Original Article

Research of Markers for the Genes of the Heavy Chain of Cardiac β-Myosin and Myosin Binding Protein C in Relatives of Patients with Hypertrophic Cardiomyopathy

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Objective

To study the molecular markers for the genes of the heavy chain of cardiac beta-myosin and the myosin binding protein C in relatives of carriers of hypertrophic cardiomyopathy.

Methods

Twelve families who had anamnesis, physical exam, electrocardiogram, echocardiogram and blood collection for the genetic study through the chain reaction of polymerase.

Results

From the 227 relatives, 25% were ill-taken, with 51% men, with an average age of 35±19 (2 to 95) years old. The genetic analysis showed a connection with the gene of the cardiac β-myosin in a family and, in another, a connection with the gene of the myosin-binding protein C. In five families, the connections with the two genes were excluded; in two, the connection with the gene of the myosin-binding protein C, but for the β-myosin gene the results were non-conclusive; in two families, the results were non-conclusive for both genes and in one the connection for the β-myosin gene was excluded. The results were non-conclusive for the gene of the myosin-binding protein C.

Conclusion

In our environment, other genes, different from those described in the literature, may prevail, or there are other genetic differences related to the origin of our population and/or environmental factors.

Key words

hypertrophic cardiomyopathy, mutation, β-myosin, gene of the myosin-binding protein C, genetics

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The hypertrophic cardiomyopathy is a primary cardiac disease, characterized by the hypertrophy of the left ventricle, without dilatation, in the absence of any other cardiac or systemic disease that may lead to the myocardial hypertrophy[1]. The estimate prevalence of the disease in the general population is about 0.2% (1:500) and 0.5% among the cardiopathy carriers[2]. The yearly mortality described in reference centers (selected patients) I from 3% to 4% in adults and 6% in children, which is significantly greater than in non-selected patients (0.5% to 1%)[3,4]. It is caused by mutations in codifier genes for proteins of the cardiac sarcomere and transmitted is a dominant autosomal way in 50% to 60% of the cases[5,6]. Since 1989, when a disease-related gene was demonstrated for the first time, approximately 270 mutation have been described, most of them of *missense* (the exchange of a simple aminoacid for another in the protein chain) kind, in ten genes that codify proteins of the cardiac sarcomer[7-10] with regulatory, structural and contractile functions. The mutant genes of the heavy chain of the cardiac β-myosin, of the myosin-binding protein C and of the troponin T represent more than 50% of the cases. The other genes are more rarely affected. Recently mutations were described in two genes that codify proteins that do not belong to the sarcomere, of non-frequent occurrence. Changes in the mitochondrial DNA have also been associated to the hypertrophic cardiomyopathy[7].

Sudden death can happen at any age, although it is more common between 12 and 35 years old, being the hypertrophic cardiomyopathy regarded as the most frequent cause of such event in young athletes. Patients with family history of sudden death are considered as greater risk and some mutations have been associated to a greater incidence of that outcome[1,11-14].

The identification of the mutations responsible for the hypertrophic cardiomyopathy is an important diagnostic resource, currently defined by the echocardiogram, which shows the myocardial hypertrophy (phenotype). However, in many cases, that cannot be evident until puberty. The genetic diagnostic is possible at any time, regardless of the symptoms or the presence of myocardial hypertrophy, being useful in the differentiation between hypertrophic cardiomyopathy and left ventricular hypertrophic secondary to the hypertension, as well as the hypertrophy of the athletes, which can provide prognostic information related to the evolution and the sudden death.

So, we have investigated molecular markers for the genes of the heavy chain of the cardiac β-myosin and the myosin-binding protein C (the most frequently changed) in relatives of patients

with hypertrophic cardiomyopathy and we assessed the incidence of assailed individuals (phenotype) in the families and their relation with the demographic, clinical, and laboratorial variables.

Methods

From the 310 patients carriers of hypertrophic cardiomyopathy under medical care follow-up, 12 families were consecutively selected with relatives who were carriers of the disease or that reported sudden death in young individuals (age < 40 years old). The diagnosis of hypertrophic cardiomyopathy was based on the echocardiographic findings of hypertrophy without dilatation of the left ventricle, with ventricular wall inspissation >15 mm among the patients and >13 mm among the relatives, in the absence of other cause of ventricular hypertrophy[13].

