Received: 2 May 2018 | Revised: 15 August 2018 | Accepted: 17 August 2018

DOI: 10.1002/ajmg.a.40630

WILEY AMERICAN JOURNAL OF medical genetics PART A

RESEARCH ARTICLE

Unmasking familial CPX by WES and identification of novel clinical signs

Bénédicte Demeer[1,2,3] | Nicole Revencu[1,4] | Raphael Helaers[1] | Bernard Devauchelle[3,5] | Geneviève François[6] | Bénédicte Bayet[7] | Miikka Vikkula[1]

[1]Human Molecular Genetics, de Duve Institute, University of Louvain, Brussels, Belgium

[2]Center for Human Genetics, CLAD nord de France, CHU Amiens-Picardie, Amiens, France

[3]EA CHIMERE, Université Picardie Jules Verne, Amiens, France

[4]Center for Human Genetics, Cliniques universitaires Saint-Luc, University of Louvain, Brussels, Belgium

[5]Department of Maxillofacial Surgery and Stomatology, centre de compétence fentes et malformations faciales MAFACE, CHU Amiens-Picardie, Amiens, France

[6]Department of Pediatrics, Cliniques universitaires Saint-Luc, University of Louvain, Brussels, Belgium

[7]Centre Labiopalatin, Division of Plastic Surgery, Cliniques universitaires Saint-Luc, University of Louvain, Brussels, Belgium

Correspondence
Miikka Vikkula, Human Molecular Genetics, de Duve Institute, University of Louvain, Brussels 1348, Belgium.
Email: miikka.vikkula@uclouvain.be

Funding information
Belgian Science Policy Office Interuniversity Attraction Poles (BELSPO-IAP), Grant/Award Number: IAP P7/43-BeMGI; BridgeIris RBC/2013-PFS-EH-11; Genomics Platform of Université catholique de Louvain for Next Generation Sequencing, la Biobanque de Picardie, Grant/Award Number: BB-0033-00017; Région Hauts-de-France; Fonds de la Recherche Scientifique - FNRS, Grant/Award Number: CdR: J.0080.16 ; Belgian Science Policy Office Interuniversity Attraction Poles, Grant/Award Number: RBC/2013-PFS-EH-11

Mutations in the T-Box transcription factor gene *TBX22* are found in X-linked Cleft Palate with or without Ankyloglossia syndrome (CPX syndrome). In addition to X-linked inheritance, ankyloglossia, present in the majority of CPX patients, is an important diagnostic marker, but it is frequently missed or unreported, as it is a "minor" feature. Other described anomalies include cleft lip, micro and/or hypodontia, and features of CHARGE syndrome. We conducted whole exome sequencing (WES) on 22 individuals from 17 "a priori" non-syndromic cleft lip and/or cleft palate (CL/P) families. We filtered the data for heterozygous pathogenic variants within a set of predefined candidate genes. Two canonical splice-site mutations were found in *TBX22*. Detailed rephenotyping of the two probands and their families unravelled orofacial features previously not associated with the CPX phenotypic spectrum: choanal atresia, Pierre-Robin sequence, and overgrowths on the posterior edge of the hard palate, on each side of the palatal midline. This study emphasizes the importance of WES analysis in familial CLP cases, combined with deep (reverse) phenotyping in "a priori" non-syndromic clefts.

KEYWORDS

choanal atresia, non-syndromic CLP, Pierre-Robin sequence, reverse phenotyping, *TBX22*, WES

1 | INTRODUCTION

Orofacial clefts (OFCs) –including cleft lip with or without cleft palate (CL/P) and cleft palate (CP)- are among the most common birth defects, with an approximate incidence of 1/700 live births, depending on ethnicity, gender, and cleft type (Mossey & Modell, 2012). Although not associated to a consequent rate of mortality in developed countries, they represent a significant lifelong morbidity, requiring multidisciplinary treatment and care. CL/P and CP have historically been considered as distinct disorders with separate etiologies based on the different embryological origin of the lip and the palate, and epidemiology (Fogh-Andersen, 1942; Fraser, 1955). However, both CL/P

and CP can occur within the same family, suggesting some etiological overlap (Kondo et al., 2002). Approximately 30% of clefts are syndromic, and in general follow a Mendelian-inheritance with an important variability in expressivity and incomplete penetrance. The remaining 70% are non-syndromic (ns) (isolated), meaning that they do not occur in combination with other developmental, structural or cognitive anomalies. Isolated clefts were recognized to have an inherited component by studies of Fogh-Andersen (Fogh-Andersen, 1942). Non-syndromic clefts, of which 80% are sporadic and 20% are multiplex (familial) cases (Mossey & Modell, 2012), are considered to have a complex etiology with multiple predisposing genetic variants acting in concert with environmental factors.

