ORIGINAL ARTICLE

Application of a clinical and laboratory protocol for the investigation of inborn errors of metabolism among critically ill children

Maria T.V. Sanseverino[1], Moacir Wajner[1,2], Roberto Giugliani[1,3]

Abstract

Objective: the aim of this work was to evaluate a protocol for investigation of Inborn Errors of Metabolism (IEM) in children who are acutely ill.

Methods: forty six children with clinical suspicion of a metabolic disorder were studied during 2 years. They were selected through request for investigation of IEM from Pediatrics or Neonatal Intensive Care Units located in the metropolitan area of Porto Alegre. Criteria for inclusion were presence of one or more of the following clinical alterations, without defined etiology: Metabolic acidosis, electrolyte disturbances, hypoglycemia, seizures, lethargy, liver disfunction, family history suggestive of IEM. The protocol included clinical evaluation, compulsory tests (performed in all patients) and optional tests (performed selectively according to the results from the first tests or through specific clinical hypothesis).

Results: six cases of IEM were identified: galactosemia, non-ketotic hyperglycinaemia, propionic acidemia, isovaleric acidemia, 3-hydroxy-3-methylglutaric acidemia and deficiency of 3-ketothiolase deficiency.

Conclusions: the frequency of organic acidurias in this group was 4/46 (8.7%), which justifies the inclusion of organic acids analysis among the first line exams in acutely and severely ill children with undefined etiology. The relatively high frequency of IEM (6/46 or 13%), which is comparable to the ones observed in other studies within high risk groups, indicates that the protocol suggested is efficient and justifies the systematic investigation of IEM in not explained critically ill children.

J Pediatr (Rio J) 2000; 76(5): 375-82. inborn errors of metabolism, organic acidurias, aminoacidopathies, hyperam-monemia, galactosemia.

Introduction

Inborn errors of metabolism are monogenic disturbs, usually with a recessive autosomal heritage pattern. Therefore, they present a possibility of 25% of reoccurrence in the affected patient's brothers/sisters. Some inborn errors of metabolism present a dominant autosomal heritage pattern, such as familial hypercholesterolemia; other inborn errors of metabolism present a heritage pattern linked to the chromosome X, as the Fabry disease, and the transcarbamylase ornithine deficiency, for instance.[1]

Inborn errors of metabolism form a heterogeneous group of more than 500 pathologies originated from an alteration in the genetic material that determines deficiency or absence of a protein, generally an enzyme, and loss of its metabolic function.[2]

1. Medical Genetics Service, Hospital de Clínicas de Porto Alegre (HCPA).
2. Medical Genetics Service, HCPA, and Department of Biochemistry, Universidade Federal do Rio Grande do Sul (UFRGS).
3. Medical Genetics Service, Hospital de Clínicas de Porto Alegre (HCPA).

Several inborn errors of metabolism have an early onset, frequently in the neonatal period. The main groups of inborn errors of metabolism with acute and severe presentation in infancy include: defects in the metabolism of carbohydrates (classic galactosemia), defects in the urea cycle (deficiency of transcarbamylase ornithine), organic aciduria (propionic aciduria), aminoacidopathies (nonketotic hyperglycinemia), defects in the metabolism of fatty acids (deficiency of medium-chain acyl-CoA dehydrogenase).[3]

Recent advances in the diagnosis and treatment of inborn errors of metabolism have significantly improved the prognostic for many of these diseases. Thus, it is essential that the pediatrician is familiar with the clinical presentation of these disorders, with a more adequate laboratory investigation, with the best emergency handling for the stabilization of critically ill patients, and with the identification of those children who may benefit from specific evaluation and treatment.[4,5]

Although individually rare, the conjunct frequency of inborn errors of metabolism in preselected high-risk groups may be up to 200 times higher than that identified in the general population.[6] For example, in a study by Arens et al. (1993), performed at the Children's Hospital Los Angeles with 166 children who were referred to metabolic investigation for apnea, seven cases of inborn errors of metabolism were found (4.2%).[7] We estimate that an experienced laboratory that counts on the collaboration of a specialized pediatrician obtains the diagnosis of inborn errors of metabolism in approximately 6% of the referred cases.[8] In a previous survey by our reference laboratory for the diagnosis of inborn errors of metabolism, 10,000 selected patients were investigated from the clinical suspicion, and 647 cases of inborn errors of metabolism were diagnosed (6.5%).[9]

