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REVIEW ARTICLE

Neurological manifestations, thrombosis, and cancer in Arg393His antithrombin Hanoi

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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.

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

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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 thrombophilis, 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 microscopy[19] 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) mutation[26] 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.

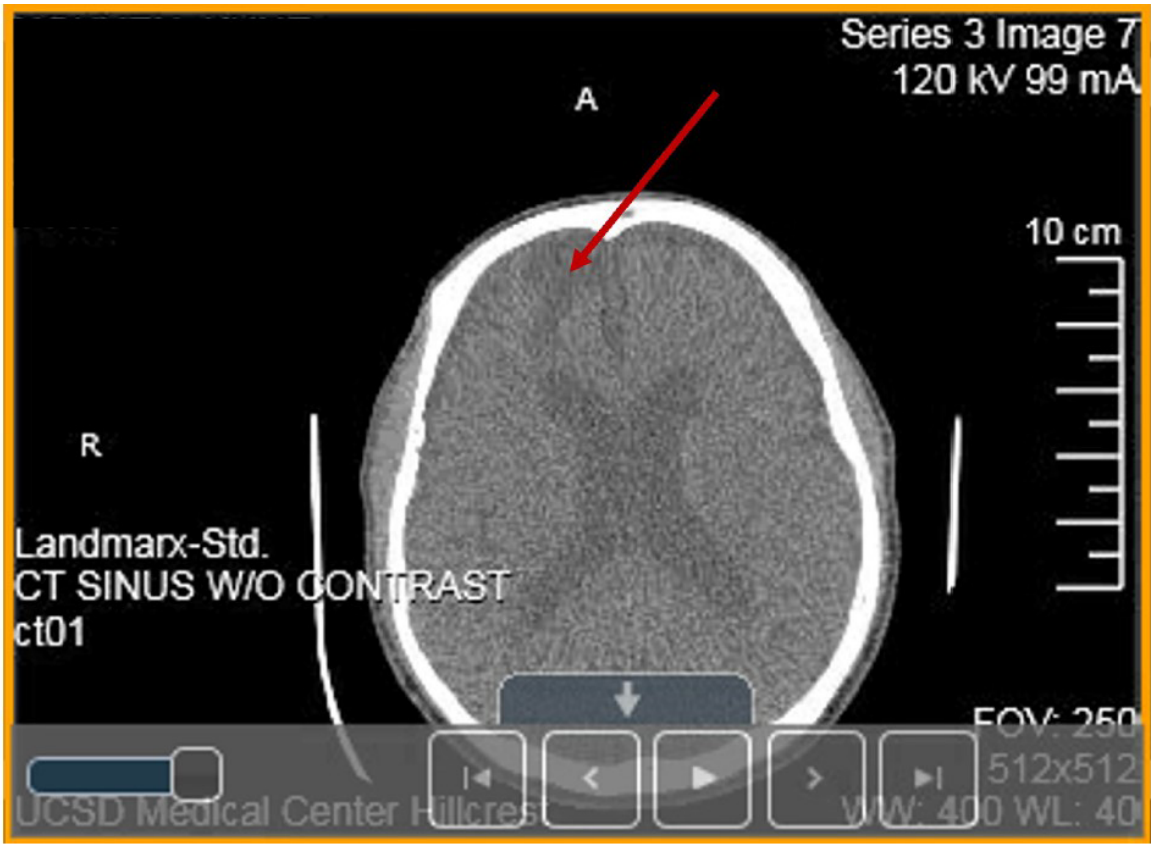

Figure 1. Non-contrast cerebral computed tomography scans of the paranasal sinuses, acquired with 0.625 mm axial slices, and reformatted in the coronal and sagittal planes. Limited portions of the brain are visualized, showing encephalomalacia/gliosis in the anterior right frontal lobe, suggestive of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

