Prevalence and phenotype of subjects carrying rare variants in the Italian registry for alpha1-antitrypsin deficiency

I Ferrarotti, J Baccheschi, M Zorzetto, C Tinelli, L Corda, B Balbi, I Campo, E Pozzi, G Faa, P Coni, G Massi, G Stella, M Luisetti

Alph1-antitrypsin deficiency (AATD) is a genetic condition associated with an increased risk of developing chronic obstructive pulmonary disease (COPD) early in life and, to a lesser extent, liver disease. Significant advances have been made during the last decades in understanding its epidemiology and it has been recently suggested that AATD is one of the commonest inherited disorders not only in Caucasians but also among other ethnic groups worldwide.

Although AAT is a highly pleomorphic glycoprotein, with approximately 100 variants having been identified, two major deficient variants, namely Z and S, account for most cases of AATD, since the vast majority of such individuals carry the PI*ZZ or PI*SZ genotype, coding for approximately 15% and 25%, respectively, of normal AAT plasma levels. The establishment of international registries, including large series of AATD individuals, has allowed not only better definition of the epidemiology of AATD, but also more precise definition of the associated clinical phenotypes.

Nevertheless, there are at least 30 AAT alleles other than the PI*Z and PI*S alleles which are associated with significantly reduced or absent plasma AAT levels.

The Italian Registry for Severe AATD was established in 1996 as a result of a nationwide screening programme sponsored by the two major Italian scientific respiratory societies. Although Italy is considered a country with a medium-low prevalence of AATD (mean PI*Z gene frequency: 0.0013), the programme succeeded in identifying a relatively large cohort of AATD individuals. During the development of the screening programme, we noticed that, in addition to the groups of AATD individuals carrying the PI*ZZ and PI*SZ genotypes, there was an unexpectedly large group of subjects carrying at least one rare AATD allele. We therefore decided to study this group of subjects, focusing particularly on characterising their clinical phenotypes.

METHODS
Screening programme
The targeted screening programme, based on dried blood spots, has already been described in detail. Briefly, paper filters and questionnaires were distributed to respiratory physicians throughout the country. Recommendations for AATD screening were the presence of the following: early-onset COPD, familial clustering of COPD, reduced levels of alph1-globulins on electrophoresis, serum levels of AAT <80 mg/dl (nephelometry) or <150 mg/dl (immunodiffusion), or a family history of AATD. Paper filters containing the blood spots were shipped to the Central Phenotyping Laboratory in Rome and submitted to isoelectric focusing. In the case of an abnormal isoelectric focusing pattern, the referring physician was asked to ship a serum sample and a frozen whole blood sample. If the abnormal isoelectric focusing pattern was confirmed, the sample was then investigated at a molecular level. The subject’s demographic and clinical data were retrieved from questionnaires filled in by the referring physicians and shipped together with the specimens. It was recommended that the diagnosis of COPD follow international guidelines.

All subjects gave their consent to undergo the genetic investigation, which was approved by the ethical committees of the institutions involved.

Key points
• Most subjects affected by alph1-antitrypsin deficiency (AATD) carry the PI*ZZ or PI*SZ genotype. Nevertheless, there are at least 30 AAT alleles other than PI*Z or PI*S associated with reduced or absent plasma AAT levels. Little is known about their epidemiology or associated clinical phenotypes.
• Over 98 months, 2922 subjects enrolled by the Italian Registry for AATD, which conducts a screening programme based on dried blood spots, were screened. A total of 155 subjects with severe AATD were identified (132 index cases), together with 152 individuals with intermediate AATD (84 cases).
• Among subjects with severe AATD, we recorded an 11% prevalence of deficient genotypes other than the common PI*ZZ or PI*SZ (15 out of 132 deficient index subjects identified). Among subjects with intermediate AATD, we recorded a 15% prevalence of deficient genotypes other than the common PI*MZ (13 out of the 84 index cases).
• As the cohort of subjects carrying rare AATD variants was relatively large, we were able investigate their characteristics in terms of associated clinical phenotypes, pulmonary lung function, smoking habit, and geographic distribution. We found that these rare variants had a special position within the spectrum of genotype-phenotype correlations in alph1-antitrypsin deficiency.
• The prevalence of rare alph1-antitrypsin variants found in Italy is the highest so far recorded worldwide and, interestingly, occurs in a country with a medium-low prevalence of the common PI*ZZ genotype.