Table 1 presents clinical manifestations that suggest inborn errors of metabolism when there is no other etiology defined.[10]

Diverse factors contribute to make the diagnosis of inborn errors of metabolism difficult, such as the large number of disturbs, the diversity of involved defects, and the absence of specific signs and symptoms in most of the cases.[10]

The necessary techniques for the diagnosis of inborn errors of metabolism range from simple urine and plasma metabolic tests to enzymatic assays and DNA analysis in leukocytes, cultivated fibroblasts, and other tissues. Except for the simple tests, the remaining part of the investigation is usually concentrated in reference laboratories. The most efficient way to systemize the investigation of inborn errors of metabolism is to direct it according to the clinical presentation - acute or chronic.[11]

Thus, considering the technical difficulties and the involved costs, an adequate managing of the laboratory investigation starting from the main clinical and laboratory

Table 1 - Characteristics that suggest the presence of inborn errors of metabolism in the absence of a clear etiology (Giugliani, 1988)

1. Newborn presenting coma, hypotonia, irritability, convulsions, acidosis, hydroelectrolytic disturbance, sepsis, hypoglycemia, jaundice, vomits, or diarrhea.
2. Delayed neuromotor development and/or mental deficiency.
3. Neurological regression with loss of previously acquired abilities.
4. Hepatomegaly and/or splenomegaly, cholestatic jaundice, or chronic diarrhea.
5. Growth deficiency and/or osteo-articular alterations.
6. Recurrent episodes of hypoglycemia, metabolic acidosis, hydroelectrolytic unbalance.
7. Report of early death of brother/sister without a defined diagnosis.
8. Consanguinity between the parents.

findings becomes important. Aiming at systemizing the investigation for inborn errors of metabolism in acute and severe conditions during the neonatal period and in infancy, and at optimizing the diagnoses, a protocol for the investigation of inborn errors of metabolism diagnosis in critically ill children was elaborated and applied.

Methods

Studied sample

The sample was selected from cases referred to metabolic investigation at the Laboratório Regional de Erros Inatos do Metabolismo (Inborn Errors of Metabolism Regional Laboratory), from the Medical Genetics Service, Hospital de Clínicas de Porto Alegre, coming from the Neonatal or Pediatric Intensive Care Units in the metropolitan region in Porto Alegre, RS, Brazil. Forty-six children who presented one or more of the following abnormalities without a defined etiology were studied: a) metabolic acidosis; b) hypoglycemia; c) hydroelectrolytic unbalance; d) seizures; e) alteration in the level of consciousness; f) acute hepatic alteration; g) familial history suggesting inborn errors of metabolism in a severe and unexplained status. In Table 2, the main reason for investigation is indicated. We must stress that many patients presented more than one reason.

Protocol

The elaboration of the protocol for the investigation of metabolic diseases applied in these patients was based on several previously published studies regarding the investigation of inborn errors of metabolism in critically ill

Table 2 - Distribution of the sample as for the main clinical or laboratory alteration that motivated the investigation

Reason for reference	Number of patients	%
Seizures	15	32.6
Metabolic acidosis	8	17.4
Alteration in the consciousness level	7	15.2
Hypoglycemia	6	13.0
Acute hepatic alteration	6	13.0
Familial history suggestive of inborn error of metabolism 8	3	6.5
Serious hydroelectrolytic unbalance	1	2.2
Total	46	100

Note: several patients presented more than one of the signs exposed above. In these cases, the most important clinical or laboratory manifestation was considered.

children[3,12-20] and adapted to the availability of resources and reference facilities of the laboratory where the study was carried out. The investigation protocol applied is explained in Table 3.

Material collection

We collected 3 ml of blood from every patient in a heparinized syringe (for ammonia dosage and amino acid chromatography), and 2 ml of blood immediately precipitated in 4 ml of frozen perchloric acid (for lactate dosage), transported to the laboratory on ice, for centrifugation. About 30 ml of occasional urine was also collected from each patient. According to the laboratory alterations detected, new samples of blood, urine, or skin were collected to complete the investigation.

Results

Of the 46 patients investigated, 28 (60.9%) were male and 18 (39.1%) were female. As for the age, most of the patients were in their 1st year of life (37 patients, 80.5%), and 13 were newborns (28.3%).

