- 大学病院医療情報ネットワーク研究センター〔UMIN-CTR〕
  http://www.umin.ac.jp/ctr/
- 日本医薬情報センター〔JAPIC〕
  http://www.japic.or.jp/
- 日本医師会治験促進センター〔JMACCT CTR〕
  http://www.jmacct.med.or.jp/
- ClinicalTrials.gov
  http://clinicaltrials.gov
- EU Clinical Trials Register
  https://www.clinicaltrialsregister.eu

ウェブサイトについて現状を把握するための項目を以下のように設定した。

ウェブサイトのアクセシビリティの調査を実施するにあたり，調査の基準として「行政情報の電子的提供に関する基本的な考え方（指針）」に記載されている JIS X 8341-3 を利用した。JIS X 8341-3（高齢者・障害者等配慮設計指針－情報通信における機器，ソフトウェア及びサービス－第三部：ウェブコンテンツ）とは，World Wide Web Consortium (W3C)によって勧告されているウェブアクセシビリティに関する国際的なガイドライン Web Content Accessibility Guidelines 2.0 (WCAG 2.0)をベースとした日本工業規格である。JIS X 8341-3 では高齢者・障害者がウェブコンテンツを利用するときに情報アクセシビリティを確保させるために，ウェブコンテンツの企画，設計，開発，制作，保守および運用の全ての工程において配慮すべき一般的原則を示している。JIS X 8341-3 には等級 A, AA, AAA の 3 つのランクが定められており，AAA が最も達成基準が厳しい等級である。今回の調査では等級 A を満たすための 25 個の細分箇条について調査を実施した(表 1)。

細分箇条の達成条件のうち，自動チェックが可能な項目はチェックツールで調査し，自動チェックが不可能な項目は手動（主に目視）で調査を実施した。自動チェックツールには総務省が無償提供している「みんなのアクセシビリティ評価ツール」を使用して調査を行った。

調査対象は，日本国内の JPRN：(UMIN-CTR, JAPIC, JMACCT CTR, NIPH)の 4 機関および米国，欧州において先進的に取り組んでおり，臨床研究・治験登録数が多く，情報検索ポータルサイトを運営している組織 ClinicalTrials.gov (CT.gov)，European Medicines Agency EU Clinical Trials Register (EU CTR)，2 機関の臨床研究情報公開ウェブサイトとした。各機関のウェブサイト内ではトップページと臨床研究情報を検索するための条件を入力するページを対象とし，検索条件指定ページが複数存在している機関については複数を対象とした。細分箇条にはそれに適合するための複数の達成基準が設けられており，本調査では自動チェックツールで適合判定可能な項目については全基準を調査し，手動チェックが必要な基準については音声ブラウザでの表示時に差が発生するなど，健常者以外にとって使用しやすいウェブサイトになっているかを判定する達成基準を中心にチェック対象とした。適合判定基準は以下とした。自動チェックツールで適合と判定された達成基準：適合，自動チェックツールで不適合と判定された達成基準：不適合，自動チェックツールで適合項目 0 と判定された達成基準：該当無し，自動チェックツールで手動確認と判定された達成基準：目視または W3C が提供する HTML 構文チェックツール(The W3C Markup Validation Service)にて確認し，不適合箇所が 1 箇所以上存在している場合は不適合，不適合箇所が 0 箇所の場合は適合，適用するべき項目が 0 個だった場合は該当なしとした。

JIS X 8341-3	WCAG 2.0	項目
7.1.1.1	1.1.1	非テキストコンテンツに関する達成基準
7.1.2.1	1.2.1	収録済みの音声しか含まないメディア及び収録済みの映像しか含まないメディアに関する達成基準
7.1.2.2	1.2.2	収録済みの音声コンテンツのキャプションに関する達成基準
7.1.2.3	1.2.3	収録済みの映像コンテンツの代替コンテンツ又は音声ガイドに関する達成基準
7.1.3.1	1.3.1	情報及び関係性に関する達成基準
7.1.3.2	1.3.2	意味のある順序に関する達成基準
7.1.3.3	1.3.3	感覚的な特徴に関する達成基準
7.1.4.1	1.4.1	色の使用に関する達成基準
7.1.4.2	1.4.2	音声制御に関する達成基準
7.2.1.1	2.1.1	キーボード操作に関する達成基準
7.2.1.2	2.1.2	フォーカス移動に関する達成基準
7.2.2.1	2.2.1	調整可能な制限時間に関する達成基準
7.2.2.2	2.2.2	一時停止，停止及び非表示に関する達成基準
7.2.3.1	2.3.1	3回のせん(閃)光又はいき(閾)値以下に関する達成基準
7.2.4.1	7.2.4.1	ブロックスキップに関する達成基準
7.2.4.2	7.2.4.2	ページタイトルに関する達成基準
7.2.4.3	2.4.3	フォーカス順序に関する達成基準
7.2.4.4	2.4.4	文脈におけるリンクの目的に関する達成基準
7.3.1.1	3.1.1	ページの言語に関する達成基準
7.3.2.1	3.2.1	オンフォーカスに関する達成基準
7.3.2.2	3.2.2	ユーザインタフェースコンポーネントによる状況の変化に関する達成基準
7.3.3.1	3.3.1	入力エラー箇所の特定に関する達成基準
7.3.3.2	3.3.2	ラベル又は説明文に関する達成基準
7.4.1.1	4.1.1	構文解析に関する達成基準
7.4.1.2	4.1.2	プログラムが解釈可能な識別名，役割及び設定可能な値に関する達成基準

表 1　日本工業規格 JIS X 8341-3 等級 A の細分箇条と Web Content Accessibility Guidelines 2.0

(倫理面への配慮)

該当なし

C.研究結果

日本，米国，欧州におけるウェブサイト・ポータルサイトについて現状を把握するための項目を比較および検討を行った。

1）トップページについて

日本ウェブサイトおよび欧米ウェブサイトの適合数は，9 から 13 であった。JMACCT CTR と CT.gov が多く，NIPH, UMIN-CTR と JAPIC は少なかった。他のウェブサイトで不適合が多かった細分箇条 7.3.1.1 と 7.2.4.1 については，NIPH ポータルサイトでは適合であった。一方，他のウェブサイトで適合が多かった 3 つの細分箇条 7.2.1.2, 7.2.4.4, 7.3.2.1 については NIPH ポータルサイトでは不適合であった(表 2)。

JIS X8341-3	NIPH	UMIN-CTR	JAPIC	JMACCT CTR	CT.gov	EU CTR
7.1.1.1	適合	不適合	不適合	適合	適合	適合
7.1.2.1	–	–	–	–	–	–
7.1.2.2	–	–	–	–	–	–
7.1.2.3	–	–	–	–	–	–
7.1.3.1	適合	不適合	適合	適合	不適合	適合
7.1.3.2	適合	適合	適合	適合	適合	不適合
7.1.3.3	適合	適合	適合	適合	適合	適合
7.1.4.1	適合	不適合	適合	適合	適合	適合
7.1.4.2	–	–	–	–	–	–
7.2.1.1	適合	適合	適合	適合	適合	適合
7.2.1.2	不適合	適合	適合	適合	適合	適合
7.2.2.1	–	–	–	–	–	–
7.2.2.2	–	–	–	–	–	–
7.2.3.1	–	–	–	–	–	–
7.2.4.1	適合	適合	不適合	不適合	不適合	適合
7.2.4.2	適合	適合	不適合	適合	適合	適合
7.2.4.3	適合	適合	適合	適合	適合	適合
7.2.4.4	不適合	適合	適合	適合	適合	適合
7.3.1.1	適合	不適合	不適合	不適合	適合	不適合
7.3.2.1	不適合	適合	適合	適合	適合	適合
7.3.2.2	–	–	–	適合	–	–
7.3.3.1	–	–	–	適合	適合	–
7.3.3.2	–	–	適合	–	不適合	–
7.4.1.1	不適合	不適合	不適合	不適合	不適合	不適合
7.4.1.2	不適合	不適合	不適合	不適合	不適合	不適合
機関対象ウェブアドレス	http://rctportal.niph.go.jp/	http://www.umin.ac.jp/ctr/index-j.htm	http://www.clinicaltrials.jp/user/cte_main.jsp	https://dbcentre3.jmacct.med.or.jp/jmactr/	http://clinicaltrials.gov/ct	https://www.clinicaltrialsregister.eu/

表 2　JIS 規格細分箇条と各機関トップページの対応

2）検索ページについて

日本ウェブサイトおよび欧米ウェブサイトの適合数は，10 から 14 であった。適合したウェブサイトについては，各機関によってバラツキがあり，同じ機関であっても検索ページによって適合細分箇条が異なるところもあった。適合数は，CT.gov が多く，UMIN-CTR，JAPIC，JMACCT CTR は少なかった。他のウェブサイトで不適合が多かった細分箇条 7.2.4.1 と 7.3.3.2 については，NIPH ポータルサイトでは適合であった。一方，他のウェブサイトで適合が多かった 3 つの細分箇条 7.1.3.2，7.2.1.2，7.3.2.1 については NIPH ポータルサイトでは不適合であった(表 3)。

