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Alpha-1-antitrypsin deficiency screening program at the Pneumonology Department of Hospital Tránsito Cáceres de Allende

Correspondence:
Gregorio Kevorkof
gregorioke@fibertel.com.ar

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Authors: Kevorkof G. V.[1, 2, 3, 4], Tellechea P. E.[1], Acosta M. A.[1, 3], Najo M. A.[1, 2, 4], Oviedo E. E.[1, 2, 4], Peyrani C.[1], Granado M.R.[1], Fernández S.G.[1, 2], Yapur Bassani N.[1], Balbo N.[1]

Pneomonology Department
[1]Hospital Tránsito Cáceres de Allende,
[2]Clínica Universitaria Reina Fabiola, FM, UCC
[3]Cátedra de Clínica Médica II, UHMI Nº5, FCM, UNC
[4]Cátedra de Semiología A, FM, UCC

Abstract

Alpha-1-antitrypsin deficiency (AATD) is a rare genetic disease associated with an increased risk of suffering from pulmonary emphysema and chronic hepatopathy in children and adults alike. It is often underdiagnosed, with long periods elapsing between the onset of symptoms and a definite diagnosis. Alpha-1-antitrypsin (AAT) is the most abundant protease inhibitor in the human body. Scientific literature considers severe deficiency to be associated with the following phenotypes: SZ, ZZ and Null. Screening programs are required for early detection, this is why an easy and specific method has been described and validated. Through this method, AAT values are quantified using nephelometry in blood drop samples on blotting paper, then genotyping of the Z and S variants is quickly performed.

Objectives: To determine the number of individuals with AATD within a population of patients with chronic respiratory diseases. To identify and define those with AAT deficiency.

Materials and Method: Observational, descriptive and cross-sectional study of AAT deficiency screening, between January 2nd, 2014 and March 30th, 2015. Out of 80 individuals who fulfilled the inclusion criteria and who spontaneously attended or were referred to the Pneumonology Department of Hospital Tránsito Cáceres de Allende, Córdoba, Argentina, 62 patients who agreed to the study were analyzed. A test to determine the concentration of alpha-1-antitrypsin was performed to the patients who met all the inclusion criteria using blood drops on blotting paper. Only patients with alpha-1-antitrypsin levels < 1.8 mg/dL were requested a spirometry, a high-resolution computed tomography of the chest and quick genotyping tests.

Results: A total of 62 patients was evaluated in this study, 28 (45.2%) were females and 34 (54.8%) were males, 37 (59.7%) had alpha-1-antitrypsin levels ≥ 1.8 mg/dL and 25 (40.3%) < 1.8 mg/dL. Genotype elicitation using the dried-droplet method in 25 (40.3%; 25:62) patients with values < 1.8 mg/dL showed that: 22 (88%; 22:25) were Non-S Non-Z, 2 (8%; 2:25) were heterozygote for Z and 1 (4%; 1:25) was heterozygote for S. According to ATS/ERS criteria, the predominant spirometric pattern was obstructive (88%). The HRCT pattern corresponded to emphysema in 22 patients (88%): 7 (31.8%) centrilobular, 8 (36.4%) paraseptal, 7 (31.8%) panlobular. There were 2 patients (8%) with bronchiectasis and 1 (4%) was normal.

Conclusion: In a population selected by symptoms and/or history, patients with AATD can be identified using the dried-droplet method. Severe AATD is uncommon in Argentina, probably because it is underdiagnosed, and the amount of heterozygote PIS and PIZ carriers is higher. Early AATD diagnosis is uncommon.

It is difficult to draw conclusions about the alpha-1-antitrypsin group below 1.8 mg/dL without severe deficiencies in connection with the variables analyzed in the sample due to the lack of studies and bibliography on this subject.
We consider that patients with non-S non-Z genotypes and the ones with discrepancies must be quantitatively confirmed and their phenotype defined in serum samples using isoelectric focusing and, occasionally, they must have a molecular gene analysis to look for uncommon, new or null allelic variants.

Key words: Alpha-1-Antitrypsin deficiency. Screening. Alpha-1-Antitrypsin concentration measurement

Introduction

Alpha-1-antitrypsin (AAT) is the most abundant protease inhibitor in the human body, with plasma values ranging between 103-200 mg/dL under normal conditions[1, 2].