Acute neurological alterations motivated the metabolic investigation in 22 patients (47.8%), and they were found in all patients who were definitively diagnosed with inborn errors of metabolism (Table 2).

In six of the 46 investigated patients (13%), an inborn error of metabolism was identified. The most frequent group of inborn errors of metabolism in our sample was that of organic aciduria, corresponding to 4 to 6 cases of inborn errors of metabolism detected (Table 4).

Table 3 - Protocol applied for the detection of inborn errors of metabolism in critically ill children

Clinical information: records related to age, evaluation reason, clinical status, familial history, nutritional aspects, drugs used, physical examination.

1 Mandatory tests: performed in every patient.
  1.1 Triage tests for inborn errors of metabolism in urine:
    a) Benedict's reaction (reducing sugars)
    b) Ferric chloride reaction (phenylalanine and tyrosine metabolites)
    c) Dinitrophenylhydrazine reaction (ketoacids)
    d) Nitrosonaphtol reaction (tyrosine metabolites)
    e) Cyanide-nitroprussiate reaction (sulfhydryl groups)
    f) Para-nitroaniline reaction (methylmalonic acid)
  1.2 Determination of creatinine in urine
  1.3 Chromatography of amino acids in blood and urine
  1.4 Dosage of lactate and pyruvate in blood
  1.5 Dosage of ammonia in plasma

2. Optional tests: performed according to a specific clinical suspicion or alterations in the mandatory tests.
  2.1 Chromatography in saccharide tenuous layers in urine
  2.2 Colorimetric dosage of orotic acid in urine
  2.3 Quantitative analysis of amino acids in plasma and urine
  2.4 Gas chromatography for the survey on organic acids in urine
  2.5 17-OH-progesterone dosage

3. Other tests: enzymatic dosage or molecular analysis, for example, based on specific abnormalities detected in previous procedures.

Table 4 - Innate errors of metabolism diagnosed in the studied sample

Propionic acidemia
Isovaleric aciduria
3-hydroxy-3-methylglutaric aciduria
3-ketothiolase deficiency
Classic galactosemia
Nonketotic hyperglycinemia

In 23 cases (50%), we established the diagnosis of a nonmetabolic pathology, which justified the clinical abnormalities that motivated the investigation of inborn errors of metabolism. The most frequent diagnosis was congenital or acquired infection (13 cases).

In 17 cases (36.9%), it was not possible to clearly establish the etiology of the disturbs that led to the investigation of a metabolic defect; in at least 12 cases (26.8%), the suspicion of a subjacent inborn error of metabolism that was not identifiable by the employed techniques remained.

The main clinical and laboratory findings for the six cases in which inborn errors of metabolism were diagnosed are now briefly summarized:

Case 9: Male, white patient, referred to metabolic investigation on the 12th day of life for presenting convulsive crises and coma. He was the fifth child of consanguine parents, first cousins, who had two other children who died during the neonatal period, with a similar clinical status. Forty-eight (48) hours after birth he was admitted to the Neonatal Intensive Care Unit presenting respiratory difficulty, incoercible vomits. On the 12th day, he started presenting convulsive crises, resulting in respiratory arrest and coma, with the need for mechanical ventilation. He presented persistent severe metabolic acidosis. The application of the protocol showed hyperammonemia (12.1 mcg/ml - normal up to 2), as well as increase of glycine and proline in the amino acid chromatography of blood and urine. The survey for organic acids in the urine evidenced elevated levels of hydroxy-propionic acid, propionylglycine, and methylcitrate. The patient died on the 14th day of life. Conclusion: propionic acidemia.

Case 19: Patient referred to metabolic investigation on the 2nd month of life presenting sepsis, cataract, hepatosplenomegaly, and jaundice. With the application of the protocol, we obtained positive Benedict's reaction and the presence of galactose in the chromatography of saccharides in urine. The dosages of ammonia and lactate were slightly elevated. The deficiency of galactose-1-phosphate-uridylyltransferase in erythrocytes was confirmed; the parents presented levels of enzymatic activity compatible with those of heterozygotes. The patient was assisted at the Pediatric Gastroenterology Service, at Hospital de Clínicas de Porto Alegre, presenting severe neuropsychomotor retardation and blindness; the family had a low socioeconomic level, which made the treatment difficult. Conclusion: classical galactosemia.

