Clinical Biochemistry 64 (2019) 49–52

Contents lists available at ScienceDirect

Clinical Biochemistry

journal homepage: www.elsevier.com/locate/clinbiochem

Case Report

An unusual case of alpha-1-antitrypsin deficiency: SZ/Z

Speevak M.D.[a,*], DeMarco M.L.[b], Wiebe N.S.[c], Chapman K.R.[d]

[a] Department of Laboratory Medicine and Pathobiology, University of Toronto, Canada
[b] Department of Pathology and Laboratory Medicine, Providence Health Care, University of British Columbia, Canada
[c] Division of Respiratory Medicine, Department of Medicine, University of Toronto, Canada
[d] Division of Respiratory Medicine, Department of Medicine, University of Toronto, Canadian Registry for Alpha-1 Antitrypsin Deficiency, Canada

ARTICLE INFO

Keywords:
Alpha-1 antitrypsin deficiency
Molecular sequence data
Alleles
Gene conversion

ABSTRACT

A female patient was first seen at age 65 due to a diagnosis of alpha-1-antitrypsin deficiency (AATD). She was a lifelong non-smoker, with no significant history of second hand smoke exposure. There was no prior family history of AATD or liver disease. Her serum AAT concentration was measured on two occasions and in both cases, concentration was < 0.21 g/L. The patient was referred for genetic testing to determine her *SERPINA1* (the gene responsible for AATD) genotype. Three deficiency alleles were identified: she was heterozygous for S, a mild deficiency allele, and homozygous for Z, a severe deficiency allele. This case represents unusual convergence of three pathogenic *SERPINA1* variants in a single individual. We report the investigations used to clarify her unusual genotype and propose non-crossover gene conversion as the likely mechanism.

1. Introduction

The α1-antitrypsin (AAT) protein is a member of the serine protease inhibitor (Serpin) superfamily of proteins. Upon contact with its substrate, AAT undergoes an irreversible conformational change that isolates and eliminates the targeted protease (reviewed, [1]). Due to its unique structure and associated function, it is acutely susceptible to mutations that adversely affect its ability to trap and inactivate its target [2]. Failure of defective AAT to protect tissues from inflammatory damage is a major cause of AATD-associated disease (reviewed, [3]). The clinical characteristics of AATD include increased risk for chronic obstructive pulmonary disease (COPD) in adults, most commonly emphysema, liver disease (pediatric as well as adult), panniculitis and c-ANCA positive vasculitis (Wegener granulomatosis) [4]. Since AAT is expressed in the blood stream, serum concentration represents a useful biomarker for determining AAT deficiency in individuals with symptoms suggestive of the disorder. AATD is a codominant, autosomal Mendelian disorder, with each allele having a serum concentration ranging from 0 (null allele) to 0.6–1.25 g/L (normal range for a single, functional allele). Penetrance is incomplete, as many individuals with severe deficiency (< 0.2 g/L) do not necessarily experience health issues during their lifetime. Disease onset in adults is significantly influenced by harmful environmental exposures such as smoking and alcohol use and by unknown, independent genetic factors. Historic descriptions of abnormal AAT are based upon isoelectric focussing patterns and specific nomenclature guidelines standardized in 1978 [5]. The classical standardized terminology describes many of the currently recognized deficiency alleles, including the most common Z and S alleles. These two variants are found among individuals of European ancestry with allele frequencies averaging 1.8% and 3.7% respectively (gnomAD data, [6]), with the most common disease genotypes being the homozygous ZZ and the compound heterozygous SZ. As rare null and partially functional alleles are also disease associated, molecular methods (PCR, sequencing) are often performed to improve accuracy in the diagnosis [7]. Here we report an unusual case of a patient who was referred for AATD testing due to a personal history of emphysema and extremely low circulating serum AAT (< 0.2 g/L). Her genotype was found by routine testing to be a combination of heterozygosity for the S allele and homozygosity for the Z allele (denoted herein as SZ/Z). As the S and Z alleles have only previously been reported *in trans*, this finding is unique, and demonstrates the role of non-Mendelian mechanisms in human disease.