All components of the families were assessed in ascending, descending and horizontal, under the phenotype point of view, between January 1999 and July 2001. After a detailed explanation on the procedures necessary to the study, the participants agreed and signed the free and clear consent term (according to resolution by the National Health Council 196, of 10/10/96).

The relatives were called, the heredogram was elaborated and each individual was submitted to an anamnesis, physical exam, rest electrocardiogram, Doppler echocardiogram and blood collection for the genetic study.

The anamnesis and the physical exam were made with emphasis on the symptoms and signs; dyspnea, palpitations, thoracic pain, syncope, heart rate, blood pressure and presence of systolic murmur in the lower sternal rim and in the mitral area. Due to the intensity of the symptoms, the patients were classified according to the criteria of the *New York Heart Association* (NYHA).

The rest electrocardiogram was done in the 12 classic derivations, with an emphasis in the following variables: presence of atrial fibrillation, overload in the left atrium, and left ventricular overload, when ≥5 points by the criteria of modified Romhilt-Estes[15,16].

The Doppler echocardiogram was done for the assessment of the structure and function of the cardiac chambers (M, unidimensional, bidimensional modes, spectral analysis of cardiac flow with the pulsatile and continuous Doppler), in accordance to the recommendations of the American Echocardiography Society[17,18]. The echocardiographic variables studied were: thickness of the interventricular septum and the posterior wall of the left ventricle, maximum diameter of the left atrium, diastolic and systolic diameters of the left ventricle, fraction of ejection of the left ventricle through the cube formula, and maximum systolic gradient in the outlet way of the left ventricle. The previously established normality criteria were used for the measurements of the ventricular dimensions[19]. The ejection fraction was considered as normal for values > 0,60. The gradient at the outlet way of the left ventricle was considered as significant when >30 mmHg.

For the genetic study, the technique of the polymerase chain reaction (PCR) for the amplification of the sequences of the genes of the heavy chain of the cardiac β-myosin (chromosome 14) and the myosin-binding protein C (chromosome 11)[20] was used. Two pairs of *primers* were used for the first gene and 4 for the second. After the end of the reaction, the PCR samples were analyzed through electrophoresis in gel of polyacrylamide[21, 22].

The data are described using mean, standard deviation (SD) and proportions. The chi-square association test was used to compare the distribution in relation to the sex, among the ill-taken and non-ill-taken. In the comparison between the average thickness of septum and age among the ill-taken patients, with or without symptoms, the Mann-Whitney test was used.

Results

Two hundred and twenty-seven individuals belonging to 12 families (average age of 26±19 years old, variation: 1 to 95 years olds) were assessed, with 117 (51.5%) men and 110 (48.5%) women. The number of ill-taken individuals was 57 (25%), being 29 (51%) men and 28 (49%) women (average age of 35± 19 years old; variation: 2 to 95 years old). From the total of ill-taken individuals, only six were not assessed (deceased), but they were reported because, according to their relatives' information and supplementary exams provided, their ill-taking was confirmed.

Regarding the sex there was no significant difference when we compare the ill-taken and non-ill-taken individuals, as 48% were women in both groups (p=0.90). No significant difference was observed between the average thickness of the interventricular septum in the ill-taken individuals, with or without symptoms (p=0.57). There was not also any important variability in the average thickness of the septum among the families, and the same happened in relation to the age of the beginning of the symptoms.

Family 1 – Three generations with 22 individuals were studied, with 19 being assessed (fig. 1). Three were not assessed because they had died. Reports from relatives concerning the presence of the disease that caused the death were reliable. The relatives III.1 and III.10 were, respectively, 16 and 21 years old, and died after physical exertion (laying football) and the relative III.9 died at 19 during her sleep. Along the study, there were another two deaths, in a total of 5 (23%). From those, 54% (12/22) showed phenotype of the disease. A genetic study of the ill-taken individuals was performed, which evidenced the connection with the gene of the heavy chain of the β-myosin.