JIS X8341-3	NIPH	UMIN-CTR	JAPIC	JAPIC	JMACCT CTR	CT.gov	CT.gov	EU CTR
7.1.1.1	適合	不適合	不適合	不適合	不適合	適合	適合	適合
7.1.2.1	–	–	–	–	–	–	–	–
7.1.2.2	–	–	–	–	–	–	–	–
7.1.2.3	–	–	–	–	–	–	–	–
7.1.3.1	不適合	不適合	適合	適合	不適合	適合	適合	不適合
7.1.3.2	不適合	適合	適合	適合	適合	適合	適合	適合
7.1.3.3	適合	適合	適合	適合	適合	適合	適合	適合
7.1.4.1	適合	適合	適合	適合	適合	適合	適合	適合
7.1.4.2	–	–	–	–	–	–	–	–
7.2.1.1	適合	適合	適合	適合	適合	適合	適合	適合
7.2.1.2	不適合	適合	適合	適合	適合	適合	適合	適合
7.2.2.1	–	–	–	–	–	–	–	–
7.2.2.2	–	–	–	–	–	–	–	–
7.2.3.1	–	–	–	–	–	–	–	–
7.2.4.1	適合	適合	不適合	不適合	不適合	不適合	不適合	適合
7.2.4.2	適合	適合	適合	適合	適合	適合	適合	適合
7.2.4.3	適合	適合	不適合	適合	適合	適合	適合	不適合
7.2.4.4	適合	不適合	適合	適合	適合	適合	不適合	適合
7.3.1.1	適合	不適合	不適合	不適合	不適合	適合	適合	不適合
7.3.2.1	不適合	適合	適合	適合	適合	適合	適合	不適合
7.3.2.2	–	–	適合	適合	–	–	–	–
7.3.3.1	適合	適合	適合	適合	適合	適合	適合	適合
7.3.3.2	適合	不適合	不適合	不適合	不適合	適合	不適合	不適合
7.4.1.1	不適合	不適合	不適合	不適合	不適合	不適合	不適合	不適合
7.4.1.2	不適合	不適合	不適合	不適合	不適合	不適合	不適合	適合
機関対象ウェブアドレス	http://rctportal.niph.go.jp/search	https://upload.umin.ac.jp/cgi-open-bin/ctr/ctr.cgi?function=search&action=input	http://www.clinicaltrials.jp/user/cteSearch.jsp	http://www.clinicaltrials.jp/user/ctrSearch.jsp	https://dbcentre3.jmacct.med.or.jp/jmactr/App/JMACTRS03/JMACTRS03.aspx?kbn=14	http://clinicaltrials.gov/ct2/search	http://clinicaltrials.gov/ct2/search/advanced	https://www.clinicaltrialsregister.eu/ctr-search/search;jsessionid=ac10292dd310afecff18ee84bad8f68bb0f62d4bdd6.rlmOa3eInljyqA4IpR9BcxaNbNq

表 3　JIS 規格細分箇条と各機関検索ページの対応

トップページのみで比較すると，国内ウェブサイトよりも欧米ウェブサイトの適合率が高い細分箇条は 7 個，国内ウェブサイトのほうが適合率が高い細分箇条は 3 個となっている。また，検索条件入力ページのみで比較すると国内ウェブサイトよりも欧米ウェブサイトの適合率が高い細分箇条は 7 個，国内ウェブサイトのほうが適合率が高い細分箇条は 3 個となっており，いずれも日本のウェブサイトのほうが適合数が少なく，その差も全体で比較した内容よりも広がっている。

D.考察

Chiang MF らの研究をはじめとするウェブアクセシビリティに関する W3C を用いた調査研究がいくつかあるが，一般の健康に関する情報に関して，視覚障害者がアクセスできる箇所（ページ）が制限されているため，規格に準拠したウェブサイトを構成することやウェブアクセシビリティを向上させることが必要と指摘している。臨床研究・治験に関するウェブサイトについては，情報アクセシビリティを考慮したウェブサイトを構築・運営しているところは少ない。臨床研究・治験情報提供機関においては，ドイツの Primary Register である German Clinical Trials Register (DRKS)で，情報アクセシビリティを考慮したウェブサイトを構築・運営しており，先進的な取り組みがされている。

本研究では，トップページは検索条件入力ページよりも内容が簡易な機関も多いが，トップページの適合数と検索条件入力ページの適合数には大きな差は見られなかった。このことから，ページ内容の影響を受けにくい箇所で不適合となっているのではないかと考えられる。NIPH のポー

タルサイトで不適合となった項目については，Tabキーでウェブページ内の項目をフォーカス移動したときに，特定の項目からフォーカスが移動しなくなり，Tabキーでフォントのサイズが変更されてしまうなどの問題点があった。これは健常者が利用する時であっても操作性を損なうものであり，改善が必要だと考えられる。また，ウェブサイトの表示内容を決定する要素であるスタイルシートを未使用にした場合に，見栄えだけではなくウェブページ内の各ラベルや入力項目の並び順が不正になってしまう問題もあり，視覚障害者が利用する音声ブラウザでウェブサイトを参照した場合に，ウェブサイトの情報の読み上げ順序が不正になり正しく情報が伝わらない可能性が高い。今回の調査ではJIS X 8341-3の中で最低レベルである等級Aを調査項目としており，より上位レベルの等級AA，等級AAAでは不適合率がさらに高くなることが予想される。よって，視覚障害者への情報発信のための音声ブラウザへの対応や，マウスを使用せずにキーボードでの全操作を可能にするなど，全日本国民を対象ユーザとしても情報が容易に正確に伝えられるように，少なくとも欧米のウェブサイトと同レベルの規格準拠度に合わせる必要があると考えられる。

E.結論

先進的な取り組みや工夫を行っているウェブサイトについて，特に技術的な面で参考なる点を活かし，日本の臨床研究・治験の情報が一元的に集約されている国立保健医療科学院「臨床研究（試験）情報検索ポータルサイト」を含めたJPRNの情報が活かせるような改善，機能追加と普及啓発を促進していくことが必要となる。

今後，障害者らがウェブサイトを利用することに配慮したデザインや機能を取り入れ，アクセシビリティ向上の観点からウェブサイトの作成を行っていくことが課題である。

F.研究発表

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G.知的財産権の出願・登録状況（予定を含む）

1. 特許取得
   なし

2. 実用新案登録
   なし

3. その他
   なし

平成25年度厚生労働科学研究費補助金/医療技術実用化総合研究事業（臨床研究・治験推進研究事業）
国民・患者への臨床研究・治験の普及啓発に関する研究

医療情報サイト横断検索の実証システムの構築

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研究要旨

臨床試験および関連する医療情報を求める一般人、患者などのためのワンストップポータルを試験的な構築を試みた。

「国民・患者への臨床研究・治験の普及啓発に関する研究」において、本研究の目的のための限定的な公開の合意が得られた各研究機関等のサイトのコンテンツ、データベースを検索結果として返す横断検索システムを作成し、利用者が疾患名や医薬品名での検索によって、既存の治験情報だけでなく、他機関が運営しているサイトのコンテンツの結果を一覧にして閲覧できるようにした。

A.研究目的

現在運用されている「臨床研究（試験）情報[2]」においては、WHOのICTRP (International Clinical Trials Registry Platform) に情報を提供するための日本の治験・臨床研究登録機関として、国内の3つの治験・臨床研究の登録センター[3]より収集したデータを検索可能なものとして提供している。

現在は医療従事者、研究者など、どちらかといえば専門家向けとなっており、一般人、患者が利用するものとしては使い勝手に問題がある。

このサイトの改善のための一つの課題として「横断検索」機能を実装することとした。この横断検索機能は、現在収集している臨床研究（試験）・治験情報だけでなく、関連する医療情報もまとめて検索ができるようにするものである。

臨床研究関連のサイトにおいて検索を実行する時には治験の情報だけでなく、既に医療現場にて一般に実施されている治療法や医薬品についての情報、また、疾病そのものについての基礎情報なども同時に得られると利便性が高いのではないかと考え、それら関連情報の横断検索機能を実装することを目標の一つとした。

検索サイト

検索対象とする医療情報は関連する各組織のサイトにおいて提供されているものを一度に検索ができるようするというものである。

そのような横断検索が実行できるワンストップポータルサイトを構築するのにあたっては自ら専門のスタッフにより情報を集めて編纂し、データ化するという方法も考えられる。

しかし、既存の研究機関において整理され、提供されている情報がインターネット上で利用可能であり、重複した作業をあらたに今回構築するサイトにおいて作業を行うのは無駄である。

現時点においては、そのような複数のサイトからの検索を行うためには、グーグルなどの汎用のウェブ検索エンジンを用いるのが一般的である。その検索操作の結果、多くの情報が得られはするのであるが、精度の問題もあり、数多くの情報源から必要とする情報を選ぶには自ら取捨選択を行わなければならない。これは利用者にとっては必ずしも利便が高いものとは言えない。

そこで、あらかじめ絞り込んだ既存サイトの情報を定期的に自動収集、あるいは、既存のサイトの検索機能を用いて必要な情報を利用者の求めに応じて集約して情報を提供できるような

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2 http://rctportal.niph.go.jp/

3 大学病院医療情報ネットワーク研究センター、日本医薬情報センター、日本医師会治験促進センター

システムとして実装することとした。

その開発の過程において、解決すべき課題となることがないか、その洗い出しも同時に行うこととした。

B.研究方法

「国民・患者への臨床研究・治験の普及啓発に関する研究」に参加の各機関のサイトよりいくつかを選び出し、今回の研究目的に限って情報の再利用の許諾を得られたものについて検索対象とした。

それらの対象のサイトにおいて全ページを取得可能なものについては全件を取得して新たに構築した検索システムにて検索を行うようにした。全ページが取得できないものに対してはシステム側にて利用者の代理として検索を実行して、その結果を取得して解析を行った。

以上、２つの対象群からの検索結果を同時に利用者に提示して、横断検索として見えるよう、適宜整形を行うシステムを構築した。

(倫理面への配慮)

利用者の情報の収集は行わず、そのプライバシーに充分に配慮する。

C.研究結果

情報の収集、解析（情報抽出)、独自の検索エンジンの構築、他の既存サイトへの検索の中継(代理検索)といった、今回目標とする横断検索の機能を実現するにあたって最低限必要となる機能はひととおり実現できた。

各機関のサイトより取得したページから、必要となる情報を抽出する処理についてはサイトごとに設定ファイルを作成し、横断検索機能にとっては不要となる情報を削減できるようにした。この設定ファイルはXSLT[4]という文書変換のためのインターネット標準の言語により記述したものを今回は採用している。

検索エンジンは Apache Solr [5]というオープンソースの検索エンジンを採用した。日本語の解析のための機能も実装されており、同意語・類義語や、文字の表記揺れなどのための機能も実現されている。

提携サイトよりページの内容を全件取得できない場合においては利用者の指定する検索語を提携サイトに対して中継する機能も実装を行った。中継を行い、その結果を検索者に対して必要な情報を抽出して提示するようになっている。

