The Clinical Course of Severe Alpha-1-Antitrypsin Deficiency: Registry-Based National Studies

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LUND
UNIVERSITY

DOCTORAL DISSERTATION

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Title and subtitle: The Clinical Course of Severe Alpha-1-Antitrypsin Deficiency: Registry-Based National Studies

Abstract:

Background: Hereditary alpha-1-antitrypsin deficiency (AATD) predisposes to emphysema and liver disease.

Aims and Methods: To study the clinical course and prognosis in severe AATD individuals (Pi*ZZ) by:

- estimating the rate of annual decline in FEV_1 (ΔFEV_1) and by identifying the risk factors involved in the rapid decline in lung function, through random-effects modelling, adjusting for age and FEV_1 at baseline (Paper I);
- investigating the health status, clinical course, and prognosis in the Pi*ZZ individuals identified by screening (Paper II);
- evaluating the risk and the risk factors for incident cancer, and analysing survival after a cancer diagnosis compared with controls from the general population, by using proportional hazards and Cox regression models, adjusting for age, sex, smoking status, and the presence of liver disease (Paper III);
- investigating the risk factors for, and the occurrence of, gastrointestinal diseases, compared with controls from the general Swedish population (Paper IV).

The study population in papers I-IV was selected from the Swedish National Registry of Individuals With Severe AATD. All information on the study population was collected from the Swedish national registries, by cross-linkage using the Swedish system of personal identity numbers, between the Swedish National AATD Registry, the Swedish National Patient Registry, the Swedish National Cancer Registry, and the Swedish National Cause of Death Registry. For papers III-IV, 6,000 control individuals were selected from three population-based cohorts within the Obstructive Lung Disease in Northern Sweden studies.

Results: Paper I: Active smoking, age, respiratory symptoms at baseline and repeated severe exacerbations of COPD are factors associated with an accelerated ΔFEV_1. Paper II: Never-smoking Pi*ZZ individuals identified by screening have better lung function, fewer symptoms, and better survival compared with the ever-smokers. Paper III: Pi*ZZ individuals have an increased risk of developing both hepatic and non-hepatic cancer, compared with the general population. Paper IV: Pi*ZZ individuals have a higher risk of developing Crohn's disease, ulcerative colitis, noninfectious gastroenteritis, and diverticular disease, compared with the general population.

Conclusions: The thesis emphasises the importance of early identification of AATD. Regular follow up of severe AATD individuals is important for prompt diagnosis and effective disease management.

Key words: alpha-1-antitrypsin deficiency, lung function, natural course, registry, screening.

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To the individuals with severe alpha-1-antitrypsin deficiency

Table of Contents

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Populärvetenskaplig sammanfattning

Det kliniska förloppet vid svår alfa-1-antitrypsinbrist. Registerbaserade nationella studier

Alfa-1-antitrypsin (AAT) är ett viktigt skyddsprotein som bildas huvudsakligen i levern. AAT spelar en central roll för inaktivering av neutrofilelastas (NE) som bryter ner byggbeståndsdelen elastin i lungvävnad.

Alfa-1-antitrypsinbrist (AAT-brist) är ett ärftligt genetiskt tillstånd. Många individer är bärare av anlagen för AAT-brist utan att själva vara medvetna om det. Diagnosen av AAT-brist är oftast försenad till den punkt då sjukdom i luftvägarna redan hunnit etableras.

Halten av AAT mäts i blodet. Vid svår AAT-brist (anlagstyp Pi∗ZZ) är AAT-halten i blodet endast 10–20% av den normala nivån. I Sverige är prevalensen av svår AAT-brist 1/1600 individer och tillståndet är underdiagnostiserat.

Svår AAT-brist innebär en ökad risk för att utveckla lungsjukdom (kroniskt obstruktiv lungsjukdom, KOL och emfysem), särskilt hos rökare, på grund av obalans mellan NE och AAT till fördel för NE. Hos individer med svår AAT-brist ackumuleras AAT i levern med ökad risk för cirros (skrumplever) och hepatocellulär cancer.

Det är okänt om svår AAT-brist också innebär ökad risk för andra sjukdomar. Tidigare studier om förloppet hos individer med svår AAT-brist har varit få. Dessutom har dessa studier främst inkluderat rökare och individer som identifierats med AAT-brist på grund av lungsjukdom. Dessa studier har visat att AAT-brist är ett allvarligt tillstånd som leder till en för tidig död.

Sverige har sedan 1991 ett väletablerat nationellt register över vuxna med säkerställd svår AAT-brist (AAT-registret). Alla nydiagnostiserade fall rapporteras till registret av avdelningen för Klinisk kemi, Skånes Universitetssjukhus i Malmö, som är det enda laboratoriet i Sverige som utför analys av anlagen för AAT-brist (så kallat Pi-typning). Någon systematisk screening för AAT-brist görs idag inte i Sverige. De bristindivider som ger sitt samtycke till det, inkluderas i AAT-registret och följs upp vartannat år med frågeformulär, leverprover och lungfunktionsundersökning.

Genom AAT-registret erbjuder Sverige världsunika möjligheter att studera det kliniska förloppet vid svår AAT-brist. Det övergripande syftet med denna avhandling var att studera det kliniska förloppet och prognosen hos personer med svår AAT-brist (Pi∗ZZ).

Avhandlingen består av fyra delarbeten baserade på data från AAT-registret. All data i delarbetena I-IV har samlats in från svenska nationella register, genom samkörning med det svenska personnummersystemet, mellan AAT-registret,

patientregistret, cancerregistret och dödsorsaksregistret. För delarbetena III-IV valdes 6000 kontrollindivider med kända rökvanor ut från tre slumpvis utvalda populationsbaserade kohorter inom OLIN-studierna (Obstructive Lung Disease in Northern Sweden) i Norrbotten.

I delarbete I var den genomsnittliga minskningen av FEV_1 i Pi*ZZ individer lägre än vad som tidigare rapporterats. Ingen signifikant skillnad hittades i den årliga minskningen av FEV_1 mellan aldrig- och före-detta-rökare. Aktiva rökare, män, medelålders individer, individer med luftvägssymtom, de som hade rökt tio paketår eller mer, de med KOL och de med upprepade exacerbationer av KOL visade sig ha en snabbare minskning i FEV_1, när man justerade för ålder och FEV_1 vid baslinjen.

I delarbete II hade de Pi*ZZ-individer som identifierats genom screening och som diagnostiserades i tidig ålder (dvs ≤14 år) bättre lungfunktion och lägre lungfunktionsnedsättning (minskning i FEV_1) jämfört med motsvarande som identifierats efter 14 års åldern. Dessutom var andelen aldrig-rökare större bland de screenade Pi*ZZ-individer identifierade innan 14-årsåldern, än bland de som identifierades efter 14-årsåldern. Diagnosen KOL var vanligare bland aktuella rökare och före detta rökare, än bland de som aldrig rökt. Majoriteten av aktiva rökare och före detta rökare identifierade genom screening hade slutat röka efter att de diagnosticerades med svår AAT-brist, eller efter inklusion i AAT-registret. Av de screenade Pi*ZZ-individer som identifierats ≤14 år rapporterade 39% yrken med exponering för luftvägsirriterande ämnen. Fler dödsfall inträffade bland rökare än bland aldrig-rökare.

Resultaten av delarbete III visar att Pi*ZZ-individer har en ökad risk för att utveckla cancer jämfört med den svenska befolkningen, oavsett rökstatus. Risken för både lever- och icke-levercancer var förhöjd även efter justering för riskfaktorerna ålder, kön, rökstatus och förekomst av leversjukdom. Överlevnadstiden efter diagnos av cancer var signifikant kortare bland Pi*ZZ-individer jämför med kontrollgruppen.

Resultaten av delarbete IV indikerar att Pi*ZZ-individer, även efter justering för riskfaktorer (ålder, kön, rökstatus och förekomst av KOL), har en signifikant högre risk för att utveckla specifika gastrointestinala sjukdomar (SGID; Crohns sjukdom, ulcerös kolit, icke-infektiös gastroenterit och divertiklar) jämfört med kontroller från den svenska befolkningen. Betydligt fler dödsfall inträffade bland Pi*ZZ-individer med SGID än bland motsvarande kontroller. Den främsta dödsorsaken bland Pi*ZZ-individer med någon SGID var andningsrelaterad.

Några klara riktlinjer om uppföljning av AAT-bristindivider saknas i dagsläget. KOL hos dessa behandlas enligt gängse behandlingsrekommendationer, liksom hos individer utan AAT-brist. Den viktigaste förebyggande insatsen hos individer med svår AAT-brist för att minska risken för framtida utveckling av lungsjukdom är att inte börja röka, samt att sluta röka om man är aktiv rökare. Det är därför viktigt att bristindividerna har kännedom om sin AAT-brist. Screening för AAT i tidig ålder,

innan den potentiella rökdebuten, är viktig för att förhindra rökning och dess skadliga effekter på hälsan. Dessutom, är det viktigt att AAT-bristindividerna tidigt ges råd gällande yrkesval och övrig miljöexponering, som skulle kunna vara skadlig för deras hälsa.

Avhandlingen har bidragit till att ytterligare klargöra naturalförloppet vid svår AAT-brist och har klargjort riskfaktorer för att utveckla klinisk sjukdom. Individer med svår AAT-brist bör följas upp regelbundet för att tidigt upptäcka förekomst av cancer eller andra sjukdomar, för att således förbättra deras prognos.

List of papers

Paper I

Hiller AM, Piitulainen E, Jehpsson L, Tanash H. 'Decline in FEV_1 and hospitalized exacerbations in individuals with severe alpha-1 antitrypsin deficiency.' *Int J Chron Obstruct Pulmon Dis*. 2019 May 23;14:1075-1083. doi: 10.2147/COPD.S195847.

Paper II

Hiller AM, Piitulainen E, Tanash H. 'The Clinical Course of Severe Alpha-1-Antitrypsin Deficiency in Patients Identified by Screening.' *Int J Chron Obstruct Pulmon Dis*. 2022 Jan 5;17:43-52. doi: 10.2147/COPD.S340241.

Paper III

Hiller AM, Ekström M, Piitulainen E, Lindberg A, Rönmark E, Tanash H. 'Cancer risk in severe alpha-1-antitrypsin deficiency.' *Eur Respir J*. 2022 Oct 27;60(4):2103200. doi: 10.1183/13993003.03200-2021.

Paper IV

Hiller AM, Piitulainen E, Ekström M, Lindberg A, Rönmark E, Tanash H. 'Severe alpha-1-antitrypsin deficiency has an increased risk of gastrointestinal diseases.' Submitted.

Author's contribution to the papers

Paper I

Adriana-Maria Hiller is the first author. I contributed to the conceptualisation, methodology (shared with co-authors), and statistical analysis, writing the original draft, and reviewing and editing it. I took independent responsibility for all the stages of the publication process.

Paper II

Adriana-Maria Hiller is the first author. I contributed to the conceptualisation, methodology (shared with co-authors), statistical analysis, writing the original draft, reviewing and editing it, and had full responsibility for all stages of the publication process.

Paper III

Adriana-Maria Hiller is the first author. I contributed to the conceptualisation of the paper. I shared the methodology with the other co-authors, and the statistical analyses with my supervisor Dr. Hanan Tanash. I took complete and independent responsibility for writing the original draft, reviewing and editing it, and for all the stages of the publication process.

Paper IV

Adriana-Maria Hiller is the first author. I contributed to the conceptualisation, methodology (shared with co-authors), and statistical analyses, writing the original draft, and reviewing and editing it. I took independent responsibility for all the stages of the publication process.

Abbreviations

AAT	Alpha-1-antitrypsin
AATD	Alpha-1-antitrypsin deficiency
ATS/ERS	American Thoracic Society/European Respiratory Society
ΔFEV_1	Annual decline in FEV_1, expressed in $mL \cdot yr^{-1}$
COPD	Chronic obstructive pulmonary disease
CI	Confidence interval
FEV_1	Forced expiratory volume in one second
FVC	Forced vital capacity
GOLD	Global Initiative for Chronic Obstructive Lung Disease
HR	Hazard ratio
ICD	International Classification of Disease system
IQR	Interquartile range
L	Litre
LT	Lung transplantation
LVRS	Lung volume reduction surgery
mL	millilitre
NE	Neutrophil elastase
n	Number
OLIN	Obstructive lung disease in northern Sweden
Pi	Protease inhibitor
SGID	Specific gastrointestinal diseases of interest
SD	Standard deviation
SNPR	Swedish National Patient Registry
VC	Vital capacity
WHO	World Health Organization
yr	Year

Unless otherwise stated, the term ‘severe AATD’ denotes the phenotype Pi∗ZZ in the text.

Introduction

Historical background

Alpha-1-antitrypsin deficiency was discovered by Professor Carl-Bertil Laurell at the Department of Clinical Chemistry in Malmö General Hospital, Sweden in 1962 (1-3). Due to his genuine interest in plasma proteins, he had by that time made substantial contributions to protein research, including his doctoral thesis on the finding and naming of transferrin, the main protein in the blood that transports iron throughout the body (3, 4).

In the 1920s, Theodor (The) Svedberg developed an ultracentrifuge for the separation of proteins in Uppsala, Sweden (2, 4, 5). During the 1930s, Arne Tiselius developed electrophoresis, the technique of protein separation in an electrical field, which allowed separation of the plasma proteins into albumin and globulins (α-, β- and γ) (6). Laurell was interested in the usage of the protein separation technique as a tool in clinical settings (2-4). Laurell refined and improved the electrophoresis technique (2-4).

During the 1960s, the electrophoresis technique gained interest among Swedish physicians (2, 4). Thus, the clinical chemistry laboratory in Malmö General Hospital became a referral centre for serum protein analyses, and samples from laboratories throughout Sweden were sent to Malmö for analysis (2, 4).

In 1961, Harald Nilsson, chief physician at the Chest Clinic (former tuberculosis sanatorium) in the city of Eksjö in southern Sweden, sent serum samples from his patients to Malmö for electrophoretic analysis, in order to identify any abnormalities (2, 4). Laurell noted that two of the samples lacked a visible α_1-globulin band. These individuals suffered from pulmonary emphysema.

Laurell invited his Ph.D. student Sten Eriksson to further evaluate the potential clinical features of the patients with the missing α_1-globulin band (2-4). Eriksson found five cases with the typical missing pattern of the α_1-globulin band among approximately 1,500 samples from diseased individuals. These cases constituted the first reported cases of alpha-1-antitrypsin deficiency (1). Three of these patients had widespread pulmonary lesions, with bronchiectasis, emphysema, and chronic bronchitis as the main diagnoses. The remaining two did not have any respiratory

symptoms (1). One of the patients came from a family with an extensive medical history of respiratory diseases (7).

Laurell and Eriksson deduced that the missing α_1-band corresponded to the protein identified by Kjell Jacobsson in 1955, which Schultze *et al.* had identified in 1962 as the protein with the ability to inhibit proteinase trypsin activity, labelled 'antitrypsin' (1, 8).

Laurell and Eriksson called their observation of 'the new type of dysproteinemia' as alpha-1-antitrypsin deficiency (AATD) (1). They also noted that the plasma concentration of the protein was low and, in addition, that the electrophoretic mobility of the protein was decreased (1). They further observed that other family members of one of the initial five AAT-deficient cases suffered from the same pulmonary disease as the index case. They suggested an association between AATD and degenerative pulmonary disease, with a structural abnormality in the protein as the primary cause (1, 2). The family studies also suggested that the deficiency was inherited (1).

Further studies by Eriksson confirmed the hereditary nature of AATD, and its clinical features were described in his Ph.D. thesis 'Studies in α_1-Antitrypsin Deficiency' from 1965 (9). These findings reinforced previous observations that individuals with low AAT levels were predisposed to onset of pulmonary disease at an early age, on a probable genetic basis, thus defining the major characteristics of alpha-1-antitrypsin deficiency (9).

During the following years, further characteristic features of AATD were documented. In 1969, the US pediatrician Harvey Sharp in Minneapolis described the association of liver disease with AATD in children, and Berg and Eriksson reported liver cirrhosis and fibrosis at autopsy in eight of 13 adults with AATD in 1972 (10, 11). Christer Larsson, a co-worker of Eriksson and Laurell documented in 1978 the relationship between lung emphysema and cigarette smoking, and demonstrated its importance as a risk factor in AATD individuals (12).

In parallel, progress was made regarding the diagnostic techniques of AATD, in order to distinguish between the normal and the abnormal protein. The initial electrophoretic variants of AAT observed by Laurell were later defined by Laurell and his Norwegian colleague, Magne Fagerhol, in a nomenclature system, the protease inhibitor (Pi) system for the AAT variants (13). Later, Diane Cox from Canada proposed different classifications for slow- and rapidly-migrating variants of AATD (14). The molecular pathologies causing different migration patterns of AAT were reported by Robin Carell and by Laurell's co-worker, Jan-Olof Jeppsson (15-17). Jan-Olof Jeppsson developed the isoelectric method focusing on Pi phenotyping of the different AAT variants. Pi phenotyping is still the main diagnostic method in Sweden for diagnosing the Pi phenotypes of AAT.