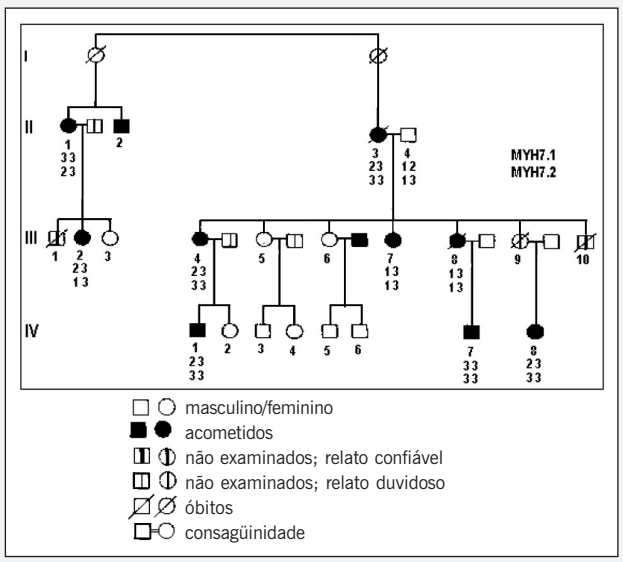

Fig. 1 - Heredogram showing the ill-taken individuals and the markers used for the gene of the heavy chain of the β-myosin.

Family 2 – Consisting of 3 generations with 9 individuals assessed (fig. 2). A genetic study was carried out in all individuals of the 2nd generation and the I.2. The individual I.1 was not counted for not being assessed. From those, 22% (2/9) had the phenotype of the disease, without occurrence of death. The genetic analysis showed a connection with the myosin-binding protein C in the chromosome 11.

Family 3 – It is an example of a non-conclusive result. Consisting of 3 generations with 13 individuals, 12 of them assessed; one deceased, but there was a reliable report he was a carrier of the disease, which had evolved to the dilated form, and died while he waited for heart transplant. Another relative, include in the study, died suddenly in her sleep. A patient had been submitted to a myectomy and an implant of definite pacemaker due to total atrioventricular blocking and currently is in CF IV (NYHA), waiting for heart transplant, and another was submitted to a therapeutic implant of a pacemaker to decrease the gradient in the outlet way of the left ventricle. The percentage of individuals with the phenotype was 46% (6/13) and the dead was 15% (2/13). The genetic analysis did not show any connection with the gene of the myosin-binding protein C and, for the gene of the β-myosin, the result was non-conclusive.

Family 12 – It is an example in which the genetic study did not show any connection with any of the two genes. Four generations were studied, with 48 individuals assessed. A relative was not counted as he had died and there was no reliable report on him. Thirteen were clinically assessed, with electrocardiogram and blood collection used for the genetic study; the echocardiograms were not performed as they lived in cities in the Northeast, which made impossible the realization *in loco* of such exams. The percentage of individuals with the phenotype was 12.5% (6/48) and no deaths were recorded.

The results from the genetic study are on the chart I and the demographic, clinical and laboratorial variables of the ill-taken individuals are on table I.

Chart I - Results of the generic study

Gene/Family	β-myosin	Binding PTC
1	Connection	-
2	-	Connection
3	Non-conclusive	Excluded
4	Excluded	Excluded
5	Excluded	Excluded
6	Non-conclusive	Non-conclusive
7	Non-conclusive	Excluded
8	Non-conclusive	Non-conclusive
9	Excluded	Excluded
10	Excluded	Non-conclusive
11	Excluded	Excluded
12	Excluded	Excluded

Discussion

Since the confirmation of the genetic etiology of the hypertrophic cardiomyopathy, in 1989 [20], many genes and a great number of mutations have been described as responsible for the disease and the interest for the study of the families has been growing. In the literature, the mutation has been related to the sudden death (malignity), the most feared manifestation, with a not totally clear mechanism, generally occurring in young patients.

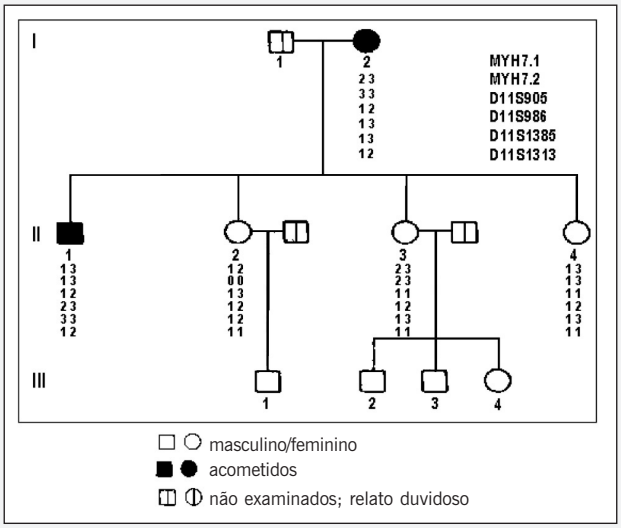

Fig. 2 - Heredogram showing the ill-taken individuals and the markers used for the gene of the myosin-binding protein C.

