggests that it may play a more disproportionately important role than α -AT in controlling thrombotic events.³¹

3.1.4. AT deficiency

Antithrombin deficiency may be inherited (autosomal dominant) or acquired.²⁵ Numerous mutations in the AT gene can lead to inherited AT deficiency, most commonly in the heterozygous state. Homozygous AT deficiency is extremely rare and usually lethal, presenting as neonatal thrombosis.²⁵ There are two types of inherited AT deficiency. Type I, which is quantitative, resulting from heterozygous point mutation or major gene deletions leading to low AT antigen and activity levels. Type II, the more common, is qualitative and is characterized by normal AT levels and reduced function (typically below 70% of normal). Mutations affecting the N-glycosylation pathway (up to 27%) can also lead to hypoglycosylation, resulting in reduced AT levels and anticoagulant activity due to an increased proportion of 3-N-glycan AT in plasma.²⁵ Because of this genetic heterogeneity, genetic testing is not feasible in routine clinical practice, and AT deficiency is often identified only after recurrent VTE and PE. The three main causes of acquired AT deficiency are liver failure (e.g., liver cirrhosis because AT is made in the liver), kidney disease (e.g., nephrotic syndrome, causing urinary loss of AT), and disseminated metastatic cancer. The association with additional genetic variants, such as factor V Leiden mutation or factor II mutation (prothrombin G20210A), further increases thrombotic risk. Other acquired thrombotic factors, including pregnancy, obesity, smoking, and cancer, also increase thrombotic risk.^{25,32-34} All forms of AT deficiency increase the risk for VTE and, much less commonly, arterial thrombosis.

3.1.5. Diagnosis

Diagnosis of AT deficiency is based on blood testing. The preferred initial test is the “AT activity” or “functional AT” assay, which determines whether circulating AT is functionally active and helps avoid missing type II deficiency.³⁵ If the AT function is abnormal, AT antigen levels are subsequently measured (AT antigen assay determines how much of AT is present in the blood) to distinguish between type I and type II deficiencies.

3.1.6. Treatment

Heparin is the initial treatment for thrombosis in patients with AT deficiency, followed by a maintenance treatment, usually with an oral anticoagulant. Like all medications, heparin can cause severe side effects,³⁶ including irritation, pain, redness, or sores at the injection site, as well as allergic reactions such as hives, chills, and fever.³⁶ Administration of heparin requires careful consideration of the patient's clinical condition, weight, and indication for treatment.³⁷ For example, in patients weighing less than 50 kg, a common

intravenous heparin loading dose is 80 units/kg, followed by a maintenance intravenous dose of 18 units/kg/h. In patients weighing more than 100 kg, a typical loading dose is 10,000 units, followed by a maintenance intravenous dose of 1,800 units/h. Dose adjustments are guided by regular blood test results, such as activated partial thromboplastin time³⁸ or heparin anti-Factor Xa assay.³⁹ For oral anticoagulants, vitamin K antagonists, such as warfarin, remain commonly used.²⁵ Low-molecular-weight heparins (LMWHs) and direct oral anticoagulants (DOACs) appear to be similarly effective.^{25,28} In patients with inherited AT deficiency who experience an acute thrombotic event and show inadequate response to intravenous heparin, direct thrombin inhibitors (e.g., dabigatran) and factor Xa inhibitors (inhibition of factor Xa prevents thrombin generation and thrombus development, e.g., rivaroxaban, apixaban, and edoxaban) are recommended. Correction of AT levels using AT concentrate is recommended for planned major surgery.^{25,28}

3.2. Host defense and inflammation

Antithrombin (both AT isoforms) contributes to the host defense mechanism through antimicrobial activity mediated by its ability to bind lipopolysaccharide, also known as endotoxin, from Gram-negative bacteria.^{40,41} While intact AT itself lacks direct antimicrobial activity, degradation of AT by endogenous and bacterial enzymes generates AT-derived antimicrobial peptides from the D-helix region, which exhibit antimicrobial activity against bacteria (Gram-negative and Gram-positive bacteria), fungi, and parasites such as *Plasmodium falciparum*, the causative agent of cerebral malaria.⁴¹ AT also appears to possess anti-viral properties against viruses such as herpes simplex virus-1 and human immunodeficiency virus-1,⁴¹ although the underlying mechanisms remain incompletely understood and may involve heparin-mediated interactions.⁴¹ In addition to host defense mechanisms, AT exerts anti-inflammatory activity by stimulating the release of molecules that reduce inflammation. Syndecan-4 has been proposed for such an effect.⁴⁰

3.3. Anti-angiogenesis and tumorigenesis

Many cancers exploit angiogenic mechanisms to promote tumor growth.⁴² AT exhibits potent anti-angiogenic and anti-tumor activities. However, these effects are not observed with native AT. Only cleaved AT and L-AT of AT display anti-angiogenic properties.⁴³⁻⁴⁵ The prelatent form of AT has demonstrated anti-angiogenic activity *in vitro*, although this effect has not yet been confirmed in experimental animal models. The precise mechanisms underlying AT-mediated anti-angiogenesis are not fully defined, but endothelial cells are likely primary targets.⁴⁶

4. Discussion

4.1. Two-way association

People with AT deficiency may have disturbances in the balance between clot formation and bleeding, and are consequently predisposed to cancer development. Indeed, in addition to the presence of encephalomalacia/gliosis (Figure 1), thromboembolic manifestations such as DVT of the leg can be a presenting sign of cancer (as observed in the proband II-2 with kidney cancer, which came to light following warfarin-associated bleeding at the tumor site^{25,47}), or may even be asymptomatic in individuals with AT deficiency (as in case III-4 with melanoma) (Figure 2). These findings, for the first time via a defined genetic trait, demonstrate a “two-way association” between cancer development and thrombosis related to AT deficiency caused by the Arg393His AT-Hanoi mutation, resulting in provoked blood clots and reflecting the close relationship between clot formation and cancer development.^{9,11-14,25,48} In this context, several anticoagulants have been reported for the prevention and treatment of VTE in patients with cancer, including LMWHs such as enoxaparin,⁴⁹ asparaginase-based chemotherapy,⁵⁰ and DOACs such as edoxaban and rivaroxaban,⁵¹ and apixaban.⁵² With regard to safety, common side effects include bleeding and nausea. Further development of cancer-associated anticoagulant therapy to improve efficacy while reducing side effects remains necessary.

