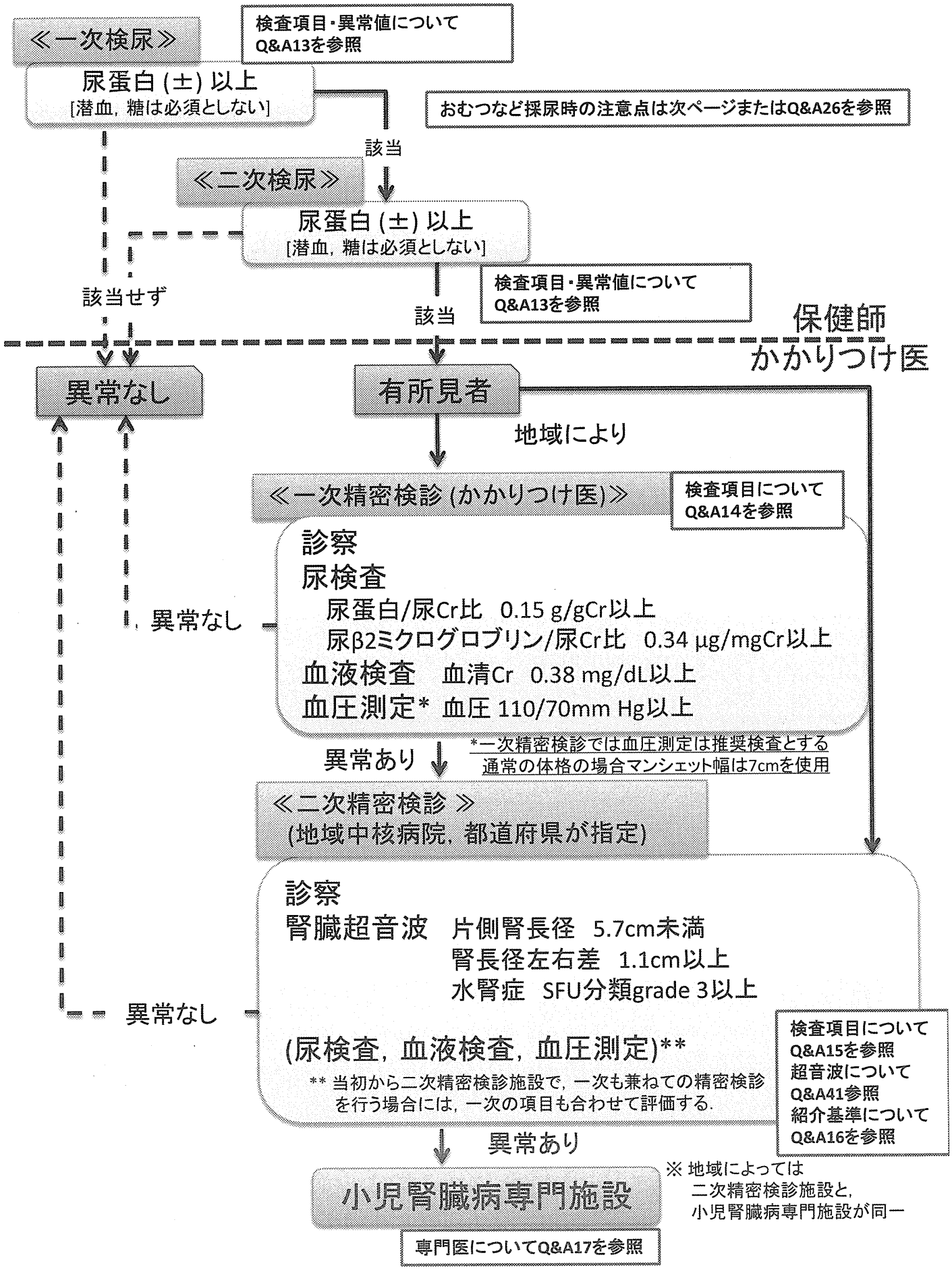
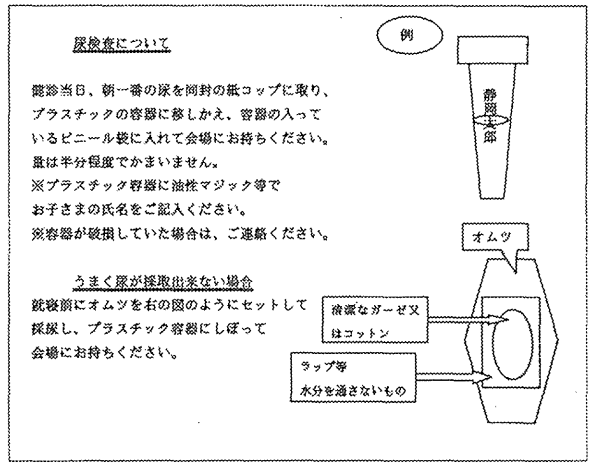
Ⅲ. 資　料

【3歳児検尿フローチャート】



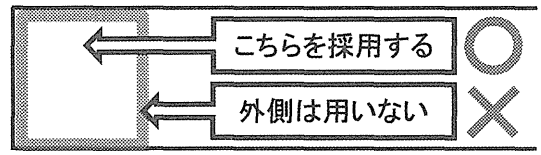
参考資料

① 正しい尿の取り方

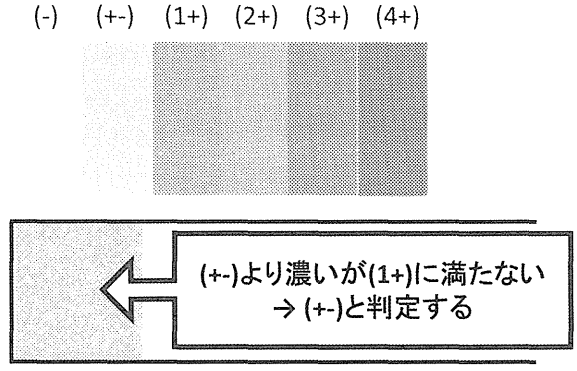


② 試験紙による尿蛋白判定の注意点

- 早朝尿が採れない場合は随時尿でもよい.
- 試験紙の中央部の呈色で判定する.



