Thalidomide Embryopathy: Follow-Up of Cases Born between 1959 and 2010

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Background: Thalidomide is a known teratogen and it is estimated that more than ten thousand babies were affected by thalidomide embryopathy (TE), which is characterized mainly by limb defects, but can involve many organs and systems. Most people with TE were only evaluated at birth and it is not well established if thalidomide exposure during embryonic development leads to later effects. We analyzed the clinical history of adults with TE to better understand this gap in the clinical findings of TE. **Methods:** Brazilian individuals with TE were invited to answer a clinical questionnaire which considered family history, social information, medical history, and current clinical and psychological health status. A clinical examination was also performed, including on the infant subjects to evaluate congenital anomalies. The characterization of the features was analyzed using descriptive statistics and Chi-square or Fisher's exact test. **Results:** The congenital anomalies caused by thalidomide were reviewed in 28 Brazilian individuals, and the questionnaire was applied to the 23 adult subjects with TE (aged 19 to 55). Progressive deafness and dental loss were reported. From the comparison of TE individuals with the general Brazilian population, the early onset of cardiovascular diseases ($p = 0.009$) and a higher frequency of psychological disorders ($p = 0.011$) were observed. **Conclusion:** Although there is no sufficient evidence that thalidomide exposure caused or worsened the described events, this approach helps to better understand the TE phenotype, improves the clinical diagnosis, and can lead to adequate health support for these individuals.

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Key words: thalidomide; teratogenesis; embryopathy; limb reduction defects; late effects

Introduction

Thalidomide was synthesized in East Germany in 1954 and introduced into the market in 1957 (Smithells and Newman, 1992). It was originally considered to be a new, virtually nontoxic, sedative hypnotic drug, due to its low absorption and negative results in toxicity tests on rodents (Somers, 1960). Later, it was licensed to treat a wide variety of ailments, including morning sickness in early pregnancies (Lenz, 1988). In 1961, Dr. Lenz, a German physician, reported that thalidomide was probably responsible for the epidemic of children born with limb defects in those years, a suggestion that was simultaneously made by Dr. McBride in Australia (Smithells and Newman, 1992). The drug was removed from the market in Germany in the same year and then by many other countries in 1962 (Lenz, 1988).

Thalidomide had been marketed in more than 46 countries, including Brazil, where it began being sold in 1958. It was withdrawn at the end of 1962 (Schmidt and Salzano, 1980; Saldanha, 1994). Thalidomide's history would probably be different if not for the discovery by Sheskin (1965) regarding its effectiveness in the treatment of erythema nodosum leprosum (ENL). ENL is an inflammatory reaction that occurs frequently in leprosy, a neglected disease caused by the bacillum *Mycobacterium leprae*. Leprosy occurs in Brazil at an estimated rate of 1.51 per 10,000 inhabitants (Basic Data Indicators, Brazilian Health Ministry, 2012), with endemic areas reaching a prevalence of 7.69 per 10,000 inhabitants (Basic Data Indicators, Brazilian Health Ministry, 2012). Thalidomide has been used in Brazil to treat this condition since the second half of the 1960s (Oliveira et al., 1999; Paumgartten and Chahound, 2006). More recently, the drug has also been designated to treat other conditions, such as multiple myeloma, lupus erythematosus, idiopathic ulceration from AIDS, and graft versus host disease, due to its important immunomodulatory and antiangiogenesis properties (Sampaio et al., 1991; Moreira et al., 1993; D'Amato et al., 1994). Because of these properties, the use of thalidomide was increased in Brazil, but under restrict legislation (RDC no. 11, March 22, 2011), implemented by the Brazilian National Health Surveillance Agency (ANVISA). Despite its restricted use and the need for a prescription, several cases of TE in Brazil have been reported over the last few decades (Castilla et al., 1996; Schuler-Faccini et al., 2007; Vianna et al., 2013b). This indicates that Brazilians of varying ages are

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affected by TE, from 50 year olds, born during the worldwide tragedy, to infants.