Abbreviations: AATD, alph1-antitrypsin deficiency; COPD, chronic obstructive pulmonary disease; OR, odds ratio; 95% CI, 95% confidence interval
Detection of AATD variants

Genomic DNA was extracted from whole blood cells using the standard technique. The S and Z variants were genotyped by a commercially available amplification-reverse hybridisation test kit (Symbiosis, Cocconato, Italy) and by PCR-RFLP using TaqI as the restriction enzyme. The genomic DNA was sequenced after PCR amplification of all coding exons (II–V). All sequencing products were obtained using the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems, Arrington, UK) and were analysed in an automatic ABI 377 DNA sequencer.

Statistical analysis

Separate files were created for the different genotypes. Subjects homozygous or compound heterozygous for deficiency variants were assigned to the severe AATD group, whereas individuals heterozygous for a deficiency allele and the normal M allele were assigned to the intermediate AATD group. Shapiro-Wilk’s test was used to test the normal distribution for quantitative variables and data values are presented as mean (SD) values. Comparisons between means were performed with analysis of variance, using the Scheffé test for post-hoc comparison. Associations between genotypes and disease status were compared with Fisher’s exact test. To detect associations between FEV₁/FVC (forced vital capacity) and cigarette smoke exposure (current/former) was detected in 100% of R/D individuals in 67% of PI*ZZ subjects and 52% of PI*SZ subjects (p = 0.008 and p = 0.0017, respectively), as well as in 85% of M/R subjects in 52% of PI*MZ subjects (p = 0.03). As far as the plasma AAT levels were concerned, the R/D subset had a lower mean (SD) level of 29 (18) mg/dl, similar to that of the PI*SZ group (28 (11) mg/dl), but significantly lower than that of the PI*ZZ group (62 (16) mg/dl; p<0.0001). The M/R subset had a mean (SD) AAT level of 61 (20) mg/dl, which is significantly lower than that of the related PI*MZ group (93 (23) mg/dl; p<0.0001).

RESULTS

During the 98 months from February 1996 to April 2004, 2922 subjects were screened, thanks to more than 250 physicians throughout the country who shipped at least one paper filter. A total of 155 individuals (5.3%) with severe AATD were detected (table 1). Of these, 132 were index cases, while the other 23 were non-index cases, that is, subjects identified during family screening. Individuals homozygous for the Z allele (PI*ZZ) formed the large majority (114, 74% of the severe AATD; index cases: 96; 73%), whereas individuals heterozygous for a deficiency allele and the normal M allele were assigned to the intermediate AATD group. The number of subjects in each group is shown in table 1. The PI*ZZ group (n = 114) was significantly larger than the PI*SZ group (n = 25); R/D group (n = 16); PI*MZ group (n = 131) and M/R group (n = 21). The R/D (rare/deficient) subset identifies subjects with severe AATD (homozygous for the rare AAT variant or compound heterozygous with the common Z allele) and the M/R (M/rare) subset consists of subjects with intermediate AATD (one rare AAT variant in combination with the normal M allele). Non-index individuals were identified during family screening.

Table 1: Individuals with severe and intermediate AATD identified during the Italian screening programme

<table>
<thead>
<tr>
<th>Severe AATD</th>
<th>Intermediate AATD</th>
</tr>
</thead>
<tbody>
<tr>
<td>PI*ZZ (n = 114)</td>
<td>PI*SZ (n = 25)</td>
</tr>
<tr>
<td>R/D (n = 16)</td>
<td>PI*MZ (n = 131)</td>
</tr>
<tr>
<td>M/R (n = 21)</td>
<td></td>
</tr>
<tr>
<td>Index cases, n (%)</td>
<td>96 (84)</td>
</tr>
<tr>
<td>Non-index cases, n</td>
<td>18</td>
</tr>
</tbody>
</table>

The R/D (rare/deficient) subset identifies subjects with severe AATD (homozygous for the rare AAT variant or compound heterozygous with the common Z allele) and the M/R (M/rare) subset consists of subjects with intermediate AATD (one rare AAT variant in combination with the normal M allele). Non-index individuals were identified during family screening.

