AUTISM WITH OPHTHALMOLOGIC MALFORMATIONS: THE PLOT THICKENS

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ABSTRACT

Purpose: To review the association of autism spectrum disorder (ASD) in individuals manifesting thalidomide embryopathy and Möbius sequence and compare them with three new studies in which ASD was also associated with ocular and systemic malformations: (1) a Swedish study of individuals with CHARGE association (***C***oloboma, ***H***eart, choanal ***A***tresia, developmental or growth ***R***etardation, ***G***enital anomaly, and ***E***ar involvement); (2) a Swedish study of Goldenhar syndrome; and (3) Brazilian Möbius syndrome (sequence) study.

Methods: In the Swedish CHARGE study, 31 patients met the inclusion criteria (3+ or 4 of the common characteristics of the CHARGE syndrome). The same team of investigators also evaluated 20 Swedish patients with Goldenhar syndrome. In the Brazilian Möbius study, 28 children with a diagnosis of Möbius sequence were studied; some children had a history of exposure during their mother's pregnancy to the abortifacient drug misoprostol in an unsuccessful abortion attempt

Results: In the CHARGE study, five patients had the more severe autism disorder and five had autistic-like condition. In the Goldenhar study, two had autism disorder and one had autistic-like condition. In the Brazilian Möbius study, the systemic findings of the misoprostol-exposed and misoprostol-unexposed patients were almost undistinguishable, and ASD was present in both groups (autism disorder in five and autistic-like condition in three).

Conclusion: Autism spectrum disorder has been reported in two conditions with known early pregnancy exposure to the teratogenic agents thalidomide and misoprostol. In the Brazilian Möbius study, autism also occurred in both the misoprostol-exposed and misoprostol-unexposed groups. Autism also was present in patients with both CHARGE association and Goldenhar syndrome.

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INTRODUCTION

The beginning of the story was an unanticipated finding of autism while the investigators were engaged in the study of 86 Swedes who had been identified in the early 1960s as having the typical findings of thalidomide embryopathy and a maternal history of thalidomide intake during early pregnancy.[1-3] Because of the large number of affected individuals described in the literature, including many informative cases in which the time of drug intake was known, it has been previously determined that the teratogenic-sensitive period extended from day 20 to day 36 after fertilization.[4-6] The literature also indicated that early exposure to the drug (days 20 to 25) resulted in involvement of the cranial nerves (especially VI and VII) and external ear, abnormal ocular movement such as Duane syndrome, aberrant lacrimation, and thumb anomalies.[7-9] The four cases of autism in the Swedish study manifested the characteristic effects of early exposure.[10]

Autism spectrum disorders (ASDs) are neurodevelopmental disorders characterized by impairment in social interaction and communication and associated with repetitive behaviors and interests. There are several clinical ASD phenotypes, including autistic disorder, childhood autism, Asperger syndrome, and atypical autism (also referred to as autistic-like condition and pervasive devel-

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opmental disorders not otherwise specified). The pathophysiology of ASD remains elusive, despite clues from genetic studies, neurochemistry, autopsy reports, functional research, radiologic imaging, research on environmental influences, and many other approaches.[11-14]

A subset of individuals with ASD have associated medical conditions (eg, syndromes, chromosomal anomalies).[11] Insight into these comorbid conditions may give some insight into the pathophysiology of autism, and the thalidomide association has provided unique information into the timing factors of the developmental insult in some cases.[11,12]

Intrigued by the association of autism with an uncommon type of strabismus (Duane syndrome) and facial nerve palsy in the thalidomide-exposed individuals**,** and by a few case reports of a connection between Möbius syndrome with its involvement of cranial nerves VI and VII and autism,[15-17] a multidisciplinary team further explored these associations in another Swedish study.[18] Möbius syndrome has more recently been designated "Möbius sequence," since the term "sequence" defines a cascade of secondary events that occur after a single embryonic insult from heterogeneous causes. Although many functional anomalies may coexist in Möbius sequence, the most accepted clinical criterion is evidence of congenital sixth and seventh cranial nerve involvement. The systemic and ocular findings of the 25 Swedish patients in the 1995-1998 study were similar to those described in the Möbius literature, except for the remarkable finding that 7 of 22 (32%) had autistic disorder or autistic-like condition.[18,19] As in the thalidomide study, the neuropsychiatric evaluations were performed by child psychiatrists. No consistent etiologic event could be ascertained through history of other tests, except that there appeared to be more than the usual adverse pregnancy events, such as bleeding (8 patients), a chronic villi sampling procedure (1), and history of drug use (1).[18]

After the Swedish Möbius study, the multidisciplinary team decided to study other conditions in which ASD had been noted with craniofacial syndrome. They selected Goldenhar syndrome (oculoauriculovertebral dysplasia syndrome) and the CHARGE association because of case reports of autism in these conditions[20-22] and the similarity of some of the craniofacial malformations. The findings of these two studies will be described here.

Another related chapter to the ophthalmology-autism connection was reported in the early 1990s in the literature from South America, especially from Brazil. A number of papers described a group of children who had limb anomalies and whose mothers had taken an abortifacient drug, misoprostol (Cytotec), early in pregnancy.[23-28] Alone, misoprostol appears to be a poor abortifacient drug; it often only causes some bleeding or uterine contraction but with the pregnancy continuing to term. In addition to limb anomalies, many of these affected individuals had cranial nerve malformations and manifested the characteristic findings of Möbius sequence.[29-32] Most of these cases were from Brazil, where abortion is illegal, except for a few reasons, but where there are a large number of self-induced attempts at abortion, often with the use of misoprostol early in pregnancy. To further investigate this misoprostol-Möbius connection, a prospective study of patients with the diagnosis of Möbius syndrome/sequence was undertaken by Ventura[33] in Pernambuco, Brazil. The purpose of her study was to describe systemic, neuropsychiatric, and ophthalmic findings of these patients with Möbius sequence. The study included children with Möbius sequence from pregnancies both with and without misoprostol exposure. The results will be described.

