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Research Report

Ancestral Origin of the First Indian Families with Myotonic Dystrophy Type 2

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Abstract.
Background: Myotonic dystrophy type 2 (DM2) is caused by a CCTG repeat expansion in intron 1 of the CCHC-Type Zinc Finger Nucleic Acid Binding Protein (*CNBP*) gene. Previous studies indicated that this repeat expansion originates from separate founders.
Objective: This study was set out to determine whether or not patients with DM2 originating from European and non-European countries carry the previously described European founder haplotypes.
Methods: Haplotype analysis was performed in 59 DM2 patients from 29 unrelated families. Twenty-three families were from European descent and 6 families originated from non-European countries (India, Suriname and Morocco). Seven short tandem repeats (CL3N122, CL3N99, CL3N59, CL3N117, CL3N119, CL3N19 and CL3N23) and 4 single nucleotide polymorphisms (SNP) (rs1871922, rs1384313, rs4303883 and CGAP_886192) in and around the *CNBP* gene were used to construct patients' haplotypes. These haplotypes were compared to the known DM2 haplotypes to determine the ancestral origin of the *CNBP* repeat expansion.
Results: Of 41 patients, the haplotype could be assigned to the previously described Caucasian haplotypes. Three patients from Morocco and Portugal had a haplotype identical to the earlier reported Moroccan haplotype. Twelve patients from India and Suriname, however, carried a haplotype that seems distinct from the previously reported haplotypes. Three individuals could not be assigned to a specific haplotype.
Conclusions: The ancestral origin of DM2 in India might be distinct from the Caucasian families and the solely described Japanese patient. However, we were unable to establish this firmly due to the limited genetic variation in the region surrounding the *CNBP* gene.

Keywords: Myotonic dystrophy type 2, haplotypes, India, European continental ancestry group

INTRODUCTION

Myotonic dystrophy type 2 (DM2) is a progressive autosomal dominant multisystem disease with muscle dysfunction as a main feature, including proximal muscle weakness, myotonia and pain [1]. In addition, cataracts, cardiac conduction defects, endocrine disorders, gastrointestinal involvement, autoimmune diseases, cerebral involvement, and hearing impairment are common in patients with DM2 [1–10].

DM2 is caused by the expansion of a CCTG repeat in intron 1 of the CCHC-Type Zinc Finger Nucleic Acid Binding Protein (*CNBP)* gene located on chro-

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Marker	Ancestry	*Centromere ←*	CL3N122	CL3N99	CL3N59	EXP	rs1871922	CL3N117	CL3N119	CL3N19	CL3N23	*→ Telomere*
Location kb from CCTG repeat			*- 396*	*- 348*	*- 119*		*+ 11*	*+ 103*	*+ 216*	*+ 292*	*+ 365*	
Liquori et al. Haplotype A [13]	Europe		216	185	157			241	236	208	NA	
Liquori et al. Haplotype B [13]	Europe		216	181	157			241	240	190	238	
Liquori et al. Haplotype C [13]	Europe		218	175	150			241	240	190	226	
Liquori et al. [13]	Afghanistan		214	191	157	*CCTG expansion*		241	232 | 236	214	NA	
Coenen et al. [14]	Morocco		210	194	150		C	241	238	186	234	
Saito et al. [15]	Japan		NA	NA	137		A | A	245	226	NA	NA	

Fig. 1. Previous reported haplotypes in literature. Reported marker lengths of Liquori et al. and Saito et al. are recalculated to make them comparable to our lengths and the lengths previously reported by Coenen et al. [13–15]. NA: not analysed.

mosome 3q21.3 [11]. The estimated age of the DM2 mutation in the European haplotype is 4000–11,000 years [12].

Previous studies show that patients with DM2 from European descent share a common founder as the DM2 mutation in these patients share identical haplotypes. In all these patients, the region in and around the *CNBP* gene can be classified into the same disease linked haplotypes based on repeat lengths (Fig. 1) [12–14]. Liquori et al. found the majority of the 70 European families from Northern European descent carried one of the three consensus haplotypes and 1 family from Afghanistan with a haplotype with great similarity to the core haplotype [13]. Bachinski et al. also found one single founder mutation in 17 unrelated DM2 pedigrees from both Northern and Southern Europe [12]. Coenen et al. reported 14 European families and 1 Moroccan family with a haplotype similar to this European or Caucasian haplotype [14]. This suggests that the CCTG repeat expansion in *CNBP* in these patients has the same ancestral origin. However, Saito et al. reported a Japanese patient with DM2 carrying a haplotype different from the common European haplotype [15]. These studies indicate that the CCTG repeat originates from at least two separate founders.

Apart from the above-mentioned studies, there is a general lack of research in patients with DM2 originating from non-European countries. This study was set out to determine whether patients with DM2 originating from European and non-European countries carry the same European founder haplotype by investigating the haplotypes of patients with ancestries that have not been examined earlier.

MATERIALS AND METHODS

Patients with DM2

All 67 newly diagnosed patients with DM2 living in the Netherlands since 2010 were identified using the Dutch neuromuscular database [16]. Inclusion criteria were age $\geq$ 18 years and a genetically confirmed diagnosis of DM2.

The study protocol was approved by the institutional review board of the Radboud university medical center (Commissie Mensgebonden Onderzoek Regio Arnhem-Nijmegen, protocol-number 2016-2535) and the procedures performed this study were in accordance with the Helsinki Declaration of 1975.

