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SHORT REPORT

Next generation phenotyping in Emanuel and Pallister-Killian syndrome using computer-aided facial dysmorphology analysis of 2D photos

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High throughput approaches are continuously progressing and have become a major part of clinical diagnostics. Still, the critical process of detailed phenotyping and gathering clinical information has not changed much in the last decades. Forms of next generation phenotyping (NGP) are needed to increase further the value of any kind of genetic approaches, including timely consideration of (molecular) cytogenetics during the diagnostic quest. As NGP we used in this study the facial dysmorphology novel analysis (FDNA) technology to automatically identify facial phenotypes associated with Emanuel (ES) and Pallister-Killian Syndrome (PKS) from 2D facial photos. The comparison between ES or PKS and normal individuals expressed a full separation between the cohorts. Our results show that NPG is able to help in the clinic, and could reduce the time patients spend in diagnostic odyssey. It also helps to differentiate ES or PKS from each other and other patients with small supernumerary marker chromosomes, especially in countries with no access to more sophisticated genetic approaches apart from banding cytogenetics. Inclusion of more facial pictures of patient with sSMC, like isochromosome-18p-, cat-eye-syndrome or others may contribute to higher detection rates in future.

KEYWORDS

Emanuel syndrome; OMIM 609029, Face2Gene, facial dysmorphology novel analysis technology, Pallister-Killian syndrome; OMIM 601803, small supernumerary marker chromosome

1 | INTRODUCTION

Before genetics diagnostics can be initiated for a specific patient in a reasonable, responsible and cost-efficient way, human genetic counseling of the patient and/or his family has to be done. Thus, a clinician meeting a patient has to undertake the critical process of phenotyping and gathering clinical information.[1] Only based on this, decisions on further diagnostic procedures can be made, which should also consider gross chromosomal aberrations. The latter are easy to be diagnosed by approaches like banding and molecular cytogenetics. The mentioned approaches are straightforward and much less costly than, for example, molecular karyotyping or "next generation sequencing (NGS)".[2] Moreover, (molecular) cytogenetic analyses are single-cell-directed, and thus even low-level mosaics can be detected easily.[3] Good examples for such syndromes are the well-known trisomies (like Down syndrome)[4] and also carriers of small supernumerary marker chromosomes (sSMCs; for review see Ref. 5). Particularly prenatal cases with de novo sSMCs are not easy to correlate with a clinical outcome.[6] It has been established that substantial parts of sSMC lead to 5 major syndromes: isochromosome-18p [OMIM-614290], inverted-duplication-chromosome-15- [OMIM-608636], cat-eye- [OMIM-115470], Pallister-Killian- [OMIM-601803], and Emanuel syndrome also known as derivative-chromosome-22 syndrome [OMIM-609029]. Recently, a sixth syndrome was identified as der(13 or 21)t(13 or 21;18)-syndrome.[7]

Emanuel syndrome (ES) is caused by a balanced translocation of chromosomes 11 and 22 in one of the parents. As summarized in our

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previous work[6]: "This translocation, cytogenetically described as t(11;22)(q23;q11.2), is the most frequently occurring recurrent non-Robertsonian constitutional translocation in humans. (…) Carriers of the balanced constitutional t(11;22) translocation are phenotypically normal. However, there is a 2% to 6% risk of their having live-born progeny with ES. (…) In literature, more than 300 ES cases have been reported. The ES-causing der(22)t(11;22)(q23;q11.2) is detected in cytogenetic analysis as a centric minute-shaped sSMC. Clinical manifestations can be microcephaly, cardiac defects, cleft palate, renal malformations, anal atresia or stenosis, hip dysplasia, other skeletal complications, (…) and other manifestations. Life-threatening congenital malformations (…) may lead to early death (…). Because medical care has improved survival chances, adult ES cases are reported nowadays."[6]