4.2. Biomarkers of hypercoagulability and cancer

A number of candidate biomarkers of hypercoagulable state have been developed for VTE prediction in cancer, including platelet and leukocyte counts, duplex ultrasound, and the Khorana score.⁵³⁻⁵⁶ However, the results obtained thus far remain challenging and insufficient for routine clinical implementation.

4.3. Epistasis and gene–environment interactions

Epigenetic processes may partially explain the highly variable thrombotic manifestations observed in AT deficiency, ranging from asymptomatic individuals (case of I-2, deceased at age 97) to DVT, CVA, and even cancer (cases of proband II-2 and III-4) (Figure 2). To date, however, no proven epigenetic abnormalities involving DNA methylation, histone modification, or microRNA-associated silencing have been identified in AT deficiency. Nonetheless, the role of epistasis (gene–gene interactions), such as the presence of factor V Leiden and prothrombin G2010A mutations in AT deficiency (co-existence of mutations)^{25,34,57-60} as well as gene–environment interactions, including cancer, surgery, pregnancy, hospitalization, diet,

obesity, physical activity, and air pollution,^{25,32,33,61-63} should be emphasized as additional risk factors for VTE and severe thrombotic events. Understanding how epistasis and gene–environment interactions contribute to disease phenotype remains challenging,^{64,65} particularly in VTE, but is essential for targeted prevention and treatment strategies. In this context, the *APP* gene could act as a modifier gene and, via alternative splicing, may be qualitatively and quantitatively altered in the presence of the Arg393His mutation. This mechanism could contribute to the development of neurological features, thrombotic events, and cancer, thereby explaining differences in disease severity among individuals with AT deficiency.^{25,66-75} Furthermore, the roles of arginine-to-histidine substitution⁷⁶ and the *BRAF* V600E missense mutation²⁶ in cancer development, particularly melanoma, in relation to the Arg393His (R393H) in AT-Hanoi warrant further investigation.

4.4. Chimeric antigen receptor T-cell therapy

Chimeric antigen receptor T-cell therapy is a personalized form of immunotherapy that uses a patient's own immune cells to recognize and destroy cancer. It represents a powerful therapeutic option for certain hard-to-treat blood cancers, particularly when conventional treatments are no longer effective. The standard approach involves harvesting T cells from a patient's blood, followed by genetic modification to express a chimeric antigen receptor (CAR) that specifically binds an antigen expressed on the patient's tumor cell but not on healthy cells. This genetic modification is typically achieved using vectors derived from engineered lentiviruses, such as human immunodeficiency virus.⁷⁷ Large numbers of CAR T cells are subsequently expanded *in vitro* and reinfused into the patient to target and attack tumor cells.⁷⁸ These modified T cells function as a “living drug” by selectively recognizing antigens on cancer cells.⁷⁹ CAR T cells can be administered as autologous products derived from the patient's own T cells or as allogeneic products from a donor. Several CAR T-cell therapies have been approved by the United States Food and Drug Administration for indications such as acute lymphoblastic leukemia and diffuse large B-cell lymphoma,^{80,81} while others remain under investigation in clinical trials. CAR T-cell therapy offers advantages, including high response rates in refractory cancers, the potential for longer-term remission due to persistence of engineered CAR T-cells, and a highly personalized treatment approach because it uses the body's own T cells. However, it may also cause severe side effects, including cytokine release syndrome, immune effector cell–associated neurotoxicity syndrome, and insertional oncogenesis risk associated with retroviral vector use.⁷⁹

5. Perspectives

Antithrombin exerts multiple roles beyond hemostasis, including functions in host defense, inflammation, anti-angiogenesis, and tumorigenesis, and can therefore be defined as a pleiotropic protein.⁸² This study represents a valuable example of a genetic trait resulting in pleiotropy, in which a single gene exerts effects on multiple phenotypic traits. From this perspective, the following approaches merit further investigation.

5.1. Exploration of the potential implications of the β -amyloid precursor protein gene in the development of neurological manifestations, thrombotic events, and cancer

Understanding the multifaceted roles of AT in intracellular functions related to cell signaling beyond its anticoagulant function, such as in host defense, inflammation, anti-angiogenesis, and tumorigenesis, in relation to *APP*, could lead to novel therapeutic approaches.^{25,66-75} To this

end, exploration of intermolecular interactions between AT and *APP* is needed. Accordingly, the construction of expression vectors for AT and *APP* using a GPI anchor has been performed as described previously²⁵ (Figure 5). In addition, the construction of expression vectors for AT and *BRAF* via the GPI anchor would be useful for investigating the impact of the *BRAF* V600E mutation²⁶ on cancer development, particularly melanoma, in relation to AT deficiency.

5.2. Selection of anti-cancer medication

5.2.1. For use in immunotherapy via an immune checkpoint inhibitor

Cancer treatment includes several modalities such as surgery, chemotherapy, radiation therapy, immunotherapy, and targeted drug therapy. Depending on specific clinical factors, including cancer type, stage, and patient health status, one or a combination of these approaches is selected. Conventional chemotherapeutic agents are typically

