

## The TREAT-NMD DMD Global Database: Analysis of More than 7,000 Duchenne Muscular Dystrophy Mutations

Catherine L. Bladen,<sup>1,\*†</sup> David Salgado,<sup>2†</sup> Soledad Monges,<sup>3</sup> Maria E. Foncuberta,<sup>3</sup> Kyriaki Kekou,<sup>4</sup> Konstantina Kosma,<sup>4,5</sup> Hugh Dawkins,<sup>6</sup> Leanne Lamont,<sup>6</sup> Anna J. Roy,<sup>7</sup> Teodora Chamova,<sup>8</sup> Velina Guergueltcheva,<sup>8</sup> Sophelia Chan,<sup>9</sup> Lawrence Korngut,<sup>10</sup> Craig Campbell,<sup>11</sup> Yi Dai,<sup>12</sup> Jen Wang,<sup>13</sup> Nina Barišić,<sup>14</sup> Petr Brabec,<sup>15</sup> Jaana Lahdetie,<sup>16</sup> Maggie C. Walter,<sup>17</sup> Olivia Schreiber-Katz,<sup>17</sup> Veronika Karcagi,<sup>18</sup> Marta Garami,<sup>18</sup> Venkatarman Viswanathan,<sup>19</sup> Farhad Bayat,<sup>20</sup> Filippo Buccella,<sup>21</sup> En Kimura,<sup>22</sup> Zaïda Koeks,<sup>23</sup> Janneke C. van den Bergen,<sup>23</sup> Miriam Rodrigues,<sup>24</sup> Richard Roxburgh,<sup>24</sup> Anna Lusakowska,<sup>25</sup> Anna Kostera-Pruszczyk,<sup>25</sup> Janusz Zimowski,<sup>26</sup> Rosário Santos,<sup>27</sup> Elena Neagu,<sup>28</sup> Svetlana Artemieva,<sup>29</sup> Vedrana Milic Rasic,<sup>30</sup> Dina Vojinovic,<sup>30</sup> Manuel Posada,<sup>31</sup> Clemens Bloetzer,<sup>32</sup> Pierre-Yves Jeannot,<sup>32</sup> Franziska Joncourt,<sup>33</sup> Jordi Díaz-Manera,<sup>34</sup> Eduard Gallardo,<sup>34</sup> A. Ayşe Karaduman,<sup>35</sup> Haluk Topaloğlu,<sup>36</sup> Rasha El Sherif,<sup>37</sup> Angela Stringer,<sup>38</sup> Andriy V. Shatillo,<sup>39</sup> Ann S. Martin,<sup>40</sup> Holly L. Peay,<sup>40</sup> Matthew I. Bellgard,<sup>41</sup> Jan Kirschner,<sup>42</sup> Kevin M. Flanigan,<sup>43</sup> Volker Straub,<sup>1</sup> Kate Bushby,<sup>1</sup> Jan Verschuuren,<sup>23</sup> Annemieke Aartsma-Rus,<sup>1,44</sup> Christophe Bérout,<sup>2,45</sup> and Hanns Lochmüller<sup>1</sup>

<sup>1</sup>The John Walton Muscular Dystrophy Research Centre, MRC Centre for Neuromuscular Diseases Institute of Genetic Medicine, University of Newcastle, Central Parkway, Newcastle upon Tyne, UK; <sup>2</sup>Aix Marseille Université, Inserm, GMGF UMR\_S 910, Marseille, France; <sup>3</sup>Hospital de Pediatria J. P. Garrahan, Pichincha, Argentina; <sup>4</sup>Department of Medical Genetics, Medical School, University of Athens, Athens, Greece; <sup>5</sup>Choremio Research Laboratory, St. Sophia's Children's Hospital Thinoon and Levadia Goudi, Athens, Greece; <sup>6</sup>Department of Health, Office of Population Health Genomics, Perth, Western Australia, Australia; <sup>7</sup>WIV-ISP, Juliette Wytsman 14, Brussels, Belgium; <sup>8</sup>Department of Neurology, Medical University-Sofia, Sofia, Bulgaria; <sup>9</sup>Department of Paediatrics and Adolescent Medicine, Queen Mary Hospital, University of Hong Kong, Pok Fu Lam, Hong Kong; <sup>10</sup>Department of Clinical Neurosciences and Hotchkiss Brain Institute, University of Calgary, Calgary, Canada; <sup>11</sup>Department of Paediatrics, Clinical Neurological Sciences & Epidemiology, Western University, London, Ontario, Canada; <sup>12</sup>Department of Neurology, Peking Union Medical College Hospital, Peking Union Medical College and Chinese Academy of Medical Sciences, Beijing, P. R. China; <sup>13</sup>Department of Neurology, China Dolls, the General Hospital of Chinese Armed Police Forces, Haidian, Beijing, P. R. China; <sup>14</sup>Division of Paediatric Neurology, University Hospital Centre Zagreb (KBC Zagreb), University of Zagreb Medical School, Croatia; <sup>15</sup>Institute for Biostatistic and Analyses, Masaryk University, Brno, Czech Republic; <sup>16</sup>Department of Child Neurology, Turku University Central Hospital, Turku, Finland; <sup>17</sup>Department of Neurology, Friedrich-Baur-Institute, Ludwig-Maximilians-University of Munich, Munich, Germany; <sup>18</sup>Department of Molecular Genetics and Diagnostics, NIEH, Budapest, Hungary; <sup>19</sup>CHILDS Trust Medical Research Foundation and Apollo Children's Hospital, Chennai, India; <sup>20</sup>Pasteur Institute of Iran, Karaj complex, Tehran, Iran; <sup>21</sup>Parent Project Onlus, Via Nicola Coviello n. 12, Roma, Italy; <sup>22</sup>4-1-1 Ogawa-Higashi, Kodaira, Tokyo, Japan; <sup>23</sup>Department of Neurology, Leiden University Medical Center, Leiden, The Netherlands; <sup>24</sup>Department of Neurology, Auckland DHB, Auckland, New Zealand; <sup>25</sup>Department of Neurology, Medical University of Warsaw, Warsaw, Poland; <sup>26</sup>Department of Genetics, Institute of Psychiatry and Neurology, Warsaw, Poland; <sup>27</sup>Centro de Genética Médica Jacinto Magalhães, Praça Pedro Nunes 88, Porto, Portugal; <sup>28</sup>National Institute of Legal Medicine, Genetics Laboratory 9, Vitan Barzesti, Bucharest, Romania; <sup>29</sup>Moscow Institute of Pediatrics, Taldomskayastr, Moscow, Russia; <sup>30</sup>Clinic for Neurology and Psychiatry for Children and Youth, Faculty of Medicine, University of Belgrade, Belgrade, Serbia; <sup>31</sup>Institute of Rare Diseases Research, Spain RDR and CIBERER, Institute of Health Carlos III, Madrid, Spain; <sup>32</sup>Neurorehabilitation Unit, Lausanne University Hospital, Lausanne, Switzerland; <sup>33</sup>Division of Human Genetics, Children's University Hospital, Inselspital, Bern, Switzerland; <sup>34</sup>Unitat de Malalties Neuromusculars, Servei de Neurologia, Hospital de la Santa Creu i Sant Pau de Barcelona, Barcelona, Spain; <sup>35</sup>Department of Physiotherapy and Rehabilitation, Faculty of Health Sciences, Hacettepe University, Ankara, Turkey; <sup>36</sup>Hacettepe Children's Hospital, Ankara, Turkey; <sup>37</sup>Neurology and Neurogenic Unit, Egypt Air Hospital, Ain Shams University, Egypt; <sup>38</sup>Action Duchenne, Epicentre, London, UK; <sup>39</sup>Institute of Neurology, Psychiatry and Narcology of NAMS, Kharkiv, Ukraine; <sup>40</sup>Duchenne Connect, Hackensack, New Jersey; <sup>41</sup>Centre for Comparative Genomics, Murdoch University, Murdoch, Western Australia; <sup>42</sup>University Medical Center Freiburg, Freiburg, Germany; <sup>43</sup>Center for Gene Therapy, The Research Institute, Nationwide Children's Hospital, Columbus, Ohio; <sup>44</sup>Department of Human Genetics, Leiden University Medical Center, Leiden, The Netherlands; <sup>45</sup>Département de Génétique Médicale et de Biologie Cellulaire, INSERM APHM, Hôpital d'Enfants de la Timone, Marseille, France