The second sSMC related disorder covered in this paper is Pallister-Killian syndrome (PKS). "Clinical features of PKS include profound mental retardation, hypotonia, facial anomalies summarized as 'coarse' facial appearance, including prominent forehead with sparse anterior scalp hair, flat occiput, hypertelorism, (…) seizures, and (others). On average, patients can live up to two decades. (…) The problem of cytogenetic diagnostics in PKS is the presence of the sSMC in a low-grade mosaic in peripheral blood cells and also in amnion and chorion."[6]

Even though the previous outlined definitions seem to describe 2 easily recognizable phenotypes, clinical diagnosis is normally not straightforward. Patients may differ in phenotype due to ethnic or even family/individual-specific genetic properties. Thus, the ability to correctly phenotype and gather clinical information is as important as ever. Unfortunately, educating young MDs in nosology for recognizing rare genetic disorder became more and more unfashionable.[8] Also, the shear amount of nowadays recognizable syndromes is amplifying, as visible by growing numbers of such disorders being listed in OMIM (https://www.ncbi.nlm.nih.gov/omim). Thus, new approaches to phenotyping of patients are needed, which became available now by so called Next Generation Phenotyping (NGP).[9]

As a form of NGP, we used in this study the facial dysmorphology novel analysis (Face2Gene; FDNA inc., Boston, MA, USA) technology to automatically identify facial phenotypes associated with ES and PKS from 2D facial photos. The discriminatory power of such technology to recognize the phenotype of patients was recently and repeatedly described.[10–13]

2 | MATERIAL AND METHODS

2.1 | Photos

This study is part of the study on sSMC patients approved by the Ethical commission of the Friedrich Schiller Universität (FSU) Jena, German (1457-12/04). As bases for this study, only frontal images (facial gestalt) were used in a completely anonymized way from individuals having either a definite diagnosis or are considered as clinically normal. For this study we used:

- 81 frontal images of 59 children (81/59) with molecularly diagnosed ES
- 92/70 with molecularly diagnosed PKS
- 1000/973 unaffected children ("Normals")
- 1000/973 children affected with 1 of 100 other syndromes, characterized by dysmorphic features with a wide range of facial phenotypes ("Others").

Group ES was compared to groups PKS, "Normals" and "Others"; group PKS was compared to groups ES, "Normals" and "Others". Control group "Others" was used to prevent an inherent bias due to the different imaging conditions and to address any concerns that FDNA's technology simply detects images from dysmorphic patients vs normal controls, without insights into the specific nature of the ES or PKS phenotype.

3 | FDNA TECHNOLOGY AND STATISTICAL ANALYSIS

The FDNA technology combines facial recognition software with clinical knowledge (feature annotation and anthropometric measurements), enabling detection of dysmorphic features and recognizable patterns of human malformations from 2 dimensional facial photographs. The undertaken measurements form a descriptor of the face that can be readily compared to other such descriptors and graphically displayed as a mask (Figure 1A). In Figure 1B, 2 heat maps, showing most distinguishable regions of ES, is depicted.

The comparison and separation quality between all 4 groups was evaluated by measuring the Area Under the Curve (AUC) of the Receiver Operating Characteristic (ROC) curve as well as for cluster analysis, plotting the true positive rate as function of the false positive rate. To estimate the statistical power of FDNA technology distinguishing affected individuals from controls, a cross validation scheme was used. For the latter, the data is split randomly multiple times, into training sets and test sets. Each such set contained half of the samples, and this random process was repeated 10 times. In addition to these binary comparisons, an experiment testing the technology's ability to identify the correct syndrome in a multi-class manner was conducted (Table 1).

4 | RESULTS

Results for all comparisons done between the 4 groups are summarized in Table 2 as mean AUCs of the ROC curve. Aggregated AUC and ROC curves are provided in Figure S1 (Supporting information). The comparison between ES and "Normals" yielded an AUC of 1 (STD 0.00) and expressed a full separation between these 2 cohorts. A slightly lower mean AUC (0.99, STD 0.005) was obtained when comparing between ES and "Others". Similar results were obtained for PKS compared to "Others". Also, when comparing images of both syndromes (ES vs PKS) a high level of discrimination ability is shown (0.99, STD 0.018), although with a slightly higher STD. The cluster analysis shown in Figure 1C also indicates the levels of separation.