Case 20: The patient is the second child of a nonconsanguineous couple, referred to investigation of inborn errors of metabolism at the age of 2 due to episodes of metabolic acidosis and alteration of the sensorium in the presence of infection, without any correlation with the clinical status. In the mandatory examinations of the protocol, we obtained a doubtful reaction for para-nitroanilines, normal at repetition, and the presence of glycine in the chromatography of amino acids in the first urine sample (during the crisis). The survey on organic acids in urine showed the presence of elevated levels of 3-hydroxy-butyrate, acetoacetate, 3-hydroxy-3-methyl-butyrate, tiglylglycine and 3-hydroxy-isovalerate, compatible with the deficiency of the mitochondrial enzyme 3-acetoacetyl-CoA thiolase (3-cetotiolase). The activity of the enzyme in fibroblasts was absent, confirming the diagnosis. The parents presented levels of enzymatic activity that were compatible to those of heterozygotes. The molecular study revealed the presence of a specific mutation.[21] The patient is being assisted at the Genetics Service, at Hospital de Clínicas de Porto Alegre, receiving a hypoproteic diet (1.5-2 g of protein/kg/day), with adequate physical and neuropsychomotor development. Conclusion: 3-ketothiolase deficiency.

Case 24: Patient referred to investigation due to convulsive crises and respiratory insufficiency in the neonatal period. On the complementary examinations, he presented persistent metabolic acidosis and severe leukopenia. The application of the protocol showed several inconclusive alterations; the survey for organic acids in urine, which was performed retrospectively, showed high levels of isovalerylglycine and isovaleric acid. On the 6th month of life, the patient was readmitted with extensive bronchopneumonia, sepsis, severe metabolic acidosis, and leukopenia; he presented severe psychomotor retardation, and died a few hours later. Conclusion: isovaleric aciduria.

Case 32: A 1-year-and-9-month old patient referred to investigation for presenting recurrent episodes of hypoglycemia and sensorium alteration in the presence of severe metabolic acidosis. The chromatography of amino acids showed the presence of valine, leucine, and isoleucine in a first blood sample, resulting normal during repetition. The survey for organic acids in urine showed elevated levels of 3-hydroxy-3-methyl-glutaric acid, and an increase of 3-methyl-glutaric acid. The activity of the enzyme 3-hydroxy-3-methyl-glutaryl-CoA lyase in fibroblasts was absent, confirming the diagnosis. The patient is being assisted at Hospital de Clínicas de Porto Alegre, following a protein and lipid diet, rich in carbohydrates, with frequent intervals of administration. Conclusion: glutaric 3-hydroxy-3-methyl aciduria.

Case 41: Patient referred to investigation for coma and convulsive crises in the neonatal period with unknown cause. The chromatography of amino acids showed an increase of glycine in blood and urine. The survey for organic acids in urine resulted normal. The quantitative analysis of amino acids showed high levels of glycine in urine, blood, and liquor, the last of them presenting really elevated concentrations. Up to the 3rd month of life, the child presented a reasonable evolution. The follow-up for this case was lost at this moment. Conclusion: nonketotic hyperglycinemia.

Discussion

The diagnosis of inborn errors of metabolism depends mainly on the clinical suspicion and on the adequate requirement and performance of complementary examinations. The establishment of protocols for the investigation of certain groups of inborn errors of metabolism has proved important for a more efficient diagnosis of these pathologies.[22]

Several studies have pointed the main clinical and laboratory characteristics that lead to the suspicion of inborn errors of metabolism in children presenting critical statuses, as well as the best way to investigate them.[9,11-20] The typical symptoms include lethargy, coma, seizures, food refusal, apnea or tachypnea, and recurrent vomits. Metabolic acidosis, hypoglycemia, and hyperammonemia are also observed in many cases. Although in newborns these symptoms have usually led to the initial hypothesis of sepsis, inborn errors of metabolism must be adequately investigated in children with these clinical or laboratory alterations.[3,4]

In the present work, acute neurological alterations motivated the metabolic investigation in 22 patients (47.8%), and they were present in every patient who had inborn errors of metabolism as the definitive diagnosis. Such findings are in accordance with the current observation that most of inborn errors of metabolism (including defects in the urea cycle, organic aciduria and some aminoacidopathies) are present in infancy with a clinical status of acute or chronic encephalopathy.[4]