Communicated by William S. Oetting

Received 18 November 2014; accepted revised manuscript 13 January 2015.

Published online 21 January 2015 in Wiley Online Library (www.wiley.com/humanmutation). DOI: 10.1002/humu.22758

† This author contributed equally to this work.

‡ Lawrence Korngut present address is Clinical Neurosciences, South Health Campus, 4448 Front Street SE, Calgary, Alberta, Canada.

\* Correspondence to: Catherine L. Bladen, The John Walton Muscular Dystrophy Research Centre, MRC Centre for Neuromuscular Diseases, Institute of Genetic Medicine, University of Newcastle, Times Square, Newcastle

upon Tyne, Newcastle NE13BZ, United Kingdom. E-mail: catherine.bladen@ncl.ac.uk

Contract grant sponsor(s): TREAT-NMD (FP6LSHM-CT-2006-036825, 20123307 UNEW\_FY2013, and AFM 16104); European Union Seventh Framework Programme (FP7/2007-2013) ( 305444 [RD-Connect] and 305121 [Neuromics]).

© 2015 The Authors. \*\*Human Mutation published by Wiley Periodicals, Inc.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

**ABSTRACT:** Analyzing the type and frequency of patient-specific mutations that give rise to Duchenne muscular dystrophy (DMD) is an invaluable tool for diagnostics, basic scientific research, trial planning, and improved clinical care. Locus-specific databases allow for the collection, organization, storage, and analysis of genetic variants of disease. Here, we describe the development and analysis of the TREAT-NMD DMD Global database ([http://umd.be/TREAT\\_DMD/](http://umd.be/TREAT_DMD/)). We analyzed genetic data for 7,149 DMD mutations held within the database. A total of 5,682 large mutations were observed (80% of total mutations), of which 4,894 (86%) were deletions (1 exon or larger) and 784 (14%) were duplications (1 exon or larger). There were 1,445 small mutations (smaller than 1 exon, 20% of all mutations), of which 358 (25%) were small deletions and 132 (9%) small insertions and 199 (14%) affected the splice sites. Point mutations totalled 756 (52% of small mutations) with 726 (50%) nonsense mutations and 30 (2%) missense mutations. Finally, 22 (0.3%) mid-intronic mutations were observed. In addition, mutations were identified within the database that would potentially benefit from novel genetic therapies for DMD including stop codon read-through therapies (10% of total mutations) and exon skipping therapy (80% of deletions and 55% of total mutations).

*Hum Mutat* 36:395–402, 2015. Published 2015 Wiley Periodicals, Inc.\*

**KEY WORDS:** DMD; Duchenne muscular dystrophy; TREAT-NMD; rare disease registries

## Introduction

Duchenne muscular dystrophy (DMD) is a severe, X-linked, progressive neuromuscular disease caused by mutations in the *DMD* gene (DMD; MIM #310200) [Hoffman et al., 1988]. Mutations in this gene give rise to two forms of muscular dystrophy depending on whether the translational reading frame is lost or maintained: severe DMD, due to out of frame mutations leading to loss of protein function, or a milder form of muscular dystrophy known as Becker muscular dystrophy (BMD; MIM #300376), caused by a reduction in the amount and/or size of dystrophin protein due to frame maintaining mutations [Koenig et al., 1989]. The *DMD* gene is the largest known gene in humans, spanning 2.3 Mb of genomic DNA. The coding sequence spans 11 Kb and is made up of 79 exons [Ahn and Kunkel, 1993]. Many different types of mutation have been described for DMD including large deletions and duplications, point mutations, and small rearrangements.