- 基準色調表と比較し，満たす色調の最大濃度を採用する.
- ※一定に達さない場合には切り上げない.

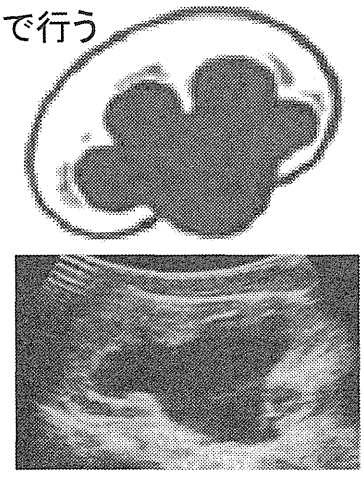


・判定は十分に明るい場所（1000ルクス程度の光源下）で行う

③腎臓超音波検査の異常所見

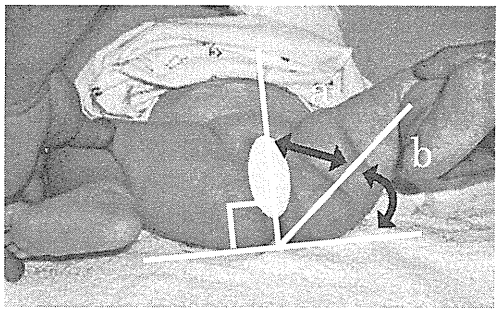
- 片側腎長径　5.7 cm未満
- 腎長径左右差　1.1 cm以上
- 水腎症　SFU分類grade 3以上

(腎盂拡張＋腎杯の拡張所見あり，皮質菲薄化なし)



乳児股関節健診の推奨項目と二次検診への紹介

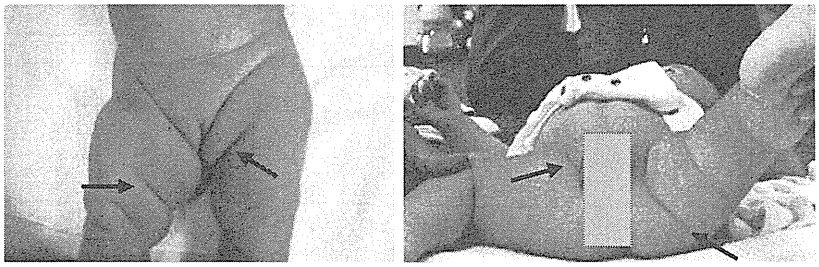
① 股関節開排制限（開排角度）



開排制限の見方：股関節を 90 度屈曲して開く。
開排角度（右図の a）が 70 度以下、すなわち、開排制限角度（右図の b）が 20 度以上、の時に陽性とする。

特に向き癖の反対側の開排制限や左右差に注意する

② 大腿皮膚溝または鼠径皮膚溝の非対称



大腿皮膚溝の位置、数の左右差、 鼠径皮膚溝の深さ、長さの左右差に注意

③ 家族歴：血縁者の股関節疾患

④ 女児

⑤ 骨盤位分娩（帝王切開時の肢位を含む）

二次検診への紹介について

- 股関節開排制限が陽性であれば紹介する
- または②③④⑤のうち 2 つ以上あれば紹介する
- 健診医の判断や保護者の精査希望も配慮する

その他：秋冬出生児に多く、股関節開排時の整復感（クリック）や股関節過開排にも注意が必要。
問診、身体所見のみで乳児股関節異常をもれなくスクリーニングすることはできない。

日本整形外科学会・日本小児整形外科学会

＜乳児股関節脱臼健診チェック項目と診断・治療の指針＞

日本小児整形外科学会、日本整形外科学会

・一次健診（主に小児科医が対応）のチェック項目と二次検診への紹介指針

① 開排制限	＋（右　　度：左　　度）	－
② 大腿・鼠径皮膚溝の非対称	＋（大腿部・鼠径部）	－
③ 家族歴	＋（関係：　　）	－
④ 女児	＋	－
⑤ 骨盤位分娩	＋	－

指針： 開排制限は股関節を 90 度屈曲して開き、開排 70 度以下（床から 20 度以上）の時に陽性とする。
特に向き癖の反対側の開排制限や左右差に注意する。
開排制限陽性の場合、あるいはその他の 4 項目中 2 項目以上が(＋)の場合は二次検診へ紹介する。

・二次検診（整形外科医が対応）のチェック項目と診断・治療の指針

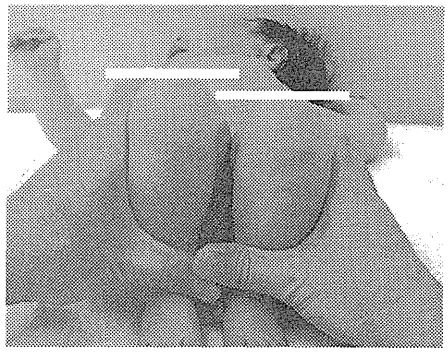
1）身体所見： 股関節開排制限、開排時のクリックサイン（骨頭が臼蓋を出入りする感触；無理に行うと骨頭傷害の危険性があり繰り返しは避ける）、Allis サイン（脱臼側の下肢短縮のサイン：図 1）など