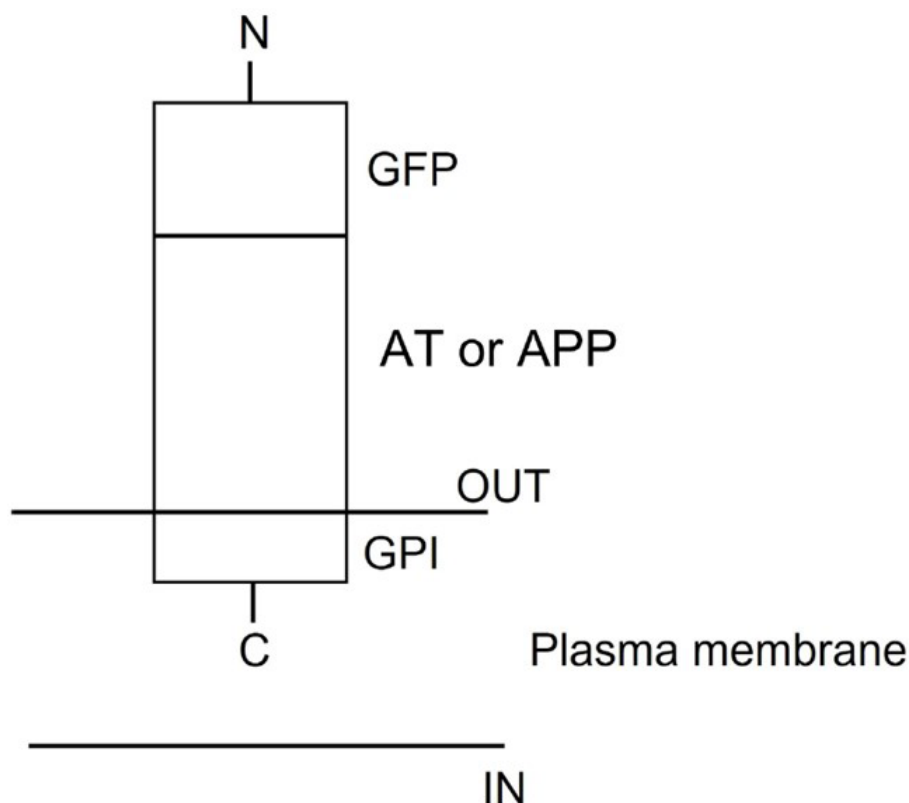


Figure 5. Schematic presentation of the membrane topology of the expression vectors for human antithrombin (AT) and β -amyloid precursor protein (APP). The mammalian expression vector pcDNATM 3.1 (+) was used as the backbone, with all genes of interest inserted in the right frame into the pcDNATM 3.1 (+) vector. The construct comprises the C-terminal glycosyl-phosphatidylinositol (GPI) anchor sequence derived from the human folate receptor protein, and the entire coding sequence (CDS) of the *AT* or *APP* gene fused to the CDS of the green fluorescent protein (GFP) gene. Image adopted from a study published by Nguyen.²⁵

cytotoxic drugs that indiscriminately affect rapidly dividing cells.⁸³ In contrast, targeted therapy focuses on molecular alterations specific to cancer cells, such as proteins or genes that drive cancer growth, thereby offering a highly precise approach leading to fewer side effects. The two most common forms of targeted therapy are monoclonal antibodies and small-molecule inhibitors. However, the success of targeted therapy largely depends on the presence of certain biomarkers for early cancer detection and treatment, making genetic testing an essential part of the treatment plan. Despite these advances, substantial toxicities, including bleeding, hair discoloration, dry skin, and skin yellowing, are still observed, and many cancers develop resistance to targeted agents over time.⁸³ Immunotherapy is a distinct yet related class of targeted therapy aimed at enhancing host response to the tumor by enabling more effective cancer recognition and elimination. It has become a cornerstone of modern cancer treatment. Recently, six approved monoclonal antibodies for ICI

therapies—pembrolizumab and nivolumab targeting the programmed cell death 1 protein; atezolizumab, durvalumab, and avelumab targeting the protein programmed death-ligand 1; and ipilimumab targeting the cytotoxic T lymphocyte-associated antigen 4 (allowing the cytotoxic T cells of the adaptive immune system to kill cancer cells)—as well as combination therapies involving these drugs, have been reported.⁸³⁻⁸⁷ Although ICI therapies have shown improved outcomes in many cancer patients, increasing evidence indicates increased risks of toxic side effects related to thromboembolic events, referred to as cancer-associated thrombosis.⁸³⁻⁸⁵ In this context, the construction of expression vectors for immune checkpoint proteins via a GPI anchor, as previously described,⁸⁸ would be useful for selecting anticancer medications for ICI-based immunotherapy (Figure 6). In addition, such expression systems could serve as models for targeted-therapy drug selection and for evaluating interactions between CARs and tumor-associated antigens in CAR T-cell therapy.