The genotyping details of the 37 individuals carrying at least one rare AATD variant are given in table 2. In this series we found 16 individuals carrying the rare variant in a homozygous fashion or in compound heterozygosity with the common AATD variant Z (hereinafter referred to as the rare/deficient (R/D) subset). Twenty one individuals carried the rare variant in heterozygosity with a normal M variant (hereinafter referred to as the M/rare (M/R) subset). The majority of the subjects carried the Mmalton and Mprocida variants. Four subjects carried the Plowell variant, two subjects carried the I variant, and one subject each the Mvarallo, Mheerlen, Qprocida, Qcairo, and Qclayton variants.

Table 2: Details of the genotype in the individuals with rare AAT variants

<table>
<thead>
<tr>
<th>R/D subset</th>
<th>M/R subset</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td>Number of subjects</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>PI<em>malton/PI</em>malton</td>
<td>5 (5)</td>
</tr>
<tr>
<td>Z/PI*malton</td>
<td>3 (3)</td>
</tr>
<tr>
<td>Mprocida/Mprocida</td>
<td>2 (2)</td>
</tr>
<tr>
<td>Z/I</td>
<td>2 (2)</td>
</tr>
<tr>
<td>Z/Plowell</td>
<td>2 (1)</td>
</tr>
<tr>
<td>Z/Qprocida</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Qclayton</td>
<td>1 (1)</td>
</tr>
</tbody>
</table>

Index cases are in brackets.
Among subjects with severe AATD, COPD (alone or in combination with chronic liver disease) was diagnosed more frequently in the R/D group (100%), than in the two other related groups, PI*ZZ (79%), and PI*SZ (38%, p = 0.004). In seven cases out of 96 PI*ZZ (7%) and in two out of 15 R/D (13%), COPD and liver disease were diagnosed simultaneously. Prevalence of chronic liver disease did not differ among subjects carrying severe AATD; it was absent in M/R subjects, but it was detected in 11% of PI*MZ individuals. A number of other associated conditions were reported: with the exception of one PI*MZ subject diagnosed with Wegener’s granulomatosis, which has been repeatedly associated with AATD, the other conditions are most likely to be chance associations. The percentage of healthy subjects increased significantly among the two groups with intermediate AATD (42% in the PI*MZ group, and 46% in the M/R group).

With reference to the functional phenotype (table 5), the mean basal FEV1 value (% predicted) was significantly lower in the R/D group as a whole than in the related PI*SZ group (p = 0.0095), but not significantly lower than in the PI*ZZ group. The mean FEV1/FVC among the five genotype groups was consistently different (table 5). However, when individuals with normal lung function (healthy, liver disease without COPD, other conditions) were disaggregated from the whole group, the FEV1 and FEV1/FVC in the remaining patients with COPD no longer differed among the R/D, PI*ZZ, and PI*SZ subgroups. The difference between PI*MZ and M/R subjects for FEV1 was significant, but it may have been influenced by the low number of M/R subjects with COPD.

The effect of smoking habit and genotype on FEV1/FVC is summarised in table 6. Logistic regression and multivariate analysis showed that smoke and PI*ZZ and PI*RD genotypes act as independent risk factors for an FEV1/FVC<0.70.