Its production is codified by the SERPINA1 gene, located in the long arm of chromosome 14, region q31-32.3.[1] This gene is passed on by autosomal codominant inheritance through two alleles, each inherited from one parent. AAT is characterized for having significant electrophoretic polymorphism, more than 100 variants were identified using Isoelectric Focusing (IEF). The set of variants is called Pi (protease inhibitor) system, many of them do not have clinical value. The incorporation of PCR techniques has increased their number to 125; the normal allele in more than 90% of normal subjects is called PI M, and the most common deficiency involves alleles PI S and PI Z. At present, it is recommended to use Pi* to identify the phenotype and PI* to identify the genotype[3-5].

The most common genotypes are: MM, MS, SS, MZ, SZ and ZZ, and they are linked to 100%, 80%, 60%, 55%, 40% and 15% of the activity of alpha-1-antitrypsin[5].

Scientific literature considers severe deficiency of alpha-1-antitrypsin (AATD) to be associated with the following phenotypes: SZ, ZZ and Null[7]. Intermediate AAT deficiency is most commonly caused by MS and MZ phenotypes[3].

Clinically, it can be associated with emphysema, COPD, asthma with irreversible airway obstruction, bronchiectasis, liver cirrhosis and, less frequently, with systemic panniculitis and vasculitis, specifically C-ANCA[5-8].

Pulmonary emphysema associated with AATD is typically an early-onset disease (35-45 years old), with no risk factors or baseline and panlobular distribution. In general, it is associated with ZZ phenotypes (96%) and, less frequently, with SZ, rare and null phenotypes (4%). Penetration (percentage of ZZ subjects who develop emphysema) is approximately 60%.

Although it is possible that it could favor the development of bronchial asthma and bronchiectasis, there is no definite evidence it influences in the frequency or severity of these diseases[5, 6, 9, 10].

The WHO and the European, Canadian and American Associations recommend COPD patients to have a test to determine the concentration of AAT in their blood, whether if they are smokers or not, at least once during their lifetime, and specifically if they have early-onset COPD[5, 11], or if they have a family history of alpha-1-antitrypsin deficiency[5, 12].

AATD is frequently underdiagnosed in clinical practice[13]. Diagnostic delay has been estimated to be around 5 and 10 years after COPD diagnosis[14], due to a lack of suspicion and since it is usually only attributed to tobacco use.

An easy and specific screening method has been described and validated. Through this method, AAT values are quantified using immunonephelometry in blood drop samples on blotting paper, after which genotyping of the Z and S variants is quickly performed[15, 16]. Samples of dried blood drops have been used for the genetic screening and diagnosis of different disorders[17-19]. This method is specific, reproducible and correlates with standard techniques for fresh blood samples[15].

Through this procedure, 1.8 mg/dL values correspond to 100 mg/dL values according to the standardization performed at the Hospital Italiano de Buenos Aires Laboratory for this technique, since this value may change depending on the reference laboratory[20].

It cannot be inferred, although the possibility exists, that when alleles Z and S are not identified, then it ought to be a normal PIMM allele[5].

AATD cases diagnosed with screening methods must be confirmed determining the serum concentration of AAT and the phenotype in serum samples or the genotype in whole blood[5].

The ratio differs according to the study population; in Western Europe and the United States, it is estimated at approximately 1:2500 and 1:5000 newborns. It is highly dependent on Scandinavian lineage and it is five times lower in Latin America. The most frequent deficiency alleles in the North of Europe are PI Z and PI S, and most of these individuals are PI ZZ[5, 19, 21].

In Argentina, SZ and ZZ genotypes are uncommon; their estimated ratios range between 1:2400 and 1:26000 for 17,000 and 1,500 subjects, respectively. However, data were obtained using indirect theoretical estimates; therefore, further epidemiologic studies must be performed to obtain reliable results[5, 22].

Nevertheless, since the incidence of AATD is unknown among patients with COPD, a few studies that enable to calculate it between 1% and 3% were taken as reference[6].

This study performed in Córdoba City at a Pneumonology Department was conducted to generate local epidemiological data.

Objectives

To determine the number of individuals with AATD within a population of patients with chronic respiratory diseases. To identify and define those with AAT deficiency.

Material and Methods

Study Design: Observational, descriptive and cross-sectional study of AAT deficiency screening.