全件取得したデータを保持する検索システムからの検索と、代理検索にて他機関からの検索では処理速度に違いがあるため、順番に検索するのでは利用者に処理が終わるまで待たせてしまうことになる。

検索処理において、文字表記・表現の揺れによって検索結果が限定されてしまうのを防ぐために同意語のデータベースを使った検索処理機能も可能なように配慮を行った。これはApache Solr に標準で備わっている機能を利用している。ただ、同意語のデータベースは独自に構築しなくてはならず、今回は限定的なものとなった。

文字表記の揺れ、たとえば、全角と半角の混在についても同様にデータベースを作成して、文字の表記の揺れを吸収する Apache Solr のフィルタ機能を利用した。

D.考察

- 各サイトのページから情報を抽出する際にはXSLTという技術を採用したが、機能的には強力ではあるが、ある程度のプログラミング経験を要するものであり、XSLTによる設定を記述するのは簡単であるとは言えない。より簡易な設定方法を独自に開発するか、あるいは、自動的にプログラムによる内容推測を行う抽出技術で利用可能なものがあれば採用したい
- 検索システムにおいては類義語を処理するための同意語のデータベース(シソーラス)の充実が重要となる。このシソーラスについては現状では運用者によりメンテナンスすることが必要であり、適宜充実させるための運用体制を構築しなくてはならない。また、既存のシソーラスがあればそれを利用することも検討する
- あらかじめ全件を取得しておいた結果からの検索した結果と、代理検索処理の結果から必要な情報を抽出した結果を

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4 http://www.w3.org/TR/xslt

5 http://lucene.apache.org/solr/

どのように統合して見せるのかについて課題がある。

- 検索結果を提示するにあたっては、何らかの基準で並べ替える必要がある。そのための重みづけは各検索システムで異なっており、統一して扱うのが難しい
- 検索結果がある一定数を超えた場合には複数のページに分割する必要がある。その場合のページ分割処理が、複数のデータベースからの検索の場合には簡単ではない。全件取得済のデータからの検索結果を数ページにわたって表示した後に代理検索の結果を並べるようにしたのでは利便性に問題がある。しかし、同時に表示する場合にはどのように重みづけをして並べ替えるのかに依存してしまう。

- 本研究により構築したシステム運用を行うにあたっては、各研究機関のサイトにて提供する情報の権利関係の処理が重要となる。平成21年の著作権法の改正において、検索サービスにおいてはある一定の限度において複製が認められるようになったが、本横断検索サイトのように、ポータルサイト自らの「コンテンツ」として見せるものにおいては適切な権利関係の処理が重要となる。権利関係の処理においては直接に連携する機関の許諾だけでは不十分で、たとえば、医薬品情報においては医薬関連企業の持つ情報(添付文書など)も利用することとなり、利用許諾を得る対象が広くなる。実現にあたっては何らかの枠組みが必要となるだろう。
- 治験情報の検索結果においては治験の責任者の情報を得ることはできるが、実際にどの医療機関において治験を実施しているか、といった情報は現在の枠組みでは得ることが難しい。治験情報の収集のための枠組みを拡張し、どの治験がどこの医療機関で実際に実施されているかの情報を得られるためのシステムを別途構築することも必要となるだろう。
- 連携サイトは本研究に関係する機関のサイトの一部に限定されており、網羅的とはいえない。医療関係の各分野を網羅的にカバーするための枠組みが必要である。

E.結論

本研究において、現在の収集している治験情報にとどまらず、ワンストップポータルとして各種疾病や医薬品の情報も横断的に検索ができるためのシステムを構築することができた。
しかし、実用的にサービスを提供するためには分野を拡げること、情報の権利処理を行うことといった課題が存在する。今後はそれらの課題を解決するための枠組みを探っていきたい。

F.研究発表

1. 論文発表
   なし

2. 学会発表
   なし

G.知的財産権の出願・登録状況（予定を含む）

1. 特許取得
   なし

2. 実用新案登録
   なし

3. その他
   なし

研究成果の刊行に関する一覧表

英文原著・症例報告

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藤井仁，野口都美，荻野大助，高橋邦彦，佐藤元	臨床試験段階の医薬，一般的な医薬の情報源についての観察研究－患者団体，通院患者，一般住民の差異	臨床医薬	30(1)	39-46	2014
荻野大助，野口都美，藤井仁，佐藤元	臨床研究・治験情報提供および情報検索ウェブサイトにおけるJIS規格への適合状況と課題	臨床医薬	30(3)	249-255	2014

ORPHANET JOURNAL OF RARE DISEASES

RESEARCH Open Access

Characteristics of Japanese Duchenne and Becker muscular dystrophy patients in a novel Japanese national registry of muscular dystrophy (Remudy)

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Abstract

Background: Currently, clinical trials for new therapeutic strategies are being planned for Duchenne and Becker muscular dystrophies (DMD/BMD). However, it is difficult to obtain adequate numbers of patients in clinical trials. As solutions to these problems, patient registries are an important resource worldwide, especially in rare diseases such as DMD/BMD.

Methods: We developed a national registry of Japanese DMD/BMD patients in collaboration with TREAT-NMD. The registry includes male Japanese DMD/BMD patients whose genetic status has been confirmed by genetic analysis. The registry includes patients throughout Japan.

Results: As of February 2012, 583 DMD and 105 BMD patients were registered. Most individuals aged less than 20 years. In terms of genetic mutations of registrants of DMD and BMD, deletion of exons was the most frequent (61.4% and 79.0%) followed by point mutations (24.5% and 14.3%) and duplications (13.6% and 4.8%), respectively. 43.6% of DMD are capable of walking, and 76.2% of BMD registrants are able to walk. 41.1% of DMD registrants in the database were treated using steroids. 29.5% of DMD and 23.8% of BMD registrants were prescribed one cardiac medicine at least. 22% of DMD used ventilator support, and non-invasive support was common. Small numbers of DMD and BMD registrants, only 3.9% and 1.0% of them, have received scoliosis surgery. 57 (9.8%) patients were eligible to clinical trial focused on 'skipping' exon 51.

Conclusions: The Remudy has already demonstrated utility in clinical researches and standardization of patients care for DMD/BMD. This new DMD/BMD patient registry facilitates the synchronization of clinical drug development in Japan with that in other countries.

Keywords: Duchenne and Becker muscular dystrophy, Neuromuscular disorder, National registry, TREAT-NMD, Registry of muscular dystrophy (Remudy), Japan

Introduction

Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD) are X-linked recessive forms of muscular dystrophy caused by mutations in the dystrophin gene (*DMD*) on chromosome Xp21.2 [1]. The *DMD* gene is the largest gene identified in human and contains 79 exons. Mutations in this gene cause deficiency of normal dystrophin protein [2]. DMD is the most frequently inherited muscular disease, affecting approximately 1 out of 3500 live male new-borns. DMD patients commonly lose their ability to walk before the age of 12 years, coupled with deterioration in respiratory and cardiac functions. DMD is usually fatal in the third decade because of either cardiac or respiratory failure. On the other hand, the course and severity of BMD is more variable [3]. Since the discovery of the *dystrophin gene*, many efforts have been made to develop effective therapeutic strategies for DMD/BMD.

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Clinical trials are now being planned and conducted for DMD/BMD [4-8]; however, many challenges exist with regard to both planning and conducting clinical trials for such rare diseases. Because of limited epidemiological data, the total number of patients, natural history of the disease and clinical outcome measures are unclear. In addition, adequate numbers of patients are needed to achieve significant results in clinical trials. As solutions to these problems, patient registries are an important resource especially in case of rare diseases such as DMD/BMD.

In Europe, Translational Research in Europe–Assessment and Treatment of Neuromuscular Diseases (TREAT-NMD) [9], a research network for neuromuscular disorders, developed a global database for DMD and spinal muscular atrophy (SMA) to obtain epidemiological data; examine the total number of patients; determine the natural history of the disease; determine appropriate clinical outcome measures and collect adequate numbers of patients needed to achieve significant results in clinical trials and inform patients of new drug development as soon as possible.

To date, several Japanese DMD/BMD databases have been developed [10-12]; however, these have not been on a broad national scale. For instance, some were on a single-centre basis and others encompassed only a small local area or several hospital sites. Some others were restricted to inpatients only. Despite these early efforts, no national registry has been developed with the purpose of focusing on clinical trials. In 2009, we developed a national registry of Japanese DMD/BMD patients (REgistry of MUscular DYstrophy; Remudy. http://www.remudy.jp/) in collaboration with TREAT-NMD. The purpose of this registry was to effectively recruit eligible patients to new clinical trials and provide timely information to patients about upcoming trials. Registry data also provides more detailed knowledge about the natural history and epidemiology of the disease, as well as information about clinical care. In this paper, we introduce Remudy, the Japanese DMD/BMD registry, and describe the clinical and molecular genetic characteristics of Japanese dystrophinopathy patients.

Materials and methods

Institution, organization and leadership

Remudy is supported by a Research Grant (20B-12, 23–4) for Nervous and Mental Disorders from the Ministry of Health, Labour and Welfare. The development and management of the registry is led by the principal investigator of the Japanese muscular dystrophy research group. Steering committee members include scientists, clinicians and representatives of patient organizations. The office of the registry of muscular dystrophy was set up within the National Center of Neurology and Psychiatry (NCNP), Tokyo, Japan. This project includes Japanese DMD/BMD patients and was made possible by collaboration with the Japan Muscular Dystrophy Association.

Patients

The database includes male Japanese DMD/BMD patients whose genetic status has been confirmed by genetic analysis. The database includes patients throughout Japan. The cost of sequencing analysis of the *DMD* gene is not covered by the system of the public health insurance in Japan. For patients who intend to register but whose genetic status is not confirmed using multiplex ligation-dependent probe amplification (MLPA), Remudy provides free service of sequencing analysis of the *DMD* gene.

Method of registration and data collection

Information about the registry was provided to interested individuals and their informed consent was obtained. Provision of all data by patients is voluntary and is not shared with any third party without the permission of the committee responsible for disclosing the information. Inclusion in the database confers no obligation for the patient, and they may be removed from the registry immediately on request. It was stated that refusal to participate would not affect the subsequent medical care of the patient. This study was approved by Institutional Review Board of National Center of Neurology and Psychiatry, Japan.