**DISCUSSION**

Our finding of a 11% prevalence of subjects with severe AATD carrying genotypes other than PI*ZZ and PI*SZ is, to our knowledge, the highest reported so far. The NHLBI Registry for severe AATD with 1021 subjects includes 1.7% with genotypes other than PI*ZZ or PI*SZ.17 The Alpha One Foundation Research Network Registry also includes subjects with intermediate AATD: individuals with rare AATD variants accounted for 5.7% of the total.18 However, in our series including subjects with both severe and intermediate AATD increased the prevalence to 13%. Thus, our large series of rare AATD variants, detected within the relatively small sized Italian registry, prompted us to investigate the characteristics of these subjects more closely.

Molecular characterisation showed that the majority of subjects with rare AATD variants, including both index and non-index cases, carried at least one Mmalton allele (16/37 individuals, 21/74 alleles). This mutation is raised on the M2 base allele and consists of the deletion of an entire TTC codon in exon II, and subsequent deletion of the Phe51 or Phe52 residue of the mature protein.19 20 Ten of the 44 subjects carried at least one Mprocida allele, based on M1(Val213), a T→C point mutation at codon 41 exon II, leading to a proline for leucine substitution.21 The I allele, found in two individuals, and the Psewell allele (also referred to as QO*Cardiff), found in four individuals, are both raised on the M1(Val213) base allele.22 The I allele is characterised by a C→T point mutation at codon 39 exon II, leading to a cysteine for arginine substitution, whereas the Psewell allele is characterised by an A→T transversion at codon 256 exon III, leading to a valine for asparagine substitution. The Msewellen variant, found in one subject, is raised on the M1(Ala213) base allele and it is characterised by a C→T point mutation at codon 369 exon V, leading to a leucine for proline substitution.23 The Q0procida allele (also referred to as Nullprocida or Nullsola di procida)24 is a null variant characterised by a 17 kb deletion encompassing exons II–V. The Q0layouto a null variant raised on the M1(Val213) base allele, more recently identified,25 is characterised by a C insertion in exon V, causing a 3′ frameshift mutation, in turn resulting in a stop codon at residue 376. Finally, two AATD variants were recently found in our series of subjects: the Mvarallo allele was first described in a family from a Northern Italian village.
This allele is characterised by a 30 bp deletion accompanied by a 22 bp fragment insertion at the 41–51 codon region in exon II,25 whereas the Q0cairo is a null variant raised on the by a 22 bp fragment insertion at the 41–51 codon region in (100%) (table 4). Accordingly, the magnitude of FEV1/FVC than the related PI subjects with intermediate AATD, M/R subjects smoked more increasing prevalence of COPD according to the hierarchy among the groups revealed interesting findings. We found an proportion of former smokers among R/D individuals is carriers of such variants. Taken as a whole and looking at large series prompted us to examine the phenotypic profile of rare AAT variants in Italy, which will be discussed later, our relatively index cases only, there was a higher proportion of men (12/15) and a higher proportion of current or former smokers (100%) among carriers of rare variants than in the other groups with severe AATD (PIZZ and PI*SZ) (table 3). Among subjects with intermediate AATD, M/R subjects smoked more than the related PI*MZ subjects (table 3). The higher proportion of former smokers among R/D individuals is intriguing: perhaps they stopped smoking because of the lung disease.

Analysis of COPD prevalence and respiratory function data among the groups revealed interesting findings. We found an increasing prevalence of COPD according to the hierarchy PI*MZ (38%) and PI*SZ (38%)< M/R (46%)< PI**ZZ (79%)< R/D (100%) (table 4). Accordingly, the magnitude of FEV1/FVC ratio impairment followed the same hierarchy: PI*MZ (0.74)< PI*SZ (0.71)< M/R (0.67)< PI**ZZ (0.57)< R/D (0.39) (table 5).