DMD has a prevalence of 21.2/100,000 school aged boys [Mah et al., 2014]. Current care recommendations (specifically, the use of corticosteroids, cardiac medications, and assisted ventilation) improve outcomes and quality of life but do not modify the underlying progression of the disease [Sejerson and Bushby, 2009; Hoffman et al., 2012]. Potential treatment strategies center primarily on targeted mitigation of the causative genetic mutation. One example of a genetic-based potential therapy is nonsense stop codon read-through therapy [Howard et al., 2000; Wagner et al., 2001; Hirawat et al., 2007; Welch et al., 2007]. These treatments include aminoglycosides and ataluren (previously PTC124), and work by selectively inducing ribosomal read-through of premature stop codons but

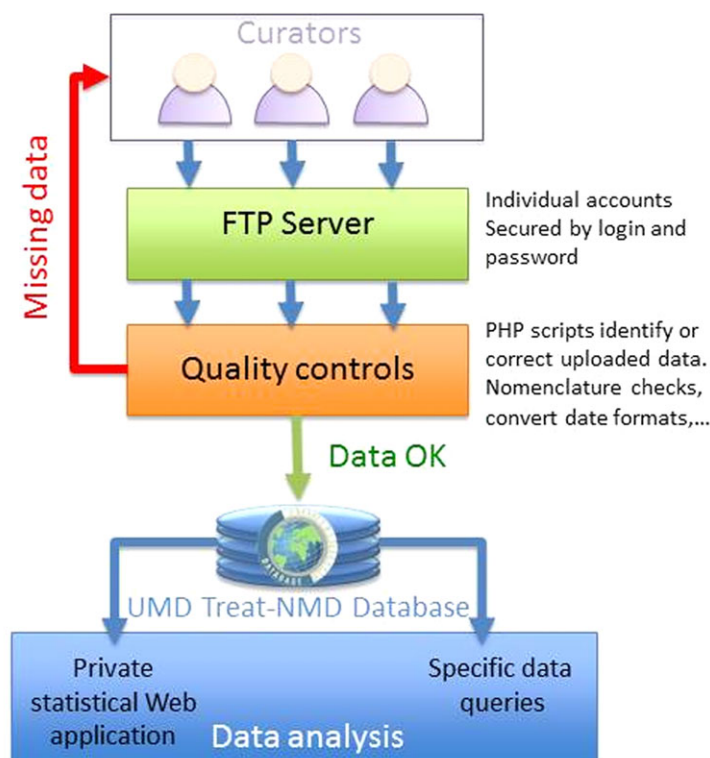
not normal stop codons. Specific nonsense mutations exist in DMD patients leading to premature stop codons (TGA, TAG, and TAA) and would potentially benefit from this therapy. Translarna recently obtained conditional marketing authorization from the European Medicine Agency for use in ambulant Duchenne patients over 5 years of age, and as such is the first drug to be approved for DMD.

A further example is the exon skipping approach. Exon skipping aims to moderate disease progression by taking advantage of the knowledge that internally deleted dystrophins (seen in BMD) can be partially functional [Bérout et al., 2007; Aartsma-Rus et al., 2009; van Ommen and Aartsma-Rus, 2013]. Significant research has been undertaken in the field of exon skipping to restore the open reading frame of dystrophin transcripts resulting in the production of partly functional dystrophin protein [van Ommen et al., 2008; Aartsma-Rus et al., 2009]. Exon skipping is achieved by the use of antisense oligonucleotides (AONs) that specifically bind to and hide exons from the splicing machinery, leading to an in-frame mRNA without this exon and giving rise to internally deleted dystrophin proteins as seen in BMD patients [Takeshima et al., 2001; Aartsma-Rus et al., 2003, 2004; Suroño et al., 2004; McClorey et al., 2006; Archavala-Gomez et al., 2007; Gurvich et al., 2008]. Since DMD has a relatively high rate of new mutations (one in three mutations is new), most patients have unique mutations [Aartsma-Rus et al., 2006; Tuffery-Giraud et al., 2009]. However, approximately 60%–65% of all DMD patients carry a deletion of one or more exons, with a tendency to cluster between exons 45 and 55 [Aartsma-Rus et al., 2006; Tuffery-Giraud et al., 2009]. Furthermore, while the location of the breakpoints in introns will differ for patients with a deletion of exon 48–50, these deletions will give rise to identical transcripts. Therefore, the skipping of certain exons would be applied to relatively large numbers of patients.

Understanding the type and frequency of patient-specific mutations that give rise to DMD-associated phenotypes is an invaluable tool for genetic diagnosis, basic scientific research, and improved clinical care, potentially leading to new treatments for the disease. Currently, the TREAT-NMD DMD Global database contains over 7,000 (7,149 as of November 2013) mutations ([http://umd.be/TREAT\\_DMD/](http://umd.be/TREAT_DMD/)). Locus-specific databases (LSDBs) allow for the collection, organization, storage, and analysis of genetic variants of disease. LSDBs collect all published and unpublished mutations for a specific gene along with complete clinical and phenotypic information. Additional confidence exists in these data sets due in part to the role of experts or “curators.” Curators validate the data held within the database and significantly reduce error rates [Bérout et al., 2005; Cotton et al., 2008]. In the case of LSDBs for DMD, a number of databases exist including, the Leiden muscular dystrophy pages (<http://www.dmd.nl/>) in the Netherlands [Aartsma-Rus et al., 2006], and the UMD-DMD (<http://www.umd.be/DMD/>) in France [Cotton et al., 2008]. We here report a new global mutation database for DMD, and outline how this can be used for genetic analysis and development of genetic therapies.

## Methods

A new global database for DMD (TREAT-NMD DMD Global database) based on the French UMD-DMD system has been developed with TREAT-NMD collaboration. TREAT-NMD was initially established as an EU-funded “network of excellence” with the remit of “reshaping the research environment” in the neuromuscular field ([<http://www.treat-nmd.eu/>], 2013; Bushby et al., 2009). Standardized mutation (DMD mutations) specific data based on TREAT-NMD mandatory and highly encouraged items from the national TREAT-NMD DMD registries [Bladen et al., 2013] were



**Figure 1.** Upload of data from national TREAT-NMD DMD registries to Global database. Standardized aggregate data from the national TREAT-NMD DMD registries was transferred to the global DMD database via a secure File Transfer Protocol transfer in order to provide a single cohort of genetic and clinical variants.