METHODS

Swedish CHARGE Study (1998-2002)

A multidisciplinary study in Sweden evaluated patients referred by the medical profession with a diagnosis of CHARGE association or with a registered diagnosis of CHARGE. Although the literature is not consistent as to what clinical characteristics are necessary to make the diagnosis, the original description of CHARGE association by Pagon and associates[34] (***C***oloboma, ***H***eart, choanal ***A***tresia, ***R***etarded growth and/or development, ***G***enital hypoplasia, and ***E***ar anomalies and/or deafness) was used to establish the key characteristics. Most patients had four characteristics, but a few had three with other frequently associated systemic anomalies. Thirty-one patients were considered to meet the minimal requirements for CHARGE association. The ophthalmic and systemic evaluations were performed with predetermined protocols similar to those of the Swedish Möbius study, and by essentially the same multidisciplinary team. In the CHARGE and Goldenhar studies, the criteria for autistic disorder from the *Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV*) were used, in addition to the Childhood Autism Rating Scale (CARS), the Autistic Behavior Checklist (ABC), and the Autism Diagnostic Interview—Revised (ADIR).[35-38]

Goldenhar/Hemifacial Microsomia Study (oculoauriculovertebral dysplasia spectrum) (1998-2002)

The Goldenhar/hemifacial microsomia (HFM) study of 20 patients was done concurrently with the CHARGE study, and by the same team. Certain modifications were necessary because of the differences in clinical characteristics of these two entities. Vertebral anomalies, ocular dermoids, and mandibular hypoplasia are characteristic of the

Goldenhar syndrome but not as frequent in the CHARGE association. In contrast, ocular coloboma and genitourinary and cardiovascular malformations are more frequently noted in the CHARGE syndrome. However, a comprehensive physical examination was done on all patients. In the neuropsychiatric evaluation, the methodology used was the same as in the CHARGE study.

In the neuropsychiatric evaluations of all individuals reported here, care was taken to remove items and criteria in diagnostic instruments when scoring for autism spectrum disorders might be affected by cranial nerve palsy and severe visual and hearing impairment (eg, items and criteria concerned with facial mimicry, eye contact, and intonation of speech).

The relevant ethics committees approved all Swedish studies.

Brazilian Möbius Study (2000-2001)

A prospective multidiscipline study was performed in Pernambuco, Brazil, an area in northeastern Brazil. Detailed pregnancy and social history was collected, with particular attention to the timing, dosage, and method of taking misoprostol or other abortifacient drugs. The inclusion criterion of Möbius sequence was evidence of involvement of the sixth and seventh cranial nerves.

The study was initiated in August 2000, and most of the patients were recruited in the first 6 months. Of the 31 patients presented with a possible diagnosis of Möbius, 28 met the criteria of the study. The patients were divided into two groups. Group 1 was composed of children without known exposure to misoprostol, and group 2 consisted of children with a history of misoprostol exposure by their mothers in early pregnancy. Detailed social, demographic, and psychological data were obtained on 26 patients and ophthalmologic data on 28 patients. Initially, the mother was interviewed and a database of information about the pregnancy, including medical history, genetic background, and drug exposure, was created. The importance of truth and confidentiality and the purpose and methods of the study were explained to the mother, and an informed consent was signed. The investigators had some concern that there might be reluctance on the part of the mother to admit to taking the drugs. However, the observation of the team was that most mothers were very desirous of discussing their problems with nonjudgmental medical staff. On separate days the children were examined by a multidisciplinary team representing the specialties of pediatrics, ophthalmology, neurology, cardiology, otolaryngology, dentistry, genetics, psychiatry, speech and language, and radiology. All examinations were performed using a constant database similar to that of the Swedish Möbius study.

Psychiatric and intellectual evaluations were performed on children old enough to be formally evaluated. These included an interview utilizing the *DSM IV* and CARS.

The study met the requirements established by the Brazilian National Health Council for Research in Humans.

RESULTS

Swedish CHARGE Study

Table 1 indicates the systemic and functional abnormalities in 31 study patients with CHARGE association and compares them to estimates reported in the literature. Colobomas, often with microphthalmia, were prominent and observed in about 90% of the patients, which is slightly higher than reports in the literature and may represent some study ascertainment bias. In 19 patients the colobomas were bilateral but showed great variation in type, ranging from an isolated iris or disk coloboma to complex colobomas involving all uveal tissues (Table 2). Severe visual impairment was common. Sixteen patients had cardiovascular anomalies, with persistent ductal arteriosus being the most frequent type. Vestibular symptoms were surprisingly frequent. The ear anomalies and hearing loss included a "characteristic CHARGE ear," other external ear malformation, and involvement of the inner ear structures. Although not part of the diagnostic criteria, the fairly frequent findings of facial nerve palsy, cleft lip or palate, and short stature have been documented also in the literature.[39-41]

Five individuals manifested characteristics of the full autistic disorder, and five had fewer characteristics and thus a diagnosis of autistic-like condition. Other patients showed only autistic traits but are not reported here. A comprehensive neuropsychiatric evaluation will be reported elsewhere (Johansson M, et al, "Autism spectrum disorders and underlying brain pathology in CHARGE association," unpublished data, 2004). Table 3 summarizes the diagnostic characteristics and other findings in these 10 patients. They do not appear to have a unique set of systemic malformations compared to the other study patients, and more detailed evaluations will be reported elsewhere.[42]

Goldenhar/HFM Syndrome Study

Table 4 summarizes the systemic findings of the 20 study patients. The observed malformations and functional findings were as expected in the diagnostic characteristics, such as ear tags, microtia, lipodermoids, epibulbar dermoids, and vertebral malformations. The many associated malformations underline that this is a multisystem and not only a craniofacial syndrome.

