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Diagnostic distinctions and genetic analysis of patients diagnosed with Moebius syndrome

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Abstract

Objective—To improve diagnostic assessment in Moebius syndrome by (1) creating more selective diagnostic subgroups and (2) conducting genetic evaluation in a large patient cohort.

Design—Prospective, observational study.

Participants—Attendees of 3 consecutive Moebius Syndrome conferences held in the United States, with a prior diagnosis of Moebius syndrome were invited to participate.

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Methods—Participants underwent standardized ophthalmologic examination for Moebius syndrome minimum diagnostic criteria (MDC) (congenital, nonprogressive facial palsy and abduction deficit) and genetic testing for *HOXA1*, *HOXB1*, and *TUBB3* mutations.

Main Outcome Measures—Number of patients meeting MDC and number with confirmed genetic mutation.

Results—A total of 112 participants from 107 families enrolled. Nineteen percent of participants (21/112) did not meet accepted MDC for Moebius syndrome because they had abduction deficits without facial palsy or facial palsy with full ocular motility. All five families with two affected individuals had at least one family member in this category, including two siblings with comitant strabismus who harbored a *HOXB1* mutation. Four unrelated participants, also not meeting MDC, had large-angle exotropia, vertical gaze deficiency, and ptosis consistent with congenital fibrosis of the extraocular muscles type 3 (CFEOM3); 1 harbored a novel and 3 harbored previously reported *de novo* *TUBB3* mutations. Three percent of participants (3/112) met MDC but also had restricted vertical gaze. The remaining 88 participants (79%) met MDC and had full vertical gaze. This group had relatively homogeneous findings and none had a family history of Moebius syndrome. Two previously undescribed phenomena were observed in this category: 1) volitional Bell's phenomenon, and 2) intorsion with fixation.

Conclusions—While the genetic contributors to classic Moebius syndrome remain elusive, accuracy in clinical evaluation will properly subdivide patients to facilitate genetic testing as new candidate genes are identified. Failure to test ocular motility may lead to misdiagnosis of Moebius syndrome, especially in patients who have facial palsy with full ductions. Patients with exotropia, vertical gaze limitation, and ptosis do not have classic Moebius syndrome and may have *TUBB3* mutations associated with CFEOM3. To optimize genetic analysis, we propose adding “full vertical motility” to the minimum diagnostic criteria for Moebius syndrome.

INTRODUCTION

Moebius syndrome is a complex, rare developmental anomaly of the hindbrain that has been described historically as the combination of congenital palsies of the abducens and facial nerves,¹ frequently with additional features including orofacial malformations, limb defects, and musculoskeletal, behavioral, and cognitive abnormalities.²⁻⁷ While a compilation of clinical,⁸⁻¹² neuropathological,¹³⁻¹⁸ and radiological¹⁹⁻²¹ reports suggest that Moebius syndrome is not a single disease entity,²² these and many other studies of the condition are confounded by variable diagnostic criteria.^{22, 23} This lack of standardized diagnostic criteria complicates the clinical assessment, determination of prognosis, and genetic analysis of Moebius syndrome patients. To address this concern, a group of clinicians and researchers met in 2007 at the bi-annual Moebius Syndrome Foundation research meeting and defined the minimum diagnostic criteria for classical Moebius syndrome as “congenital, uni- or bilateral, non-progressive facial weakness and limited abduction of the eye(s).”¹¹

The purpose of the current report is to characterize the ocular and facial phenotypes of a large number of individuals diagnosed with Moebius syndrome in order to determine whether they meet minimum diagnostic criteria. We also screened participants' DNA for

mutations in the *HOXA1*, *HOXB1* and *TUBB3* genes (reported to cause atypical forms of Moebius syndrome)²⁴⁻²⁷ in an attempt to identify genetic etiologies underlying the disorder.

METHODS

Research participants with a prior diagnosis of Moebius syndrome and their family members were recruited from three consecutive international Moebius Syndrome Conferences organized by the Moebius Syndrome Foundation (8th, 9th, and 10th conferences held in Parsippany, New Jersey, Broomfield, Colorado, and Philadelphia, Pennsylvania respectively) into an ongoing study of the genetics of strabismus approved by the Boston Children's Hospital institutional review board. The study was compliant with the Health Insurance Portability and Accountability Act and adhered to the tenets of the Declaration of Helsinki.