2）X 線所見（生後 3 か月以降）；

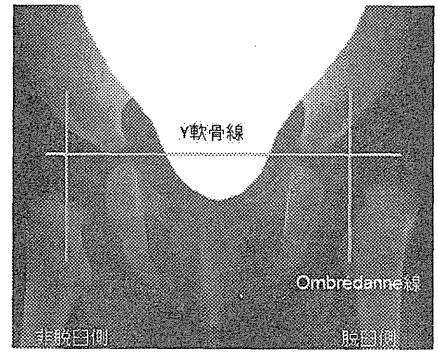
	良	不良
骨頭核の位置	図 2 の(a)の領域にある	上方や外方へ逸脱している
Shenton 線、Calvé 線（図 3）	連続している	連続していない
臼蓋角（α角）　（図 4）	30 度未満	30 度以上
臼蓋の形態	凹型で外側縁が角張っている	直線状あるいは下方凸型で外側縁が丸い～欠損している

注）正確な評価のため、骨盤の傾き（回旋）に注意して正しい正面像を撮影することが重要

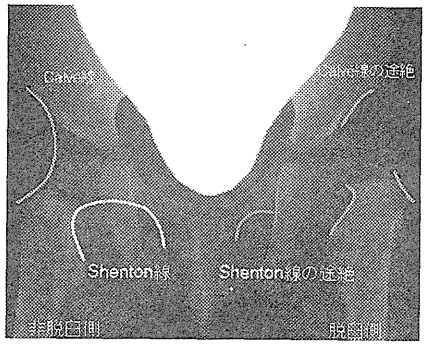
診断、治療の指針： 身体所見と X 線(または超音波)検査所見を総合的に評価する。異常な身体所見を認める場合や、X 線所見で骨頭核の位置や Shenton 線、Calvé 線などが不良の場合は股関節脱臼や亜脱臼が疑われ、治療が必要である。脱臼や亜脱臼は否定的であるが臼蓋角や臼蓋形態が不良な場合は X 線の経過観察が必要である。状況により乳幼児股関節脱臼紹介可能施設（三次施設）への紹介を検討する。



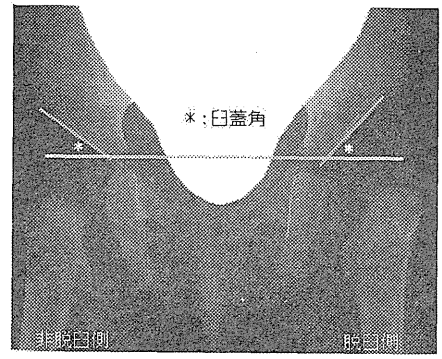
（図 1）
Allis サイン：両踵を床面につけ両膝の高さの差をチェック



（図 2）
(a)：Y 軟骨線の下、Ombrédanne 線より内側の領域
（骨頭核が出現していない場合は出現位置をイメージして評価する）



（図 3）



（図 4）

先天性股関節脱臼紹介可能施設

北海道		北海道大学付属病院、札幌医科大学付属病院、JR 札幌病院、旭川医科大学付属病院、旭川豊岡中央病院、北海道立子ども総合医療療育センター
東　北	秋田県	秋田県立医療療育センター
	青森県	弘前大学医学部附属病院、青森県立あすなろ療育福祉センター、青森県立はなます医療療育センター、つがる西北五広域連合つがる総合病院
	山形県	日本海病院、置賜総合病院、県立新庄病院、山形県総合療育センター、山形済生病院、山形大学
	岩手県	岩手医科大学、岩手医科大学花巻温泉病院、岩手県立療育センター
	宮城県	東北大学病院、仙台赤十字病院、宮城県拓桃医療療育センター、気仙沼市立病院、大崎市民病院、仙台市立病院、松田病院、くらた整形外科クリニック
	福島県	福島県立医科大学付属病院、会津中央病院、福島県総合療育センター
	新潟県	新潟大学病院、はまぐみ小児療育センター、西新潟中央病院、ほんま整形外科医院、亀田第一病院
関　東	茨城県	愛正会記念茨城福祉医療センター、筑波大学病院、茨城西南医療センター病院
	栃木県	自治医科大学とちぎ子ども医療センター、済生会宇都宮病院
	群馬県	伊勢崎市民病院、群馬県立小児医療センタ - 、太田記念病院、群馬大学病院
	埼玉県	埼玉県立小児医療センター、さいたま市立病院、獨協医科大学越谷病院
	千葉県	千葉県こども病院、千葉こどもとおとなの整形外科、成田赤十字病院、松戸市立病院、慈恵柏病院
	東京都	心身障害児総合医療療育センター、東京大学医学部附属病院、東京医科歯科大学医学部附属病院、水野記念病院、都立小児総合医療センター、国立成育医療センター、慈恵医大本院、慈恵第三病院、慶應義塾大学病院
	神奈川県	神奈川県立こども医療センター、横浜市大医学部付属病院、おうぎや整形外科、かただ整形外科
	山梨県	山梨大学附属病院、山梨県立あけぼの医療福祉センター
中　部	静岡県	西部：浜松医科大学医学部附属病院、聖隷浜松病院、磐田市立総合病院 中部：静岡県立こども病院 東部：順天堂大学医学部附属静岡病院
	愛知県	愛知県青い鳥医療福祉センター、聖霊病院、名古屋大学附属病院、愛知県心身障害者コロニー中央病院、愛知県立心身障害児療育センター第二青い鳥学園、あいち小児保健医療総合センター、東海市民病院、豊田市こども発達センター、名古屋市立大学、総合青山病院、藤田保健衛生大学坂文種報徳會病院整形外科、藤田保健衛生大学病院整形外科
	三重県	国立病院機構三重病院、三重大学
	石川県	金沢こども医療センター、やわたメディカルセンター
	富山県	富山県立高志学園