Period: January 2nd, 2014 to March 30th, 2015.

Population: Out of 80 individuals who fulfilled the inclusion criteria and who spontaneously attended or were referred to the Pneumonology Department of Hospital Tránsito Cáceres de Allende, Córdoba, Argentina, 62 patients who agreed to the study were analyzed.

Inclusion Criteria:

- Individuals over the age of 18.
- Symptoms: coughing, expectoration, dyspnea, frequent exacerbations.
- Early-onset emphysema (≤45 years old), emphysema in the absence of a known risk factor (smoking, occupational exposure, etc.).
- COPD.
- Asthma with irreversible airway obstruction.
- Bronchiectasis in adults without apparent reason.
- Individuals with a family history of COPD or liver disease that could be attributed to AATD.

Exclusion Criteria:

- Pregnancy.
- Drug or any other type of immunosuppression.
- Acute infection.
- Oncological patients.
- Use of oral contraceptives.

Each doctor's office was equipped with a kit that consisted of blotting paper, a lancet, dressing pads, numbered envelope, an informed consent form and a brief questionnaire to collect patient data.

Sample screening process using the dried-droplet method

Capillary blood drops were collected via finger stick and placed on blotting paper discs; afterwards, they were sent by mail to the Hospital Italiano de Buenos Aires central laboratory. Samples were processed to quantitatively determine alpha-1-antitrypsin values using nephelometry (1.8 mg/dL correspond to 100 mg/dL in serum form)[20] and the genotype was determined from the PCR reaction in real time. The latter uses 2 pairs of *primers* to amplify two regions: one of 177 pb that corresponds to the PI S allele variant (protease S inhibitor) and another fragment of 229 pb from the PI Z variant (protease Z inhibitor). This was conducted in every patient with alpha-1-antitrypsin values < 1.8 mg/dL (Cut point: 1.8 mg/dL). In addition, a spirometry and a high-resolution computed tomography (HRCT) of the chest were requested aiming to determine spirometric and CT patterns: emphysema and bronchiectasis.

Analyzed variables:

Quantitative: Age (years), FEV_1/FVC (%) before and after bronchodilation, FEV_1 (L and %) before and after bronchodilation, FVC (%), alpha-1-antitrypsin blood levels (mg/dL), age at symptom onset (years), No. of packets/year.

Qualitative: Gender (females or males), smoking habit (yes, no, former smoker), spirometric pattern according to ATS/ERS[23] guidelines, high-resolution computed tomography of the chest (predominant pattern), genotypes (S and Z). COPD and the severity of airflow limitation were defined according to the 2015 GOLD criteria[11].

Data collection, processing and analysis: Data were collected from the medical history of the patients who were enrolled (secondary data source); the information was documented in a datasheet created using an Excel® spreadsheet, then it was analyzed using UNC's statistical software InfoStat®. Data were analyzed using categorical methods, as well as their results, frequencies/percentages; and measurable variables were studied with central statistical (mean, median) and statistical dispersion methods (standard deviation, coefficient of variation, maximum and minimum values, median), and they were presented in graphs and/or tables, as appropriate, for a better interpretation.

Ethical considerations: Data collection was confidential. The Bioethics and Qualification / Teaching Committee from Hospital Tránsito Cáceres de Allende and the Health Research Ethics Committee provided their authorization under Provincial Registration for Health Research N° 29/2013. All the patients signed an Informed Consent Form.

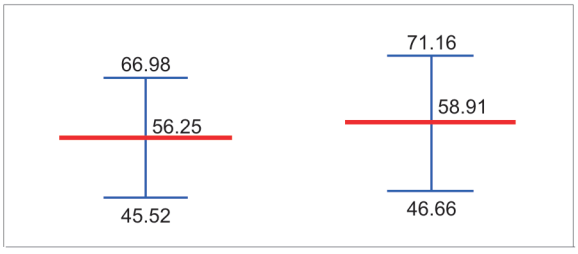
Results

Out of a total of 320 patients who attended the Pneumonology consulting rooms at the HTCA, generating 3150 consultations within the study period, 80 patients met the criteria to be included in this study and only 62 (19.4%) agreed to participate in the study.