Autistic disorder was present in two cases and autis-

TABLE 1. SWEDISH CHARGE STUDY: SUMMARY OF MOST FREQUENT MALFORMATIONS AND FUNCTIONAL PROBLEMS, WITH COMPARISON TO A LITERATURE SUMMARY (n = 31)

PROBLEM	NO. (%)	LITERATURE ESTIMATES*
Inclusion criteria anomalies		
Coloboma (19 bilateral, 4 unilateral)	28/31 (90)	80%-90%
Microphthalmos (13)†	27/31 (87)	80%-90%
Heart	16/31 (52)	75%-80%
Choanal atresia	11/31 (35)	59%-60%
Retardation (severe 12)	21/28 (75)	100%
Genital hypoplasia: cryptorchism (8), micropenis (8), small labia (1)	12/31 (39)	70%-80%
External ear	28/31 (90)	90%
Other anomalies		
Short stature	15/31 (48)	70%
Facial nerve	11/31 (35)	Significant
Cleft lip/palate	6/31 (19)	15%-20%
Spine	8/31 (26)	Rare
Renal	4/31 (13)	15%-25%
Functional problems		
Eating	25/31 (81)	
Vestibular (balance)	21/24 (88)	
Speech	21/26 (81)	
Autism spectrum disorders‡ (AD or ALC)	10/26 (38)	
Visual impairment§	11/19 (58)	
Ear malformations¶	23/31 (74)	
Severe developmental delay	12/28 (43)	

AD, autistic disorder; ALC, autistic-like condition.
*Blake et al,[39] Byerly and Pauli.[40]
†Clinical estimate; no ultrasound done. In four patients there was obvious unilateral colobomas, but fellow microphthalmic eye was too small to evaluate formally. In a few, it was not clear whether there was an associated coloboma.
‡Six patients could not be evaluated for autism spectrum disorders; three were too young and three were completely deaf and blind.
§Corrected visual acuity in best eye ≤0.3; 12 patients unable to test because of mental disability, although clinical impression was that eight of these had poor visual acuity from structural anomalies.
¶Eleven had inner ear malformations.

tic-like condition in one case (Table 5). The associated anomalies did not seem to distinguish this subgroup from the rest of the study patients. More details of the psychiatric evaluations will be reported elsewhere.

Brazilian Möbius Study

Table 6 summarizes the major systemic findings of the 28 patients; findings are not separated by groups because there was minimal difference between groups 1 and 2. In the 17 patients with misoprostol exposure (group 2), 13 mothers had taken misoprostol only and four had taken misoprostol plus tea, which was a culturally popular drug felt (but not proven) to induce abortion. Three patients took misoprostol plus injection of an unidentified medication. One patient in the unexposed group (group 1) also had taken tea. A few patients in each group took other unidentifiable medications, which may have been misoprostol. Misoprostol was taken both orally and vaginally alone or together. The average number of pills ingested was 4.8 (each pill was 200 mg). In the group with misoprostol exposure, 15 patients had a history of bleeding early in pregnancy, compared to four in the unexposed group. The average duration of bleeding was approximately 9 to 10 days in both groups. Not surprisingly, bleeding was more frequent in the "attempted abortion" group than the "etiology unknown" group.

Common associated anomalies were micrognathia and posterior rotated ear; there was no difference in prevalence between the two groups. Limb anomalies were present in 22 of the 28 patients in the study, with clubfoot and clinodactyly the most frequent. Abnormal tearing was present in both groups. Many patients had oral or dental malformations, including cleft palate, abnormal tongue anatomy, altered tongue tone, and poor sucking. There were only slight differences in a few malformations. A detailed analysis of this study is reported elsewhere by Ventura.[33]

Radiologic imaging was done on 25 of the 28 patients. The main findings were brain-stem calcification in six patients, Dandy-Walker or variant in two, arachnoid cyst in two, hydrocephalus in three, cerebral atrophy in four, and a variety of other single anomalies. There did not seem to be a significant difference between the misoprostol-exposed and the unexposed groups.

TABLE 2. SWEDISH CHARGE STUDY: DESCRIPTION OF TYPES OF COLOBOMA AND MICROPHTHALMOS

CONDITION	NO. OF EYES
Coloboma	46
(27 patients, 19 bilateral)	
Iris	4
Iris + uvea	2
Iris + uvea + optic nerve	15
Uvea	5
Uvea + optic nerve	10
Optic nerve	10
Microphthalmos	21
(13 patients, 8 bilateral)	
Microphthalmos + coloboma	15
Iris + uvea	2
Iris + uvea + optic nerve	10
Uvea + optic nerve	1
Optic nerve	2
Microphthalmos	4
(coloboma indeterminable)	

Of the 28 patients, 23 had an evaluation for ASD (Table 7). In 23 patients examined, five met the diagnostic criteria for autistic disorder according to *DSM-IV* and two had autistic-like condition. There was a positive history of misoprostol in three of the five with autism disorder and in one of the two with autistic-like condition. In the "etiology unknown" group, two had autistic disorder and one had autistic-like condition. Because the misoprostol cohort had more cases than the group with unknown diagnosis (17 versus 11), the percentage of ASD seems to be comparable in the two groups. Bandim and associates[43] have reported the detailed psychiatric evaluation.

Although the number of patients with ASD in groups with and without exposure to misoprostol is not sufficient for accurate statistical comparison of subgroups, clinodactyly, equinovarus, mitral prolapse, and involvement of cranial nerves IX and X occurred in each group. This finding provides support for a final common pathophysiology that produces the manifestations of the condition designated as Möbius sequence.

Other malformations and functional disorders also showed a fairly similar percentage in both the misoprostol and the "etiology unknown" group. Psychosocial data, economic information, and more detailed clinical reports are found in Ventura's thesis.[33]

DISCUSSION

Autistic disorder is not a rare condition; estimated prevalence is about 1 to 2 individuals per 1,000. If one expands the diagnosis to include all variants of ASDs, the estimate increases to about 0.5% to 1% of the general population.[44-46] Even if we consider only the patients with the full autism syndrome (autistic disorder), the rates of autism in thalidomide exposure, Möbius sequence, CHARGE association, and Goldenhar syndrome are unquestionably significant.

The diagnosis of ASD is based on clinical characteristics and an established interview questionnaire with parents. The clinical behavioral characteristics include difficulties in social interaction, often with impaired verbal and nonverbal communication. Often individuals with ASD show very restrictive habits of behavior and interests and a need for routine schedules. There is often a lack in cognitive play and poor interpersonal sensitivity. Mental retardation is common, but a subset of those with ASD have normal or about average intelligence (Asperger syndrome).

