ORIGINAL ARTICLE

From gestalt to gene: early predictive dysmorphic features of PMM2-CDG

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ABSTRACT

Introduction Phosphomannomutase-2 deficiency (PMM2-CDG) is associated with a recognisable facial pattern. There are no early severity predictors for this disorder and no phenotype–genotype correlation. We performed a detailed dysmorphology evaluation to describe facial gestalt and its changes over time, to train digital recognition facial analysis tools and to identify early severity predictors.

Methods Paediatric PMM2-CDG patients were evaluated and compared with controls. A computer-assisted recognition tool was trained. Through the evaluation of dysmorphic features (DFs), a simple categorisation was created and correlated with clinical and neurological scores, and neuroimaging.

Results Dysmorphology analysis of 31 patients (4–19 years of age) identified eight major DFs (strabismus, upslanted eyes, long fingers, lipodystrophy, wide mouth, inverted nipples, long philtrum and joint laxity) with predictive value using receiver operating characteristic (ROC) curveanalysis (p<0.001). Dysmorphology categorisation using lipodystrophy and inverted nipples was employed to divide patients into three groups that are correlated with global clinical and neurological scores, and neuroimaging (p=0.005, 0.003 and 0.002, respectively). After Face2Gene training, PMM2-CDG patients were correctly identified at different ages.

Conclusions PMM2-CDG patients' DFs are consistent and inform about clinical severity when no clear phenotype–genotype correlation is known. We propose a classification of DFs into major and minor with diagnostic risk implications. At present, Face2Gene is useful to suggest PMM2-CDG. Regarding the prognostic value of DFs, we elaborated a simple severity dysmorphology categorisation with predictive value, and we identified five major DFs associated with clinical severity. Both dysmorphology and digital analysis may help physicians to diagnose PMM2-CDG sooner.

INTRODUCTION

Phosphomannomutase-2 deficiency (PMM2-CDG) or Jaeken syndrome (MIM#601785), caused by pathogenic variants in the *PMM2* gene, is the most frequent congenital disorder of N-linked glycosylation.[1,2] Clinically, PMM2-CDG presents as a multisystem disorder (mainly development disability, cerebellar atrophy, failure to thrive, enteropathy, coagulopathy and liver dysfunction), fat pads and inverted nipples.[3,4] Over time, the phenotype expands to include retinopathy, osteopenia, peripheral neuropathy, hypergonadotropic hypogonadism and stroke-like episodes, among other pathologies.[2,3]

The classic phenotype is a severe multisystem disease with neonatal onset, while a milder phenotype is associated with a predominantly neurological manifestation.[5,6] The classic form is rather easier to identify but the milder, oligosymptomatic and atypical forms can be challenging to diagnose.[7–10]

Previous studies on dysmorphism in PMM2-CDG patients have not clearly characterised the dysmorphic findings to date.[3,4] These findings include strabismus, prominent forehead, large ears, thin upper lip, prominent jaw, and long and slender fingers and toes.[3,4,11] The most typical features of PMM2-CDG are an abnormal fat distribution and inverted nipples.[12,13] However, as in other dysmorphic conditions, these features may change with age.[13,14] Moreover, there is a clear benefit in characterising recognisable features over time as they may facilitate earlier and more precise diagnosis in such a pleomorphic disorder. In the era of next-generation sequencing and next-generation phenotyping, there remains a lack of detailed PMM2-CDG phenotypic description based on a quantitative approach comparing to normal measures. Extensive descriptions through automated facial analysis software such as Face2Gene (https://www.face2gene.com/) may be very helpful to clinicians.[15] It should be noted that pattern recognition through facial images in inborn errors of metabolism is a growing field.[16–18]

Many pathogenic variants have been identified in the *PMM2* gene, but their correlation with phenotype severity is controversial.[19,20] No biological predictors have been identified.

In this study of a large sample of PMM2-CDG patients, we describe dysmorphic features (DFs) with the aim of facilitating diagnostic recognition by distinguishing between major and minor DFs, and of providing online free training tools to create recognisable facial patterns. We also explore qualitative and quantitative correlations between DFs and neurological, radiological and molecular findings to identify predictors of disease severity.

Patients and methods

We designed an ambispective, observational study that included PMM2-CDG patients followed in tertiary care hospitals in the Spanish CDG Network. Patients were recruited and evaluated from March 2015 to December 2017. Clinical, biological, radiological and molecular data were obtained from patient medical records.

Healthy controls included sex-matched and age-matched children of the same ethnicity, excluding children with any confirmed or suspicious neurological or genetic disease. Controls were recruited from June 2017 to December 2017 with open advertisement in nearby regular schools, high schools and universities and received informed consent.

DF classification

Thirty-two DFs were initially selected based on a priori team expertise and previous publications. Face and body measures were compared with reference values for age and gender from the Hall's Handbook of Normal Physical Measurements.[21] For qualitative analysis, the presence or absence of facial and extra-facial DFs was compared with a sample of healthy controls to assess the possibility of obtaining a morphology clinical score.

DFs presented by PMM2-CDG patients showing a prevalence >50% over the control group prevalence were classified a priori as *major DFs* for subsequent analysis. Conversely, DF with a statistically greater prevalence compared with controls that were not >50% were classified as *minor DFs*. To evaluate whether a number of major DFs or the total number of DFs could predict the probability of a patient having PMM2-CDG, a receiver operating characteristic (ROC) curve analysis was performed.

