

1 **Diagnostic distinctions and genetic analysis of patients diagnosed with Moebius**
2 **syndrome**

3 **Sarah MacKinnon, MSc, OC(C)^{1*} Darren T. Oystreck, MMedSci, OC(C)^{1,2,3* +}**
4 **Caroline Andrews, MS^{4,8,9} Wai-Man Chan, MS^{4,8,9} David G. Hunter, MD, PhD^{1,7#} and**
5 **Elizabeth C. Engle, MD^{1,4-9#}**

6 From the ¹Department of Ophthalmology, Boston Children's Hospital, Boston, MA,
7 ²Department of Ophthalmology, College of Medicine, King Saud University, Riyadh
8 Saudi Arabia; ³Division of Ophthalmology, Faculty of Health Sciences, University of
9 Stellenbosch, Tygerberg, South Africa; ⁴Department of Neurology, ⁵F.B. Kirby
10 Neurobiology Center, and the ⁶Manton Center for Orphan Disease Research, Boston
11 Children's Hospital, Boston, USA; Departments of ⁷Ophthalmology and ⁸Neurology,
12 Harvard Medical School, Boston, USA; ⁹Howard Hughes Medical Institute, Chevy
13 Chase, Maryland, USA.

14 + Current address: Eye Clinic, IWK Health Centre, 5850 University Avenue, PO Box
15 9700, Halifax, Nova Scotia, B3K 6R8 Canada

16 *These authors contributed equally.

17 #Co-corresponding authors

18 **Meeting presentations:** American Association of Pediatric Ophthalmology and
19 Strabismus, National Meeting, Boston, MA April 2013.

20 **Financial Support:** National Eye Institute, National Institutes of Health, Bethesda,
21 Maryland: R01-EY012498, and the Moebius Syndrome Foundation, Pilot Grove,
22 Missouri. The sponsor and funding organization had no role in the design or conduct of
23 this research.

24 **Conflict of Interest:** No conflicting relationship exists for any of the authors.

25 **Running head:** Diagnostic and genetic analysis in Moebius syndrome.

26 **This manuscript contains two videos as additional online-only material.**

27 **Please send correspondence to:**

28 David G Hunter, Fegan 4, Boston Children's Hospital, 300 Longwood Ave, Boston, MA
29 02115, david.hunter@childrens.harvard.edu and Elizabeth Engle, CLS14075, Boston
30 Children's Hospital, 300 Longwood Ave, Boston, MA 02115,
31 Elizabeth.Engle@childrens.harvard.edu

32 **Précis:**

33 While several patients carrying the diagnosis of "Moebius syndrome" had either TUBB3
34 or HOXB1 mutations, no patients meeting strict minimum diagnostic criteria had these
35 mutations. We propose refined diagnostic criteria for future Moebius syndrome studies.

36 **ABSTRACT**

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

40 **Design:** Prospective, observational study.

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

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

47 **Main Outcome Measures:** Number of patients meeting MDC and number with
48 confirmed genetic mutation.

49 **Results:** A total of 112 participants from 107 families enrolled. Nineteen percent of
50 participants (21/112) did not meet accepted MDC for Moebius syndrome because they
51 had abduction deficits without facial palsy or facial palsy with full ocular motility. All five
52 families with two affected individuals had at least one family member in this category,
53 including two siblings with comitant strabismus who harbored a *HOXB1* mutation. Four
54 unrelated participants, also not meeting MDC, had large-angle exotropia, vertical gaze
55 deficiency, and ptosis consistent with congenital fibrosis of the extraocular muscles type
56 3 (CFEOM3); 1 harbored a novel and 3 harbored previously reported *de novo* *TUBB3*
57 mutations. Three percent of participants (3/112) met MDC but also had restricted

58 vertical gaze. The remaining 88 participants (79%) met MDC and had full vertical gaze.
59 This group had relatively homogeneous findings and none had a family history of
60 Moebius syndrome. Two previously undescribed phenomena were observed in this
61 category: 1) volitional Bell's phenomenon, and 2) intorsion with fixation.

