A recessive ataxia diagnosis algorithm for the next-generation sequencing era

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Abstract

Objective: Differential diagnosis of autosomal recessive cerebellar ataxias can be challenging. A ranking algorithm that predicts the molecular diagnosis based on the clinical phenotype of a patient has been developed to guide genetic testing and to align genetic findings with the clinical context.

Methods: An algorithm that follows clinical practice, including patient history, clinical, MRI, electromyography and biomarker features, was developed following a review of the literature on 67 autosomal recessive cerebellar ataxias and personal clinical experience. Frequency and specificity of each feature were defined for each autosomal recessive cerebellar ataxia, and corresponding prediction scores assigned. Clinical and paraclinical features of patients are entered into the algorithm, and a patient's total score for each autosomal recessive cerebellar ataxia is calculated, producing a ranking of possible diagnoses. Sensitivity and specificity of the algorithm were assessed by blinded analysis of a multinational cohort of 834 patients with molecularly confirmed autosomal recessive cerebellar ataxia. The performance of the algorithm was assessed versus a blinded panel of autosomal recessive cerebellar ataxia experts.

Results: The correct diagnosis was ranked within the top 3 highest-scoring diagnoses at a sensitivity or specificity of >90% for 84% and 91% of the evaluated genes, respectively. Mean sensitivity and specificity of the top 3 highest-scoring diagnoses were 92% and 95%, respectively. The algorithm outperformed the panel of ataxia experts (P=0.001).

Interpretation: Our algorithm is highly sensitive and specific, accurately predicting the underlying molecular diagnoses of autosomal recessive cerebellar ataxias, thereby guiding targeted sequencing or facilitating interpretation of next-generation sequencing data.

Keywords: Ataxia; Molecular genetics; Cerebellar function; Genetics: movement disorders; Clinical practice

Introduction

Clinical heterogeneity and rarity of some neurological disorders may challenge the ability of clinicians to make timely diagnoses. This is true for autosomal recessive cerebellar ataxia (ARCA), a complex group of rare disorders [1-4]. The revolution in molecular genetics, especially next-generation sequencing (NGS) [5], has led to the identification of new ARCA-causing genes or novel phenotypes of known ARCA-causing genes, improving our understanding of these disorders and our ability to diagnose them [6, 7]. However, NGS generates a huge amount of data, including variants of unknown significance (VUS) that may be difficult and time-consuming to correctly interpret and establish relationships between potential pathogenic variants and observed phenotypes [8].

There is therefore an unmet need for a tool to assist physicians and geneticists in providing a comprehensive and balanced differential diagnosis (DD) of ARCAs. A DD tool that predicts the gene responsible for a phenotype by converting clinical and paraclinical data into a shortlist of likely molecular diagnoses could significantly increase the speed and yield of diagnosis for ARCAs, subsequently improving patient care, particularly in cases where treatments are available.

Our study aimed to create and validate a tool for the DD of patients with suspected ARCA.

Materials and methods

The Recessive Ataxias ranking differential DIagnosis ALgorithm (RADIAL)

A diagnostic algorithm for ARCA based on literature and expert opinion has been produced to guide neurologists who may encounter patients with ataxia in clinical practice (**Fig. 1**). Recessive disorders (herein referred to as entities, each defined by mutations in specific gene(s)) with ataxia as a common, but not necessarily an initial or prominent feature, were identified from the literature according to previously published recommendations [1, 2].

Publications were identified by PubMed search for English language articles between January 1995–January 2016, using the following terms: “recessive cerebellar ataxia”, “recessively inherited cerebellar ataxia”, or “inherited cerebellar ataxia”, and screened for reports of molecularly confirmed cases of recessively inherited disorders with cerebellar ataxia in order to include the majority of the most common ARCA; 281 manuscripts were used to describe the entities included in the algorithm (**Supplementary Table 2**). To describe entities, 124 individual clinical and paraclinical findings including routine biomarkers (so-called features), were identified and refined by expert opinion.