From the 12 families studied with markers for the genes described, the family 1 had a connection with the gene of the β-myosin, and the family 2, with the gene of the myosin-binding protein C. The two genes were excluded in the families 4, 5, 9, 11 and 12, and in the families 3 and 7, the connection with the gene of the protein C was excluded; the study was non-conclusive for the gene of the β-myosin. In the family 10, the connection with the gene of the β-myosin was excluded; the study was non-conclusive for the gene of the protein C. In the families 6 and 8, the markers were non-conclusive for both genes.

Our findings may seem different from those in the literature, in which the changes in the gene of the β-myosin take place in 35% to 50% of all cases HCM and the gene of the myosin-binding protein C comes in second, occurring in 20% to 25%[9]. We can speculate that, in Brazil, whose population results from the miscegenation of people from many continents (Western Europe, Africa, and natives) and that had many influences in the environment, there could be other mutations with different phenotypes from those already described in North America, Europe and Japan and, for that reason, the mortality in our environment would be lower than the described [2,23, 24].

On the other hand, if we think that the genetic etiology of the hypertrophic cardiomyopathy takes place in 50% to 60% of the cases, that other 40% to 50% do not have defined etiology[5,6] yet, and that the results were non-conclusive (which means the possibility of a connection with one of the two) in five families, our data are close to those in the literature.

Family 1 showed a connection with the gene of the β-myosin, 54% of ill-taken and 23% of deaths. There will possibly be, in the sequencing of the gene, a malignant mutation, maybe already described, as Arg403Gln (in which arginine was exchanged for glycine at the position 403 in the chain of the protein)[14,25], Arg719Trp[26] or Arg453Cys[14,25] whose studies show a high penetrance, early beginning of the symptoms, high mortality in young individuals and important changes at the electrocardiogram and echocardiogram. Marian and Roberts[25] studied 14 patients with hypertrophic cardiomyopathy and Arg403Gln mutation, who had an average septal thickness of 18 mm, which was similar to the average found in this family (17 mm). In the sequencing of the gene we may find a new mutation, yet not described, but with the same

Table I - Average values, standard deviation, and proportions of continuous and descriptive variables of the ill-taken patients in each family

Family No.	Age (y.o.) M/SD	Sex M n	Sex M %	Sex F n	Sex F %	Symptoms n	Symptoms %	Murmur n	Murmur %	LVO N	LVO %	Septum M/SD	Ill-taken n	Ill-taken %	Deaths n	Deaths %
1	23/14	4	33	8	67	7	78	4	44	4	44	17/4	12	54	5	23
2	41/16	1	50	1	50	0	0	1	50	1	50	19/3	2	22	0	0
3	39/17	5	83	1	17	2	40	3	60	1	20	17/8	6	46	2	15
4	30/14	2	33	4	67	4	67	6	100	4	67	17/4	6	26	0	0
5	33/16	3	100	0	0	2	67	1	33	1	33	19/3	3	11	0	0
6	23/5	1	33	2	67	2	100	1	50	1	50	18/5	3	33	2	22
7	23/14	2	67	1	33	3	100	2	67	2	67	20/4	3	43	0	0
8	41/27	3	75	1	25	1	33	2	67	1	33	20/4	4	80	1	20
9	43/18	2	40	3	60	3	60	3	60	1	20	16/4	5	17	0	0
10	47/13	1	25	3	75	4	100	3	75	0	0	17/3	4	19	0	0
11	40/14	2	67	1	33	3	100	1	33	0	0	15/3	3	21	0	0
12	53/18	3	50	3	50	5	83	3	50	5	83	23/4	6	12.5	0	0
Total	35/19	29	51	28	49	36	63	30	53	21	37	18±5	57	25	10	17.5

F- female; M- male; M/DP- mean/standard deviation; n– number of patients; LVO– left ventricle overload

characteristics of malignity. Another important characteristic, which compromises the prognostic of such family even more, is the existence of two ill-taken children (two years old, septum = 21 mm and obstructive shape, and another 11-year-old child). The literature shows worse prognostic in children and teenagers, with a higher mortality rate that in the adults[3].