Applying the FDNA technology, the correct syndrome out of the 2 test cohorts and both control groups yielded a mean accuracy of

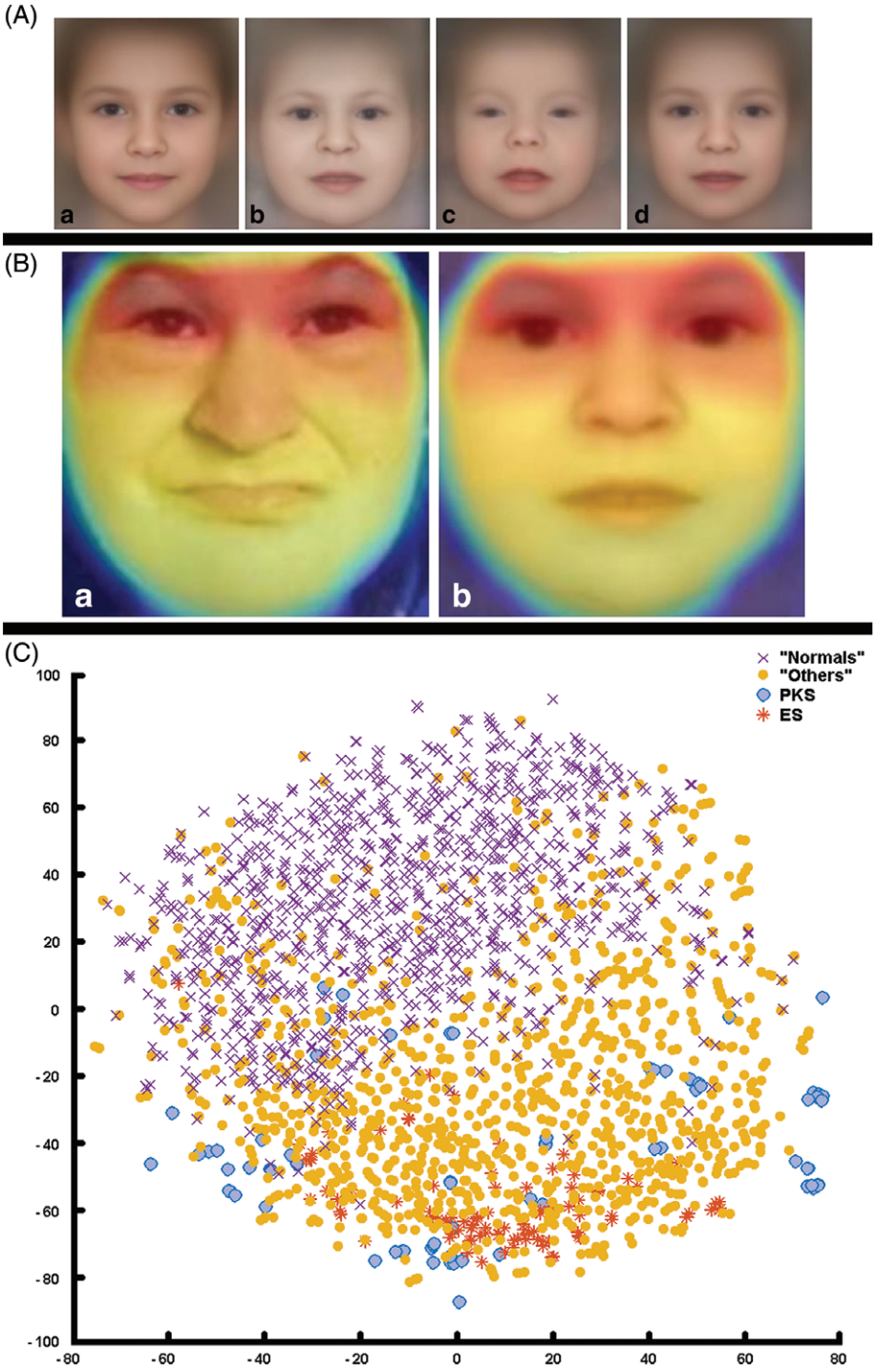

FIGURE 1 A, Syndrome mask—a robust template depicting an average appearance of (a) "Normals", (b) ES patients, (c) PKS patients, and (d) "Others". B, Heat maps displaying the most distinguishable regions of ES, drawn over a patient's facial image (a) and compared to the matched syndrome's mask as suggested by the FDNA technology analysis (b); the latter is based on 81 frontal images of 59 children with molecularly diagnosed ES. C, The cluster analysis indicates that there is some level of separation between the cohorts. The "Normals" aggregate at the upper, the "Others" at the bottom area, and so do the 2 test cohorts (ES and PKS), however, in a more scattered way

89.6% (STD = 3.3) and best split accuracy of 92.8%. This result is more than 3 times higher than the random chance of 25% (Table 1).

5 | DISCUSSION

Nowadays, a majority of clinical genetic publications is related to sequencing technologies and DNA-variant interpretation; much progress was achieved thereby during the past few years.[14–17] Still "old fashioned" approaches can and should not be forgotten and they should not be abandoned because of these fascinating new technologies.

TABLE 1 Multi-class confusion matrix. Multi-class confusion matrix: The highlighted diagonal represents here the True Positive (TP) values. The other values represent the errors. For instance, 62 images from the ES cohort where indeed classified as ES (TP value), 19 photos from "Others", were classified as ES (false positive) and 7 photos from the ES were classified as having any other syndrome but ES or PKS (false negative)

		Predicted			
Actual		ES	PKS	"Others"	"Normals"
	ES	*62*	0	19	0
	PKS	0	*61*	30	0
	"Others"	7	5	*898*	90
	"Normals"	0	0	74	*926*

TABLE 2 Summary of experiments. Summary of comparisons conducted and AUC values of ROC curve received, including standard deviation and best performing split AUC