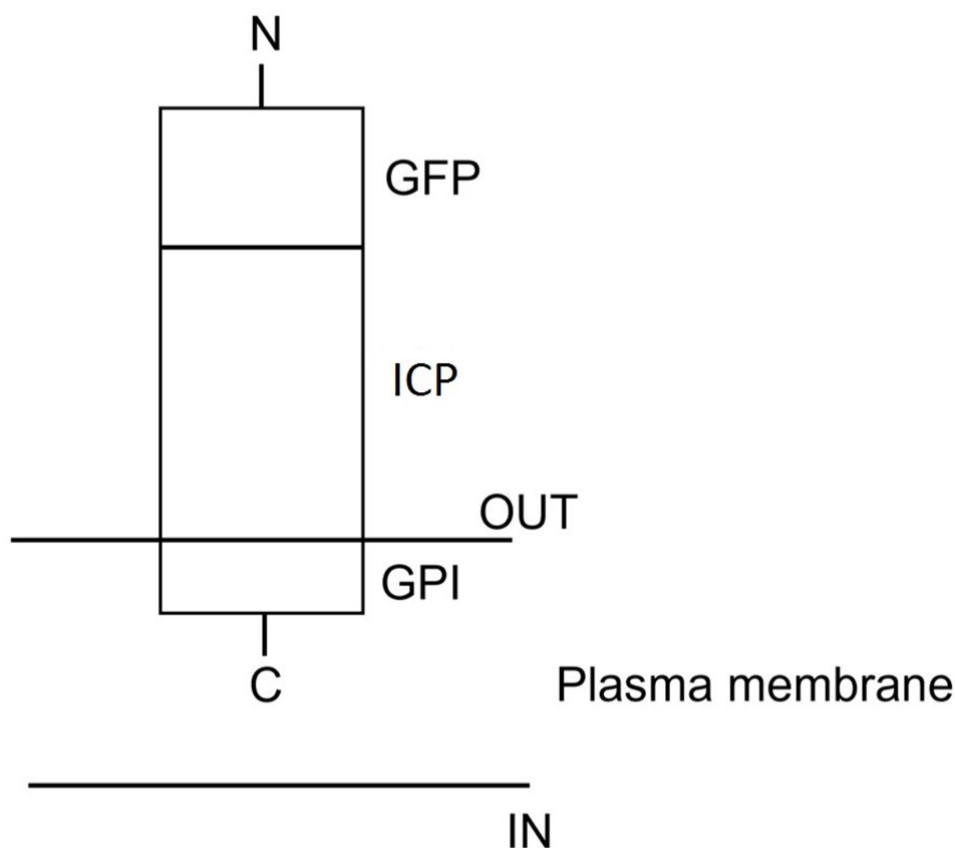


Figure 6. Schematic presentation of the membrane topology of the expression vector for immune checkpoint protein (ICP). The mammalian expression vector pcDNATM 3.1 (+) was used as the backbone, with all genes of interest inserted in the right frame into the pcDNATM 3.1 (+) vector. The construct comprises the C-terminal sequence of the glycosylphosphatidylinositol (GPI) anchor derived from the human folate receptor 1 (FOLR1) protein, and the entire coding sequence (CDS) of the *ICP* gene fused with the CDS of the green fluorescent protein (*GFP*) gene. Image adapted from a study published by Nguyen.⁸⁸

5.2.2. Use of anticoagulant (warfarin)

Warfarin, a competitive inhibitor of vitamin K epoxide reductase, inhibits blood clot formation and may also interfere with tumor growth and dissemination in cancer patients. Warfarin has been suggested to possess additional anti-tumor effects by modulating host immune defense mechanisms,^{48,89} as observed in the case of the proband II-2.²⁵ However, large-scale randomized controlled clinical trials evaluating warfarin use in cancer patients are still required. While anticoagulation remains a cornerstone of treatment, cancer-associated thrombosis management remains complex.

5.2.3. Combination therapy

Tumor cells can evolve new mechanisms to evade immune surveillance following initial immune responses, such as secreting immunosuppressive factors or altering antigen expression, thereby limiting recognition and elimination by conventional T cells. Consequently, combination therapy involving warfarin, engineered CAR T cells, and ICIs may provide therapeutic benefit for cancer patients. For example, combination strategies using CAR T-cell therapy with ICIs have already been reported.⁹⁰

5.3. Sonogenetics

Sonogenetics, a field that combines genetic engineering and ultrasound technology to activate sonosensitive mediators (SSMs) in specific cells, has recently emerged as a novel approach for controlling biomolecular functions such as gene expression and cellular signaling. This strategy enables precise modulation of downstream biological effects and remodeling outcomes.⁹¹⁻⁹³ Identifying SSMs that exhibited both structural and functional compatibility with sonogenetics responsiveness is crucial for developing sound-sensitive protein toolkits. For this purpose, the construction of expression vectors via a GPI anchor, as described previously⁸⁸ would be valuable. Sonogenetics holds considerable potential for non-invasive therapy of neurological disorders, cardiovascular diseases, cancer, and other conditions.⁹¹⁻⁹³ (Figure 7). However, like many emerging technologies, sonogenetics remains at an experimental stage, and clinical translation has been limited by ongoing challenges, including an incomplete understanding of the ultrasound mechanisms underlying cellular excitability.

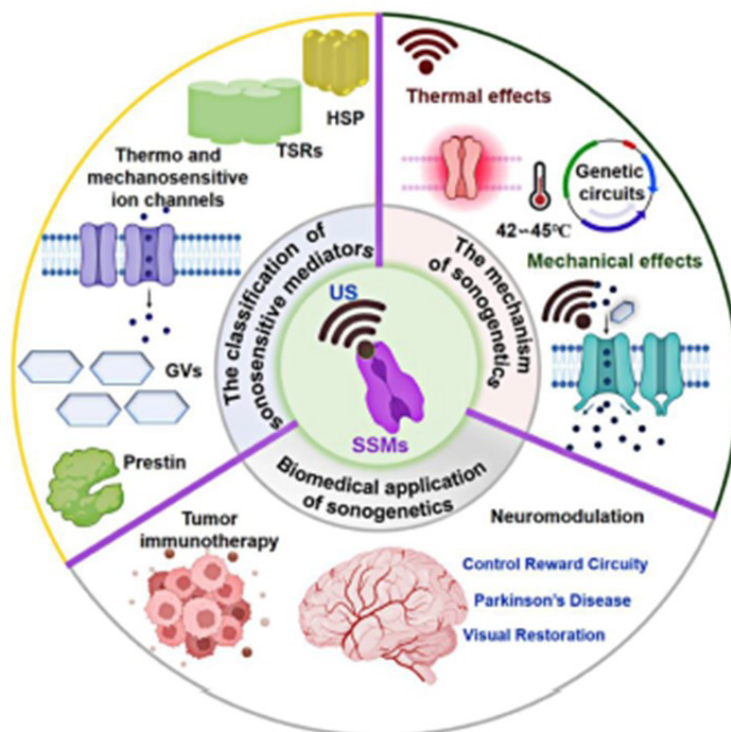


Figure 7. Schematics illustrating the classification, generation, mechanism, and biomedical application of sonogenetics. Image adopted from a study published by Wu et al.⁹¹

Notes: Prestin: an auditory-sensing protein found exclusively in outer cochlear hair cells. Prestin deficiency is associated with hearing loss in both humans and mice.

Abbreviations: GVs: Gas vesicles; HSP: Heat shock promoter; SSMs: Sonosensitive mediators; TSRs: Temperature-sensitive repressors; US: ultrasound.