There is no recognized biomarker, no consistent radiologic imaging abnormalities, and no consistent evidence of a time or location of developmental disturbance of the brain responsible for ASD. The evidence for a genetic component is high but complex, with many genes implicated as risk factors. Whereas most individuals with ASD do not have obvious systemic malformations, there is a subset of individuals with autism that have recognizable associated conditions.

This report summarizes two previous studies in the literature and three newer studies in which there is a group of individuals with the characteristic findings of ASD and also the systemic and functional findings indicating an early insult in embryonic development. This does not imply or mean to suggest that most cases of ASD result from a similar developmental disturbance, but the observations add a few more pieces of the puzzle of this complex neuropsychiatric disorder.

The research interest in this area was initiated by a somewhat serendipitous observation of four individuals with thalidomide embryopathy who demonstrated the classic behavior of severe autistic disorder. Although this Swedish study of 86 individuals with thalidomide embryopathy was prospective, it was ophthalmologically oriented and psychiatric data were not collected in an organized manner; psychiatric consultation was required to confirm autism in these four individuals.[1,3,10] A search of the literature on thalidomide embryology indicated that teratogenicity occurred only in days 20 to 36± after fertilization and that the cranial nerve involvement and autism by association were in the early sensitive period of about days 20 to 25 after fertilization.[4,5,7,8,10] Some literature evidence of ASD in a condition with similar findings, Möbius sequence, prompted a follow-up prospective

TABLE 3. SWEDISH CHARGE STUDY: FINDINGS ASSOCIATED WITH PATIENTS WITH AUTISM SPECTRUM DISORDER

CASE	AGE (YR)/SEX	AUTISM	OCULAR FINDINGS COLOBOMA*	OCULAR FINDINGS MICROPHTHALMOS†	HEART	CHOANAL ATRESIA†	DEVELOPMENT DELAY‡	GENITAL†	EAR§ EXT	EAR§ HEARING	OTHER ANOMALIES/ FUNCTIONAL PROBLEMS
1	5M	AD	++	+	0	0	++	Cryptorchism micropenis	+	+	Short stature, spine, hand, dysphagia
2	6F	AD	++	+	PDA, Fallot	+	++	Labial hypoplasia	+	+	Short stature, spine, dysphagia
3	7F	AD	++	+	ASD, VSD, PDA	0	++	0	+	0	Cleft palate, anal atresia, renal, spine, dental, dysphagia
4	13M	AD	++	0	PDA	+	++	Cryptorchism	+	+	Cleft palate, trachea esophageal fistula, dental, short stature, dysphagia
5	16F	AD	++	+	PDA, ASD	0	+	0	+	0	Cleft lip/palate, short stature, dental, dysphagia
6	4M	ALC	+	0	0	0	+	0	+	+	Short stature, dysphagia, facial nerve
7	5F	ALC	++	0	PDA, VSD, PS	+	++	0	+	+	Facial nerve, TE fistula, anal atresia, limb, dysphagia
8	14M	ALC	0	0	0	0	0	Cryptorchism, micropenis	+	+	Facial nerve palsy, delayed puberty, balance, short stature, spine
9	17F	ALC	++	+	0	+	++	0	+	+	Craniosynostosis, balance
10	18F	ALC	++	0	0	+	++	Labial, hypoplasia	+	+	Delayed puberty, limb, dysphagia

AD, autistic disorder; ALC, autistic-like condition; ASD, atrial septal defect; EXT, external; PDA, patent ductus arteriosis; PS, pulmonary stenosis; TE, tracheoesophageal; VSD, ventricular septal defect.

*++ = bilateral; + = unilateral; 0 = absent.

†+ = present; 0 = absent. - =

‡++ = severe; + = mild; 0 = not present.

§+ = malformation or functional deficit present; 0 = no malformation or functional deficit present.

multidisciplinary study, which revealed a surprisingly high presence of ASDs.

The three studies reported here are a natural extension of interest in syndromes/sequences with characteristic evidence of early developmental errors of ocular structures, cranial nerves, and systemic organs and more than chance presence of autism syndrome disorders. The Swedish team involved in the Möbius study decided to continue and selected CHARGE association and Goldenhar syndrome because of a few case reports of ASD in these conditions.

An "association" is a characteristic group of anomalies observed in patients seemingly more than by chance, but usually without a definite etiologic diagnosis. One example is the CHARGE association initially described by Pagon and associates.[34] They proposed the acronym "CHARGE" be used to describe the characteristic findings (***C***oloboma, ***H***eart, choanal ***A***tresia, ***R***etardation,

TABLE 4. GOLDENHAR/HFM STUDY: SUMMARY OF MOST FREQUENT MALFORMATIONS AND FUNCTIONAL PROBLEMS (N = 20)

PROBLEM	NO. (%)
Cranial nerve involvement	
Facial nerve palsy	8/20 (40)
Neurosensory deafness	3/20 (15)
Systemic/ocular malformations	
Microsomia	15/20 (75)
Ear tags	14/20 (70)
Ocular dermoids	13/20 (65)
Lipodermoid	13/20 (65)
Epibulbar dermoids	10/20 (50)
Gastrointestinal	11/20 (55)
Microtia	10/20 (50)
Vertebral anomaly	10/20 (50)
Genitourinary	7/20 (35)
Developmental delay	7/20 (35)
Cardiovascular	6/20 (30)
Fistula	4/20 (20)
Functional problems	
Hearing	14/20 (70)
Severe developmental delay	4/19 (21)
Autism spectrum disorder	3/18 (17)

HFM, hemifacial microsomia.