DNA analysis

Genomic DNA of the participants was extracted from blood lymphocytes via standard Chemagen procedures (PerkinElmer, Groningen, The Netherlands). DNA was analysed as described in Coenen et al. with slight modifications [14]. Primers (Applied Biosystems, Nieuwerkerk aan den IJssel, The Netherlands) for 7 short tandem repeats (STRs) (CL3N122, CL 3N99, CL3N59, CL3N117, CL3N119, CL3N19 and CL3N23) were used to investigate patients' haplotypes. In addition, four single nucleotide polymorphisms (SNPs) in and around the *CNBP* gene were used to investigate patients' haplotypes. They include rs1871922 (hg19 chr3:g. 128902599G > T/C, NG_011902.1:g.5212C > A), rs1384313 (hg19 chr 3:g. 128896074C > T, NG_011902.1:g.11737G > A), rs4303883 (hg19 chr3:g. 128890350G > A/T, NG_ 011902.1:g.17461C > T). The position of the fourth SNP (CGAP_886192) was not exactly known, therefore the genomic region was extracted from a previous publication [12].

Rs1384313 and rs4303883 were genotyped using Taqman SNP genotyping (assay id C___8684563_10 and C___25473588_10 respectively) according to the instructions of the manufacturer (ThermoFisher, Nieuwerkerk aan den IJssel, The Netherlands). An overview of the sequences of the used STR primers and the other 2 SNPs is presented in Supplemental File 1. For the STR markers, GeneMarker software

(v.2.6.7, SoftGenetics LLC, State College, Pennsylvania, USA) was used to determine PCR lengths. SNP rs1871922 genotyping was performed by Restriction Fragment Length Polymorphism analysis (RFLP) as described previously [14]. SNP CGAP 886192 was genotyped using sequencing. In brief, genomic DNA (10ng) was amplified using AmpliTaq Gold 360 Master Mix (Applied Biosystems, Foster City, California, United States)), with 0.3μM forward and the same amount of reverse primer. PCR amplification was performed using an annealing temperature of 54 °C. Sequencing was performed using the PCR primers described previously. Sequence results were analyzed using Vector NTI software from Thermo Fisher. All genotypes were independently confirmed by a second person. Positive controls with known haplotypes (3 most common haplotypes (A, B and C) in Caucasians) were included to make sure that reported repeat lengths agreed with previous reports.

Analysis methods

Haplotypes were constructed using Phase v2.1 [17, 18]. Affected haplotypes were established by determining which alleles co-segregated in the families. In cases where DNA from only the proband was available, we assigned (if possible) the haplotype that was most like to those present in other patients with DM2 published previously [12–15]. If this was not possible, the haplotype with the highest probability (reflecting the likelihood that this haplotype is carried by the patient) from the Phase analysis was chosen.

RESULTS

Sixty-seven patients with DM2 were identified by searching the Dutch neuromuscular database. Two patients were excluded because they were younger than 18 years old. Two registered patients were found to be deceased and 4 patients had DNA that was not available for research; leaving DNA of 59 DM2 patients from 29 unrelated families for analysis, including 14 probands (patient 1, 7, 10, 22, 23, 25, 26, 41, 52, 53, 55–59).

The mean age of genetically confirmed diagnosis was 47.2 years. Twenty-three families were from European descent. Six families (2 families from each country) ethnically originated from non-European countries, namely India, Suriname and Morocco (Table 1).

Table 1
Characteristics of the study population

N (families)	59 (29)	
Female sex (*n* (%))	34 (58)	
Age of genetically confirmed diagnosis (yrs)		
Mean (SD)	47.2 (14.4)	
Range	20–74	
Country of origin	Patients (*n* (%))	Families (*n* (%))
The Netherlands	39 (66)	18 (62)
India	8 (14)	2 (7)
Suriname	4 (7)	2 (7)
Morocco	2 (3)	2 (7)
Belgium	2 (3)	2 (7)
Ukraine	2 (3)	1 (3)
Greece	1 (2)	1 (3)
Portugal	1 (2)	1 (3)

Abbreviations: SD, standard deviation.

Haplotype analysis

Results of the haplotype analysis are shown in Figs 2 and 3. SNPs rs1384313, rs4303883 and CGAP_886192 are not included as these did not show genetic variation in our study population. Most of the identified STR marker lengths are in the same range as those reported earlier [13, 14]. In 19 of the 59 patients, only one haplotype with high probability was constructed by Phase (≥0.99). For the other 40 patients, multiple haplotypes were identified. An overview of all haplotypes constructed using Phase is presented in Supplemental File 2. All family members were assigned to the haplotype linked to the DM2 causing *CNBP* repeat expansion based on the family structure.

Comparison of our data to the previously reported data showed that 41 patients could be directly assigned to the haplotypes described by Liquori et al. [13]. Though, in 3 patients (1, 26 and 27) the exact haplotype is not clear. Patient 1 fits with haplotype B based on all markers, except for the CL3N59 marker closest to the CCTG repeat expansion in *CNBP*. Patient 26 fits within haplotype A based on the markers closest to the DM2 repeat (CL3N59 and CL3N117), but the lengths of the other markers are in line with haplotype group C. For patient 27, the length of the markers closest to the *CNBP* repeat expansion (CL3N59 and CL3N117) fits with haplotype C, but the other markers are more difficult to assign to a haplotype.