Each participant provided written informed consent and completed a family, medical, and ophthalmic history questionnaire, with emphasis on known associations with Moebius syndrome. Medical, systemic, or neurological abnormalities and developmental or behavioral disorders were identified. Prior ocular or facial surgeries were documented. Each participant also donated a blood or salivary specimen for DNA extraction.

Probands and, when available, parents and family members underwent ophthalmological evaluation, testing of facial movement and strength, and assessment for a subset of additional anomalies associated with Moebius syndrome. Ophthalmological exam included visual acuity testing using developmentally appropriate symbols (letters or HOTV test), non-cycloplegic autorefraction (Retinomax K-Plus 2, Right Manufacturing, Tokyo, Japan), pupil, external and anterior segment evaluation by handlight, and sensorimotor evaluation. External examination of the participants involved measurement of eyelid position and facial nerve function. Sensorimotor evaluation included evaluation of eye movements (ductions and versions), prism-and-cover testing, and sensory testing (stereopsis and Worth 4-dot). To quantify ocular motility a standard 0-8 scale was used.²⁸ On this scale 0 indicated full duction (no limitation), and -4 indicated that the eye was able to reach, but not move past, midline. In rare cases, if the eye was fixed in the opposing gaze, a score of > -4 (maximum -8) was given. Duction limitation was then categorized as "mild" (-1 limitation), "moderate" (-2 limitation), or "severe" (>-2 limitation). Convergence was classified as "normal" (near point of convergence (NPC) $\leq$ 10 cm), limited (NPC 11 cm to 50 cm), or "remote" (> 50 cm). Slit lamp biomicroscopy, cycloplegic refraction, and dilated fundus examination were not possible at time of enrollment; however, past ophthalmological records were obtained when available.

Genomic DNA was extracted from each participant's blood or saliva sample using Qiagen Gentra Puregene DNA isolation kits (Valencia, CA) or DNA Genotek Oragene saliva and assisted saliva swab kits (Kanata, Ontario, Canada). Mutation analysis was performed by PCR amplifying all exons and exon-intron boundaries of the *HOXA1*, *HOXB1*, and *TUBB3* genes. All the resulting amplicons were Sanger sequenced, and primer sequences and screening conditions are available upon request. Subsets of the mutation positive participants have been reported previously.^{25, 26}

RESULTS

A total of 112 affected individuals from 107 families were enrolled and examined. Of these, 5 families included 2 affected members, while 102 affected participants were simplex cases. Age ranged from 6 months to 62 years (median 8.5 years). Individuals were from multiple ethnic backgrounds and geographical regions (over 4 continents). All individuals had been given a diagnosis of Moebius syndrome prior to their enrollment in this study.

Participants were grouped based upon presence or absence of abduction limitation and facial palsy, which define the currently accepted minimum diagnostic criteria (MDC) for Moebius syndrome. MDC were present in 91/112 participants, while 21/112 participants did not meet MDC despite having been given the clinical diagnosis of Moebius syndrome by their physician (Figure 1).

Participants not meeting MDC for Moebius syndrome

Twenty-one participants lacked either facial palsy or abduction limitation and thus did not meet MDC (Figure 1A). One participant had an abduction deficit but no facial palsy and did not harbor a mutation in any of the genes sequenced (Figure 2 B, F-H).

Sixteen participants had facial palsy but full ocular motility. Of these, 5 had no strabismus (2 of whom were mother and son), while 11 had comitant strabismus (Figure 2 A, C-E). One of the 11 had a *history* of unilateral limited abduction, but full abduction was observed on examination (after strabismus surgery); considering that extraocular muscle surgery never improves abduction in Moebius patients, the participant was felt to have had a pseudo-deficit preoperatively and did not meet minimum diagnostic criteria. Of the remaining 10 with strabismus, full motility, and facial palsy, one had a sibling with ophthalmoplegia (see below) and one had a cousin who met MDC. Four of the 10 were two pairs of siblings, and we previously reported that one of those pairs harbored a homozygous c.619C>T mutation (R207C) in the *HOXB1* gene.²⁵ None of the remaining 14 participants in this group had a mutation in any of the sequenced genes. Thus, while five enrolled families had two affected individuals, in all five at least one member did not meet MDC.