In 1972, Carl-Bertil Laurell described a simplified screening test for AATD from dried blood on filter paper (18). He initiated the Swedish National Neonatal Screening Study, that was performed during 1972-1974 by the paediatrician Tomas Sveger (19). All 200,000 newborn children were screened for AATD. The aims of the screening programme were to study liver and lung diseases during infancy and adolescence, and to protect children from cigarette smoke exposure and, later on, from starting to smoke (20).

Records have been kept at the laboratory in Malmö, Sweden for all subjects with severe deficiency of AAT, identified at the Department of Clinical Chemistry in Malmö. In 1991, the Swedish National Registry of Severe AATD was started by Sten Eriksson and my co-tutor, Eeva Piitulainen. All adult individuals with severe AATD are asked to participate in the registry (21). The present thesis is the result of the follow-up of the AATD individuals included in the Swedish National AATD Registry.

Structure, synthesis, and function of alpha-1-antitrypsin

Alpha-1-antitrypsin is a glycoprotein, 6.7 nm by 3.2 nm, of about 52.000 Dalton molecular weight (22-24). It consists of a single polypeptide chain of 394 amino acids and three carbohydrate side chains, globularly structured into nine alpha-helices (A-I), three beta-sheets (A-C) and one loop (the reactive centre, Met^{358}–Ser^{359}), as depicted in Figure 1 (22, 23, 25, 26). Two internal salt bridges between Glu^{342} – Lys^{290} and Glu^{264} – Lys^{387} help in the stabilisation of the molecule's structure (22).

AAT is mainly synthesised in the parenchymal cells of the liver (hepatocytes), and to a lesser extent in monocytes, alveolar macrophages, enterocytes of the gastrointestinal tract, and corneal epithelial cells (24, 27, 28). Following synthesis, it is released into the circulation and reaches all tissues (22, 23, 27). Humans produce daily approximately 34 mg AAT per kilo of body weight, corresponding to a plasma concentration of 1-2 $g \cdot L^{-1}$ (29).

AAT is an acute phase protein, which is why the circulating level increases during inflammatory conditions, infections, cancer, and pregnancy (27). The half-life of the AAT molecule in blood is estimated to 4-5 days (29).

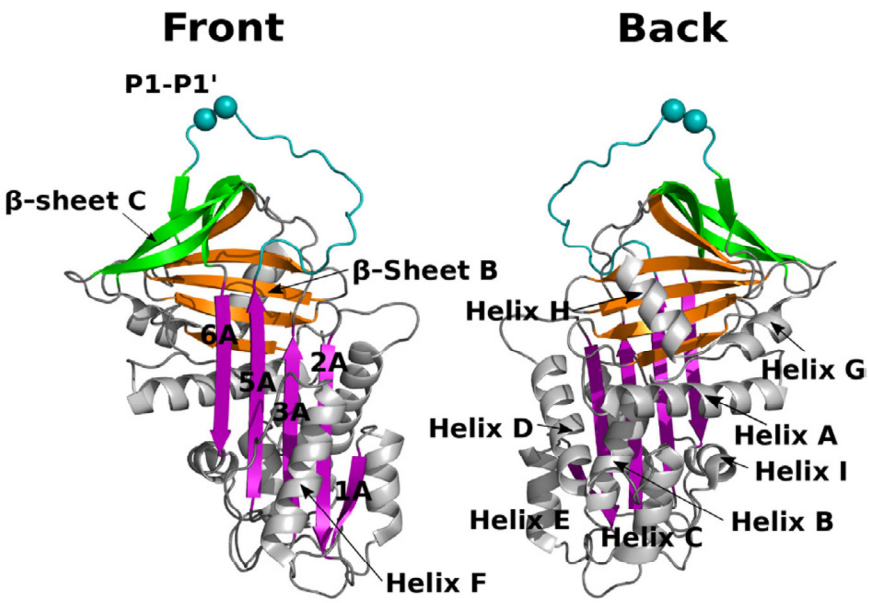

Figure 1.
The structure of the alpha-1-antitrypsin molecule, including the beta (β) sheets A, B and C (purple, orange and green, respectively), the helices A–I (grey) and the reactive center loop (cyan). (The original published figure has been cropped for the purpouse of the present thesis). Reprinted with permission from Elsevier from Jagger A., *et al*. 'Alpha1-Antitrypsin: structure and dynamics in health, disease and drug development' in: N Kalsheker, R Stockley (Eds.), *Alpha-1-Antitrypsin Deficiency*, Academic Press, Boston, 2017, p. 49–80, Copyright © 2017 Elsevier Inc. All rights reserved.

AAT is a member of the superfamily of serpins (**ser**ine **p**rotease **in**hibitors) and inactivates proteases that have a serine residue at their active site (serine proteases) (24). Proteases are proteins that act as enzymes, and cleave and degrade other proteins in the human body (30). AAT is the major serpin in human plasma inhibiting serine proteases neutrophil elastase (NE), proteinase 3, cathepsin G, and trypsin (22, 24, 31, 32). Other properties of AAT include anti-inflammatory, immunomodulatory, anti-apoptotic, tumour suppressor, anti-microbial, and tissue-repair regulator (29, 33-37).

The predominant protease inhibited by AAT is NE, released by neutrophils. NE is a single-chain protein, consisting of 218 amino acid residues, with two carbohydrate side chains, and a molecular weight of 29 kDa (38). It is synthesised in the bone marrow during the stem cell differentiation into mature neutrophilic granulocytes, and is stored in the azurophilic granules of the mature neutrophil, until it is released in the tissues when the neutrophil is activated or dies (23, 38).

Neutrophils are the first line of defence against invading microorganisms, which they degrade by the action of the elastases they release (39). Thus, the normal

physiologic function of NE is defence against invading microbes. However, NE is also capable of cleaving various components of the connective tissue and extracellular matrix proteins, including elastin, collagen, fibronectin, laminin, and proteoglycan, thus damaging tissues (23, 39-41). Also, the role of NE in cancer development has been described (37).

The mechanism by which AAT inhibits NE is complex and depends upon the ability of AAT to undergo conformational changes in its structure, as depicted in Figure 2 (25, 26, 42). The Met^{358}–Ser^{359} on the reactive centre loop of AAT is highly attractive to the proteolytic site of the NE (His^{41}-Asp^{88}-Ser^{173}). The attraction between AAT and NE is high, with an association rate constant of AAT for NE of $6.5x10^7$ $M^{-1}s^{-1}$, which is almost 25-fold greater than for the interaction of AAT with any other protease (23).

When they interact, NE docks at the reactive centre loop of AAT, and an irreversible 1:1 complex bond between AAT and NE is formed. Following this conformational transition, described as a 'mousetrap' action, both the NE and the AAT are inactivated and removed from circulation (22, 43).

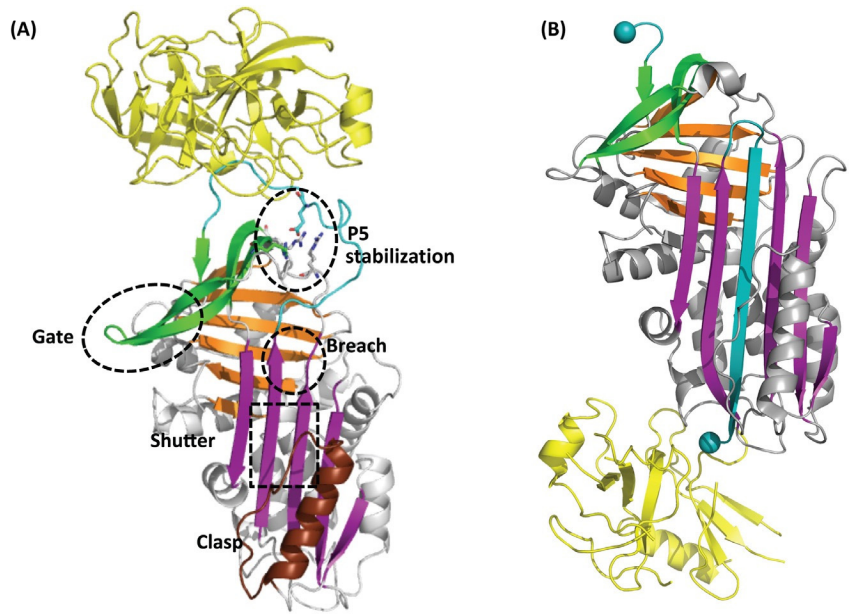

Figure 2.
Figure depicting the encounter between alpha-1-antitrypsin and protease (A), and the final complex between these two, where the protease after cleavage is inserted as the central strand of beta (β)-sheet A (B). Reprinted with permission from Elsevier from Jagger A., *et al.* 'Alpha1-Antitrypsin: structure and dynamics in health, disease and drug development' in: N Kalsheker, R Stockley (Eds.), *Alpha-1-Antitrypsin Deficiency*, Academic Press, Boston, 2017, p. 49–80, Copyright © 2017 Elsevier Inc. All rights reserved.

By inhibiting the proteolytic activity of NE, AAT protects the lung parenchyma. However, the Met^{358} residue at the active site of the AAT is vulnerable to oxidation by oxidising agents (44-47). These are highly reactive oxygen metabolites that can impair and damage proteins (48). Intrapulmonary oxidants are produced by inflammatory cells, or are inhaled from the environment (cigarette smoke and air pollution particles) (49, 50). Oxidants in cigarette smoke can oxidate methionine to methionine sulfoxide (44, 51). This action reduces the association rate constant of AAT for NE (by a factor of 2,000), thereby resulting in loss of inhibitory activity toward NE, which, when uninhibited, can exert its proteolytic effects and destruct pulmonary tissue (52). Cigarette smoke also recruits and activates neutrophils and macrophages in the lung (53). Increased levels of proteases along with decreased functional capacity of AAT leads to pulmonary tissue injury (reviewed later in this thesis) (47, 54, 55).

Genetics

Deficiency of alpha-1-antitrypsin is a hereditary condition, inherited in an autosomal co-dominant fashion (9, 56).

The alpha-1-antitrypsin protein is coded by a single 12.2 kilobase long gene on the chromosome 14 within the q31-32.3 region (the SERPINA1 gene) (22). The expression of AAT is complex and not entirely understood (57). The gene is polymorphic, and approximately 150 alleles have been identified to date (58-62). Each AAT variant has the prefix "Pi" indicating "protease inhibitor", followed by an ∗ (asterisk) and a capital Roman letter (58).

The AAT variants are categorised based on the plasma levels of AAT and function in the following groups (also reviewed in Table 1 under the section of 'Diagnosis of alpha-1-antitrypsin deficiency') (16, 22, 63-65):

- **Normal**: **M** allele, the most common AAT allele. Homozygous individuals (Pi∗MM) have normal plasma level and function of AAT.
- **Deficient**: characterised by lower AAT levels than normal and decreased functional activity, due to point mutations that give rise to amino acid shifts. The **Z** and **S** variants are most common alleles with AAT deficiency.

  The **Z**-variant accounts for 95% of the individuals with severe AATD. In the **Z**-variant, Glu^{342} is substituted by Lys^{342}. Homozygosity for the Z-alleles (**Pi∗ZZ)** is consistent with a plasma level approximately 11-20% of the normal level. In the **S**-variant Glu^{264} is substituted by Val^{264}, leading to a less stable AAT molecule than the M-variant.

- **Null**: characterised by the absence of circulating AAT in the plasma due to transcriptional or translational errors interrupting the synthesis of AAT. These variants are rare.
- **Dysfunctional**: characterised by normal levels, but with abnormal AAT function, as in the **F**-variant, which has reduced binding to NE, or in the **Pittsburgh**-variant, which converts the AAT into a thrombin inhibitor by a mutation in the active site of AAT from Met^{358} to Arg^{358}.

Pathogenesis of alpha-1-antitrypsin deficiency

Normally, in Pi∗MM individuals, following transcription in the nucleus of the AAT-synthesising cell in the liver and translation in the rough endoplasmic reticulum (ER), the synthesised AAT molecule folds, and is translocated to the Golgi apparatus for complete forming of its side chains (22). This process yields a mature AAT protein which is secreted to the circulation.

Several mechanisms have been described that could result in AATD (66). These include deletion of the AAT gene, degradation of AAT during transcription, accumulation of the newly synthesised AAT in the rough ER due to conformational changes that prevent its translocation from the ER to the Golgi apparatus, degradation of the AAT protein within the synthesising cell because of incorrect folding and, secretion of dysfunctional AAT protein which is not able to act normally.

The clinical manifestations of AATD in liver and lung disease can briefly be explained by the mechanisms of 'gain of function' and 'loss of function' (67, 68). These mechanisms reflect an intracellular accumulation of AAT in the hepatocytes and, on the other hand, low AAT levels in the circulation, respectively (56, 69).

Intracellular accumulation

Aggregation of the Z molecules in the endoplasmic reticulum of the liver cells leads to the formation of a chain of polymers of aberrant proteins, in a process called loop-sheet polymerisation, Figure 3 (70). Polymerisation leads to the accumulation of the AAT polymers in the hepatocytes, and decreased secretion into the circulation (67, 68).

Accumulated AAT polymers are the histopathological hallmark of AAT-related liver disease, and can be identified by electron microscopy as diastase-resistant periodic acid-Shiff (PAS) stained inclusion bodies within the endoplasmic reticulum of hepatocytes (56).

The retention of accumulated, misfolded AAT molecules initiates a series of pathologic events in the hepatocyte that culminate in hepatic injury, due to the 'gain of function' mechanism (71). However, there is limited understanding in the exact mechanism/s by which the cellular injury occurs, though, it is presumed that liver injury and liver disease occur when the cell-protective mechanisms are overcome (68). Some of these mechanisms involve proteasomal degradation of misfolded AAT molecules, in a process called ER-associated degradation (ERAD) and autophagic degradation, in which the aberrant AAT molecules are degraded through engulfment within vesicles that fuse with lysosomes (68).

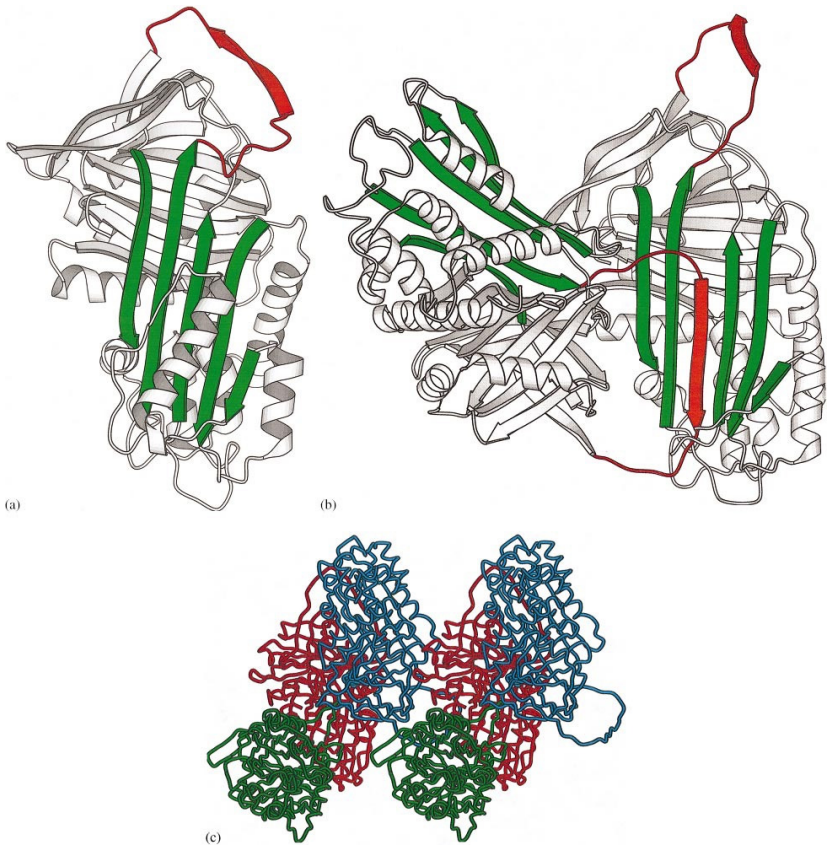

Figure 3.
The figure depicts the molecular basis of loop-sheet polymerisation, where the reactive loop of alpha-1-antitrypsin (red) (a) can insert into β-sheet A (green) of a second alpha-1-antitrypsin molecule (b). (c) depicts a dimer of alpha-1-antitrypsin where the reactive centre loop of one alpha-1-antitrypsin molecule inserts into a β-sheet A of a second alpha-1-antitrypsin molecule. Reprinted with permission from Elsevier from Lomas D.A. 'Loop-sheet polymerization: the mechanism of alpha1-antitrypsin deficiency.' *Respiratory Medicine* 2000;94(Supplement C):S3-S6.

Approximately 70% of the Z-variants are recognised by protein-folding quality-control mechanisms and degraded through the ERAD pathway within the hepatocytes (26). Fifteen per cent of the Z-variants that form polymers accumulate within the ER as intracellular inclusions and cause cellular damage (72). The remaining 15% are secreted through the Golgi apparatus into the circulation (26, 73).