Structure of the registry form

Data obtained via the registry form included clinical symptoms, results of biochemistry, muscle biopsy and other laboratory analysis and description of the genetic mutation. Epidemiological information provided includes walking capability, cardiac and respiratory functions, creatine kinase levels, history of scoliosis surgery and steroid therapy status. All these data should be confirmed by molecular and clinical curators in Remudy (three active molecular and three active clinical curators). The structure of the Case Report Form and registry items are shown in Table 1. Information was annually updated by registrant's self report after their physician's confirmation following reminder from registration office. To decide whether a patient was classified as DMD or BMD, first, attending physician made a diagnosis whether a patient was DMD or BMD by the clinical and molecular information. Then, when our clinical and genetic curators double-checked their classification by reviewing clinical information, and also data from pathological (including dystrophin immuno-staining, if applicable) and genetic analysis.

Results

As of February 2012, 876 Japanese patients across Japan had sought registration, and 583 DMD and 105 BMD patients were registered based on their eligibility as

Table 1 The report form for registry to Remudy

Contact	Clinical data
-Name	-Data
-ID number	-Muscle biopsy
-Hospital	•Examed/not examed
-Date of birth	•Dystrophin immunostain
-Address	-Walking capability
-Phone	•Ambulant /wheelchair
-E-mail	-Use steroid therapy
-Signed up for other registries	-Cardiac function
-Attending any clinical trials	•LVEF(%)
-Registering other database	•Medication
Diagnosis	-Respiratory function
-DMD/BMD/IMD	•FVC
-Proof of the diagnosis	•Mechanical support
•Genetic confirmed	-Scoliosis surgery
•Muscle biopsy	-CK level
•Suspected from family history	-Weight
•Others	Molecular genetic data (certificated report should be attached)
	-Method
	•MLPA/Multiplex PCR/southern blot/RT-PCR/ Direct sequencing of exons
	-Type of mutation
	•Deletion/duplication/others
	•Details of the mutation

DMD, Duchenne muscular dystrophy; BMD, Becker muscular dystrophy; IMD, Intermediate muscular dystrophy; MLPA, Multiplex ligation-dependent probe amplification; LVEF, Left ventricular ejection fraction; FVC, Forced vital capacity; CK, Creatine kinase.

confirmed by clinical and molecular genetic data (Figure 1, Figure 2 and Table 2). Most individuals aged less than 20 years; however, several registered individuals were aged over 35 years. There are five patients between 45 and 49 years old in DMD. The molecular data of four patients are consistent with reading frame rule, and these patients became bedridden with tracheotomy in muscular dystrophy care ward. A molecular data of another patient isn't consistent with DMD mutation (in-frame mutation, del 10–42), however he lost his walking ability in childhood and had a tracheotomy. His attending doctor clinically diagnosed him as DMD. In terms of genetic mutations of registrants of DMD and BMD, deletion of exons was the most frequent (61.4% and 79.0%) followed by point mutations (24.5% and 14.3%) and duplications (13.6% and 4.8%), respectively. Most registered patients lived in cities, namely Tokyo, Osaka and Nagoya (Figure 3).

Tables 3 describe the clinical characteristics of Japanese DMD/BMD patients included in the registry. The information collected was similar to that for the core items required for the TREAT-NMD global registry, and thus, we will be able to compare Japanese data with those contained in the global registry.

Among the DMD registrants, 43.6% of them are capable of walking. On the other hand, 76.2% of BMD registrants are able to walk. In terms of cardiac and respiratory function, 67.2% of DMD and 72.4% of BMD have normal cardiac function and respiratory functional examination was not performed among half of DMD registrants. It was suggested that many registrants were too young to be tested when they were registered. 41.1% of DMD registrants in the database were treated using steroids. 29.5% of DMD and 23.8% of BMD registrants were prescribed one cardiac medicine at least, and ACE–inhibitor was the most common medicine for cardiac failure. One fifth of DMD used ventilator support, and among the registrants with

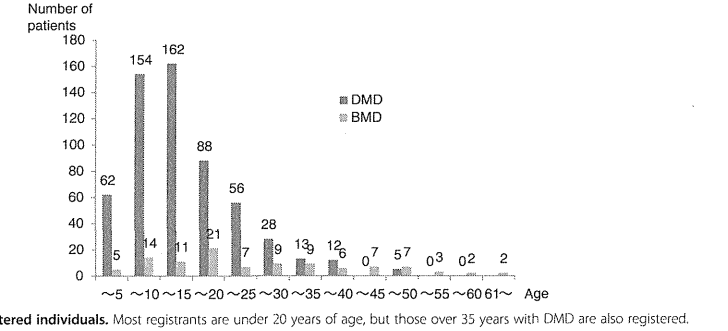

Figure 1 Ages of registered individuals. Most registrants are under 20 years of age, but those over 35 years with DMD are also registered.

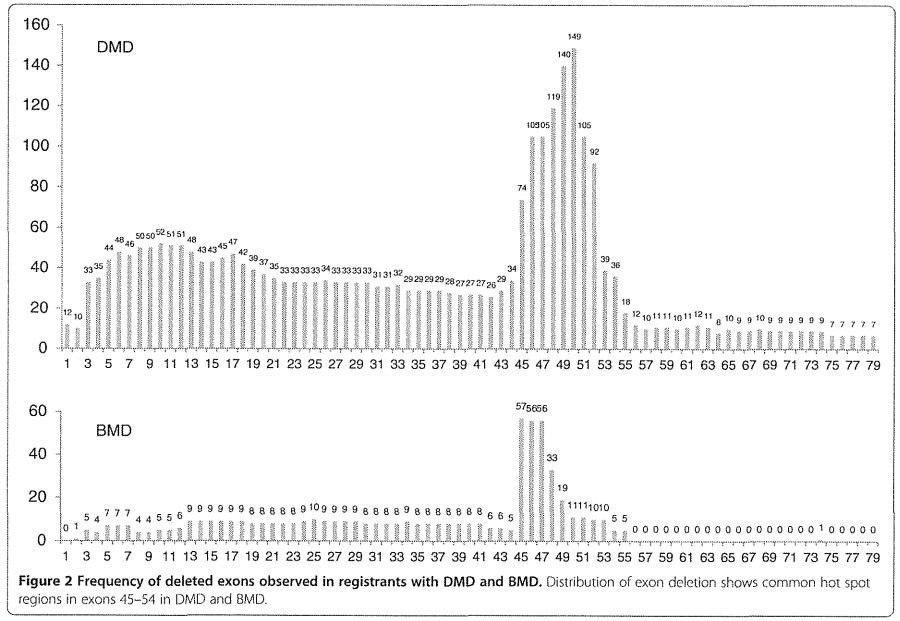

Figure 2 Frequency of deleted exons observed in registrants with DMD and BMD. Distribution of exon deletion shows common hot spot regions in exons 45–54 in DMD and BMD.

mechanical ventilation, non-invasive support was common. Small numbers of DMD and BMD registrants, only 3.9% and 1.0% of them, have received scoliosis surgery. The Remudy registry has already demonstrated utility in clinical trials for DMD/BMD. A clinical trial focused on 'skipping' exon 51 using antisense oligonucleotides has been recently conducted in Japan. From the Remudy registry data, 57 (9.8%) patients have mutations theoretically addressable by skipping of exon 51 (Table 4).

Table 2 Distribution of mutations in the registrants with DMD and BMD

	DMD patients		BMD patients	
	No. of case	% of cases	No. of case	% of cases
Distribution of mutation				
Deletion	358	61.4%	83	79.0%
Duplication	79	13.6%	5	4.8%
Deletion and Duplication	1	0.2%	0	0.0%
Others *	144	24.7%	15	14.3%
No mutation found**	1	0.2%	2	1.9%
	583	100.0%	105	100.0%

* Others include nonsense mutations, small insertion/deletion mutations, deep intronic mutations, and splice site mutations.
** Their diagnosis was confirmed based on their pathological findings in muscle biopsy including a negative immunohistochemical staining against dystrophin.

Discussion

The DMD/BMD registry run by Remudy is the one of its kind in Japan specifically targeting neuromuscular diseases. It is the first national registry in Japan to include both clinical and molecular genetic data, and its utility in promoting clinical trials has been demonstrated. Prior to the development of Remudy, some DMD/BMD databases existed in Japan [10-12]; however, they were limited in their coverage and utility. This is the first Japanese DMD/BMD database aimed at facilitating clinical trials and the first to coordinate with a global database.

Since 2011, a worldwide phase III, randomized, double-blind, placebo-controlled clinical study is being conducted for patients with DMD who have a *DMD* gene mutation amenable to an exon 51 skip. Partially because of the development of the Remudy database, this trial now includes Japanese sites and is thus the first global clinical trial to become available to Japanese DMD patients. Up until now, clinical trials for DMD under new International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use–Good

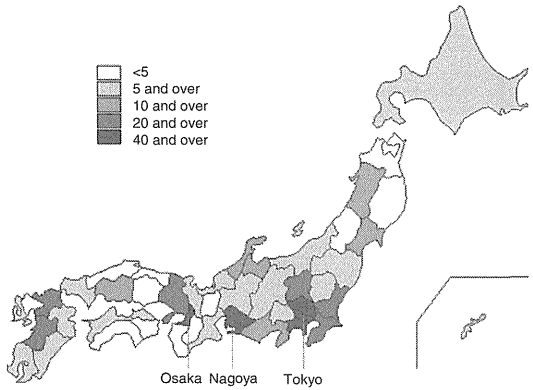

Figure 3 Prefectural distribution of the registrants. There are 47 prefectures in Japan. Individuals from all rural areas in Japan were registered but most registrants were concentrated in big cities, namely Tokyo, Osaka and Nagoya.

Clinical Practice (ICH-GCP) have not been conducted in Japan. Registry information concerning eligible Japanese individuals was sought by TREAT-NMD and pharmaceutical companies and has been provided with permission. This example demonstrates the usefulness of the registry for facilitating patient access to clinical trials, as well as enhancing recruitment for such trials.