	Number of individuals	Number of images	Mean AUC	STD AUC	Best split AUC
ES vs "Normals"	59 vs 973	81 vs 1000	1	0	1
ES vs "Others" controls	59 vs 936	81 vs 1000	0.99	0.005	0.99
PKS vs "Normals"	70 vs 973	92 vs 1000	1	0	1
PKS vs "Others"	70 vs 936	92 vs 1000	0.98	0.004	0.98
ES vs PKS	59 vs 70	81 vs 92	0.99	0.018	1

In this study, we sought to use the FDNA technology to automatically identify facial phenotypes associated with 2 of the yet 6 known sSMC syndromes, that is, ES and PKS. The here presented results support the evidence, that computer aided facial recognition can help in the clinic and could possibly reduce the time patients spend in the diagnostic odyssey in general.[10–13] Specifically, the FDNA technology may now also help to differentiate ES or PKS from each other and from other patients with sSMCs. This is especially important in countries with no access to more sophisticated genetic approaches apart from banding cytogenetics.[18] Clinicians involved in the diagnosis of a child affected by a genetic disorder and known to carry an sSMC in those countries, can now also check for PKS or ES by performing NGP. Also, clinicians in many developed countries are urged to reduce the use of expensive NGS testing. Thus, NGP could be the tool to indicate that a less expensive cytogenetic testing could end the diagnostic quest.

Thus, the FDNA technology is paramount for clinicians who like to rule out specific syndrome suggestions before embarking on more expensive molecular approaches. For sSMC, the training of the technology by including more facial images of patients with sSMC, like isochromosome-18p-, cat-eye-syndrome or others, like neocentric ones, is the authors next step in the near future.

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Conflict of interest

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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