The remaining four participants in this category had incomitant strabismus but did not meet MDC because they did not have abduction limitation. Each had ptosis and a large angle exotropia with severe deficiencies of both adduction and vertical gaze typical of congenital fibrosis of the extraocular muscles type 3 (CFEOM3; Figure 3). These participants had complete absence of adduction with minimal or absent ipsilateral abduction deficits (Figure 3 A-C), inability to elevate either eye (with eyes anchored below the midline in 2 cases) (Figure 3E), mild weakness of eye depression, and severe ptosis. Two participants also had irregular pupils (Figure 3 D and E). All four participants in this group harbored heterozygous *TUBB3* missense mutations.²⁷ We previously reported that two of the participants had *de novo* E410K substitutions.^{26, 27} A third participant was not previously reported, but we found to harbor the previously reported R262H substitution,²⁶ which also arose *de novo*. The fourth participant was found to have a previously unreported c.1229A>T *TUBB3* mutation, predicted to result in a TUBB3 E410V substitution, thus altering the same amino acid residue as E410K. This novel mutation was also *de novo*, and was not present in

dbSNP 132 (<http://www.ncbi.nlm.nih.gov/SNP>, accessed 11/18/13), the Exome Variant Server (<http://evs.gs.washington.edu/EVS>, accessed 11/18/13), nor the 1000 Genomes Project (<http://browser.1000genomes.org/index.html>, accessed 11/18/13).

Participants meeting minimum diagnostic criteria for Moebius syndrome

Ninety-one participants met MDC. Among these, 3 also had limited vertical eye movements, a pattern of ocular dysmotility not typical of Moebius syndrome; these participants were thus categorized separately. One of these participants had an exotropia with a downgaze deficiency; the second had a unilateral frozen globe with ipsilateral complete ptosis; the third (the sibling a participant with facial weakness and comitant strabismus) had a complete lack of both horizontal and vertical eye movements with both eyes essentially fixed in straight ahead position. None of these three participants had a mutation in any of the screened genes.

Eighty-eight participants met full MDC for Moebius syndrome and had normal vertical eye movements, which we refer to as ‘classic Moebius syndrome’ (Table 1, Figure 4). Among these, one had a unilateral facial palsy while the remaining 87 had a bilateral facial palsy, which was recorded as symmetric in 54 and asymmetric in 33. Twenty-nine were excluded from further detailed characterization of ocular alignment and motility due to prior strabismus surgery, leaving 59 with unaltered motility allowing for analysis. Limitation of abduction was severe and bilateral in all 59 participants. A co-existing adduction deficit not exceeding the ipsilateral abduction deficit was present in 49/59 (83%). In 16/59 (27%) the adduction deficit was mild, while in 33/59 individuals (56%) it was severe, resulting in a motility pattern most consistent with horizontal gaze palsy. Convergence was normal in 34/59 (58%) and partially limited in the remainder; no subject had absent convergence. Esotropia was present in more than half of individuals, with the remainder having no manifest strabismus in straight-ahead gaze, and 30/59 (51%) had at least some binocular vision. Dysinnervation was present in 24 subjects (41%) evident most commonly by excessive tearing when eating (crocodile tears) or adduction of both eyes with attempted lateral gaze (synergistic convergence.) Pupils were of normal shape and reactivity in all cases.

Two novel findings were discovered in our participants with classic Moebius syndrome. The first was *intorsion with fixation*, which was not observed until the final year of enrollment and was confirmed in 14/33 participants evaluated (42%, or 18% of the entire cohort). In these participants, we observed an intorsion movement of the non-fixating eye as it became the fixating eye (Video 1, available at <http://aaojournal.org>). Individuals demonstrating this phenomenon had minimal to no strabismus. The second phenomenon was a volitional Bell’s phenomenon Figure 5, which was noted in 40/88 (43%) participants. In these cases, participants appeared to intentionally and routinely exert an eyelid closure effort; while eyelid closure did not occur due to facial palsy, the accompanying Bell’s phenomenon elevated the eye above the upper eyelid margin (Video 2, available at <http://aaojournal.org>). We presume that this volitional ocular elevation served to lubricate the ocular surface to compensate for the lack of a normal blink.