	福井県	福井県こども療育センター
	長野県	信濃医療福祉センター、長野赤十字病院、長野県立こども病院
	岐阜県	岐阜県立希望が丘学園
近　畿	滋賀県	滋賀県立小児保健医療センター、今津病院
	京都府	京都府立医科大学、京都第二赤十字病院、京都第一赤十字病院、綾部市立病院、公立南丹病院、京都府立北部医療センター（旧与謝の海病院）
	大阪府	大阪府立母子保健総合医療センター、大阪市立総合医療センター、国立病院機構 大阪医療センター、大阪赤十字病院附属、大手前整肢学園、大阪発達療育センター、南大阪小児リハビリテーション病院、大阪医科大学附属病院、大阪大学医学部附属病院、済生会吹田病院、みどりが丘病院、関西医科大学付属病院、森之宮病院
	兵庫県	兵庫県立こども病院、神戸大学、兵庫医大、加古川東病院、川崎病院
	奈良県	奈良県：奈良県立医大、東大寺福祉療育病院
	和歌山県	和歌山県立医大、日赤和歌山医療センター、愛徳医療福祉センター、済生会和歌山病院、綿貫整形外科
中国四国	鳥取県	鳥取県立中央病院、鳥取大学病院整形外科、山陰労災病院
	島根県	島根大学病院、西部島根医療福祉センター
	岡山県	岡山大学病院、旭川荘療育・医療センター、川崎医科大学病院、津山中央クリニック
	広島県	広島県立障害者リハビリテーションセンター、中電病院、福山医療センター
	山口県	山口労災病院、山口大学病院、鼓ヶ浦こども医療福祉センター
	香川県	かがわ総合リハビリテーションセンター、独立行政法人国立病院機構四国こどもとおとなの医療センター
	愛媛県	愛媛県立子ども療育センター、南愛媛療育センター、愛媛大学
	徳島県	徳島赤十字ひのみね総合療育センター
	高知県	高知県立療育福祉センター
九　州	福岡県	福岡市立こども病院、北九州市立総合療育センター、九州大学病院、福岡大学病院
	佐賀県	佐賀整肢学園こども発達医療センター、佐賀大学医学部附属病院
	長崎県	長崎県立こども医療福祉センター、長崎大学病院
	大分県	別府発達医療センター、大分大学医学部附属病院
	熊本県	熊本県こども総合療育センター、天野整形外科皮ふ科医院
	宮崎県	宮崎県立こども療育センター、宮崎大学医学部附属病院
	鹿児島県	鹿児島市立病院、鹿児島共済会南風病院
	沖縄県	沖縄県立南部医療センター・こども医療センター、琉球大学医学部附属病院、沖縄赤十字病院、沖縄県立中部病院

ー赤ちゃんが股関節脱臼にならないよう注意しましょうー

＊生後の赤ちゃんの扱い方が大切です！

「股関節脱臼」は脚の付け根の関節がはずれる病気です。その発生はまれですが（1000人に1～3人）、抱き方やおむつの当て方など、赤ちゃんの扱い方を注意することにより、発生をさらに減少させ、また、悪化を防止することができます。

以下の1)～5)のうち、複数の項目があてはまる場合はとくに正しい扱い方を心がけ、必ず3～4か月の健診を受けるようにしましょう。 **1) 向き癖がある 2) 女の子（男の子より多い） 3) 家族に股関節の悪い人がいる 4) 逆子（骨盤位）で生まれた 5) 寒い地域や時期（11月～3月）に生まれた（脚を伸ばした状態で衣服でくるんでしまうため）**

いつも顔が同じ方ばかり向いている**「向き癖」**は、向いている側の反対の脚がしばしば立て膝姿勢となってしまい、これが股関節の脱臼を誘発することがあります。

赤ちゃんの脚は、両膝と股関節が十分曲がったM字型で、外側に開いてよく動かしているのが好ましく（図1）、立て膝姿勢をとったり、脚が内側に倒れた姿勢をとったりすると（図2）、股関節が徐々に脱臼してくることがあるとされています。

両脚がM字型に開かず伸ばされたような姿勢も同様で、要注意とされています（図3）。

＊この紙を壁に貼って、いつも注意しましょう！

ー歩き始めるまで、次の点に注意しましょうー

仰向けで寝ている時は；M字型開脚を基本に自由な運動を

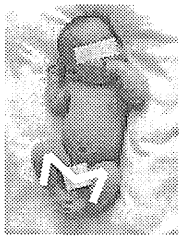
両膝と股関節を曲げてM字型に開脚した状態を基本として（図1）、自由に脚を動かせる環境をつくりましょう。両脚を外から締めつけて脚が伸ばされるような、きついオムツや洋服はさけましょう（図3）。

抱っこは；正面抱き「コアラ抱っこ」をしましょう

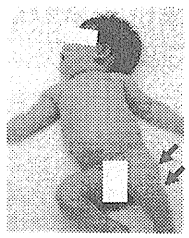
赤ちゃんを正面から抱くと、両膝と股関節が曲がったM字型開脚でお母さん（お父さん）の胸にしがみつく形になります。この正しい抱き方は、あたかもコアラが木につかまった形であることから「コアラ抱っこ」とも呼ばれています（図4）。同様に、両膝と股関節がM字型に曲がって使える「正面抱き用の抱っこひも」の使用も問題ありません（図5）。横抱きのスリングは開脚の姿勢がとれず、また、両脚が伸ばされる危険もあるため、注意が必要です（図6）。