Another novel aspect is patient participation in the registration process. Previously, database information was supplied by clinicians. In contrast, the Remudy system enables patients themselves to provide their information to the registry in collaboration with their clinicians. The registry consists of valuable clinical and genetic data, which yields valuable epidemiological information including walking capability, cardiac and respiratory functions, creatine kinase levels, history of scoliosis surgery and steroid therapy status, all of which are needed to plan clinical trials and determine the eligibility of individuals for such trials. This has resulted in Remudy becoming one of the largest and most reliable rare disease registries in Japan.

Remudy also reveals the structure of mutations in Japanese DMD/BMD patients. The distribution of mutations and the frequency of individual exon deletion found in our study are consistent with previous reports. In DMD, distribution, duplication and point mutations comprise 61.4%, 13.6% and 24.5%, respectively. Distribution of exon deletions reveals significant region in exons 42–54 (Figure 2). This is similar to the findings of other databases [13] and those of a Japanese study in a single referral centre [11].

Because of improvements in respiratory and cardiac complications, life expectancy of Japanese DMD patients has been prolonged [14]. Our findings support this, with the Remudy registry including several individuals over 40 years of age (though most were aged <20 years). 41.1% of DMD patients in the database were treated using steroids. Importantly, this ratio may be relatively low compared to that in western countries, considering that the family guide for the diagnosis and management of DMD became available to download in Japanese in 2011 [15]. Given that this treatment is recommended in the guide, the number of individuals being treated with steroids for DMD may increase in future.

Remudy is intended to be used as a public service for the benefit of patients living with neuromuscular diseases. Through participation in the database, patients may be identified as candidates for upcoming clinical trials. In addition, Remudy has provided additional useful resources for patients through a website and newsletter. The information provided via these methods includes the development of new therapeutic compounds, information regarding care standards (known as the Family Guide for the Diagnosis and Management of Duchenne Muscular Dystrophy [15]) and other relevant medical information. In addition, Remudy has facilitated the genetic diagnosis and detailed sequencing analysis of individuals, thereby promoting genetic counseling in family and also providing important information required to determine eligibility for clinical trials. As a result, more dystrophinopathy patients have become aware of the necessity of confirming their diagnosis via analysis of the *DMD* gene.

The development of Remudy is part of a global trend toward database development for neuromuscular disorders.

Table 3 Clinical manifestations, medications and intervention characteristics in the registrants with DMD and BMD

	DMD patients		BMD patients	
	No. of case	% of cases	No. of case	% of cases
Walking capability				
Normal walking	254	43.6%	80	76.2%
Not able to walk, and sit without support	184	31.6%	19	18.1%
Not able to sit without support	145	24.9%	6	5.7%
	583	100.0%	105	100.0%
Cardiac function				
Normal	392	67.2%	76	72.4%
Dysfunction	180	30.9%	28	26.7%
Not performed	11	1.9%	1	1.0%
	583	100.0%	105	100.0%
Respiratory function				
Normal	65	11.1%	45	42.9%
Dysfunction	202	34.6%	18	17.1%
Not performed	316	54.2%	42	40.0%
	583	100.0%	105	100.0%
Steroid use				
Current	171	29.3%	6	5.7%
Used to	69	11.8%	6	5.7%
Never	343	58.8%	93	88.6%
	583	100.0%	105	100.0%
Cardiac medication				
Prescribed	172	29.5%	25	23.8%
Not prescribed	411	70.5%	80	76.2%
	583	100.0%	105	100.0%
Drug				
β-blocker	94	54.7%	16	64.0%
ACE-inhibitor	140	81.4%	19	76.0%
ARB	12	7.0%	5	20.0%
Diuretics	43	25.0%	7	28.0%
Other	29	16.9%	7	28.0%
	172*1	100%	25*1	100%
Mechanical ventilation				
No	455	78.0%	103	98.1%
Yes	128	22.0%	2	1.9%
	583	100.0%	105	100.0%
Invasive	20	15.6%	2	100.0%
Non-invasive	108	84.4%	0	0.0%
	128*2	100.0%	2*2	100.0%
Temporal	71	55.5%	0	0.0%
Continuous	57	44.5%	2	100.0%
	128*2	100.0%	2*2	100.0%
Scoliosis surgery				
Yes	23	3.9%	1	1.0%
No	560	96.1%	103	98.1%
Not described	0	0.0%	1	1.0%
	583	100.0%	105	100.0%

*1; The number of registrants who were prescribed with cardiovascular medicines.
*2; The number of registrants using ventilator support.

TREAT-NMD is an excellent network in the field of neuromuscular disorders and has been leading international registry collaboration for many diseases including DMD, spinal muscular atrophy, myotonic dystrophy type 1 and others. Close collaboration with TREAT-NMD has allowed Japan to enhance the management and care of Japanese DMD and BMD patients and facilitated research in neuromuscular disorders. One of the most important outcomes of this collaboration is the development of the Japanese national registry for DMD described in this paper. By collecting common information, the registry data can easily be compared between Japan and other countries. TREAT-NMD has helped to support these efforts by providing an infrastructure that continues to accelerate research, therapy development, and trial readiness in addition to increasing collaboration and improving patient care. Japanese patients, families, physicians and anyone affected by a neuromuscular disorder play a key role in the worldwide neuromuscular community by participating in this collaboration with TREAT-NMD, which is Remudy.

The TREAT-NMD global registry has been focused on European countries and the United States; however, it is currently expanding to other countries. Rare disease registries are emerging in Asian countries [16] and Japanese experiences in collaboration with TREAT-NMD and registry development should be able to provide more information about these activities to Asian countries. A global registry

Table 4 Applicable individuals for exon 51 skipping clinical trial

Deleted exons	No. of individuals	% in registrants
45-50	17	2.9%
47-50	0	0.0%
48-50	9	1.5%
49-50	18	3.1%
50	6	1.0%
52	7	1.2%
52-63	0	0.0%
total	57	9.8%

for patients with myotonic dystrophy type 1 will be launched in the near future [17]. Several other registries for neuromuscular diseases have been developed worldwide, such as the international congenital muscular dystrophy registry (CMDIR) run by the US patient organization 'Cure CMD'. Remudy has been developing Japanese registries for other neuromuscular diseases including glucosamine (UDP-N-acetyl)–2-epimerase/N-acetylmannosamine kinase (GNE) myopathy and myotonic dystrophy type 1 (DM1).

We have thus demonstrated how this registry can enhance the readiness for clinical trials in Japan, and how this unique form of infrastructure can be used to accelerate international efforts in fighting orphan diseases.

Conclusions

The Remudy registry has already demonstrated utility in clinical trials and standardization of patients care for DMD/BMD. This new DMD/BMD patient registry will facilitate the synchronization of clinical drug development in Japan with that in other countries.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
HN and EK participated in planning this study, analysis and interpretation, generation of the tables and figures, in writing of the manuscript. MY and HK participated in curating clinical data and data collection. YM, KG, and YH participating in genetic analysis, curating the molecular data and data collection. IN, ST and MK supervised in planning this study. All authors read and approved of the final manuscript.

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ORIGINAL COMMUNICATION

Prednisolone improves walking in Japanese Duchenne muscular dystrophy patients

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Abstract We evaluated the long-term efficacy of prednisolone (PSL) therapy for prolonging ambulation in Japanese patients with genetically confirmed Duchenne muscular dystrophy (DMD). There were clinical trials have shown a short-term positive effect of high-dose and daily PSL on ambulation, whereas a few study showed a long-term effect. Especially in Japan, "real-life" observation was lacking. We utilized the national registry of muscular dystrophy in Japan for our retrospective study. We compared the age at loss of ambulation (LOA) between patients in PSL group and those in without-PSL group. Out of 791 patients' in the Remudy DMD/BMD registry from July 2009 to June 2012, 560 were matched with inclusion criteria. Of the 560, all were genetically confirmed DMD patients, 245 (43.8 %) of whom were treated with PSL and 315 (56.2 %) without PSL. There was no difference between the two groups regarding their mutational profile. The age at LOA was significantly greater (11 month on average) in the PSL group than in the without-PSL group (median, 132 vs. 121 months; $p = 0.0002$). Although strictly controlled clinical trials have shown that corticosteroid therapies achieved a marked improvement in ambulation, discontinuation of the drug due to intolerable side effects led to exclusion of clinical trial participants, which is considered as unavoidable. In our study, patients were not excluded from the PSL group, even if they discontinued the medication shortly after starting it. The results of our study may provide evidence to formulate recommendations and provide a basis for realistic expectations for PSL treatment of DMD patients in Japan, even there are certain limitations due to the retrospectively captured data in the registry.

Keywords Duchenne muscular dystrophy · Prednisolone · Walking · National registry · Natural history

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Introduction

Duchenne muscular dystrophy (DMD) is a rare disease linked to the X-chromosome that affects 1 in 5,000–6,000 newborn males [1]. The disorder follows a progressive course of muscle weakness and also involves cardiac and respiratory muscles. DMD is caused by mutations in the *DMD* gene, which results in severe reduction or complete elimination of the dystrophin protein. Although the molecular origins of DMD have been known for several years, there is still no curative treatment for the disease. It has been nearly four decades since the potential benefits of glucocorticoids (GCs) for DMD were first reported by Drachman et al. [2]. In the years since, several randomised controlled trials (RCTs) have concluded that GCs increase short-term muscle strength and improve muscle function (from 6 months to 2 years) [3–7] with frequent but not severe adverse effects [6]. In contrast, the long-term benefits and adverse events of GCs have not yet been assessed by an RCT [4], although non-RCTs have suggested functional benefits for over 5 years in some GC-treated patients [8–17]. However, these studies were conducted in small numbers of patients. While PSL has been available for DMD patients since 1990s, there has been very little literature regarding the regimens of PSL for DMD in Japan. Some Japanese experts have a vague idea that the adequate dose could be lower than the one recommended (0.75 mg/kg/day) based on their expert experiences. Deflazacort has not been available yet in Japan [18].We used a large national registry of DMD patients in Japan to conduct a retrospective study on the long-term clinical efficacy of PSL therapy for maintenance of unassisted ambulation in DMD patients.