Low plasma concentration

Retention of AAT molecules in the hepatocytes results in low level of AAT in plasma (56). This leads to the 'loss of function' theory, denoting an imbalance between proteases and antiproteases in the lung parenchyma (74).

When there is a deficiency of antiproteases, the proteases remain free to exert their proteolytic action. In the lungs, this results in degradation of pulmonary components (75, 76). In the Pi∗ZZ state, the Z-variant of AAT fails to reach the lung in adequate quantities and, thus, there is reduced inhibition of the serine proteases released by the neutrophils, particularly NE. Unopposed NE activity results in cleavage of the components of connective tissue including elastin (a matrix protein important for the lung tissue structure and function), collagen, proteoglycans, and fibronectin, contributing to emphysema. NE can also induce increased mucus secretion and hyperplasia of mucus-secreting cells (goblet cells); impair the mucociliary clearance, leading to defective bacterial clearance; and stimulate the release of inflammatory mediators which act chemotacticly for further neutrophil recruitment (40).

Epidemiology of alpha-1-antitrypsin deficiency

Previous studies indicate that severe AATD might have arisen in northern Europe, more precisely in southern Scandinavia, and was then spread to other countries following the Vikings' expeditions (77, 78). The highest Pi∗ZZ prevalence is found in southern Sweden, Denmark, Norway, the Baltics, Finland, the Netherlands, Belgium, western France, England, and Spain (29). The Pi∗ZZ prevalence decreases gradually from north-western to south-eastern countries (78).

The prevalence of Pi∗ZZ reported in Sweden is 1 in 1,575 individuals (56). This was established by the nation-wide neonatal screening between 1972-1974, when 127 Pi∗ZZ were identified among 200,000 new-borns (19). In Denmark, among 9,187 randomly-selected individuals from the Copenhagen City Heart Study, a prevalence of approximately one Pi∗ZZ in 1,500 individuals was found (79). In the USA, the prevalence of Pi∗ZZ has been estimated to approximately one in 3,500 individuals. In 1987 in the St. Louis area, Silverman and his colleagues reported a high Pi∗ZZ prevalence of one in 2,857 individuals, following screening of 20,000

blood donors (80). In contrast, a screening programme in Oregon from 1978 following testing of 107,038 new-borns, reported a lower prevalence of one in 5,097 individuals (81).

In a global survey comprising 373 cohorts from 58 countries around the world, de Serres estimated that there are 116,000,000 AATD carriers (Pi∗MS and Pi∗MZ) and as many as 3.4 million individuals with Pi∗SS, Pi∗SZ, and Pi∗ZZ (82). In a more recent review of 224 cohorts (90 European, 42 American, 66 Asian, 17 African, eight Australian and one from New Zealand) comprising 65 countries in the world, Blanco and colleagues estimated that there are a total of 253,404 individuals with the Pi∗ZZ phenotype worldwide (83).

Severe AATD appears to be a relatively common disorder that is rarely diagnosed, and not a rare disease as previous anticipated (84). However, only a minority of those with severe AATD are recognised (78). Under-recognition delays the time to diagnosis, the time to disease management and to the identification of family members at risk of having severe AATD. The mean time between experiencing the first symptoms and the initial AATD diagnosis (the diagnostic delay interval) is estimated to 5.6±8.5 years (85). Several health care providers are sought until a diagnosis is made. In a survey of 300 Pi∗ZZ individuals, Stoller *et al.* reported that 44% met at least three physicians before the AATD diagnosis was made, and 12% reported seeing 6-12 physicians (86).

The literature elaborates some of the reasons for AATD under-recognition (78). The clinical presentation of AATD is variable, as emphysema, bronchitis, and liver disease are not specific for AATD. The genetic penetrance is incomplete, as at most 60% of Pi∗ZZ individuals develop COPD (87). Due to lack of awareness among physicians, only a minority of them actually adhere to the recommendations of testing for AATD in all individuals with COPD (88). The AATD individuals might be misdiagnosed and classified as having COPD related to smoking and liver disease related to alcohol (29). The ‘therapeutic nihilism’ of the physicians is also mentioned in the literature, regarding the effectiveness of the available therapy and its costs (85).

Diagnosis of alpha-1-antitrypsin deficiency

Determination of alpha-1-antitrypsin concentration

The concentration of AAT in serum or plasma is measured by means of nephelometry or turbidimetric immunoassay (89-91). These techniques are based on light scatter for the measurement of an AAT-antibody complex (90-92). Turbidimetry measures the intensity of the transmitted light through the sample, whereas nephelometry measures the scattered light (91). In Scania region (Region

Skåne), Sweden, nephelometry is used to determine the AAT concentration (Dr. Magnus Förnvik Jonsson, personal communication).

The average plasma concentration of AAT in healthy individuals approximates 1.32 $g \cdot L^{-1}$ (reference interval 0.86 – 1.75 $g \cdot L^{-1}$ in men; 0.94 – 1.94 $g \cdot L^{-1}$ in women) (93, 94). As AAT is an acute-phase protein, its concentration levels may fluctuate, and be elevated during inflammatory conditions (56, 92). Simultaneous quantification of the C-reactive protein (CRP) level can be performed to recognise any acute phase response by the time of testing (89, 92).

AAT levels vary for different AAT phenotypes, and may even overlap (Table 1) (93).

Phenotyping by isoelectric focusing

According to the American Thoracic Society/European Respiratory Society statement on standards for diagnosis and management of AATD, the gold standard for diagnosing AATD is phenotyping (56).

In Sweden, phenotyping is done by isoelectric focusing (IEF), at the accredited laboratory, at the Department of Clinical Chemistry at Skåne University Hospital in Malmö. Thus, samples are sent from all over Sweden to Malmö for Pi phenotyping.

Table 1.
Plasma concentration of AAT, prevalence in the Swedish population, and the estimated risk of emphysema in different AAT phenotypes. Adapted with permission from Studentlitteratur, from Jonsson M, Hansson L-O, Larsson A, Grubb A. 'Plasmaproteiner, inflammation och amyloidos' in Theodorsson E, Berggren Söderlund M (Eds.), *Laurells klinisk kemi i praktisk medicin*, Studentlitteratur, Lund, 2018, p. 87-130.

Phenotype	Concentration±2SD		Prevalence in the Swedish population	Risk of pulmonary emphysema
	($g \cdot L^{-1}$)	% of normal concentration (1.32 $g \cdot L^{-1}$)		
Pi∗MM	0.92 – 1.72	70 – 130	93/100	Normal
Pi∗MZ	0.53 – 0.99	40 – 75	4.6/100	Normal
Pi∗SZ	0.34 – 0.65	26 – 49	1/750	Increased in smokers
Pi∗ZZ	0.15 – 0.26	11 – 20	1/1600	High
Pi∗Null Null	-	-	0.0001	Very high

Phenotyping is performed through isoelectric focusing, by which plasma proteins are separated according to their isoelectric point (95, 96). Mobility of the proteins is obtained by subjecting them to an electric field on a gel plate with a fixed pH

gradient (95, 97). The protein with the lowest isoelectric point will migrate towards the anode, while the protein with the highest isoelectric point will migrate towards the cathode (97). The protein will migrate until its overall charge reaches zero, which is when it reaches its isoelectric point in the pH gradient of the gel (90, 92, 95, 97). When that happens, the protein variants are 'focused' and after staining can be visualised as banding patterns; see Figure 4 (97).

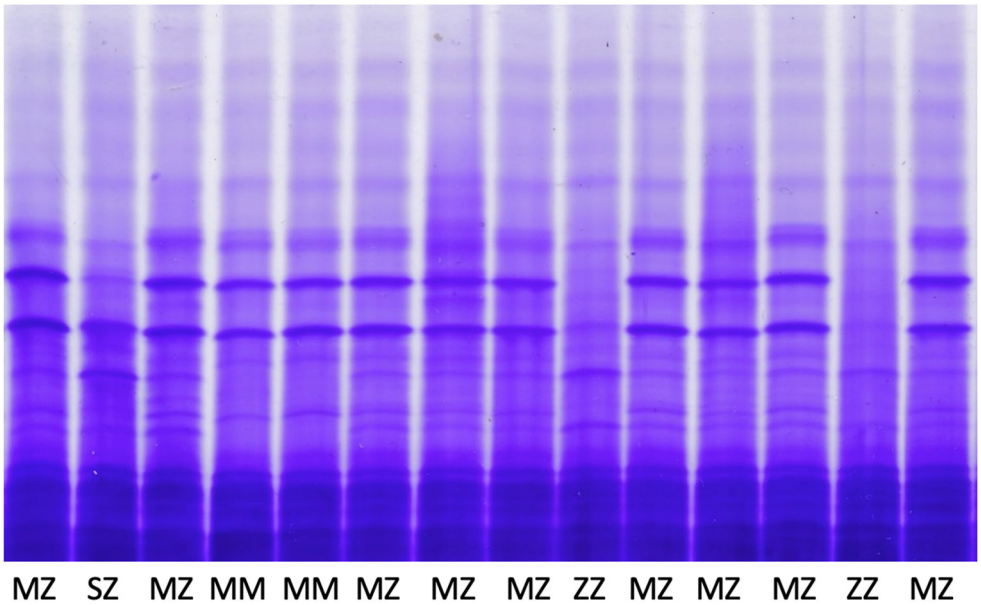

Figure 4.
Electrofocusing of various alpha-1-antitrypsin phenotypes at pH 4-5 after Coomassie Brilliant Blue R-250 staining. Anode at the top. The capital letters correspond to the electrophoretic mobility of the AAT molecules from cathode to anode. (Courtesy of Dr. Magnus Förnvik Jonsson).

The nomenclature of the protein's electrophoretic mobility in the gel includes letters from the beginning of the alphabet to characterise the most anodal variants, while the cathodal variants are designated by letters from the end of the alphabet (Figure 4) (13, 14, 58). The heterogenic mobility is related to the protein charge differences, as a result of the amino acid sequences (13, 97). Pi phenotyping cannot distinguish homozygous variants from heterozygosity with a null variant (*i.e.* Pi*MM, SS, ZZ from Mnull, Snull, Znull).

Genotyping

Genotyping by DNA sequencing can determine all AAT variants, and is used to identify rare allelic variants, including the null allele (98). It is also useful whenever an individual's phenotype does not correspond to the measured AAT plasma or

serum level (98). Genotyping can determine the specific allelic variants through polymerase chain reaction (PCR) (89, 90, 98, 99).

Who should be tested for AATD?

The World Health Organization (WHO) and medical societies in the US and Europe have developed recommendations regarding three types of testing for AATD: diagnostic testing, predispositional testing, and screening (56, 100). The recommendations are ranged A to D, with A recommending testing, B opting for discussing testing, C neither encouraging nor recommending testing, and D discouraging testing.

- ***Diagnostic testing*** evaluates the presence of AATD in individuals with signs or symptoms that could indicate an AATD-related disease. Type A recommendation includes symptomatic adults with emphysema, COPD, and severe asthma, asymptomatic individuals with pulmonary dysfunction combined with smoking and occupational exposure, individuals with unexplained liver disease, and adults with necrotizing panniculitis. Type B recommendation includes symptomatic adults with bronchiectasis and multisystemic vasculitis, and adolescents with pulmonary dysfunction.
- ***Predispositional testing*** aims to identify asymptomatic individuals who might be at risk of having a genetic susceptibility to develop AATD-related diseases. The single type A recommendation regards individuals with a family member with severe AATD (siblings). The type B recommendation includes individuals with a severe AAT-deficient family member (offspring, parents, and distant relatives), individuals with a family history of COPD or liver disease, and individuals having a family member with heterozygous AATD (Pi∗MZ).
- ***Screening*** in populations for individuals with predisposition to AATD-related disease, with no previous suspicion that any of them has the condition. Type B recommendation for screening of adolescents and adults exists only for countries with high prevalence of AATD, combined with high smoking rates and access to suitable counselling services.

Clinical manifestations of severe alpha-1-antitrypsin deficiency

Pulmonary disease

Emphysema

Emphysema is the main clinical manifestation associated with severe AATD, and is characterised by early onset during the fourth decade of life (56). Emphysema is a condition of the lung characterised by abnormal, permanent enlargement of air spaces distal to the terminal bronchiole, accompanied by destruction of their walls (101, 102). In severe AATD, emphysema is often most accentuated at the lung bases, characterised by a panacinar pattern (extending to the alveolar tissue) (63, 103, 104). In contrast, in non-AATD individuals with emphysema the pattern is usually apical (upper lobes), with a centriacinar pattern (involving the walls of the respiratory bronchioles, sparing the alveoli) (105). However, the distribution and type of emphysema in individuals with severe AATD may vary (104, 106, 107).

Chronic obstructive pulmonary disease (COPD)

COPD is defined as:

> a heterogeneous lung condition characterized by chronic respiratory symptoms (dyspnea, cough, sputum production and/or exacerbations) due to abnormalities of the airways (bronchitis, bronchiolitis) and/or alveoli (emphysema), that cause persistent, often progressive, airflow obstruction (108).

Definition of chronic bronchitis is productive cough on most days of a month for at least three months of a year in two or more consecutive years (108).

Airflow obstruction in COPD is caused by destruction in the lung parenchyma, and/or by inflammation of the airways. The following structural changes, with thickened bronchial walls, narrowing of the airways, and the loss of elastic recoil due to destruction of the components in the alveolar walls, diminish the ability of the airways to remain open during expiration and to empty the lungs during forced expiration (108).

Diagnosis

The airflow obstruction in COPD is assessed with dynamic spirometry (a lung function test), from which the values of vital capacity (VC), the air volume exhaled

from the point of maximal inspiration, and forced expiratory volume in one second (FEV_1) result. The role of spirometry in COPD is diagnostic and prognostic, and it is also used in the follow-up assessment (108, 109). In clinical practice, forced vital capacity (FVC), the air volume forcibly exhaled from the point of maximal inspiration, is often used.

In a reversibility test, spirometry is performed before and 15 minutes after administration of a short-acting bronchodilator. A post-bronchodilator FEV_1/FVC ratio <0.7 is the spirometry criterion for diagnosis of COPD (108). Age, height, sex, and race determine the reference values for the results of spirometry (110). The severity of airflow obstruction is based on post-bronchodilator FEV_1 values (% of predicted) and comprises the following four severity groups according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD): GOLD 1 (mild) $FEV_1 \geq 80\%$ of predicted value, GOLD II (moderate) $50\% \leq FEV_1 < 80\%$ of predicted, GOLD III (severe) $30\% \leq FEV_1 < 50\%$ predicted, and GOLD IV (very severe) $FEV_1 <$ 30% of predicted (108).

Clinical presentation

The clinical presentation of COPD due to severe AATD shares common features with usual, non-AAT replete COPD, including the characteristic symptoms of dyspnea, wheezing, cough, and sputum production. The onset of respiratory symptoms and decreased lung function has been reported to already occur in the fourth decade of life in Pi*ZZ smokers, compared to non-smoking Pi*ZZ individuals, in whom the onset can be delayed to the fifth decade (12, 56, 107, 111-114).

During the course of COPD, affected individuals might experience acute worsening of their usual respiratory symptoms, called exacerbations, that result in additional therapy other than their regular medication (108).

The GOLD 2024 defines an exacerbation of COPD (ECOPD) as:

> an event characterised by dyspnea and/or cough and sputum that worsens over <14 days, which may be accompanied by tachypnea and/or tachycardia and is often associated with increased local and systemic inflammation caused by infection, pollution, or other insult to the airways (108).

The severity grades of the ECOPD are divided into the categories of mild, moderate, and severe (108). In severe ECOPD, the individual requires hospitalisation or visits the emergency room. ECOPD contributes negatively to the disease progression and prognosis and constitutes a negative impact on health status (108, 115).

Lung function decline

The value of FEV_1 is useful in the diagnosis and prognosis of COPD (116). Also, the initial value of FEV_1 has been reported as an accurate predictor of respiratory mortality (117). Since the natural history of FEV_1 was described by Fletcher and Peto, the rate of decline in FEV_1 serves as a marker of the degree of airway obstruction and so of COPD progression (117, 118). Also, in healthy individuals, FEV_1 changes with age, increasing during childhood to a stage through early adulthood and decreasing thereafter due to physiological lung aging (108, 116, 118). FEV_1 declines normally with aging in healthy adults who do not smoke by approximately 20-30 mL annually (116, 119). A range of lung function trajectories throughout life have been described in the normal population (108).

The effect of smoking on the annual decline in FEV_1 (ΔFEV_1) has previously been investigated, with results showing that smokers have a steeper decline in FEV_1 than do the never smokers (118, 120). Cessation of smoking may normalise the decline in FEV_1 to the rate of never smokers (109, 120). Age, occupational dust exposure, smoking, years of smoking, and bronchodilator response are some of the risk factors reported for decline in FEV_1 in individuals with 'usual' COPD (121). Exacerbations of COPD are risk factors for an accelerated decline in FEV_1 (122-124).

Asthma

The Global Initiative for Asthma (GINA) describes asthma as:

> a heterogenous disease, usually characterised by chronic airway inflammation, defined by the history of respiratory symptoms such as wheeze, shortness of breath, chest tightness, and cough, that vary over time and in intensity, together with variable expiratory airflow limitation (125).