that caveat that the most frequent malformations reported in this association are colobomas, choanal atresia, and heart defects, there is a strong suggestion from a recent review of cases by Källén and associates[47] that microphthalmia/anophthalmia be included in the "C"; that penis hypoplasia was frequently associated; that "R" includes other brain malformations; and that growth retardation or developmental delay was common and heart defects were frequent but nonspecific. Also, cleft lip or palate occurred in 15% to 20% of CHARGE patients. Blake and associates[39] estimated the prevalence of CHARGE at about 1 in 10,000. Byerly and Pauli[40] brought attention to the many children with CHARGE association who have facial nerve palsies and feeding and swallowing difficulties and reported a case of CHARGE association with Möbius sequence. Blake and associates[39] proposed that cranial nerve dysfunction (anosmia, facial nerve palsy, sensorineural deafness and vestibular problems, swallowing difficulties) be considered a major criterion. The involvement of cranial nerves may be a thread that exists with CHARGE association, thalidomide embryopathy, and Möbius sequence. A common pathway for CHARGE and oculoauriculovertebral spectrum has been suggested by Van Meter and Weaver.[53]

Whereas most cases of CHARGE are sporadic with unknown etiology, there are some familial cases and some associated with chromosomal anomalies.[54-56] Many features suggest defects in neural crest cell development or migration, which led some investigators to suggest that

TABLE 5. GOLDENHAR/HFM STUDY: FINDINGS ASSOCIATED WITH PATIENTS WITH AUTISM SPECTRUM DISORDER

CASE	AGE(YR)/SEX	ABNORMAL EAR (EXTERNAL)	DECREASED HEARING	DERMOID	AUTISM TYPE	OTHER MALFUNCTIONS/ SYMPTOMS
1	4/M	0	+	Epibulbar lipodermoid	AD	Cardiovascular, gastrointestinal anomalies, mandibular hypoplasia, microphthalmos
2	16/F	+	+	Epibulbar lipodermoid	AD	Limb, gastrointestinal, genitourinary, vertebral
3	6/M	(Microtia, ear tags, fistula)	+	Epibulbar lipodermoid	ALC	Vertebral, facial palsy, gastrointestinal

AD, autistic disorder; ALC, autistic-like condition; OAV, oculoauriculovertebral dysplasia syndrome; symbols: + = present, 0 = normal.

Genital and ***E***ar anomalies). The diagnostic criteria in this CHARGE study were the presence of four of these six characteristics, or three characteristics plus other malformations that have been frequently reported by other investigators. Subsequent reports in the CHARGE literature have suggested some modifications of the core diagnostic group.[39,40,47-52] Since the findings cover multiple disciplines of medicine, there is often a bias of ascertainment in any series reflecting the population evaluated, the interest of the investigators, or the sophistication of the examination of any given organ or structure. Even with CHARGE association should be considered in the group of neurocristopathies.[57] Why ASD exists in a significant number of cases is still a mystery. However, the time of initial embryonic insult is necessarily early, because ocular colobomas, a significant characteristic, are caused by failure of closure of the embryonic fetal fissure by about the sixth week, although according to the thalidomide study, an earlier insult (25 to 27 days ±) can result in an ocular coloboma.

Hemifacial microsomia is a descriptive term used by Gorlin[58,59] and others to characterize a group of patients

TABLE 6. BRAZILIAN MÖBIUS STUDY: SUMMARY OF MOST FREQUENT SYSTEMIC MALFORMATIONS AND FUNCTIONAL PROBLEMS* (n = 28)

PROBLEM	NO. (%)
Cranial nerve involvement	
Abducens (sixth)	28/28 (100)†
Facial (seventh)	28/28 (100)†
Trigeminal (fifth)	5/28 (18)
Hypoglossal (twelfth)	5/28 (18)
Tearing symptoms‡	21/28 (75)
Systemic malformations	
Cleft lip/palate/uvula	13/28 (46)
Micrognathia	18/28 (64)
Limb	22/28 (79)
Tongue (microglossia/asymmetry/function)	14/25 (56)
Poland syndrome	1/28 (4)
Functional problems	
Mental retardation	14/23 (61)
Hearing	8/28 (29)
Sucking in infancy	18/28 (64)
Dysphagia	9/28 (32)
Autism spectrum disorder (AD, ALC)	7/23 (30)
Seizures	12/27 (44)

AD, autistic disorder; ALC, autistic-like condition.

*Some patients did fail appointments for some examinations, and some were too young or difficult to examine.

†Inclusion criteria.

‡A few were suggestive of abnormal innervation of the lacrimal system, and some may have been secondary to facial nerve palsy.

who manifest a spectrum of malformations involving the ear, mandible, mouth, eye, and, often, the cervical spine. The findings occur unilaterally in most, but not all, patients. It is usually sporadic, but family occurrences, especially with only a few anomalies, have been reported. Goldenhar syndrome has been proposed to represent a variant of this entity.[59] Initially, Goldenhar[60] described a number of patients with a combination of epibulbar dermoids, lipodermoids, and preauricular skin tags and fistula. Later, upper lid coloboma and facial and vertebral anomalies became appreciated as part of the syndrome. Duane syndrome has been reported in a number of patients with Goldenhar syndrome but is not a common characteristic. Although no consistent evidence of pathophysiology or etiology is accepted, disruption of embryonic vasculature has been suggested as one mechanism to explain the observed findings. The time of embryonic insult is more difficult to pinpoint in Möbius sequence or Goldenhar syndrome than it is in thalidomide embryopathy, but best estimates are early in pregnancy, probably around 4 to 6 weeks of development. Lam[61] proposed that ectodermal nondisjunction involving the otic placode could produce the malformations seen in Goldenhar syndrome. If correct, this would explain the multisystem findings and also place the time early in the fourth week. Another suggestion is that Goldenhar syndrome can be a result of "reproductive wastage" in high-risk conceptions, based on one case of possible monozygotic twins conceived by in vitro fertilization and embryo transfer.[62] There were a few in vitro fertilization cases in our series also. These observations support the concept of a nonspecific early embryonic event resulting in a malformation complex. If correct, this makes the association with ASD even more intriguing. Although the number of cases of autistic disorder or autistic-like condition in our Goldenhar group (3 in 18) was not as high as in the Möbius studies, it certainly is more than chance, with the estimated prevalence of autism being 1 to 2 per 1,000.