向き癖がある場合は；反対側の脚の姿勢に注意しましょう

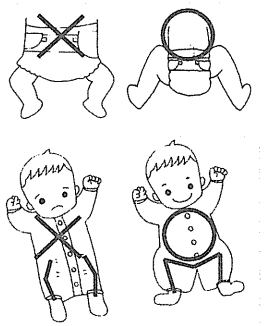
向き癖方向と反対側の脚が立て膝姿勢にならず、外側に開脚するような環境を作ってあげるよう留意しましょう。赤ちゃんには常に向き癖の反対側から話しかける、向き癖側の頭から身体までをバスタオルやマットを利用して少し持ち上げる（図7）などの方法が提唱されています。それぞれの赤ちゃんに合った方法を工夫してみましょう。



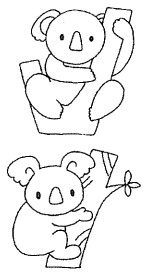
（図1）好ましい姿勢：両脚をM字型に曲げて開き、よく動かしている



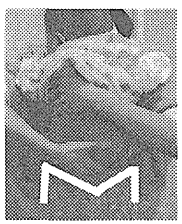
（図2）右への向き癖：左脚が立て膝～内倒れになっている



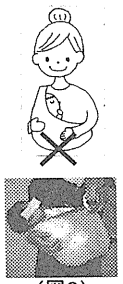
（図3）好ましいオムツや洋服：両脚をM字型に曲げる余裕がある（外側がきついと脚が伸びてしまう）



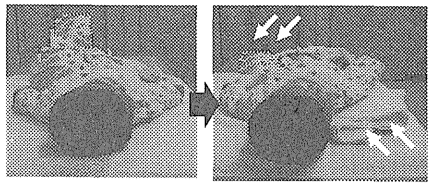
（図4）コアラの姿勢とコアラ抱っこ：両脚が十分曲がりM字型をしている（注：首が座るまでは必ず頭部を支えてあげましょう）



（図5）抱っこひもを利用したコアラ抱っこ



（図6）



（図7）右への向き癖の場合、右側の頭～身体を少し持ち上げて斜めにして、左脚が外側に倒れて開くように工夫する。

＊1か月と3～4か月の健診でチェックを受け、異常を疑われた場合は整形外科を受診することになりますが、気になる点がある時はいつでも整形外科を受診下さい。

（日本整形外科学会，日本小児整形外科学会）

Ⅳ. 研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

書　籍

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ
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Ⅴ. 研究成果の刊行物・別冊

CLINICAL REPORT

AMERICAN JOURNAL OF medical genetics A

Identification of a Novel Missense Mutation of *MAF* in a Japanese Family With Congenital Cataract by Whole Exome Sequencing: A Clinical Report and Review of Literature

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Congenital cataracts are the most important cause of severe visual impairment in infants. Genetic factors contribute to the disease development and 29 genes are known to cause congenital cataracts. Identifying the genetic cause of congenital cataracts can be difficult because of genetic heterogeneity. V-maf avian musculoaponeurotic fibrosarcoma oncogene homolog (*MAF*) encodes a basic region/leucine zipper transcription factor that plays a key role as a regulator of embryonic lens fiber cell development. *MAF* mutations have been reported to cause juvenile-onset pulverulent cataract, microcornea, iris coloboma, and other anterior segment dysgenesis. We report on six patients in a family who have congenital cataracts were identified *MAF* mutation by whole exome sequencing (WES). The heterozygous *MAF* mutation Q303L detected in the present family occurs in a well conserved glutamine residue at the basic region of the DNA-binding domain. All affected members showed congenital cataracts. Three of the six members showed microcornea and one showed iris coloboma. Congenital cataracts with *MAF* mutation exhibited phenotypically variable cataracts within the family. Review of the patients with *MAF* mutations supports the notion that congenital cataracts caused by *MAF* mutations could be accompanied by microcornea and/or iris coloboma. WES is a useful tool for detecting disease-causing mutations in patients with genetically heterogeneous conditions. © 2014 Wiley Periodicals, Inc.

Key words: congenital cataract; *MAF*; iris coloboma; microcornea; whole exome sequencing

How to Cite this Article:
Narumi Y, Nishina S, Tokimitsu M, Aoki Y, Kosaki R, Wakui K, Azuma N, Murata T, Takada F, Fukushima Y, Kosho T. 2014. Identification of a novel missense mutation of *MAF* in a Japanese family with congenital cataract by whole exome sequencing: a clinical report and review of literature.
Am J Med Genet Part A 164A:1272–1276.

INTRODUCTION

Congenital cataracts are an ocular abnormality causing crystalline lens opacification and are the most important cause of severe visual impairment in infants. [Huang and He, 2010]. The estimated prevalence of congenital cataracts are 1–15 cases per 100,000 live births in the world [Santana and Waiswol, 2011]. Congenital cataracts are considered to occur during embryonic development. Between 8.3% and 25% of congenital cataracts are considered to be inherited as autosomal dominant, autosomal recessive, or X-linked

Conflict of interest: none.
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traits [Hejtmancik, 2008; Churchill and Graw, 2011]. To date, 29 genes associated with congenital cataracts have been identified [Huang and He, 2010]. These genes encode structural proteins, cytoskeletal proteins, gap junction channel protein, membrane associated proteins, glycolytic enzymes, and cell-signaling proteins [Huang and He, 2010]. In most cases of inherited congenital cataracts, lens manifestations occur in isolation, while the other cases exhibit other ocular anomalies or occur as part of a metabolic disease or genetic syndrome [Huang and He, 2010; Churchill and Graw, 2011]. However, patients with congenital cataracts having identical gene mutations exhibited various types of lens manifestations and also other ocular abnormalities while others with the same phenotype of lens abnormalities had different gene mutations [Hu et al., 2010; Huang and He, 2010; Sun et al., 2011]. Such genetic heterogeneity makes it difficult to identify disease-causing mutations in patients with congenital cataracts. A new approach, whole exome sequencing (WES), is a remarkable tool that can identify disease-causing mutations in genetic heterogenetic diseases [Tsurusaki et al., 2012; Aoki et al., 2013]. Kondo et al. [2013] reported two families with congenital cataracts and *CRYAA* or *CRYGC* mutations using WES.