Given the complex nature of non-syndromic clefts, identification of causal genes has been challenging. Several genetic approaches, often complementary, have been used to identify candidate regions and genes for nsCLP: identification of chromosomal abnormalities or microdeletions in individual cases, linkage analyses in families, direct sequencing, and candidate-locus and genome-wide association studies. Animal models, especially murine models, because of the similar development of their palate compared to humans, have provided candidate genes, and greatly helped in the understanding of gene function and dissection of the molecular pathways that underlie facial development.

Important findings have also emanated from studies involving syndromic forms. Some of those genes have now also been implicated in non-syndromic forms, through variable penetrance perhaps due to the action of modifiers (Schliekelman & Slatkin, 2002). Strongest evidence was obtained for the *IRF6* gene, causing van der Woude (VWS) syndrome, since the majority of the patients with VWS have only minor additional signs, and 15% have isolated CLP (Desmyter et al., 2010; Kondo et al., 2002). The second VWS gene, *GRHL3*, also demonstrated non-complete penetrance for lip pits (Peyrard-Janvid et al., 2014). Mutations in *MSX1* are associated with syndromic clefting (with hypondontia) with approximately 2% of cases having non-syndromic CL/P; and *MSX1* polymorphisms are associated with non-syndromic CL in different populations. Several other syndromic genes, studied for their role in isolated CLP include *FGFR1*, *TP63*, *TBX22*, *PVRL1*, and *PTCH*, and may contribute to up to 10% of non-syndromic CLP (Basha et al., 2018; Stanier & Moore, 2004).

Clefts of the lip and palate show a range of phenotypic expression. Yet, they are generally defined as "qualitative traits" (i.e., affected vs. unaffected). We conducted whole exome sequencing (WES) on 22 individuals with "a priori" nsCLP drawn from multiplex families (n = 17) to identify causal nsCLP variants.

2 | MATERIALS AND METHODS

2.1 | Patient selection

Samples for this study were drawn from a large cohort of nsCL/P & nsCP families, recruited over the last 25 years in collaboration with the multi-disciplinary team at the Centre Labio-Palatin (Cliniques universitaires Saint-Luc, UCL, Brussels, Belgium). Familial cases were defined as at least two affected cases within the third degree of relatedness. In order to collect samples uniformly, a standardized questionnaire was set up for the physician, notably detailing family history and clinical phenotype. A total of 22 affected patients were included, with index patients only for 12 families and an additional second or third degree relative for five families. Affected subjects were diagnosed as having: cleft lip alone: nine, cleft lip with cleft palate: four, cleft palate alone: five or Pierre Robin sequence: four. All patients had a standard karyotype and many had also been tested negative for *22q11.2* deletion and *IRF6* mutation. All participants gave informed consent for the participation in the study. The ethical committee of the medical faculty at University of Louvain, Brussels, Belgium, approved the research protocol.

2.2 | Whole exome sequencing

DNA was extracted from blood samples using Wizard® Genomic DNA Purification Kit (Promega). WES was carried out by using 1 μg of genomic DNA per sample. Exomes were captured using a commercial enrichment kit (Agilent SureSelectXT Human All Exon KitV5, Santa Clara, CA 95051 USA) and enriched libraries were sequenced on an Illumina Hiseq2000. Generated reads were aligned to the human genome build hg19 using Burrows-Wheeler Aligner (BWA 0.7.15; see URLs). Data processing and variant calling were performed with Picard 1.107, Genome Analysis Toolkit 3.3 package (GATK) respectively. The generated variant (.vcf) files were imported into a database and further analysed using Highlander 14.10.3, an in-house bioinformatics framework for variant annotation, filtering, and visualization (see URLSs) (Helaers et al., under revision).

We considered as rare variants those with a frequency below 0.5% in public databases (ExAC, 1,000 Genomes and GO-NL (genome of the Netherlands) and in our in-house control database.

As "likely pathogenic "variants, we considered those with a high impact (canonical splice-site variants and those generating a premature termination codon: out-of-frame indels and nonsense variants), or a moderate impact (non synonymous-NS), as annotated by SnpEff 4.1 and a combined annotation dependent depletion (CADD) phred score > 10. Missense variants were considered as possibly pathogenic only if predicted to be possibly/probably damaging in at least three out of seven, seven variant in silico classifiers (Sift, Polyphen, LRT, Mutation taster, Mutation assessor, FATHMM, DEOGEN). We considered a list of 89 genes implicated in both syndromic and non-syndromic CLP (Stanier & Moore, 2004), nsCLP candidate genes (Leslie & Murray, 2013) and a selection of CLP candidate genes that we gathered from publications (Conte et al., 2016; Jugessur, Farlie, & Kilpatrick, 2009; Kousa, Mansour, Seada, Matoo, & Schutte, 2017; Lough, Byrd, Spitzer, & Williams, 2017) including recently confirmed CLP genes such as *ARHGAP29* (Leslie et al., 2012) and *GRHL3* (Peyrard-Janvid et al., 2014). Gene list is available upon request.