Almost all of the 88 individuals with classic Moebius syndrome had at least one associated non-ophthalmological anomaly (Table 2), with 81/88 (92%) having more than one. The commonest features were anomalies of the tongue and limb typical of those known to be associated with Moebius syndrome. Many participants also had a history of intellectual and/or social developmental delays (46/88, 52%).

All classic Moebius syndrome cases were simplex, with no known affected family members. One participant had a cousin with facial palsy and strabismus who was given the diagnosis of Moebius syndrome but found to have full motility on our examination. Genetic testing in all 88 participants within this group revealed no mutation in any of the screened genes.

DISCUSSION

Accurate diagnosis of Moebius syndrome is essential to allow for relevant discussions of prognosis, appropriate genetic counselling, and productive genetic discovery and testing. Patients with Moebius syndrome have clinical findings that may also occur in other conditions or in isolation, making it difficult at times to correctly diagnose an individual patient. In this paper we define the steps required to confirm the diagnosis of Moebius syndrome (patients must have *both* facial palsy and motility deficit) and to distinguish classic Moebius syndrome with abduction deficit from atypical Moebius syndrome (with limited vertical gaze with or without primary position exotropia).

Only 91 of the 112 participants believed to have Moebius syndrome met minimum diagnostic criteria. This confusion in diagnosis most likely resulted from the varied phenotypic descriptions used in the literature.^{10, 19, 29-32} In addition, Moebius syndrome is rare, and the expertise of those making the diagnosis varies. For example, patients who have limb anomalies or other features often associated with Moebius syndrome combined either with facial palsy or limited abduction – but not both – might also be diagnosed with Moebius syndrome by clinicians not familiar with the minimum diagnostic criteria. Similarly, if a patient with congenital facial palsy has a coincidental diagnosis of esotropia (which is present in 1-2% of the general population³³), an examiner not experienced in assessing horizontal ductions might incorrectly diagnose Moebius syndrome. The association of congenital facial palsy with comitant esotropia may not, however, be entirely coincidental: for example, the two siblings of consanguineous parents with hereditary congenital facial palsy and comitant esotropia are one of two families with this phenotype reported to harbor a homozygous mutation in the *HOXB1* gene.²⁵ The two affected siblings in the second family were also initially misdiagnosed as having Moebius syndrome.²⁵ *HOXB1* mutations have not been found in patients with classic Moebius syndrome either in this study or the previous report.²⁵

Four participants who did not meet MDC had findings consistent with a diagnosis of CFEOM3. Two harbored the E410K TUBB3 substitution²⁷ and a third harbored a R262H substitution²⁶ previously reported to cause CFEOM3. The fourth participant had a unique TUBB3 E410V substitution and findings consistent with the E410K TUBB3 syndrome. The E410K substitution is now referred to as the ‘TUBB3 E410K syndrome’ with clinical features that include Kallmann syndrome (hypogonadotropic hypogonadism and anosmia),

stereotyped midface hypoplasia, intellectual and social disabilities and, in some cases, vocal cord paralysis, tracheomalacia, cyclic vomiting responsive to valproic acid, and distinctive MRI anomalies.²⁷ The number of probands harboring TUBB3 E410V and R262H are too few to fully define the resulting phenotype, but both TUBB3 amino acid substitutions also resulted in additional neurological signs and symptoms. It is essential that these patients be differentiated from patients with classic Moebius syndrome to allow for a search for associated findings noted above and treatment for hypogonadotropic hypogonadism and cyclic vomiting when necessary.

There were three participants with incomitant strabismus and abduction limitation who met MDC, but who also had vertical motility restriction. One had exotropia and downgaze limitation, neither of which were observed in any of the 88 Moebius syndrome participants who met MDC. The remaining two individuals had a complete ophthalmoplegia of one or both eyes. It might be argued that this phenotype represents the severe end of the Moebius syndrome spectrum, but this seems unlikely considering the lack of even mild vertical motility restriction in the entire classic Moebius group. Considering that participants with profound motility limitations and, in particular, vertical limitation and/or exotropia were rarely observed and, in 4 of 7 cases, were characteristic of CFEOM3 (with genetic confirmation), all of the participants in this group sometimes described as “atypical Moebius syndrome” likely have a pathophysiology that is distinct from that of classic Moebius syndrome. Thus, to avoid confusion, we recommend that patients with limited vertical gaze and/or exotropia in primary position should not be referred to as having either typical or atypical Moebius syndrome. Others have described patients who met MDC for Moebius syndrome and who also had vertical gaze deficits with or without ptosis.^{3, 11, 34} These include patient 1 in Cronemberger et al,¹⁰ patients 30, 35, and 36 in Verzijl et al,³ the “Moebius Pattern C” patients described by Carta et al,¹¹ as well other published cases described as “Moebius syndrome.”³⁵⁻³⁷ A subset of the patients likely had CFEOM, and in particular those who also had ptosis may harbor *TUBB3* mutations.¹¹