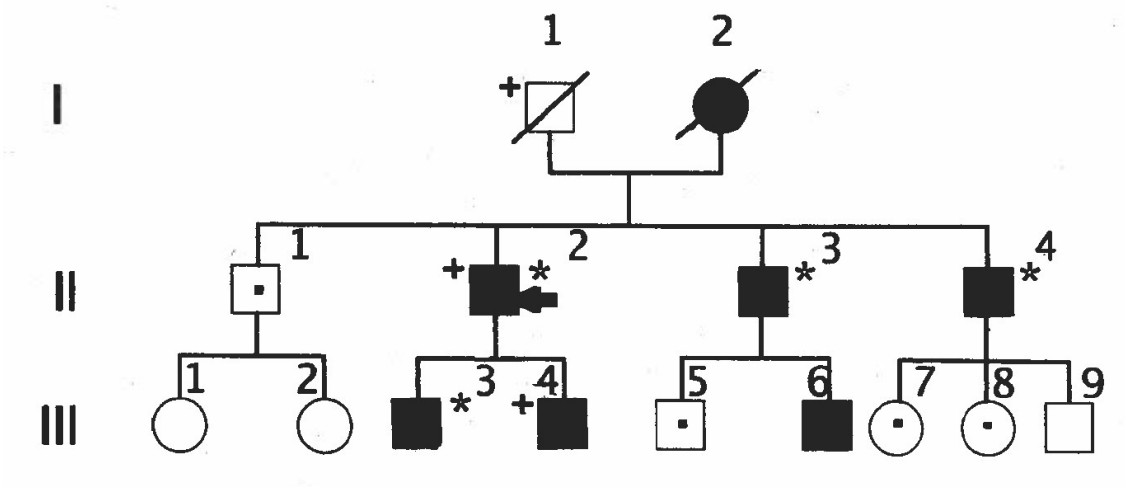

Figure 2. Pedigree of the family. The proband (II-2) is indicated by an arrow.
Notes: * denotes thrombosis; + denotes cancer; solid symbols denote presence of heterozygous Arg393His point mutation; dotted symbols denote absence of heterozygous Arg393His point mutation; open symbols denote not investigated; 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

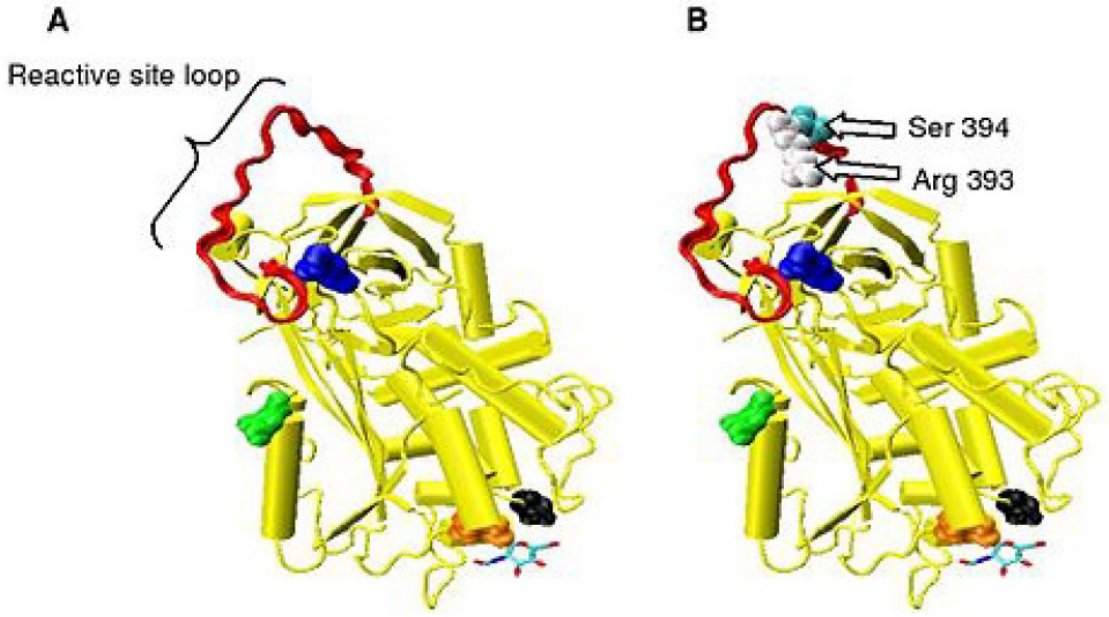

Figure 3. The reactive Arg393–Ser394 bond is located on an exposed loop at the surface of the molecule. This loop is called the reactive site loop or reactive center loop.