Automated image analysis

Face2Gene (FDNA., Boston Massachusetts, USA) was used as the tool for computer analysis based on pattern recognition of frontal photographs (https://face2gene.com).[18 22] The work was divided into three phases: (I) training of the algorithm within the tool, (II) assessment of distinctness of facial phenotypes and (III) study of facial phenotypes per age group.

Phase I

Initially, the Face2Gene CLINIC application did not assign PMM2-CDG to any of the patient facial photos as its mathematical algorithm was not yet trained to discriminate this condition. Facial photographs were uploaded to allow training with PMM2-CDG patients. Subsequently, a new group of PMM2-CDG patient images were used to test this tool.

Phase II

In order to assess how specific the facial phenotypes of PMM2-related disorder were, two cohorts of controls were selected: healthy individuals and Angelman syndrome patients. Angelman syndrome was chosen because it was the most frequently suggested syndrome by the tool. Face2Gene RESEARCH allows in silico experiments with user-defined cohorts, and the results are expressed in a confusion matrix for the original sample.[22] The area under the curve (AUC) of the ROC curve was computed. In order to measure the statistical significance, p value random permutation tests and train models were calculated 1000 times.

Phase III

The ability of the tool to recognise physical characteristics at different ages was also tested. All of the photos from children evaluated in this study were divided into three age groups: 0–5, 6–11 and 12–18 years. Through the RESEARCH application, differences between the groups, healthy controls and Prader-Willi syndrome controls from the same age group were examined. Prader-Willi syndrome was chosen as it was the most frequently suggested syndrome by the tool for younger children. The AUC of the ROC curves and p values were calculated. In addition, differentiated composite images for the three age groups were created.

Clinical and radiological evaluation

International Cooperative Ataxia Rating Scale (ICARS) involves a 100-point rating scale in which higher scores denote more evident clinical abnormalities.[23] ICARS includes subscores for posture and gait (0–34), kinetic functions (0–52), speech abnormalities (0–8) and oculomotor function (0–6). For qualitative correlations, ICARS results were stratified as mild (0–25), moderate (26–50) and severe (51–100).

All patients were assessed using the Nijmegen Paediatric CDG Rating Scale (NPCRS) to evaluate multisystem involvement using total score and severity categories for statistical analysis.[24]

To evaluate MRI cerebellar images, 2D analysis was performed using the midsagittal vermis relative diameter (MVRD). Lower percentages denote more evident cerebellar atrophy. This parameter has been validated in children with PMM2-CDG by our group.[25 26]

For principal correlation analysis, ICARS and NPCRS but not MVRD were used. Available MRIs were acquired at different ages and, due to the progression of atrophy during childhood,[23 26] were not comparable.

Molecular studies

Molecular studies were performed in CEDEM-UAM, Madrid. Pathogenic variant analysis was performed by genomic DNA analysis, both in patient and parent samples, to ensure that changes were on different alleles and to rule out the presence of large genomic rearrangements. In certain cases, the effect on splicing was examined by cDNA profile analysis. Primers used for cDNA and genomic DNA amplification were designed using the ENSEMBL database (http://www.ensembl.org/index.html, ENSG00000140650) and the GenBank accession number NM_000303.2.

Severity of different pathogenic variants was categorised depending on the potential protein alteration effects and the presence of residual enzymatic activity calculated in vitro for the recombinant protein.[27 28]

Concerning potential protein alteration effects, pathogenic variants were classified as mild–moderate (dimerisation (D) or folding with residual activity (FW)) vs severe (catalytic (C), folding without residual activity (FWO) or no protein (NP)). Compound heterozygous mutations were classified as mild (D+D, D+FW, and FW +FW), moderate (D+FWO, D+C, C+FW, FW +FWO, FW +NP), or severe (FWO +NP, C+NP, C+NO, NP +FWO, C+FWO, FWO +FWO, C+C, NP +NP).

The presence of residual enzymatic activity, calculated in vitro for recombinant proteins, was classified as mild–moderate (residual activity) versus severe (no residual activity).[27 28] Patients with uniparental disomy were excluded from this analysis.

Dysmorphology categorisation

To define a dysmorphology categorisation method, major DFs exhibiting good correlation with clinical and neuroimaging results were selected and evaluated in every patient. According to the presence of one or more DFs or the absence of those selected DFs, patients were divided into three phenotypic groups: mild,