Methods

In 2009, we developed a national registry of Japanese DMD/BMD patients (Remudy) in collaboration with the Translational Research in Europe-Assessment and Treatment of Neuromuscular Diseases (TREAT-NMD) Network of Excellence [19, 20]. The Remudy database includes clinical and molecular genetic data as well as all required items for the TREAT-NMD global patient registry. The database includes male Japanese DMD/BMD patients throughout Japan whose genetic status has been confirmed by genetic analysis. The registry data includes age at registration, birth date, area of residence, features of the muscle biopsy, genomic mutations, complicating diseases, PSL use (present use, past use or never), present functional mobility, age at LOA, cardiac function, respiratory function, spinal surgery, serum CK level, family history of DMD etc., but does not includes PSL regimes (dose, age at commencement and duration), side effects of PSL or physiotherapy. All these data were confirmed by three molecular and two clinical curators in Remudy. In this study, we used the registry data compiled from July 2009 to June, 2012 to compare the clinical course of DMD between patients with and without PSL therapy. Patients were excluded for any of the following reasons: (1) dystrophin expression remained on muscle biopsy by immunohistochemistry test; (2) in-frame, missense or unconfirmed mutation of *DMD gene* by mutation screenings; (3) comorbidities, such as adrenal hypoplasia or nephrotic syndrome; (4) current age ≤5 years or ≥40 years (because PSL therapy for DMD was not common before the 1990s) or (5) missing data on PSL use (Fig. 1). We compared the age at LOA between PSL group of patients, which was comprised of both current and past PSL-treated patients, and without-PSL group, which was comprised of patients who had never been treated with PSL (steroid naïve). The primary outcome measure was 'independent walking' defined as 'unsupported walking indoors' [11], which is one of the standardized items in the TREAT-NMD global registry format. Because LOA was not well defined in several previous studies, there is no clear consensus on the

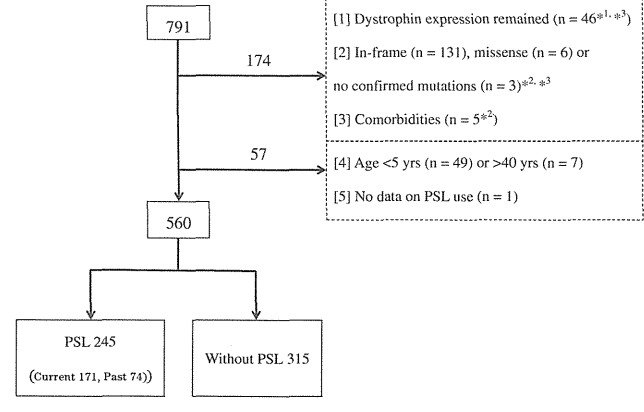

Fig. 1 Selection of DMD patients from the Remudy database for this study. *1: These 46 patients included out-of-frame mutations ($n = 7$) and nonsense mutations ($n = 7$). *2: Three patients met (2) and (3) of our exclusion criteria. *3: Twenty-three patients met (1) and (2) of our exclusion criteria. *DMD* Duchenne muscular dystrophy, *PSL* prednisolone, *Remudy* Registry of Muscular Dystrophy

definition of LOA [11]. The Kaplan–Meier method was used to analyse the age at LOA, and the log-rank test was used to compare differences between PSL group and without-PSL group. We used age at LOA as a primary outcome because the database did not contain information on the initiation or duration of PSL treatment [21]. We set 5 years as the start time for PSL therapy. We used the Cox regression model to perform univariate and multivariate analyses to assess the effect of PSL. A covariate selected for adjustment was area of residence because the registrants varied in number and frequency of PSL treatment among 6 geographical areas. In addition, we considered family history of DMD as another covariate for adjustment because it might have influenced the patients' decisions to accept PSL treatment. We calculated hazard ratios (HRs) and their 95 % confidence intervals (CIs). Statistical significance was defined as a two-sided p value <0.05. The software, SAS version 9.2 (SAS Institute Inc., Cary, NC, USA), was used to perform all statistical analyses. We also searched the PubMed database, reviewed related studies on the long-term effect of GCs on preservation of ambulation, and compared these previous results to those reported in the present study.

Results

Demographics

Of the 791 patients (from 147 hospitals, with 228 doctors' cooperation) in the Remudy database, 174 were excluded because they met at least 1 of the exclusion criteria, and dystrophin expression remained on muscle biopsy tissue was observed in 46 patients. One hundred and forty patients were excluded by *DMD gene* mutation screening, 131 had in-frame mutations, 6 had missense mutations and 3 did not show mutations detectable with standard methods (MLPA, exonic sequencing). Five had comorbid diseases, such as nephrotic syndrome and adrenodysplasia. We also excluded 57 patients because 49 were <5 years old, 7 were ≥40 years old and there was missing data on the use or non-use of PSL for 1 patient. After removing patients who fulfilled at least 1 exclusion criterion, the final group for analysis included 560 genetically confirmed DMD patients (Fig. 1).

Baseline characteristics are presented in Table 1. The mean current age of the 560 patients was 15.4 years, and the median current age was 14.0 years (interquartile range, 9–20 years). Of the 560 patients included, 245 (43.8 %) were in PSL group, and 315 (56.2 %) were in without-PSL group. The PSL group included 74 patients who had been treated with PSL in the past and 171 patients were currently on PSL (Fig. 1). Table 1 also presents the features of the *DMD gene* mutations in the PSL group and without-PSL group. Mutations included exon deletions or exon duplications (PSL patients: 183/245, 74.7 %; without-PSL patients: 230/315, 73.0 %); small frame shifts, deletions or insertions (PSL: 21/245, 8.6 %; without-PSL: 26/315, 8.3 %) and nonsense mutations (PSL: 29/245, 11.8 %; without-PSL: 41/315, 13.0 %). There was no difference in the mutation type distribution between the 2 groups. On the other hand, the geographic distribution of the 2 groups was significantly different, between 12 and 63 % of patients received PSL. We also presented distribution of the year-of-birth (per decade) in both PSL group and without-PSL group. The patients (PSL group and without-PSL group) were distributed in 2001–2010 (87/245, 35.5 %; 106/315, 33.7 %), 1991–2000 (131/245, 53.5 %; 120/315, 38.1 %), 1981–1990 (24/245, 9.8 %; 60/315, 19.0 %) and 1971–1980 (3/245, 1.2 %; 29/315, 9.2 %) respectively.

Table 1 Patient characteristics

		PSL		Without-PSL		Total
	Total	*n* 245	% 100.0	*n* 315	% 100.0	*n* 560
Mutation	Exon del/dup	183	74.7	230	73.0	413
	Frame shift or small del/ins	21	8.6	26	8.3	47
	Nonsense	29	11.8	41	13.0	70
	Others	12	4.9	18	5.7	30
Family history	Yes	60	24.9	110	34.9	170
	No	185	75.1	205	65.1	390
Region	Hokkaido and Tohoku	17	9.6	13	4.1	30
	Kanto	148	60.4	87	27.6	235
	Chubu and Tokai	33	13.5	73	23.2	106
	Kansai	25	10.2	62	19.7	87
	Chugoku and Shikoku	14	5.7	23	7.3	37
	Kyusyu and Okinawa	8	3.3	57	18.1	65
Year of birth	2001–2010	87	35.5	106	33.7	193
	1991–2000	131	53.5	120	38.1	251
	1981–1990	24	9.8	60	19.0	84
	1971–1980	3	1.2	29	9.2	32

PSL prednisolone, *del* deletion, *dup* duplication, *ins* insertion

Outcome

Of the 560 patients, we excluded three patients from the PSL group and four from the without-PSL group because ambulation status was unknown. Finally, 553 patients, 242 in the PSL group and 311 in without-PSL group were included in the analysis. LOA was reported in 190 of the 311 patients in without-PSL group and 123 of the 242 patients in PSL group. The median age at LOA was 121 months (10.1 years, interquartile range: 120–126 months) for the patients in without-PSL group and 132 months (11.0 years, interquartile range: 126–138 months) in PSL group (Fig. 2). The HR for without-PSL group versus PSL group was 0.67 (95 %CI: 0.53–0.83, $p = 0.0004$), and the adjusted HR was 0.64 (95 %CI: 0.50–0.82, $p = 0.0005$).

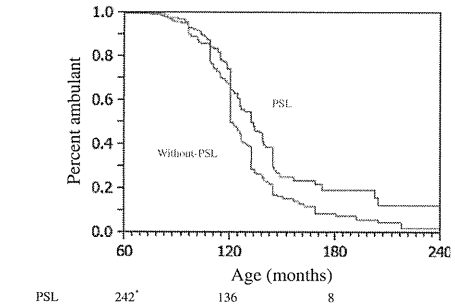

Fig. 2 Time to loss of ambulation in the PSL group and without-PSL group determined by the Kaplan–Meier method. *Three patients in the PSL group and. **Four patients in the without-PSL group were excluded because their ambulation status was unknown. The PSL group had 242, 136 and 8 ambulant patients at 60, 120 and 180 months of age, respectively. The without-PSL group had 311, 145 and 10 ambulant patients at 60, 120 and 180 months of age, respectively. *PSL* prednisolone, *HR* hazard ratio

	Without-PSL* n = 311	PSL** n = 242
Median age (months) (interquartile 25%–75%)	**121** (10.1 yrs) **(120–126)**	**132** (11.0 yrs) **(126–138)**
Log-rank test	p = 0.0002	
HR	0.67 (95% CI 0.53–0.83; p = 0.0004)	
Adjusted HR	0.64 (95% CI 0.50–0.82; p = 0.0005)	