Signs and symptoms of asthma are common in severe AATD, and asthma is often diagnosed as the first manifestation of AATD (56, 100, 126). AATD might predispose to the airway hyperresponsiveness seen in asthma (127).

Bronchiectasis

Bronchiectasis is a chronic respiratory disease, characterised by abnormal, irreversible dilation of the bronchi with impaired mucociliary clearance, featuring persistent cough with sputum production and recurrent acute exacerbations (128).

Bronchiectasis has also been associated with AATD. The mechanistic link described in the literature is incompletely opposed NE activity, which impacts bronchiectasis disease progression (129). The ATS/ERS statement recommends diagnostic testing

for AATD in individuals with bronchiectasis without evident etiology (recommendation grade type B) (56).

Risk factors for lung disease

Severe AATD is the most relevant genetic risk factor for COPD (108).

Tobacco smoking, indoor and outdoor air pollution, and occupational exposure to toxic particles, gases, and fumes are the main environmental risk factors for the development of COPD (108, 130). The adverse health effects of exposure to air pollution in humans show not only short- and long-term mortality, but also adverse respiratory effects such as accelerated lung function decline and increased risk of exacerbations in COPD (49, 131).

Cigarette smoking is the most important environmental risk factor for COPD in severe AATD (53, 132). The available data indicate that other risk factors in the environment, such as outdoor air pollution and different occupational exposures to dust, fumes, gas and smoke may worsen respiratory status and lung function in severe AATD individuals (53, 132, 133).

Cigarette smoke stimulates neutrophilic inflammation in the lung, thus, the amount of pulmonary damage accounted for by NE is larger in the AATD smokers than in the smokers with normal AAT level (53). It also has detrimental oxidising effects on AAT's reactive site, and may drive polymerisation of AAT molecules, which in turn can increase the neutrophil recruitment to the site, thus increasing the inflammation in the lungs. These actions may account for the progression of the pulmonary tissue destruction resulting in emphysema.

Treatment of pulmonary disease

Preventive management and treatment of AATD associated lung disease is similar to 'usual' COPD treatment algorithms and recommendations (56, 89, 108, 134). It includes smoking cessation, minimising exposure to respiratory irritants (tobacco smoke, gases, dusts, fumes), pharmacological treatment with inhaled bronchodilators, early antibiotic therapy for all purulent acute exacerbations of COPD, supplemental oxygen therapy when indicated by conventional criteria, preventive influenza and pneumococcal vaccinations, nutritional support, pulmonary rehabilitation for individuals with functional impairment, management of associated complications such as depression and anxiety, and surgical interventions (56, 65, 89, 135).

Augmentation therapy

The specific treatment for AATD is augmentation therapy, denoting substitution with exogen AAT, in order to raise the levels of AAT in deficient individuals (136, 137). The rationale behind substitution therapy is to raise the AAT level and the capacity to inhibit NE to a level that protects against further elastase-mediated degradation of the lung tissue and further progression of emphysema (138).

The American Thoracic Society (ATS)/European Respiratory Society (ERS) statement recommends intravenous augmentation therapy for individuals with established airflow obstruction and with FEV_1 35-60% predicted (56).

Some of the treatment options are *intravenous human plasma-derived AAT augmentation therapy* with regular weekly infusions, purified from human plasma, and *augmentation therapy by inhalation* (136, 138, 139). *Recombinant AAT augmentation therapy* and *synthetic elastase inhibitors* have been described as alternative treatment options (140). Other new treatment possibilities include gene therapy to increase the levels of AAT, and therapies aimed at preventing the accumulation of the Z-variant in the hepatocytes, or accelerating the intracellular degradation of the Z-type AAT, among others. However, these therapy approaches are still under development (65, 141).

In Sweden, two drugs are available for AAT augmentation therapy for clinical use. However, these are not included in the pharmaceutical benefit supplemented economically by the state and are thus not reimbursed. Until recently, the Swedish Respiratory Society (*Svensk Lungmedicinsk Förening*) did not generally recommend augmentation therapy, based on insufficient knowledge on the effects of such a treatment. Also, the Swedish National Board of Health and Welfare (*Socialstyrelsen*) recommends AAT substitution therapy only in connection with research (142).

However, as of 2022, the Swedish Respiratory Society has published guidelines to help clinicians consider initiating substitution therapy in AATD (143). The society also recommends having a discussion with several colleagues regarding an individual, prior to initiation of treatment, and the head of the clinical department must participate in the treatment decision, given the high costs of the treatment.

Surgical procedures

Surgical procedures include lung volume reduction surgery (LVRS) and lung transplantation (LT). LVRS implies the resection of the most severely involved areas of emphysema in individuals with advanced emphysema and dyspnea despite optimal medical and rehabilitative treatment (56). The ATS/ERS conclude that LVRS offers only short-term benefits for most AATD individuals with emphysema.

Liver disease

The liver disease in severe AATD may result from the retention and accumulation of AAT polymers within the endoplasmic reticulum of the hepatocytes, as described earlier (56, 144). Liver disease in severe AATD is variable and presents a biphasic pattern, with the first peak occurring in early childhood (neonatal cholestasis) and the second peak occurring in adulthood (cirrhosis and hepatocellular carcinoma) (145). The estimated prevalence of liver disease in Pi∗ZZ children is 17% and is 10-15% in adult Pi∗ZZ individuals (56, 146).

In the studies from the Swedish neonatal screening of 200,000 newborns, Sveger found that 22 of the 127 Pi∗ZZ newborns (17%) had clinical signs of liver disease in infancy (19). Five Pi∗ZZ children died within their first decade of life: two died of liver cirrhosis, one died in an accident (autopsy revealed liver fibrosis), one died of aplastic anemia (autopsy revealed early signs of liver cirrhosis), and one died of anaphylactic shock (147). Approximately half of the clinically healthy Pi∗ZZ children had abnormal liver function test results, which normalised during early childhood. At the follow-ups when the Pi∗ZZ children were 12-18 years of age, none of them showed clinical signs of liver disease (148). The regular long-term follow-ups of these Pi∗ZZ individuals, at 26, 30, 34, and 37 to 40 years of age, showed that they had normal liver function test results (149-152). However, at 37 to 40 years of age, the severe AATD men had higher median values of liver function tests as compared with male controls from the general population, even though these values were within the normal range.

Several studies have shown increased risk of liver cirrhosis and hepatocellular cancer in adults with severe AATD (12, 145, 153). A recent registry-based study, encompassing 1,595 Swedish adults with severe AATD, revealed that the prevalence of any liver disease among this group was 10% (146). The mean age at onset of liver disease was 61 (SD 15) years. The risk factors associated with an increased risk of developing any liver disease were male sex, age over 50 years, hepatitis virus infection, having repeated elevated liver function tests, and a diagnosis of diabetes.

The ATS/ERS task force has summarised that there are no other known risk factors than male sex for the development of liver disease in severe AATD (56).

It is not well known why some Pi∗ZZ develop clinically significant liver disease, and others do not (154). The ATS/ERS task force recommends diagnostic testing for AATD (type A recommendation) in all individuals with unexplained liver disease, including neonates, children, and adults, particularly the elderly (56).

Treatment of liver disease

Besides supportive care, there is no specific treatment for AATD associated liver disease, other than liver transplantation in end-stage liver disease (145). Liver transplantation has shown favourable results regarding survival (155, 156).

New treatment approaches are under development, some of which assess the reduction of the amount of accumulated Z-variant in the hepatocytes, likewise blocking the polymerisation of the Z-variant, among others (65, 72).

Current guidelines recommend regular assessment of the development of liver disease in individuals with severe AATD, using clinical, laboratory, and liver ultrasonography examination (56). Vaccination against viral hepatitis is recommended in Pi∗ZZ children and adults suffering from chronic liver disease (56). Other recommendations include maintaining a low to non-existent alcohol consumption, prevention of malnutrition, and avoidance of the use of NSAIDs (56, 65).

Other diseases

Skin disease

Necrotising panniculitis, although rare, is a well-recognised dermatologic complication of severe AATD (56). The WHO report describes a prevalence of less than one case in 1,000 individuals with AATD, predominantly among Pi∗ZZ individuals (100). Stone *et al.* reported a higher prevalence of panniculitis, nine in 1,000 individuals, among the Pi∗ZZ attending the UK national centre for AATD (157). In the Swedish National Registry of Individuals With Severe AATD, panniculitis was reported in two individuals (0.1%) (158).

Necrotising panniculitis is characterised by inflammatory and necrotising lesions of skin and subcutaneous tissue, the bottom fatty layer underneath the skin's surface, that start with hot, red, painful, tender nodules on thighs and/or buttocks, leading to suppurative, oily ulcerations (56). Skin biopsy and histologic evaluation may reveal a neutrophilic inflammation among focal areas of fat necrosis adjacent to areas of normal fat (159). Loss of elastic tissue in the areas of inflammation can also be observed.

In case reports, the mean age at onset was about 44 years, but the disease could occur from infancy to age 65 years, with an equal sex distribution of affected individuals (56, 159, 160). Trauma in the skin area may precipitate the disease (161). The mechanism of the disease may be due to 'loss of function', leading to unopposed proteolysis in the skin (159). Polymers of the Z-variant have been

observed in the skin of a Pi*ZZ individual with panniculitis (161). In addition to conventional therapy (corticosteroids, antibiotics, immunosuppressants), augmentation therapy with purified human AAT has shown successful results, with clinical improvement and healing of the skin inflammation (160).

The prognosis is variable, partly depending on the presence of other AATD-related complications. The ATS/ERS position document recommends a diagnostic test for AATD in adults with necrotising panniculitis (type A recommendation) (56).

Vasculitis

The literature reports an association between severe AATD and vasculitis (56, 63). Previous series estimated that the prevalence of the Z allele among anti-neutrophil cytoplasmic antibody (c-ANCA) positive individuals of 5.6 – 17.6% exceeded by three- to ninefold the frequency in normal individuals (161, 162). Individuals with the Z-allele were overrepresented among those with c-ANCA positive vasculitis, specifically the subgroup with granulomatosis with polyangiitis (GPA, with c-ANCA against proteinase 3) (56, 163, 164).

The mechanism of AATD-associated vasculitis is not completely understood, but may relate to an imbalance between proteases and antiproteases (161). Proteinase 3 (PR3) is a neutrophil elastase-like serine proteinase released by neutrophils to effect proteolytic damage on vessels. AAT is its major inhibitor. Consequently, proteolytic damage on vessels may continue due to unopposed PR3 action in the AAT-deficiency state. Also, in GPA, PR3 is the main target antigen of ANCA, and increased immune exposure of PR3 could enhance an autoimmune reaction (56, 63).

The ATS/ERS task force recommends diagnostic testing for AATD (type B recommendation) in adults with c-ANCA-positive vasculitis (56).

Cancer

Increased risk of hepatocellular carcinoma in Pi*ZZ individuals is well known (56, 146, 153, 165). Only a few studies on a possible association between severe AATD and types of cancers other than hepatocellular cancer have previously been published (37, 166-169).

Gastrointestinal diseases

Some studies have suggested that severe AATD might be implicated in the pathogenesis of inflammatory bowel disease (IBD) and colonic diverticula, due to local tissue injury in the intestines caused by the imbalance between proteases and protease-inhibitors (157, 170, 171).

Thromboembolism

Recently, data from the Swedish National AATD Registry have shown that Pi*ZZ individuals have an increased risk of developing venous thromboembolism compared with the general population, even after adjusting for potential confounding risk factors such as sex, age, the presence of COPD, cancer and liver disease (172).

Cardiovascular disease

In another study from the Swedish National AATD Registry, Pi*ZZ individuals were found to have a lower risk of developing ischemic heart diseases as compared with controls from the Swedish population, independent of sex, age, smoking status, the presence of COPD, and comorbidities (hypertension, diabetes mellitus, and hyperlipidemia) (173).

Prognosis of alpha-1-antitrypsin deficiency

Apart from early onset of emphysema, previous studies of the natural history of AATD have indicated that affected individuals might have a reduced life expectancy (12, 56, 103). For instance, Larsson reported reduced survival in Pi*ZZ individuals, especially among smokers (12). The latter were found to have a median survival time of 40 years, compared with 65 years in never-smoking Pi*ZZ individuals.

In a study comprising 568 Pi*ZZ non-smokers, Tanash *et al.* reported a median age at death of 76 years (range 24-95) and the most common underlying causes of death among the 93 deceased were emphysema (45%) and liver disease (28%) (113). In a more recent study, the Pi*ZZ individuals identified by screening, and who never smoked, had a similar life expectancy as the never smokers in the general Swedish population (174).

The most common underlying causes of death reported by Stoller in Pi*ZZ individuals included in the National Heart, Lung, and Blood Institute (NHLBI) Registry were emphysema (72%) and liver cirrhosis (10%), followed by malignancy and diverticulitis (3% each) (175). Tanash *et al.* found that Pi*ZZ individuals had increased mortality from respiratory diseases, liver carcinoma, complicated colon diverticulitis, and pulmonary embolism as compared with the Swedish general population (176).

The disease burden related to AATD has recently been reviewed (177). Miravitlles *et al.* reported that AATD individuals have a poor quality of life; that the caregivers experience loss of personal time due to stress, anxiety, and their caring responsibilities; and that AATD is also associated with high usage of healthcare resources and medical costs (177).

The Swedish National Neonatal Screening Study of Alpha-1-Antitrypsin Deficiency

Screening is:

> the presumptive identification of unrecognized disease or defect by the application of tests, examinations or other procedures which can be applied rapidly. Screening tests sort out apparently well persons who probably have a disease from those who probably do not. A screening test is not intended to be diagnostic. Persons with positive or suspicious findings must be referred to their physicians for diagnosis and necessary treatment (178).

In 1972, after Carl-Bertil Laurell had described an AATD screening test, which allowed mass screening for deficiency of AAT using a drop of blood dried on filter paper, the Swedish National Neonatal Screening Study was initiated (18, 179). The aims were to assess the prevalence of severe AATD in newborn children in Sweden, to evaluate the screening procedure, and to study lung and liver disease in AATD in early life, but also to give parents the possibility of preventing the AATD child from being exposed to parental tobacco smoke while growing up, and of preventing the child from starting to smoke (20, 180).

The screening study was performed between November 1972 – September 1974. The same blood samples that were collected after birth and sent to the PKU (phenylketonuria) laboratory for the routine Guthrie screening test were analysed for AATD (179). Whenever a sample with AAT levels less than 40% of normal was identified, it was mailed to the laboratory in Malmö, Sweden for Pi phenotyping.

Among 200,000 newborn children, 127 Pi∗ZZ, two Pi∗ZNull, 54 Pi∗SZ, and one Pi∗SNull were identified (19, 179). Five of the Pi∗ZZ and one of the Pi∗SZ children died in early life. The rest have regularly been followed up (at ½, 1, 2, 4, 8, and 12 years), every two years in adolescence and every four years in adulthood (114, 147, 149, 151, 181-188).

In a follow-up study, the majority of the severe AATD adolescents (88%) were non-smokers at 18-20 years of age, compared with 65% among control adolescents (180). At the age of 42 years, none of the 99 severe AATD individuals that participated in the follow-up was a current smoker (188). The majority of the AATD individuals identified by the Swedish National Neonatal Screening Study are included in the Swedish National Registry of Individuals With Severe AATD.

The Swedish National Registry of Individuals With Severe Alpha-1-Antitrypsin Deficiency

The Swedish National Registry of Individuals With Severe AATD was started in 1991 by my co-supervisor, Eeva Piitulainen, and her tutor, Sten Eriksson (21). It is administered at the Department of Respiratory Medicine and Allergology in Malmö, Skane University Hospital. It is approved by the Swedish Ethical Review Authority *(Etikprövningsmyndigheten*), Lund University, Sweden and by the Swedish Authority for Privacy Protection (*Integritetsskyddsmyndigheten,* previously called *Datainspektionen*).

Aims of the registry

The aims of the Swedish National Registry of Individuals With Severe AATD are to study the natural history of severe AAT deficiency, including the risk factors for lung and liver disease, to study the lung and liver function and other organ manifestations in individuals with severe AATD, to improve knowledge of severe AATD among physicians and the general population, to encourage family screenings, and to create a pool of individuals for clinical studies (158, 189).

Inclusion criteria

The inclusion criteria in the Swedish National Registry of Individuals With Severe AATD are age 18 years or older, the severe AATD phenotypes (Pi∗ZZ, Pi∗ZNull or Pi∗NullNull), and written informed consent. Since December 2020, individuals with phenotype Pi∗SZ and other, rare phenotypes are also included (143).

As of September 2023, a total of 1,922 individuals with severe AATD were included in the registry. The expansion of the Swedish National AATD Registry is shown in Figure 5.

Inclusion and follow-up

The Swedish National Registry of Individuals With Severe AATD is in co-operation with the Department of Clinical Chemistry in Malmö, which is the only laboratory performing Pi phenotyping in Sweden. Thus, all Pi phenotypes are verified by isoelectric focusing.