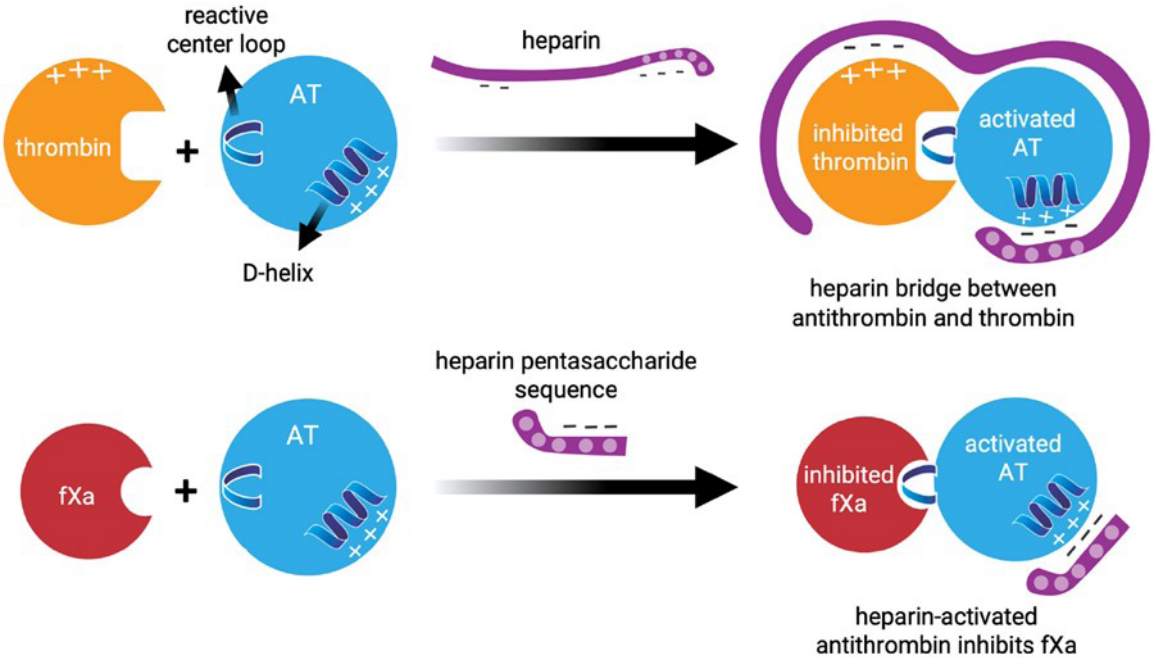

Figure 4. The anticoagulant effect of heparin occurs via the binding of antithrombin (AT) with thrombin and activated factor X (fXa). Image adopted from a study published by Rodgers and Mahajerin.[29]

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 affinity[28] 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 time[38] 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 G20210A 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 substitution[76] and the *BRAF* V600E missense mutation[26] 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 previously[25] (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 mutation[26] 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

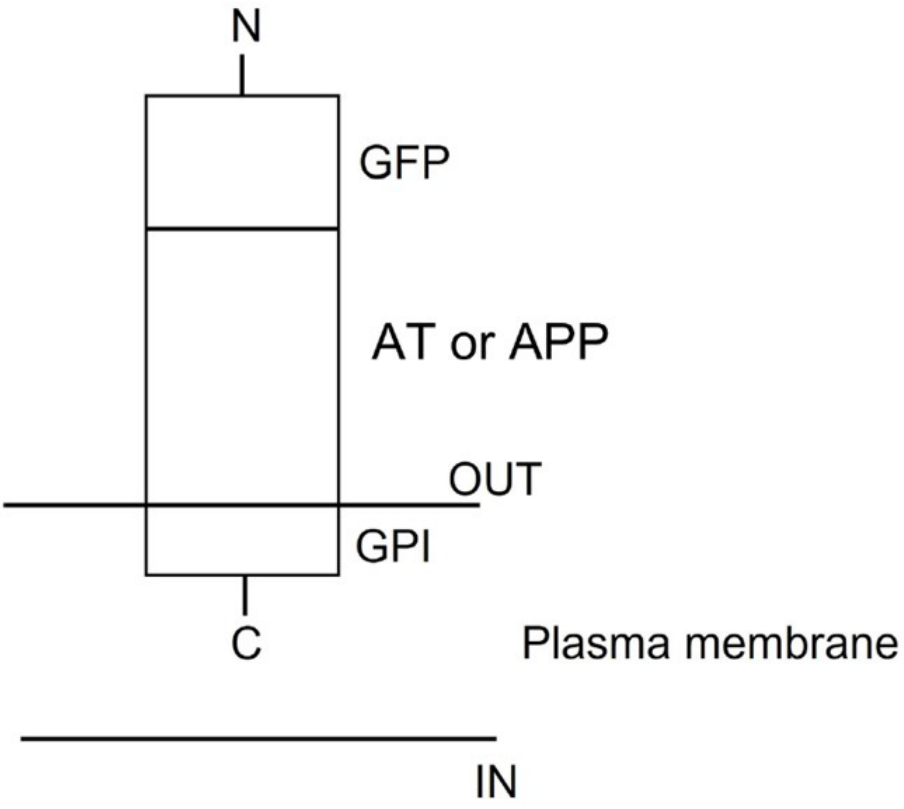

Figure 5. Schematic presentation of the membrane topology of the expression vectors for human antithrombin (AT) and β-amyloid precursor protein (APP). The mammalian expression vector pcDNA™ 3.1 (+) was used as the backbone, with all genes of interest inserted in the right frame into the pcDNA™ 3.1 (+) 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.