Outside the 41 patients assigned to the earlier reported Caucasian haplotypes, 18 patients did not fit well with these haplotypes [13]. For 6 patients

Marker		CL3N122	CL3N99	CL3N59	rs1871922	CL3N117	CL3N119	CL3N19	CL3N23	
Location kb from CCTG repeat		*369*	*348*	*119*	*11*	*103*	*216*	*292*	*365*	
Patient – Family	Ancestry									
Haplotype B (Liquori et al.)		**216**	**181**	**157 | 148**		**241 | 245**	**240**	**190**	**238**	Haplotype B
1-XVII	NL/Hungary	**216 | 218**	**181 | 175**	152 | 141	**C C**	**241 | 245**	**240 | 236**	**190 | 214**	**238 | 230**	
2-XI	NL	**216 | 218**	**181 | 171**	**157 | 148**	**C C**	**241 | 241**	**240 | 236**	**190 | 204**	**234 | 238**	
3-XI	NL	**216 | 216**	**181 | 181**	**157 | 148**	**C C**	**241 | 241**	**240 | 246**	**190 | 186**	**234 | 238**	
4-XI	NL	**216 | 218**	**181 | 173**	**157 | 148**	**C A**	**241 | 245**	**240 | 234**	**190 | 202**	**234 | 230**	
5-XVIII	Ukraine	**216 | 214**	**181 | 169**	**157 | 143**	**C C**	**241 | 243**	**240 | 240**	**190 | 204**	**234 | 222**	
6-XVIII	Ukraine	**216 | 218**	**181 | 181**	**157 | 143**	**C C**	**241 | 245**	**240 | 242**	**190 | 208**	**234 | 238**	
7-XIX	NL	**216 | 218**	**181 | 173**	**157 | 148**	**C A**	**241 | 245**	**240 | 236**	**190 | 200**	**234 | 234**	
8-XX	NL	**214** | 218	**169** | 173	**157 | 148**	**C A**	**241 | 245**	**240 | 238**	**190 | 204**	**226** | 230	
9-XX	NL	**214** | 216	**169** | 175	**157 | 148**	**C A**	**241 | 245**	**240 | 236**	**190 | 208**	**226** | 234	
10-XXI	Belgium	**216 | 220**	**181 | 173**	**157 | 148**	**C A**	**241 | 245**	**236** | 236	**208** | 204	**226** | 238	
Haplotype A (Liquori et al.)		**216**	**185**	**157**		**241**	**236** | 232	**208**	**NA**	Haplotype A
11-XXII	NL	**216 | 218**	**185 | 177**	**157 | 148**	**C A**	**241 | 245**	**236** | 238	**208** | 212	**234** | 230	
12-XXII	NL	**216 | 218**	**185 | 177**	**157 | 148**	**C A**	**241 | 245**	**236** | 238	**208** | 212	**234** | 230	
13-XXIII	NL	**216 | 216**	**185 | 167**	**157 | 148**	**C A**	**241 | 245**	**236** | 238	**208** | 206	**234** | 218	
14-XXII	NL	**216 | 216**	**185 | 175**	**157 | 148**	**C C**	**241 | 241**	**236** | 236	**208** | 190	**234** | 234	
15-XXII	NL	**216 | 214**	**185 | 165**	**157 | 148**	**C A**	**241 | 245**	**236** | NA	**208** | 204	**234** | 234	
16-XXII	NL	**216 | 212**	**185 | 171**	**157 | 148**	**C C**	**241 | 241**	**236** | 238	**208** | 206	**234** | 230	
17-XXII	NL	**216 | 218**	**185 | 177**	**157 | 150**	**C A**	**241 | 245**	**236** | 236	**208** | 208	**234** | 234	
18-XXII	NL	**216 | 218**	**185 | 177**	**157 | 150**	**C A**	**241 | 245**	**236** | 236	**208** | 208	**234** | 234	
19-IV	NL	**216 | 216**	**185 | 181**	**157 | 146**	**C A**	**241 | 245**	**236** | 238	**208** | 210	**234** | 230	
20-XXIII	NL	**216 | 218**	**185 | 177**	**157 | 148**	**C C**	**241 | 245**	**236** | 242	**208** | 204	**234** | 226	
21-XXIII	NL	**216 | 216**	**185 | 169**	**157 | 148**	**C A**	**241 | 245**	**236** | 236	**208** | 210	**234** | 238	
22-XXIV	NL	**216 | 212**	**185 | 171**	**157 | 148**	**C C**	**241 | 245**	**236** | 242	**208** | 204	**234** | 242	
23-XXV	NL	**216 | 216**	**185 | 179**	**157 | 146**	**C A**	**241 | 245**	238 | 236	**208** | 210	**230** | 230	
24-VII	NL	**216 | 218**	**189** | 171	**157 | 148**	**C A**	**241 | 245**	**236** | 244	**208** | 200	**230** | 238	
25-XXVI	NL	**216 | 218**	**189** | 177	**157 | 148**	**C C**	**241 | 241**	**236** | 240	**208** | 206	**230** | 242	
26-XXVII	NL	**214** | 218	**169** | 175	**157** | 141	**C C**	**241 | 245**	**240** | 236	**190** | 212	**226** | 230	
27-XIV	NL	**214** | 218	**192** | 175	**150** | 148	**C A**	**241** | 243	**240** | 232	**190** | 208	**226** | 242	Haplotype C
28-XIV	NL	**214** | 218	**192** | 175	**150** | 143	**C C**	**241 | 245**	**240** | 238	**190** | 200	**226** | 226	
29-XIV	NL	**214** | 218	**192** | 175	**150** | 146	**C A**	**241** | 247	**240** | 249	**190** | 200	**226** | 230	
30-XIV	NL	**214** | 218	**192** | 171	**150** | 148	**C C**	**241 | 245**	**240** | 248	**190** | 202	**226** | 238	
31-XIII	NL	**214** | 216	**192** | 173	**150** | 146	**C A**	**241 | 245**	**240** | 236	**190** | 214	**226** | 238	