It is estimated that more than ten thousand children have been born with thalidomide embryopathy (TE), which includes congenital defects, especially in limbs, the heart, ears, and eyes (Lenz, 1988). Some patterns are essential for differential diagnosis of the embryopathy: the limbs are the organs of the body most commonly affected and, therefore, bilateral limb reduction defects (LRD) are expected in TE individuals, because both sides of the body develop in parallel (Smithells and Newman, 1992); however, the degree of symmetry can vary. Two types of bilateral LRD are considered to be signals of the TE phenotype: preaxial longitudinal and intercalary transverse (Lenz, 1988; Vianna et al., 2013b). Cases with amelia are also commonly seen in TE (Newman, 1986). The majority of TE individuals frequently have the upper limbs affected, and the pattern of lower limb defects is similar to that for the upper limbs (Smithells and Newman, 1992).

Craniofacial anomalies are also commonly seen in TE individuals, affecting mainly eyes and ears (Strömland and Miller, 1993). Facial palsy has also been described in an evaluation of 86 TE subjects; its frequency being estimated in 20% of the affected individuals (Strömland and Miller, 1993), and later estimated in 13%, according to an analysis of 31 affected individuals (Sjögreen and Kiliaridis, 2012). The heart and kidneys are the internal organs mainly affected, but there are also several reports of genital and alimentary tract anomalies (Smithells and Newman, 1992; Miller and Stromland, 1999). The mortality of babies with TE is approximately 40% due to the severity of the malformations, and consequently, internal defects are not common in adult individuals (Smithells and Newman, 1992).

Although phocomelia is quite characteristic of TE, one of the main challenges in TE diagnosis is the fact that none of the congenital defects observed are unique to TE (Smithells and Newman, 1992), and also because it includes a variety of defects (Smithells and Newman, 1992).

Recently, studies of people affected by TE have been performed by some groups around the world to determine different aspects of the embryopathy and to better characterize the main clinical features. Examples of this include aberrant tearing (Miller et al., 2008), facial palsy (Sjögreen and Kiliaridis, 2012), and ocular motility (Miller and Strömland, 1991). However, it is not known if different health symptoms or medical evaluations for those individuals are occurring now as an outcome of in utero exposure to this teratogen. Thirty-one Swedish citizens affected by TE were included in a cohort study that intended to examine their dental condition by clinical and radiological evaluation. TE individuals were found to have more tooth wear than the unaffected population, probably due to the high number of subjects who suffered from regurgitation (Ekfeldt and Carlsson, 2008). Later, an Australasian group evaluated new neurological complaints, but the authors suggested that these findings could be explained by the compensatory posture used to compensate for the disability or low limb motility, and that there were no reasons to suspect a delayed effect on the embryo which had been exposed to thalidomide (Jankelowitz et al., 2013). Thus, here we aim to evaluate clinical findings for individuals with TE and search for delayed manifestations caused or anticipated by thalidomide exposure.

Materials and Methods

This study was approved by the Committee for Ethics and Research of the Hospital de Clínicas of Porto Alegre, under number 10-0244.

Several Brazilians affected by TE were invited through the Brazilian Association of Thalidomide Victims (ABPST) to answer a clinical questionnaire developed by our group. The four authors (T.W.K., F.S.L.V., M.T.V.S., and L.S.-F.) administered the questionnaires and performed clinical examinations of the participants. The research form consisted of: evaluation of physical anomalies, family history, social information, medical history, and current clinical and psychological health status. The analysis of congenital defects consisted of the determination of clinical features compatible with TE, according to the diagnosis guidelines developed by our group (Vianna et al., 2013b). The guideline was developed after a careful review of the literature of the cases of the 1960s and divides the clinical features and systems affected by frequency of occurrence in TE, which greatly facilitate the correct diagnosis. Furthermore, the diagnosis follows recommendations provided from the report after the Meeting of Experts of Thalidomide Embryopathy (Thalidomide Embryopathy - World Health Organization Report, 2014).

The sample consisted of 28 people born in 12 different federative units of Brazil, from the five regions of the country. The participants were born between 1959 and 2010, with 21 of them being born during the worldwide thalidomide tragedy (1959 to 1964). Five participants only performed the clinical evaluation and questionnaire for the congenital anomalies. The other 23 did the entire analysis. Demographic data are available in Table 1.