6. Conclusion

This AT deficiency could serve as a model for rare hereditary blood-clotting disorders to develop valuable drugs that modulate the coagulation cascade and thereby prevent the consequences of blood-clotting disorders, which partly contribute to neurological and cardiovascular diseases. Furthermore, this AT deficiency provides a useful model for studying the “two-way association” between cancer and thrombosis and may therefore contribute to the development of cancer drugs. Future research works should focus on: (i) studying intermolecular interactions to explore the potential implication of the *APP* gene in the development of neurological features, thrombotic events and cancer; (ii) investigating the roles of arginine-to-histidine substitution and the *BRAF* V600E missense mutation in cancer development, particularly melanoma, in relation to the Arg393His mutation in AT-Hanoi; (iii) selecting anti-cancer drugs for use in immunotherapy via ICIs, as well as their combinations with engineered T cells and warfarin; and (iv) exploring the potential applications of sonogenetics for non-invasive therapy of neurological disorders, cardiovascular diseases, cancer, and other conditions.

Acknowledgments

The author received no support from any organization for the submitted work.

Funding

None.

Conflict of interest

The author declares no conflict of interest.

Author contributions

This is a single-authored article.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data

Data used in this work is available from the corresponding author upon reasonable request.

References

1. Feigin VL, Vos T, Nichols E, *et al.* The global burden of neurological disorders: translating evidence into policy. *Lancet Neurol.* 2020;19(3):255-265.
doi: 10.1016/S1474-4422(19)30411-9
2. Steinmetz JD, Seeher KM, Schiess N, *et al.* Global, regional, and national burden of disorders affecting the nervous system, 1990-2021: a systematic analysis for the Global Burden of Disease Study 2021. *Lancet Neurol.* 2024;23(4):344-381.
doi: 10.1016/S1474-4422(24)00038-3
3. Sanders T, Liu Y, Buchner V, *et al.* Neurotoxic effects and biomarkers of lead exposure: a review. *Rev Environ Health.* 2009;24(1):15-45.
doi: 10.1515/reveh.2009.24.1.15
4. Boehme AK, Esenwa C, Elkind MSV. Stroke risk factors, genetics, and prevention. *Circ Res.* 2017;120(3):472-495.
doi: 10.1161/CIRCRESAHA.116.308398
5. Murphy SJx, Werring DJ. Stroke: causes and clinical features. *Medicine (Abingdon).* 2020;48(9):561-566.
doi: 10.1016/j.mpmed.2020.06.002
6. Ali N, Ayyub M, Khan SA. High prevalence of protein C, protein S, antithrombin deficiency, and factor V Leiden mutation as a cause of hereditary thrombophilia in patients of venous thromboembolism and cerebrovascular accident. *Pak J Med Sci.* 2014;30(6):1323-1326.
doi: 10.12669/pjms.306.5878
7. Mattiuzzi C, Lippi G. Current cancer epidemiology. *J Epidemiol Glob Health.* 2019;9(4):217-222.
doi: 10.2991/jegh.k.191008.001
8. Bray F, Laversanne M, Sung H, *et al.* Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin.* 2024;74(3):229-263.
doi: 10.3322/caac.21834
9. Noble S, Pasi J. Epidemiology and pathophysiology of cancer-associated thrombosis. *Br J Cancer.* 2010;102(Suppl 1):S2-S9.
doi: 10.1038/sj.bjc.6605599
10. Trousseau A. Phlegmasia alba dolens. In: *Clinique médicale de l'Hôtel-Dieu de Paris.* 2nd ed. Balliere; 1865;3:654-712.
11. Timp JF, Braekkan SK, Versteeg HH, *et al.* Epidemiology of cancer-associated venous thrombosis. *Blood.* 2013;122(10):1712-1723.
doi: 10.1182/blood-2013-04-460121
12. Razak NBA, Jones G, Bhandari M, *et al.* Cancer-associated thrombosis: an overview of mechanisms, risk factors, and treatment. *Cancers.* 2018;10(10):380.
doi: 10.3390/cancers10100380
13. Sharma BK, Flick MJ, Palumbo JS. Cancer-associated