For all variants that satisfied our criteria, we performed co-segregation analysis on all the affected and non-affected family members that were available. Variants were PCR-amplified followed by sequencing on an ABI 3130XL genetic analyzer (Applied BioSystems).

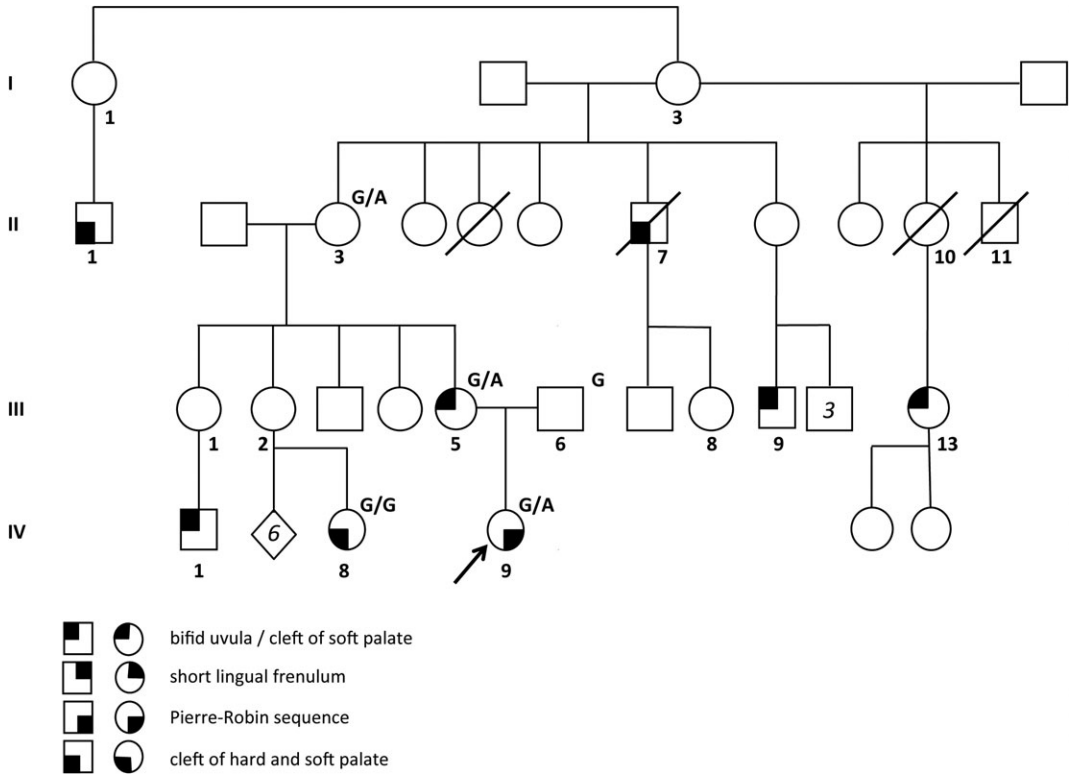

FIGURE 1 Pedigree of Family 1

3 | RESULTS

Using our filters and candidate gene list, we were able to point out two genetic variants in *TBX22* gene that we consider as causative of the respective phenotype (Family 1 and 2). Within the other 15 families, no causative variant was detected. A nonsense variation in *PDGFC* gene (c.660G > A, NM_16205, p.Trp220*, NP_057289) was found in the index case of one of the nsCP families, but did not co-segregate with the phenotype, and in one of the CL/P families, a missense variation was found in the *TULP4* gene (c.983A > G, NM_020245, p.Asn328Ser, NP_064630), but it did not co-segregate with the CL/P phenotype.

3.1 | Family 1

The proband was born at 40GW after an uneventful pregnancy, with birth weight of 2,910 kg. Diagnosis of a Pierre-Robin Sequence (PRS) due to the association of cleft palate, glossoptosis and discrete micrognathia was made. Cerebral, cardiac and renal ultrasounds were normal. Array-CGH (Agilent ISCA 184) and *IRF6*, *COL2A1*, *COL11A1*, *COL11A2* sequencing were negative. On maternal side, familial history was remarkable with her mother having a cleft of soft palate, one male cousin a bifid uvula, one female cousin a cleft of soft and hard palate, one maternal great-uncle a cleft of soft and hard palate and the son of a maternal great-aunt a cleft of soft palate (Figure 1). Following genetic results, reassessment of the familial history noted two additional, more distant relatives with cleft palate: one male (II-1) and one female (III-13) (Figure 1). According to family members, II-11 had died at birth because of "closed nares." Clinical examination confirmed that the proband and her mother had no ankyloglossia, and that the maternal grandmother was unaffected.

We identified a G-to-A transversion at the +1 position of Intron 2 (c.356 + 1G > A; NM_016954) of the *TBX22* gene in the proband. The mutation was heterozygous in the mother and the grandmother (Figure 1). It was absent in the female cousin (Figure 1 – IV-8), a phenocopy with cleft of soft and hard palate. Further mutation segregation could not be performed in the extended family. This sequence change alters the invariant GT splice consensus sequence of Exon 2 and is therefore predicted to disrupt normal splicing. It was not present in the single nucleotide polymorphism database (dbSNP), gnomAD or in control chromosomes (n = 1,128).