Variables such as level of schooling and professional occupation were determined to verify possible injuries or exposures during the person's life, besides the impact that embryopathy has had on the professional and personal life. The family history of the individuals and their first-degree relatives (parents, siblings, and children) were investigated, with questions about consanguinity marriages, history of congenital malformations, and genetic or chronic diseases to exclude the possibility of the presence of concomitant genetic syndromes or familial diseases in late-effects analysis. Moreover, the reproductive history of the participants was evaluated to identify possible

TABLE 1. *Social and Clinical Characterization for Individuals with TE Recognized after Clinical Examination*

Information	**Thalidomide embryopathy affected ID**																											
Social profile	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**
Age	19	52	52	52	32	54	49	53	53	50	51	51	15	3	51	53	51	53	51	37	42	51	53	53	51	53	38	53
Gender	F	M	F	M	F	F	F	F	F	M	M	F	M	F	F	F	M	M	F	M	F	M	M	M	M	M	M	M
Schooling									NA				NA	NA		NA	NA											
Illiterate					+																							
Elementary school		+	+				+			+											+	+	+				
High school	+			+							+								+	+					+			
Graduated								+				+			+			+								+	+	
Post-graduated						+																		+				+
Occupation									NA				NA	NA		NA	NA		NA									
Employed		+		+		+		+		+		+			+			+		+		+	+	+	+	+	+	+
Retired			+																									
Other	+				+		+				+										+							
Number of children	0	4	0	NA	0	0	1	0	NA	0	0	3	NA	NA	0	NA	NA	2	NA	2	0	0	4	2	2	NA	NA	1
Congenital anomalies*	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Upper limbs (27/28)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+
Intercalary transverse	+		+	+	+	+		+	+	+	+		+	+	+	+		+	+		+	+					+	
Preaxial longitudinal		+					+					+					+			+			+		+	+		+
Lower limbs (12/28)	+		+	+			+		+		+		+	+						+				+		+		+
Intercalary transverse	+		+	+					+				+	+						+				+		+		+
Preaxial longitudinal							+				+																	
Ears (6/28)				+	+	+							+		+			+										
Tortuosity of external auditory meatus				+											+													
Accessory auricles													+															
Stenosis															+													
Absence of auditory canal																		+										
Deafness					+	+									+			+										
Eyes (10/28)			+			+						+	+	+				+	+			+		+			+	
Pterygium			+									+																
Strabismus						+												+	+									

TABLE 1. *Continued*

Information	**Thalidomide embryopathy affected ID**																												
Social profile	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**
Microphthalmia														+														
Corneal opacification													+															
Macular degeneration																								+				
Aberrant tearing													+					+				+						
Restricted eye movements													+														+	
Craniofacial (6/28)					+	+							+								+	+	+					
Mandibular asymmetry																					+		+					
Small mandible					?																							
VIth nerve palsy						+																						
Facial palsy													+									+						
Congenital anomalies*	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Absent kidney (2/28)								?					+															
Heart (4/28)						+	+						+											+				
Extrasystole						+																						
Arrhythmia																								+				
Genital tract (3/28)						+									+											+		
Fallopian tube alteration						+																						
Bicornuate uterus															+													
Absent testis																										?		
Undescended testis																										?		
Skeletal															+				+					+		+		
Hemivertebrae															+													
Rib anomalies															+											+		
Scoliosis																			+					+		+		
Clinical findings	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Hypertension		+		+			+	+	NA				NA	NA	+	NA	NA							+	+			+
Diabetes		+							NA				NA	NA		NA	NA											+
Alimentary tract diseases																				+						+		
Gastritis																				+								