moderate and severe. Non-specific DFs, such as joint laxity, strabismus, high arched palate and pes planus, were excluded from this analysis as they are very prevalent in neuropaediatric patients regardless of the underlying cause.
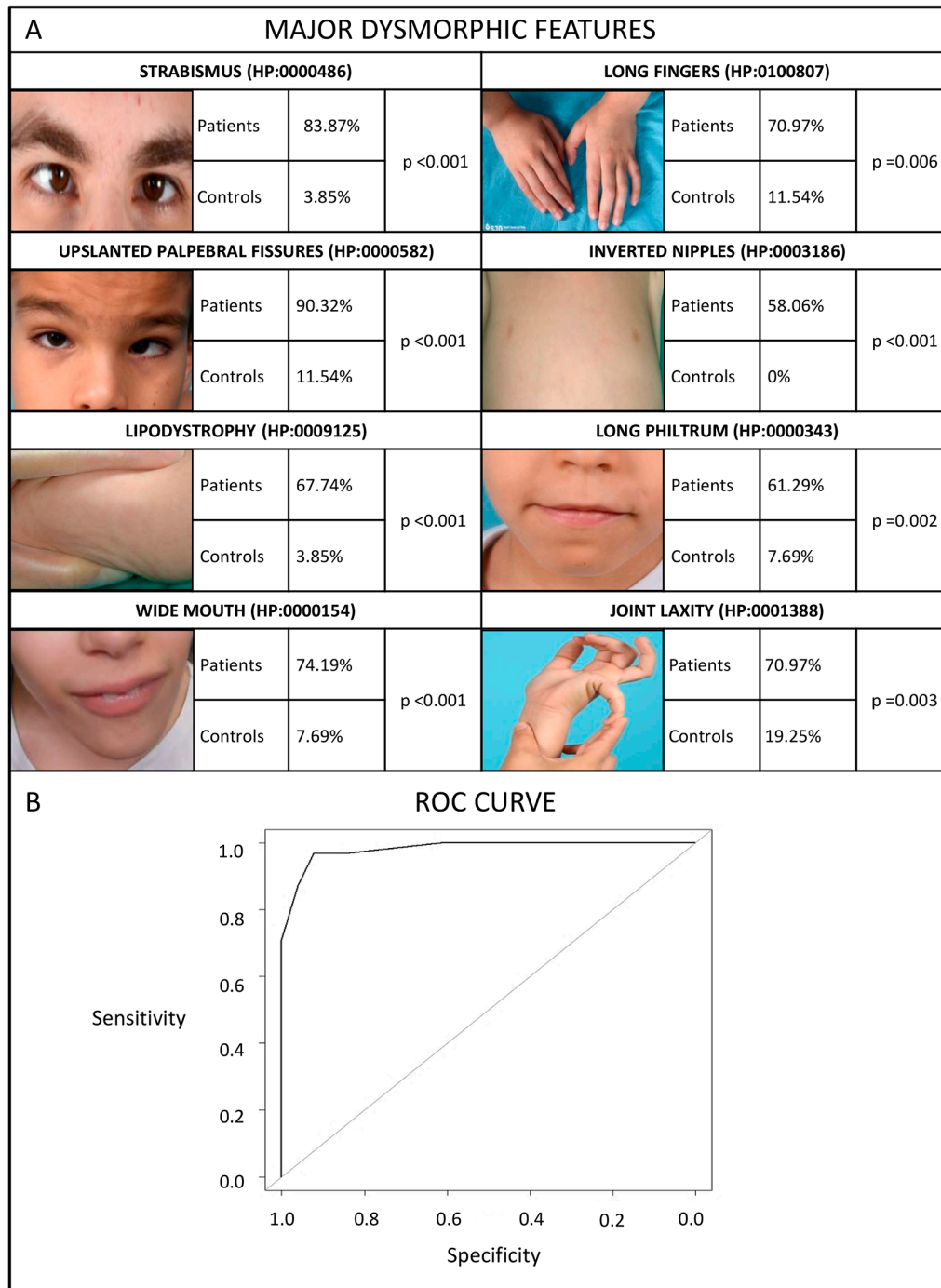
Statistical analysis

Statistical analysis was performed using SPSS V.21.0 software (IBM) and R V.3.3.2 (R Foundation for Statistical Computing, Vienna, Austria). All statistical tests were two-sided, and p values <0.05 were considered to be significant. Kruskal-Wallis test was used to assess distribution differences in neurological scores (MVRD, ICARS and NPCRS) among the three phenotypic groups. Mann-Whitney test was used to examine neurological scores and age according to the presence or absence of DFs. Principal component analysis (PCA) was used to evaluate the predictive power of the most common clinical features of PMM2-CDG with respect to disease severity.
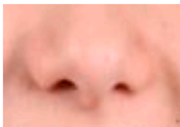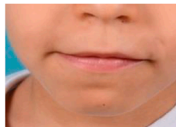
RESULTS

Thirty-one PMM2-CDG patients (13 females, 18 males; mean age: 11.5 years; SD: 4.7 years; range: 4–19) were available for

A MAJOR DYSMORPHIC FEATURES

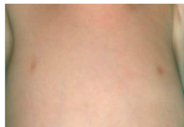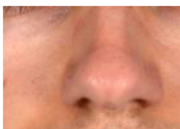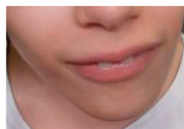| STRABISMUS (HP:0000486)			
	Patients	83.87%	p <0.001
	Controls	3.85%	

LONG FINGERS (HP:0100807)			
	Patients	70.97%	p =0.006
	Controls	11.54%	

UPSLANTED PALPEBRAL FISSURES (HP:0000582)			
	Patients	90.32%	p <0.001
	Controls	11.54%	

INVERTED NIPPLES (HP:0003186)			
	Patients	58.06%	p <0.001
	Controls	0%	

LIPODYSTROPHY (HP:0009125)			
	Patients	67.74%	p <0.001
	Controls	3.85%	

LONG PHILTRUM (HP:0000343)			
	Patients	61.29%	p =0.002
	Controls	7.69%	

WIDE MOUTH (HP:0000154)			
	Patients	74.19%	p <0.001
	Controls	7.69%	

JOINT LAXITY (HP:0001388)			
	Patients	70.97%	p =0.003
	Controls	19.25%	

B ROC CURVE

Figure 1 Prevalence of major dysmorphic features (DFs) in phosphomannomutase-2 deficiency (PMM2-CDG) patients and controls and receiver operating characteristic (ROC) curve analysis. (A) Prevalence of each DF was significantly greater in patients compared with controls (in all cases p<0.05). (B) ROC curve analysis resulted in an area under the curve (AUC) of 0.983 (p<0.001), demonstrating its accuracy to discriminate patients from controls. For instance, considering three or more major DFs in a subject, ROC curve analysis may lead to a sensitivity of 96.77% and a specificity of 92.31%.