After segregating participants who did not have Moebius syndrome and cases with vertical limitation and/or exotropia from the cohort, the clinical findings in classic Moebius participants became much more consistent. All of those with classic Moebius had significant, bilateral abduction deficits and full vertical gaze along with facial weakness. Of these 41% were orthotropic in primary position (described by Carta as “Pattern A”) while 59% presented with esotropia (“Pattern B”).¹¹ More than 80% had at least a mild adduction deficit and, in the majority, the adduction deficit was severe enough to mimic horizontal gaze palsy. Adduction deficits are frequently reported in patients with classic Moebius syndrome,^{3, 8, 9, 11, 34} with horizontal gaze palsies reported in roughly 50% of patients.^{3, 11} Orthotropia and binocular vision were observed in approximately half of the participants, slightly more than the 41% described by Carta.¹¹ Dysinnervation was also common. No participant with classic Moebius syndrome had a positive family history and none was found to have a mutation in any of the sequenced genes.

Two novel findings were also discovered in our participants with classic Moebius Syndrome. Volitional Bell’s was most likely a learned response to compensate for inadequate lid closure to lubricate the ocular surface. The cause of intorsion with fixation is

less clear. The superior oblique muscle can provide a small amount of abduction. We speculate that in participants with small-angle esotropia and limited abduction, the superior oblique muscle is recruited to abduct the esotropic eye as it attempts to fixate on the object of regard during cover testing, thus intorting the eye. Ventura and colleagues³⁴ noted excyclotorsion (rather than intorsion) during cover testing in one of their Brazilian participants, but that patient also demonstrated gaze evoked nystagmus on upward gaze, which we did not observe. Lacking video recordings of these eye movements,³⁴ it is not possible to determine whether the findings they described are related to our observations.

In this study, our primary concern was to determine whether each individual previously assigned a diagnosis of “Moebius syndrome” met the currently recognized minimum diagnostic criteria to allow for accurate genetic studies. In the process we discovered misclassification of a significant percentage of such participants. It is vital that clinicians carefully evaluate ocular ductions in individuals suspected of having Moebius syndrome, especially in patients with facial palsy who have strabismus; if horizontal ductions are full, the patient does not have Moebius syndrome. Moreover, we identified a subset of patients who met MDC but also had abnormal vertical eye movements with or without exotropia; these patients appear to form a distinct category, and should be further evaluated for the presence of other defined genetic syndromes such as CFEOM. We thus propose that the minimum diagnostic criteria for Moebius syndrome now include full vertical eye movements, as follows: “A congenital, uni- or bilateral, non-progressive facial weakness and limited abduction of the eye(s) and full vertical motility.” (Figure 1B). With this distinction made, it is clear that Moebius syndrome does not differ phenotypically worldwide.^{3, 11, 34} More accurate clinical diagnosis will help the clinician provide patients and families with accurate prognosis and genetic counseling and will empower researchers to construct clinically homogeneous subgroups in the search for a genetic etiology of Moebius syndrome.

We have determined that only 4 out of 5 participants diagnosed with Moebius syndrome have the classic manifestations of the condition; the remainder have either a disturbance of vertical gaze or do not meet the minimum diagnostic criteria for the disease. The diagnostic differentiation can be performed simply by assessing ocular ductions in patients with congenital facial palsy. Once subdivided in this manner, the distinctions between and among the groups become clear. Such careful phenotyping of patients is essential to provide proper prognosis and genetic counseling and will facilitate ongoing research into the genetic etiology of these conditions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Participant diagnostic categories for Moebius syndrome. **A:** Currently accepted minimum diagnostic criteria. **B:** New proposed minimum diagnostic criteria.