The relationship between each feature and each entity was defined by frequency and/or specificity according to the literature and clinical experience of CT and MA, as follows:

- High frequency (H) – feature occurs in ≥50% of patients with the entity
- Low frequency (L) – feature occurs in <50% of patients with the entity. Where not clearly definable, low frequency is assumed
- Specific (S) – feature presents in <10% of entities
- Not present (0) – feature not considered to be associated with the entity
- Not known (NK) – feature not reported with the entity, but with insufficient evidence to exclude association

These classifications were combined as necessary, e.g. HS, high frequency and specific; LS, low frequency and specific.

For each entity, scores were assigned based on an assessment of the frequency and specificity of features, with more frequent and specific features scoring highest, and weighted based on the perceived importance for DD. Based on the opinion of MA, NK, 0, L, H, LS, and HS were arbitrarily scored 0, −1, 1, 3, 7 and 9, points respectively, for clinical features, neuroimaging and electromyography, whereas scores were doubled for age of onset, severity of disease progression and biomarkers (**Table 1**). For example, if a patient with suspected ARCA has a *cataract*, they would score +3 points for cerebrotendinous xanthomatosis, in which *cataract* is high

frequency (H), but would score -1 points for Friedreich's ataxia, in which *cataract* is not reported (0). A second patient who does not present with *cataract* would score 0 points for both cerebrotendinous xanthomatosis and Friedreich's ataxia.

The outcome of this process was RADIAL, comprising a knowledgebase that defines the association of each clinical and paraclinical feature with each entity, with a corresponding score assigned to each feature-entity association (**Supplementary File 1**).

ARCA patient clinical features data collection

Diagnostic performance of RADIAL was assessed by applying it blindly to a population of patients with molecularly confirmed ARCA, for whom a description of clinical and paraclinical features at the time of molecular diagnosis was available.

The clinical and paraclinical features recorded at the time of molecular diagnosis of a worldwide patient cohort were collected by retrospective chart review between February–May 2016. The persons responsible for data collection from collaborating centres were blinded to the scoring and weighting of features within the algorithm.

All patient data were blinded by the contributor so that names, addresses or other identifying information were not available to any other party involved in the analysis or review of the data. All investigators adhered to local privacy laws and regulations to ensure patient confidentiality. Patients gave written consent and ethical approval for the study was provided by the local ethics committee of the Strasbourg University Hospital, France.

Statistical analysis

Performance assessment of RADIAL

Features of each patient were assessed against the knowledgebase according to the aforementioned calculation method. For each patient, the sum of scores for each feature was calculated for each entity. The total score for each entity defined its position on a ranked list of most likely (highest score) to least likely (lowest score) molecular diagnoses. Algorithm performance was a measurement of sensitivity, and specificity. Correct patient classification was defined as a ranking of the correct

diagnosis within the first, third, and fifth highest scores. The primary outcome of the performance assessment was the ability of RADIAL to correctly predict entities within the Top 3 highest scores. Other outcomes were: correctly predicting entities within the Top 1 and Top 5 highest scores; average sensitivity and specificity for all patients; the ability of RADIAL to correctly identify an entity compared to that of a panel of ARCA experts and correlation between performance and the total number and/or number of specific clinical features per entity.

Blind evaluation against an ARCA expert panel

The ability of RADIAL to correctly identify an entity within the Top 3 highest scores was compared against a blind evaluation by a panel of five ARCA experts (FM, MP, MS, CT and BW) in a sample of 100 patients for each expert, randomly selected from the patient cohort. The experts were given the same list of features provided by the investigators for the algorithm assessment, and produced a ranked shortlist of the three most likely diagnoses for each patient. The experts were able to consult the literature (e.g. PubMed) and were allowed as much time as required to complete the task. The three most likely diagnoses were compared against the Top 3 diagnoses provided by RADIAL for the same 100 patients. For mutations in *FXN* gene, the diagnosis was considered correct when Friedreich's ataxia, LOFA and v-LOFA was proposed. Statistical differences were assessed by McNemar's exact test.