Figure 2 Dysmorphology changes over time. For every dysmorphic feature, a picture is included and the significant inflexion point age is detailed.

quantitative measures. Online supplementary table S1 summarises clinical features, including anthropometric, dysmorphic, clinical, radiological and molecular findings of all patients, and online supplementary figure S1 contains representative images of PMM2-CDG facial features.

Twenty-six epidemiologically comparable healthy controls were included (12 females, 14 males; mean age 9 years; SD 3.8 years; range: 3–18).

Facial and extrafacial dysmorphology description: frequencies, DF classification, comparison to controls and changes with age

The most frequent DFs in order of prevalence in the PMM-CDG group were as follows: upslanted palpebral fissures (28/31, 90.3%), high arched palate (27/31, 87.1%), strabismus (26/31, 83.7%), anteverted nares (23/31, 74.2%), long fingers (22/31, 71.0%), wide mouth (23/31, 74.2%), lipodystrophy (21/31, 67.7%), hypertelorism (21/31, 67.7%), joint laxity (20/31, 64.5%), long philtrum (19/31, 61.3%), inverted nipples (18/31, 58.1%), thin upper lip (18/31, 58.1%), prominent forehead (17/31, 54.8%), long face (17/31, 54.8%) and pes planus (17/31, 54.8%).

The prevalence of every single DF was significantly greater in patients compared with controls (figure 1A). These differences were also significant when results were corrected for age (p<0.001). Out of the 32 initially selected DFs, 8 fulfilled the major DF criteria (prevalence >50% over control group prevalence): strabismus, upslanted palpebral fissures, long fingers, lipodystrophy, wide mouth, inverted nipples, long philtrum and joint laxity.

The average number of major DFs exhibited by patients (mean: 4.4) was higher than in controls (mean: 0.5) (p<0.001). The average number of total DFs was higher in PMM2-CDG patients (mean: 17.3) than in controls (mean: 2.2) (p<0.001). All controls possessed six or fewer DFs, and all patients had seven or more DFs.

In order to evaluate the merit of DFs as a predictive model of PMM2-CDG diagnosis, a ROC curve analysis resulted in an AUC of 0.983 (p<0.001), showing its accuracy to discriminate patients from controls. For instance, considering three or more major DFs in a subject, the ROC curve may lead to a sensitivity of 96.8% and a specificity of 92.3% (figure 1B).

Concerning the analysis of changes in DFs over time, age effect was evaluated for each DF. Major DFs and those minor DFs that change with age are represented in figures 1 and 2. Prominent jaw and prominent nose were more representative at older ages (p=0.004 and 0.005, respectively). At earlier ages, there was a greater presence of inverted nipples (p=0.009), epicanthal folds (p=0.008), anteverted nares (p=0.005), thin upper lip (p=0.024) and retrognathia (p<0.001). For these DFs, an age-related inflexion point could be identified: over time, the presence of retrognathia decreases, and the presence of prominent jaw increases. Also, the anteveres nares observed in certain patients at early ages lead to a prominent nose in older children.
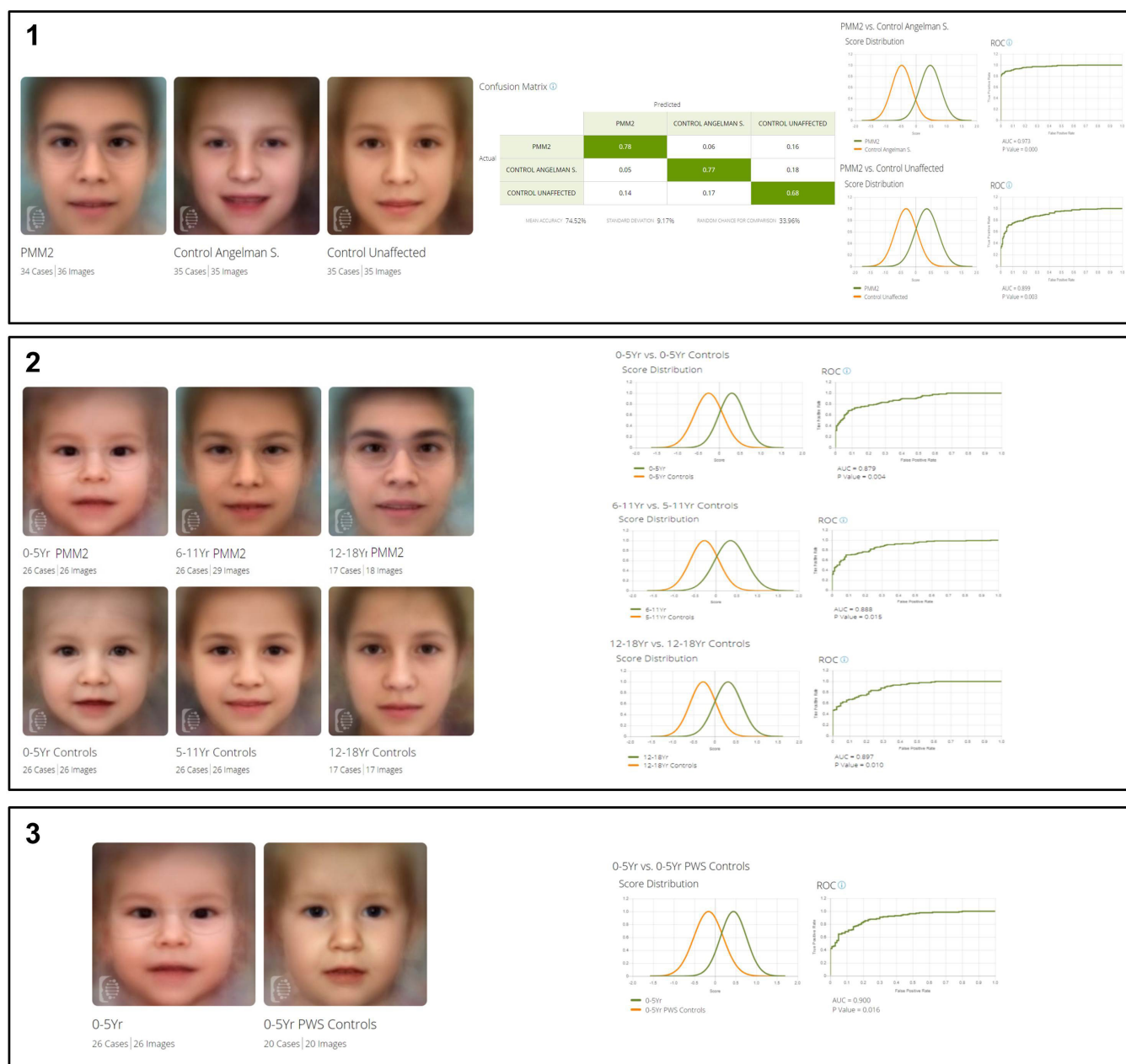
Computer facial recognition analysis and proposed gestalt picture
Phase I
The trained algorithm correctly identified 31 facial photographs of patients used for training. Forty-one photographs of new patients with a confirmed diagnosis of PMM2-CDG were added. In all cases, PMM2-CDG appeared as one of the top 10 syndrome matches offered by the tool.