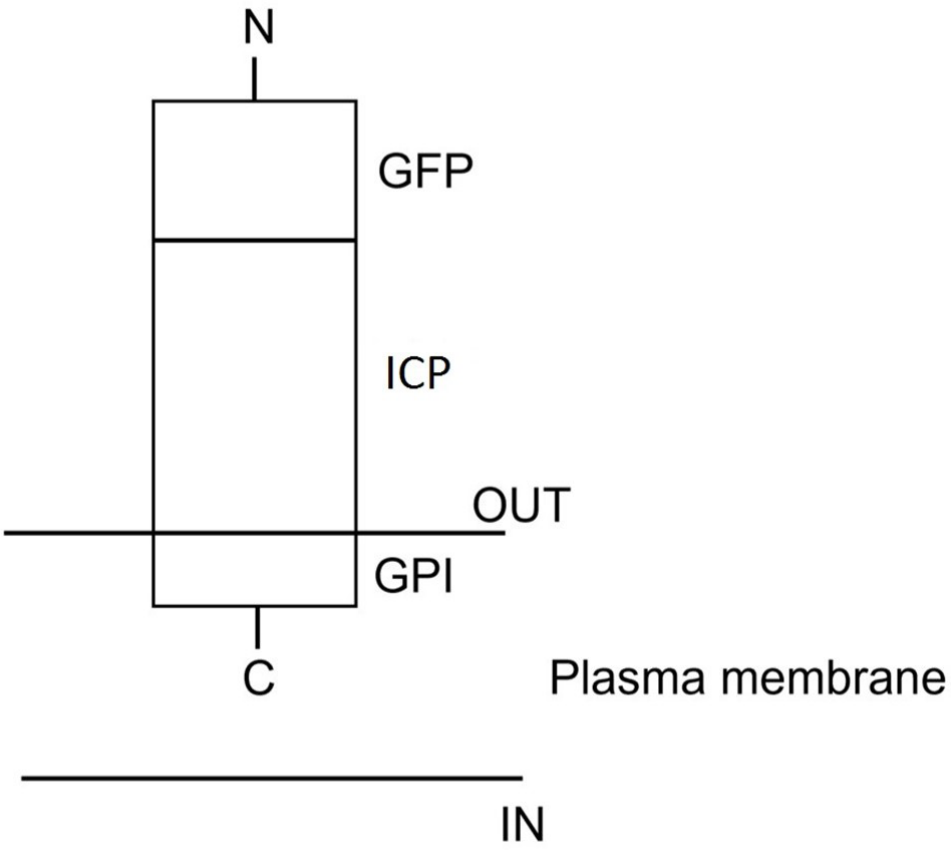

Figure 6. Schematic presentation of the membrane topology of the expression vector for immune checkpoint protein (ICP). The mammalian expression vector pcDNA™ 3.1 (+) was used as the backbone, with all genes of interest inserted in the right frame into the pcDNA™ 3.1 (+) vector. The construct comprises 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 previously[88] 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.

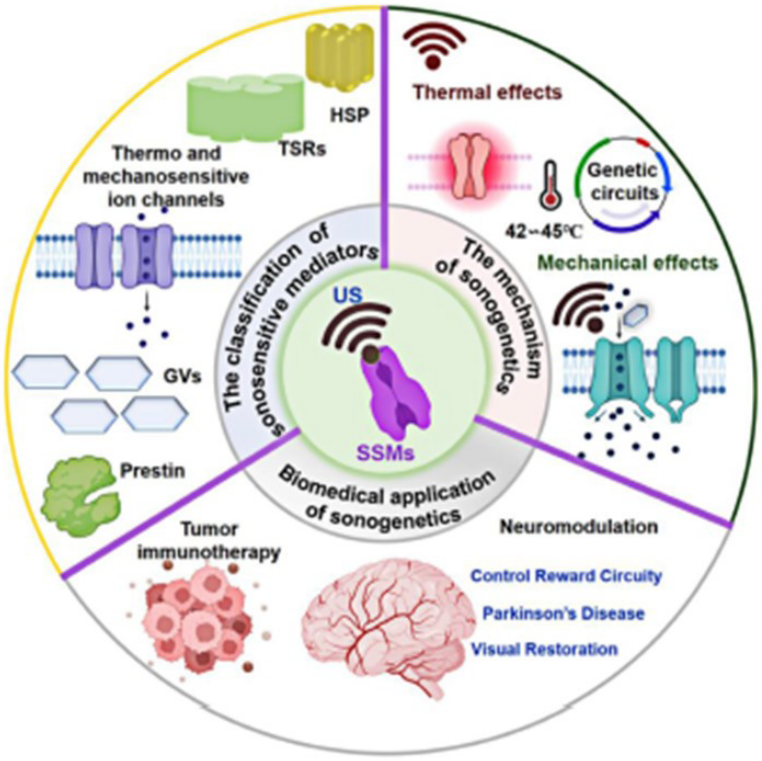

Figure 7. Schematics illustrating the classification, generation, mechanism, and biomedical application of sonogenetics. Image adopted from a study published by Wu et al.[91]

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.

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Conflict of interest

The author declares no conflict of interest.

Author contributions

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Availability of data

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

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