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

70

71 **INTRODUCTION**

72 Moebius syndrome is a complex, rare developmental anomaly of the hindbrain that has
73 been described historically as the combination of congenital palsies of the abducens
74 and facial nerves,¹ frequently with additional features including orofacial malformations,
75 limb defects, and musculoskeletal, behavioral, and cognitive abnormalities.²⁻⁷ While a
76 compilation of clinical,⁸⁻¹² neuropathological,¹³⁻¹⁸ and radiological¹⁹⁻²¹ reports suggest
77 that Moebius syndrome is not a single disease entity,²² these and many other studies of
78 the condition are confounded by variable diagnostic criteria.^{22, 23} This lack of
79 standardized diagnostic criteria complicates the clinical assessment, determination of
80 prognosis, and genetic analysis of Moebius syndrome patients. To address this

81 concern, a group of clinicians and researchers met in 2007 at the bi-annual Moebius
82 Syndrome Foundation research meeting and defined the minimum diagnostic criteria for
83 classical Moebius syndrome as “congenital, uni- or bilateral, non-progressive facial
84 weakness and limited abduction of the eye(s).”¹¹

85

86 The purpose of the current report is to characterize the ocular and facial phenotypes of
87 a large number of individuals diagnosed with Moebius syndrome in order to determine
88 whether they meet minimum diagnostic criteria. We also screened participants’ DNA for
89 mutations in the *HOXA1*, *HOXB1* and *TUBB3* genes (reported to cause atypical forms
90 of Moebius syndrome)²⁴⁻²⁷ in an attempt to identify genetic etiologies underlying the
91 disorder.

92

93 **METHODS**

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

102

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

109
110 Probands and, when available, parents and family members underwent
111 ophthalmological evaluation, testing of facial movement and strength, and assessment
112 for a subset of additional anomalies associated with Moebius syndrome.

113 Ophthalmological exam included visual acuity testing using developmentally appropriate
114 symbols (letters or HOTV test), non-cycloplegic autorefraction (Retinomax K-Plus 2,
115 Right Manufacturing, Tokyo, Japan), pupil, external and anterior segment evaluation by
116 handlight, and sensorimotor evaluation. External examination of the participants
117 involved measurement of eyelid position and facial nerve function. Sensorimotor
118 evaluation included evaluation of eye movements (ductions and versions), prism-and-
119 cover testing, and sensory testing (stereopsis and Worth 4-dot). To quantify ocular
120 motility a standard 0-8 scale was used.²⁸ On this scale 0 indicated full duction (no
121 limitation), and -4 indicated that the eye was able to reach, but not move past, midline.
122 In rare cases, if the eye was fixed in the opposing gaze, a score of > -4 (maximum -8)
123 was given. Duction limitation was then categorized as “mild” (-1 limitation), “moderate” (-
124 2 limitation), or “severe” (>-2 limitation). Convergence was classified as “normal” (near
125 point of convergence (NPC) \leq 10 cm), limited (NPC 11 cm to 50 cm), or “remote” (>

126 50 cm). Slit lamp biomicroscopy, cycloplegic refraction, and dilated fundus examination
127 were not possible at time of enrollment; however, past ophthalmological records were
128 obtained when available.

129
130 Genomic DNA was extracted from each participant's blood or saliva sample using
131 Qiagen Gentra Puregene DNA isolation kits (Valencia, CA) or DNA Genotek Oragene
132 saliva and assisted saliva swab kits (Kanata, Ontario, Canada). Mutation analysis was
133 performed by PCR amplifying all exons and exon-intron boundaries of the *HOXA1*,
134 *HOXB1*, and *TUBB3* genes. All the resulting amplicons were Sanger sequenced, and
135 primer sequences and screening conditions are available upon request. Subsets of the
136 mutation positive participants have been reported previously.^{25, 26}

137

138 **RESULTS**

139

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

146

147 Participants were grouped based upon presence or absence of abduction limitation and
148 facial palsy, which define the currently accepted minimum diagnostic criteria (MDC) for

149 Moebius syndrome. MDC were present in 91/112 participants, while 21/112 participants
150 did not meet MDC despite having been given the clinical diagnosis of Moebius
151 syndrome by their physician (**Figure 1**).