Phase II
The Face2Gene RESEARCH application for facial phenotypic analysis was used to perform multiclass and binary comparisons and to create ROC curves showing that a recognisable facial pattern in PMM2-CDG was both distinctive from healthy controls with an AUC of 0.97 (p<0.001) and different from Angelman syndrome controls with an AUC of 0.89 (p=0.003) (figure 3A).

Figure 3 Face2Gene facial recognition analysis of patients with phosphomannomutase-2 deficiency (PMM2-CDG) versus controls. (A) Composite photos computed from images of PMM2-CDG patients, Angelman syndrome patients and unaffected controls. Multiclass and binary comparisons of cross-validation experiments of 10 random splits for PMM2-CDG patients, Angelman syndrome patients and unaffected controls. Results are displayed as confusion matrix, score distribution and receiver operating characteristic (ROC) curves, respectively. (B) Composite photos computed from age-matched images of PMM2-CDG patients and unaffected controls. Results are displayed as score distribution and ROC curves. (C) Composite photos computed from images of 0–5-year-old PMM2-CDG patients and Prader-Willi patient controls. Results are displayed as confusion matrix, score distribution and ROC curves, respectively.
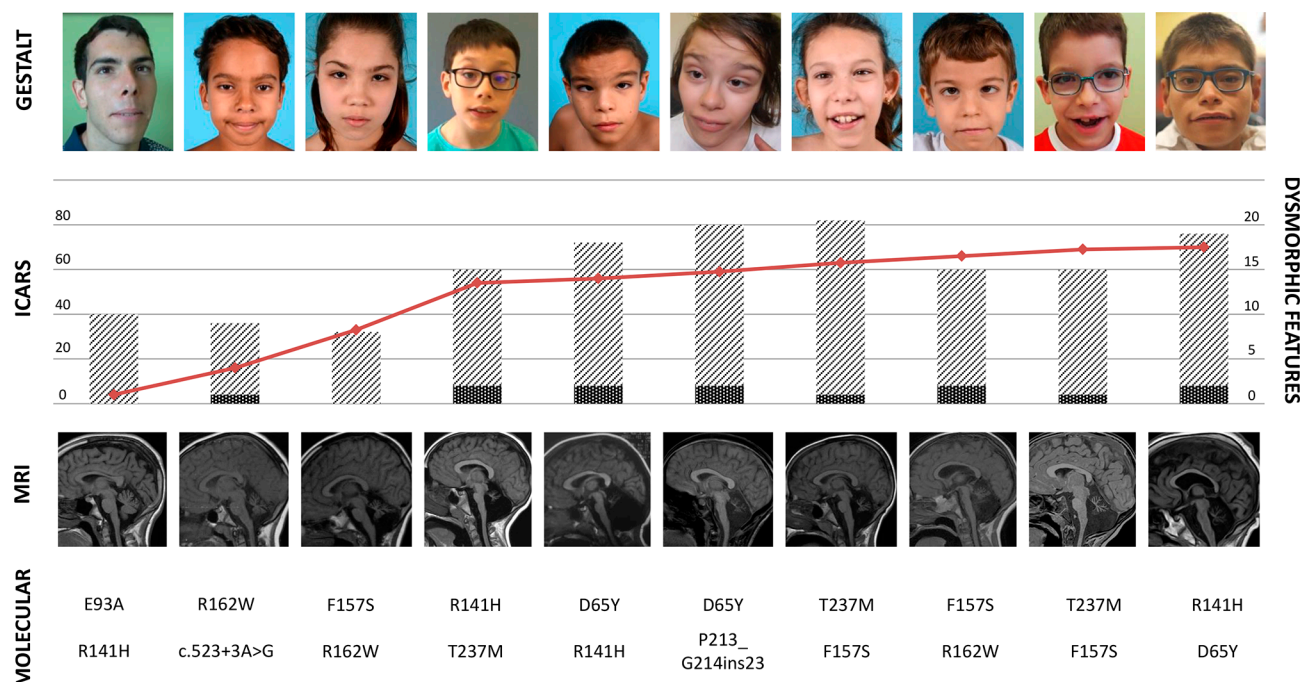
Phase III

Three different age groups were compared with aged-matched healthy controls and aged-matched Prader-Willi patient photos. The AUC was >0.90 for all comparisons, suggesting a high level of difference between the tested groups (figure 3B, C).