**Table 1. Type and Frequency of Mutations Held within the TREAT-NMD DMD Global Database**

		Percentage of total mutations
Total	7,149	
Large mutations	5,682	79
Large deletions ( $\geq 1$ exon)	4,894	68
Large duplications ( $\geq 1$ exon)	784	11
Small mutations	1,445	20
Small deletions (<1 exon)	358	5
Small insertions (<1 exon)	132	2
Splice sites (<10 bp from exon)	199	3
Point mutations	756	11
Nonsense	726	10
Missense	30	0.4
Mid-intronic mutations	22	0.3

transferred to the global DMD database via a secure File Transfer Protocol transfer in November 2013 in order to provide a single cohort of genetic and clinical variants (Fig. 1). Analysis of DMD genetic mutations was then carried out for the 7,149 patient data sets held within the TREAT-NMD DMD Global database. HGVS (Human Genome Variation Society) nomenclature was used throughout (<http://www.hgvs.org/mutnomen/>).

## Results

The TREAT-NMD DMD Global database currently contains 7,149 DMD mutations. There were 5,684 large mutations (80%), of which 4,894 (86%) were deletions (1 exon or larger) and 784 (14%) were duplications (1 exon or larger) (Table 1). There were

1,445 small mutations (20% of all mutations), of which 358 (25%) were deletions (smaller than 1 exon) and 132 (9%) were duplications (smaller than one exon); 199 (14%) splice site mutations were recorded. Point mutations totalled 756 (10% of all mutations, 52% of small mutations) with 726 nonsense mutations (10% of all mutations, 50% of small mutations) and three missense mutations (<1% of all mutations, 2% of small mutations). Finally, 22 (less than 1% of all mutations, 0.3% of small mutations) mid-intronic mutations were observed (Table 1).

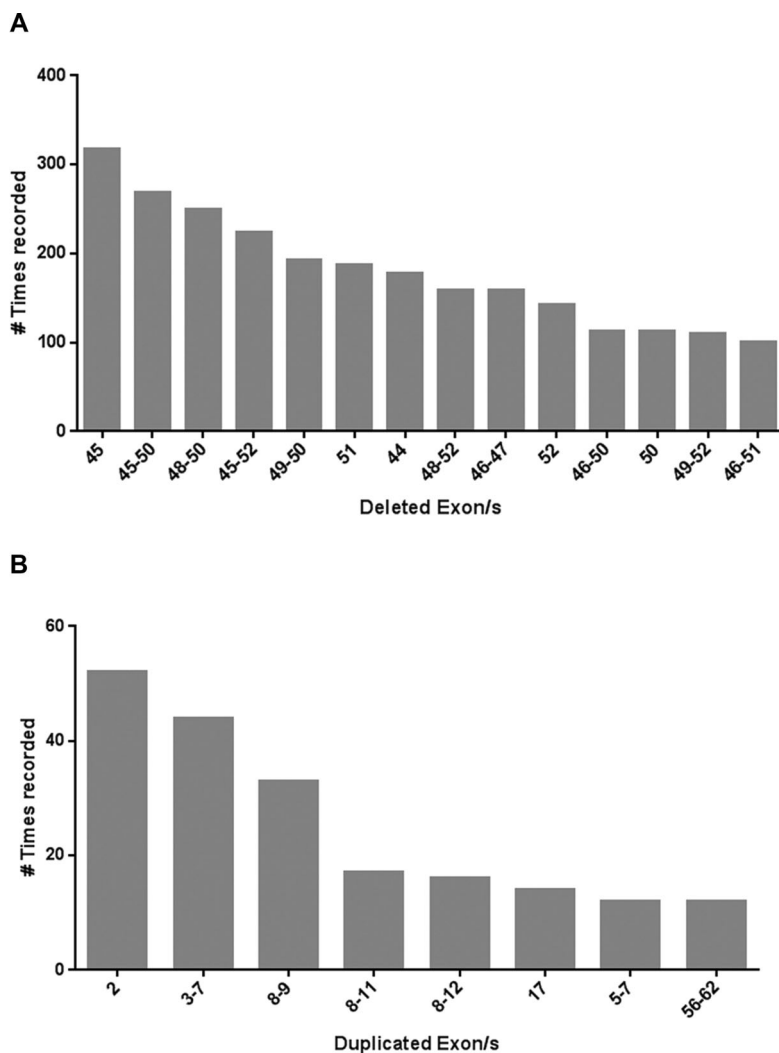
### Large Mutations

#### Large deletions

Four thousand eight hundred ninety four large deletions were reported and accounted for 68% of total mutations. Figure 2a highlights the ten most commonly reported (observed more than 100 times) large deletions within the database. The most common large deletion was a deletion of exon 45, which was recorded 316 times in the database (4% of deletions). Distribution of large deletions was nonrandom with the majority of large deletions (80%) covering either the distal region mutation hot spot of the dystrophin gene (exons 45–55) or the proximal region (exons 2–20) (Fig. 3).

#### Large duplications

Seven hundred eighty four large duplications were reported and accounted for 11% of total mutations. Figure 2b shows the eight most commonly reported large duplications (observed more than



**Figure 2.** Most commonly reported large mutations. Most commonly reported large deletions (recorded 100 times or more) (A) and large duplications (recorded ten times or more) (B) in the TREAT-NMD DMD Global database.

ten times). The most common large duplication was duplication of exon 2 (11% of duplications). Distribution of large duplications was nonrandom with most large duplications involving distal or proximal mutation hot spots (65%) (Fig. 3).

#### Single exon deletions and duplications

The five most frequent single exon deletions recorded in the database (all reported more than 100 times) were deletion of exon 45 (4%), 51 (3%), 44 (3%), 52 (3%), and 50 (2%). Single exon duplications occurred less frequently than single exon deletions. Duplication of exon 2 was the most frequent and was reported 50 times, while the second most frequent single exon duplication was duplication of exon 17 and was reported 11 times in the database.