152

153 **Participants not meeting MDC for Moebius syndrome**

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

157

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

171

172 The remaining four participants in this category had incomitant strabismus but did not
173 meet MDC because they did not have abduction limitation. Each had ptosis and a large
174 angle exotropia with severe deficiencies of both adduction and vertical gaze typical of
175 congenital fibrosis of the extraocular muscles type 3 (CFEOM3; **Figure 3**). These
176 participants had complete absence of adduction with minimal or absent ipsilateral
177 abduction deficits (**Figure 3 A-C**), inability to elevate either eye (with eyes anchored
178 below the midline in 2 cases) (**Figure 3E**), mild weakness of eye depression, and
179 severe ptosis. Two participants also had irregular pupils (**Figure 3 D and E**). All four
180 participants in this group harbored heterozygous *TUBB3* missense mutations.²⁷ We
181 previously reported that two of the participants had *de novo* E410K substitutions.^{26, 27} A
182 third participant was not previously reported, but we found to harbor the previously
183 reported R262H substitution,²⁶ which also arose *de novo*. The fourth participant was
184 found to have a previously unreported c.1229A>T *TUBB3* mutation, predicted to result
185 in a *TUBB3* E410V substitution, thus altering the same amino acid residue as E410K.
186 This novel mutation was also *de novo*, and was not present in dbSNP 132
187 (<http://www.ncbi.nlm.nih.gov/SNP>, accessed 11/18/13), the Exome Variant Server
188 (<http://evs.gs.washington.edu/EVS>, accessed 11/18/13), nor the 1000 Genomes Project
189 (<http://browser.1000genomes.org/index.html>, accessed 11/18/13).

190

191 **Participants meeting minimum diagnostic criteria for Moebius syndrome**

192

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

201
202 Eighty-eight participants met full MDC for Moebius syndrome and had normal vertical
203 eye movements, which we refer to as 'classic Moebius syndrome' (**Table 1, Figure 4**).
204 Among these, one had a unilateral facial palsy while the remaining 87 had a bilateral
205 facial palsy, which was recorded as symmetric in 54 and asymmetric in 33. Twenty-nine
206 were excluded from further detailed characterization of ocular alignment and motility
207 due to prior strabismus surgery, leaving 59 with unaltered motility allowing for analysis.
208 Limitation of abduction was severe and bilateral in all 59 participants. A co-existing
209 adduction deficit not exceeding the ipsilateral abduction deficit was present in 49/59
210 (83%). In 16/59 (27%) the adduction deficit was mild, while in 33/59 individuals (56%) it
211 was severe, resulting in a motility pattern most consistent with horizontal gaze palsy.
212 Convergence was normal in 34/59 (58%) and partially limited in the remainder; no
213 subject had absent convergence. Esotropia was present in more than half of individuals,
214 with the remainder having no manifest strabismus in straight-ahead gaze, and 30/59
215 (51%) had at least some binocular vision. Dysinnervation was present in 24 subjects

216 (41%) evident most commonly by excessive tearing when eating (crocodile tears) or
217 adduction of both eyes with attempted lateral gaze (synergistic convergence.) Pupils
218 were of normal shape and reactivity in all cases.

219

220 Two novel findings were discovered in our participants with classic Moebius syndrome.

221 The first was *intorsion with fixation*, which was not observed until the final year of
222 enrollment and was confirmed in 14/33 participants evaluated (42%, or 18% of the
223 entire cohort). In these participants, we observed an intorsion movement of the non-
224 fixating eye as it became the fixating eye (**Video 1**, available at <http://aaojournal.org>).

225 Individuals demonstrating this phenomenon had minimal to no strabismus. The second
226 phenomenon was a volitional Bell's phenomenon **Figure 5**, which was noted in 40/88
227 (43%) participants. In these cases, participants appeared to intentionally and routinely
228 exert an eyelid closure effort; while eyelid closure did not occur due to facial palsy, the
229 accompanying Bell's phenomenon elevated the eye above the upper eyelid margin
230 (**Video 2**, available at <http://aaojournal.org>). We presume that this volitional ocular
231 elevation served to lubricate the ocular surface to compensate for the lack of a normal
232 blink.