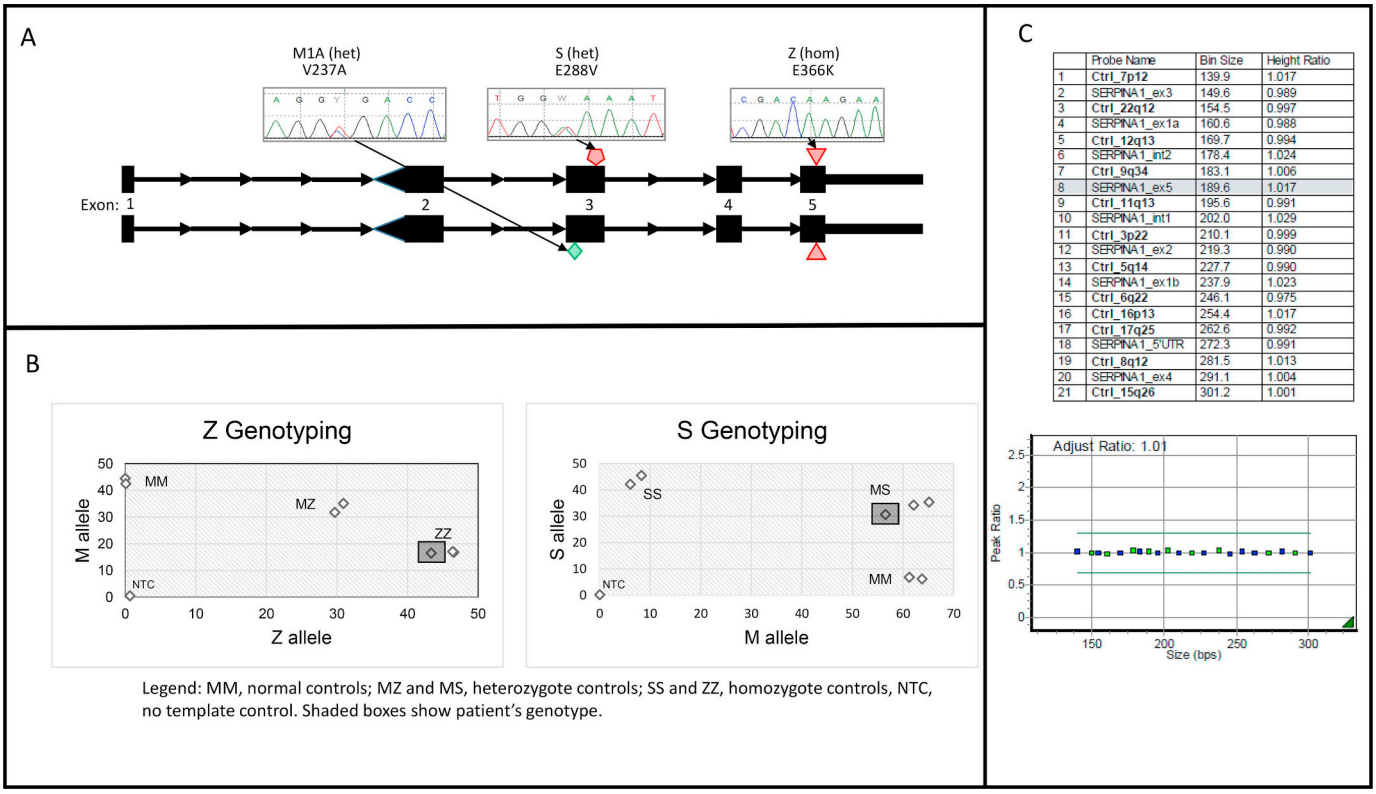
2. Materials and methods

2.1. Informed consent

Informed consent to publish this case was obtained from the patient through the authors' institutional Research Ethics Board approved process.

* Corresponding author at: Genetics Division, Trillium Health Partners, 2200 Eglinton Ave. W., Rm 2H132, Mississauga, Ontario L5M 2N1, Canada.
E-mail address: marsha.speevak@thp.ca (M.D. Speevak).

https://doi.org/10.1016/j.clinbiochem.2018.12.008
Received 5 September 2018; Received in revised form 19 November 2018; Accepted 19 December 2018
Available online 21 December 2018



	Probe Name	Bin Size	Height Ratio
1	Ctrl_7p12	139.9	1.017
2	SERPINA1_ex3	149.6	0.989
3	Ctrl_22q12	154.5	0.997
4	SERPINA1_ex1a	160.6	0.988
5	Ctrl_12q13	169.7	0.994
6	SERPINA1_int2	178.4	1.024
7	Ctrl_9q34	183.1	1.006
8	SERPINA1_ex5	189.6	1.017
9	Ctrl_11q13	195.6	0.991
10	SERPINA1_int1	202.0	1.029
11	Ctrl_3p22	210.1	0.999
12	SERPINA1_ex2	219.3	0.990
13	Ctrl_5q14	227.7	0.990
14	SERPINA1_ex1b	237.9	1.023
15	Ctrl_6q22	246.1	0.975
16	Ctrl_16p13	254.4	1.017
17	Ctrl_17q25	262.6	0.992
18	SERPINA1_5'UTR	272.3	0.991
19	Ctrl_8q12	281.5	1.013
20	SERPINA1_ex4	291.1	1.004
21	Ctrl_15q26	301.2	1.001