Discussion

To our knowledge, this is one of the largest studies worldwide on the long-term effects of PSL therapy on prolongation of independent walking ability in DMD and also the first study in Japan (Table 2). Historically, most DMD patients lose the ability to walk between 9 and 11 years of age [22], but recent improvements in care may have increased the age at LOA slightly even without the administration of steroids. In our study, the median age at LOA in patients who were never treated with PSL (without-PSL group) was 10.1 years. In a recent natural history study of 371 DMD boys, those on any steroid regimen for >6 months walked significantly longer (median age at LOA 12.0 years) than those on any regimen for <6 months or never on steroid (10.0 years) [23], which is quite similar to those without-PSL in our study. According to previous studies, patients receiving GC treatment were able to ambulate 2–5 years longer than those not treated with GCs [8, 23]. In the current study, patients treated with PSL were able to ambulate 11 months (0.9 years) longer on average than those without PSL, and the extension was relatively modest as compared to previous studies (Table 2). This may be due to one or several of the following factors: differences in ethnic origin of the treated population: small size of some of the previous studies: differences in the clinical definitions of DMD, different definitions of ambulation, variations in PSL regimens, and most importantly duration of treatment. First, previous studies only have been conducted in small numbers of patients (129 patients at most [12]), whereas the sample size in our study was 560 patients. On the other hand, Ricotti et al. [24] performed a prospective observational study in 360 patients, but their study did not compare a GC-treated group to a non-treated group. Second, the genetic and molecular criteria used to define DMD have varied between studies (Table 2). In the Leiden *DMD* mutation database, 9 % of the mutations did not follow the reading-frame rule [25]. A diagnosis based on a purely molecular genetic approach may not accurately distinguish DMD from Becker muscular dystrophy and milder dystrophinopathies, especially in young children with no family history of DMD. In these patients, a muscle biopsy can help verify dystrophin expression to confirm the existence and severity of a functional mutation in the *DMD gene* [26]. Using *DMD gene* analysis only, previous studies may have included subjects with a milder phenotype (residual dystrophin expression) with longer prolongation of independent ambulation regardless of GC treatment history. To improve the precision of diagnosis in our study, we

Table 2 Related studies on long-term effect of GC on preservation of ambulation

	Study design	Treated Non-treated Numbers	Criteria		Definition of loss of ambulation	Loss of ambulation		
			DMD gene analysis	Muscle biopsy		Treated Control Median age (Years)	Prolonged ambulation, (Years)
Our study	Ret	245 (P) 315	Exclude in-frame, missense, not confirmed mutations	Exclude residual Dys	Unable to walk, unsupported indoors	11.0 10.1	0.9
Ricotti [24]	Pro	360 (Pi191, Pd169) –	Include DMD mutation	Include Dys (−) or both	NorthStar Ambulatory Assessment	Pi12.0, Pd14.5 –	2.5[a]
Merlini [13]	Pro	4 (P + D) 3	Out of frame in 3 patients	Include Dys (−)	10 m and 6 min walk	16–18[b] –	–
Bach [15]	Ret	17 (P16, D1) 117	Unknown	Include Dys (−)	Wheelchair dependence	10.8* 9.7*	1.1
Straathof [11]	Ret	35 (Pi) 0	Unknown	Unknown	Unable to walk, unsupported indoors	10.8 –	–
Houde [10]	Ret	37 (D) 42	Include deletions	Include Dys (−)	Can no longer walk even with help	11.5* 9.6*	1.9
King [12]	Ret	91 (P36 D25) 68	Exclude BMD-like mutation and phenotype	Unknown	Functional walking without orthoses or any assistive device	12.5* 9.2*	3.3
Pradhan [14]	Pro	15 (P) 19	Include deletions	Unknown	Chair-bound stage	14.0* 11.0*	3.0
Biggar [9]	Ret	40 (D) 34	Include deletions	Include consistent with DMD and or	Unable to walk independently	–[c] 9.8*	3–5[c]
Balaban [8]	Ret	30 (P18, D12) 19	Unknown	Unknown	Unable to walk 30 feet on a level floor	P10.6–12.4*, D10.9–12.9* 8.9–9.9*	–
Yilmaz [16]	Pro	66 (P) 22	Unknown	Unknown	Loss of independent walking ability	10.0* 8.6*	1.4

Pro Prospective study, *Ret* Retrospective study, *D* Deflazacort, *P* Prednisolone (Prednisone); *Pi* Intermittent Prednisolone, *Pd* daily prednisolone, *Dys* dystrophin expression

[a] Comparison between Pd and Pi

[b] 4 Treated patients (age 16–18) were fully ambulant, able to fast walk 10 m, and to perform the 6MWT; three of them were still able to climb stairs

[c] All treated boys could walk 10 m at 10 years of age, 25 (81 %) of 31 at 12 years, 13 (76 %) of 17 at 15 years and two of six boys walked independently at 18 years of age

* Mean age, years

excluded all patients who had any residual dystrophin expression in muscle tissue. However, 303 patients in our study were diagnosed as having DMD only based on *DMD gene* analysis. Of the 303 patients, 125 (28 treated in the past, 97 currently being treated) were in PSL group (50.0 % of 250), and 178 were in without-PSL group (56.5 % of 315). Therefore, some patients with milder phenotype may have been included in both groups. Third, PSL regimes (dose, age at commencement and duration) in our study may possibly have differed from those in related studies. A few previous studies only enrolled patients treated with GC for >1 [4] or >2 [8] years before LOA. Strictly controlled clinical trials have shown a more marked improvement in ambulation. However, discontinuation of the drug due to intolerable side effects leads to exclusion of clinical trial participants, while in our study patients were not excluded from PSL group, even if they discontinued the medication shortly after starting it. The American Academy of Neurology [27] and the Cochrane review [6] evaluated all RCTs on the use of GCs in DMD and concluded that PSL administered at 0.75 mg/kg/day was effective. However, a broadly accepted GC dose–response relationship has not been defined [6]. Therefore, a large-scale prospective study using strict criteria has been started very recently to determine the optimal regime in DMD (FOR-DMD) [28].

Our study is limited because all data is retrospectively captured by the registry. The registry items does not include detailed information of PSL regimes (dose, age at commencement and duration), physiotherapy, or other additive treatments such as creatine [29, 30]. Although we adjusted for family history and area of residence in the multivariate analysis, there was still some possibility of residual confounding between the two groups, such as progression of the attitude of "the standards of DMD care" by the decades. There was no item regarding the side effects of long-term PSL administration. Thus, we did not conclude that the benefits of PSL treatment outweigh the risks. The most frequent adverse effect of long-term GC treatment was a reduction in a patient's height [6]. Weight gain was the second most frequent adverse event and the reason most often cited for discontinuing treatment [17]. However, weight gain in GC-treated DMD patients was a multifactorial effect due to pharmacological effects of GC and patients immobility, because weight gain generally was more pronounced in non-ambulatory patients [31].

However, our observational study showed actual clinical setting of GCs therapy in Japan ("real life" data). The result of our study could provide evidence to formulate recommendations and base realistic expectations for steroid treatment of DMD patients in Japan. The residential variation in PSL use, depending on the geographical region of Japan, probably due to differing practices among hospitals and doctors, suggested that PSL therapy for the DMD patients had not been standardised in Japan [18]. Clinical practice guidelines for DMD in Japan will be published by the end of 2013. (http://www.neurology-jp.org/link/index.html, accessed August 12th, 2013). Finally, our data presents the first large outcome study of DMD patients in an Asian country. Recently, well conducted natural history studies for DMD have been reported from Europe and North American countries [23, 32]. Considering feasibility of global clinical trials for DMD, it appears relevant to obtain natural history data in non-western DMD patient populations. This study could add important information of the "real life" of DMD patients.

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Conflicts of interest The authors declare that they have no conflicts of interest.

Ethical standard Approval of the study was given by the National Centre of Neurology and Psychiatry, Ethics Committee involved in the study.

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アレイ CGH を用いた DMD 遺伝子の重複変異領域の断端解析

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DMD 遺伝子の変異は Duchenne/Becker 型筋ジストロフィーを引き起こす。同遺伝子の変異領域の断端（breakpoint）解析は、同疾患に対する遺伝カウンセリング並びにエクソン・スキッピングなどの変異特異的な治療法の検討において有用な情報となる。断端解析を行うにあたっては、欠失変異では短いゲノム PCR 産物の有無により欠失を推測できるのに対し、重複変異では技術的に高度な long-range PCR が要求される。我々は重複変異における断端解析を簡易化するために、DMD 遺伝子全領域をカバーするアレイ CGH（DMD-aCGH）の有用性を検討した。MLPA 法によりあらかじめエクソン 5–7 の重複変異が同定されたサンプルを用いて検証したところ、DMD-aCGH によりゲノム上の重複の全長と断端の位置が容易に推測可能であった。最終的にはイントロン 4 から 7 にかけての 29 kb の重複領域、並びに断端接合部の位置と塩基配列を同定できた。切断点の解析から、本例における重複の発生機構として FoSTeS （Fork Stalling and Template Switching）の関与が示唆された。

Title

Identification of a Duplication Breakpoint in the *DMD* Gene using Array Comparative Genomic Hybridization

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Abstract

Mutations in the *DMD* gene cause Duchenne/Becker muscular dystrophy (DMD/BMD). Full characterization of the mutations, including the analysis of deletion or duplication breakpoints, is diagnostically and therapeutically beneficial. To identify duplication breakpoints, the array comparative genomic hybridization (aCGH) method was used, covering the whole *DMD* gene. For the previously identified duplication of exons 5–7, DMD-aCGH revealed the duplication at a higher resolution, and enabled detection of the breakpoint junction. The 29 kb duplication from intron 4 to 7, with its precise breakpoint junction sequence, was determined. Additionally we found a complicated rearrangement, fork stalling and template switching, in the breakpoint.