233

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

239

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

245

246 **DISCUSSION**

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

255

256 Only 91 of the 112 participants believed to have Moebius syndrome met minimum
257 diagnostic criteria. This confusion in diagnosis most likely resulted from the varied
258 phenotypic descriptions used in the literature.^{10, 19, 29-32} In addition, Moebius syndrome is
259 rare, and the expertise of those making the diagnosis varies. For example, patients who
260 have limb anomalies or other features often associated with Moebius syndrome

261 combined either with facial palsy or limited abduction – but not both – might also be
262 diagnosed with Moebius syndrome by clinicians not familiar with the minimum
263 diagnostic criteria. Similarly, if a patient with congenital facial palsy has a coincidental
264 diagnosis of esotropia (which is present in 1-2% of the general population³³), an
265 examiner not experienced in assessing horizontal ductions might incorrectly diagnose
266 Moebius syndrome. The association of congenital facial palsy with comitant esotropia
267 may not, however, be entirely coincidental: for example, the two siblings of
268 consanguineous parents with hereditary congenital facial palsy and comitant esotropia
269 are one of two families with this phenotype reported to harbor a homozygous mutation
270 in the *HOXB1* gene.²⁵ The two affected siblings in the second family were also initially
271 misdiagnosed as having Moebius syndrome.²⁵ *HOXB1* mutations have not been found
272 in patients with classic Moebius syndrome either in this study or the previous report.²⁵

273
274 Four participants who did not meet MDC had findings consistent with a diagnosis of
275 CFEOM3. Two harbored the E410K TUBB3 substitution²⁷ and a third harbored a R262H
276 substitution²⁶ previously reported to cause CFEOM3. The fourth participant had a
277 unique TUBB3 E410V substitution and findings consistent with the E410K TUBB3
278 syndrome. The E410K substitution is now referred to as the ‘TUBB3 E410K syndrome’
279 with clinical features that include Kallmann syndrome (hypogonadotropic hypogonadism
280 and anosmia), stereotyped midface hypoplasia, intellectual and social disabilities and, in
281 some cases, vocal cord paralysis, tracheomalacia, cyclic vomiting responsive to valproic
282 acid, and distinctive MRI anomalies.²⁷ The number of probands harboring TUBB3
283 E410V and R262H are too few to fully define the resulting phenotype, but both TUBB3

284 amino acid substitutions also resulted in additional neurological signs and symptoms. It
285 is essential that these patients be differentiated from patients with classic Moebius
286 syndrome to allow for a search for associated findings noted above and treatment for
287 hypogonadotropic hypogonadism and cyclic vomiting when necessary.

288

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

307 subset of the patients likely had CFEOM, and in particular those who also had ptosis
308 may harbor *TUBB3* mutations.¹¹

309

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

324

325 Two novel findings were also discovered in our participants with classic Moebius
326 Syndrome. Volitional Bell’s was most likely a learned response to compensate for
327 inadequate lid closure to lubricate the ocular surface. The cause of intorsion with
328 fixation is less clear. The superior oblique muscle can provide a small amount of
329 abduction. We speculate that in participants with small-angle esotropia and limited

330 abduction, the superior oblique muscle is recruited to abduct the esotropic eye as it
331 attempts to fixate on the object of regard during cover testing, thus intorting the eye.
332 Ventura and colleagues³⁴ noted excyclotorsion (rather than intorsion) during cover
333 testing in one of their Brazilian participants, but that patient also demonstrated gaze
334 evoked nystagmus on upward gaze, which we did not observe. Lacking video
335 recordings of these eye movements,³⁴ it is not possible to determine whether the
336 findings they described are related to our observations.

337

338 In this study, our primary concern was to determine whether each individual previously
339 assigned a diagnosis of “Moebius syndrome” met the currently recognized minimum
340 diagnostic criteria to allow for accurate genetic studies. In the process we discovered
341 misclassification of a significant percentage of such participants. It is vital that clinicians
342 carefully evaluate ocular ductions in individuals suspected of having Moebius syndrome,
343 especially in patients with facial palsy who have strabismus; if horizontal ductions are
344 full, the patient does not have Moebius syndrome. Moreover, we identified a subset of
345 patients who met MDC but also had abnormal vertical eye movements with or without
346 exotropia; these patients appear to form a distinct category, and should be further
347 evaluated for the presence of other defined genetic syndromes such as CFEOM. We
348 thus propose that the minimum diagnostic criteria for Moebius syndrome now include
349 full vertical eye movements, as follows: “A congenital, uni- or bilateral, non-progressive
350 facial weakness and limited abduction of the eye(s) and full vertical motility.” (**Figure**
351 **1B**). With this distinction made, it is clear that Moebius syndrome does not differ
352 phenotypically worldwide.^{3, 11, 34} More accurate clinical diagnosis will help the clinician

353 provide patients and families with accurate prognosis and genetic counseling and will
354 empower researchers to construct clinically homogeneous subgroups in the search for a
355 genetic etiology of Moebius syndrome.