32-XXVIII	NL	**216** | 220	**181** | 179	**150** | 148	**C C**	**241 | 245**	**234** | 224	**190** | 210	**214** | 222	
33-XXVIII	NL	**216** | 214	**181** | 169	**150** | 146	**C A**	**241 | 245**	**234** | 236	**190** | 190	**214** | 238	
34-XXVIII	NL	**216** | 216	**181** | 181	**150** | 137	**C A**	**241 | 245**	**234** | 236	**190** | 210	**214** | 238	
35-XXIX	NL	**216** | 214	**187** | 165	**150** | 148	**C A**	**241 | 245**	**224** | 234	**210** | 204	**226** | 234	
36-XXIX	NL	**216** | 216	**187** | 181	**150** | 148	**C A**	**241 | 245**	**224** | 240	**210** | 204	**226** | 230	
37-XXIX	NL	**216** | 218	**187** | 177	**150** | 148	**C C**	**241 | 241**	**224** | 240	**210** | 206	**226** | 247	
38-XXIX	NL	**216** | 218	**187** | 173	**150** | 148	**C A**	**241 | 245**	**224** | 234	**210** | 204	**226** | 226	
39-XXIX	NL	**216** | 216	**187** | 173	**150** | 150	**C A**	**241 | 245**	**224** | 236	**210** | 204	**226** | 234	
40-XXIX	NL	**216** | 216	**187** | 171	**150** | 150	**C A**	**241 | 245**	**224** | 236	**210** | 204	**226** | 234	
41-XXX	NL	216 | 216	169 | 185	150 | 146	C A	241 | 245	234 | 236	208 | 210	226 | 234	
Haplotype C (Liquori et al.)		**218**	**175**	**150**		**241**	**240**	**190**	**226** | 238	
Newly found haplotype		**214**	**169**	**141**		**245 | 241**	**226**	**196**	**222/226**	New haplotype
42-XXXI	India	**214 | 212**	**169 | 169**	**141 | 148**	**C C**	**245 | 241**	**226 | 246**	**196 | 204**	**226** | 242	
43-XXXI	India	**214 | 212**	**169 | 169**	**141 | 148**	**C A**	**245 | 241**	**226 | 236**	**196 | 200**	**226** | 222	
44-XXXI	India	**214 | 214**	**169 | 169**	**141 | 148**	**C A**	**245 | 241**	**226 | 236**	**196 | 206**	**226** | 234	
45-XXXI	India	**214 | 216**	**169 | 173**	**141 | 146**	**C C**	**245 | 241**	**226 | 242**	**196 | 204**	**226** | 238	
46-XXXI	India	**214 | 212**	**169 | 169**	**141 | 148**	**C C**	**245 | 241**	**226 | 246**	**196 | 204**	**226** | 242	
47-XXXI	India	**214 | 216**	**169 | 169**	**141 | 148**	**C A**	**245 | 241**	**226 | 238**	**196 | 208**	**226** | 234	
48-XXXI	India	**214 | 214**	**169 | 185**	**141 | 148**	**C C**	**245 | 241**	**226 | 236**	**196 | 206**	**226** | 210	
49-XXXII	Suriname	**214 | 220**	**169 | 192**	**141 | 159**	**C C**	**245 | 241**	**226 | 240**	**196 | 192**	**222** | 234	
50-XXXII	Suriname	**214 | 212**	**169 | 169**	**141 | 146**	**C A**	**245 | 241**	**226 | 238**	**196 | 192**	**222** | 234	
51-XXXII	Suriname	**214 | 225**	**169 | 173**	**141 | 146**	**C A**	**245 | 241**	**226 | 228**	**196 | 206**	**222** | 242	
52-XXXIII	Suriname	**214 | 212**	**169 | 169**	**141 | 146**	**C A**	**245 | 241**	**226 | 246**	**196 | 192**	**222** | 226	
53-XXXIV	India	**216 | 218**	**169 | 169**	**141 | 150**	**C A**	**245 | 241**	**240** | 236	**190** | 214	**222** | 234	
54-XII	NL/Austria	216 | 218	181 | 175	155 | 148	**C A**	241 | 245	234 | 236	210 | 210	222 | 238	
55-XXXV	Belgium	216 | 220	185 | 179	146 | 148	C A	241 | 245	238 | 238	210 | 204	234 | 230	
56-XXXVI	Greece	218 | 218	173 | 175	148 | 143	C A	241 | 241	238 | 238	204 | 202	230 | 234	
57-XXXVII	Portugal	**210 | 218**	**194 | 173**	**150 | 150**	**C A**	**241 | 245**	**238 | 236**	**NA | NA**	**226** | 234	Variant haplotype
58-XXXVIII	Morocco	**210 | 218**	**190 | 173**	**150 | 152**	**C C**	**241 | 241**	**238 | 238**	**186 | 190**	**222** | 238	
59-XXXIX	Morocco	**210 | 216**	**192 | 173**	**150 | 148**	**C A**	**241 | 245**	**236** | 234	**186 | 204**	**234** | 246	
37-XV Coenen et al. [14]	Morocco	**210 | 210**	**194 | 198**	**150 | 139**	**A C**	**241 | 241**	**238 | 242**	**186 | 200**	**234** | 222	
38-XV Coenen et al. [14]	Morocco	**210 | 218**	**194 | 177**	**150 | 146**	**C C**	**241 | 245**	**238 | 236**	**186 | 206**	**234** | 242	
39-XV Coenen et al. [14]	Morocco	**214 | 216**	**194 | 173**	**150 | 143**	**C C**	**241 | 241**	**238 | 236**	**186 | 190**	**234** | 226	
Saito et al. [15] (*)	Japan	**NA | NA**	**NA | NA**	**137 |**	**A A**	**245 |**	**226 |**	**NA |**	**NA |**	
Liquori et al. [13] (*)	Afghanistan	**214 |**	**191 |**	**157 |**		**241 |**	232 | 236	**214 |**	**NA |**	