#### Small Mutations

The database contained 1,445 small lesions and included deletions (smaller than 1 exon; 355, 25%), duplications (smaller than

one exon; 132, 9%), 199 (14%) splice site mutations and 756 (52%) point mutations, 726 (50%) nonsense mutations, 30 (2%) missense mutations, and 22 (0.3%) mid-intronic mutations (Table 1). Small deletions and mutations ranged in size from two nucleotides (occurring 85 times) to 111 nucleotides (occurring twice).

#### Nonsense mutations

Nonsense mutations represented 50% of the small mutations in the database and 10% of total mutations. Transition mutational events (70%) were more common than transversions (30%), with the C-to-T substitution being the most frequent (90%).

#### Potential DMD therapies

Nonsense stop codon read-through therapy has obtained conditional marketing authorization [Howard et al., 2000; Wagner et al., 2001; Hirawat et al., 2007; Welch et al., 2007]. This treatment selectively induces ribosomal read-through of premature stop codons but not normal stop codons. Mutations were identified

**Table 2. Overview of DMD Exons**

Real <sup>a</sup>	Mutation (%)	Deletion (%)	Adjusted <sup>b</sup>	Mutation (%)	Deletion (%)
Exon 51	14.0	20.50	Exon 51	14.0	20.5
Exon 53	10.1	14.7	Exon 45	9.0	13.1
Exon 45	9.0	13.1	Exon 53	8.1	11.8
Exon 44	7.1	11.1	Exon 44	7.6	11.1
Exon 43	7.5	11.0	Exon 50	3.8	5.6
Exon 46	4.6	6.7	Exon 43	3.1	4.5
Exon 50	3.8	5.6	Exon 8	2.00	2.9
Exon 52	3.6	5.3	Exon 55	1.7	2.5
Exon 55	2.7	3.9	Exon 52	0.9	1.3
Exon 8	2.0	2.9	Exon 11	0.9	1.3

<sup>a</sup>Overview of exons for which single exon skipping would be applicable to the largest groups of patients.

<sup>b</sup>Adjusted overview of applicability of single exon skipping.

within the database that would potentially benefit from this therapy. These included 317 mutations (4% of overall mutations) with a premature TGA stop codon, 215 (3%) with a TAG stop codon, and 194 (3%) with a TAA stop codon.

Exon skipping technology takes advantage of the fact that inter-nally deleted dystrophins, often seen in BMD, can be partially functional. Mutations were identified within the database that would potentially benefit from exon skipping therapy. The top ten exon skips that would be applicable to the largest group of patients were skipping of exon 51 (14% of total mutations/21% of deletions), 53 (10%/15%), 45 (9%/13%), 44 (7%/11%), 43 (7%/11%), 46 (5%/7%), 50 (4%/6%), 52 (4%/5%), 55 (3%/4%), and 8 (2%/3%), respectively, as shown in Table 2. It is important to point out that the applicability of exon skipping of certain exons reduces once AONs targeting other exons have been developed. For example, the reading frame of exon 52 deletions can be restored by skipping exon 51 or by skipping exon 53. However, once an AON for exon 51 has been developed and approved for clinical use, the additional applicability of exon 53 skipping is then lower than the a priori applicability, since it now only applies to 8% of mutations rather than 10%, because the exon 52 deletion has already been rescued by exon 51 skipping. The top 10 of exon skips and their added applicability (i.e., taking this adjustment into account) is shown in Table 2.

### CpG sites

Substitutions involving a CpG dinucleotide accounted for 31% (233/756) of point mutations within the database. The CpG dinucleotide has been shown to undergo oxidative deamination of 5-methyl cytosine resulting in a mutational “hot spot” and mutation rates an order of magnitude higher than normally expected [Akalin et al., 1994; Krawczak et al., 1998; Flanigan et al., 2009].

### Geography and DMD mutations

Regardless of geographical location (determined by continent), large deletions were by far the most commonly observed (64% in Oceania to 88% in Africa) mutation followed by large duplications (5% in Africa to 12% in Europe). The number of small mutations was generally more variable (7% in Africa to 22% in Oceania) but this variability is likely explained by the fact that not all countries routinely assay for point mutations and other small lesions and indeed numbers of patients were significantly smaller, for example, in Africa compared to Europe (Fig. 4).

## Discussion

### Databases

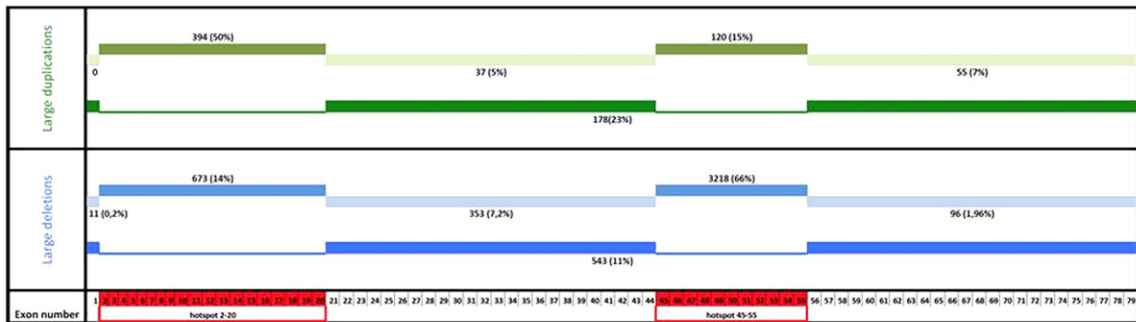
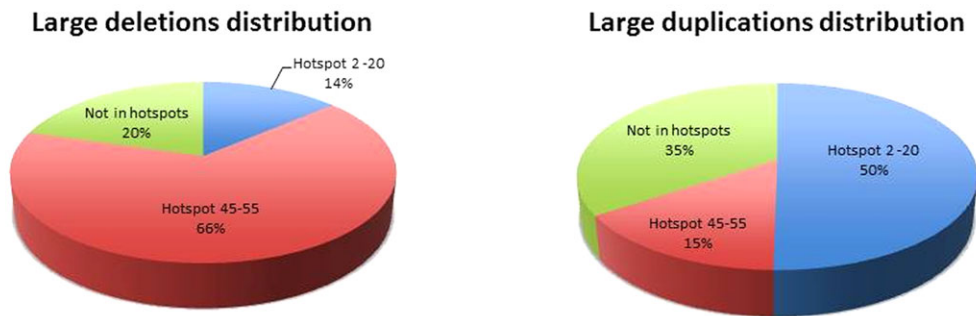
Resources existed for DMD prior to the creation of the TREAT-NMD DMD Global database, the Leiden muscular dystrophy pages (<http://www.dmd.nl/>) in the Netherlands [Aartsma-Rus et al., 2006], and the UMD-DMD (<http://www.umd.be/DMD/>) in France [Cotton et al., 2008]. For both the previously existing databases, bias of recorded mutations has been an inherent problem due in part to the method used to determine the mutation. Historically, detection of deletions and duplications was easier than detection of point mutations and other small rearrangements leading to an overrepresentation of such mutations in the literature and indeed a possible underrepresentation of point mutations and other small rearrangements. However, this bias is becoming less of an issue due to current diagnostic techniques that are widely available [Prior and Bridgeman, 2005; Flanigan et al., 2009]. Also, while initially, the most commonly occurring mutations were recorded; now there is potentially a bias toward only novel mutations being recorded in the Leiden database. In addition to this, the UMD-DMD database is specific to France and could potentially include a bias for mutations observed with higher or lower frequencies only in France. The new TREAT-NMD DMD Global database houses what we believe to be the single largest cohort of verified DMD mutations in the world and was established to collect and compare and molecular mutations found within this patient group. Mutational analysis of the database illustrates the allelic heterogeneity of the *DMD* gene. Indeed, one-third of all DMD mutations occur de novo [Laing, 1993].