Correlation was tested using Pearson correlation coefficient. All statistical analyses were performed using the statistical package SAS v9.3.

Results

The RADIAL

The algorithm knowledgebase contains 67 individual ARCAs (**Supplementary Table 3**), and was populated using the clinical findings of a total of 2,906 patients from the 281 manuscripts (**Supplementary Table 2**) and clinical experience. The well-described variants LOFA and v-LOFA were considered a single entity distinct from Friedreich's ataxia within the knowledgebase and these analyses [9, 10]. The RADIAL knowledgebase contains 8,308 individual correlations between 67 entities and 124 features (**Supplementary File 1**).

Details of the patient cohort

Data of 834 patients from 18 different countries and representing 45 distinct entities were collected (**Supplementary Table 4**). Among these 834 patients, 618 (74%) were reported in the literature used to create the knowledgebase, corresponding to 21% of the 2,906 patients used to create the knowledgebase.

A broad symptomatology was reported in the patient cohort; a summary of the symptomatology observed in the entire cohort and in the 8 entities with ≥30 patients is presented in **Supplementary Table 5.**

Algorithm performance

Of the 45 entities tested, 91% (41/45) were ranked within the Top 3 highest-scoring diagnoses at a specificity of >90% and 84% (38/45) at a sensitivity of >90% (**Supplementary Table 6**). Among these entities, the correct diagnosis was the highest scoring in 23 entities (51%), and was always found within the Top 16 highest scoring entities. The Top 3 highest scoring diagnoses had an average sensitivity and specificity of 92.2% and 95.4%, respectively (**Supplementary Table 7 and Fig. 2**). The highest scoring diagnosis had an average sensitivity and specificity of 77.1% and 99.3% respectively. The Top 5 highest scoring diagnoses had an average sensitivity and specificity of 96.8% and 91.1%. Average sensitivity and specificity plots including all tested disorders are shown in **Fig. 2**. *ADCK3* [11], *OPA1* [12], *PNPLA6* [13], *STUB1* [14] and *SYNE1* [15] and LOFA/vLOFA [9, 10] were not identified within the Top 3 scores with a sensitivity >90%.

Assessment of the correlation between the number of specific features associated with each entity and RADIAL performance, and between the total number of features associated with each entity and RADIAL performance, showed that neither the number of total signs (r=0.052, *P*=0.730) nor specific signs (r=−0.033, *P*=0.830) influence discriminatory ability (**Fig. 3**).

RADIAL versus expert panel challenge

RADIAL performed well compared with a panel of 5 ARCA experts. In five series of 100 patients randomly selected from the patient cohort, RADIAL placed the correct

diagnosis in the Top 3 highest scoring entities in 95.2% of patients versus 77.8% identified by the experts (P<0.001; McNemar's exact test) and as the highest scoring entity in 80.6% of patients versus 67.6% identified by the experts (P<0.001; McNemar's exact test). The experts required an average of 7.2 hours [range: 6–10] to complete their 100 cases.

Discussion

This study describes RADIAL, an algorithm that aims to improve the DD approach towards ARCA by using patients' features to predict the underlying responsible gene. Sensitivity and specificity of the algorithm in correctly identifying the diagnosis within the Top 3 highest scoring entities was excellent, even outperforming a panel of ARCA experts.

In clinical practice, use of RADIAL should be considered in all patients suspected with ARCA. The diagnostic workup outlined in **Fig. 1** takes a stepwise approach to the patient with ataxia [1, 2, 16, 17], also indicating how the point of suspecting ARCA is reached.