CCTG expansion

Fig. 2. Haplotypes of 59 DM2 patients. *(both color and black/white version):*NA: not analysed. The length of the analysed markers is expressed in base pairs. Haplotypes are based on earlier reported haplotypes [13–15]. Repeat lengths as reported by Liquori et al. are shown in the first row [13]. The families related to earlier reported patients by Coenen et al. are shown in italic [14]. Patients have been assigned to a haplotype based on the observed repeat lengths. The repeat lengths of the disease-linked alleles are shown in bold. For some individual patients, it was not possible to determine this with certainty. Genotypes shared among the different haplotypes are shaded. * Recalculated reported marker lengths of previous studies to make them comparable to our lengths.

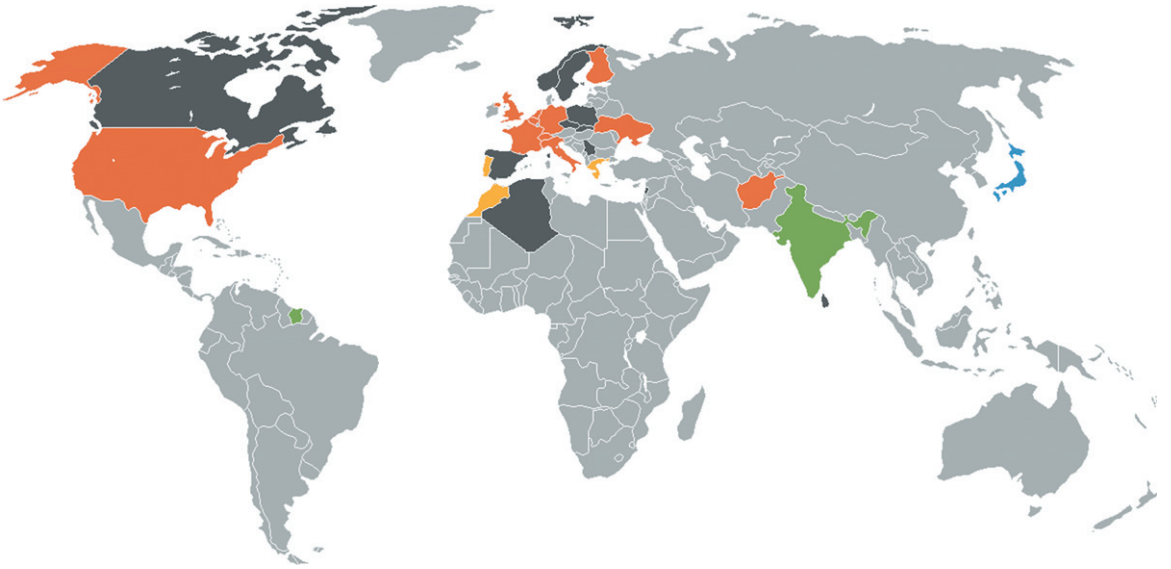

Fig. 3. Geographic distribution of the distinct DM2 haplotypes. *(color version):* Patients with DM2 throughout the world as reported in literature (Canada, USA, Suriname, UK, Norway, Sweden, Finland, The Netherlands, Belgium, France, Spain, Portugal, Germany, Switzerland, Italy, Malta, Poland, Czech Republic, Slovakia, Ukraine, Serbia, Greece, Morocco, Algeria, Lebanon, Afghanistan, India, Sri Lanka and Japan) [11, 12, 14, 15, 24, 25] Orange: European haplotype. Blue: Japanese haplotype. Green: Indian haplotype. Yellow: haplotype does not exactly fit with European haplotype. Dark grey: reported patients with DM2, but no haplotyping study is done. *(black/white version):* Patients with DM2 throughout the world as reported in literature (Canada, USA, Suriname, UK, Norway, Sweden, Finland, The Netherlands, Belgium, France, Spain, Portugal, Germany, Switzerland, Italy, Malta, Poland, Czech Republic, Slovakia, Ukraine, Serbia, Greece, Morocco, Algeria, Lebanon, Afghanistan, India, Sri Lanka and Japan) [11, 12, 14, 15, 24, 25] Striped pattern: European haplotype. Checkered pattern: Japanese haplotype. Dotted pattern: Indian haplotype. Dark grey: haplotype does not exactly fit with European haplotype. Black: reported patients with DM2, but no haplotyping study is done.