### Analysis and Comparisons

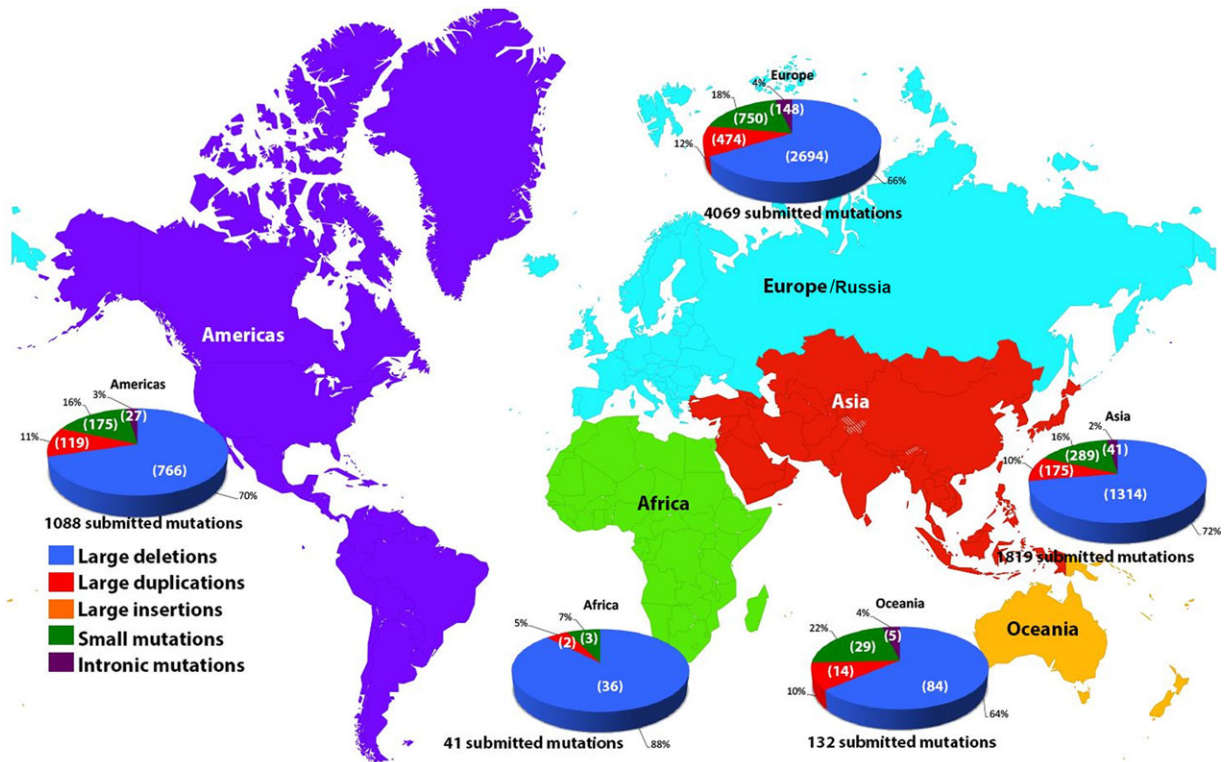
Analysis of the TREAT-NMD DMD Global database revealed that large deletions were the most prevalent genetic mutation recorded and accounted for 68% of the total mutations analyzed, deletion of exon 45 being the single most common large deletion (reported 316 times). These results are similar to both the French UMD database [Tuffery-Giraud et al., 2009] with 62% of the mutations being large deletions and the Leiden database [Aartsma-Rus et al., 2006], where 72% of the mutations are large deletions. In the Leiden database and the TREAT-NMD DMD Global databases, deletion of exon 45 was the most common deletion, making up 4% of the mutations in the TREAT-NMD DMD Global database and 2% of the Leiden database. Large duplications accounted for 11% of mutations in the TREAT-NMD DMD Global database compared to 13% in the French UMD database and 8% in the Leiden database. The most commonly occurring large duplication was duplication of exon 2 in all three databases. Small rearrangements accounted for 20% of the TREAT-NMD DMD Global database that was similar to the French UMD database (26%) and the Leiden database (20%). Point mutations and nonsense mutation were the most prevalent small rearrangements with nonsense mutations accounting for 50% of the small rearrangements in the TREAT-NMD DMD Global database, compared to 40% in the French UMD database and 50% in the Leiden database. Large deletions and duplications follow a nonrandom distribution with 78% of them including either the proximal or distal mutation hot spots [Koenig et al., 1989; Prior and Bridgeman, 2005; Aartsma-Rus et al., 2006].