In the early 1990s there were a number of case reports in the Brazilian literature of infants born with malformations involving limbs, cranial nerves, and other anomalies following self-induced but failed abortions.[23-28] The abortifacient drug used was misoprostol, a prostaglandin type E analogue. In some of these reports, the children exhibited the typical findings of Möbius syndrome with and without limb anomalies.[29-32]

Misoprostol as a drug for self-induced abortions has gained much popularity in South America, especially Brazil, where abortions are not legal except in a few situations.[27,63] It was estimated to be used in more than 50% of attempted abortions in some areas of Brazil. Misoprostol was cheap and readily available because of its accepted use in medical conditions such as gastric ulcers and arthritis.[64,65] Medically induced abortions have advantages over clandestine abortion from unlicensed "professionals." They avoid risk of anesthesia and surgical complications in unclean environments and, perhaps most important, can be done in privacy. Misoprostol is also utilized for planned abortions, conducted by medical professionals in many countries, but almost always combined with another drug, such as mifepristone.[66,67] However, misoprostol alone is a poor abortifacient drug, and many pregnancies continue to term. It appears to be a fairly weak teratogen, since the reported percentage of malformation is low, but because of its tremendous popularity as an abortifacient drug for self-induced abortions, even low-incidence complications such as Möbius sequence occurred in sizable numbers.

The Swedish study of Möbius, along with reports in the Brazilian literature of the association of Möbius sequence and misoprostol, prompted a prospective multidisciplinary study in Brazil by Ventura and associates.[33] It was designed to be descriptive of malformations and func-

TABLE 7. BRAZILIAN MÖBIUS STUDY: FINDINGS IN PATIENTS WITH AUTISM SPECTRUM DISORDER

CASE	AGE (YR) /SEX	MISOPROSTOL EXPOSURE	STRABISMUS PRIMARY POSITION	FACIAL NERVE PALSY	OTHER CRANIAL NERVES	AUTISM TYPE	CARS SCORE*	MENTAL RETARDATION†	OTHER ANOMALIES/ FUNCTIONAL PROBLEMS
1	4F	+	Straight	+	9th, 10th	AD	39	+	Cleft palate, micrognathia, clindactyly, calcification of brain stem
2	2F	0	ET	+	9th, 10th	ALC	38	+	Arthrogryposis, micrognathia, club foot, clindactyly, arachnoid cyst, hydrocephaly, polymicrogyria, cerebral atrophy, cleft uvula
3	11M	+	ET	+	9th - 12th	AD	45	+	Cleft palate, club foot, arthrogryposis, normal MRI
4	9F	0	XT	+	9th, 10th	AD	46.5	+	Mitral valve prolapse, clindactyly, club foot, Dandy-Walker anomaly
5	3M	0	ET	+	9th, 10th	AD	47.5	+	Cerebral atrophy, calcification of brain stem, cleft uvula
6	2M	+	ET	+	9th, 10th	ALC	29	+	Cleft uvula, micrognathia, club foot, normal MRI
7	2F	+	Straight	+	9th, 10th	AD	38	+	Clindactyly, stenosis of aqueduct of Sylvus, hydrocephalus

AD, autism disorder (*DSM IV* criteria); ALC, autistic-like condition; CARS, Childhood Autism Rating Scale; ET, esotropia; MRI, magnetic resonance imaging; XT, exotropia; symbols: + = anomaly present, 0 = absent.
*Median score for Möbius cases without autism, 18.4.
†Per Wechsler Intelligence Scale for Children.

tional disorders in all study patients and also to compare the findings of those whose mothers took misoprostol early in pregnancy with the findings of those with no exposure history. Additionally, there was interest to see if ASD occurred in some patients.

The Brazil study is the pièce de résistance with new, key pieces to the puzzle of autism, reaffirming observations in the other four studies of ASD associated with cranial nerve, ophthalmic, and craniofacial malformations. In addition to thalidomide, it introduced a second teratogen, misoprostol, associated with ASD. It supports the conclusions of the Swedish Möbius study that ASD has a significant association with Möbius sequence, but in another ethnic group with another research team.[18] And perhaps the most exciting observation in the South America study was that ASD occurred in individuals with and without known exposure to misoprostol during their mother's pregnancy.

Another very interesting finding is the marked increase in cases of Möbius sequence in some areas of South America after misoprostol became a commonly used abortifacient drug. The fact that Ventura[33] and her multidisciplinary team were able to recruit 28 cases of Möbius sequence in 6 months from only one surrounding region is remarkable. It is probably explained by the 17 cases that had known misoprostol exposure.

A number of questions come to mind when looking at these three present studies and the previous thalidomide and Swedish Möbius study. The first might be whether the cases with ASD in these conditions were chance occurrences or represented some study bias. To answer this concern, the prevalence of autism must be analyzed along with the methodology of all studies. In the thalidomide study, the rate of ASD was the lowest, with four of 86 proven by formal neuropsychiatric examinations to have the full-blown autistic disorder.[2] Since only five individuals with severe neuropsychiatric behavior noted by the pediatric ophthalmologists doing the study were referred for psychiatric evaluation, these numbers could easily be an underestimation of what might be present if the study would have included neuropsychiatric behavior data in all study patients. Even so, this rate of 46 per 1,000 is significant. The Swedish Möbius study in the literature was more dramatic, reporting six of 23 study patients showing the full autistic disorder and one with autistic-like condition (a prevalence of 260 in 1,000). The Brazilian Möbius/misoprostol study had five of 23 patients meet the criteria for the full autistic disorder and two for autistic-like condition. The Swedish CHARGE association indicated that in the individuals old enough to be evaluated, five had autistic disorder and five autistic-like condition. In the 18 patients able to be tested in the

Goldenhar/HFM study, two had autistic disorder and one had autistic-like condition. The diagnosis in all studies was made by experienced child psychiatrists utilizing more than one of the accepted diagnostic tools of the *DSM-III-R* and *DSM-IV* criteria and the *International Statistical Classification of Diseases and Related Health Problems, tenth revision* (*ICD-10*) criteria as operationalized in the ADI-R, CARS, and ABC. The recruitment was based on the diagnostic characteristics of the condition and not autism, so there seems little bias of ascertainment.