The laboratory continuously reports data to the Swedish National AATD Registry of all newly identified individuals with severe AATD, by sending a copy of the Pi phenotyping where the analysis has shown the severe AATD phenotypes.

The registry contacts the attending physician who ordered the AAT analysis by sending information about the AATD Registry, together with information to the AAT-deficient individual, and two questionnaires (one to be answered by the attending physician, see Appendix I, and one to be answered by the AAT-deficient

individual, see Appendix II). The attending physician is encouraged to ask the individual for participation in the registry. In 1999, the questionnaire for the attending physicians was partly modified to facilitate their administrative work (Appendix III).

Figure 5.
The expansion of the Swedish National Registry of Individuals With Severe Alpha-1-Antitrypsin Deficiency between 1991-2023. (Courtesy of Associate Professor, Dr. Hanan Tanash).

Briefly, these questionnaires regard data on the characteristics of the severe AATD individual, smoking status, indication for the original AAT analysis, results of dynamic spirometry and liver function tests (AST, ALT, ALP, GGT), medical treatment, data regarding lung or liver transplantation, and occupation.

After returning the signed written informed consent form and the completed questionnaires, the severe AATD individual is included in the registry. Initially, after inclusion, the severe AATD individual was followed up every year until 1999, and every two years thereafter. The AATD Registry sends a follow-up questionnaire to the attending physician, the severe AATD individual undergoes physical examination, including spirometry and the same blood samples as at inclusion. All visits are performed at the individual's local hospital or in the primary care setting, and the results are reported to the AATD Registry via a questionnaire.

The Obstructive Lung Disease in Northern Sweden (OLIN) studies

The Obstructive Lung Disease in Northern Sweden (OLIN) studies were initiated in 1985. The overall aim was to prevent obstructive airways diseases by investigating the incidence and prevalence of obstructive lung diseases and associated risk factors including lifestyle, occupational, and environmental factors (190).

The epidemiological studies have been carried out in Norrbotten, the northernmost province of Sweden, which makes up 24% (105,886 km^2) of Sweden's area, but only around three per cent of the Swedish population (approximately 250,000 inhabitants). Today, more than 60,000 individuals, both children and adults, have participated in the OLIN studies.

At study entry, the participants answered a questionnaire sent by regular mail. The questionnaire included questions on respiratory symptoms, diseases, smoking status (current smoker, former smoker, or never smoker), current and previous occupation, and physician diagnoses of asthma, chronic bronchitis or emphysema. The questions on respiratory symptoms included attacks of breathlessness, wheezing, longstanding cough, and sputum production, whether the symptoms occurred in special circumstances or after specific exposures (for instance tobacco smoke, dust, air pollution and strong scents).

Following the questionnaire, a random sample of the responders was invited to clinical examinations including a structured interview containing a large number of questions about respiratory symptoms, factors provoking these symptoms, data on diagnoses and comorbidities, medication used, smoking status, parental smoking status and occupations, other potential risk factors, and lung function testing. The question about physician diagnosis of asthma has been validated by clinical examinations both among children and adults (190, 191).

Aims of the present papers

The overall aim of this thesis was to study the clinical course and prognosis in severe AATD individuals, as described in the following papers:

Paper I

To estimate the rate of decline in lung function in individuals with severe AATD and to identify factors involved in the rapid decline in lung function.

Paper II

To investigate the health status, clinical course, and prognosis in the individuals with severe AATD identified by screening.

Paper III

To evaluate the relative risk and risk factors for incident cancer in Pi*ZZ individuals, compared with a random general population sample from the general Swedish population, and to evaluate survival after cancer diagnosis.

Paper IV

To investigate the potential association between severe AATD and risk of developing gastrointestinal diseases, excluding liver diseases. The specific gastrointestinal diseases of interest (SGID) include Crohn's disease, ulcerative colitis, noninfectious gastroenteritis, and diverticular disease, encompassing symptomatic diverticulosis and its complications.

Methods

Study population

Individuals with severe AATD (papers I-IV)

The individuals with severe AATD (phenotype Pi∗ZZ) included in studies I-IV were selected from the Swedish National Registry of Individuals With Severe AATD, in which they were included.

All individuals were ≥ 18 years of age, had a diagnosis of severe AATD (phenotypes Pi∗ZZ and Pi∗ZNull) performed by isoelectric focusing, and had given their written informed consent to participate in the registry.

In Paper I, the severe AATD individuals who reported having symptoms like cough, phlegm, dyspnea, or wheeze were defined as having respiratory symptoms. Based on their age at inclusion in the AATD Registry, they were stratified into three age subgroups: 'young' denoting the individuals aged 18-39 years, 'middle-aged' denoting the individuals aged 40-59 years and 'old' denoting the individuals ≥60 years of age.

In Paper II, the term 'symptomatic individuals' referred to those who had any of the following at inclusion in the Swedish National AATD Registry: respiratory symptoms or respiratory diseases including asthma, COPD or emphysema, reported either by their attending physician or obtained from the Swedish National Patient Registry (see below). 'Asymptomatic individuals' was the term used for the severe AATD individual without any respiratory symptoms or disease at inclusion in the Swedish National AATD Registry.

Controls (papers III-IV)

In the studies III-IV, controls were randomly chosen from three population-based cohorts within the Obstructive Lung Disease in Northern Sweden (OLIN) studies.

For the studies included in the current Ph.D. thesis, data from three adult OLIN-cohorts, in total comprising around 17,000 individuals, have been included. These three adult OLIN-cohorts were initially recruited in 1992, 1996, and 2006 (192, 193). The participants were between the ages 20-69 years at recruitment. The participation rates were n=4,851 (85% of those invited) in 1992, n=7,420 (85% of those invited) in 1996, and n=6,165 (77% of those invited) in 2006 (192, 193). Two

thousand individuals from each of the three adult OLIN-cohorts (a total of 6,000 controls), were randomly selected and matched according to sex with the severe AATD individuals.

Data collection

After inclusion in the Swedish National AATD Registry, the individuals with severe AATD have been regularly followed up through questionnaires, clinical examination, and lung and liver function tests by means of spirometry and blood tests, respectively. These were performed either at the individuals' local hospitals or in the primary care setting.

The results of the physical examination, including the lung and liver function tests, were then reported every two years to the Swedish National AATD Registry via the returning questionnaires (Appendices I-III).

All data emerging from the questionnaires were assembled and transcribed by research assistants into a dedicated Excel file, lock-secured, at the Department of Respiratory Medicine and Allergology in Malmö, Sweden. This file was also completed with data on the severe AATD individuals emerging from other Swedish national registries (see below), followed by transformation into a SPSS (the Statistical Package for the Social Sciences) file using an automatic merge function in the software.

In order to obtain general information on the individuals with severe AATD and the controls needed for the purpose of the analyses in the studies I-IV, their data was cross-linked with other Swedish national registries through the unique personal identity number of the individuals.

National registries

Data used in the papers included in the present thesis were obtained from Swedish national registries by cross-linkage using the Swedish system of personal identity numbers.

The following national registries were used: the Swedish National Patient Registry (SNPR) (papers I-IV), the Swedish National Cause of Death Registry (papers I-IV) and the Swedish National Cancer Registry (Paper III).

The SNPR was established in 1964, and contains patient-related data (personal identity number, age, sex, and residence), data about caregivers, administrative data (admission date, discharge date, duration of admission, mode of admission and of discharge) and medical data (diagnoses and procedures) (194, 195). It covers more than 99% of all somatic and psychiatric hospital discharges since 1987, when it became nationwide, and has about 80% coverage of hospital-based outpatient care

since 2001 nationwide. Missing information is estimated to be less than one per cent per year regarding hospital admissions.

The SNPR was used in Paper I for data regarding severe exacerbations, in which hospital admission diagnoses after inclusion in the Swedish National AATD Registry due to respiratory diseases were included; in Paper II for data regarding diagnoses at inclusion in the Swedish National AATD Registry and during follow-up; in Paper III for data regarding diagnoses of COPD and liver disease; and in Paper IV for data regarding the diagnoses of Crohn's disease, ulcerative colitis, noninfectious gastroenteritis, diverticular diseases encompassing symptomatic diverticulosis and its complications, and COPD, and for information regarding hospitalisations among the study population.

The Swedish National Cause of Death Registry was established in 1952 (196). Among the many variables it includes is information on vital status, and the date and the cause of death. From this registry, the causes of death and the time of death were used for the papers I-IV.

The Swedish National Cancer Registry was established in 1958 (197). It is of good quality, with more than 99% of the cases being morphologically verified, and has a relatively high overall completeness (198). This registry was used in Paper III for information regarding data of cancer diagnoses.

The questionnaires

The following data can be obtained from the questionnaires (Appendices I-III): (a) details of the attending physician (name, clinic/hospital, telephone number, address), and the date of examination, (b) characteristics of the individual (date of birth, biological sex, body height, body weight, number of siblings), (c) smoking status (age at onset, duration, quantity and quality [cigarettes or cigars or pipe], age when stopped smoking), (d) occupation and workplace environment, (e) original indication for the initial AAT or plasma protein analysis (lung disease, liver disease, other diseases [renal diseases, joint symptoms, repeated infections other than respiratory, a high sedimentation rate or any other sign or symptom for which the analysis has been performed as part of the clinical investigation] or screening [neonatal, family or population screening]), (f) phenotype, (g) date of diagnosis of AATD, (h) diagnoses of lung disease (type of diagnose [emphysema, chronic bronchitis, asthma, bronchiectasis, lung fibrosis or other]), (i) age at onset of respiratory symptoms, (j) the main symptom at onset (dry cough, cough with phlegm, shortness of breath at rest or at exertion, attacks of breathlessness), (k) other diseases, (l) previous pneumonia or colds and the amount of pneumonias/colds, (m) individual's estimation of their health (including symptoms such as cough, phlegm, wheezy chest, exertion capacity and breathlessness), (n) data on medical interventions (lung and/or liver and/or heart transplantation, lung volume reduction surgery and date of the intervention), (o) present treatment (for lung disease or for

other conditions), (p) chest X-ray or CT scan of thorax (date), (q) spirometry results (previous and/or current) and (r) blood tests for liver function tests.

Smoking status

Information on smoking status in the study population was obtained from the questionnaires in the Swedish National Registry of Individuals With Severe AATD (papers I-IV) and from the OLIN studies for the controls (papers III-IV). The smoking status was based on the individual's self-reports.

A never-smoker was defined as an individual who had never smoked, or who had reported having smoked less than 100 cigarettes during his or her lifetime. An ex-smoker was defined as an individual who had smoked more than 100 cigarettes in his or her lifetime, and ceased smoking at least 12 months prior to inclusion in the Swedish National AATD Registry (for severe AATD individuals) or prior to answering the survey in the OLIN studies (for the controls in papers III and IV). A current smoker was defined as an individual who currently smoked more than one cigarette daily by the time of inclusion in the AATD Registry or the OLIN studies. The smoking status was available for all individuals in the studies.

In Paper I, the smoking status was divided into three groups: 'never-', 'ex-' and 'current smokers'. A smoker who stopped smoking during the follow-up period was defined as a 'quitter'.

In Paper II, the smoking status was divided into the two groups 'never-smokers' and 'ever-smokers', where the term 'ever-smokers' included the ex-smokers and the current smokers.

In papers III-IV, the smoking status was divided into three groups: 'never-smoker', 'ex-smoker' and 'current smoker'. The ex-smokers and the current smokers were together categorised as 'ever-smokers'.

Lung function tests

The pulmonary lung function tests were performed at the individuals' local hospitals or in the primary care setting, using European standards for lung volume measurements. The spirometry results were expressed in absolute numbers in litres (L), and were calculated in papers I-II as percentage of the predicted European values accordingly (110).

The first reported FEV_1 and FVC measurements at inclusion in the Swedish National AATD Registry were used as baseline values. In the individuals who had undertaken lung volume reduction surgery (LVRS) or transplantation of the lung(s) (LT), only the spirometries prior to the surgical procedure were included in the analyses.

Only pre-bronchodilator lung function values were analysed because a reversibility test was not consistently performed. In individuals with airflow obstruction

(consistent with a value of FEV_1/FVC ratio below 0.70) the airflow obstruction was graded into one of the four previously described GOLD severity stages (108).

Decline in FEV_1 (ΔFEV_1)

In papers I-II, the annual decline in FEV_1 was analysed in those individuals that had undergone at least three lung function tests at two-year intervals or longer during the whole follow-up time.

The annual decline in FEV_1 was expressed in $mL \cdot yr^{-1}$ and abbreviated ΔFEV_1. Only the values of FEV_1 measured prior to any surgical treatment (LVRS or lung transplantation(s)) were included in the statistical analyses. Pre-bronchodilator values were analysed.

Exacerbations of COPD

In Paper I, the information on severe exacerbations of COPD in the severe AATD individuals with a diagnosis of COPD at inclusion in the Swedish National AATD Registry was obtained from the Swedish National Patient Registry by cross-linkage using the individual's unique personal identity numbers.

The term 'frequent exacerbations' referred to experiencing more than the median annual exacerbation rate, while 'infrequent exacerbations' referred to experiencing less than the median annual exacerbation rate.

Occupations

Details of the individuals' occupation were obtained from the questionnaires in the Swedish National AATD Registry. In Paper II, the reported occupations were classified into nine groups, conferring to the Swedish Standard Classification of Occupations of 2018, which is adapted from the International Standards Classification of Occupations (199).

The nine occupation groups include: (1) managers, (2) professionals, (3) technician and associate professionals, (4) clerical support workers, (5) service, care and sales workers, (6) skilled agricultural, gardening, forestry, and fishery workers, (7) craft and related trades workers, (8) plant and machine operators and assemblers, and (9) elementary occupations.

The groups five to nine were the occupation categories in which the study participants were assessed as having a risk of occupational exposure to airway irritants.

Diagnoses

The diagnoses of interest for the analyses of papers I-IV were coded according to the WHO International Classification of Disease (ICD) system (the 9th and the 10th revisions) (200, 201). The diagnoses were obtained from the SNPR, from the

Swedish National Cause of Death Registry and from the Swedish National Cancer Registry. The ICD codes are presented in papers I-IV as ICD-9 and ICD-10 accordingly.

In Paper I, the diagnoses due to respiratory diseases after inclusion in the Swedish National AATD Registry were included. Exacerbations were identified through the ICD-9 and ICD-10 codes with either the diagnosis of COPD in the first position (491-492; J43-44) and the diagnosis code for acute exacerbation of COPD (466; J440-441), or for respiratory failure (786A, 799B; J96) or pneumonia (480-488; J189) in the second position, or the diagnoses of acute exacerbation or respiratory failure or pneumonia in the first position and the diagnosis of COPD in the second position.

In Paper II, the ICD codes were categorised as (ICD-9; ICD-10): respiratory diseases (emphysema (492; J43), COPD (496; J44), asthma (493; J45), chronic bronchitis (490–491; J40-42)), hepatic diseases (520–579; K70-93), cardiovascular diseases (401–429; I10-52), neurological conditions (320–389, 430–438; G12.2, I60-69), cancer affecting the respiratory organs (162; C34), and cancer affecting the female breast (174; C50.9).

In Paper III, the ICD-7, ICD-9 and ICD-10 codes were included. The cancer diagnoses in Paper III were grouped as 'hepatic cancer' (156; C22) and 'non-hepatic cancer'. The subgroup 'non-hepatic cancer' included cancer in the digestive organs (150-155, 157-159; C15-21, C23-26), reproductive and genitourinary organs (171-179, 181; C51-58, C60-68), respiratory organs (162-163; C30-39), hematological and lymphoproliferative (198, 201-204; C81-96), breast (170; C50), skin (190-191; C43-44), and other cancers involving the thyroid gland, the parathyroid gland, the pituitary gland, the thymus, the adrenal glands, the nervous system, and oropharyngeal (140-148, 160-161, 164-165, 180, 192-197, 199-200, 205-207; C00-14, C40-41, C45-49, C69-97).

In Paper IV, the diagnoses regarding the specific gastrointestinal diseases of interest (SGID) were Crohn's disease (555; K50), ulcerative colitis (556; K51), noninfectious gastroenteritis (558; K52), diverticular disease of the intestines (562; K57), and COPD (496; J44).

Statistical analysis

All data at inclusion (baseline data) were tabulated using frequencies and percentages for categorical variables. For continuous data, means with standard deviation (SD) were used when the distribution was normal, and median with interquartile range (IQR) was used when a non-normal distribution occurred.

Categorical data were compared through the χ^2 -test and the Fisher's exact test. Continuous variables with normal distribution were analysed using the parametric tests independent sample's t-test and ANOVA and the Kruskal-Wallis and the Mann Whitney non-parametric tests for non-normally distributed data. The post hoc Tukey test was used for multiple comparisons.

In Paper I, ΔFEV_1 was calculated as the difference between the results of the individual's last and first reported FEV_1 measurement (in mL) in the registry, and divided by the calculated time between the measurements (in years), and was analysed by random effects modelling (202, 203). This included ΔFEV_1 ($mL \cdot yr^{-1}$) as the dependent variable, with covariates being the age at baseline, the FEV_1 at baseline (the individual's first reported FEV_1 measurement at inclusion in the registry), and the follow-up time, while the fixed factors included gender, smoking status, presence of respiratory symptoms and of COPD, and the individual patients as random effects factors. The follow-up time for the analysis of ΔFEV_1 was the time from the date of the spirometry at inclusion to lung transplantation (LT), lung volume reduction surgery (LVRS), death, or study end (01 June 2016), whichever occurred first.