### Reading Frame Rule

The majority of the reported (DMD) mutations in the TREAT-NMD DMD Global database resulted in frame-shift mutations



**Figure 3.** Distribution of the most common large deletions and duplications on the *DMD* gene.



**Figure 4.** Geography and DMD mutations. Distribution of DMD mutation types stratified by continent.

(93%). Mutations not following the reading-frame rule in the TREAT-NMD DMD Global database accounted for 7% of total mutations compared with 4% in the UMD-DMD database and 9% in the Leiden database.

## Potential DMD Therapies

Several potential novel DMD therapies exist and are focused on the mitigation of the underlying genetic defect. The two most promising examples are nonsense read-through and exon skipping. Mutations were identified within the database that would potentially benefit from this stop codon read-through therapy (10% of mutations). Exon skipping mutations were identified within the database that would potentially benefit from exon skipping therapy (55% of total mutations and 80% of deletions).

Understanding the type and frequency of patient-specific mutations that give rise to DMD-associated phenotypes will potentially lead to personalized (targeted/precision) therapies. DMD essentially serves as a paradigm for this type of treatment and ultimately could lead the way to similar approaches in other rare diseases and indeed in more common disorders.

## Acknowledgments

The authors would like to acknowledge the families of those living with DMD who have been instrumental in the formation of the DMD national registries. We also acknowledge former and current members of the TREAT-NMD office at the Institute of Genetic Medicine in Newcastle including Stephen Lynn, Emma Heslop, and Rachel Thompson. We also extend our thanks to the members of the TREAT-NMD executive committee: Hanns Lochmuller, Annemieke Aartsma-Rus, Anna Ambrosini, Filippo Buccella, Kevin Flanigan, Eric Hoffman, Janbernd Kirschner, Eugenio Mercuri, Ichizo Nishino, Kathy North, Jes Rahbek, and Thomas Sejersen. We also acknowledge the members of the current TGDOC: Jan Verschuuren (Chair), Hugh Dawkins (Chair elect), Anna Ambrosini, Svetlana Artemieva, Alexander N. Baranov, Farhad Bayat, Christophe Bérout, Ria Broekgaarden, Filippo Buccella, Craig Campbell, Nick Catlin, Monica Ensini, Pat Furlong, Kevin Flanigan, Ole Gredal, Lauren Hache, Seraplnal, Jacqueline Jackson, Pierre-Yves Jeannot, Anna Kaminska, A. Ayse Karaduman, Veronika Karcagi, En Kimura, Janbernd Kirschner, Jaana Lähdetie, Hanns Lochmüller, Vitaliy Matyushenko, Vedrana Milic-Rasic, Violeta Mihaylova, Marie-Christine Ouilade, Ian Murphy, Miriam Rodrigues, Rosario dos Santos, Pascale Saugier-Verber, Inge Schwersenz, Thomas Sejersen, Rasha El Sherif, Eduardo Tizzano, Isabela Tudorache, Sylvie Tuffery-Giraud, Jen Wang, Simon Woods, W. Ludo van der Pol, Peter Van den Bergh, and Petr Vondráček.

*Disclosure statement:* Professor Hanns Lochmuller was elected chair of the TREAT-NMD Alliance in April 2012 and is the previous chair of the oversight committee. Professor Lochmuller has a financial interest/arrangement with Pfizer, Ultragenyx, and GlaxoSmithKline (Research grant investigator).

## References

Aartsma-Rus A, Fokkema I, Verschuuren J, Ginjaar I, van Deutekom J, van Ommen GJ, den Dunnen JT. 2009. Theoretic applicability of antisense-mediated exon skipping for Duchenne muscular dystrophy mutations. *Hum Mutat* 30:293–299.

Aartsma-Rus A, Janson AA, Kaman WE, Bremmer-Bout M, den Dunnen JT, Baas F, van Ommen GJ, van Deutekom JC. 2003. Therapeutic antisense-induced exon skipping in cultured muscle cells from six different DMD patients. *Hum Mol Genet* 12:907–914.

Aartsma-Rus A, Janson AA, Kaman WE, Bremmer-Bout M, van Ommen GJ, den Dunnen JT, van Deutekom JC. 2004. Antisense-induced multiexon skipping for Duchenne muscular dystrophy makes more sense. *Am J Hum Genet* 74:83–92.

Aartsma-Rus A, Van Deutekom JC, Fokkema IF, Van Ommen GJ, den Dunnen JT. 2006. Entries in the Leiden Duchenne muscular dystrophy mutation database: an overview of mutation types and paradoxical cases that confirm the reading-frame rule. *Muscle Nerve* 34:135–144.

Ahn AH, Kunkel LM. 1993. The structural and functional diversity of dystrophin. *Nat Genet* 3:283–291.

Akalin N, Zietkiewicz E, Makalowski W, Labuda D. 1994. Are CpG sites mutation hot spots in the dystrophin gene? *Hum Mol Genet* 3:1425–1426.

Arechavala-Gomez V, Graham IR, Popplewell LJ, Adams AM, Aartsma-Rus A, Kinali M, Morgan JE, van Deutekom JC, Wilton SD, Dickson G, Muntioni F. 2007. Comparative analysis of antisense oligonucleotide sequences for targeted skipping of exon 51 during dystrophin pre-mRNA splicing in human muscle. *Hum Gene Ther* 18:798–810.

Bérout C, Hamroun D, Collod-Beroud G, Boileau C, Soussi T, Claustres M. 2005. UMD (Universal Mutation Database): 2005 update. *Hum Mutat* 26:184–191.

Bérout C, Tuffery-Giraud S, Matsuo M, Hamroun D, Humbertclaude V, Monnier N, Moizard MP, Voelckel MA, Calemard LM, Boisseau P, et al. 2007. Multiexon skipping leading to an artificial DMD protein lacking amino acids from exons 45 through 55 could rescue up to 63% of patients with Duchenne muscular dystrophy. *Hum Mutat* 28:196–202.

Bladen CL, Rafferty K, Straub V, Monges S, Moresco A, Dawkins H, Roy A, Chamova T, Guergueltcheva V, Korngut L, et al. 2013. The TREAT-NMD Duchenne muscular dystrophy registries: conception, design, and utilization by industry and academia. *Hum Mutat* Nov;34(11):1449–57.