3.2 | Family 2

The proband is the second child of non-consanguineous parents, with a history of absent uvula on the maternal side. At birth, he presented with a short lingual frenulum that was cut during neonatal period. A diagnosis of submucous cleft palate was made at 2,5 years of age. Following the genetic results, clinical reassessment of the family detected that his mother, who had a short frenulum cut soon after birth, and two more times later on, had two bony overgrowths on the posterior edge of the hard palate, on each side of the palatal midline, in addition to the absent uvula (Figure 2b). His maternal grandmother had a short lingual frenulum cut at the age of 15 years, and a surgical repair of a unilateral choanal atresia at 31 years of age. She also had two bony palatal overgrowths, and an underdeveloped soft palate. His sister had two bony overgrowths similar to her mother and maternal grandmother (Figure 2b). His maternal-great grandfather was described to

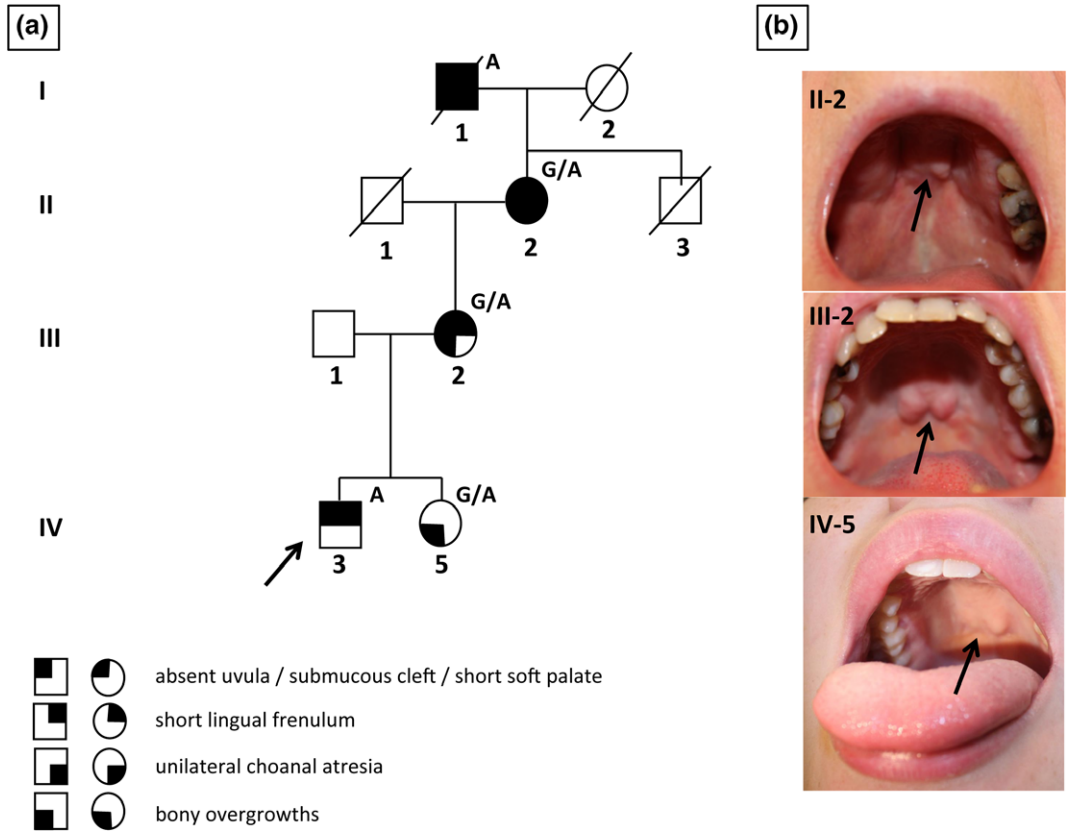

FIGURE 2 (A) Family 2 pedigree; (B) Endobuccal pictures of II-2, III-2, IV-5. Arrows show bony overgrowths

have an absent uvula, associated with a short lingual frenulum, nasal speech, and unilateral choanal atresia.

The proband and his maternal grandmother were found to have a G-to-A transversion at the +1 position of Intron 4 (c.633 + 1G > A; NM_016954) in *TBX22*. Co-segregation studies confirmed that his mother was carrier, and found that his sister, who only had the bony overgrowths, was a carrier too. The substitution affects the invariant GT splice consensus sequence. It is the same variant reported already twice in classic CPX, once in a Brazilian (Braybrook et al., 2001) and once in a white European family (Marcano et al., 2004; Pauws et al., 2013).