356

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

365 **ACKNOWLEDGEMENT:**

366 We thank Marilyn Miller, MD, for her insightful input over the past three Moebius
367 Syndrome Foundation meetings and for bringing one of the previously unreported
368 findings in this paper, the volitional Bell's phenomenon, to our attention.

369

370 **REFERENCES**

- 371 1. Möbius PJ. Ueber angeborene doppelseitige Abducens-
372 Facialis-Lahmung. MMW Munch Med Wochenschr 1888;35:91-4.
- 373 2. Stromland K, Sjogreen L, Miller M, et al. Mobius sequence--
374 a Swedish multidiscipline study. Eur J Paediatr Neurol
375 2002;6:35-45.
- 376 3. Verzijl HT, van der Zwaag B, Cruysberg JR, Padberg GW.
377 Mobius syndrome redefined: a syndrome of rhombencephalic
378 maldevelopment. Neurology 2003;61:327-33.
- 379 4. Rogers GL, Hatch GF Jr, Gray I. Mobius syndrome and limb
380 abnormalities. J Pediatr Ophthalmol 1977;14:134-8.
- 381 5. Miller MT, Stromland K, Gillberg C, et al. The puzzle of
382 autism: an ophthalmologic contribution. Trans Am Ophthalmol Soc
383 1998;96:369-85; discussion 385-7.
- 384 6. Gadoth N, Biedner B, Torok G. Mobius syndrome and Poland
385 anomaly: case report and review of the literature. J Pediatr
386 Ophthalmol Strabismus 1979;16:374-6.
- 387 7. Briegel W. Neuropsychiatric findings of Mobius sequence --
388 a review. Clin Genet 2006;70:91-7.
- 389 8. Miller MT, Stromland K. The Mobius sequence: a relook. J
390 AAPOS 1999;3:199-208.
- 391 9. Amaya LG, Walker J, Taylor D. Mobius syndrome: a study and
392 report of 18 cases. Binocular Vis Q 1990;5:119-32.
- 393 10. Cronemberger MF, de Castro Moreira JB, Brunoni D, et al.
394 Ocular and clinical manifestations of Mobius' syndrome. J
395 Pediatr Ophthalmol Strabismus 2001;38:156-62.
- 396 11. Carta A, Mora P, Neri A, et al. Ophthalmologic and systemic
397 features in Mobius syndrome an Italian case series.
398 Ophthalmology 2011;118:1518-23.

- 399 12. de Souza-Dias CR, Goldchmit M. Further considerations about
400 the ophthalmic features of the Mobius sequence, with data of 28
401 cases. *Arq Bras Oftalmol* 2007;70:451-7.
- 402 13. Pitner SE, Edwards JE, McCormick WF. Observations on the
403 pathology of Moebius syndrome. *J Neurol Neurosurg Psychiatry*
404 1965;28:362-74.
- 405 14. Towfighi J, Marks K, Palmer E, Vannucci R. Mobius syndrome.
406 Neuropathologic observations. *Acta Neuropathol* 1979;48:11-7.
- 407 15. Verzijl HT, van der Zwaag B, Lammens M, et al. The
408 neuropathology of hereditary congenital facial palsy vs Mobius
409 syndrome. *Neurology* 2005;64:649-53.
- 410 16. Sudarshan A, Goldie WD. The spectrum of congenital facial
411 diplegia (Moebius syndrome). *Pediatr Neurol* 1985;1:180-4.
- 412 17. Meyer E, Ludatscher RM, Zonis S. Mobius syndrome. Electron
413 microscopic study of the extraocular muscles. *Ophthalmic*
414 *Paediatr Genet* 1984;4:91-5.
- 415 18. Traboulsi EI, Maumenee IH. Extraocular muscle aplasia in
416 Moebius syndrome. *J Pediatr Ophthalmol Strabismus* 1986;23:120-2.
- 417 19. Saint-Martin C, Clapuyt P, Duprez T, et al. Mobius sequence
418 and severe pons hypoplasia: a case report. *Pediatr Radiol*
419 1998;28:932.
- 420 20. Sano M, Kaga K, Takeuchi N, et al. A case of Mobius
421 syndrome--radiological and electrophysiological findings. *Int J*
422 *Pediatr Otorhinolaryngol* 2005;69:1583-6.
- 423 21. Matsunaga Y, Amamoto N, Kondoh T, et al. A severe case of
424 Moebius syndrome with calcification on the fourth ventricular
425 floor. *J Hum Genet* 1998;43:62-4.
- 426 22. Heidary G, Traboulsi EI, Engle EC. The genetics of
427 strabismus and associated disorders. In: Traboulsi EI, ed.
428 *Genetic Diseases of the Eye*. 2nd ed. New York: Oxford University
429 Press; 2012:657-86.