Fig. 1. A The predicted phasing of the patient's SZ/Z alleles is shown. The E288V (S, shown as a pentagon) is *in cis* with E366K (Z, shown as a triangle) on one allele and the V237A (M1A, shown as a diamond) is predicted to be retained *in cis* with Z on the second allele. B Genotyping assay to confirm presence of one S allele and two Z alleles. The patient's PCR results are shown in the shaded boxes, sorting with ZZ (left) and MS (right). C *SERPINA1* MLPA assay showing normal, 1:1 dosage along the entire gene (exon 5, location of Z, shaded).

2.2. Case presentation

The patient was diagnosed with AATD at the age of 65. She was a lifelong non-smoker, with no significant history of second hand smoke exposure. There was no prior family history of AATD or liver disease. She had eight siblings, one of whom had a history of asthma. The patient reported progressive shortness of breath on exertion over the previous eight years since her diagnosis. Her pulmonary function tests, performed at the age of 73 showed FEV_1/FVC ratio of 51%, FEV_1 1.3 L (58% of predicted), FVC 2.6L (89% of predicted), and post-bronchodilator FEV_1/FVC ratio of 51% with FEV_1 of 1.43 L. Her TLC was 6.6 L (126%), RV/TLC 58 (132%), and DLCO 10.7 (57%). Overall, there was moderate airflow obstruction with no significant improvement post-bronchodilator. There was evidence of hyperinflation and gas trapping. Her serum AAT concentration was measured on two occasions and in both cases, was < 0.21 g/L (Tina-quant ver.2, Cobas c701, Lifelabs). The patient was referred for genetic testing to determine her *SERPINA1* genotype.

2.3. Molecular analyses

The SZ/Z genotype was first determined via blood spot testing through the Alpha-1 Targeted Detection Program (GeneAidyx, Genetics Laboratory, Fl, USA). Following this, a fresh peripheral EDTA blood sample was obtained from the patient from which DNA was extracted. Sanger sequencing was performed on exons 2 through 5 of *SERPINA1* (GRCh38, NM_000295.4). Primer sequences were as follows: 2aF: 5′-TGATTCTTTCAGTGTTAC-3′; 2aR: 5′-CGTGAGGTTGAAATTCAG-3′; 2bF: 5′- TGATCAGGATCACCCAAC-3′; 2bR: 5′-AACTGATGGTTTGAG AAT-3′; 3F: 5′-CTTGGATGGTCAGTTTCAGC-3′; 3R: 5′-ACAGAGTAGCA GTGACCC-3′; 4F: 5′-AGGAGGTGGCATTTCAA-3′; 4R: 5′-GGGTCTTCAT TTGTTTCCCTC-3′; 5F: 5′-TGTCCACGTGAGCCTTGC-3′; 5R: 5′-CCTCA GCAGGCAAAGGG-3′. Sequencing was performed on the ABI 3500 Genetic Analyzer (Thermo Fisher Scientific). Allele-specific PCR was performed on the LC480 LightCycler (Roche Scientific) using published Taqman Genotyping primers and probes specific to S (dbSNP rs17580) and Z (dbSNP rs28929474) as per the Taqman SNP Genotyping Assay Procedure (Thermo Fisher Scientific). Multiplex Ligation-dependent Probe Amplification (MLPA) was performed on the ABI 3130 Genetic Analyzer (Thermo Fisher Scientific) using the SALSA MLPA probemix P459-A1 (MRC-Holland) as per the manufacturer's directions. The products were analyzed using GeneMarker software (SoftGenetics).

2.4. AAT structure

To assess the impact of the identified sequence variants on AAT structure and stability, the variants, i.e. V237A (M1A), E288V (S), and E366K (Z), were examined in the context of a crystal structure of wild-type human AAT (PDBID: 3NE4; [8]). The 1.8 Å resolution structure contained residues 72-442 (note that sequencing numbering of AAT used throughout includes the 24-residue signal sequence). Protein structural images were created using UCSF Chimera [9].