We have found a v-maf avian musculoaponeurotic fibrosarcoma oncogene homolog (*MAF*) mutation in a large family with congenital cataracts by WES. *MAF* is one of the causative genes for congenital and juvenile cataracts. Thirty-three patients from five families have been reported typically with pulverulent or cerulean types of cataracts accompanied by microcornea [Jamieson et al., 2002, 2003a,b; Vanita et al., 2006; Hansen et al., 2007, 2009]. For further delineation of clinical characteristics of patients with *MAF* mutations, we will describe detailed clinical information of affected individuals in our current family and review all the patients reported to date.

MATERIALS AND METHODS

Clinical Report

A Japanese boy (IV-1), now 5 years old, was the first child born to nonconsanguineous parents. His mother (III-2) had bilateral congenital cataracts, requiring lens removal at age 3 months. His maternal grandmother (II-2) had bilateral cataracts requiring surgery in her high-school period (Fig. 1A).

During the neonatal period, he was diagnosed with lamellar-type cataract without other eye malformations. Bilateral lens removal was performed at age 3 months. Throughout infancy and childhood, his motor development was normal. However, his language development was delayed and he was diagnosed with autism spectrum disorder at 4 years of age. He also had abnormal lower incisors and a bifid uvula. Nance-Horan syndrome (NHS), an X-linked disorder characterized by congenital cataract, dental anomalies, dysmorphic features, and mild to moderate intellectual disability was suspected, but molecular analysis for the causative gene NHS showed no mutation. G-banded chromosomes was normal. Microarray comparative genomic hybridization using CGX-3 cytogenetics arrays (Roche NimbleGen, Inc., Madison, WI) showed no pathogenic genomic copy number abnormalities.

IV-2, a Japanese girl now 3 years old, was the first child born to nonconsanguineous parents. She was a maternal cousin of IV-1. She had bilateral lamellar and anterior polar type of congenital cataract with bilateral microcornea and iris coloboma. Her visual acuity was 20/100 OD, 20/100 OS, and 20/50 OU with myopic astigmatism. Her developmental milestones were normal.

IV-3, a younger brother of IV-2, now 1 year and 2 months old, had bilateral nuclear and anterior subcapsular type of cataract with bilateral early cataract surgery at 3 months of age, and secondary surgery for glaucoma in the left eye at 11 months of age. After cataract removal, he was found to have bilateral mild macula hypoplasia. Aphakic glasses were prescribed and his grating visual acuity with correction was 20/190 OU. He also had inguinal hernia, which was surgically repaired. His developmental milestones were normal. His mother (III-3) is a sister of III-2 and was diagnosed with bilateral cataracts and microcornea. She underwent a surgery for bilateral cataracts and a second surgery for retinal detachment in the right eye during junior high school period.

Exome Sequencing

Library preparation. After informed consent was obtained, genomic DNA for II-2, III-1, III-2, and IV-1 was extracted from peripheral blood using the Gentra PureGene Blood kit (QIAGEN, Inc., Valencia, CA). Genomic DNA for II-1, III-3, and IV-3 was extracted from saliva using the Oragene DNA collection Kit (DNA Genotek, Inc., Ottawa, Ontario, Canada) according to the manufacturers instructions.

Target selection and sequencing. Exome sequencing was conducted for three DNA samples (II-2, III-2, and IV-1). The genomic DNA was shared into approximately 150–200 base pair (bp) fragments, and used to make a library for multiplexed paired-end sequencing (Illumina, San Diego, CA). The constructed library was hybridized to biotinylated cRNA oligonucleotide baits from the SureSelect Human All Exon 50 Mb Kit (Agilent Technologies, Inc., Santa Clara, CA) for exome capture. Targeted sequences were purified by magnetic beads, amplified, and sequenced on an Illumina HiSeq2000 platform in a paired-end 101 bp configuration.

Mapping and SNV/indel calling. After quality control tests, the reads were mapped to the reference human genome (UCSC Genome Browser hg19) using Burrows-Wheeler Aligner (ver. 0.5.9). The mapping results were corrected using Picard (ver. 1.49) to remove duplicates and Genome Analysis Toolkit (GATK, ver. 1.1–31) for local alignment and quality score recalibration. SNV and Indel calls were performed with multi-sample calling using GATK. The annotations of SNVs and Indels were based on dbSNP131, CCDS (NCBI, Sep 2009), RefSeq (UCSC Genome Browser, Oct 2010), and Encode (UCSC Genome Browser, ver. 4).

Verification of variants. The segregated variants in the present family were confirmed by Sanger sequencing. Primers were designed flanking the candidate loci based on the genomic sequence of the human genome (UCSC Genome Browser hg19). The genomic DNA of the patients in the present study was amplified by polymerase chain reaction (PCR) for each gene. After purification, the PCR samples were directly sequenced using the ABI BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA). Reactions were analyzed on an ABI 3100 semi-automated sequencing analyzer (Applied Biosystems). The DNA sequences