430 23. Graeber CP, Hunter DG, Engle EC. The genetic basis of
431 incomitant strabismus: consolidation of the current knowledge of
432 the genetic foundations of disease. *Semin Ophthalmol*
433 2013;28:427-37.

434 24. Tischfield MA, Bosley TM, Salih MA, et al. Homozygous *HOXA1*
435 mutations disrupt human brainstem, inner ear, cardiovascular and
436 cognitive development. *Nat Genet* 2005;37:1035-7.

437 25. Webb BD, Shaaban S, Gaspar H, et al. *HOXB1* founder mutation
438 in humans recapitulates the phenotype of *Hoxb1*(-/-) mice. *Am J*
439 *Human Genet* 2012;91:171-9.

440 26. Tischfield MA, Baris HN, Wu C, et al. Human *TUBB3* mutations
441 perturb microtubule dynamics, kinesin interactions, and axon
442 guidance. *Cell* 2010;140:74-87.

443 27. Chew S, Balasubramanian R, Chan WM, et al. A novel syndrome
444 caused by the E410K amino acid substitution in the neuronal
445 beta-tubulin isotype 3. *Brain* 2013;136:522-35.

446 28. Mehendale RA, Dagi LR, Wu C, et al. Superior rectus
447 transposition and medial rectus recession for Duane syndrome and
448 sixth nerve palsy. *Arch Ophthalmol* 2012;130:195-201.

449 29. MacDermot KD, Winter RM, Taylor D, Baraitser M.
450 Oculofacialbulbar palsy in mother and son: review of 26 reports
451 of familial transmission within the 'Mobius spectrum of
452 defects'. *J Med Genet* 1991;28:18-26.

453 30. Graziadio C, Lorenzen MB, Rosa RF, et al. New report of a
454 familial case of Moebius syndrome presenting skeletal findings.
455 *Am J Med Genet A* 2010;152A:2134-8.

456 31. Kumar D. Moebius syndrome. *J Med Genet* 1990;27:122-6.

457 32. Baraitser M. Genetics of Mobius syndrome. *J Med Genet*
458 1977;14:415-7.

459 33. Greenberg AE, Mohny BG, Diehl NN, Burke JP. Incidence and
460 types of childhood esotropia: a population-based study.
461 Ophthalmology 2007;114:170-4.

462 34. Ventura BV, Miller MT, Danda D, et al. Profile of ocular
463 and systemic characteristics in Mobius sequence patients from
464 Brazil and Italy. Arq Bras Oftalmol 2012;75:202-6.

465 35. Abid F, Hall R, Hudgson P, Weiser R. Moebius syndrome,
466 peripheral neuropathy and hypogonadotropic hypogonadism. J
467 Neurol Sci 1978;35:309-15.

468 36. Rubinstein AE, Lovelace RE, Behrens MM, Weisberg LA.
469 Moebius syndrome in Kallmann syndrome. Arch Neurol 1975;32:480-
470 2.

471 37. Brackett LE, Demers LM, Mamourian AC, et al. Moebius
472 syndrome in association with hypogonadotropic hypogonadism. J
473 Endocrinol Invest 1991;14:599-607.