Performance of RADIAL depends on accurate identification of the patient's features, and could be impaired in the absence of sufficiently detailed information. In such cases, the algorithm could be used to guide clinical investigations based on the features of the highest scoring entities from the knowledgebase. The knowledgebase could also clarify the clinical phenotype in a 'genotype-first' [18, 19] (i.e. genotyping before phenotyping), or 'reverse phenotyping' (i.e. phenotyping following genotyping according to genetic results) method [20]. However, due to the risk of missing a correct diagnosis, we would not recommend a 'genotype-first' approach, and 'reverse phenotyping' should be considered with caution. Regardless of where in the diagnostic workup it occurs, a sufficiently detailed phenotypic evaluation is always mandatory. RADIAL could also be used to identify one high likelihood entity which can be confirmed by single gene sequencing. Thus, in this 'phenotype-first' approach, the algorithm could also guide molecular analyses. Whether the 'phenotype-first' or 'genotype-first' approach is followed, the entity ranking provided by RADIAL supports identification of the gene responsible for the phenotype. Indeed, RADIAL

should be considered as an interface between phenotype and genotype, enabling each one to complement the other.

Beyond its efficacy to predict the underlying molecular defect based on clinical data, RADIAL will provide guidance on best-practice for the diagnostic work-up of patients with suspected ARCA, including medical history, clinical examination, and paraclinical signs, serving as a reminder to assess many features that may otherwise be overlooked.

Given the performance of RADIAL, it could facilitate the interpretation of large volumes of data provided by NGS (panel gene sequencing, whole exome or whole genome sequencing), and pathogenicity of VUS could be more easily determined [21]. For instance, the probability of a VUS being pathogenic when the affected gene is not within the Top 16 entities should be very low, whereas a VUS in a high-ranking gene, especially one within the Top 3, is much more likely, facilitating interpretation of NGS data and guiding searches for a second mutation in the same gene. The good sensitivity of RADIAL is important to avoid a missed diagnosis. Conversely, the good specificity provides confidence in the identified top scoring genes, especially when use of RADIAL follows molecular analyses. In such cases, RADIAL could be helpful to identify which VUS are pathogenic mutations responsible for the phenotype and which VUS are polymorphisms.

RADIAL also represents an up-to-date knowledgebase comprising clinical descriptions of 67 individual ARCAs based on the integration of numerous references and expert clinical experience. The 67 entities described in the knowledgebase include the majority of the most common ARCAs, but are not an exhaustive list since there are several other recessively inherited diseases that may include cerebellar ataxia. Some of these diseases have not been included in the current knowledgebase as they lack sufficiently detailed information on their clinical features (e.g. *KIAA0226)* [22, 23]. Several other entities included in the knowledgebase could be viewed as controversial, as cerebellar ataxia is not the initial or most prominent feature of the disease. However, the entities were included in the knowledgebase because cerebellar ataxia was sufficiently well-described as a clinical feature. Following this initial study, periodic updates of the knowledgebase as the literature grows could add or redefine associations between features and entities further improving performance of

RADIAL, particularly for entities that are currently poorly characterised, and allowing addition of new entities. One may hypothesize that increasing the number of entities covered by the algorithm, may increase its superiority to the experts.