4 | DISCUSSION

We conducted a whole exome sequencing study on non-syndromic CLP families to search for rare causal variants. We identified two clear mutations in *TBX22* within the 17 non-syndromic CLP families screened. This result is consistent with the literature in favour of a 10% contribution of syndromic gene mutations to non-syndromic forms of clefting (Basha et al., 2018; Stanier & Moore, 2004).

Mutations in *TBX22* transcription factor are known to cause X-linked cleft palate (CPX) with ankyloglossia (MIM 303400) (Andreou et al., 2007; Braybrook et al., 2001; Braybrook et al., 2002; Chaabouni et al., 2005), as well as contributing to the general prevalence of orofacial clefting (Marcano et al., 2004; Suphapeetiporn, Tongkobpetch, Siriwan, & Shotelersuk, 2007). Patients with variable other anomalies including CL, micro and/or hypodontia (Kaewkhampa, Jotikasthira, Malaivijitnond, & Kantaputra, 2012; Kantaputra et al., 2011), and features of CHARGE syndrome (Pauws et al., 2013) have also been reported. TBX22 linked CPX phenotype most likely results from a loss of protein function (Andreou et al., 2007; Braybrook et al., 2001). Other T-Box related diseases, such as ulnar mammary syndrome and Holt-Oram syndrome, have been linked to loss-of-function mutations in *TBX3* (Bamshad et al., 1997) and *TBX5* (Basson et al., 1997), respectively.

Canonical splice-site donor mutations affecting Exon 4 (Braybrook et al., 2001; Marcano et al., 2004; Pauws et al., 2013) and Exon 6 (Braybrook et al., 2001) have been associated with classical CPX phenotype. The c.356 + 1G > A mutation identified in Family 1 is the first description of a canonical splice site donor mutation affecting Exon 2. Like the previously described causal splice-site mutations, we predict that this variant would result either in aberrant splicing of Exon 2, and/or in nonsense-mediated mRNA decay and haploinsufficiency. (Braybrook et al., 2001).

In classical CPX, a high inter- and intra-familial phenotypic variation exists, ranging from ankyloglossia alone to submucous cleft, bifid uvula or cleft of the secondary palate, all with or without ankyloglossia. With the X chromosomal localization, milder phenotype is usually exhibited by haploinsufficient females. Penetrance is complete in males and calculated to be 62% in females. Males frequently present cleft palate and ankyloglossia together (79%). While ankyloglossia is commonly seen (45%) in affected females, cleft palate (CP) is present as the sole feature in only 6% (Marcano et al., 2004).

The Family 1 proband had isolated PRS, which has not been associated with classical CPX phenotype or to a *TBX22* mutation (a targeted PRS screen) (Marcano et al., 2004). Moreover, the limited pedigree size, the presence of a female phenocopy with CP, and family history of CP with similar expression in males and females prevent to define an X-linked inheritance pattern in this family. The absence of ankyloglossia, a useful diagnostic feature, underscores the difficulty to distinguish familial non syndromic CP from familial CPX. This patient and family do not a priori evoke need for screening *TBX22*.

The *TBX22* mutation is the most likely cause of the cleft/bifid uvula in Family 1. As II-3, III-5, and IV-9 do not have ankyloglossia, the penetrance is lower than expected for CPX. Although minor signs could have been overlooked in the five unaffected obligate female carriers and four affected males, *TBX22* seems to have retained some "protective" activity or other (genetic) protective factors segregate in this family (Frischmeyer & Dietz, 1999). However IV-8 being a phenocopy and the unavailability of many affected members to be tested also raise the possibility of a more complicated cause or causes to run within the family.

In Family 2, two clinical orofacial signs, previously not associated with CPX, were described: choanal atresia and bony overgrowths on the posterior edge of the hard palate, on each side of the palatal midline. Choanal atresia, a common craniofacial defect characterized by obstruction of the posterior nasal passages, has an approximate incidence of one case in 8,000 live births. Although the pathogenesis is still unknown, it is generally believed to be caused by the persistence of the buccopharyngeal membranes or by failure of the oronasal membrane to rupture. Additional malformations have been previously reported in up to 49% of individuals with choanal atresia and stenosis (CA/S) (Burrow et al., 2009; Keller & Kacker, 2000). Craniofacial expression of human and murine *TBX22* correlates with the cleft palate and ankyloglossia observed in patients (Braybrook et al., 2002). *Tbx22*^{null} mice have multiple craniofacial defects, which are all consistent with its known expression pattern, including submucous cleft palate with a small minority developing overt clefts, reduced vomer and ankyloglossia. *Tbx22*^{null} mice were also found to have persistent oronasal membrane or, in some mice, a partial rupture, resulting in choanal atresia (Pauws et al., 2009). This first description of choanal atresia in two CPX patients expands the orofacial phenotype associated with TBX22. Interestingly, in Family 1, II-11 apparently died at birth because of "closed nares," which evokes bilateral choanal stenosis. Specific investigation will be required in the future to determine the variability and penetrance of choanal atresia in TBX22.