3. Results

Sanger sequencing showed the presence of a homozygous Z and a heterozygous S allele. In current molecular terminology (HGVS), these are described as c.1096G > A, p.E366K and c.863A > T, p.E288V respectively. The Z allele typically exists in equilibrium with a benign, single nucleotide variant (SNV) in exon 3 known as M1A (alanine at codon position p.237) and the S allele is typically found in equilibrium with the GRCh38 genome reference sequence, M1V (valine at codon p.237). Whereas a ZZ result is expected to occur *in cis* with homozygous M1A alleles, the patient was found to be heterozygous M1A/M1V

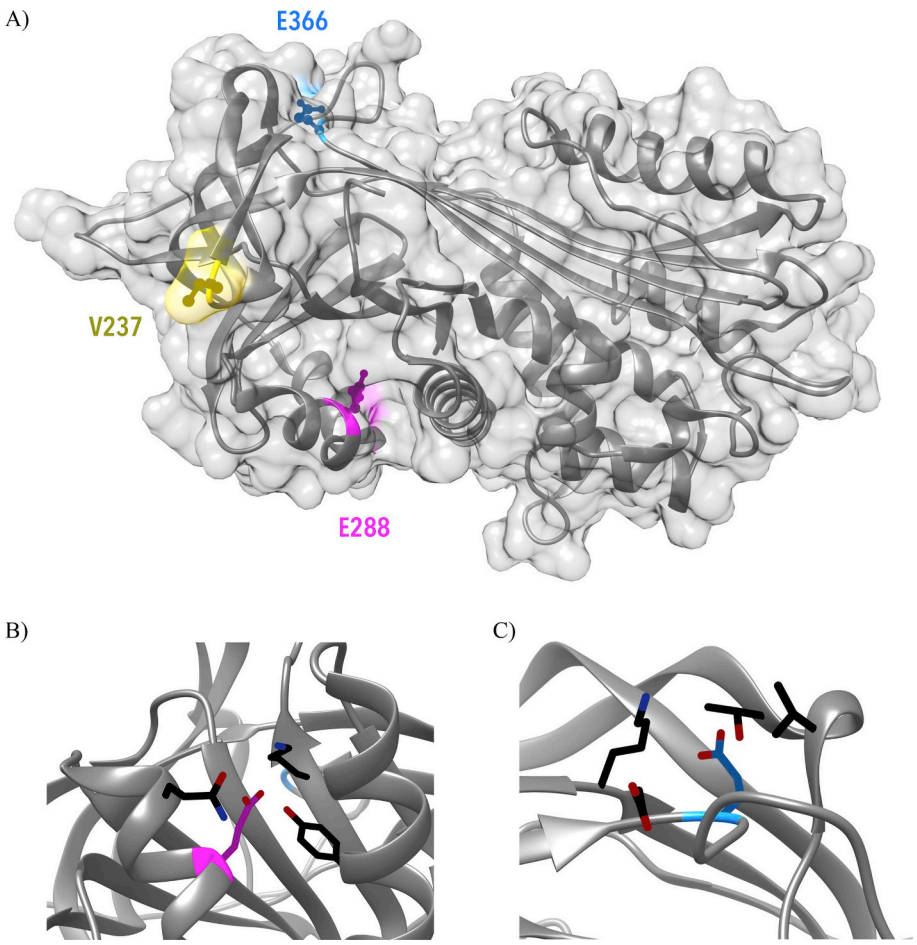


Fig. 2. (A) Three-dimensional structure of wild-type AAT (backbone structure and transparent space-filling model shown) with the benign variant position V237A (yellow), and the pathogenic variant positions E288V (magenta) and E366K (light blue), noted. Close-up view of the native salt-bridge and hydrogen bonding networks of (B) E288 (magenta) and (C) E366 (light blue), with the side-chains of relevant proximal residues displayed (carbons, black; oxygens, red; nitrogens, blue; hydrogens not shown). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(Fig. 1A). To rule out PCR based allele drop out at the Z locus, real-time PCR was performed using primers and probes designed to directly detect the S and Z alleles. This analysis confirmed heterozygous S and homozygous Z in the sample (Fig. 1B). Furthermore, to rule out allele drop out due to an exon 5 deletion on the M1A allele, multiplex ligation-dependent probe amplification (MLPA) was performed. The MLPA assay is designed to quantify each exon of the target gene in comparison with control loci. A normal 1:1 ratio of each *SERPINA1* exon was detected, ruling out an exon 5 deletion in this patient (Fig. 1C). Thus, the combination of methods (serum concentration, Sanger sequencing, allele-specific PCR and MLPA) all agreed with the finding of SZ/Z in this patient.