RADIAL performance is limited by the quality and completeness of data published in the literature. The knowledgebase should be helpful to any physician facing ataxia, or interested in becoming more specialised within this field. The extensive list of features allows a very precise description of each entity (124 features equates to over 2.1 $x10^{37}$ pairwise combinations) leading to good differentiation between entities, even those with similar but non-identical phenotypes. The performance and flexibility of RADIAL means that an exhaustive assessment of all the signs in patients suspected with ARCA is not required for good performance, and that accurate diagnoses for many patients are possible with few clinical features. A second class of entities, including *ADCK3* [11], *OPA1* [12], *PNPLA6* [13], *STUB1* [14] and *SYNE1* [15], are still poorly recognised by RADIAL. These entities are unlikely to appear near the top of the ranked lists, but should be considered as DD when genetic analyses are inconclusive and the clinical phenotype does not clearly match the highest scoring entities. The poorer performance of RADIAL in recognising these entities might be attributable to an intrinsic difficulty in their identification due to pleiotropic, overlapping clinical phenotypes (e.g. pure cerebellar ataxia in *ADCK3* and *SYNE1*) [11, 15], the lack of helpful biomarkers, or limitations in our classification of their specific clinical features due to their novelty (e.g. *PNPLA6* and *STUB1*) [24, 25]. RADIAL was not able to recognise vLOFA and LOFA as effectively as Friedreich's ataxia. However, given that the former are variants of Friedreich's ataxia, the performance of RADIAL is successful at identifying *FXN* gene mutations. Moreover, Friedreich's ataxia, including LOFA and v-LOFA, is not diagnosed by NGS, therefore validating a VUS on the basis of such diagnoses is not pertinent.

The use of real-life clinical data to test the accuracy of RADIAL addresses many of the limitations discussed above, as the excellent diagnostic performance suggests that the knowledgebase provides a good representation of the clinical characteristics of each entity. This is reinforced by the algorithm outperforming an ARCA expert panel in correctly identifying diagnoses in both the Top 1 and Top 3 positions, a method commonly used to validate the performance of diagnostic algorithms, despite recent

evidence showing that the superiority of the physician is maintained over several algorithms [26]. In the same way, the potential interest of computer based evaluation of photos to diagnose facial dysmorphism has been recently studied [27].

The true incidence of ARCAs observed in clinical practice is not reflected in the patient cohort. We were particularly interested in testing the algorithm with a broad sample of entities, especially those that are not well known and/or difficult to diagnose, which is not the case for Friedreich's ataxia, the most frequent ARCA [1]. Since the ranking calculation does not take into account the prevalence/incidence of a disease we do not believe that this negatively impacted the validation. To obtain a sufficient quantity of data to robustly validate RADIAL performance, it was necessary to obtain previously reported patient data with clear-cut molecular diagnoses by retrospective chart review. Unfortunately the main limitation of this approach is that many patients who were used to assess the final algorithm were also used to construct the knowledgebase; this is more problematic with the rarer entities for which the published cases may represent the majority of the global patient population. Regardless, many more patients whose data were used in construction of the knowledgebase were not assessed in the study. It is also of worth considering that the data reported in the literature are generally less precise and detailed that the clinical data provided by the collaborators. Thus, even if we used the same patients, their data to create the tool and to evaluate the tool were not identical. Moreover, the strength of RADIAL is not only to identify a specific disorder, but rather to identify a specific disorder amongst 67 other entities, which is much more difficult. Finally, that the expert panel were able to correctly identify the diagnosis in the majority of cases also supports the accuracy of the phenotypic definitions of each entity that were used to construct the knowledgebase. Taken together, these arguments support the genuinely very good diagnostic performance of RADIAL.

Another consideration, is the difficulty in accurately determining the frequency and specificity of clinical features, especially in rare entities where reported patient numbers are low. Future large-scale prospective real-world validation of RADIAL should be undertaken to address these concerns, further validate and, if necessary, refine RADIAL. A prospective study would also allow the opportunity to assess utility of RADIAL for interpretation of NGS-derived data, and assess whether

RADIAL can predict the pathogenicity of novel VUS. For this purpose, RADIAL is available to all healthcare professionals as a free-to-use electronic application. RADIAL can be accessed online at http://radial-ataxia-algorithm.com/ or as an offline version in the manuscript supplementary material (**Supplementary File 2**).

This algorithmic approach may be of further interest for many other diseases with inherent diagnostic difficulties, including neurological (e.g. autosomal dominant cerebellar ataxias, hereditary spastic paraplegias, neuropathy, myopathy, complex dystonia, and early dementia) and non-neurological disorders.