In *Tbx22*^{null} mice, Pauws and coworkers showed that Tbx22 is a major determinant for intramembranous bone formation in the posterior hard palate, rather than being involved in palatal shelf closure. The knock-out mice had marked reduction in bone formation of the vomer and the posterior hard palate on analysis of the craniofacial skeleton. Interestingly, three of the affected patients of Family 2 (Figure 2b) have two bony overgrowths on the posterior edge of the hard palate, on each side of the palatal midline, raising the question of an associated (reactive) abnormal ossification of the posterior palate. This has not been reported before, and it would be interesting to know if it is an overlooked or even specific *TBX22*-caused CPX sign. In Family 2, I-1 and II-2 have a comparable, severe CPX phenotype. It contrasts to the less severe phenotypic variation in generation III and IV implicating additional genetic and/or environmental factors to play a role.

Family recruitment in research studies is commonly based on the phenotype of the proband and a limited family history. This study provides support for "deep phenotyping" of probands and family members a posteriori. WES can guide the needed meticulous clinical evaluation regarding minor orofacial signs and endophenotypes, which may go unnoticed, as the least severe end of the spectrum of syndromic forms can mimic non-syndromic forms and a non-syndromic proband not always comes from a non-syndromic family. This study also highlights the contribution of "deep reverse phenotyping" to help refining clinical and molecular entities.

The recurrence risk is substantially enhanced for each a non-syndromic CLP patient with a causal mutation in a single-gene CLP-disorder, from the empiric 4–5% for non-syndromic CL/P and 3% for non-syndromic CP to the more personalized 25–50%.

Substantial evidence is accumulating on rare variants in syndrome-causing genes to account for Mendelian transmission of both syndromic and non-syndromic familial clefting. Thus, one should consider to use NGS in clinics. This should not only be limited to syndromic CLP cases, but also used in familial cases of a priori non-syndromic CLPs for genetic counselling indications. Two effective approaches could be used: targeted NGS (T-NGS) or WES. WES enables to investigate more genes throughout the exome, which might be relevant given the genetic heterogeneity of clefts. However, compared to T-NGS, it provides a weaker horizontal coverage, and it is more complex in terms of data analysis, less cost-effective, and raises the concern of incidental findings in genes not related to the phenotype (Christenhusz, Devriendt, & Dierickx, 2013). A candidate-gene strategy applied to WES would likely be the most relevant, with WES data being filtered through a fixed list of candidate genes, list that can evolve with biological knowledge. Clinical guidelines have still to be defined.

5 | CONCLUDING REMARKS

Our study supports WES as an efficient tool for studying non-syndromic familial CLPs, as up to 10% of syndromic gene mutation may contribute to non-syndromic CLP. WES can thereby provide a precise diagnosis, accurate risk assessment and help genetic counselling. It also emphasizes the important collaboration between clinicians and researchers to refine clinical entities and further improve our understanding of the genetic heterogeneity underlying non-syndromic CLP.

CONTRIBUTORS

BD and MV prepared the manuscript. BD performed patient selection for WES, WES data analysis and validation/co-segregation of identified variants. BD, NR, and BB were in charge of enrolment of subjects and collecting samples. RH helped in bioinformatics analyses of WES data. BD, NR, BD, GF, and BB collected the clinical data. All authors contributed to re-drafting of the manuscript. MV conceived and

coordinated the project and is responsible for the overall content. All authors have seen and approved the final manuscript.

COMPETING INTERESTS

None declared.

ACKNOWLEDGMENTS

We are grateful to all the family members for their invaluable contributions. The authors thank the Genomics Platform of Université catholique de Louvain for Next Generation Sequencing, la Biobanque de Picardie -BB-0033-00017-, and the Foundation against Cancer, Belgium.

WEB RESOURCES

Highlander: http://sites.uclouvain.be/highlander/

Online Mendelian Inheritance in Man (OMIM): http://www.omim.org

1,000 Genomes: http://www.1000genomes.org/.

Genome of the Netherlands (GoNL): http://www.nlgenome.nl/.

NHLBI Exome Sequencing Project (ESP): http://evs.gs.washington.edu/EVS/

Exome Aggregation Consortium (ExAC): http://exac.broadinstitute.org/.

Genome Aggregation Database (gnomAD): http://gnomad.broadinstitute.org/.

The Human Gene Mutation Database (HMGD): http://www.hgmd.cf.ac.uk/.

Polyphen2: http://genetics.bwh.harvard.edu/pph2/

SIFT: http://sift.jcvi.org/.

Mutation Taster: http://www.mutationtaster.org/.

Mutation Assessor: http://mutationassessor.org/.

Functional Analysis through Hidden Markov Models (FATHMM): http://fathmm.biocompute.org.uk/.

denovo-db: http://denovo-db.gs.washington.edu/denovo-db/

Mutalyzer: https://mutalyzer.nl/.