TABLE 1. *Continued*

Information	**Thalidomide embryopathy affected ID**																											
Social profile	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**
Gastroesophageal reflux																										+		
Respiratory diseases																			+									
Respiratory insufficiency																			+									
Psychological disorders	+			+		+	+	+	NA		+		NA	NA		NA	NA	+	+	+	+		+				+	
Anxiety	+						+	+			+							+	+									
Depression						+	+	+												+								
Phobia				+															+		+							
Stress disorder																											+	
Bipolar disorder							+																					
Musculoskeletal diseases		+		+					NA		+	+	NA	NA		NA	NA	+	+			+						
Osteopenia				+							+																	
Arthrosis		+										+																
Osteoporosis																		+										
Fasciitis																			+									
Scoliosis																						+						
Chronic spine pain																						+						
Cardiovascular diseases				+				+	NA		+		NA	NA		NA	NA								+	+	+	
Acute myocardial stroke																									+	+		
Hypercholesterolemia											+																+	
Hemorrhagic cerebrovascular accident								+																				
Angina				+																								
Auto-immune diseases									NA				NA	NA	+	NA	NA											
Uveitis															+													
Endocrine diseases									NA				NA	NA		NA	NA		+									
Hypothyroidism																			+									

NA, Not answered; (+), presence of clinical aspect; (?), presence of clinical aspect not confirmed; *, includes structural anomalies or functional consequences.

consequences (not diagnosed at birth) of thalidomide exposure in the reproductive system.

Medical interventions, such as surgeries and chronic use of medications, were determined to access the clinical history of the individuals affected by TE. The clinical file contained questions about diseases in different systems (cardiovascular, immune, respiratory, gastric, genitourinary, and musculoskeletal); dental, visual, and auditory deficiencies; psychological disorders; and chronic common diseases in our populations, including cancer (previous or present), diabetes, and hypertension.

The characterization of the features and differences were analyzed using descriptive statistics and Chi-square or Fisher's exact test. All tests were two-tailed and the significance level was set at 0.05. The analyses were performed using the SPSS® program, version 20 (SPSS, www.spss.com, IBM, USA).

Results

CONGENITAL ANOMALIES

The description and frequencies of the congenital anomalies in limbs and other systems are described in Table 1. All participants have LRD. Only one individual did not have anomalies in the upper limbs. Seventeen cases involved intercalary transverse defects (61%), 10 participants had preaxial longitudinal LRD (36%), while there was no case of amelia in this sample. Almost half of the individuals (46.5%) also had the lower limbs affected, with 11 cases of intercalary transverse LRD (39%) and 2 with preaxial longitudinal defects (7%). Half of all the individuals had the left side of the body more affected than the right side. Six had both sides equally affected (21%).

Ten subjects in our research (36%) did not have any other organ or system affected. Four individuals stated to never have been evaluated for internal anomalies. Three of them are included in this group with malformations of the limbs only.

SOCIAL INFORMATION AND FAMILIAL HISTORY

Only one individual was not literate, due to the occurrence of congenital deafness together with phocomelia of the four members, which precluded the learning of sign language. All of them receive financial restitution from the Brazilian government. Occupation and schooling data are summarized in Table 1.

In regard to familial history, there was one case of achondroplasia, one case of webbed neck, and one case of epilepsy, each in a different family. The number of siblings ranged from one to thirteen, with only one of the people interviewed being an only child (4%). Twelve (12 of 19) stated that they have tried to have children and 9 of them (7 male) had at least 1 child (75%). Two males (2 of 12) stated that their wives have suffered abortions, but each of them subsequently fathered 2. None of the 21 children was born with congenital anomalies or genetic diseases. A similar finding was encountered in the study performed by Strömland et al. (2002), which reaffirms that thalidomide is not mutagenic. Another three individuals tried to have children (two of them female) without success. One of these women was born with a bicornuate uterus and the other one reported to have fallopian tube alteration. No other individual in this sample referred to any anomaly in the reproductive system.

MEDICAL HISTORY AND CURRENT HEALTH STATUS

The diseases reported by the adult individuals with TE are described in Table 2, in accordance with the systems affected. Glaucoma was the only ocular disease reported; however, many were affected by refractive errors such as myopia and astigmatism. Only six individuals (26%) stated to have perfect vision, without use of corrective lenses.

Besides the four individuals with congenital deafness, six individuals (27%) reported progressive deafness after the age of 40. Because deafness may cause social withdrawal and other psychological consequences, we compared the individuals who have hearing loss with all psychological disorders reported in the query ($p = 0.22$), as well as depression ($p = 0.59$) and anxiety ($p = 0.64$); however, no statistical significance was found.