(54–59) the lengths of the markers closest to the repeat were not comparable with previously reported lengths, however some of the markers located more distant from the repeat expansion are in line with the previously reported haplotypes. Thus, based on our results we cannot assign these patients to one of the previously reported haplotypes. The 3 probands from Portugal and Morocco (patient 57, 58 and 59) have a haplotype comparable with the Moroccan patients (family XV) reported by Coenen et al. [14]. The probability of the haplotypes of these patients was low for patient 57 (0.35) and higher for the other 2 individuals (>0.83).

Eleven patients were related to 6 families earlier reported by Coenen et al. (family IV, VII, XI-XIV) [14]. In our results, we used the same numbering for these families as reported earlier to compare the haplotype of our patients with their relatives. The length of the repeat markers of our 11 patients are the same as those of their earlier reported relatives.

Four families (XXXI, XXXII, XXXIII and XX XIV) from India and Suriname showed a haplotype different from the common haplotypes, with six markers shared within family XXXI, XXXII and XXXIII over a 661-kb interval. The other patient of Indian descent (patient 53, family XXXIV) shares three markers with these families.

The SNP rs1871922, located 11kb telomeric from the CCTG expansion in intron 1 of the *CNBP* gene, was analysed in 59 patients. All patients carried at least one C allele. All except for patient 55 and 56, confirmed that the expansion is linked to the C allele. For patient 55 and 56 it remained unclear whether the DM2 causing *CNBP* repeat expansion is linked to the C or A allele of the SNP. However, as both patients are from Caucasian origin it is highly likely that the expansion is linked to the C allele.

DISCUSSION

In this study, we suggest that the DM2 linked repeat expansion in *CNBP* may originate from more founders than previously had been thought. Through our research with the Indian and Suriname families, a new haplotype may have been identified. The lengths of the markers of these families do not show a high

overlap with either the European or Japanese haplotypes [13–15].

The similarity between the haplotype of these 4 families (Indian and Suriname families XXXI-XXXIV) suggests that the Surinamese patients are connected to the Indian family XXXI. Indeed, it is highly plausible that these are from Indian descent, as the Indians form 27% of the population of Suriname. The Indians in Suriname are descendants of 19th-century contract workers from British India [19]. A previous study established 1 Afghan family carrying a haplotype strikingly similar to the core region of haplotype A. This DM2 linked *CNBP* repeat expansion may have been introduced when the ancient Aryan tribes of Indo-European extraction settled in ancient Afghanistan [13]. Based on the migration of the Indo-Europeans, it is likely that patients from North Indian descent have similarity to this Afghan haplotype and therefore to haplotype A.

Genetic studies have shown that most Indian groups are descendants of two major genetically divergent populations [20]. The first, Ancestral North Indians, are (genetically) related to Central Asians, Middle Easterners, Caucasians and Europeans (West Eurasians). Ancestral South Indians are restricted to South Asia and are not closely related to groups outside their region, but to indigenous Andaman Islanders. Both groups are also distinct from East Asians (including Japan).

The 4 families included in this study from Indian descent do not have haplotypes similar to the Afghan patients or European patients. This suggests that the haplotype of the Indian patients differs from that of the European haplotype, and therefore possibly originate from Ancestral South India. Unfortunately, exact data regarding the geographic origin of the family of the Indian patients is missing.

In addition, genotype analysis of SNP rs1871922 showed that all patients, including the Indian families, carry at least one C allele. Thus, it might be possible that the Indian families carry a short common haplotype between the markers CL3N59 and CL3N117. Unfortunately, the three additional SNPs (located close to the repeat expansion) did not show variation in any of the patients. We specifically selected all SNPs from literature and databases with high frequency differences between populations. Based on these analyses, we cannot conclude, with certainty, that the Indian families carry a non-Caucasian haplotype as in the case of the reported Japanese founder haplotype (associated with the A allele) [12, 15, 21]. However, based on the previous described migration history, an absence of an A allele and different lengths of the other analysed markers, we propose that the DM2 linked repeat expansion in Indian patients originates from an ancestral origin different from the European and Japanese families.

The similarity between the patients with Indian ancestries and the previously reported Japanese patient is the length of the CL3N117 and CL3N119 markers, which is respectively 245 and 226 bp. The 226 bp allele has a frequency of 10% in Caucasians, in Asia the prevalence is probably equal or higher [13].

We included 14 patients with DM2 that did not have diagnosed family members with DM2 in The Netherlands. In those cases, it is more difficult to determine which alleles are linked with DM2. The 3 individuals (patient 54–56) that do not fit within a European haplotype all have at least 1 parent originating from outside The Netherlands. Patient 54 has a mother from Austria and patients 55 and 56 have a Belgian and Greek parent respectively. These differences in ancestry may explain the wider variations in the length of the analysed STR repeat markers. It would be necessary to analyse relatives of these patients to pinpoint the haplotype linked to DM2 in these patients.