Thus, within the spectrum of prevalence of lung disease associated with AATD, carrying a rare AAT variant(s) places R/D individuals at the highest risk, in the same position as PI**ZZ individuals, whereas M/R individuals are in an intermediate position, with PI*SZ and PI*MZ individuals at the end with lower risk.8 Since it is widely accepted that phenotypes result from the interaction between genetic determinants (in this case, the plasma levels of AAT) and environmental determinants (in this case, cigarette smoking), then the phenotypic ranking seems to result from the interaction between the AAT plasma levels reported in our series, with the R/D and PI**ZZ groups at the lowest end (29 and 28 mg/dl, respectively), followed by PI*SZ (62 mg/dl) and M/R (61 mg/dl), and with PI*MZ individuals at the highest end (93 mg/dl), and the smoking prevalence, ranking R/D (100%)> M/R (85%)> PI**ZZ (67%)> PI*SZ (52%) and PI*MZ (52%). These findings further support the concept that the genetic risk factor for COPD is significantly related to the AAT level,8 and that cigarette smoking may influence the risk rate.26

Chronic liver disease, which is the second most common feature associated with AATD, was detected in 13% of R/D subjects, 13% of PI**ZZ subjects, 19% of PI*SZ subjects, and 11% of PI*MZ subjects (table 4). Diagnosis of chronic liver

Table 5 Mean (SD) lung function of individuals with severe and intermediate AATD

<table>
<thead>
<tr>
<th>Genotype</th>
<th>FEV1†</th>
<th>FEV1/FVC*</th>
</tr>
</thead>
<tbody>
<tr>
<td>PI**ZZ</td>
<td>Total, n = 93</td>
<td>57 (32)</td>
</tr>
<tr>
<td></td>
<td>COPD, n = 73</td>
<td>45 (21)</td>
</tr>
<tr>
<td>PI*SZ</td>
<td>Total, n = 17</td>
<td>82 (36)</td>
</tr>
<tr>
<td></td>
<td>COPD, n = 7</td>
<td>48 (29)</td>
</tr>
<tr>
<td>R/D</td>
<td>Total, n = 16</td>
<td>39 (18)*</td>
</tr>
<tr>
<td></td>
<td>(all COPD)</td>
<td></td>
</tr>
<tr>
<td>PI*MZ</td>
<td>Total, n = 70</td>
<td>94 (29)</td>
</tr>
<tr>
<td></td>
<td>COPD, n = 37</td>
<td>69 (24)</td>
</tr>
<tr>
<td>M/R</td>
<td>Total, n = 12</td>
<td>78 (35)</td>
</tr>
<tr>
<td></td>
<td>COPD, n = 5</td>
<td>40 (17)**</td>
</tr>
</tbody>
</table>

Three PI**ZZ, four PI*SZ, one PI*MZ, and one M/R subjects were excluded because of insufficient data.

*p = 0.00095 v PI*SZ; **p = 0.009 v PI**SZ; ***p = 0.0034 v PI*MZ.

†Pre-bronchodilator, % predicted.

Table 6 Odds ratio and 95% confidence interval (95% CI) for FEV1/FVC<0.70 according to smoking habit and AATD genotype

<table>
<thead>
<tr>
<th>Variable</th>
<th>Multivariate * OR (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoke (yes v no)</td>
<td>3.9 (1.9 to 8.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Genotype</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MZ</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>MR v MZ</td>
<td>1.6 (0.3 to 9.4)</td>
<td>0.557</td>
</tr>
<tr>
<td>SZ v MZ</td>
<td>0.8 (0.2 to 2.9)</td>
<td>0.709</td>
</tr>
<tr>
<td>ZZ v MZ</td>
<td>7.4 (3.4 to 15.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>RD v MZ</td>
<td>16 (1.9 to 133.9)</td>
<td>0.011</td>
</tr>
</tbody>
</table>

*Adjusted for smoke and AATD genotype.
intracellular accumulation of the AAT protein.\textsuperscript{12} In our
variant, is known to be significantly associated, like the
Genetica, Clinica di Malattie dell'Apparato Respiratorio, Pavia, Italy
regions, such as Sardinia, with lower PI
displayed a different geographic distribution, peaking in
areas (fig 1). Interestingly, however, the rare AATD variants
their probable southern Italian origin (M procida, M palermo,
subject (Z/Plowell) and in association with COPD in two
series, isolated liver disease was detected in one non-index
years and, therefore, the condition might be underestimated
Secondly, chronic liver disease may be asymptomatic for
years and, therefore, the condition might be underestimated
at the time of patient assessment by the physicians
participating in the screening programme (who are mostly
panmoulmonologists).