Musculoskeletal complaints were recurrent in the inquiry, with seven individuals affected (30%). Two conditions were mentioned twice (9%): osteopenia and arthrosis, one in the hip and the other one in the femur. According to the people interviewed, the arthrosis occurred due to loss of bone mass, and in both cases a prosthesis was going to be inserted. The type of congenital anomalies in the upper and lower limbs did not explain the later diseases in the musculoskeletal system either ($p = 0.643$ and 0.175, respectively). The need for surgery over the course of their lives was also queried, and only 5 of 22 (23%) had never undergone any surgical procedure. Ten (45.5%) reported to have had at least one corrective surgery performed on the upper or lower limbs. Four (18%) had already had a spine procedure performed.

Only one participant (4%) reported having a congenital dental anomaly, due to an asymmetric jaw. A second individual underwent dental surgery to pull out some teeth due to a small dental arcade. Four other individuals (17%) declared to have loose or weak teeth. One of these had lost all her teeth by the age of 12 and another individual had lost 12 teeth from the upper dental arcade. Two more participants reported to have undergone dental surgeries (9%) and another one told of frequent dental decay and osteomyelitis (4%).

In the sample, two individuals (9%) were current smokers and three others (13%) had quit smoking. If considering the individuals who have ever smoked, it is possible to visualize a statistical correlation between tobacco use and hypertension ($p = 0.033$); tobacco use is a known risk factor for this disease.

TABLE 2. *Description of Chronic Diseases Reported for TE Individuals, Together with Frequencies in the Group and in General Brazilian Population, Including Individuals Older Than 18*

Chronic disease	Frequency in TE individuals (%)	Frequency in Brazilian population (%)	*p* Value
Hypertension	8/23 (35%)	35%	0.586
Diabetes	2/23 (9%)	10%	0.592
Alimentary tract diseases	2/23 (9%)	NA	–
Gastritis	1/23 (4%)	NA	–
Gastroesophageal reflux	1/23 (4%)	NA	–
Respiratory diseases			
Respiratory insufficiency	1/23 (4%)	NA	–
Psychological disorders	12/23 (52%)	25%–30%	**0.011**
Anxiety	6/23 (26%)	12%	**0.05**
Depression	4/23 (17%)	13%–20%	0.487
Phobia	3/23 (13%)	NA	–
Stress disorder	2/23 (9%)	NA	–
Bipolar disorder	1/23 (4%)	NA	–
Musculoskeletal diseases	7/23 (30%)	NA	–
Osteopenia	2/23 (9%)	NA	–
Arthrosis	2/23 (9%)	NA	–
Osteoporosis	1/23 (4%)	NA	–
Fasciitis	1/23 (4%)	NA	–
Scoliosis	1/23 (4%)	NA	–
Chronic spine pain	1/23 (4%)	NA	–
Cardiovascular diseases	6/23 (26%)	NA	–
Acute myocardial stroke	2/23 (9%)	10.3%*	0.207*
Hypercholesterolemia	2/23 (9%)	24%	0.061
Hemorrhagic cerebrovascular accident	1/23 (4%)	NA	–
Angina	1/23 (4%)	NA	–
Uveitis	1/23 (4%)	NA	–
Endocrine diseases			
Hypothyroidism	1/23 (4%)	NA	–
Total	15/23 (65%)	31.8%	**0.001**

[a]Frequency and analysis of the prevalence all heart diseases in Brazil (data obtained from Basic Data Indicators, 2014, Brazilian Health Ministry).

NA, not available.

Discussion

Thalidomide teratogenesis was elucidated in 1961 and despite the molecular mechanism which leads to the embryopathy remaining unclear, the efforts of many physicians at the time of the thalidomide tragedy were very important to understanding some characteristics concerning its teratogenicity. Thanks to their careful clinical evaluation of thalidomide victims, it is known that the dosage consumed is not related to the embryopathy, but there is a time-specific pattern of congenital anomalies according to the day the drug was ingested (Newman, 1986; Shardein, 1993; Miller and Strömland, 1999).