Out of the 62 patients from the sample, 28 (45.2%) were females and 34 (54.8%) were males, 37 (59.7%) had alpha-1-antitrypsin levels ≥ 1.8 mg/dL and 25 (40.3%) < 1.8 mg/dL. The overall mean age was 57.7 ± 11.57 years, with a minimum and a maximum value of 18 and 85 years; values arranged by gender are shown in figure 1.

The number of AATD cases, as mentioned above, was 25 (40.3%) using the dried-droplet method with values < 1.8 mg/dL, the characteristics of their genotypes are shown in table 1.

Out of these patients, with a concentration < 1.8 mg/dL, 60% (n = 15) were females and 40% (n = 10) were males. Mean age ranged between 56.1 ± 14.27 with a minimum and maximum value of 18 and 85 years; 7 (28%) of them were active smokers, 3 (12%) were non-smokers and 15 (60%) were former smokers (Figure 2). The average of packets/year (p/y) was 34.9 ± 28.76 with a minimum and maximum value of 0 and 100 p/y.



Sex	n	Alf	D.E.	Mín	Máx
Females	28	56.25	10.73	30.00	85.00
Males	34	58.91	12.25	18.00	80.00

Figure 1. sample distribution by gender and age, values are presented as average and standard deviation. (n = 62)

TABLE 1. Determination and genotype of AAT cases < 1.8 mg/dL (n = 25)

AAT N	AAT %	Genotype	n	%
25	40,3	Z heterozygote	2	8
		S heterozygote	1	4
		Non-S Non-Z	22	88

References: AAT: alpha-1-antitrypsin; n: number of cases; %: percentage of cases. Values are presented as frequencies and percentages.

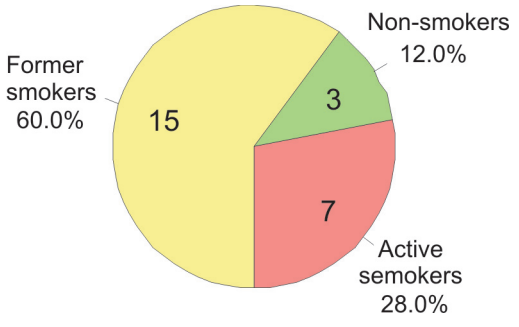


Figure 2. Distribution of patients with an alpha-1-antitrypsin value < 1.8 mg/dL by smoking habit, values are presented as frequencies and percentages. (n = 25)

The predominant spirometric pattern according to ATS/ERS criteria was the obstructive pattern (88%), of which 8 (32%) had a slight obstruction, 2 (8%) a moderate obstruction, 4 (16%) moderately severe, 2 (8%) severe and 6 (24%) highly severe (Figure 3). In only one patient (4%), the spirometry suggested restriction and it was within normal values in 2 (8%).

There were 13 patients with COPD (52%): 2 (15.4%) were GOLD 1; 7 (53.8%) were GOLD 2; 1 (7.7%) was GOLD 3; and 3 (23.1%) were GOLD 4. There were 2 (8%) patients with bronchiectasis; 1 (4%) with asthma; 1 (4%) had a family history of AATD and 8 (32%) had symptoms.

Mean age at symptom onset was 45.5 ± 18.57 years.

The HRCT pattern corresponded to emphysema in 22 patients (88%): 7 (31.8%) centrilobular, 8 (36.4%) paraseptal, 7 (31.8%) panlobular. There were 2 patients (8%) with bronchiectasis and 1 (4%) was normal (Figure 4).

The mean concentration of alpha-1-antitrypsin was 1.4 ± 0.27 mg/dL, with a minimum of 0.57 mg/dL and a maximum of 1.72 mg/dL.

Out of the cases with allele Z, one was a 30-year-old male with an alpha-1-antitrypsin value of 1.15 mg/dL; the reason for his inclusion was his family history of AATD, he was asymptomatic and spirometry values were within normal ranges, the chest HRCT evidenced subpleural bullae in both apices and in the anterior segment of the right lower lobe, measuring 11.7 mm. The other patient was a 61-year-old female with an alpha-1-antitrypsin value of 1.31 mg/dL; the reason for her inclusion was her history of COPD, she had symptoms since age 19, the spirometry showed a slight obstructive pattern (GOLD 1) and the HRCT evidenced a bilateral centrilobular emphysema in both upper lobes.