In Paper II, the ΔFEV_1 was calculated in the same manner as in Paper I, but the fixed factors were smoking status, identification with severe AATD before the age of 14 years, occupational exposure to airway irritants, and the presence of respiratory symptoms.

The rate of the annual exacerbations in Paper I was calculated for each severe AATD individual, as reported in other studies on exacerbations of COPD (204, 205). The mathematical formula was: (the total number of exacerbations the individual experienced/the total number of days the individual participated in the study * 365).

In Paper III, the incidence rate of cancer, expressed as n/per 1,000 person-years, where *n* denoted any case of cancer observed during the follow-up time, was calculated by the fraction between the numerator *n* and the sum of the follow-up years (the time at risk) as denominator, and multiplied by 1,000. In Paper IV, the incidence rate of any SGID was calculated in the same manner as the incidence rate of cancer in Paper III. The incidence rates of cancer (Paper III) and of SGID (Paper IV) were calculated separately for the severe AATD individuals and for the controls.

COX proportional hazards regression analyses were used to investigate risk factors for having a diagnosis of COPD at study-end (Paper II), risk factors for developing cancer (Paper III), and SGID (Paper IV), and the risk of hospitalisations among the study participants with SGID (Paper IV). The estimates were expressed as hazard ratios (HR) with 95% confidence interval (CI), and as adjusted hazard ratios (aHR) with 95% CI, when the analyses were adjusted for different risk factors.

In Paper III, the analysis model was adjusted for the baseline age of the individuals, sex, the smoking status of the individuals, and the presence of liver disease at

inclusion in the Swedish National AATD Registry for the severe AATD individuals and the OLIN studies for the controls. The analyses were made separately for the severe AATD individuals and the controls, and for hepatic and non-hepatic cancer, and were stratified by sex, smoking status, and the presence of liver disease at inclusion.

In Paper IV, the analysis model was adjusted for the baseline age of the individuals, sex, smoking status at inclusion and the presence of a diagnosis of COPD at inclusion.

In Paper III, the Kaplan-Meier method was used to investigate survival after the first cancer diagnosis, where the follow-up time for mortality was from the date of the first cancer diagnosis to the date of death or study end on 01 January 2015, whichever occurred first. Differences in survival time were analysed using the log-rank test. In Paper IV, the Kaplan-Meier method was used to illustrate the cumulative incidence of any SGID in the whole study population.

A p-value of <0.05 was considered statistically significant.

The statistical analyses were performed with the Statistical Package for the Social Sciences (SPSS), version 23.0 (IBM Corporation, Armonk, NY, USA) in Paper I, version 27.0 in papers II-III, and version 29.0 in Paper IV.

Results

Study population

In Paper I, of the 1,640 severe AATD individuals included in the Swedish National AATD Registry up to 01 June 2016, 508 individuals were excluded since they had undergone less than three spirometries. Thus, 1,132 severe AATD individuals were included in the analyses. Of these, 30 underwent LVRS and 86 underwent LT. These individuals' data were analysed up to the date of LVRS or LT.

In Paper II, of the 1,585 severe AATD included in the Swedish National AATD Registry up to 01 June 2016, 671 individuals were identified due to respiratory symptoms or diseases and 537 individuals were identified due to liver and other symptoms or diseases. These individuals were excluded from the analyses. The remaining 377 severe AATD individuals identified by screening were thus included in the analyses.

In Paper III, of the 1,595 severe AATD individuals included in the Swedish National AATD Registry up to 01 January 2015, 10 individuals were excluded since they had undergone lung transplantation prior to inclusion in the Swedish National AATD Registry and 15 individuals were excluded since they suffered from cancer within five years prior to inclusion in the Swedish National AATD Registry. Of the 6,000 controls, one was excluded due to incorrect personal identification number and 48 individuals were excluded since they suffered from cancer within five years prior to inclusion in the OLIN studies. Thus, 1,570 severe AATD individuals and 5,951 controls were included in the analyses.

In Paper IV, of the 1,595 severe AATD individuals included in the Swedish National AATD Registry up to 01 January 2015, 10 individuals were excluded due to lung transplantation prior to inclusion in the Swedish National AATD Registry, three were excluded due to liver transplantation prior to inclusion in the Swedish National AATD Registry and 21 were excluded because they had any SGID prior to inclusion in the registry. Of an initial 6,000 controls, one control was excluded due to incorrect personal identification number, and 35 were excluded because they had any SGID prior to inclusion in the OLIN studies. Thus, 1,561 severe AATD individuals and 5,964 controls were included in the analyses.

Paper I

Of the 1,132 severe AATD individuals, 43% were never-smokers, 48% were ex-smokers and 9% were current smokers. Of the 104 current smokers, 73 (70%) stopped smoking during the follow-up period. The individuals' mean age at baseline was 45 (SD 16) years. The mean follow-up was 15 (SD 6) years for the whole study population.

Sixty-one per cent (n=696 individuals) experienced respiratory symptoms. Fifty-two per cent (n=584 individuals) of the study population had a diagnosis of COPD at inclusion, consistent with $FEV_1/FVC<0.7$. Never-smokers had better lung function compared with the ex-smokers and current smokers; see Table 2.

Table 2.
Lung function in the study population.

Characteristics	All, n=1,132	Never-smokers, n=489	Ex-smokers, n=539	Current-smokers, n=104
FEV_1 at baseline, L, mean (SD)	2.58 (1.38)	3.22 (1.30)∗,∗∗	2.03 (1.20)	2.40 (1.36)
FEV_1 % predicted, mean (SD)	74 (32)	91 (25)∗	61 (31)	66 (32)
FVC % predicted, mean (SD)	96 (23)	101 (19)∗	92 (25)	91 (26)
FEV_1/FVC, mean (SD)	0.63 (0.22)	0.75 (0.17)∗∗∗	0.53 (0.20)	0.60 (0.21)

∗ p<0.001 between never-smokers *versus* ex-smokers and between never-smokers *versus* current smokers; ∗∗ p<0.05 between ex-smokers and current smokers; ∗∗∗ p<0.05 between all smoking categories. FEV_1 = forced expiratory volume in 1 second; FVC = forced vital capacity; L = liter; n = number; SD = standard deviation.

Sixty-one per cent (n=355 individuals) of the severe AATD individuals with COPD experienced hospitalised exacerbations of COPD. The median (IQR) annual exacerbation rate for the whole group was 0.66 (1.4). One hundred seventy-six (30%) of the severe AATD individuals with COPD experienced frequent exacerbations of COPD.

The mean ΔFEV_1 was 40 (95% CI 36-44) $mL \cdot yr^{-1}$ in the whole study population.

Adjusting for FEV_1 and age at baseline yielded the following results:

- The ΔFEV_1 was significantly higher:

  - In men than in women: 45 (95% CI 39-51) *versus* 36 (95% CI 30-42) $mL \cdot yr^{-1}$.
  - In the middle-aged individuals compared with the young individuals: 48 (95% CI 41-55) *versus* 32 (95% CI 18-45) $mL \cdot yr^{-1}$.

- In those with respiratory symptoms compared with those without: 46 (95% CI 40-52) *versus* 30 (95% CI 22-38) $mL \cdot yr^{-1}$.
- In those with COPD at inclusion compared with those without: 47 (95% CI 40-54) *versus* 33 (95% CI 26-41) $mL \cdot yr^{-1}$.
- In current smokers than in ex- and never-smokers: 70 (95% CI 56-83) v*ersus* 42 (95% CI 36-48) and *versus* 32 (95% CI 25-38) $mL \cdot yr^{-1}$ respectively.
- In the individuals that have smoked ≥10 pack-years compared with those that have smoked less than 10 pack-years: 50 (95% CI 43-58) *versus* 33 (95% CI 24-41) $mL \cdot yr^{-1}$.
- In the frequent exacerbators than in those with infrequent exacerbations of COPD: 57 (95% CI 47-68) *versus* 27 (95% CI 17-37) $mL \cdot yr^{-1}$.

- No statistically significant difference in the ΔFEV_1 was found between the individuals with COPD at baseline when their airflow obstruction was stratified into the severity groups according to GOLD: 44 (95% CI 28-61) $mL \cdot yr^{-1}$ in GOLD I *versus* 49 (95% CI 38-60) $mL \cdot yr^{-1}$ in GOLD II *versus* 49 (95% CI 36-61) $mL \cdot yr^{-1}$ in GOLD III *versus* 40 (95% CI 24-56) $mL \cdot yr^{-1}$ in GOLD IV.

Paper II

The 240 severe AATD individuals identified through family screening, together with the 109 individuals identified through neonatal screening and the 28 individuals identified by other types of screening, made up the 377 severe AATD individuals included in Paper II.

Their median follow-up was 18 (range 0-23) years. The majority were never-smokers, n=221 (59%). Compared with the ever-smokers, the never-smokers had better lung function, and a lower proportion had airflow obstruction and a diagnosis of COPD at inclusion in the Swedish National AATD Registry; see Table 3.

At inclusion in the Swedish National AATD Registry, eight individuals (2%) reported respiratory symptoms, consisting of attacks of dyspnea, dyspnea on exertion, phlegm, and dry cough. Other reported diseases were: diabetes mellitus in two individuals (0.5%), hyperlipidemia in five individuals (1%), and hypertension in four individuals (1%), without any statistically significant difference between never-smokers and ever-smokers. None of the individuals had any liver disease, ischemic heart disease, or cancer at inclusion.

Forty-six per cent (n=72) of the ever-smokers stopped smoking prior to being diagnosed with AATD, and 42 individuals (27%) stopped smoking after receiving

the diagnosis of AATD. Forty-two (27%) individuals were current smokers by the time of inclusion in the Swedish National AATD Registry. During the follow-up period, 19 (45%) of the 42 current smokers stopped smoking.

Table 3.
The characteristics at inclusion in the Swedish National AATD Registry of the individuals identified through screening, stratified by their smoking status.

Characteristics	All, n=377	Never-smokers, n=221	Ever-smokers, n=156	p-value
FEV_1 % predicted, mean (SD)	93 (24)	98 (18)	85 (28)	<0.001
FVC % predicted, mean (SD)	92 (16)	94 (14)	90 (19)	<0.001
FEV_1/FVC, mean (SD)	0.76 (0.15)	0.81 (0.12)	0.70 (0.18)	<0.001
Individuals with FEV_1/FVC<0.70, n (%)	82 (22)	25 (11)	57 (37)	<0.001
Asymptomatic individuals, n (%)	268 (71)	177 (80)	91 (58)	<0.001
Diagnosis of COPD, n (%)	60 (16)	17 (8)	43 (28)	<0.001
Diagnosis of asthma, n (%)	41 (11)	22 (10)	19 (12)	0.494
Occupational exposure to airway irritants, n (%)*	143/363 (39)	79/214 (37)	64/149 (43)	0.247
Individuals identified with severe AATD ≤ 14 years, n (%)	145 (38)	116 (52)	29 (19)	<0.001

* The occupation was known in 363 severe AATD individuals. AATD = alpha-1-antitrypsin deficiency; COPD = chronic obstructive pulmonary disease; FEV_1 = forced expiratory volume in 1 second; FVC = forced vital capacity; n = number; SD = standard deviation.

Among the 145 individuals identified with severe AATD at an age ≤14 years, the proportion of never-smokers was higher than among the 232 individuals identified with severe AATD at an age older than 14 years, (80% *versus* 45%), p<0.001. The characteristics of the screened individuals stratified by the age at identification of severe AATD are presented in Table 4.

Table 4.
The characteristics of the individuals identified by screening at inclusion in the Swedish National AATD Registry, stratified by the age at identification of severe AATD.

Characteristics	Individuals identified with severe AATD ≤ 14 years of age, n=145	Individuals identified with severe AATD > 14 years of age, n=232	p-value
FEV_1 % predicted, mean (SD)	101 (14)	87 (27)	<0.001
Asymptomatic individuals, n (%)	125 (86)	143 (62)	<0.001
Diagnosis of COPD, n (%)	1 (1)	59 (25)	<0.001
Diagnosis of asthma, n (%)	18 (12)	23 (10)	0.448
Occupational exposure to airway irritants, n (%)*	55/141 (39)	88/222 (40)	0.904

* The occupation was known in 363 severe AATD individuals. AATD = alpha-1-antitrypsin deficiency; COPD = chronic obstructive pulmonary disease; FEV_1 = forced expiratory volume in 1 second; n = number; SD = standard deviation.

The annual ΔFEV_1 was calculated in 275 (73%) severe AATD individuals with three or more lung function tests available. Adjusted for age and FEV_1 at baseline, the overall ΔFEV_1 in the whole study population was 47 (95% CI 42-51) $mL \cdot yr^{-1}$. The ΔFEV_1 was significantly lower:

- In the never-smokers compared with the ever-smokers: 42 (95% CI 36-47) *versus* 53 (95% CI 47-60) $mL \cdot yr^{-1}$ respectively, p=0.011.
- In the individuals identified with severe AATD before the age of 14 years compared with those identified with severe AATD at an age older than 14 years: 33 (95% CI 24-42) *versus* 55 (95% CI 49-62) $mL \cdot yr^{-1}$ respectively, p<0.001.
- In asymptomatic ever-smokers compared with symptomatic ever-smokers: 39 (95% CI 27-50) *versus* 74 (95% CI 61-87) $mL \cdot yr^{-1}$ respectively, p<0.001.

The annual ΔFEV_1 was similar in individuals with occupational exposure to airway irritants compared with individuals without such an exposure: 50 (95% CI 42-57) *versus* 51 (95% CI 44-59) $mL \cdot yr^{-1}$ respectively, p=0.744.

Of the 268 asymptomatic individuals, 12% (n=31 individuals) developed COPD. Five individuals with COPD underwent lung transplantation during follow-up.

The risk factors for having a diagnosis of COPD at study-end were the identification of severe AATD at an age older than 14 years (HR 9.21; 95% CI 2.79-30.38) and the presence of respiratory symptoms or diseases (HR 4.79; 95% CI 2.96-7.73).

Forty-five individuals (12%) died during the follow-up period. There were 13 (6%) deaths among the never-smokers, compared with 32 (21%) among the ever-smokers, p<0.001. The main causes of death were cardiac (29%), followed by respiratory (24%), other (24%) including shock, sepsis, drowning and unknown causes, cerebrovascular (9%), hepatic (7%), and neoplastic (7%).

Paper III

The characteristics of the study population at baseline are presented in Table 5.

Table 5.
Characteristics of the study population (individuals with severe AATD and controls) at baseline.

Characteristics	Individuals with severe AATD, n=1,570	Controls, n=5,951	p-value
Smoking status, n (%)			
Never-smokers	722 (46)	3,154 (53)	<0.001
Ex-smokers	718 (46)	1,253 (21)	
Current smokers	130 (8)	1,544 (26)	
Presence of liver disease, n (%)	58 (4)	18 (0.3)	<0.001
Presence of COPD, n (%)	723 (46)	219 (4)	<0.001

AATD = alpha-1-antitrypsin deficiency; COPD = chronic obstructive pulmonary disease; n = number.

The follow-up period was 17,669 person-years in the severe AATD individuals and 89,202 person-years in the controls. The incidence of cancer was higher among the severe AATD individuals as compared with the controls, as presented in Table 6.

Adjusting for risk factors at inclusion (sex, age, smoking status, and the presence of liver disease), the severe AATD individuals still had a significantly higher risk of developing any type of cancer compared with the controls, (aHR 1.50; 95% CI 1.26-1.79), as well for hepatic cancer (aHR 23.39; 95% CI 9.88-55.39) as for non-hepatic cancer (aHR 1.27; 95% CI 1.05-1.53).

In the severe AATD individuals, the only independent risk factor for developing any type of cancer was age (HR 1.06; 95% CI 1.05-1.08), while the smoking status (ever-smoking *versus* never-smoking HR 1.11; 95% CI 0.81-1.51), the sex (men *versus* women HR 1.30; 95% CI 0.95-1.76) and the presence of liver disease at inclusion (yes *versus* no HR 1.42; 95% CI 0.58-3.47) were not.

Table 6.
The incidence of different types of cancer among the severe AATD individuals and the controls.

	Cancer events, n (n per 1,000 person-years)		HR (95% CI)**	p-value
	Individuals with severe AATD	**Controls**	**Individuals with severe AATD *versus* controls**	
Any type of cancer	178 (10.07)	591 (6.63)	1.48 (1.24-1.76)	<0.001
Hepatic cancer	28 (1.58)	7 (0.08)	20.34 (8.83-46.86)	<0.001
Non-hepatic cancer*	150 (8.49)	584 (6.55)	1.25 (1.04-1.51)	0.018

* Includes cancer in the digestive organs, in the reproductive and genitouritary organs, in breast, pulmonary, skin, lymphoma, oropharyngeal, thyroid, parathyroid, pituitary, nervous system, adrenal gland and in the thymus; ** unadjusted. AATD = alpha-1-antitrypsin deficiency; CI = conficence interval; HR = hazard ratio; n = number.