In summary, we have developed a tool that facilitates the differential diagnosis of autosomal recessive cerebellar ataxias. RADIAL uses patient's features to produce a list of potential diagnoses ranked by likelihood, and which may be used to inform further confirmatory clinical or genetic testing, and assist the interpretation of next-generation sequencing data.

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Author contributions

MA, CT, MR, MK and JVTM contributed to conception and design of the study; MR, CT, JVTM, FM, MS, BvdW, MP, MK, SAK, MA contributed to the acquisition and analysis of the data; MR, CT, JVTM, FM, MS, BvdW, MP, MK, SAK, MA contributed to drafting the text, preparing figures and approving the final manuscript.

Members of the RADIAL working group (**Supplementary Table 1**) who were not authors, contributed patient data but did not have substantial involvement in drafting the manuscript.

Potential conflicts of interests

MA reports travel grants and personal fees from Actelion Pharmaceuticals Ltd.

SAK is an employee of Actelion Pharmaceuticals Ltd.

MP reports a patent *Direct molecular diagnosis of Friedreich's ataxia* with royalties paid. This molecular diagnosis is included in RADIAL.

JVTM of Syntax for Science Ltd., performed statistical analyses paid for by Actelion Pharmaceuticals Ltd.

MK, FM, MR, MSy, CT, BvdW do not have anything to report

Supplementary material

7 Supplementary Tables and 2 Supplementary File

References

1. Anheim M, Fleury M, Monga B, et al. Epidemiological, clinical, paraclinical and molecular study of a cohort of 102 patients affected with autosomal recessive progressive cerebellar ataxia from Alsace, Eastern France: implications for clinical management. Neurogenetics 2010;11:1-12

2. Anheim M, Tranchant C, Koenig M. The autosomal recessive cerebellar ataxias. N Engl J Med 2012;366:636-646

3. Fogel BL, Perlman S. Clinical features and molecular genetics of autosomal recessive cerebellar ataxias. Lancet Neurol 2007;6:245-257
4. van de Warrenburg BP, van Gaalen J, Boesch S, et al. EFNS/ENS Consensus on the diagnosis and management of chronic ataxias in adulthood. Eur J Neurol 2014;21:552-562
5. Koboldt DC, Steinberg KM, Larson DE, Wilson RK, Mardis ER. The next-generation sequencing revolution and its impact on genomics. Cell 2013;155:27-38
6. Nemeth AH, Kwasniewska AC, Lise S, et al. Next generation sequencing for molecular diagnosis of neurological disorders using ataxias as a model. Brain 2013;136:3106-3118
7. Marelli C, Guissart C, Hubsch C, et al. Mini-exome coupled to read-depth based copy number variation analysis in patients with inherited ataxias. Hum Mutat 2016;37:1340-1353
8. Precone V, Del Monaco V, Esposito MV, et al. Cracking the Code of Human Diseases Using Next-Generation Sequencing: Applications, Challenges, and Perspectives. Biomed Res Int 2015;2015:161648
9. Lecocq C, Charles P, Azulay JP, et al. Delayed-onset Friedreich's ataxia revisited. Mov Disord 2016;31:62-69
10. Reetz K, Dogan I, Costa AS, et al. Biological and clinical characteristics of the European Friedreich's Ataxia Consortium for Translational Studies (EFACTS) cohort: a cross-sectional analysis of baseline data. Lancet Neurol 2015;14:174-182
11. Mignot C, Apartis E, Durr A, et al. Phenotypic variability in ARCA2 and identification of a core ataxic phenotype with slow progression. Orphanet J Rare Dis 2013;8:173
12. Bonifert T, Karle KN, Tonagel F, et al. Pure and syndromic optic atrophy explained by deep intronic OPA1 mutations and an intralocus modifier. Brain 2014;137:2164-2177
13. Synofzik M, Gonzalez MA, Lourenco CM, et al. PNPLA6 mutations cause Boucher-Neuhauser and Gordon Holmes syndromes as part of a broad neurodegenerative spectrum. Brain 2014;137:69-77
14. Shi Y, Wang J, Li JD, et al. Identification of CHIP as a novel causative gene for autosomal recessive cerebellar ataxia. PLoS One 2013;8:e81884