Family 2 showed connection with the gene of the protein C, 22% of ill-taken and no deaths, which seemed to be a benign mutation. However, the fundamental characteristic of the mutations in that gene is de reduced penetrance up to the 5th decade of life, late beginning of the symptoms, lower ventricular hypertrophy and favorable prognostic up to 40 years of age[27-31]. In that family we noted an average age of the ill-taken of 41 years old, all symptomless, 50% with left ventricular overload at the electrocardiogram, which coincides with the literature. The average of the septal thickness (19 mm), above of what the literature shows (13 mm), can be explained by the following fact: in the literature, the carriers of the mutation without hypertrophy (genotype without phenotype) are reported and in our series, we considered as ill-taken those with the phenotype. Studies show that the mutations in the gene of the myosin-binding protein C are usually more benign than those of the β-myosin[28,29]. However, that family must be followed, as it is known that the apparently normal individual to the echocardiogram can develop late hypertrophy, sometimes with mutations as malignant as those of the β-myosin[29].

Family 3 had a high percentage of ill-taken individuals (46%). In the 2nd generation there was an onset of 100% and global mortality of 15%. The connection with the gene of the protein C was excluded, but for the β-myosin the study was non-conclusive (which could be connected to that gene), possibly because the family had few components. However, we noticed that the characteristics are malignant, whatever the mutation is, as an individual had sudden death and two evolved with the dilated form of the disease. Besides, before joining our study, two ill-taken individuals had already been submitted to a surgical treatment, as they were refractory to the clinical treatment. In the literature, the Gly716Arg and Arg403Leu mutations in the heavy chain of the β-myosin have high penetrance, develop early hypertrophy and premature sudden death, besides evolving to cardiac insufficiency in adulthood[32,33]. The genes of α-tropomyosin[34,35], of actine[36,37] and troponin T[38] can also have mutations that lead to the ventricular dilatation, cardiac insufficiency and high incidence of sudden death.

In family 4, with 26% of ill-taken individual, booth genes were excluded. Despite the absence of disease-related deaths, there are two cases with the obstructive form, one with the dilated form, two in CF III/IV and a child (8 years old), showing that the mutation cannot be regarded as benign. The same did not happen in the family 5, in which the two genes were also excluded, but that showed a low percentage of ill-taken individuals (11%), all little symptomatic, without occurrence of deaths.

In the families 6 and 8 the results were non-conclusive for both genes, probably because they had few components, which made the markers little informative. Family 6 showed some malignant characteristics, as an ill-taken relative had sudden death; the other two were little symptomatic at the time of the study and one of them evolved quickly to the dilated form, and died two years later. The average age of those patients, when they died, was 23 years old, different from the family 8, in which the percentage of ill-taken was high (80%), with the death of a 70-year-old patient with the dilated form of the disease. The other relatives are little symptomatic, which suggests a benign mutation.

The study in the family 7 was non-conclusive for the β-myosin, and the connection with the gene of the protein C was excluded. The percentage of ill-taken was high (43%), there were no deaths and all the ill-taken individual were symptomatic, suggesting a mutation with not so malignant characteristics, despite having an ill-taken 7-year-old child, which could indicate a worse prognostic.

In families 9 and 11, the two genes were excluded, with a moderate percentage of ill-taken individuals, without any occurrence of sudden death, suggesting benign mutations.

Family 10 was the only one that had exclusion of the gene of the β-myosin, with a non-conclusive genetic analysis for the gene of the protein C, but with some indicating characteristics of that gene: the average age of the ill-taken (47 years old), all symptomatic, normal electrocardiogram and no occurrence of deaths [27-31].

In the family 12, with 12% of ill-taken individuals, there were no deaths and in it we recorded the oldest ill-taken in the study (95 years old). It was observed that three (50%) from the ill-taken had syncope, a non-frequent symptom in the other families. The presence of syncope indicates a worse prognostic[11,12,39], but it is very little reported in genetic studies[7].