The patients from Portugal (patient 57) and Morocco (patient 58 and 59) have marker lengths comparable with previously reported Moroccan patients [14]. These patients may have another haplotype, or at least variations of the major Caucasian haplotypes, due to potential recombination and microsatellite instability events in flanking regions. Their haplotype has probably arisen from the C haplotype, as the 222 kb region surrounding the *CNBP* gene of these patients is comparable with this haplotype.

Our study included patients originating from European countries that have not been examined before, namely Ukraine, Belgium, Greece and Portugal. We show that DM2 patients from Ukraine and Belgium carry the European common haplotype and that patients originating from Greece, Portugal and Morocco may have a variant of this haplotype (Figs 2 and 3). These results are in line with previous reports showing that patients from European ancestry carry a common haplotype [12, 13]. Further research should be undertaken to obtain more insight in the worldwide haplotypes of DM2 as data from other regions are lacking [15].

In addition, it would be interesting to investigate whether these haplotypes differ in phenotypic presentation and severity of DM2. For example, research regarding the presence of additional symptoms such as cataracts, cardiac conduction defects and autoimmune diseases in DM2 patients with different haplotypes would be worthwhile to investigate whether these are linked to specific haplotypes. The mean age of genetically confirmed diagnosis of our study population corresponds to literature with a mean onset between the third and fourth decade [1, 22, 23].

Based on the haplotype studies performed thus far, including this study, we can conclude that the European haplotype is associated with a 241 bp length of the CL3N117 marker, as all tested European patients carry this allele [13, 14]. This repeat length occurs at a frequency of 12% in a healthy Caucasian population in which a length of 245 bp is much more prevalent (82%) [13]. Besides, the DM2 region appears to be highly conserved allele [13]. This all indicates that the European founder *CNBP* repeat expansion developed on the less frequent 241 bp length. In the Japanese patient, the CL3N117 marker length associated with DM2 was 245 bp. In the Indian haplotype, it is most likely that the *CNBP* repeat expansion is also linked to the repeat length of 245 bp of CL3N117, as this was the haplotype constructed with highest probability for all members of family XXXI. For 4 patients (43, 45, 47 and 48) the probability was even 1.0. More research in patients with DM2 from India is necessary to validate this observation. Research regarding the most common haplotypes in India would also be interesting and informative.

Our study describes the first Indian patients with DM2, all currently living and diagnosed in The Netherlands. DM2 is probably (significantly) underdiagnosed in Indians. Our research describes 4 different families with a DM2 causing *CNBP* repeat expansion just inside The Netherlands. The fact that India has a population twice as big as Europe, genetic testing for DM2 in Indian population might indeed show that DM2 is underdiagnosed in this population.

In conclusion, our data shows that patients from Indian descent may carry a haplotype different from the haplotypes identified before. This might suggest a third founder in the etiology of myotonic dystrophy type 2. We also show that most European patients with DM2 carry the common founder haplotype. Further studies in European and especially non-European and Asian DM2 patients are required to confirm our findings.

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CONFLICT OF INTEREST

The authors have no conflict of interest to report

SUPPLEMENTARY MATERIAL

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REFERENCES

[1] Day JW, Ricker K, Jacobsen JF, Rasmussen LJ, Dick KA, Kress W, et al. Myotonic dystrophy type 2: Molecular, diagnostic and clinical spectrum. Neurology. 2003;60(4):657-64. doi:10.1212/01.wnl.0000054481.84978.f9

[2] Meola G. Clinical and genetic heterogeneity in myotonic dystrophies. Muscle Nerve. 2000;23(12):1789-99. doi:10.1002/1097-4598(200012)23:12<1789::aid-mus2>3.0.co;2-4

[3] Wahbi K, Meune C, Becane HM, Laforet P, Bassez G, Lazarus A, et al. Left ventricular dysfunction and cardiac arrhythmias are frequent in type 2 myotonic dystrophy: A case control study. Neuromuscul Disord. 2009;19(7):468-72. doi:10.1016/j.nmd.2009.04.012

[4] Tieleman AA, van Vliet J, Jansen JB, van der Kooi AJ, Borm GF, van Engelen BG. Gastrointestinal involvement is frequent in Myotonic Dystrophy type 2. Neuromuscul Disord. 2008;18(8):646-9. doi:10.1016/j.nmd.2008.05.010

[5] Hilbert JE, Barohn RJ, Clemens PR, Luebbe EA, Martens WB, McDermott MP, et al. High frequency of gastrointestinal manifestations in myotonic dystrophy type 1 and type 2. Neurology. 2017;89(13):1348-54. doi:10.1212/WNL.0000000000004420

[6] Tieleman AA, den Broeder AA, van de Logt AE, van Engelen BG. Strong association between myotonic dystrophy type 2 and autoimmune diseases. J Neurol Neurosurg Psychiatry. 2009;80(11):1293-5. doi:10.1136/jnnp.2008.156562

[7] Peric S, Mandic-Stojmenovic G, Stefanova E, Savic-Pavicevic D, Pesovic J, Ilic V, et al. Frontostriatal dysexecutive syndrome: A core cognitive feature of myotonic dystrophy type 2. J Neurol. 2015;262(1):142-8. doi:10.1007/s00415-014-7545-y

[8] Meola G, Sansone V, Perani D, Scarone S, Cappa S, Dragoni C, et al. Executive dysfunction and avoidant personality trait in myotonic dystrophy type 1 (DM-1) and in proximal myotonic myopathy (PROMM/DM-2). Neuromuscul Disord. 2003;13(10):813-21. doi:10.1016/s0960-8966(03)00137-8

[9] Minnerop M, Weber B, Schoene-Bake JC, Roeske S, Mirbach S, Anspach C, et al. The brain in myotonic dystrophy 1 and 2: Evidence for a predominant white matter disease.