Table 1[Click here to download Table: Table 1 with legend.docx](#)

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.

Figure 1

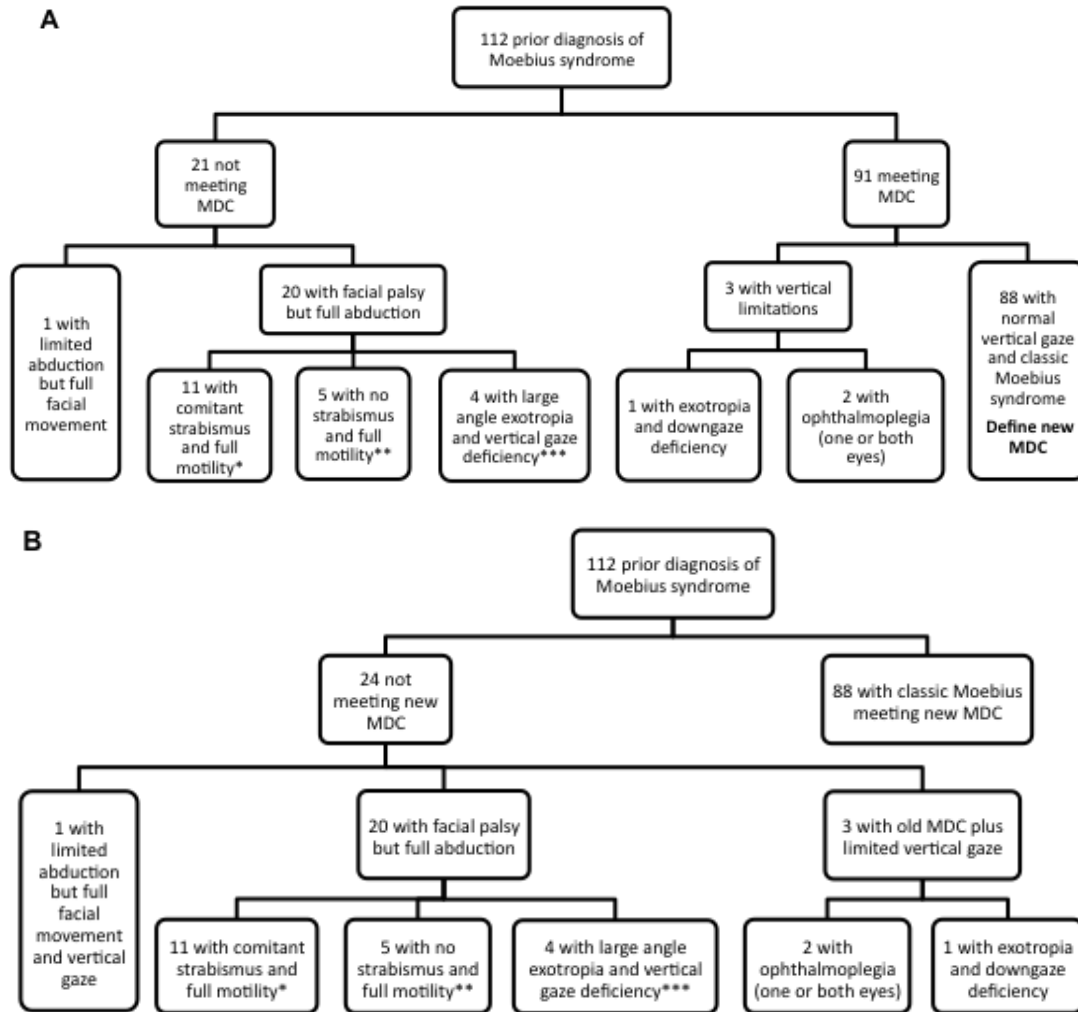


Figure 2
[Click here to download high resolution image](#)