Lipodystrophy, inverted nipples, joint laxity and long fingers (not fulfilling arachnodactyly criteria) were the most common extrafacial DFs in PMM2-CDG patients. The gestalt facial pattern based on the qualitative and quantitative dysmorphology approach following a systematic clinical examination includes long face, strabismus, hypertelorism, upslanted palpebral fissures, short nose, anteverted nares, long and smooth philtrum, a thin vermillion of the upper lip, wide mouth, high-arched palate, retrognathia or prominent jaw, and variable dysplastic and/or large ears.

Correlation between DFs, neurological involvement and the prediction model

Cerebellar atrophy was more evident in those with higher ICARS. Furthermore, these subjects exhibited clearer DFs (figure 4).

Figure 4 Gestalt, dysmorphic features, ICARS, cerebellar atrophy and molecular findings in phosphomannomutase-2 deficiency (PMM2-CDG) patients. Patients who exhibited increased numbers of Total dysmorphic features presented with a more severe cerebellar syndrome and higher ICARS, as well as greater cerebellar atrophy on MRI. In bars, the presence of inverted nipples and/or lipodystrophy is detailed at the bottom with dots.

Through Mann-Whitney comparison tests, the presence of three major DFs (strabismus, inverted nipples and lipodystrophy) significantly correlated with both ICARS (p=0.005, 0.002 and 0.02, respectively) and NPCRS assessments (p=0.002, 0.007 and <0.001, respectively).

With respect to neuroimaging, the presence of five DFs (four major DF—strabismus, inverted nipples, lipodystrophy and long fingers—and one minor DF—prominent jaw) exhibited a significant inverse relationship with MVRD (p=0.007, 0.003, 0.002, 0.005 and 0.01, respectively).

Inverted nipples and lipodystrophy were selected to define the dysmorphology categorizations. Fifteen PMM2-CDG patients presenting both were included in the 'severe group'. Nine PMM2-CDG patients showing one of these DFs were included in the 'moderate group'. Finally, seven patients with neither lipodystrophy nor inverted nipples were sorted into the 'mild group' (figure 4).

Statistical comparisons of the three dysmorphology severity groups and their clinical assessments and neuroimaging revealed significant correlations (figure 5A– C).

Genotype–phenotype analysis

The distribution of patients based on potential protein alterations of their molecular findings was as follows: 1 severe, 17 moderate, 1 mild and 11 unknown due to lack of information regarding at least one of their pathogenic variants.

Categorisation of patients based on molecular findings and residual enzymatic activity (online supplementary table S1) did not reveal stratification of the samples by differing severity as almost all patients possessed both one severe pathogenic variant and one mild pathogenic variant.

Statistical analysis of dysmorphological, clinical and neuroradiological features did not to reveal any statistically significant associations with the molecular severity classification.

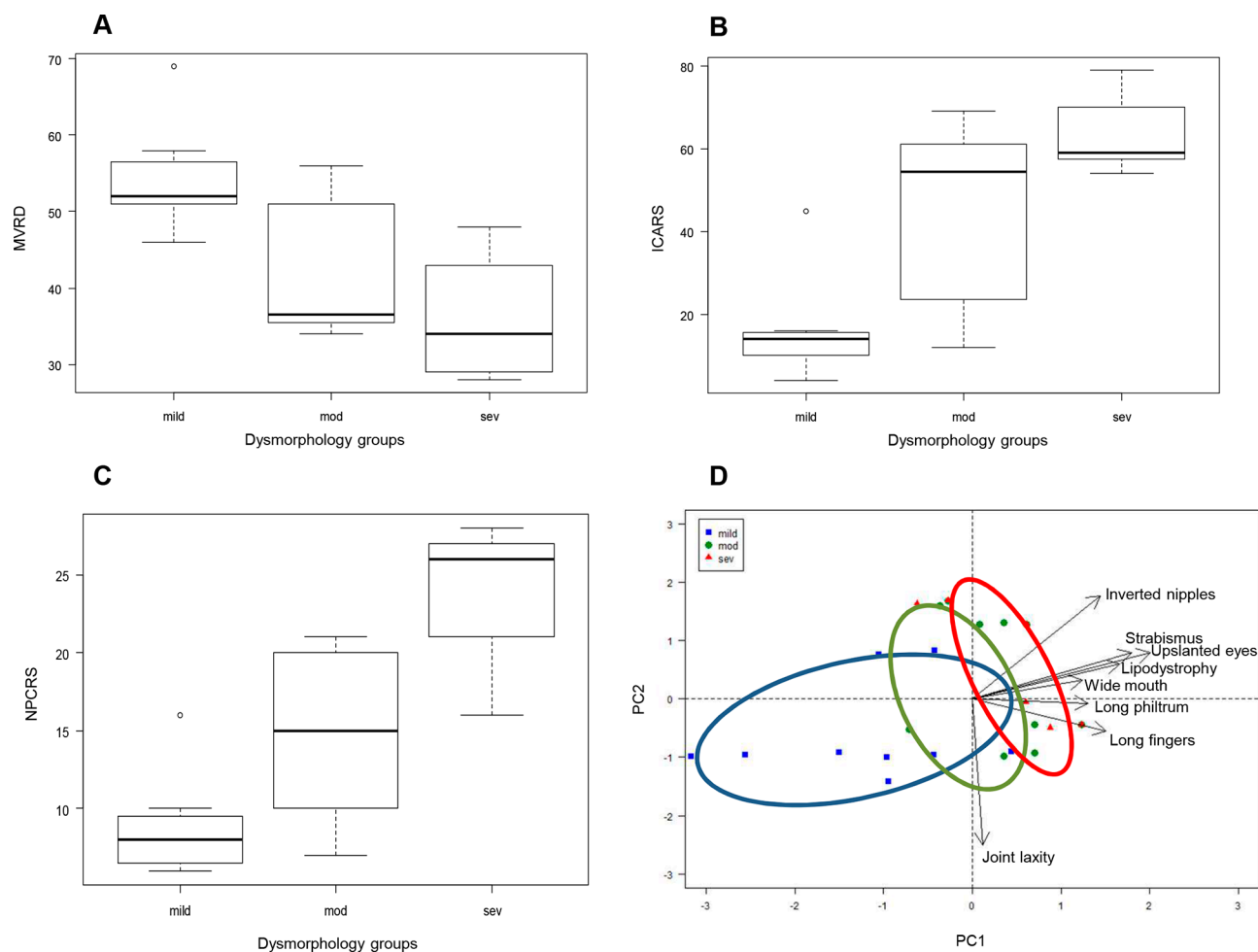### Identifying factors with severity predictive value