- thrombosis: a two-way street. *Semin Thromb Hemost.* 2019;45(6):559-568.
doi: 10.1055/s-0039-1693472
14. Pavlovic D, Niciforovic D, Markovic M, *et al.* Cancer-associated thrombosis: epidemiology, pathophysiological mechanisms, treatment, and risk assessment. *Clin Med Insights Oncol.* 2023;17:1-14.
doi: 10.1177/11795549231220297
 15. White RH. Identifying risk factors for venous thromboembolism. *Circulation.* 2012;125(17):2051-2053.
doi: 10.1161/CIRCULATIONAHA.112.102814
 16. Murchison JT, Wylie L, Stockton DL. Excess risk of cancer in patients with primary venous thromboembolism: a national, population-based cohort study. *Br J Cancer.* 2004;91(1):92-95.
doi: 10.1038/sj.bjc.6601964
 17. Sundén P, Svensson PJ, Själander A. Venous thrombosis and cancer risk. *J Thromb Thrombolysis.* 2017;43(1):68-73.
doi: 10.1007/s11239-016-1411-y
 18. Hägg L, Ehres F, Lind M, *et al.* Cancer incidence and mortality after a first-ever venous thrombosis: a cohort study in northern Sweden. *Thromb J.* 2024;22(1):77.
doi: 10.1186/s12959-024-00646-z
 19. Marcuello C, Lim KS, Nisini G, *et al.* Nanoscale analysis beyond imaging by atomic force microscopy: molecular perspectives on oncology and neurodegeneration. *Small Sci.* 2025;5(11):2500351.
doi: 10.1002/smcs.202500351
 20. Wang Y, Liao W, Wang L, *et al.* Advance and prospect of positron emission tomography in Alzheimer's disease research. *Mol Psychiatry.* 2025;30(10):4899-4909.
doi: 10.1038/s41380-025-03081-2
 21. Maruyama K, Kokame K. Carrier frequencies of antithrombin, protein C, and protein S deficiency variants estimated using a public database and expression experiments. *Res Pract Thromb Haemost.* 2020;5(1):179-186.
doi: 10.1002/rth2.12456
 22. Marco-Rico A, Marco-Vera P. Antithrombin deficiency and thrombosis: a wide clinical scenario reported in a single institution. *J Blood Med.* 2023;14:499-506.
doi: 10.2147/JBM.S416355
 23. de Moerloose P, Boehlen F. Inherited thrombophilia in arterial disease: a selective review. *Semin Hematol.* 2007;44(2):106-113.
doi: 10.1053/j.seminhematol.2007.01.008
 24. Cornelia E, Königsbrügge O, Nopp S, *et al.* Antithrombin activity and association with risk of thrombosis and mortality in patients with cancer. *Int J Mol Sci.* 2022;23(24):15770.
doi: 10.3390/ijms232415770
 25. Nguyen KV. Encephalomalacia/gliosis, deep venous thrombosis, and cancer in Arg393His antithrombin Hanoi and the potential impact of the β -amyloid precursor protein (APP) on thrombosis and cancer. *AIMS Neurosci.* 2022;9(2):175-215.
doi: 10.3934/Neuroscience.2022010
 26. Davies H, Bignell GR, Cox C, *et al.* Mutations of the BRAF gene in human cancer. *Nature.* 2002;417(6892):949-954.
doi: 10.1038/nature00766
 27. Viskov C, Elli S, Urso E, *et al.* Heparin dodecasaccharide containing two antithrombin-binding pentasaccharides. *J Biol Chem.* 2013;288(36):25895-25907.
doi: 10.1074/jbc.M113.485268
 28. Rezaie AR, Giri H. Antithrombin: an anticoagulant, anti-inflammatory and anti-bacterial serpin. *J Thromb Haemost.* 2020;18(3):528-533.
doi: 10.1111/jth.14724
 29. Rodgers GM, Mahajerin A. Antithrombin therapy: current state and future outlook. *Clin Appl Thromb Hemost.* 2023;29:1-16.
doi: 10.1177/10760296231205279
 30. Roth R, Swanson R, Izaguirre G, *et al.* Saturation mutagenesis of the antithrombin reactive center loop p14 residue supports a three-step mechanism of heparin allosteric activation involving intermediate and fully activated states. *J Biol Chem.* 2015;290(47):28020-28036.
doi: 10.1074/jbc.M115.678839
 31. Frebelius S, Isaksson S, Swedenborg J. Thrombin inhibition by antithrombin III on the subendothelium is explained by the isoform AT beta. *Arterioscler Thromb Vasc Biol.* 1996;16(10):1292-1297.
doi: 10.1161/01.ATV.16.10.1292
 32. Crous-Bou M, De Vivo I, Camargo CA Jr, *et al.* Interactions of established risk factors and a GWAS-based genetic risk score on the risk of venous thromboembolism. *Thromb Haemost.* 2016;116(4):705-713.
doi: 10.1160/TH16-02-0172
 33. Crous-Bou M, Harrington LB, Kabrhel C. Environmental and genetic risk factors associated with venous thromboembolism. *Semin Thromb Hemost.* 2016;42(8):808-820.
doi: 10.1055/s-0036-1592333
 34. Gemmati D, Longo G, Franchini E, *et al.* Cis-segregation of c.1171C>T stop codon (p.R391*) in SERPINC1 gene and c.1691G>A transition (p.R506Q) in F5 gene and selected GWAS multilocus approach in inherited thrombophilia.

- Genes*. 2021;12(6):934.
doi: 10.3390/genes12060934
35. Maclean PS, Tait RC. Hereditary and acquired antithrombin deficiency: epidemiology, pathogenesis and treatment options. *Drugs*. 2007;67(10):1429-1440.
doi: 10.2165/00003495-200767100-00005
 36. Alban S. Adverse effects of heparin. *Handb Exp Pharmacol*. 2012;207:211-263.
doi: 10.1007/978-3-642-23056-1_10
 37. Rodgers R, Coelho C. Clinical Guideline: Heparin dose adjustment in adult patients with very high or low body weight. NHS Greater Glasgow and Clyde. Updated May 2025.
 38. Bates SM, Weitz JI. Coagulation assays. *Circulation*. 2005;112:e53-e60.
doi: 10.1161/CIRCULATIONAHA.104.478222
 39. Newall F. Anti-factor Xa (anti-Xa) assay. *Methods Mol Biol*. 2013;992:265-272.
doi: 10.1007/978-1-62703-339-8_19
 40. Parija SC. *Textbook of Microbiology & Immunology*. India: sevier; 2009. Available from: <https://books.google.com/books?id=HcgGLfxDJQC>
 41. Schlommer C, Brandtner A, Bachler M. Antithrombin and its role in host defense and inflammation. *Int J Mol Sci*. 2021;22(8):4283.
doi: 10.3390/ijms22084283
 42. Liu ZL, Chen HH, Zheng LL, *et al*. Angiogenic signaling pathways and anti-angiogenic therapy for cancer. *Signal Transduct Target Ther*. 2023;8(1):198.
doi: 10.1038/s41392-023-01460-1
 43. O'Reilly MS, Pirie-Shepherd S, Lane WS, *et al*. Antiangiogenic activity of the cleaved conformation of the serpin antithrombin. *Science*. 1999;285(5435):1926-1928.
doi: 10.1126/science.285.5435.1926
 44. O'Reilly MS. Antiangiogenic antithrombin. *Semin Thromb Hemost*. 2007;33(7):660-666.
doi: 10.1055/s-2007-991533
 45. Luengo-Gil G, Calvo MI, Martin-Villar E, *et al*. Antithrombin controls tumor migration, invasion and angiogenesis by inhibition of enteropeptidase. *Sci Rep*. 2016;6(1):27544.
doi: 10.1038/srep27544
 46. Olgasi C, Assanelli S, Cucci A, *et al*. Hemostasis and endothelial functionality: the double face of coagulation factors. *Haematologica*. 2024;109(7):2041-2048.
doi: 10.3324/haematol.2022.282272
 47. Wallis CJD, Juvet T, Lee Y, *et al*. Association between use of antithrombotic medication and hematuria-related complications. *JAMA*. 2017;318(13):1260-1271.
doi: 10.1001/jama.2017.13890
 48. Zacharski LR, Henderson WG, Rickles FR, *et al*. Rationale and experimental design for the VA cooperative study of anticoagulation (warfarin) in the treatment of cancer. *Cancer*. 1979;44(2):732-741.
doi: 10.1002/1097-0142(197908)44:2%3C732::AID-CNCR2820440246%3E3.0.CO;2-Y
 49. Enoxaparin sodium. The American Society of Health-System Pharmacists. Available from: <https://www.drugs.com/monograph/enoxaparin-sodium.html>
 50. Zwicker J, Wang TF, DeAngelo DJ, *et al*. The prevention and management of asparaginase-related venous thromboembolism in adults: guidance from the SSC on hemostasis and malignancy of the ISTH. *J Thromb Haemost*. 2020;18(2):278-284.
doi: 10.1111/jth.14671
 51. Key NS, Khorana AA, Kuderer NM, *et al*. Venous thromboembolism prophylaxis and treatment in patients with cancer: ASCO clinical practice guideline update. *J Clin Oncol*. 2020;38(5):496-520.
doi: 10.1200/JCO.19.01461
 52. Key NS, Khorana AA, Kuderer NM, *et al*. Venous thromboembolism prophylaxis and treatment in patients with cancer: ASCO guideline update. *J Clin Oncol*. 2023;41(16):3063-3071.
doi: 10.1200/JCO.23.00294
 53. Khorana AA, Connolly GC. Assessing risk of venous thromboembolism in patients with cancer. *J Clin Oncol*. 2009;27(29):4839-4847.
doi: 10.1200/JCO.2009.22.3271
 54. Syrigos K, Grapsa D, Sangare R, *et al*. Prospective assessment of clinical risk factors and biomarkers of hypercoagulability for identification of patients with lung adenocarcinoma at risk for cancer-associated thrombosis: the observational ROADMAP-CAT study. *Oncologist*. 2018;23(11):1372-1381.
doi: 10.1634/theoncologist.2017-0530
 55. Connors JM. Fine tuning venous thromboembolism risk prediction in patients with cancer. *J Clin Oncol*. 2023;41(16):2881-2883.
doi: 10.1200/JCO.23.00100
 56. Dragan A, Dragan AS. Novel insights in venous thromboembolism risk assessment methods in ambulatory cancer patients: from the guidelines to clinical practice. *Cancers*. 2024;16(2):458.
doi: 10.3390/cancers16020458
 57. International Human Genome Sequencing Consortium.