The location of the three variants: benign SNV V237A and pathogenic SNVs E288V (S) and E366K (Z), were mapped onto the known three-dimensional structure of wild-type AAT with V237 (Fig. 2A). These three residues are not in proximity with one another, in terms of both primary and tertiary structure. Residue 237 is solvent exposed, whereas residues 288 and 366 have minimal solvent exposure. Residue E288 is found on one of the α-helices of AAT and stabilizes secondary and tertiary structure as it participates directly in a salt-bridge and a hydrogen bonding network (Fig. 2B). The E288V variant results in a non-conservative substitution of a negatively charged residue (E) for a non-polar residue (V), which would disrupt the interactions noted, likely negatively affecting secondary and tertiary structure and stability. Similarly, E366 participates in a hydrogen bonding network (Fig. 2C) contributing to stabilizing the secondary and tertiary structure of AAT. For E366K, the non-conservative substitution of a negatively charged residue (E) for a positively charged residue (K) would necessarily alter the native hydrogen bonding network in this region.

4. Discussion

The detection of three AAT deficiency alleles in this patient was surprising and unique. As far as we are aware, an *in cis* SZ allele occurring in any single individual has not been reported previously. Z and S have been stably retained *in cis* with M1A and M1V haplotypes respectively, in the general European population over dozens of generations. The M1A, Z allele is thought to have had a single ancestral origin no earlier than 2000 years ago [10,11,12]. The M1V, S allele is thought to have arisen 15000–20000 years ago [13]. The only explanation for this patient's *SERPINA1* genotype is that the S and Z alleles underwent genetic recombination in a parent/ancestor or possibly as an early zygotic event. Sibs and other family members were unavailable for genetic testing to explore this further.

Genetic recombination is a programmed exchange of DNA between homologous chromosomes, occurring at meiosis I during gametogenesis. It relies on double strand break (DSB) repair with a crossover (CO) of DNA strands between the homologues. When the two strands at the site of the DSB are non-identical due to sequence differences, the DSB repair may lead to non-crossover (NCO) gene conversion, where the exchange of DNA is small and unidirectional. Germline gene conversions are usually difficult to detect since they only involve a few hundred basepairs as opposed to CO recombinations which are typically many megabases in length. Although NCO gene conversions are evolutionarily important, and occur at a much higher frequency per chromosome as compared to CO recombinations, they are not thought to contribute significantly to the human germline mutation rate [14,15].

SERPINA1 is genetically stable with consistent and ancient heritable haplotypes [16]. Under what circumstances our patient arose as a SZ/Z

individual is unknown. A CO may have occurred at meiosis I in a compound heterozygous, SZ parent or ancestor. However, in our opinion, a more probable mechanism would be a NCO gene conversion due to a DSB downstream of exon 3 and upstream of exon 5. In NCO DSB repair, either the S allele or opposing Z allele could have served as a template for repair since the M1V, S haplotype is very tightly linked. It is difficult to prove CO versus NCO DNA exchange, since to do so, extensive haplotype analyses extending beyond the *SERPINA1* gene would be required involving multiple first degree family members.

As AATD phenotypes are interpreted from the perspective of the concentration of AAT in serum – reflective of translation, native folding and structural stability of AAT – we also explored the potential structural consequences of the two known pathogenic variants, reflective of S and Z genotypes respectively, occurring in *cis*. As residues 288 and 366 occur in distinct regions of the AAT structure (Fig. 2), the destabilizing pathogenic variants are anticipated to have a compounding deleterious effect on AAT structure and stability. This is consistent with the observed AAT serum concentration below the detectible limit in this case. With respect to the V237A variant, this residue is on an unstructured loop and is solvent exposed; thus, a conservative amino acid substitution at this position is not anticipated to significantly alter the structure or dynamics of AAT, consistent with this being a benign polymorphism.

5. Conclusion

In summary, we have a unique case of an AATD patient with a triple SZ/Z genotype. The patient's very low serum AAT concentration and clinical history are consistent with this finding. The SZ/Z genotype has implications for her descendants, since inheritance of the *in cis* SZ allele could be misinterpreted to be the *in trans,* affected state of AATD. This case illustrates the importance of phasing pathogenic variants, particularly when predicting likelihood of disease in late onset disorders, or when attempting to predict disease likelihood based on genotyping results. This case also illustrates how disease alleles can be transmitted in a non-Mendelian manner; a rare, but possibly under-recognized mechanism of inheritance with implications for biallelic disorders.