To evaluate the predictive power of major DFs in PMM2-CDG with regard to clinical severity, an initial PCA with varimax rotation created two composite variables (or principal components (PCs)) from the eight original major DFs using the tetrachoric correlation matrix between them as they were binary variables. Using the screen test, the set of eight composite variables was reduced to two. PCA of the data set is visualised as a biplot of PC1 and PC2, which explained 34% and 27%, respectively, of the variance in the data set (figure 5D, online supplementary table S2). A differential distribution of PMM2-CDG patients can be observed in the biplot. The major DF vectors with direction and sense point towards the localisation of more severe patients and are closely associated, likely explaining the largest variance in the data set and could be considered disease severity predictors. These are five of the eight major DFs: strabismus, lipodystrophy, upslanted palpebral fissures, inverted nipples and wide mouth.

DISCUSSION

This report describes the first and largest cohort of PMM2-CDG patients from the point of view of next generation phenotyping, comprising computer facial recognition analysis association with the study of the diagnostic and prognostic value of DFs.

This condition was first described in 1980 by Jaeken et al.[29] Inverted nipples and lipodystrophy were later described,[30 31] and additional DFs have been subsequently added to the literature.[5 6 32 33] Among these DFs, the most frequently reported facial features are dysplastic ears, high forehead, triangular face and thin upper lip, but these characteristics have never been related to age or clinical severity. Although there is great diversity in the phenotypic spectrum of PMM2-CDG patients,[9 10 19 20] differences in DF prevalence between patients and controls were statistically significant.

Figure 5 Dysmorphology and neurological correlations in phosphomannomutase-2 deficiency (PMM2-CDG) patients. (A) Box plot representing midsagittal vermis relative diameter (MVRD) from different phenotypic groups (mean: mild 54.6%, moderate 42.0%, severe 36.5%) (p=0.002) showing lower MVRD in those with more severe dysmorphic phenotypes. (B) Box plot representing ICARS from different phenotypic groups (mean: mild 16.3, moderate 44.9, severe 63.9) (p=0.003) showing higher ICARS in those with more severe dysmorphic phenotypes. (C) Box plot representing Nijmegen Paediatric CDG Rating Scale (NPCRS) from different phenotypic groups (mean: mild 8.9, moderate 14.9, severe 24.1) (p=0.005) showing higher NPCRS in those with more severe dysmorphic phenotypes. (D) Visualisation of initial principal component analysis (PCA) where biplot is, according to NPCRS categorisation, divided into three groups. Each dot represents a patient with PMM2-CDG and is coloured according to assigned disease severity (black: mild, green: moderate, red: severe). Each of the eight major DFs is represented by a vector. Length and direction of the vectors indicate their contribution to the data set's variance, represented as PC1 (35% variance) and PC2 (23% variance). PC1 seems to differentiate patients according to severity, and PC2 seems to be related to severity in minor degree, while also differentiating those who have joint laxity from those who do not. A division of patients is observed in three distributions, from minor to greater severity. Major dysmorphic feature (DF) vectors pointing towards the location of more severe patients could be considered as disease severity predictors, namely inverted nipples, strabismus, ascending palpebral fissures, lipodystrophy and wide mouth.

The frequencies of DFs in our sample are similar to those reported.[34] Although lipodystrophy is a well-known early indicator of PMM2-CDG, its prevalence is scarcely detailed in previous papers. In our sample its frequency is elevated and closer to that reported in studies focusing on dermatological features of CDG.[35–37] Moreover, its change with time could be a source of bias.

Eight DFs, denoted here as major DFs, have been identified as being suggestive and particularly prevalent among patients compared with controls. Our study demonstrates that the increased incidence of DFs offers a high sensitivity and specificity to clinicians faced to a patient with a suspicion of PMM2-CDG. This is especially true when the patient presents at least three major DFs or at least seven total DFs.