Keywords

Duchenne muscular dystrophy, duplication mutation, array comparative genomic hybridization, fork stalling and template switching

Introduction

Mutations in the *DMD* gene (MIM 310200) cause DMD/BMD. Deletions of one or more exons are most frequent, occurring in approximately two-thirds of all patients. Large duplications have been reported in approximately 6% of all DMD/BMD cases[1, 2]. Genetic testing of the *DMD* gene is the initial method for confirming the diagnosis. Multiplex ligation-dependent probe amplification (MLPA) analysis, which is used to examine every exon for deletion and/or duplication, has contributed to a marked improvement in the mutation detection rate[3]. However, full characterization of the mutational spectrum, including the analysis of deletion and duplication breakpoints, is desirable for genetic counseling and eligibility assessment, ultimately leading to mutation-specific therapy, such as an antisense mediated exon-skipping[4, 5]. It is reported that the length of the flanking introns affects the dynamics of splicing; therefore, a determination of the breakpoint junction and the length of an intron is important in an antisense therapy[6]. We planned an antisense mediated exon-skipping assay in the duplicated *DMD* gene; therefore, we performed a breakpoint analysis before the assay. Compared with the characterization of deletion breakpoints, duplication breakpoint analysis is more challenging. It usually requires a technically laborious long-range PCR to obtain a PCR fragment containing the duplication breakpoint[6]. The aCGH method has been widely used to identify chromosomal copy number and structural changes, at a high resolution. To identify the *DMD* duplication breakpoint, we chose the CytoSure DMD Array, a commercially available "DMD-aCGH" with comprehensive coverage of the whole *DMD* gene, which was used in a previous study of copy number variations in *DMD* gene[7]. This paper reports the rapid characterization of the duplication breakpoint, utilizing high-resolution aCGH, customized for the *DMD* gene.

Materials and Methods

Samples

As the reference normal human genome, TIG-119 fibroblasts were obtained from the Health Science Research Resource Bank (Osaka, Japan). As the DMD patient genome, GM04327 fibroblasts (clinically diagnosed DMD, exons 5–7 duplications identified by MLPA) were obtained from the Coriell Cell Repositories (Camden, NJ, USA). DNA was prepared from each of the fibroblast samples using the Wizard SV Genomic DNA Purification system (Promega, Fitchburg, WI, USA).

DMD-aCGH

The CytoSure DMD Array (Oxford Gene Technology, Oxford, UK) was used in this study. It comprises 44,000 probes of 60mer oligonucleotides that cover the whole *DMD* gene on a single array. The average probe spacing is 10 bp within the exons and 106 bp within the introns. The restriction digestion of genomic DNA, the labeling of the DNA, the hybridization of arrays with labeled target and the scanning of arrays was performed according to the manufacturer's instructions. Briefly, patient samples and normal reference samples were labeled with Cy5 and Cy3, respectively. After

hybridization on the DMD-aCGH, the scanned fluorescent signal was analyzed (**Figure 1A**). Data analysis was performed using CytoSure Interpret Software ver. 3.4.6 (Oxford Gene Technology).

Breakpoint PCR

Two intron 7 forward primers and two intron 4 reverse primers were designed based on the DMD-aCGH result (primer sequences are available on request). Each primer pair flanked the duplication breakpoint junction, and was expected to yield PCR products within the range of 1–15 kb. PCR was performed using KOD FX (Toyobo, Osaka, Japan), and the cycling program was set to yield 15 kb products with a program of 35 cycles of 98°C for 10 sec, 60°C for 30 sec, and 72°C for 450 sec. One of the four primer pairs yielded a 3 kb PCR fragment. Primer walk sequencing was performed on the fragment (Operon Biotechnologies, Tokyo, Japan). Human genome sequence NCBI Build 36.1 was used as the reference genomic sequence.

Results

In the chromosomal overview, high-density probes were mapped to the Xp21.2 region. Magnification of the region indicated an at least 24 kb gain aberration from intron 4 to intron 7, consistent with the result of MLPA analysis (**Figure 1B**). We selected four representative probes near the breakpoint junction. Probes 1 and 2 were from intron 4, and probes 3 and 4 were from intron 7. From the mean signal ratio of these probes, we speculated that probes 1 and 4 were positioned in the non-duplicated region and probes 2 and 3 were positioned in the duplicated region (**Figure 2A**). The tandem duplication model was used; the tandem duplication of introns 7 and 4 was a maximum of 131 kb, and probes 1–4 were mapped twice (1p–4p: primary, 1s–4s: secondary). Probes 4p and 1s were speculated to be in the region missing from the tandem intron; therefore, the 104 kb region between probes 4p and 1s was excluded from design of the breakpoint primers. We designed an intron 7 forward primer between probes 3p and 4p, and an intron 4 reverse primer between probes 1s and 2s (**Figure 2B**). A primer pair flanking the breakpoint junction yielded a 3kb PCR product, which revealed that the rearranged concatenation from intron 7 to intron 4 was 15 kb (**Figure 2C**). The sequence analysis of the 3 kb PCR fragment revealed the genotype; arr Xp21.1(32,731,239-32,760,260)×2 , and the duplication size was 29 kb. In the breakpoint, we identified a 19 bp insertion "GTATCTAGTTAAAATCATA". (**Figure 2D**). "TTAAA" is common between intron 4 and 7. The inserted sequence of "TCTAGTTAAAATCA" is also found in intron 4. This result suggested that the fork stalling and template switching (FoSTeS) mechanism had mediated the genomic rearrangement in this case.

Discussion

The characteristics of a breakpoint provide an insight into complex chromosomal rearrangements[6]. In determining a breakpoint junction, the correct design of PCR primers covering the breakpoint regions is essential. In this case, if the conventional long-range PCR approach was chosen, candidate primer regions would be spread throughout the duplicated region, and a trial-and-error approach would be necessary for successful PCR. The DMD-aCGH technique narrowed the candidate primer region from

131 kb to 17 kb, which dramatically accelerated the design of primers, quickly achieved successful PCR, and provided a definitive sequence of the breakpoint.

In the *DMD* gene, the duplications are evenly spread throughout the gene. An exception is the duplication of exon 2, which is the most common single-exon duplication[2]. In the Leiden Muscular Dystrophy Database (http://www.dmd.nl/), we found only 17 cases of exons 5-7 duplication, compared to 113 cases of exon 2 duplication[8]. Among the 17 cases, only one case had a reported breakpoint determined by custom-designed aCGH, and was genotyped as arr Xp21.1(32,725,684-32,765,708) ×2; the minimum duplication size was 40.0 kb[9]. Compared with our result, the duplication size of this case was 11.0 kb longer, and the intron size was 5.5 kb longer for introns 4 and 7. A study of 11 cases of exon 2 duplication showed that the breakpoints in intron 1 were relatively scattered, whereas the 10 breakpoints in intron 2 were clustered in the first 40 kb region[10]. The apparent clustering of breakpoints within intron 2 suggested a breakpoint hotspot in that particular intron; however, analysis of the two cases of exons 5–7 duplication provided no apparent bias in breakpoint distribution within intron 4 and 7.

The breakpoint analysis suggested that the FoSTeS mechanism had mediated the genomic arrangement in this case. FoSTeS is proposed as a microhomology-mediated replication error mechanisms and has been found previously in a *DMD* gene rearrangement [11,12]. The revealed rearrangement was a tandem duplication, but contained 5 bp of microhomology and 16 bp of replication from intron 4. A large-scale breakpoint analysis study demonstrated that microhomology-mediated processes, including FoSTeS, account for 28% of observed rearrangements[13].

Conclusion

The DMD-aCGH platform was used to detect genomic rearrangements involving the *DMD* gene. It revealed a 29 kb duplication from intron 4 to 7 and a complicated FoSTeS rearrangement at the breakpoint.

Declarations

The authors declare no financial conflict of interest.

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Figure Legends

Figure 1 - Copy number changes in the *DMD* gene detected on the DMD-aCGH platform

(A) Overview of the DMD-aCGH procedure. (B) Each unique 60-mer oligonucleotide probe (gray dot) is represented by a data point along the X-axis, based on its physical position at theXp21.1 *DMD* locus. In the magnified view of the Xp21.1 locus, probe (gray dot with black circle) copy number gains are shown at the duplication involving *DMD* exons 5–7.

Figure 2 - Detection of breakpoint junction in the exons 5–7 duplication

(A) Each probe (black dot) is displayed horizontally along with its genomic position and vertically by the signal. Probes 1–4 (gray vertical bar) were speculated to be positioned near the breakpoint junction. (B) In a tandem duplication model, probes 1–4 were mapped twice (1p–4p: primary, 1s–4s: secondary). Probe 4p and 1s (gray dashed vertical bar) were speculated to be in the region missing from the genome. Breakpoint detection primers: an intron 7 forward primer (i7 fwd) and an intron 4 reverse primer (i4 rev) were designed between probes 3p and 4p, and 1s and 2s, respectively. (C) The rearranged concatenation from intron 7 to intron 4 was 15 kb. The duplication size was 29 kb. (D) Bases written in upper case with a gray background are the sequence of the breakpoint junction. "TTAAA" (solid line) is common between introns 7 and 4, and is inserted into the breakpoint. "TATCTAGTTAAAATCA" (dashed line) is common between the inserted sequence and the breakpoint of intron 4.

Figure 1

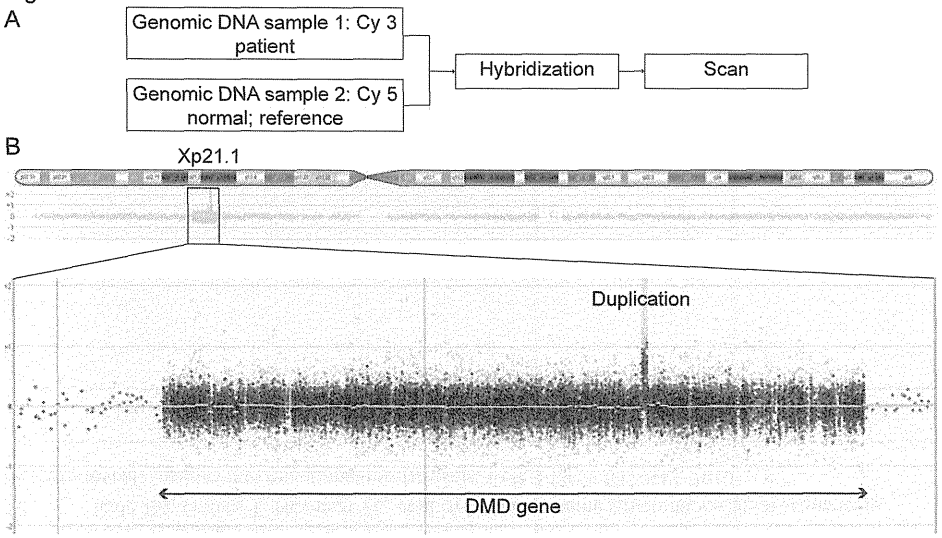

Figure 2