In a separate analysis stratified by sex and smoking status, and adjusted for age and the presence of liver disease at inclusion, the ever and never smoking severe AATD individuals had significantly higher risk of developing any type of cancer, as well hepatic as non-hepatic cancer, when compared with the corresponding controls; see Table 7.

Table 7.
The results of the separate analyses, stratified by sex and smoking status, comparing the adjusted hazard ratios for incident cancer in the severe AATD individuals and the controls.

Individuals with severe AATD *versus* controls:	**Any type of cancer aHR (95% CI)**	**Hepatic cancer aHR (95% CI)**	**Non-hepatic cancer aHR (95% CI)**
Men*	1.95 (1.52-2.51)	46.94 (13.60-162.01)	1.49 (1.12-1.97)
Women*	1.22 (0.95-1.56)	6.34 (1.62-24.90)	1.15 (0.94-1.41)
Ever-smokers**	1.59 (1.21-2.09)	86.39 (11.00-678.35)	1.27 (0.95-1.71)
Never-smokers**	1.43 (1.13-1.80)	12.65 (4.58-34.89)	1.27 (0.99-1.62)

* Adjusted for age, the smoking status and the presence of liver disease at inclusion; ** adjusted for age, sex and the presence of liver disease at inclusion. aHR=adjusted hazard ratio; AATD = alpha-1-antitrypsin deficiency; CI = conficence interval.

There were 102 (57%) deaths among the 178 severe AATD individuals who developed cancer during the follow-up, 25 (89%) deaths among the 28 individuals with hepatic cancer, and 77 (51%) among the 150 individuals with non-hepatic cancer. The corresponding figures among the 591 controls with cancer were 262 (44%) deaths, 7 (100%) in the 7 controls with hepatic cancer and 255 (44%) in those 584 with non-hepatic cancer.

After a diagnosis of any type of cancer, the severe AATD individuals had a shorter survival than the controls, median 6 (95% CI 4-8) years in the severe AATD

individuals *versus* median 12 years (95% CI 9-15) years in the controls, p=0.003. There were no statistically significant differences in the median survival time in severe AATD individuals compared with the controls after a diagnosis of hepatic cancer (0.4; 95% CI 0.1-0.7 years *versus* 1.0; 95% CI 0.0-2.9 years) and non-hepatic cancer (10; 95% CI 5-14 years *versus* 13; 95% CI 10-16 years).

Paper IV

The characteristics of the study population at baseline are presented in Table 8.

Table 8.
Characteristics of the individuals with severe AATD and the controls at baseline.

Characteristics	Individuals with severe AATD, n=1,561	Controls, n=5,964	p-value
Smoking status, n (%)			
Never-smokers	716 (46)	3,163 (53)	<0.001
Ex-smokers	716 (46)	1,256 (21)	
Current smokers	129 (8)	1,545 (26)	
COPD at inclusion, n (%)	712 (46)	219 (4)	<0.001

AATD = alpha-1-antitrypsin deficiency; COPD = chronic obstructive pulmonary disease; n = number.

The follow-up period was 14,740 person-years for the severe AATD individuals and 77,848 person-years for the controls. The incidence of any SGID among the study population is presented in Table 9.

Table 9.
The cumulative incidence of any SGID (Crohn's disease, ulcerative colitis, noninfectious gastroenteritis and diverticular disease encompassing symptomatic diverticulosis and its complications) among the severe AATD individuals and the controls.

	Cumulative incidence, n (%)		Cumulative incidence rate, n per 1,000 person-years (95% CI)		HR (95% CI)∗
	Individuals with severe AATD	**Controls**	**Individuals with severe AATD**	**Controls**	**Individuals with severe AATD *versus* controls**
Any SGID	156 (10)	281 (5)	10.6 (9.0-12.4)	3.6 (3.2-4.1)	2.9 (2.4–3.5)
Crohn's disease	16 (1)	22 (0)	1.1 (0.6-1.8)	0.3 (0.2-0.4)	3.5 (1.8–6.7)
Ulcerative colitis	19 (1)	27 (1)	1.3 (0.8-2.0)	0.4 (0.2-0.5)	3.4 (1.9–6.1)
Noninfectious gastroenteritis	22 (1)	31 (1)	1.5 (0.9-2.3)	0.4 (0.3-0.6)	3.6 (2.1-6.3)
Diverticular disease	99 (6)	201 (3)	6.7 (5.5-8.2)	2.6 (2.2-3.0)	2.6 (2.1–3.3)

∗ Unadjusted. AATD = alpha-1-antitrypsin deficiency; CI = conficence interval; HR = hazard ratio; n = number.

Adjusting for risk factors at inclusion (age, sex, smoking status, and the presence of COPD), the severe AATD individuals still had a significantly higher risk of developing any SGID compared with the controls, (aHR 2.7; 95% CI 2.1-3.4), including Crohn's disease (aHR 4.0; 95% CI 2.0-8.3), ulcerative colitis (aHR 4.5; 95% CI 2.4-8.5), noninfectious gastroenteritis (aHR 2.5; 95% CI 1.3-5.1) and diverticular disease (aHR 2.1; 95% CI 1.5-2.9).

Irrespective of the smoking status, when adjusting for sex, age at inclusion and the presence of COPD at inclusion, the severe AATD individuals had significantly higher risk than the controls for developing any SGID (aHR 2.9; 95% CI 2.0-4.2 between ever-smokers with severe AATD and ever-smoking controls, and aHR 2.4; 95% CI 1.7-3.4 between never-smoking severe AATD individuals and never-smoking controls). Age at inclusion was the only independent risk factor in the severe AATD individuals for developing any SGID.

One hundred and thirty (83.3%) severe AATD individuals compared with 167 (59.4%) controls with any SGID experienced any hospitalisation, $p<0.001$. The median hospital stay length was 11 days in the severe AATD individuals (IQR 28) *versus* 5 (IQR 11) in the controls, $p<0.001$. COPD was present in 71 (54.6%) of the hospitalised severe AATD individuals versus 9 (5.4%) controls, $p<0.001$.

There were 61 (39.1%) deaths among the severe AATD individuals with SGID *versus* 57 (20.3%) in the controls, $p<0.001$. In the severe AATD individuals with any SGID the main causes of death were: respiratory diseases (n=37; 61%), cardiovascular diseases (n=7; 11%), malignancy (n=6; 10%), gastrointestinal cause (n=6; 10%), and other causes (n=5; 8%). The main death causes among the controls with any SGID were: malignancy (n=25; 44%), cardiovascular diseases (n=13; 23%), other causes (n=11; 19%), respiratory causes (n=6; 11%), and gastrointestinal causes (n=2; 4%).

Discussion

The present thesis is a result of the long follow-up of the Swedish National Registry of Individuals With Severe AATD, in which most of the individuals are never-smokers identified on the basis of indications other than respiratory symptoms. The overall aim of the present thesis was to study the clinical course and prognosis in severe AATD.

Decline in FEV_1

The results from Paper I showed that the adjusted mean decline in FEV_1 was notably higher in current smokers compared to ex-smokers and never-smokers (70 *versus* 42 and *versus* 32 $mL \cdot yr^{-1}$). Additionally, middle-aged individuals had a higher decline than younger individuals (48 *versus* 32 $mL \cdot yr^{-1}$), and those with respiratory symptoms at inclusion showed a higher decline compared to asymptomatic individuals (46 *versus* 30 $mL \cdot yr^{-1}$). Moreover, individuals with frequent exacerbations experienced a greater decline compared to those with infrequent exacerbations (57 *versus* 27 $mL \cdot yr^{-1}$).

Previous studies of lung function in severe AATD individuals have reported a high variability in the rate of decline in FEV_1, greater decline in current smokers than in ex- and never-smokers, and various results on the yearly decline in FEV_1 in never-smokers (206-210). Some studies have included only a limited number of never-smokers. The analysis in Paper I extends the previously published studies by including a large number of never-smokers and individuals identified for reasons other than respiratory symptoms.

In Paper I, no significant difference was found in the annual decline in FEV_1 between the never- and ex-smokers, consistent with the results of previous analysis from the data in the Swedish National AATD Registry (207). These results suggest that in smoking individuals with severe AATD, the decline in lung function returns to a similar level as never-smokers after smoking cessation.

Moreover, a greater annual decline in FEV_1 was found in the subjects with mild and moderate COPD (GOLD stages I and II) compared to those with severe COPD. However, the difference became insignificant after adjustment for FEV_1 and age at baseline. This implies that the decline in lung function is dependent on the initial FEV_1. Individuals with severe AATD with initial, well preserved lung function appear to experience a sharper decline in FEV_1 than those with low initial FEV_1.

Hospitalised exacerbations of COPD in severe AATD

Up to 52% of the study population in Paper I had a diagnosis of COPD at inclusion. The majority of the individuals with COPD had experienced at least one severe exacerbation during the follow-up period. Their median annual rate of severe exacerbations of COPD of 0.66 was lower than previously reported (124, 211). However, the limitations of previous studies were that the exacerbations were self-reported by the individuals and only a limited number of never-smokers were included.

The clinical course in severe AATD individuals identified by screening

In Paper II, the screened severe AATD individuals diagnosed at an early age (*i.e.* ≤14 years) were more often never-smokers, had better lung function, and a lower lung function decline than those identified after the age of 14 years.

The never-smoking severe AATD individuals had better lung function and a lower rate of lung function decline compared with the ever-smokers. Also, the proportion of asymptomatic severe AATD individuals identified by screening was higher among never-smokers than among ever-smokers.

The majority of the ever-smoking, screened, severe AATD individuals in Paper II had quit smoking after the diagnosis of AATD, or after inclusion in the Swedish National AATD Registry. This finding supports the importance of early identification of AATD, before the assumed smoking debut, in order to improve the prognosis, by promoting early lifestyle options and preventive measures such as abstaining from smoking.

Thirty-nine per cent of the severe AATD individuals identified before the age of 14 years reported occupations with exposure to airway irritants. This finding suggests that there is still a need for information and instruction at an early age in the individuals with severe AATD not only concerning smoking but also their choice of occupation.

A diagnosis of COPD was more prevalent among the ever-smokers than among the never-smokers identified through screening. Twenty-two per cent of the screened severe AATD individuals had airflow obstruction at inclusion in the Swedish National AATD Registry. These findings suggest that early identification of AATD is important in preventing individuals from starting to smoke and deteriorating their lung function, and thus preventing the development of respiratory symptoms.

The main cause of death among the symptomatic ever-smokers was respiratory related. More deaths occurred among the ever-smokers with severe AATD identified by screening than among the never-smokers. The never-smoking AATD individuals had better survival compared with the ever-smokers. However, since only a small number of deaths occurred, the median survival time could not be calculated.

Cancer in individuals with severe AATD

The results of Paper III indicate that individuals with severe AATD may have an increased risk of developing cancer compared with the general Swedish population. The risk of both hepatic and non-hepatic cancer was increased after controlling for the potential risk factors of age, sex, smoking status, and the presence of liver disease at inclusion. Irrespective of smoking status, the risk of developing cancer was significantly higher among the severe AATD individuals than among the corresponding controls.

The association between the Pi∗ZZ phenotype and hepatic cancer is already well-established (56, 153). However, few studies have been published on the association between severe AATD and types of cancers other than hepatic (37, 166-169). These studies were case-control studies including only a few AAT-deficient individuals, with various AAT alleles *(e.g.* Z, S and I).

Although several mechanisms have been proposed as underlying the role of AAT in the development and progression of cancer, there is a need for further studies in order to elucidate the role of AATD in the pathogenesis of cancer (37). However, the findings in Paper III extend the knowledge on excess cancer risk in severe AATD individuals as, to the knowledge of the authors, Paper III is the first population-based, longitudinal study to examine the risk of cancer in severe AATD individuals compared with controls from a general population sample with known smoking status.

Age was the only independent risk factor for developing any type of cancer in severe AATD individuals. Sex, smoking status, or the presence of liver disease at inclusion were not factors associated with increased risk of developing any type of cancer. This finding emphasises the importance of early identification of individuals with severe AATD, in order to monitor them regularly and reduce the burden of cancer.

The survival time after diagnosis of any type of cancer was significantly shorter in the severe AATD individuals compared with the controls, but was not significant after diagnosis of non-hepatic cancer. The survival time after a diagnosis of hepatic cancer was reduced to less than two years, as previously reported (146). These findings show the importance of regular follow-up of the severe AATD individuals using imaging techniques which improve the early detection of liver cancer and thus its prognosis.

Gastrointestinal diseases in individuals with severe AATD

The results of Paper IV showed that individuals with severe AATD have an increased risk of developing gastrointestinal diseases compared to the general population. In particular, they were up to three times more prone to develop Crohn's disease, ulcerative colitis, noninfectious gastroenteritis and diverticular disease.

The results of previously published studies regarding development of gastrointestinal diseases in severe AATD have been controversial (157, 170, 171, 174, 176). These studies have included few cases with severe AATD. Some studies have suggested that severe AATD might be implicated in the pathogenesis of inflammatory bowel diseases (*i.e.* Crohn's disease and ulcerative colitis) and diverticular disease, due to local tissue injury in the intestines caused by the imbalance between proteases and protease-inhibitors (157, 170).

Neither the diagnosis of COPD at inclusion nor smoking were significant risk factors for the development of any SGID. This finding suggests that severe AATD *per se* influences the risk for SGID.

More than half (55%) of the hospitalised severe AATD individuals with SGID had COPD. They had longer hospital stays compared with the controls. It is possible that the hospitalisations in the severe AATD individuals with SGID were driven by the presence of COPD. Also, the majority of the deaths among the severe AATD individuals with SGID were respiratory-related.

Paper IV is the first longitudinal study to examine the risk of gastrointestinal diseases in severe AATD individuals compared with controls with known smoking status. The findings extend the knowledge of excess risk for gastrointestinal diseases (i.e. Crohn's disease, ulcerative colitis, noninfectious gastroenteritis and diverticular disease) in severe AATD individuals, with a negative prognostic trend.

The strengths and limitations in the papers included in the thesis

The main strength of the papers in the present thesis is the accuracy of the data, assembled from the well-established Swedish National Registry of Individuals With Severe AATD, with a correct diagnosis of AATD based on isoelectric focusing and confirmed at the accredited laboratory.

A large proportion of the individuals with severe AATD in the registry are never-smokers and have been identified for reasons other than respiratory symptoms, which increases the accuracy of the data.

The follow-up period in the papers was long, from 1991 to 2015/2016, during which the possible development of different types of illnesses could be followed.

Other analysed data in the papers were obtained from well-established Swedish national registries with high coverage, by cross-linkage with the Swedish system of personal identity numbers.

Another strength was the ability to accurately compare the risk of cancer and of specific gastrointestinal diseases of interest in papers III-IV, between severe AATD individuals and a control group with known smoking status.

The identification rate of AATD in Sweden and the coverage of the Swedish National AATD Registry is high, as 20-30% of all Swedish adults with a severe

AATD phenotype are identified and included in the national registry (158). This is partly owing to the good collaboration with the Department of Clinical Chemistry in Malmö, which reports all the individuals with severe AATD to the registry. Also, the participation rate in the registry is relatively high among Swedish AATD individuals.

When an individual is diagnosed with severe AATD (the 'index' individual), the registry generously offers voluntary diagnostic testing for AATD in family members of the individual ('non-index' individual(s); for instance descendants and siblings), preceded by adequate informed consent. This action contributes to high family screening of AATD in Sweden, to the expansion of the registry, and also to the possible identification of individuals with AATD prior to development of respiratory diseases.

Physicians in all parts of Sweden contribute to the expansion of the registry by their willingness and efforts to report data to the registry. The analysis of plasma proteins is commonly utilised in Sweden as an investigation tool in many pathological medical conditions. The diagnosis of AATD is accurate, and all included individuals have been phenotyped prior to inclusion.

The regular follow-up of individuals with severe AATD in the Swedish National Registry contributes to the possibilities of a long follow-up of the individuals, early identification of lung and liver diseases as well as of other diseases, and a regular reminder to the individuals to avoid potential harmful environments (for instance, exposure to fumes, dust, vapours, and occupational exposures to airway irritants).

The majority of the Swedish severe AATD individuals included in the registry (57%) are identified due to conditions other than respiratory related (158). The majority of them are never-smokers (45%), and only a minority of the severe AATD individuals are current smokers (8%). Thus, the Swedish National AATD Registry is a large national AATD registry, with a history of long follow-up, and is less biased than other registries because few individuals are identified because of respiratory symptoms or diseases (158).

Even if the detection rate of AATD is high in Sweden, and a high proportion of individuals included in the registry are identified by screening or for reasons other than respiratory disease or symptoms, the individuals included in the registry do not represent a random sample of adult severe AATD individuals in Sweden. Only results obtained for individuals identified through population screening can avoid bias. Thus, as the majority of the individuals with severe AATD are unidentified and not included in the Swedish National AATD Registry, their health status remains unknown, and therefore the analyses of registry data cannot be considered as adequately-performed epidemiological studies.