ORCID

Bénédicte Demeer https://orcid.org/0000-0001-5266-7710

REFERENCES

Andreou, A. M., Pauws, E., Jones, M. C., Singh, M. K., Bussen, M., Doudney, K., … Stanier, P. (2007). TBX22 missense mutations found in patients with X-linked cleft palate affect DNA binding, sumoylation, and transcriptional repression. *American Journal of Human Genetics*, *81*(4), 700–712. https://doi.org/10.1086/521033

Bamshad, M., Lin, R. C., Law, D. J., Watkins, W. C., Krakowiak, P. A., Moore, M. E., … Jorde, L. B. (1997). Mutations in human TBX3 alter limb, apocrine and genital development in ulnar-mammary syndrome. *Nature Genetics*, *16*(3), 311–315. https://doi.org/10.1038/ng0797-311

Basha, M., Demeer, B., Revencu, N., Helaers, R., Theys, S., Bou Saba, S., … Vikkula, M. (2018). Whole exome sequencing identifies mutations in 10% of patients with familial non-syndromic cleft lip and/or palate in genes mutated in well-known syndromes. *Journal of Medical Genetics*, *55*, 449–458. https://doi.org/10.1136/jmedgenet-2017-105110

Basson, C. T., Bachinsky, D. R., Lin, R. C., Levi, T., Elkins, J. A., Soults, J., … Seidman, C. E. (1997). Mutations in human TBX5 [corrected] cause limb and cardiac malformation in Holt-Oram syndrome. *Nature Genetics*, *15*(1), 30–35. https://doi.org/10.1038/ng0197-30

Braybrook, C., Doudney, K., Marcano, A. C., Arnason, A., Bjornsson, A., Patton, M. A., … Stanier, P. (2001). The T-box transcription factor gene TBX22 is mutated in X-linked cleft palate and ankyloglossia. *Nature Genetics*, *29*(2), 179–183. https://doi.org/10.1038/ng730

Braybrook, C., Lisgo, S., Doudney, K., Henderson, D., Marcano, A. C., Strachan, T., … Lindsay, S. (2002). Craniofacial expression of human and murine TBX22 correlates with the cleft palate and ankyloglossia phenotype observed in CPX patients. *Human Molecular Genetics*, *11*(22), 2793–2804.

Burrow, T. A., Saal, H. M., de Alarcon, A., Martin, L. J., Cotton, R. T., & Hopkin, R. J. (2009). Characterization of congenital anomalies in individuals with choanal atresia. *Archives of Otolaryngology – Head & Neck Surgery*, *135*(6), 543–547. https://doi.org/10.1001/archoto.2009.53

Chaabouni, M., Smaoui, N., Benneji, N., M'Rad, R., Jemaa, L. B., Hachicha, S., & Chaabouni, H. (2005). Mutation analysis of TBX22 reveals new mutation in Tunisian CPX family. *Clinical Dysmorphology*, *14*(1), 23–25.

Christenhusz, G. M., Devriendt, K., & Dierickx, K. (2013). Disclosing incidental findings in genetics contexts: A review of the empirical ethical research. *European Journal of Medical Genetics*, *56*(10), 529–540. https://doi.org/10.1016/j.ejmg.2013.08.006

Conte, F., Oti, M., Dixon, J., Carels, C. E., Rubini, M., & Zhou, H. (2016). Systematic analysis of copy number variants of a large cohort of orofacial cleft patients identifies candidate genes for orofacial clefts. *Human Genetics*, *135*(1), 41–59. https://doi.org/10.1007/s00439-015-1606-x

Desmyter, L., Ghassibe, M., Revencu, N., Boute, O., Lees, M., Francois, G., … Vikkula, M. (2010). IRF6 screening of syndromic and a priori non-syndromic cleft lip and palate patients: Identification of a new type of minor VWS sign. *Mol Syndromol*, *1*(2), 67–74. https://doi.org/10.1159/000313786

Fogh-Andersen, P. (1942). *Inheritance of harelip and cleft palate; contribution to the elucidation of the etiology of the congenital clefts of the face*. Copenhagen: Afhandling - Copenhagen.

Fraser, F. C. (1955). Thoughts on the etiology of clefts of the palate and lip. *Acta Genetica et Statistica Medica*, *5*(4), 358–369.

Frischmeyer, P. A., & Dietz, H. C. (1999). Nonsense-mediated mRNA decay in health and disease. *Human Molecular Genetics*, *8*(10), 1893–1900.

Jugessur, A., Farlie, P. G., & Kilpatrick, N. (2009). The genetics of isolated orofacial clefts: From genotypes to subphenotypes. *Oral Diseases*, *15*(7), 437–453. https://doi.org/10.1111/j.1601-0825.2009.01577.x

Kaewkhampa, A., Jotikasthira, D., Malaivijitnond, S., & Kantaputra, P. (2012). TBX22 mutation associated with cleft lip/palate, hypodontia, and limb anomaly. *The Cleft Palate-Craniofacial Journal*, *49*(2), 240–244. https://doi.org/10.1597/10-208

Kantaputra, P. N., Paramee, M., Kaewkhampa, A., Hoshino, A., Lees, M., McEntagart, M., … Stanier, P. (2011). Cleft lip with cleft palate, ankyloglossia, and hypodontia are associated with TBX22 mutations. *Journal of Dental Research*, *90*(4), 450–455. https://doi.org/10.1177/0022034510391052

Keller, J. L., & Kacker, A. (2000). Choanal atresia, CHARGE association, and congenital nasal stenosis. *Otolaryngologic Clinics of North America*, *33*(6), 1343–1351 viii.