A final point should be made on the geographic
distribution of the AATD variants. It is well known that the
prevalence of the PI\textsuperscript{TZ} gene in Europe is higher in north-
western countries, with a decreasing gradient towards the
south-east.\textsuperscript{13} This gradient was respected in Italy, where
most PI\textsuperscript{TZ}Z individuals in our series were found in northern
areas (fig 1). Interestingly, however, the rare AATD variants
displayed a different geographic distribution, peaking in
regions, such as Sardinia, with lower PI\textsuperscript{TZ}Z prevalence. Since
the nomenclature of many rare AATD variants reflects their
probable southern Italian origin (M\textsubscript{procida}, M\textsubscript{palermo}
QO\textsubscript{dada} di procida, QO\textsubscript{asterve}), this raises the intriguing question
of whether rare AATD variants are more prevalent in areas
with a lower PI\textsuperscript{Z} gene frequency.

In conclusion, from the limited data obtained from the
Italian Registry for Severe AATD, it seems that individuals
carrying rare AATD variants are characterised by a rather
peculiar phenotypic profile, placing them in a precise position
within the spectrum of genotype-phenotype correlations in
AATD. These data, as well as the geographic distribution of
rare variants, need verification using larger, international
registries.

ACKNOWLEDGEMENTS
The authors are deeply indebted to all physicians who are
participating in the Italian AATD screening program, to members
of the Gruppo IDA, to Dr Daniela Medicina, and to Mrs Nuccia Gatta
and the Associazione x1-AT. We greatly acknowledge manuscript
editing by Dr Rachel Stenner. ML is a member of the Council of the
Alpha One International Registry (A.I.R.).

Authors' affiliations
I Ferrarotti, J Baccheschi, M Zorzetto, I Campo, E Pozzi, G Stella,
Luisetti M, Centro di Diagnosi e di Coordinamento del Registro Italiano
for the Deficit Severe di AAT; antitripsina, Laboratorio di Biochimica e
Genetica, Clinica di Malattie dell'Apparato Respiratorio, Pavia, Italy
to Daniela Medicina, and to Mrs Nuccia Gatta
A. T. We gratefully acknowledge manuscript
editing by Dr Rachel Stenner. ML is a member of the Council of the
Alpha One International Registry (A.I.R.).

G Massi, Laboratorio per la Diagnosi del Defetto Congenito di AAT-
antitripsina, Università Cattolica del Sacro Cuore, Rome, Italy
This study was supported by IRCCS Policlinico San Matteo Ricerca
Corrente grants, MIUR Progetti di Interesse Nazionale 2002, the Alpha-
1 Foundation, Fondazione Carlos, Bay EUR, and Alanta.
Competing interests: none declared

Correspondence to: Dr Maurizio Luisetti, Clinica di Malattie
dell'Apparato Respiratorio, IRCCS Policlinico San Matteo, Università di
Pavia, via Taramelli 5, 27100 Pavia, Italy; m.luisetti@matteo.pv.it