Declarations of interest

None.

References

[1] G.A. Silverman, P.I. Bird, R.W. Carrell, F.C. Church, P.B. Coughlin, P.G.W. Gettins, J.A. Irving, et al., The serpins are an expanding superfamily of structurally similar but functionally diverse proteins. Evolution, mechanism of inhibition, novel functions, and a revised nomenclature, J. Biol. Chem. 276 (2001) 33293–33296.

[2] B. Gooptu, B. Hazes, W.S. Chang, T.R. Dafforn, R.W. Carrell, R.J. Read, D.A. Lomas, Inactive conformation of the serpin alpha(1)-antichymotrypsin indicates two-stage insertion of the reactive loop: implications for inhibitory function and conformational disease, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 67–72.

[3] F. De Serres, I. Blanco, Role of alpha-1 antitrypsin in human health and disease, J. Intern. Med. 276 (2014) 311–335.

[4] J.K. Stoller, F.L. Lacbawan, L.S. Aboussouan, Alpha-1 antitrypsin deficiency. 2006 Oct 27 [Updated 2017 Jan 19], in: M.P. Adam, H.H. Ardinger, R.A. Pagon, et al. (Eds.), GeneReviews® [Internet], University of Washington, Seattle, Seattle (WA), 1993–2018Available from: https://www.ncbi.nlm.nih.gov/books/NBK1519/.

[5] D.W. Cox, Genetic variation of alpha 1-antitrypsin, Am. J. Hum. Genet. 30 (1978) 660–662.

[6] M. Lek, K.J. Karczewski, E.V. Minikel, K.E. Samocha, E. Banks, T. Fennell, A.H. O'Donnell-Luria, et al., Analysis of protein-coding genetic variation in 60,706 humans, Nature 536 (2016) 285–291.

[7] R.P. Graham, M.A. Dina, S.C. Howe, M.L. Butz, K.S. Willkomm, D.L. Murray, M.R. Snyder, K.M. Rumilla, K.C. Halling, W.E. Highsmith Jr., SERPINA1 full-gene sequencing identifies rare mutations not detected in targeted mutation analysis, J. Mol. Diagn. 17 (2015) 689–694.

[8] A.O. Patschull, L. Segu, M.P. Nyon, et al., Therapeutic target-site variability in α1-antitrypsin characterized at high resolution, Acta. Crystallogr. Sect. F Struct. Biol. Cryst. Commun. 67 (2011) 1492–1497 Pt 12.

[9] E.F. Pettersen, T.D. Goddard, C.C. Huang, G.S. Couch, D.M. Greenblatt, E.C. Meng, T.E. Ferrin, UCSF Chimera—a visualization system for exploratory research and analysis, J. Comput. Chem. 25 (13) (2004 Oct) 1605–1612.

[10] D.W. Cox, S.L.C. Woo, T. Mansfield, DNA restriction fragment associated with α1-antitrypsin indicate a single origin for the deficiency allele PI Z, Nature 316 (1985) 79–81.

[11] B. Lace, T. Sveger, A. Krams, G. Cernevska, A. Krumina, Age of SERPINA1 gene PI Z mutation: Swedish and Latvian population analysis, Ann. Hum. Genet. 72 (2008) 300–304 Pt 3.

[12] M. Luisetti, N. Seersholm, α1-Antitrypsin deficiency: epidemiology of α1-antitrypsin deficiency, Thorax 49 (2004) 164–169.

[13] S. Seixas, O. Garcia, M.J. Trovoada, et al., Patterns of haplotype diversity within the serpin gene cluster at 14q32.1: insights into the natural history of the α1-antitrypsin polymorphism, Hum. Genet. 108 (2001) 20–30.

[14] J.-M. Chen, D.N. Cooper, N. Chuzhanova, C. Ferec, G.P. Patrinos, Gene conversion: mechanisms, evolution and human disease, Nat. Rev. Genet. 8 (2017) 762–775.

[15] V.M. Narasimhan, R. Rahbari, A. Scally, A. Wuster, D. Mason, Y. Xue, J. Wright, et al., Estimating the human mutation rate from autozygous segments reveals population differences in human mutational processes, Nat. Commun. 8 (2017) 303.

[16] S. Denden, J. Ben Chibani, A. Haj Khelil, Origins and spreads of Alpha 1 antitrypsin variants in world human populations: a synthetic review, Int. J. Mod. Anthropol. 5 (2012) 40–58.