Brain. 2011;134(Pt 12):3530-46. doi:10.1093/brain/awr299

[10] van Vliet J, Tieleman AA, van Engelen BGM, Bassez G, Servais L, Behin A, et al. Hearing impairment in patients with myotonic dystrophy type 2. Neurology. 2018; 90(7):e615-e22. doi:10.1212/WNL.0000000000004963

[11] Liquori CL, Ricker K, Moseley ML, Jacobsen JF, Kress W, Naylor SL, et al. Myotonic dystrophy type 2 caused by a CCTG expansion in intron 1 of ZNF9. Science. 2001;293(5531):864-7. doi:10.1126/science.1062125

[12] Bachinski LL, Udd B, Meola G, Sansone V, Bassez G, Eymard B, et al. Confirmation of the type 2 myotonic dystrophy (CCTG)n expansion mutation in patients with proximal myotonic myopathy/proximal myotonic dystrophy of different European origins: A single shared haplotype indicates an ancestral founder effect. Am J Hum Genet. 2003;73(4):835-48. doi:10.1086/378566

[13] Liquori CL, Ikeda Y, Weatherspoon M, Ricker K, Schoser BG, Dalton JC, et al. Myotonic dystrophy type 2: Human founder haplotype and evolutionary conservation of the repeat tract. Am J Hum Genet. 2003;73(4):849-62. doi:10.1086/378720

[14] Coenen MJ, Tieleman AA, Schijvenaars MM, Leferink M, Ranum LP, Scheffer H, et al. Dutch myotonic dystrophy type 2 patients and a North-African DM2 family carry the common European founder haplotype. Eur J Hum Genet. 2011;19(5):567-70. doi:10.1038/ejhg.2010.233

[15] Saito T, Amakusa Y, Kimura T, Yahara O, Aizawa H, Ikeda Y, et al. Myotonic dystrophy type 2 in Japan: Ancestral origin distinct from Caucasian families. Neurogenetics. 2008;9(1):61-3. doi:10.1007/s10048-007-0110-4

[16] van Engelen BG, van Veenendaal H, van Doorn PA, Faber CG, van der Hoeven JH, Janssen NG, et al. The Dutch neuromuscular database CRAMP (Computer Registry of All Myopathies and Polyneuropathies): Development and preliminary data. Neuromuscul Disord. 2007;17(1):33-7. doi:10.1016/j.nmd.2006.09.017

[17] Stephens M, Smith NJ, Donnelly P. A new statistical method for haplotype reconstruction from population data. Am J Hum Genet. 2001;68(4):978-89. doi:10.1086/319501

[18] Stephens M, Scheet P. Accounting for decay of linkage disequilibrium in haplotype inference and missing-data imputation. Am J Hum Genet. 2005;76(3):449-62. doi:10.1086/428594

[19] General Statistics Bureau of Suriname. Censusstatistieken 2012. 2012. pp. 76.

[20] Reich D, Thangaraj K, Patterson N, Price AL, Singh L. Reconstructing Indian population history. Nature. 2009;461(7263):489-94. doi:10.1038/nature08365

[21] Vallo L, Bonifazi E, Borgiani P, Novelli G, Botta A. Characterization of a single nucleotide polymorphism in the ZNF9 gene and analysis of association with myotonic dystrophy type II (DM2) in the Italian population. Mol Cell Probes. 2005;19(1):71-4. doi:10.1016/j.mcp.2004.09.003

[22] Hilbert JE, Ashizawa T, Day JW, Luebbe EA, Martens WB, McDermott MP, et al. Diagnostic odyssey of patients with myotonic dystrophy. J Neurol. 2013;260(10):2497-504. doi:10.1007/s00415-013-6993-0

[23] Papadopoulos C, Kekou K, Xirou S, Kitsiou-Tzeli S, Kararizou E, Papadimas GK. Early onset posterior subscapular cataract in a series of myotonic dystrophy type 2 patients. Eye (Lond). 2018;32(3):622-5. doi:10.1038/eye.2017.280

[24] Udd B, Meola G, Krahe R, Thornton C, Ranum L, Day J, et al. Report of the 115th ENMC workshop: DM2/PROMM and other myotonic dystrophies. 3rd Workshop, 14-16 February 2003, Naarden, The Netherlands. Neuromuscul Disord. 2003;13(7-8):589-96. doi:10.1016/s0960-8966(03)00092-0

[25] Radvanszky J, Surovy M, Polak E, Kadasi L. Uninterrupted CCTG tracts in the myotonic dystrophy type 2 associated locus. Neuromuscul Disord. 2013;23(7):591-8. doi:10.1016/j.nmd.2013.02.013