The statistic analysis of the clinical variables (sex, symptoms, hypertrophy level and age at the beginning of the symptoms) did not evidence differences that could help us to recognize the individuals with potential of malignity and worse prognostic. There was a great variation concerning the presence of cardiac murmur in each family. The obstructive form was present in eight (14%) of the ill-taken individuals, which was less than that was observed in the literature (25% in selected patients)[1,9]. The dilated form with systolic dysfunction was observed in 8% of the ill-taken, a value close to the one described in the literature for selected patients (10%)[40].

During that study we noted that the heredogram makes evident not only the percentage of ill-taken individuals, but also those who died suddenly, which gives us the idea if the family had a tendency to the malignant form of the disease. So, for us, it was very valuable in the guidance of the treatment and prognostic, not only among the ill-taken, but also those who does not have the phenotype. For example, in the family 1, the percentage of ill-taken individuals (54%) and the mortality rate of the disease (23%) were high (tab. I), different from family 2, in which the heredogram (fig. 2), gave us the impression of benignity of the disease, as the penetrance was low, all ill-taken individuals were symptomless and there were no deaths.

So, the construction of the heredogram is a practical and simple way to know what is happening to the penetrance (number of ill-taken) and to the lethality of a certain gene (unknown), by helping in the clinical behavior of the patient and the familial counseling.

This is the first study performed in Brazil, with an emphasis on the genetic changes of the familial forms of the hypertrophic cardiomyopathy. There are few centers in the world that perform that kind of study and the reasons of its occurrence. Firstly, the hypertrophic cardiomyopathy is a complex disease, with many ill-taken genes, incomplete penetrance (carriers of the mutation that do not develop hypertrophy), age-dependant, heterogeneity, and great clinical variability of the genetic expression, which makes that every family be studied individually, making the connection study complex. Besides, we have been working with genes that codify big sarcomeric proteins, with difficult sequencing and of high cost, which requires qualified personnel.

The ideal would be finding the mutation in all families and related them to their malignity. However, due to the aspects described, unfortunately the genetic study of the families with hypertrophic cardiomyopathy will not become a routine exam, at least in middle term. The routine use of that practice would surely help in the diagnosis from the birth and in the differentiation with the hypertrophies of the athletes and the hypertensive people.

Our initial results show that there are no significant differences related to the variables studied, whether they are demographic, clinical or laboratorial, which lead us to think that the genetic factor is really important, but that possibly other genetic or environmental factors are also related. The modifying genes were described to explain the variability of the phenotypic expression, as they alone could not cause the disease, but they would affect its seriousness. It is possible that the polymorphism of the converter enzyme of the angiotensin-1, variants of the endothelin-1 and alpha factor of tumorous necrosis are also modulators of the phenotype of the hypertrophic cardiomyopathy[41, 42].

Small families, small number of families with identical mutations, variability of the phenotypic expression among the ill-taken individuals from the same family or among different families with the same mutation, low frequency of each mutation and the influence of modifying genes, as well as non-genetic factors in the phenotype, are described in the literature as limiting factors in the studies of genotype-phenotype correlation. Regardless of those limitations, everyone agrees that the mutations affect the phenotypic expression of the hypertrophic cardiomyopathy, especially the cardiac hypertrophy and the risk of sudden death. The mutations in the gene of the β-myosin are generally associated to a greater level of hypertrophy in young patients and a greater risk of sudden death, when compared with mutations in the genes of the myosin-binding protein C and of the α-tropomyosin. However, for each gene benign and malignant mutations are described, each with particular characteristics, which makes difficult the generalization of those findings for the individuals or for the whole family.

The mortality rate of selected patients[24] is similar to the casuistic ones of non-selected patients [43] and lower than the one described in reference centers[3]. Maybe what can be explained in our environment, due to the prevalence of other genes that are not those described in the literature, as being the most frequent ones, or that there may be other genetic or environmental differences interfering in the phenotype of our population.

However, it is very early to talk about the prevalence of other genes in our environment, having in mind that this is an initial work and it must be extended, with the involvement of other families, use of markers for other genes, sequencing of genes found to find the mutations, and the search for modifying factors that can explain the differences in our population, if they really exist. Besides, it is very important the realization of researches in other centers, in order to make possible to assess the genetic prevalence in many regions in the country, and to make us arrive to definite conclusions.

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