The emerging data in the registry is self-reported by the individual participants; for instance, all smoking status. No objective test was analysed to verify the smoking status, such as analysing the level of cotinine in plasma, saliva or urine.

The lung function tests are performed at the individual's local hospital or in the primary care setting throughout Sweden, with the possible use of different spirographs and different calibration modalities performed by different operators. Also, reversibility tests are not always performed in all individuals on all occasions.

The use of FEV_1/FVC ratio <0.7 was used as definition of airflow obstruction instead of the lower limit of normal (LLN). As the study population is relatively young at inclusion in the registry, it cannot be excluded that the proportion of individuals with airflow obstruction was underestimated.

In Paper I, other risk factors for decline in lung function, such as occupational exposure to airway irritants, was not performed.

Since the questionnaires contain data on diagnoses reported by the attending physicians, there is a possibility that some diagnoses are under-reported. Lastly, the registry does not hold data on all possible variables that could potentially influence the course of the AATD-related diseases; for instance, alcohol consumption or any kind of quantification of environmental exposure to gas, dust, or fumes, or indoor exposure to mould and dampness.

In Paper III, the diagnosis of COPD was not included in the multivariate analysis of risk factors for cancer, because the diagnosis of COPD might have been underestimated in the controls. The majority of the severe AATD individuals are referred, treated, and followed up by respiratory physicians or specialists in internal medicine at hospitals where spirometry is regularly performed, which was not the case for all the controls.

The severe AATD individuals were generally recruited from all parts of Sweden while the controls in papers III-IV were recruited from the northern part of Sweden, and they were not recruited specifically for the purpose of the studies in papers III-IV.

The diagnosis of cancer or of gastrointestinal diseases may be underestimated in the controls, because they did not undergo regular medical follow-ups, and thus possibly the diagnosis of their cancer or gastrointestinal diseases was delayed.

The quantitative burden of other potential risk factors (alcohol consumption, quantity of tobacco, medications, body mass index, presence of metabolic syndrome, environmental exposure to dust, gas, or fumes) was not analysed for cancer or for gastrointestinal diseases, as this was not available in all study participants.

The clinical implications of the papers in the present thesis

Overall, the thesis emphasises the importance of early identification of severe AATD. Regular follow up of severe AATD individuals is important for prompt diagnosis and effective disease management. The most important intervention is smoking prevention.

The individuals with severe AATD must be informed about their deficiency state. They are entitled to meet qualified doctors, with good knowledge of AATD in order not to delay their visits to the healthcare system. They should also be monitored regularly, regarding the development of symptoms that can later turn into irreversible illnesses.

Guidelines for the monitoring of individuals with severe AATD are available to every doctor, and are updated regularly by the holders of the Swedish National AATD Registry. The available information must be disseminated.

In Paper II, the never smoking severe AATD individuals identified by screening had better clinical outcomes than the corresponding ever-smokers. In Sweden, screening for AATD was not continued after the termination of the screening programme in 1974, because of the perceived negative psychological consequences for the families (212).

The Swedish National Neonatal Screening Study and Follow-Up Programme is unique, and Sweden is the only country in the world that has conducted a screening programme of such a magnitude. It has contributed to the knowledge of the natural history of lung and liver disease in childhood and adolescence, and to a successful anti-smoking status in the AATDs. Thus, is there a future for screening for AATD at an early age?

To motivate screening in the absence of an effective treatment for AATD might be difficult in Sweden. Yet, for many adults with severe AATD, the identification is made too late to prevent the development of pulmonary disease. A rationale would be to recommend screening in early adolescence, before these young adolescents may be inclined to start smoking, and when they are competent enough to participate in the decision to undergo screening owing to more mature thinking.

Many Swedish teenagers start smoking between the ages of 13-15 years (213). Thus, identification of AATD close to that age would provide early information about the consequences of smoking and could discourage the initiation of smoking. Providing adequate counselling would allow the affected individuals to avoid exposure to environmental hazards, not only in terms of cigarette smoke, but also through their choice of occupation. ‘There is no statistical refuge for Pi*ZZ smokers, and they should know this” (214)!

Conclusions

Paper I

In severe AATD individuals, the mean decline in FEV_1 and the annual rate of exacerbations were lower than previously reported. The factors associated with an accelerated decline of lung function were sex, age, active smoking, the presence of respiratory symptoms, the presence of COPD, the number of pack-years, and experiencing repeated exacerbations of COPD.

Paper II

Never-smoking severe AATD individuals identified through screening had better lung function, fewer respiratory symptoms, lower annual lung function decline, and better survival as compared with the ever-smokers. Severe AATD individuals diagnosed at an early age (*i.e.* ≤14 years) were more often never-smokers, with better lung function and lower annual lung function decline compared to those identified after the age of 14 years.

Paper III

Compared with the general population, the severe AATD individuals had an increased risk of developing cancer, as well for hepatic as for non-hepatic cancer. The risk was increased even after adjustment for the risk factors of age, sex, smoking status, and the presence of liver disease at inclusion. The survival time after a diagnosis of any type of cancer was significantly shorter in the severe AATD individuals compared to the controls.

Paper IV

Severe AATD individuals had an increased risk of developing any gastrointestinal diseases (Crohn's disease, ulcerative colitis, noninfectious gastroenteritis, and diverticular disease) compared to the general population, even after adjustment for the risk factors of smoking status, presence of COPD, age, and sex. The severe AATD individuals diagnosed with gastrointestinal diseases had more frequent hospitalisations related to their gastrointestinal conditions and longer hospital stays compared to the controls. Their main cause of death was respiratory related.

Future perspectives

Studies III and IV show that severe AATD individuals may have an elevated risk of developing cancer and gastrointestinal diseases. Further studies are needed to understand the mechanisms of AATD in the pathogenesis of these diseases.

The Swedish National AATD Registry is growing continuously and, combined with the control group from the OLIN studies, provides good opportunities to study the risk of developing different diseases in AATD. For instance, as AATD is described as a model for conformational diseases, it could be associated with neurodegenerative disorders (31). As AAT possesses anti-inflammatory and immunomodulatory properties, studies need to be performed on the subject to elaborate the impact of severe AATD in the development of inflammatory and immunologic diseases, and whether individuals with severe AATD are more prone to infectious diseases compared with the general population (34).

There appears to be limited understanding of the exact mechanism/s by which the hepatic cellular injury occurs in severe AATD. Although liver transplantation has shown promising results regarding survival, more research is needed in order to study the clinical course in individuals with severe AATD that have received a liver transplant. Is their risk of developing pulmonary disease decreased, or are they still at risk of developing respiratory diseases? There are individuals in the Swedish National AATD Registry that have received a liver transplant and thus, their clinical course could be analysed.

More research is needed to elucidate the impact of environmental factors on the clinical course in severe AATD. How does the lung and liver function of individuals with severe AATD relate to occupational exposure for airway irritants? Does exposure to mould and dampness impact negatively on the health of individuals with severe AATD? Which AATD individuals are at risk of being exposed to airway irritants? Are there any risky jobs for individuals with severe AATD, and which ones should they avoid? How do polluted particles in the air deposit in the lungs of the AATD individuals, and what is their interaction with AAT?

In papers I, III and IV, approximately half of the included severe AATD individuals had airflow obstruction. Why is the clinical presentation so variable in individuals with severe AATD? Might there be other factors of importance; for instance, the environment, or are there other genetic factors that warrant further elucidation?

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Appendix I

1. ATTENDING PHYSICIAN

Name: …… Tel no: …… (give area code)

Clinic: …… Hospital: ……

Address: ……

Date of examination: ……

2. PATIENT

A. Name: …… **Date of birth:** ……

B. Patient alive ☐ YES ☐ NO ☐ UNKNOWN

a: If No, date of decease: b. Post-mortem performed? ☐ YES ☐ NO ☐ UNKNOWN

C. Original indication for protein analysis (electrophoresis):

a ☐ Lung disease
b ☐ Liver disease
c ☐ Increased ERS
d ☐ Infection
e ☐ Family study
f ☐ Screening
g ☐ Other reason:

D. Is the patient still followed-up at your clinic?

☐ If YES, continue to question no. 3

☐ NO, REFERRED TO AN OTHER CLINIC: .....

3. DIAGNOSIS (one or more alternatives)

A. LUNG DISEASE ☐ YES ☐ NO

a ☐ Emphysema
b ☐ Chronic bronchitis
c ☐ Asthma
d ☐ Lung fibrosis
e ☐ Bronchiectasis
f ☐ Other lung disease:
.....

B. OTHER DISEASE ☐ YES ☐ NO

Diagnosis (ICD No.)

....

....

....

....

4. Latest chest X-ray

Date: ☐ UNKNOWN

Radiology unit: .....

5. BLOOD TESTS (Liver function)

Date for the latest test:

Copy of the results attached? ☐ YES ☐ NO

If No, give recorded values available:

ASAT ..... (μkat/l)

ALAT ..... (μkat/l)

GT ..... (μkat/l)

ALP ..... (μkat/l)

6. LUNG FUNCTION

A. SPIROMETRY AT INCLUSION IN THE REGISTER

Date: ....
year month day

Height …… Weight: ……

Clinic ……..

Copy of results attached? ☐ YES ☐ NO

If NO, give results available

before / after β2-agonist

$FEV_{1.0}$ ..... /.....L

VC ...../.....L

PEF ..... /.....L/min

B. HAS SPIROMETRY BEEN PERFORMED EARLIER?
☐ YES ☐ NO ☐ UNKNOWN

If YES, record the date and values of the first spirometry:

Height: Weight:

Clinic:

Copy of results attached? ☐ YES ☐ NO

If NO, give results available

before / after β2-agonist

$FEV_{1.0}$ ..... /.....L

VC ...../.....L

PEF ..... /.....L/min

B. HAVE INTERMEDIATE SPIROMETRIES BEEN PERFORMED?

☐ YES ☐ NO ☐ UNKNOWN

If YES, record the

Clinic: ……..

7. TREATMENT

A. FOR LUNG DISEASE ☐ YES ☐ NO

If YES, indicate type

a ☐ Inhaled β2-agonist
b ☐ Oral β2-agonist
c ☐ Inhaled anticholinergic
d ☐ Theophylline
e ☐ Inhaled steroids
f ☐ Oral steroids
g ☐ Continuous O_2
h ☐ Diuretics
i ☐ Other:

B. FOR OTHER DISEASE ☐ YES ☐ NO
If YES, record the treatment:

.....

.....

C. Human AAT replacement therapy? ☐YES ☐ NO
If YES, give date of starting:

D. VOLUME REDUCTION SURGERY ☐ YES ☐ NO
If YES, give date:

E. HEART/LUNG TRANSPLANTATION ☐ YES ☐ NO
If YES, give date:

F. LIVER TRANSPLANTATION ☐ YES ☐ NO
If YES, give date:

G. FAMILY STUDIES

☐ YES ☐ NO ☐ UNKNOWN

Is severe deficiency (PiZZ) known in any 1st degree relative?

☐ YES ☐ NO ☐ UNKNOWN

Any further comments on the patient:
(if possible record the type of spirometer used)

Appendix II

8. NUMBER OF SIBLINGS.....

How many of them are alive? .....

How many of them have been tested for AAT deficiency?

..... ☐ Don't know

9. OCCUPATION: .....

Are you employed? ☐ YES ☐ NO

If NO, give reason ☐ age

☐ disease

☐ other

10. WORKPLACE ENVIRONMENT

A. Have you ever regularly been exposed to dust, fumes, gas at work for at least three months?
☐ YES ☐ NO ☐ Don't know

If YES, for how many years?: ……..

11. SMOKING HABITS

A. Have you ever regularly smoked?

☐ YES ☐ NO

If YES: ☐ cigarettes?

☐ pipe?

☐ cigars?

When did you start? Age: …… years

B. If you have stopped smoking, when did you stop? Age: ……. **years**
C. Your current smoking habits

..... cigarettes/day

..... g tobacco/week (1 packet = 50 g)

..... cigars/week

D. During the time you smoked, how much on average?

..... cigarettes/day

..... g tobacco/week (1 packet = 50 g)

..... cigars/week

12. STATE OF HEALTH

A. Estimate your general state of health by marking an x on the line below:

|----------|

BAD (10 cm) GOOD

B. How often do you have colds?

☐ At most once a year ☐ At least twice a year

C. How often have you had pneumonia?

Number of times: …….. ☐ Don't know

D. **Do You usually have cough?** ☐YES ☐ NO

If YES, indicate when:

☐ in the morning

☐ during the rest of the day and night

E. Do you usually daily cough for at least three consecutive months a year?

☐ NO If YES, for how many years? .....

F. Do you usually bring up phlegm from your chest? ☐ YES ☐ NO

If YES, indicate when:

☐ in the morning

☐ during the rest of the day and night

G. Do you usually bring up phlegm from your chest for at least three consecutive months a year? ☐ YES ☐ NO

If YES, for how many years? .....

H. Does your chest ever sound wheezy or whistle? ☐ YES ☐ NO

If YES, indicate when:

☐ apart from cold/damp weather
☐ in the presence of strong odours
☐ when you have colds
☐ in other circumstances:

If YES, for how many years? .....

I. Estimate your exertion capacity by marking an X on the line below:

|----------|

LOW (10 cm) HIGH

J. Are you troubled by shortness of breath when walking 100 m on the level

when walking quickly ☐ YES ☐ NO

„ „ normally ☐ YES ☐ NO

„ „ slowly ☐ YES ☐ NO

K. Do you become breathless at the slightest exertion?

☐ YES ☐ NO

If YES to any question above, for how many years have you had breathlessness on exertion?

.....years

Appendix III

1 ATTENDING PHYSICIAN

Name: …… Tel no: …….

Clinic: ..... Hospital: ……

Address: ……

2 Date of examination: ……

PATIENT

3 Date of birth: ..... 4 **Sex:** ……

5 Body height (cm): ..... 6 Body weight (kg): .....

Smoking habits
7 Ever smoked? ..... **8 Age started: .....**

Stopped smoking? ..... **9 Age stopped: .....**

10 Av number of cigarettes per day: .....
11 Av number of cigars per day: .....
12 Pipe smoking-g/weeks: .....

13 Original indication for protein analysis (electrophoresis):
14 ☐ Lung disease 17 ☐ Family study
15 ☐ Liver disease 18 ☐ Screening
16 ☐ Other disease 19 ☐ Other reason:
14 Phenotype ☐ **Z**
15 Date of diagnosis of AAT deficiency: .....

DIAGNOSIS (one or more alternatives)

16 LUNG DISEASE ☐ YES ☐ NO
17 ☐ Emphysema 20 ☐ Bronchiectasis
18 ☐ Chronic bronchitis 21 ☐ Other lung disease
19 ☐ Asthma

22 Age at onset of respiratory symptoms? years:…… months: …….

23 Main symptom: ……?
24 ☐ Dry cough
25 ☐ Cough with phlegm
26 ☐ Shortness of breath at rest
27 ☐ Shortness of breath at exertion
28 ☐ Attacks of breathlessness

29 OTHER DISEASE ☐ YES ☐ NO
Diagnosis (ICD No.)

....

30 LUNG TRANSPLANTATION □ YES □ NO

If YES, give date:

31 VOLUME REDUCTION SURGERY □ YES □ NO

If YES, give date

32 LIVER TRANSPLANTATION □ YES □ NO

If YES, give date:

33 Has patient ever had pneumonia?

□ YES □ NO

34 Number of times: .....

CT-Scan

35 CT-scan of thorax? □ YES □ NO

If YES give date

Date: ....
year month day

Present treatment

37 For lung disease □ YES □ NO

38 Long-term oxygen treatment

□ YES □ NO

Augmentation therapy

39 Ever augmentation therapy □ YES □ NO

40 If YES, give date for first therapy

Date..... ....
year month day

41 Stopped treatments? □ YES □ NO

If YES, give date for last therapy

4 Date: ....
year month day

LUNG FUNCTION

43 HAS SPIROMETRY BEEN PERFORMED EARLIER?

Date: ....
year month day

Pre-bronchodilator	Post-bronchodilator
44 FEV_1 .....,..... L	45 FEV_1 .....,..... L
46 FVC .....,..... L	47 FVC .....,..... L
48 VC .....,..... L	49 VC .....,..... L

50 SPIROMETRY AT INCLUSION IN THE REGISTER

Date: ....
year month day

Pre-bronchodilator	Post-bronchodilator
51 FEV_1 .....,..... L	52 FEV_1 .....,..... L
53 FVC .....,..... L	54 FVC .....,..... L
55 VC .....,..... L	56 VC .....,..... L

Liver enzymes

57 Liver enzymes analysis

58 If YES, give date

Date: ....
year month day

Elevated

59 ASAT	□ YES	□ NO
60 ALAT	□ YES	□ NO
61 GGT	□ YES	□ NO
62 ALP	□ YES	□ NO

63 Occupational status

64 Regularly working □YES □ NO

:

If NO, specify reason: .....

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Severe alpha-1-antitrypsin deficiency (AATD) is a hereditary condition that predisposes to lung and liver disease. The thesis emphasises the importance of early identification and regular follow-up of severe AATD individuals.

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