- Finishing the euchromatic sequence of the human genome. *Nature*. 2004;431:931-945.
doi: 10.1038/nature03001
58. van Boven HH, Reitsma PH, Rosendaal FR, *et al.* Factor V Leiden (FV R506Q) in families with inherited antithrombin deficiency. *Thromb Haemost*. 1996;75(3):417-421.
doi: 10.1055/s-0038-1650289
 59. Gemmati D, Serino ML, Moratelli S, *et al.* Coexistence of antithrombin deficiency, factor V Leiden and hyperhomocysteinemia in a thrombotic family. *Blood Coagul Fibrinolysis*. 1998;9(2):173-176.
doi: 10.1097/00001721-199803000-00008
 60. van Boven HH, Vandenbroucke JP, Briet E, *et al.* Gene-gene and gene-environment interactions determine risk of thrombosis in families with inherited antithrombin deficiency. *Blood*. 1999;94(8):2590-2594.
doi: 10.1182/blood.V94.8.2590.420k40_2590_2594
 61. Signorelli SS, Ferrante M, Gaudio A, *et al.* Deep vein thrombosis related to environment. *Mol Med Rep*. 2017;15(5):3445-3448.
doi: 10.3892/mmr.2017.6395
 62. Renzi M, Stafoggia M, Michelozzi P, *et al.* Long-term exposure to air pollution and risk of venous thromboembolism in a large administrative cohort. *Environ Health*. 2022;21(1):21.
doi: 10.1186/s12940-022-00834-2
 63. Lutsey PL, Misialek JR, Yong MT, *et al.* Air pollution is associated with increased risk of venous thromboembolism: the multi-ethnic study of atherosclerosis. *Blood*. 2025;145(10):1089-1096.
doi: 10.1182/blood.2024026399
 64. Sun H, Lan X, Ma L, *et al.* Revealing modifier variations characterizations for elucidating the genetic basis of human phenotypic variations. *Hum Genet*. 2022;141(6):1223-1233.
doi: 10.1007/s00439-021-02362-4
 65. Virolainen SJ, VonHandorf A, Viel KCMF, *et al.* Gene-environment interactions and their impact on human health. *Genes Immun*. 2023;24(1):1-11.
doi: 10.1038/s41435-022-00192-6
 66. Selkoe DJ, Podlisny MB, Joachim CL, *et al.* β -Amyloid precursor protein of Alzheimer disease occurs as 110-135-kilodalton membrane-associated proteins in neural and nonneural tissues. *Proc Natl Acad Sci USA*. 1988;85(19):7341-7345.
doi: 10.1073/pnas.85.19.7341
 67. Turner PR, O'Connor K, Tate WP, *et al.* Roles of amyloid precursor protein and its fragments in regulating neural activity, plasticity and memory. *Prog Neurobiol*. 2003;70(1):1-32.
doi: 10.1016/s0301-0082(03)00089-3
 68. Nguyen KV. Epigenetic regulation in amyloid precursor protein and Lesch-Nyhan syndrome. *Biochem Biophys Res Commun*. 2014;446(4):1091-1095.
doi: 10.1016/j.bbrc.2014.03.062
 69. Nguyen KV. Epigenetic regulation in amyloid precursor protein with genomic rearrangements and the Lesch-Nyhan syndrome. *Nucleosides Nucleotides Nucleic Acids*. 2015;34(10):674-690.
doi: 10.1080/15257770.2015.1071844
 70. Pandey P, Sliker B, Peters HL, *et al.* Amyloid precursor protein and amyloid precursor-like protein 2 in cancer. *Oncotarget*. 2016;7(15):19430-19444.
doi: 10.18632/oncotarget.7103
 71. Canobbio I, Visconte C, Momi S, *et al.* Platelet amyloid precursor protein is a modulator of venous thromboembolism in mice. *Blood*. 2017;130(4):527-536.
doi: 10.1182/blood-2017-01-764910
 72. Zhao L, He D, Jiao M, *et al.* Overexpression of histone deacetylase and amyloid precursor protein in hepatocellular carcinoma. *Technol Cancer Res Treat*. 2017;16(5):586-594.
doi: 10.1177/1533034616661664
 73. Nguyen KV, Nyhan WL. Quantification of various APP-mRNA isoforms and epistasis in Lesch-Nyhan disease. *Neurosci Lett*. 2017;643:52-58.
doi: 10.1016/j.neulet.2017.02.016
 74. Gao L, Zhao H, Zhang D. Role of APLP2 in the prognosis and clinicopathology of renal cell carcinoma. *Oncol Lett*. 2018;17(1):508-513.
doi: 10.3892/ol.2018.9577
 75. Nguyen KV. Epigenetic modulation of human neurobiological disorders: Lesch-Nyhan disease as a model disorder. *AIMS Neurosci*. 2025;12(2):58-74.
doi: 10.3934/Neuroscience.2025005
 76. White KA, Ruiz DG, Szpiech ZA, *et al.* Cancer-associated arginine-to-histidine mutations confer a gain in pH sensing to mutant proteins. *Sci Signal*. 2017;10(495):eaam9931.
doi: 10.1126/scisignal.aam9931
 77. Srivastava S, Riddell SR. Engineering CAR-T cells: design concepts. *Trends Immunol*. 2015;36(8):494-502.
doi: 10.1016/j.it.2015.06.004
 78. Fox M. New gene therapy for cancer offers hope for those with no option left. NBC News. July 12, 2017. Available from: <https://www.nbcnews.com/health/health-news/new-gene-therapy-cancer-offers-hope-those-no-options-left-n741326>
 79. Sadelain M, Brentjens R, Rivière I. The basic principles of chimeric antigen receptor design. *Cancer Discov*.