Why is the coexistence of ASD and systemic conditions not more widespread in a large body of autism literature? The thalidomide observation may be explained by the fact that most of the literature involved infants or young children in whom the extensive limb and other systemic anomalies were more pertinent issues for the individual. There were a few multidiscipline studies of adults, but although there were psychological symptoms, the diagnosis of autism was not made.

Autism coexisting in individuals with Möbius sequence, CHARGE association, and Goldenhar syndrome is reported in the literature, but not in the degree noted in these four studies. However, after attention had been drawn to the possibility of this association, we have been informed of a number of unreported cases, especially with Möbius sequence. For example, there is a Möbius support group, and requests have been made to address the subject of autism at their meeting because of a number of parents with concerns about the behavior of their children.

Another question might be, Is there a common thread or pathway that ties the patients with Möbius sequence, CHARGE association, and Goldenhar syndrome together? The most common characteristic of the anomalies associated with autism in these studies is that they result from an early adverse embryonic event. The literature can be confusing and must be read carefully when it relates to embryonic timing issues. "Gestational age," used by obstetricians and many others, is calculated from the last menstrual period. A gestational age of 1 month of an embryo is actually 2 weeks postconception/fertilization. These 2 weeks are certainly not trivial when looking at early embryonic events. The final studies described events by the actual age of development of the fetus. The most reliable timing is developmental insult, as in the thalidomide data, in which the four individuals with autism were exposed to thalidomide 20 to 25 days postfertilization because of their associated malformations (Duane syndrome, facial nerve palsy, ear anomalies). The next evidence of embryonic timing is from the misoprostol group. Although less precise, it appears that exposure was at 4 to 6 weeks (6 to 8 weeks from the last menstrual cycle). In Möbius patients with unknown etiology, there is no definite information except that it seems consistent with the misoprostol group to be early (eg, 4 to 6 weeks). There are cases reported with later adverse pregnancy events associated with Möbius sequence, so one has to be careful not to make absolute conclusions. The least established timing is in the CHARGE group, although we know the ocular embryonic fissure is closed in around the sixth week of embryogenesis, so the insult must be some time before that date. From the thalidomide timetable it could be as early as the late fourth week. The Goldenhar group seems to be at 4 to 6 weeks of embryogenesis, based on associated anomalies.

Although the coexistence of ASD with these reported conditions is quite convincing, the proposed pathophysiology mechanisms are not clear. Comprehensive reviews of possible pathophysiology in thalidomide have not resolved the issue. Stephens and associates[68] noted that although there were 2,000 papers published in the last 40 years concerning thalidomide teratogenicity, the mechanism of action still remains elusive.

In the sporadic cases of Möbius sequence, there is some agreement in the literature as to possible causal mechanisms. A popular theory is that it belongs in a group of "disruption syndromes," although there is disagreement as to the causes of the embryonic disruption. The most frequently stated cause is that of a vascular disruption in the early embryonic period. Some investigators refer to it as the "subclavian disruption syndrome."[69,70] They postulate a primary vascular disruption causing hypoxia, ischemia,[71-73] edema, and hemorrhage, followed by secondary events that may affect other organs. The timing extent of this hypoxic event will determine the ultimate malformations based on the sensitive tissues at the time of the hypoxia.

There are clinical case reports that support the vascular disruption concept. For example, malformations suggestive of Möbius sequence have been reported in fetuses exposed to cocaine, presumably causing vasoconstriction of the uterine vessels.[74,75] Also, chorionic villi sampling has been suggested as an occasional cause of limb anomalies and of Möbius sequence, although there are reports both supporting and refuting this association.[76-79] Möbius cases have been associated with hypovolemia in a splenic bleed during pregnancy and inadvertent exposure to ergotamine with apparent uterine constriction.[80-82] A few children with Möbius sequence have a history of polyhydramnios in pregnancy.[72] In the Swedish Möbius study and other studies, there was an apparent increase of bleeding in early pregnancy reported without known precipitating causes, and also one case with a history of chorionic villi sampling procedure. Courtens and associates[83] reported a case associated with a history of exposure

to benzodiazepines. The apparent common characteristic of all these cases in the literature is an early adverse pregnancy event resulting in a possible short period of hypoxia brought on by disturbance in the blood supply from uterine constriction. This line of reasoning received support by the association of misoprostol taken early in pregnancy in failed abortion attempts resulting in infants with characteristic findings of Möbius sequence.

Another type of disruption was proposed by Bamforth,[84] who described a process termed "organizational disruption" (blastogenic disruption) as an explanation for some of the observed malformations, suggesting that it is a better explanation for some phenotypes. He proposes that there are a group of organizational molecules (morphogenes), highly conserved and determined by chromosomes in a sequential manner, that are important in the early stage of organization. This organization is imposed on embryonic cells by activation determined by homebox genes. If something interferes with the organization of these morphogenes, higher or lower concentrations may cause activation of genes at inappropriate times, which could result in malformation of organs or histologic development. This theory is perhaps compatible with the observations that some of the *HOX* gene defects in the animal models result in the same brain-stem malformations that are sometimes associated with autism in humans.

How do we get from early-onset insult that seems to affect multiple brain-stem structures to autism disorders that involve higher centers not yet formed? Is there a group of unidentified cells that are even at this time programmed for a higher brain center, which are damaged, or is there an interruption in a series of connections ultimately crucial for higher centers to develop correctly? These are key questions, but we may only be able to speculate about answers at this time on some evidence from other studies of the associated conditions or malformations.

Rodier and associates[85] noted almost complete absence of facial nuclei and shortening of brain stem in a patient with autism. In magnetic resonance imaging (MRI) studies by Hashimoto and associates[86] and Cody and associates,[14] the MRI findings in individuals with autism are noted. Radiologic abnormalities, albeit not consistent or conclusive, have also been reported in Möbius syndrome. Some include abnormalities of the brain stem.[87-89] There are a number of literature reports of central hypoventilation, brain-stem changes, and, in some, Möbius sequence.[90,91] Marques-Dias and associates[92] reported neuropathologic findings in three cases of Möbius syndrome related to misoprostol, finding calcification of the brain stem involving some of the cranial nerve nuclei. They felt this was due to vascular disruption. In the Brazilian Möbius study, there was a variety of MRI abnormalities, the most common being brain-stem calcification.