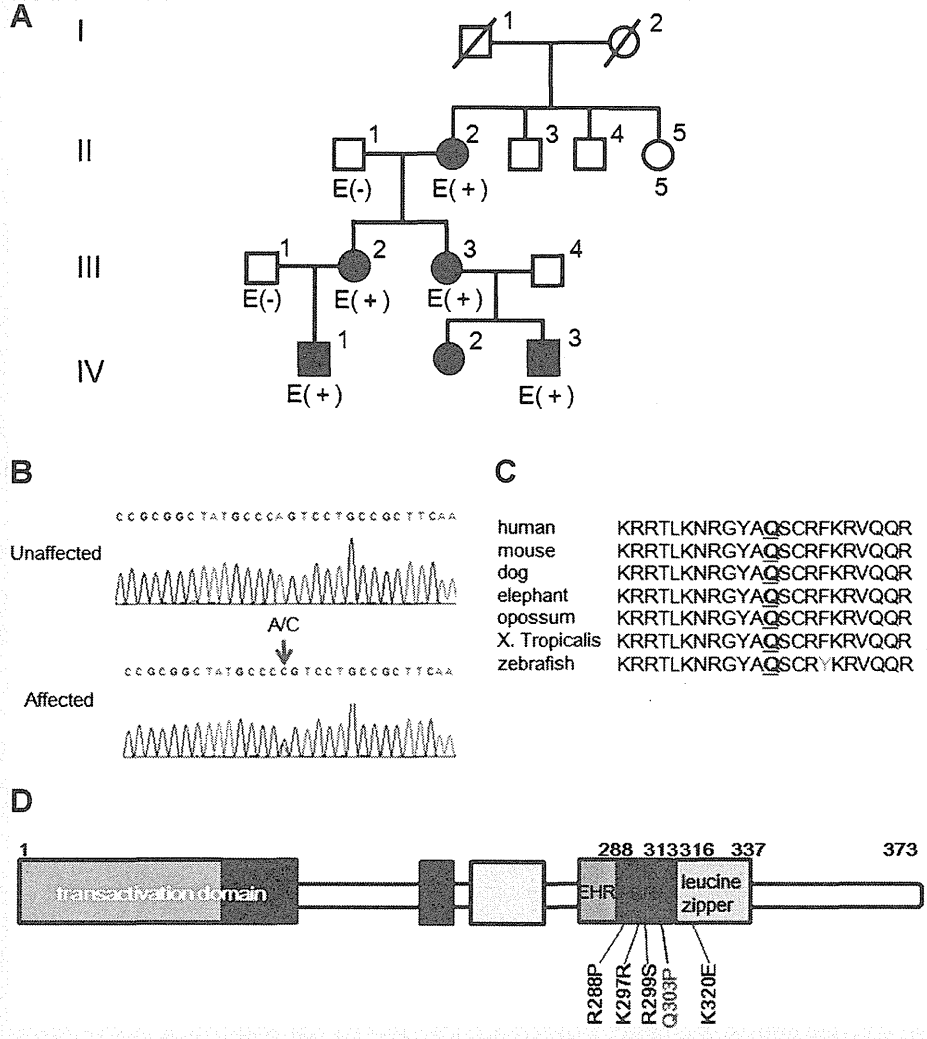

FIG. 1. The *MAF* Glu 303 Pro mutation was identified in five patients in one family A: Pedigrees of the family. Square: male; circle: female; open symbol: unaffected; filled symbol: affected; E+ mutation positive, E− mutation negative. B: Sequencing results of *MAF* from unaffected (III-1) and affected (IV-1) members is shown. The position of nucleotide substitution is indicated by a red arrow. C: Compared amino acid region of *MAF* across species. Glutamine at position 303 is in bold and underlined. Different parts from human are indicated in gray. D: Schema of *MAF* domain structure and identified mutations in previously reported and present (red letter) patients. The functional domains are indicated as follows: EHR, extended homology region; BR, basic region.

were analyzed using FinchTV version 1.4.0 (Geospiza, Inc., Seattle, WA). The comparison of sequencing data was completed by the GENETYX software (Software Development, Tokyo, Japan). This study was was approved by the ethics committee of Shinshu University School of Medicine.

RESULTS

After removing previously reported variants from the WES generated data, we identified 2,231 variants in three affected members. We screened these variants for inheritance in an autosomal dominant manner or X-linked dominant manner among these three patients. After this step, we identified six variants (*USP9X*, *XPNPEP2*, *BMP15*, *TIMP*, *CXorf59*, and *MAF*). We surveyed the mutations in the 29 known genes of congenital cataract by visual inspection. The *MAF* c.908A>C variant was predicted to convert glutamine to proline at amino acid position 303. This mutation was not found in any samples in the National Heart, Lung, and Blood Institute Exome Sequencing Project (NHLBI ESP) Exome Variant Server (Seattle, WA). The algorithms of Polymorphism Phenotyping (PolyPhen-2) and Sorting Intolerant from Tolerant (SIFT) software predicted that p. Q303L would be a damaging mutation as the PolyPhen-2 score was 1.0 (range 0–1; 0 = neutral, 1 = damaging mutation) and SIFT tolerance index score was $\leq$0.05. To confirm the variants detected in WES, all family members were screened by PCR amplification and Sanger sequencing for the *MAF*

and other five variants. A heterozygous variant in *MAF* was found in all affected members (Fig. 1B), but the other five variants were not seen in two members (III-1, IV-3). The *MAF* variant had not been identified in 200 alleles in Japanese controls and in other unaffected members (II-1, III-1). These data indicated that the c.908A>C mutation in *MAF* probably would cause cataract in this family.

DISCUSSION

We report on a Japanese family with congenital cataracts and identify *MAF* mutation by WES. The three affected members in this current family show lamellar, anterior polar, nuclear, and anterior subcapsular types of cataract accompanied by microcornea and iris coloboma. To date, missense mutations in *MAF* were identified in four families [Jamieson et al., 2002, 2003a,b; Vanita et al., 2006; Hansen et al., 2007, 2009]. Additionally, Jamieson et al. [2002] reported a family with congenital cataract and a balanced or an unbalanced translocation, one of the breakpoint of which was located in 16q23.2. Molecular cloning of the breakpoint demonstrated that the breakpoint did not disrupt the coding region of *MAF* directly but transected the the genomic-control domain of *MAF*. Totally, 39 patients from six families with *MAF* abnormalities have been reported including the current family (Table I).