- 2013;3(4):388-398.
doi: 10.1158/2159-8290.CD-12-0548
80. Kochenderfer JN, Wilson WH, Janik JE, *et al.* Eradication of B-lineage cells and regression of lymphoma in a patient treated with autologous T cells genetically engineered to recognize CD19. *Blood*. 2010;116(20):4099-4102.
doi: 10.1182/blood-2010-04-281931
 81. Kochenderfer JN, Dudley ME, Kassim SH, *et al.* Chemotherapy-refractory diffuse large B-cell lymphoma and indolent B-cell malignancies can be effectively treated with autologous T cells expressing an anti-CD19 chimeric antigen receptor. *J Clin Oncol*. 2015;33(6):540-549.
doi: 10.1200/JCO.2014.56.2025
 82. Stearns FW. One hundred years of pleiotropy: a retrospective. *Genetics*. 2010;186(3):767-773.
doi: 10.1534/genetics.110.122549
 83. Spiers L, Coupe N, Payne M. Toxicities associated with checkpoint inhibitors - an overview. *Rheumatology*. 2019;58(Suppl 7):vii7-vii16.
doi: 10.1093/rheumatology/kez418
 84. Bauer AT, Gorzelanny C, Gebhardt C, *et al.* Interplay between coagulation and inflammation in cancer: limitations and therapeutic opportunities. *Cancer Treat Rev*. 2022;102:102322.
doi: 10.1016/j.ctrv.2021.102322
 85. Cantrell R, Palumbo JS. Hemostasis and tumor immunity. *Res Pract Thromb Haemost*. 2022;6(4):e12728.
doi: 10.1002/rth2.12728
 86. Callahan MK, Kluger H, Postow MA, *et al.* Nivolumab plus ipilimumab in patients with advanced melanoma: updated survival, response, and safety data in a phase I dose-escalation study. *J Clin Oncol*. 2018;36(4):391-398.
doi: 10.1200/JCO.2017.72.2850
 87. Olson DJ, Eroglu Z, Brockstein B, *et al.* Pembrolizumab plus ipilimumab following anti-PD-1/L1 failure in melanoma. *J Clin Oncol*. 2021;39(24):2647-2655.
doi: 10.1200/JCO.21.00079
 88. Nguyen KV, Naviaux RK, Nyhan WL. Lesch-Nyhan disease: I. Construction of expression vectors for hypoxanthine-guanine phosphoribosyltransferase (HGPrt) enzyme and amyloid precursor protein (APP). *Nucleosides Nucleotides Nucleic Acids*. 2020;39(6):905-922.
doi: 10.1080/15257770.2020.1714653
 89. Zacharski LR, Henderson WG, Rickles FR, *et al.* Effect of warfarin on survival in small cell carcinoma of the lung: Veterans Administration Study No. 75. *JAMA*. 1981;245(8):831-835.
doi: 10.1001/jama.1981.03310330021017
 90. Chong EA, Melenhorst JJ, Lacey SF, *et al.* PD-1 blockade modulates chimeric antigen receptor (CAR)-modified T cells and induces tumor regression: refueling the CAR. *Blood*. 2016;129(8):1039-1041.
doi: 10.1182/blood-2016-09-738245
 91. Wu P, Liu Z, Tao W, Lai Y, Yang G, Yuan L. The principles and promising future of sonogenetics for precision medicine. *Theranostics*. 2024;14(12):4806-4821.
doi: 10.7150/thno.98476
 92. Nguyen KV. Medical imaging and perspective on sonogenetics. *J Radiat Res Imaging*. 2025;3(1):4-8.
 93. Germain P, Grillon C, Nguyen KV. A narrative review of potential therapies for the treatment of myocardial tissue in relation to heart failure. *Nucleosides Nucleotides Nucleic Acids*. 2025.
doi: 10.1080/15257770.2025.2550969