Organic acidurias are alterations of the intermediary metabolism that lead to the accumulation of organic acids in the tissues, disorders in the basic-acid balance, and intracellular biochemical alterations.[23] They form a particularly frequent group of inborn errors of metabolism, considering that they occur in at least 1/6,700 births.[24] Chalmers & Lawson[14] verified that these pathologies were the most frequent ones in critically ill children. The survey for organic acids in urine through gas chromatography coupled with mass spectrometry, is the indicated examination in these cases. Nowadays, this examination is already available in our field, with the identification of 63 cases among 1,013 patients studied.[25] Among the 46 patients studied in the present work, six cases of inborn errors of metabolism were diagnosed and confirmed later in foreign laboratories. Among these, four cases were organic acidurias: isovaleric aciduria, propionic aciduria, 3-ketothiolase deficiency, and 3-hydroxy-3-methyl-glutaric aciduria.

On the other hand, the identification and the quantification of amino acids are important for the diagnosis and the follow-up of aminoacidopathies, possibly the most well-known group of inborn errors of metabolism that occur during the neonatal period, with disorders in the urea cycle, and nonketotic hyperglycinemia.

The dosage of ammonia in the plasma, though technically complex, is important for the diagnosis of disorders in the urea cycle, and it may also be increased in other errors of metabolism, such as organic acidurias. It is usually elevated in critically ill newborns too, with asphyxia and infections.[26] In this work, most patients presented slightly elevated ammonia levels, possibly related to the seriousness of the clinical status. However, significantly elevated levels were only identified in the patient with propionic aciduria.

A growing number of inborn errors of metabolism may be adequately treated, although the long-term prognosis is not established yet for many of them. Therapeutic measures usually suggested during emergency include rehydration, administration of glucose, suspension of the administration of proteins, and correction of metabolic acidosis. The therapy with vitamin cocktails in high doses, considered empirical, must be used until the establishment of a definitive diagnosis. The removal of toxic metabolites through dialysis procedures is indicated in many acute cases that are resistant to habitual measures.

As the necessary complementary investigation for the diagnosis of inborn errors of metabolism involves a series of sophisticated equipment and high-cost procedures, as well as experienced investigators, the international tendency has been the establishment of regional centers of reference and a larger specialization by the laboratories concerning a given group of pathologies[9,11,29]

Sometimes, patients with inborn errors of metabolism present critical statuses, with eventual death. Even when a treatment is not available, the identification of inborn errors of metabolism is important for the genetic counseling and for making the pre-natal diagnosis possible in a future gestation.[27] Table 5 shows an orientation about the samples that should be collected and sent to reference laboratories when there is suspicion of inborn errors of metabolism, even when patients die.[28]

The protocol applied in the present study proved efficient for the detection of inborn errors of metabolism in critically ill children who presented suggestive findings. This sample allowed the detection of six cases of inborn errors of metabolism in 46 evaluated patients (13%), a relatively high frequency when compared to that of 6.5% in a general sample of patients referred to metabolic evaluation.[9]

Based on the reviewed literature and on the results found with the application of the protocol for the detection of inborn errors of metabolism in critically ill children, we elaborated the following conclusions and some recommendations:

1) Urine triage tests are subject to false-positive (interference of drugs and other factors) and false-negative (detection of a limited number of pathologies) results. Nevertheless, they present low cost and, in some cases, allow us to suggest a more specific investigation, such as Benedict's positive reaction in the galactosemia case, and the presence of ketoacids (dinitrophenylhydrazine reaction) in organic acidurias, for instance.

Table 5 - Samples to be collected from critically ill patients with suspicion of inborn errors of metabolism (Giugliani, 1990)

The most important samples (always collect):

1. **Urine**: 30 ml or more, and it may be the conjunct of several micturitions; do not add preservatives; keep it at -20 ℃.

2. **Plasma or serum**: at least 3 ml; keep it at -20 ℃; collect it before blood transfusion.

Very useful samples (collect whenever possible):

1. **Heparinized blood**: collect 8 to 10 ml in heparinized syringe, and keep it at 4 ℃ (do not freeze it); be sure that the samples are collected before blood transfusion; this sample is essential for enzymatic assays in leukocytes and erythrocytes.