The TE phenotype has been well described in the literature (Lenz, 1988; Smithells and Newman, 1992), although the people affected were only evaluated at birth. The only reported lasting effect related to thalidomide exposure in the uterus, is autism, which is present in 5% of the individuals examined in a Swedish study (Strömland et al., 1994). Research of other teratogens, such as alcohol, has demonstrated that many structural changes caused by its prenatal

exposure may go undetected for many years (Reynolds et al., 2011).

In this evaluation, we reviewed the congenital anomalies caused by thalidomide exposure in 28 Brazilian individuals. Also, a questionnaire was administered to 23 adult subjects with TE, between 19 and 55 years of age, in an attempt to understand TE in adults. Brazilian children and adolescents with TE are still being followed-up by our group to evaluate their development and health status. However, because we identified new cases of TE, it is imperative to ensure strict measures to control the distribution and dispensing the medication allied to educational campaigns directed to health professionals and general population. Complementary, monitoring the occurrence of compatible phenotypes of the embryopathy is fundamental to check the effectivity of the safety procedures. Our group has conducted epidemiological surveillance studies to identify and prevent new babies of being affected by thalidomide teratogenesis (Schuler-Faccini et al., 2007; Vianna et al., 2011; Vianna et al., 2013b). These studies have helped to make the regulation of thalidomide more restrictive and rigid in Brazil (RDC).

It is a challenge to identify which conditions may be a consequence of thalidomide exposure. As in the Australasian cohort study (Jankelowitz et al., 2013), we reported a high frequency of musculoskeletal diseases in TE individuals, but there is no suggestion that these conditions were caused by uterine exposure to thalidomide. They are probably caused by compensatory postures and difficulties in executing normal daily tasks due to limb anomalies. Additionally, the suggestion of the early onset of cardiovascular diseases and the frequency of psychological disorders encountered led to a deeper analysis together with data from the general Brazilian population.

In our sample, 15 individuals (65%) with TE suffer from at least one chronic disease. According to the 2003 PNAD questionnaire from the Brazilian Institute of Geography and Statistics (2003), 31.8% of Brazilians between 15 and 59 years of age have at least one chronic disease, a rate that is far below that encountered in the sample (65%, $p = 0.001$). This frequency seems to occur mostly due to the high number of TE individuals with some kind of chronic psychological disturbance (12 of 23 or 52%), especially anxiety (26%). Individual reports of sporadic episodes of depression, anxiety or other psychological disorders were not included in the analysis. Table 2 shows a comparison between the data obtained in the current study and frequencies in the general Brazilian population for the most common health problems. Despite the small sample size is a limitation of our study, this statistical analysis helps to demonstrate the importance of the follow-up of individuals with TE in their adult life. Even if these findings could not evidence an increased risk for the described diseases, this approach helps in the management of these subjects and in the formation of strategies for prevention and treatment of other individuals with TE.

A recent study by a Japanese group evaluated the psychological and mental health of TE individuals. It was concluded that despite there being no evident influence of thalidomide in encephalographic abnormalities, these individuals seem to have a decreased working memory capacity (Imai et al., 2014). They also observed that in TE individuals there is evidence of somatic suffering, such as anxiety and insomnia (Imai et al., 2014). Similar to our research, the group was only able to compare these conditions with a general population of individuals without congenital anomalies. A deeper analysis should also be done of a group of Brazilians with congenital anomalies not related to thalidomide to evaluate thalidomide's role in psychological disorders and neurological development.

CRBN, the primary target of thalidomide binding (Ito et al., 2010), is primarily known for its abundant presence in the human brain and putative role in cerebral development (Higgins et al., 2004). Studies involving rats also reported a large presence in the hippocampus (Jo et al., 2005), an area with a role that has recently been associated with the working memory (Axmacher et al., 2010). Despite the reported findings, none of the participants had autism, a condition reported to be more frequent in TE individuals (5%) than in the general population (1%) (Strömland et al., 1994). Nevertheless it is important to accentuate that autism is hard to detect in the general population and even harder in a group like this, where the patients usually do not come for a neuropsychiatric consultation and diagnosis. Moreover, it will be interesting to see if there is a relationship between the time of exposure to thalidomide in the uterus and the reported outcomes.