Kondo, S., Schutte, B. C., Richardson, R. J., Bjork, B. C., Knight, A. S., Watanabe, Y., … Murray, J. C. (2002). Mutations in IRF6 cause Van der Woude and popliteal pterygium syndromes. *Nature Genetics*, *32*(2), 285–289. https://doi.org/10.1038/ng985

Kousa, Y. A., Mansour, T. A., Seada, H., Matoo, S., & Schutte, B. C. (2017). Shared molecular networks in orofacial and neural tube development. *Birth Defects Res*, *109*(2), 169–179. https://doi.org/10.1002/bdra.23598

Leslie, E. J., Mansilla, M. A., Biggs, L. C., Schuette, K., Bullard, S., Cooper, M., … Murray, J. C. (2012). Expression and mutation analyses implicate ARHGAP29 as the etiologic gene for the cleft lip with or without cleft palate locus identified by genome-wide association on chromosome 1p22. *Birth Defects Research. Part A, Clinical and Molecular Teratology*, *94*(11), 934–942. https://doi.org/10.1002/bdra.23076

Leslie, E. J., & Murray, J. C. (2013). Evaluating rare coding variants as contributing causes to non-syndromic cleft lip and palate. *Clinical Genetics*, *84*(5), 496–500. https://doi.org/10.1111/cge.12018

Lough, K. J., Byrd, K. M., Spitzer, D. C., & Williams, S. E. (2017). Closing the gap: Mouse models to study adhesion in secondary palatogenesis. *Journal of Dental Research*, *96*(11), 1210–1220. https://doi.org/10.1177/0022034517726284

Marcano, A. C., Doudney, K., Braybrook, C., Squires, R., Patton, M. A., Lees, M. M., … Stanier, P. (2004). TBX22 mutations are a frequent cause of cleft palate. *Journal of Medical Genetics*, *41*(1), 68–74.

Mossey, P. A., & Modell, B. (2012). Epidemiology of oral clefts 2012: An international perspective. *Frontiers of Oral Biology*, *16*, 1–18. https://doi.org/10.1159/000337464

Pauws, E., Hoshino, A., Bentley, L., Prajapati, S., Keller, C., Hammond, P., … Stanier, P. (2009). Tbx22null mice have a submucous cleft palate due to reduced palatal bone formation and also display ankyloglossia and choanal atresia phenotypes. *Human Molecular Genetics*, *18*(21), 4171–4179. https://doi.org/10.1093/hmg/ddp368

Pauws, E., Peskett, E., Boissin, C., Hoshino, A., Mengrelis, K., Carta, E., … Stanier, P. (2013). X-linked CHARGE-like Abruzzo-Erickson syndrome and classic cleft palate with ankyloglossia result from TBX22 splicing mutations. *Clinical Genetics*, *83*(4), 352–358. https://doi.org/10.1111/j.1399-0004.2012.01930.x

Peyrard-Janvid, M., Leslie, E. J., Kousa, Y. A., Smith, T. L., Dunnwald, M., Magnusson, M., … Schutte, B. C. (2014). Dominant mutations in GRHL3 cause Van der Woude syndrome and disrupt oral periderm development. *American Journal of Human Genetics*, *94*(1), 23–32. https://doi.org/10.1016/j.ajhg.2013.11.009

Schliekelman, P., & Slatkin, M. (2002). Multiplex relative risk and estimation of the number of loci underlying an inherited disease. *American Journal of Human Genetics*, *71*(6), 1369–1385. https://doi.org/10.1086/344779

Stanier, P., & Moore, G. E. (2004). Genetics of cleft lip and palate: Syndromic genes contribute to the incidence of non-syndromic clefts. *Hum Mol Genet*, *13 Spec*, *1*, R73–R81. https://doi.org/10.1093/hmg/ddh052

Suphapeetiporn, K., Tongkobpetch, S., Siriwan, P., & Shotelersuk, V. (2007). TBX22 mutations are a frequent cause of non-syndromic cleft palate in the Thai population. *Clinical Genetics*, *72*(5), 478–483. https://doi.org/10.1111/j.1399-0004.2007.00891.x

How to cite this article: Demeer B, Revencu N, Helaers R, et al. Unmasking familial CPX by WES and identification of novel clinical signs. *Am J Med Genet Part A*. 2018;176A:2661–2667. https://doi.org/10.1002/ajmg.a.40630