REFERENCES
2002;346:45–53
2 de Serres FJ. Worldwide racial and ethnic distribution of x1-antitrypsin
deficiency. Summary of an analysis of published genetic epidemiology
3 Luisetti M, Saarenlinn M. Epidemiology of alpha-1-antitrypsin deficiency.
Thorax 2004;59:164–9
4 American Thoracic Society/European Respiratory Society Statement
Standards for the diagnosis and management of individuals with alpha-1
5 Alpha-I-Antitrypsin Deficiency Registry Study Group. Survival and FEV\textsubscript{1}
decline in individuals with severe deficiency of alpha-1-antitrypsin. Am J Respir
Crit Care Med 1998;158:49–58
6 Luisetti M, Miravetille M, Stedley RA. Alpha-1-antitrypsin deficiency: a report
from the 2nd meeting of the Alpha One International Registry, Rapallo
7 Seersholm N, Kok-Jensen A. Clinical features and prognosis of lifetime non-
8 Turino GM, Barker AF, Brantly ML, Cohen AB, Connelly RP, Crystal RG,
Eiden E, Schlüchter MD, Stoller JK. Clinical features of individuals with the
PI\textsuperscript{SZ} phenotype of alpha-1-antitrypsin deficiency. Am J Respir Crit Care Med
1996;154:1718–25
9 Brantly M. Alpha-1-antitrypsin genotypes and phenotypes. In: Crystal RG, ed.
10 Luisetti M, Massi G, Massobrio M, Guarneri P, Manchichi FM, Becizza M,
Balbi B. A national program for detection of x1-antitrypsin deficiency in
Italy. Respir Med 1999;93:629–72
11 de Serres FJ, Blanco I, Fernandez-Bustillo E. Genetic epidemiology of alpha-
antitrypsin deficiency in southern Europe. France, Italy, Portugal and Spain.
12 American Thoracic Society. Standards for the diagnosis and care of patients
with chronic obstructive pulmonary disease. Am J Respir Crit Care Med
1995;152:577–120
13 Zorzetto M, Tamburroli C, Mascheroti B, Massi G, Battaglia C, Medaglia S,
Luisetti M. A fast amplification-reverse hybridization assay kit to detect the
most frequent deficient variants in the alpha-1-antitrypsin gene. Respiraion
2002;69:51–6
14 Benettozzi MG, Gile LS, Bombieri C, Malerba G, Massobrio M, Pignatti PF,
Luisetti M. x1-Antitrypsin, Tarp polymorphism and x1-antitrypsin gene
mutations in patients with obstructive pulmonary disease. Respir Med
1999;93:648–54
15 Brantly M. Laboratory diagnosis of x1-AAT deficiency. In: Crystal RG, ed.
16 Fortin PR, Fraser RS, Watts CS, Esdale JM. Alpha-1-antitrypsin and systemic
17 McElvany NG, Stoller JK, Buist AS, Prokas UBS, Brantly ML, Schlüchter MD,
Crystal RG, and the x1-Antitrypsin Deficiency Registry Study Group. Baseline
characteristics of enrollees in the National Heart, Lung and Blood Institute
Registry of x1-antitrypsin deficiency. Chest 1997;111:394–403
18 Brantly M, Brantly ML, Fleming LE, Bean JA, Walsh J. Formation and current
results of a patient-organized registry for x1-antitrypsin deficiency. Chest
2000;118:843–8
19 Frazier GC, Harnold TR, Halkert MH, Cox DW. In frame single codon deletion
in the M\textsuperscript{nulla}, deficiency allele of alpha-1-antitrypsin. Am J Hum Gen
20 Graham A, Kalsheker NA, Newton CR, Bamford FJ, Powell SJ, Markham AF.
Molecular characterisation of three alpha-1-antitrypsin deficiency variants:
proteinase inhibitor (Pi) Nullisola di procida, M\textsuperscript{nulla} (hps50-1 deletion), and Pi I (ang39-to-cys). Hum Genom 1989;84:55–8
21 Takahashi H, Nukawa T, Sato K, Ogushi F, Brantly M, Fells G, Siler C,
Courtney M, Crystal RG. Characterization of the gene and protein of the
alpha-1-antitrypsin deficiency “nulla” allele M\textsubscript{prosida}. J Biol Chem
1989;263:15528–34
22 Halkert MH, Nukawa T, van Paassen HMB, Nelen M, Kramps J, Klarenz EC,
Frants RR, Crystal RG. A pro-to-leu substitution in codon 369 of the alpha-1-
23 Takahashi H, Crystal RG. Alpha-1-antitrypsin Null, a disease of x1-antitrypsin
24 Brantly M, Hwa Lee J, Hildeshiem J, Uhms CS, Prokas UBS, Staats BA,
Crystal RG. x1-Antitrypsin gene mutation hot spot associated with the
formation of a retained and degraded Null variant. Am J Respir Cell Mol Biol

www.jmedgenet.com