The most frequent disease in this sample was hypertension, affecting eight (34.8%) participants (Table 2). Considering only the individuals over 40 years of age,, the frequency of hypertension increases to 40%, which is just a little higher than the general prevalence of the disease in Brazil (35%) for individuals of this age (Basic Data Indicators, 2012, Brazilian Health Ministry); however, there is no statistical difference ($p = 0.344$). According to the 2003 PNAD questionnaire from the Brazilian Institute of Geography and Statistics, 10.3% of Brazilians between 50 and 64 years of age confirmed having some type of heart disease, a rate which is consistent with our sample of 3 of 18 or 16.7% ($p = 0.098$). For the four reports of cardiovascular diseases, the development of the disease was confirmed before 50 years of age. Case 5 developed angina at the age of 28. The occurrence rate for cardiovascular diseases for Brazilians aged between 30 and 49, is 3.7%, which is much lower than in this sample ($p = 0.009$). This data shows a tendency for the early onset of cardiovascular diseases in TE individuals. Furthermore, some genetic risk factors were known: haplotypes in the endothelial nitric-oxide synthase (eNOS) gene have already been associated with cardiovascular diseases in many populations, including Brazil (Da Costa Escobar Piccoli et al., 2012; Martinelli

et al., 2012; Guo, 2014). Recently, our group recruited individuals with TE and analyzed two functional polymorphisms in the eNOS gene (Vianna et al., 2013a), concluding that TE individuals have a higher frequency of alleles associated with lower expression of this gene, the same alleles indicated in studies with cardiovascular diseases. Thalidomide inhibits angiogenesis, a possible mechanism of its teratogenecity (D'Amato et al., 1994), and nitric oxide safeguards against the drug's teratogenicity, protecting rabbit embryos from anomalies (Siamwala et al., 2012). Also, deficiencies in eNOS in mice have been associated with the development of LRD (Gregg et al., 1998), which increases curiosity in relation to the said gene and its role in TE.

Dental loss was also noticed in the clinical evaluation. Human odontogenesis begins around the sixth week of embryogenesis and many genes have already been identified as contributing to cellular communication, which leads to tissue formation (Townsend et al., 2012). Animal studies demonstrate that the signaling pathways include the genes *Fgf, Bmp, Shh, Wnt*, and *Tnf*, with *Fgf8* being one of the first genes to be expressed and *Bmp4* the most important of the *Bmp* family in odontogenesis (Townsend et al., 2012; Jheon et al., 2013). Despite there being no assays evaluating thalidomide ingestion and odontogenesis, decreased expression of *Fgf8* and *Fgf10* was observed in the limbs of rabbit embryos treated with thalidomide (Hansen and Harris, 2004), and also in zebrafish and chick embryos, due to the reduction of ubiquitinase activity in the CRBN complex, which is an upstream controller of *Fgf8, Fgf10*, and *Bmp4* expression, and is also the first target of thalidomide binding (Ito et al., 2010). According to in vitro assays, *Tnf-alpha* is one of the main targets of thalidomide immunomodulatory activity (Sampaio et al., 1991). A Swedish group reported disturbances in teeth development of TE children, with disturbances in mineralization, numerical deviation, and shape abnormalities being observed (Axrup et al., 1966). However, a recent study, also performed in Sweden, with TE individuals aged between 45 and 49, reported that the subjects with embryopathy had no great deviations when compared with individuals from the general population (Ekfeldt and Carlsson, 2008). The present study is the first report of teeth loss in individuals with TE embryopathy.

Thalidomide teratogenesis is still surrounded by many questions. We reported some clinical features in individuals affected by TE, but there is still no evidence that the conditions encountered are caused or worsened due to uterine exposure to thalidomide. Other comparisons and studies should be performed in larger samples to clarify these relationships. This type of approach helps to better understand TE phenotypes and may contribute to studies searching for the molecular mechanism of the drug's teratogenesis and also provide better health support for these individuals.

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