The type of cataract was not available in all the patients in the current family. In previous reports, patients in a single family displayed multiple types of cataracts with microcornea, iris coloboma, and other segment dysgenesis conditions [Jamieson et al., 2002; Hansen et al., 2007]. A patient in Family CCMC0113, reported by Hansen et al. [2009], manifested only microcornea without cataract, while other patients in the family showed cataract with or without microcornea. In the current family, three of the six affected patients exhibited severe cataract, requiring lens removal at infancy. Total 26 patients underwent surgery until adulthood and at least five patients did not require lens removal. Among all the reported patients including those in the current family, 17 patients had microcornea and three had unilateral or bilateral iris coloboma.

MAF encodes a protein that belongs to a family of DNA-binding, basic region/leucine zipper transcription factors playing a key role in regulating embryonic lens fiber cell development, increasing T-cell susceptibility to apoptosis, and chondrocyte terminal differentiation [Yi et al., 2011]. *MAF* is expressed early in the developing lens vesicle and has the central regulator of gene expression in the crystalline lens during the differentiation of the primary posterior lens fibers [Kawauchi et al., 1999; Kim et al., 1999; Ogino et al., 2000; Ring et al., 2000]. Homozygous null mutant *c-Maf* mice showed defective lens formation and microphthalmia as a result of failure to elongate of the posterior lens fibers [Kim et al., 1999]. The *MAF* mutation R288P which was identified in a family with juvenile cataract [Jamieson et al., 2002] showed a reduction in the transactivation ability of *MAF* [Perveen et al., 2007]. The *MAF* mutation Q303L in the current family was found in a well-conserved glutamine residue at the basic region of DNA-binding domain (Fig. 1C,D) and was presumed to interfere with *MAF*–dependent transcriptional activation.

Patients with the *MAF* abnormalities exhibited variable types of congenital cataracts with or without microcornea and iris

TABLE I. Clinical Manifestations in *MAF* Positive Patients

	MAF mutation	Total in *MAF* positive patients	Patients with cataract	Cataract classification	Age at operation (26 patients)	Microcornea (17 patients)	Iris coloboma (3 patients)	Other ocular manifestations	Other
II-2	Q303P	6	6	NA	High school period	NA	NA	NA	–
III-2				NA	3m	NA	NA	NA	–
III-3				NA	Junior high school period	+	–	–	–
IV-1				Lamellar	3 m	–	–	–	Lower incisor, bifid uvula, ASD
IV-2				Lamellar, anterior polar	Unoperated	+	+	–	–
IV-3				Nuclear, anterior subcapsular	3 m	+	–	–	Inguinal hernia
Family1 [1,2]		5	4	Total cataract, cortical and sutural pulverulent	Infancy–Adulthood(4)	–	–	Opaque cornea, Peter anomaly, Microphthalmia	*2
Family2 [1,3]	R288P	5	5	Cortical pulverulent, nuclear pulverulent, posterior subcapsular,	25–28y(4)	(2)	(1)	Uveal melanomaUveal naevus	Hodgkin's disease
CC–277 [4]	K297R	12	12	*Cerulean*	*Childhood*—24y(8)	(6)	–	–	–
Family CCMC 0112 [5]	R299S	4	4	Posterior polar, nuclear, lamellar	1m–47y(4)	(3)	(1)	–	–
Family CCMC 0113 [6]	K320E	7	6	Nuclear	43y(1)	(3)	–	–	–

*1 The family had balanced or unbalanced translocation in 5p15.3 and 16q23.2.
*2 The patients with unbalanced translocation showed developmental delay, thin upper lip, short nose, microcephaly, brachycephaly, coarse hair, and small hands with clinodactyly.
NA, data not available; ASD, autism spectrum disorder; y.o., years old; m.o., month old. 1) Jamieson et al. [2002], 2) Jamieson et al. [2003a], 3) Jamieson et al. [2003b], 4) Vanita et al. [2006], 5) Hansen et al. [2007], 6) Hansen et al. [2009].

coloboma. Microcornea is thought to occur secondary to an arrest in corneal growth as the result of an overgrowth of the tips of the optic cup [Nischal, 2002]. Coloboma is caused by faulty closure of the optic fissure [Chang et al., 2006]. These developmental defects of these manifestations seem to be different from those of congenital cataracts. However, cataracts can be found together with iris coloboma or microcornea [Chang et al., 2006]. Mutant mice heterozygous for *c-Maf*R291Q showed pulverulent cataract, whereas other strains could show aberration in the development of the anterior segment in addition to cataract [Lyon et al., 2003]. Some genetic modifiers were suggested to contribute to the development of various ocular abnormalities in an incomplete penetrance [Lyon et al., 2003]. *MAF* abnormalities are considered to that act or coordinate with other modifiers during eye development together with other genetic modifiers resulting not only in congenital cataracts but also in anterior segment dysgenesis.

Two male members of the current family had cataracts with autism spectrum disorder, abnormal lower incisors, bifid uvula, or inguinal hernia. *MAF* has not been proposed to play a role in ASD or in inguinal hernia. From a report of a *MAF* mutation-positive patient with Hodgkin disease [Jamieson et al., 2003b], further analysis of the clinical manifestations of the *MAF* mutation in a higher number of patients is needed to evaluate the associations with other systematic diseases.

In conclusion, WES identified a novel *MAF* mutation in a family segregating with congenital cataract and microcornea and/or iris coloboma in some affected individuals. Review of the patients with *MAF* mutations supports the notion that congenital cataracts caused by *MAF* mutations could be accompanied by microcornea and/or iris coloboma. WES is a useful tool for detecting disease-causing mutations in patients with genetically heterogeneous conditions.

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