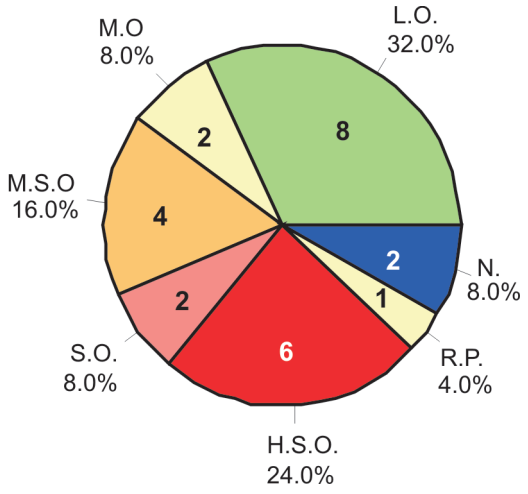


Figure 3. Distribution of patients with an alpha-1-antitrypsin value <1.8 mg/dL by spirometric pattern, values are presented as frequencies and percentages. References: SL.O.: slight obstruction, M.O.: moderate obstruction, M.S.O.: moderately severe obstruction, S.O.: severe obstruction, H.S.O.: highly severe obstruction, R.P.: restrictive pattern, N: normal. (n = 25)

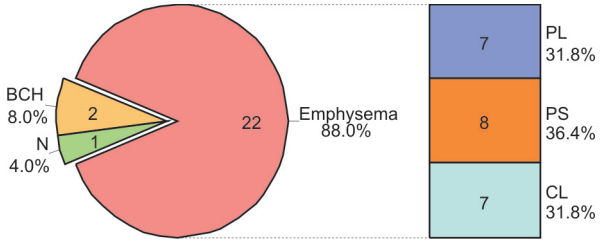


Figure 4. Distribution of patients with an alpha-1-antitrypsin value <1.8 mg/dL by HRCT pattern, values are presented as frequencies and percentages. References: Emphysema: emphysema, PL: panlobular, PS: paraseptal, CL: centrilobular, BCH: bronchiectasis, N: normal. (n = 25).

The only case involving an S allele was a 61-year-old female with an alpha-1-antitrypsin value of 0.57 mg/dL; the reason for her inclusion were respiratory symptoms, age at symptom onset was 30 years, the spirometry showed a slight obstructive pattern without spirometric criteria for COPD, HRCT evidenced a paraseptal emphysema in both apices.

It should be noted that the 3 were former smokers exposed to 20, 10 and 2 p/y, respectively.

Discussion

AATD is an underdiagnosed genetic disorder, mostly because doctors are not aware of its existence. The dried-droplet screening technique using blotting paper turned out to be a reliable, easy and accessible method to detect AATD, just as it was for other authors[5, 20, 24, 26, 27].

AAT is quantified and the presence or absence of S or Z alleles is analyzed, but this is not done with other deficient or normal alleles. Screening programs are very useful in Public Health since they enable to detect patients with AATD and promote health interventions, such as quitting tobacco use, investigating AATD in immediate relatives, providing genetic counseling and prescribing substitute therapy in selected cases[5].

In the study by Sorroche et al.[20] where they used AAT deficiency screening in dried blood samples on paper, out of a total of 1002 patients, AAT deficiency was detected in 217 patients; 15 (1.5%) were associated with a severe deficiency, 12 (1.2%) were ZZ and 3 (0.3%) SZ, 4 (0.4%) had an SS genotype, 29 (2.89%) were Z heterozygote and 25 (2.5%) S heterozygote, whereas 144 (14.37%) patients had non-S non-Z genotypes, out of whom only 7 (4.86%) had phenotyping studies performed, the rest did not due to operative reasons.

The estimated ratio of SZ and ZZ genotypes for the Argentine population was 1:2400 and 1:26,000, respectively[5]; the results are similar to other studies with a larger number of patients and in countries with a higher predominance, such as the studies by C. de la Roza et al. in Spain (n=86)[24] and Wencker et al. in Germany (n=1060), where no severe deficiency (PIZZ) was found[25].

In the study population (n=62), there were 2 cases of Z heterozygotes (3.22%) and 1 S heterozygote (1.61%), our findings differ from what is theoretically expected for the Argentine population, where the number of S heterozygotes is higher[5]. Among the studies where there was a higher number of Z than S, the ones that stand out are the study by C. de la Roza et al. from 2005, conducted in patients with COPD[24], the study by Wencker et al. from 2002, conducted in patients with COPD, asthma and bronchiectasis[25], and the study by Sorroche et al. from 2015, conducted in patients with COPD in Argentina[20].