2. **Skin biopsy**: collect it under sterile conditions and put it in a sterile flask, containing half of culture for cells (same as the kariotype); in an emergency, replace it with physiological solution; never use agar; samples collected up to 24 hours after death may be viable if they are not contaminated; keep it at 4 ℃.

3. **Liquor**: collect 2 to 3 ml; centrifuge, freeze, and store the remaining material (supernatant) at -20 ℃.

Useful samples in special situations (collect when indicated or when the indication is not clear):

1. **Blood for DNA analysis**: collect 10 to 20 ml of total blood using EDTA as an anticoagulant; freeze it at -20℃; these samples may be useful in the molecular analysis.

2. **Tissue samples**: collect it when the diagnostic suspicion may be clarified through biochemical studies of specific tissues; the most useful tissue is usually the liver, but the skeletal and cardiac muscles may also be informative; if possible, collect 2 to 3 fragments by percutaneous biopsy; samples collected up to 2 hours after death are usually viable; pack them in aluminum paper, and freeze them immediately at -20 ℃; do not add formol.

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2) Amino acid chromatography in blood and urine is really a useful semi-quantitative examination, which contributes decisively for the diagnosis of most aminoacidopathies, such as nonketotic hyperglycinemia. It is also useful in the suspicion of organic aciduria due to the increase of glycine in urine, like in the propionic aciduria and in the 2-methylcrotonyl-glycinuria in this study.

3) Slightly elevated levels of ammonia are found in most of critically ill children. Significantly elevated levels suggest the presence of a defect in the urea cycle, and may be present in organic acidurias, as well as in the patient with propionic aciduria.

4) Although it was not detected in this study, elevated levels of lactate in the absence of hypoxemia suggest primary lactic acidemia.

5) The survey on organic acids in urine through gas chromatography coupled with mass spectrometry contributes to the diagnosis of aminoacidopathies, and it allows the definitive diagnosis of an organic aciduria, as in four cases of this study. Considering the elevated frequency of such defects in critically ill children, this technique should be included among the first-line examinations for this group of patients.

6) Most specialized examinations, such as specific enzymatic assays and more complex dosages, must be reserved to the cases in which there is a specific diagnostic hypothesis, based on the preliminary laboratory and clinical findings.

7) Clinical evaluation is fundamental for the definitive diagnosis of inborn errors of metabolism, helping in the adequate managing of the investigation. Thus, detailed data on clinical history, familial history, laboratory alterations, diet, and drugs used must always be sent to the laboratory along with blood and urine samples.

8) The frequency of inborn errors of metabolism in the researched group justifies a systematic investigation of this condition in children that present any of the following clinical alterations without a reasonable clinical justification: severe or recurrent metabolic acidosis, hypoglycemia, consciousness alteration, convulsive crises, alteration of the hepatic function, hydroelectrolytic unbalance, or even in critically ill children who present a familial history suggestive of inborn errors of metabolism.

9) Follow-up of cases in which the diagnosis of inborn errors of metabolism was not ruled out is important because it allows the performance of additional examinations according to the evolution of the patient, and thus broads the possibility of a diagnostic definition. We must stress that frequently the abnormal metabolite is elevated only due to an acute crisis.

10) Whenever there is the suspicion of inborn errors of metabolism, specific therapeutic measures must be readily and quickly applied, since some of these errors, like organic acidurias, for example, may have a very favorable evolution after the resolution of the acute phase.

11) When there is clinical suspicion for a specific pathology(ies), even with negative triage tests, the directed investigation must continue with more sophisticated examinations.

12) In cases that present unfavorable evolution, the collection and storage of adequate predeath samples is important in order to try to establish the diagnosis and benefit the family.

13) The definitive diagnosis of inborn errors of metabolism is important because it may allow a specific treatment. It also prevents the performance of unnecessary examinations, allows the genetic counseling for risk-group couples, and offers prenatal diagnosis in a posterior pregnancy.

14) The protocol for the investigation of inborn errors of metabolism in critically ill children proposed in this work proved efficient, since it allowed the diagnosis of inborn errors of metabolism in more than 10% of the studied sample.

Acknowledgements

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Correspondence:
Dra. Maria Teresa Vieira Sanseverino
Serv. Genética Médica – Hosp. de Clínicas de Porto Alegre
Rua Ramiro Barcelos, 2350
CEP 90035-003 – Porto Alegre, RS, Brazil
E-mail: msanseverino@hcpa.ufrgs.br