Bushby K, Lynn S, Straub T, Network T-N. 2009. Collaborating to bring new therapies to the patient—the TREAT-NMD model. *Acta Myol* 28:12–15.

Cotton RGH, Auerbach AD, Beckmann JS, Blumenfeld OO, Brookes AJ, Brown AE, Carrera P, Cox DW, Gottlieb B, Greenblatt MS, et al. 2008. Recommendations for locus-specific databases and their curation. *Hum Mutat* 29:2–5.

Flanigan KM, Dunn DM, von Niederhausern A, Soltanzadeh P, Gappmaier E, Howard MT, Sampson JB, Mendell JR, Wall C, King WM, et al. 2009. Mutational spectrum of DMD mutations in dystrophinopathy patients: application of modern diagnostic techniques to a large cohort. *Hum Mutat* 30:1657–1666.

Gurvich OL, Tuohy TM, Howard MT, Finkel RS, Medne L, Anderson CB, Weiss RB, Wilton SD, Flanigan KM. 2008. DMD pseudoexon mutations: splicing efficiency, phenotype, and potential therapy. *Ann Neurol* 63:81–89.

Hirawat S, Welch EM, Elfring GL, Northcutt VJ, Paushkin S, Hwang S, Leonard EM, Almstead NG, Ju W, Peltz SW, et al. 2007. Safety, tolerability, and pharmacokinetics of PTC124, a nonaminoglycoside nonsense mutation suppressor, following single- and multiple-dose administration to healthy male and female adult volunteers. *J Clin Pharmacol* 47:430–444.

Hoffman EP, Fischbeck KH, Brown RH, Johnson M, Medori R, Loike JD, Harris JB, Waterston R, Brooke M, Specht L, et al. 1988. Characterization of dystrophin in muscle-biopsy specimens from patients with Duchenne's or Becker's muscular dystrophy. *N Engl J Med* 318:1363–1368.

Hoffman EP, Reeves E, Damsker J, Nagaraju K, McCall JM, Connor EM, Bushby K. 2012. Novel approaches to corticosteroid treatment in Duchenne muscular dystrophy. *Phys Med Rehabil Clin N Am* 23:821–828.

Howard MT, Shirts BH, Petros LM, Flanigan KM, Gesteland RF, Atkins JF. 2000. Sequence specificity of aminoglycoside-induced stop codon readthrough: potential implications for treatment of Duchenne muscular dystrophy. *Ann Neurol* 48:164–169.

Koenig M, Beggs AH, Moyer M, Scherpf S, Heindrich K, Bettecken T, Meng G, Muller CR, Lindlof M, Kaariainen H, et al. 1989. The molecular basis for Duchenne versus Becker muscular dystrophy: correlation of severity with type of deletion. *Am J Hum Genet* 45:498–506.

Krawczak M, Ball EV, Cooper DN. 1998. Neighboring-nucleotide effects on the rates of germ-line single-base-pair substitution in human genes. *Am J Hum Genet* 63:474–488.

Laing NG. 1993. Molecular genetics and genetic counselling for Duchenne/Becker muscular dystrophy. *Mol Cell Biol Hum Dis Ser* 3:37–84.

Mah JK, Korngut L, Dykeman J, Day L, Pringsheim T, Jette N. 2014. A systematic review and meta-analysis on the epidemiology of Duchenne and Becker muscular dystrophy. *Neuromuscul Disord* 24:482–491.

McCloy G, Moulton HM, Iversen PL, Fletcher S, Wilton SD. 2006. Antisense oligonucleotide-induced exon skipping restores dystrophin expression in vitro in a canine model of DMD. *Gene Ther* 13:1373–1381.

Prior TW, Bridgeman SJ. 2005. Experience and strategy for the molecular testing of Duchenne muscular dystrophy. *J Mol Diagn* 7:317–326.

Sejerson T, Bushby K, Excellence T-NENo. 2009. Standards of care for Duchenne muscular dystrophy: brief TREAT-NMD recommendations. *Adv Exp Med Biol* 652:13–21.

Surono A, Van Khanh T, Takeshima Y, Wada H, Yagi M, Takagi M, Koizumi M, Matsuo M. 2004. Chimeric RNA/ethylene-bridged nucleic acids promote dystrophin expression in myocytes of duchenne muscular dystrophy by inducing skipping of the nonsense mutation-encoding exon. *Hum Gene Ther* 15:749–757.

Takeshima Y, Wada H, Yagi M, Ishikawa Y, Ishikawa Y, Minami R, Nakamura H, Matsuo M. 2001. Oligonucleotides against a splicing enhancer sequence led to dystrophin

- production in muscle cells from a Duchenne muscular dystrophy patient. *Brain Dev* 23:788–790.
- Tuffery-Giraud S, Bérout C, Leturcq F, Yaou RB, Hamroun D, Michel-Calemard L, Moizard MP, Bernard R, Cossee M, Boisseau P, et al. 2009. Genotype-phenotype analysis in 2405 patients with a dystrophinopathy using the UMD-DMD database: a model of nationwide knowledgebase. *Hum Mutat* 30:934–945.
- van Ommen GJ, Aartsma-Rus A. 2013. Advances in therapeutic RNA-targeting. *N Biotechnol* 30:299–301.
- van Ommen GJ, van Deutekom J, Aartsma-Rus A. 2008. The therapeutic potential of antisense-mediated exon skipping. *Curr Opin Mol Ther* 10:140–149.
- Wagner KR, Hamed S, Hadley DW, Gropman AL, Burstein AH, Escolar DM, Hoffman EP, Fischbeck KH. 2001. Gentamicin treatment of Duchenne and Becker muscular dystrophy due to nonsense mutations. *Ann Neurol* 49:706–711.
- Welch EM, Barton ER, Zhuo J, Tomizawa Y, Friesen WJ, Trifillis P, Paushkin S, Patel M, Trotta CR, Hwang S, et al. 2007. PTC124 targets genetic disorders caused by nonsense mutations. *Nature* 447:87–91.