The absence of false negatives enables to classify patients with values over the cut point (1.8 mg/dL) as not severely deficient, according to Sorroche et al[20].

AAT values of deficient alleles found in the PIZ genotype were within the expected parameters[3, 5].

It should be noted that the PIS genotype displayed an alpha-1-antitrypsin value below expected[3, 5], which shows a discrepancy and induces a quantitative determination of AAT, a phenotype description using IEF and a potential molecular gene analysis to establish null and rare genotypes.

It has been described that the PIMZ genotype has an increased susceptibility to develop emphysema in patients who are smokers, and we found 2 studies conducted in the general population that evidence that said genotype is associated with a rapid decline in FEV_1 compared to PIMM individuals[27, 28].

Available data on the risk of developing a lung disease in PIMS are controversial and inconsistent; therefore, most authors consider that this genotype does not imply a higher risk of developing a lung disease [6, 29].

With reference to the spirometry, one was normal (male PIZ) and two showed a slight obstructive patter (PIZ and PIS). Only the PIZ female patient met the criteria for COPD[11].

Mean expected age at diagnosis (45 ± 9.5 years) was lower in the male patient and higher in the female patients, the period between symptom onset and diagnosis was widely exceeded by female patients. There are publications that state men are diagnosed earlier than women[30, 31].

The chest HRCT evidenced centrilobular emphysema in the upper lobes (female PIZ) and paraseptal emphysema in the apices (female PIS). When comparing this to reviewed literature, we observed that it had neither the typical AATD distribution (lower lobes) nor the classic emphysema pattern (panlobular)[3, 4, 6], this could be associated to the fact that it was not a severe deficiency and that both patients were former smokers.

The HRCT of the male PIZ genotype showed bullae in both apices and in the right lower lobe; it must be taken into consideration that this was one of the youngest patients enrolled in the study (30 years old).

Most of the studies reviewed on HRCT patterns and pulmonary function are conducted based on severe AATD[3, 4, 6, 8, 10, 19, 27, 32]. Thus, there was no information available connecting these variables with alpha-1-antitrypsin levels below the cut point without severe AATD in our bibliographic search.

Senn et al. evidenced there is a complex interrelation between circulating AAT, tobacco exposure, gender and pulmonary function[33].

In this study, two Z heterozygote and one S heterozygote patient was detected, in addition to 22 with non-S non-Z genotype and values <1.8 mg/dL. There were no patients with severe deficiency; we acknowledge as limitations the reduced number of cases and the lack of availability of procedures in public sector hospitals for phenotype description using isoelectric focusing and sequencing of the SERPINA1 gene in whole blood to classify uncommon, new or null genotypes; in non-S non-Z cases and in those with discrepancies. We recommend extending the program at a provincial level with the participation of different health centers, both

public and private, aiming to increase detection, avoid underdiagnosis, improve prevention and promote health.

Conclusion

In a population selected by symptoms and/or history, patients with AATD can be identified using the dried-droplet method. Severe AATD is uncommon in Argentina, probably because it is underdiagnosed, and the amount of heterozygote PIS and PIZ carriers is higher.

A special characteristic of AATD in our patients with heterozygote genotypes is the distinct variability in its clinical, CT and spirometric presentation.

Early diagnosis of AATD is uncommon and there is a significant delay between the age at symptom onset and the age at diagnosis.

Early diagnosis of patients is more associated with a family history of AATD than to clinical suspicion in COPD or symptomatic patients.

Screening is a simple and efficient technique to determine AAT deficiencies.

According to the results, it is difficult to draw conclusions about the AATD group below 1.8 mg/dL without severe deficiencies in connection with the variables analyzed in the sample and also due to the lack of studies and bibliography on this subject. We consider that patients who have a non-S non-Z genotype and the ones who have discrepancies must be quantitatively confirmed and their phenotype defined in serum samples using isoelectric focusing and, occasionally, they must have a molecular gene analysis to look for uncommon, new or null allelic variants.

Conflict of interest: The authors declare that there is no conflict of interest associated with this publication.

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