To the best of our knowledge, how DFs in PMM2-CDG patients change over time has never been investigated. It is reported that strabismus and lipodystrophy can disappear over time (sometimes therapy is a bias, as happens with strabismus), but it seems to be a slow process, and most patients present these DFs still during.[13 14] We observed that a prominent jaw develops in place of retrognathia, which is more prevalent in infancy, and antevertes nares, observed in certain patients at early ages, seems to be linked to a prominent nose in older children. A coarse facial appearance, described in older patients,[10] may be due to the confluence of a prominent jaw and a wide mouth. Its prevalence increases with age in our cohort. Our study is not longitudinal in nature. Nevertheless, it is desirable to evaluate changes over time in these patients. Thus, we propose some age cut-offs to define when certain DFs are more or less frequent in PMM2-CDG.

New technologies of facial pattern recognition, such as Face-2Gene, are useful in a wide number of genetic conditions.[22] In PMM2-CDG, a highly variable and rare disease, facial DFs may be subtle, and the diagnosis requires awareness that relies on

sialotransferrin analysis by different techniques that may sometimes be normal.[2] Therefore, the utilisation of computer tools for new generation phenotyping is a priority.[38–40] Our work has permitted Face2Gene to be trained for discrimination and suggestion of PMM2-CDG at different ages even <1 year, even in the absence of multisystem involvement and supplementary Human Phenotype Ontology (HPO).

In view of both, the potential offered by DF analysis and the capability of informatised platforms of facial recognition to aid in clinical diagnosis, two different but synergistic approaches can be integrated into a single tool to improve diagnostic rates. These modalities still need to be further explored and the design of decision algorithms based on exhaustive HPO lists corresponding to all DFs and supplemented by automatic facial analysis could achieve next-generation phenotyping.

Initial suspicion based on clinical features, including dysmorphism, which primarily depends on doctors' skills and training, would lead to subsequent laboratory studies and a final diagnosis. If this suggestion comes automatically from a computerised tool, it will be less dependent on the observer's experience, increasing the rate of diagnostic success.

At present, there are no studies describing a clear correlation between genotype and phenotype for PMM2-CDG.[16 17 41] More than 100 pathogenic variants have been described to cause PMM2-CDG.[18 19 42] and most patients are double heterozygous. This hinders the possibility of establishing correlations between phenotype and genotype[18–21 41 43] despite some previous reports suggesting milder clinical phenotypes related to some specific mutations.[10] However, at the clinical level, there are validated scores to graduate systemic involvement (NPCRS), cerebellar syndrome (ICARS) and cerebellar atrophy (MVRD). In our cohort, there was a good correlation between DFs and clinical or neuroimaging analysis. However, we could not demonstrate a correlation between molecular severity classifications based on either protein alterations or residual activity and clinical aspects, such as DFs, cerebellar syndrome or neuroimaging findings. As a limitation to our work, we have no patients with the same pair of pathogenic variants, despite having one of the largest cohorts ever reported. Moreover, the presence of polymorphisms in other genes of the N-glycosylation pathway that could alter biological processes has not been considered (eg, in *ALG6*).[44]

Moreover, cerebellar atrophy is not always evident in the newborn but rather progresses during the first decade.[25] This lack of valuable prognostic signs in infants incites clinicians to look for early informative markers. We identified a correlation between measurable DFs and neurological phenotype of patients. Moreover, based on the presence or absence of two of the most common early DFs (lipodystrophy and inverted nipples), three phenotypic severity groups were defined that showed statistical correlation with different levels of clinical and neuroradiological manifestations.

Our study suggests that when a patient belongs to the severe group, presenting lipodystrophy and inverted nipples, neurological involvement will be greater. This needs to be confirmed through evaluation of longitudinal samples in future studies.

Interestingly, factorial analysis showed that five major DFs are associated with PMM2-CDG and are sufficient (either isolated or coexisting) for predicting disease severity The five major DFs proven to predict clinical severity were strabismus, lipodystrophy, upslanted palpebral fissures, long fingers, inverted nipples and wide mouth. These DFs can be easily evaluated by physicians at early ages, helping to provide early and relevant information to the family and to guide the management of patients.

Dysmorphology analyses should be interpreted in the context of a complete clinical picture of a patient and always considering that PMM2-CDG molecular confirmed patients may associate subtle dysmorphisms as shown in our sample. When PMM2-CDG is suspected, we suggest to determine whether the child possesses three or more major DFs or at least seven of all DFs, and to upload a frontal face picture to the Face2Gene CLINIC application. Additionally, once a PMM2-CDG diagnosis is confirmed, our results offer clinicians a dysmorphology categorisation based on two major DFs to guide neurological prognosis. However, dysmorphology information should never be a substitute for biochemical tests or clinical or radiological scores but may be helpful in very young patients in whom cerebellar atrophy is subtle so that cerebellar syndrome scores are not suitable. Currently, molecular studies remain the gold standard to make a final diagnosis, and clinical assessment remains essential.

In summary, on the basis of a dysmorphology analysis of a large sample of patients, we propose (1) a classification of DFs into major and minor with diagnostic risk implications, and (2) a facial pattern recognition analysis that trained the Face2Gene mathematical algorithm to discriminate PMM2-CDG at different ages. Moreover, we elaborated a simple severity dysmorphology categorisation with predictive value, and we identified five major DFs associated with clinical severity.

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