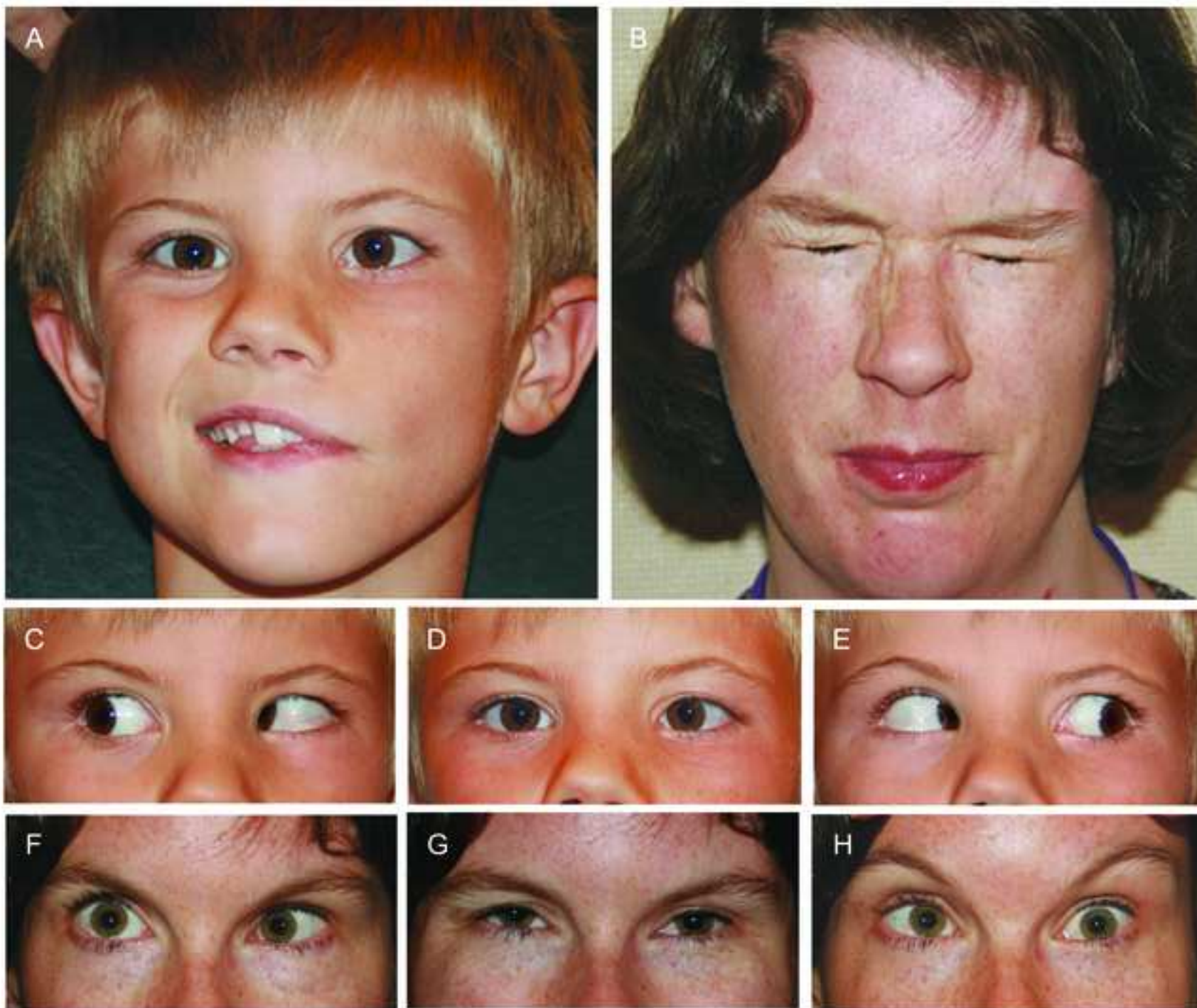


Figure 3
[Click here to download high resolution image](#)



Figure 4
[Click here to download high resolution image](#)



Figure 5
[Click here to download high resolution image](#)



FIGURE LEGENDS

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**.

Video 1. Intorsion with fixation. This video demonstrates the intorsion movement of the non-fixating eye as it became the fixating eye. This movement is most apparent in downgaze, and is absent in upgaze.

Video 2. Volitional Bell's phenomenon. The patient appears to intentionally exert an eyelid closure effort despite the absence of eyelid function. The accompanying Bell's phenomenon, observed in the video, allows the eye to move above the upper eyelid margin in order to lubricate the ocular surface.