Aberrant innervation does not occur frequently in nature, and yet examples of aberrant innervation of brain-stem structures appear throughout these studies; the most striking is in thalidomide embryopathy with Duane syndrome and paradoxical lacrimation. We note aberrant lacrimation in patients with Möbius sequence. Abnormal tearing symptoms were also present in many in the Brazilian study, in both the misoprostol related and "etiology unknown" groups. Amaya and associates[93] noted excessive lacrimation in 11 of 18 cases in a series of Möbius cases, with three patients also manifesting tearing when eating. It is interesting that early in embryogenesis the sixth and seventh cranial nerve nuclei and lacrimal nuclei are in close proximity. Destruction or failure of development of these structures might result in aberrant repair processes with inappropriate innervation. There is also a literature report of another type of paradoxical innervation, Marcus Gunn jaw winking, with CHARGE association.[94] Local vulnerability and critical time may be the necessary factors for these neurologic mismatches to occur.

In conclusion, although autism may result from a variety of mechanisms and causes, evidence from the thalidomide, Möbius, CHARGE, and Goldenhar studies seems to establish quite firmly that early insults in embryogenesis, often involving brain-stem structures, may be associated with ASD.

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DISCUSSION

Dr Gerhard W. Cibis. The lead authors of this presentation, Drs Miller and Strömland, were the first to report the association of autism in 5 percent of Thalidomide victims.[1] Most importantly, they were able to pinpoint the timing of toxicity to days 20-24 after conception based on their autism patients having external ear anomalies but no involvement of the limbs, which occurs days 25-35. In this study, the relationship of autism with ophthalmic disorders involving cranial nerves, specifically CHARGE syndrome, MOEBEUS sequence GOLDENHAR syndrome and MISOPROSTOL exposure, are added. The Brazilian cases show autism in a racially different population than the more homogenous Swedish population.

The cause of autism is unknown.[2] Very few neurons form as early as the fourth week of gestation; most that do are cranial. The brainstem in autism is shorter than normal. The facial nucleus and trapezoid body are closer to the hypoglossal nucleus and inferior olive. The superior olive is missing and the facial nucleus is smaller than normal. Such changes can occur only in early gestation. Behaviors disturbed in autism such as language and planning and interpretation of social cues are of course not brainstem but higher brain functions of the cerebral cortex and hippocampus.

This study links autism to cranial nerve disruption from early embryologic events before the higher regions of the brain are even formed. As the authors point out, presumably the evolution of unidentified cells into higher brain structures is somehow damaged or altered. It need not be the cells themselves directly that are damaged but the evolutionarily highly conserved homeobox control genes that determine embryogenesis. Rodier[2] has found variants in HOXA1 allele to be twice as common in autism patients as in their non-autistic family members. HOXA1 is thought to be only one of many genes involved in the spectrum of autism disorders. Patients with autism have a reduction in the number of neurons in the cerebellum that controls muscle movement. Parts of the cerebellum are activated during certain tasks requiring high-level cognitive processing. Some symptoms common in autism, such as lack of facial expression, hypersensitivity to touch, sound and sleep disturbances, could be associated with the more primitive brain stem functions. Our understanding of the neurologic sources of autism is still poor.

Fetal alcohol mouse model confirms a similar disruption of later brain and eye formation. In ETOH patients, the electroretinogram shows abnormalities reflecting abnormal retinal circuitry.[3] An ERG study of autism patients might be of interest in that regard.

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Dr Irene H. Ludwig. A number of years ago, I reported on a group of patients who were somewhat similar: They had congenital, central hypoventilation syndrome, which is a brain stem disorder affecting respiratory control, and they also had similar eye findings to your thalidomide groups. In the studies of those families, the pulmonologists were never able to identify a genetic cause. But, they did find geographic clustering, suggesting that there may be an environmental influence. Have you looked for environmental teratogens in your groups? The group of hypoventilation syndrome patients also had very high incidence of autism and ADD-type disorders.

Dr John T. Flynn. Marilyn Miller is like Archibald Garad, the great English teratologist, who by minute observation of these abnormalities began the whole study of teratology

in the 19th century. Marilyn has taken us on a tour of the brain stem and the malformations that occur by exposure to different kinds of teratogens. I think the central thread of your studies is similar to a generalization that John Opitz, the American teratologist, made: the organism reacts to a whole series of insults in very stereotyped ways. The insults can be many, but it is the time when the insults are delivered that causes these different anomalies.

Dr Marilyn T. Miller. Dr Ludwig brings up something interesting. A number of the Möbius patients also have difficulty with ventilation and there's no question there is a spectrum of problems that are somehow related to the time of insult. We probably did not look sufficiently for environmental factors; we queried extensively the pregnancy histories in both the Swedish study and the Brazilian study. In the Swedish study, there did not seem to be any factors except that there were more histories of bleeding early in pregnancy. The Brazilian study was also not fruitful in the pregnancy history, except in that case we were dealing with the misoprostol exposure, so obviously these women had histories of bleeding because they attempted abortions. Most of them had about 12 or 24 hours of uterine contractions and some bleeding, but they didn't abort and they went on to term.

Dr Flynn, that is my favorite quote from Opitz. There's no question that we're dealing with time sensitivity and structures that are developing. There is a lot of speculation in the literature what these reasons are, but the brain stem structures seem to be very sensitive to hypoxia at that time. That is also the time that the embryo is folding, and some people have speculated that makes it even more sensitive. It is probably a matter of the sensitivity of the area and the timing. Whether autism is related to other structures or to the brainstem is not known. In the Brazilian study, we performed MRIs and CTs on 25 of the 28 with 15 demonstrating abnormalities, mostly with signs of necrosis or calcification, but also a variety of nonspecific findings too.