MDC = minimum diagnostic criteria. *Two brothers within this group were identified with HOXB1 mutations (facial nerve palsy and comitant esotropia). **Two participants (mother and son) were diagnosed with hereditary congenital facial nerve palsy (HCFP). *** All participants were molecularly diagnosed with a TUBB3 mutation causing congenital fibrosis of the extraocular muscles.

Figure 2.

Eye alignment, horizontal ocular motility patterns, and facial strength in participants not meeting the minimum diagnostic criteria for Moebius syndrome. Images **A**, **C**, **D**, and **E** are from an 8 year old boy with a unilateral facial palsy (**A**) who also demonstrates full abduction of both the right eye (**C**) and left eye (**E**) as well as an esotropia in straight ahead gaze (**D**). Images **B**, **F**, **G**, and **H** are from an adult woman who does not have facial weakness (**B**), but does have a severe abduction deficit of the right eye (**F**) and left eye (**H**) with a small angle esotropia in straight ahead gaze (**G**).

Figure 3.

Exotropia, limited vertical movement, and ptosis in individuals with genetically confirmed congenital fibrosis of the extraocular muscles type 3. Images **A**, **B**, and **C** are from a 7 year old boy with a heterozygous E410V TUBB3 amino acid substitution. He has a marked exotropia of his right eye in straight ahead position (**B**) with nearly full abduction and markedly reduced adduction of both eyes (**A** and **C**). Aberrant innervation was also present, with left ptosis in right gaze (**A**). Vertical ductions were absent. Image **D** is a 7 year old girl with a heterozygous R262H TUBB3 substitution showing exotropia of the right eye and marked bilateral ptosis for which she has already had previous lid surgery with limited success. Image **E** shows an 8 year old girl with a heterozygous E410K TUBB3 substitution. There is exotropia with both eyes anchored below the vertical midline, and both pupils are small and irregular.

Figure 4.

Eye alignment and horizontal ocular motility patterns in two participants with classic Moebius syndrome. Images B and E are in straight ahead gaze, while images A and D are right gaze and images C and F are left gaze. Images A, B, and C show an 18 month old with a large esotropia, marked abduction deficit of each eye with good adduction. Images D, E, and F show an adult with relatively straight eyes (orthotropia) with limited horizontal gaze in both directions.

Figure 5. Volitional Bell's phenomenon. **A**, patient fixating on camera; **B**, volitional Bell's phenomenon forces upgaze. Corneas are lubricated when they bury under the immobile upper lid. See also Video 2.

Table 1

Classic Moebius syndrome characteristics. (n=88 for all participants meeting criteria, n=59 when participants post strabismus surgery were excluded from motility analysis.)

Classic Moebius Syndrome Characteristics	
Facial nerve palsy	100% (88/88)
Severe abduction deficit	100% (59/59)
Full vertical gaze	100% (59/59)
Full adduction	17% (10/59)
Mildly reduced adduction deficit	27% (16/59)
Severe adduction deficit	56% (33/59)
Orthotropia in primary gaze	41% (24/59)
Esotropia in primary gaze	59% (35/59)
Binocular vision *	51% (30/59)
Dysinnervation	41% (24/59)
Volitional Bells	43% (40/88)
Intorsion with fixation	18% (14/88)

* Includes subjects with small angle esotropia/monofixation syndrome

Table 2

Dysmorphological and non-ophthalmological anomalies in classic Moebius syndrome.

Category of Anomaly	Number of Participant Affected
Orofacial [†]	82 (93%)
Periorbital	31 (35%)
Ear shape/position	39 (44%)
Congenital hearing deficit	16 (18%)
Limb	54 (61%)
Lower limb only	23 (26%)
Upper limb only	18 (20%)
Upper and lower limbs	13 (15%)
Muscular or skeletal [‡]	30 (34%)
Medical systemic or neurological	21 (24%)
Developmental, cognitive, or behavioral	46 (52%)
Individuals having involvement of:	
None	3 (3%)
1 category (as defined above)	4 (5%)
2 categories	20 (23%)
3 categories	20 (23%)
4 categories	14 (16%)
5 categories	13 (15%)
6 categories	6 (7%)
7 categories	8 (9%)

[†]Excludes facial weakness and lid ptosis;[‡]Excludes anomalies of hands or feet that are included in 'Limb' section.