15. Synofzik M, Smets K, Mallaret M, et al. SYNE1 ataxia is a common recessive ataxia with major non-cerebellar features: a large multi-centre study. Brain 2016;139:1378-1393
16. Klockgether T. Sporadic ataxia with adult onset: classification and diagnostic criteria. Lancet Neurol 2010;9:94-104
17. Durr A. Autosomal dominant cerebellar ataxias: polyglutamine expansions and beyond. Lancet Neurol 2010;9:885-894
18. Stessman HA, Bernier R, Eichler EE. A genotype-first approach to defining the subtypes of a complex disease. Cell 2014;156:872-877
19. Mefford HC. Genotype to phenotype-discovery and characterization of novel genomic disorders in a "genotype-first" era. Genet Med 2009;11:836-842
20. Uliana V, Percesepe A. Reverse phenotyping comes of age. Mol Genet Metab 2016;118:230-231
21. Cooper GM. Parlez-vous VUS? Genome Res 2015;25:1423-1426
22. Mallaret M, Synofzik M, Lee J, et al. The tumour suppressor gene WWOX is mutated in autosomal recessive cerebellar ataxia with epilepsy and mental retardation. Brain 2014;137:411-419
23. Assoum M, Salih MA, Drouot N, et al. Rundataxin, a novel protein with RUN and diacylglycerol binding domains, is mutant in a new recessive ataxia. Brain 2010;133:2439-2447
24. Mignarri A, Gallus GN, Dotti MT, Federico A. A suspicion index for early diagnosis and treatment of cerebrotendinous xanthomatosis. J Inherit Metab Dis 2014;37:421-429
25. Hakonen AH, Isohanni P, Paetau A, Herva R, Suomalainen A, Lonnqvist T. Recessive Twinkle mutations in early onset encephalopathy with mtDNA depletion. Brain 2007;130:3032-3040
26. Semigran HL, Levine DM, Nundy S, Mehrotra A. Comparison of physician and computer diagnostic accuracy. JAMA Intern Med 2016;176:1860-1861
27. Lumaka A, Cosemans N, Lulebo Mampasi A, et al. Facial dysmorphism is influenced by ethnic background of the patient and of the evaluator. Clin Genet 2016:10.1111/cge.12948

Figure legends

Figure 1: Algorithm outline.

Figure 2: Average sensitivity and specificity for all patients related to the window size, defined as the position of the entity in the ranking list.

Figure 3: Relationship between the total number of features (x-axis), the number of specific features (y-axis), and the required window size, defined as the position of the entity in the ranking list, to reach 90% sensitivity (diameter of bubble increases as performance decreases). The figure shows the lack of association between performance of the algorithm and the number of features. Pearson correlation coefficient for total number of features vs window size: r=0.052, *P*=0.730; specific number of features vs window size: r=−0.033, *P*=0.830.

Table 1: Convention for notation and scoring for frequency and/or specificity for each category of signs and symptoms included in the algorithm.

Category	Clinical features	Neuro-imaging	Electro-myography	Age of onset	Rapid disease progression	Biomarkers
NK	0	0	0	0	0	0
0	-1	-1	-1	-2	-2	-2
L	1	1	1	2	2	2
H	3	3	3	6	6	6
LS	7	7	7	14	14	14
HS	9	9	9	18	18	18

0, No association; L, Low frequency (<50% of patients); LS, Low frequency (<50% of patients) and specific (<10% of entities); H, High frequency (≥50% of patients); HS, High frequency (≥50% of patients) and specific (<10% of entities); NK, extent of association not known.